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SECOND SYMPOSIUM ON THE ROLE OF THE VESTIBULAR ORGANS IN SPACE EXPLORATION

AMES RESEARCH CENTER

Moffett Field, California

JANUARY 25-27, 1966

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION

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SECOND SYMPOSIUM ON

THE ROLE OF THE VESTIBULAR ORGANS IN SPACE EXPLORATION

Held under the auspices of The National Academy of Sciences — National Research Council Committee on Hearing, Bioacoustics, and Biomechanics

Ames Research Center
Moffett Field, California
January 25-27, 1966

General Chairman: JORGE HUERTAS
AMES RESEARCH CENTER
Program Chairman: ASHTON GRAYBIEL
U.S. NAVAL AEROSPACE MEDICAL INSTITUTE

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Foreword

These proceedings are the record of the second symposium of a series on gravitoinertial receptor mechanisms and related systems in aerospace flight and add to information presented in the proceedings of the first symposium, NASA SP–77, entitled "The Role of the Vestibular Organs in the Exploration of Space." Symposiums to follow will at regular intervals update information in this important field of research. The long-range plan is thus apparent: to collect in one series of reports complete and current information dealing with gravitoinertial receptor mechanisms and related systems in aerospace flight.

In this volume the reports follow the order of presentation in the meeting, thereby preserving the proper continuity of the discussions. This order, which was mainly for the benefit of the speakers, tends to mask somewhat the three central themes underlying the plan for the conference, which were: (1) the presentation of practical problems posed by weightlessness and subgravity states, including the need for artificial gravity as it might be revealed by exposing animals to weightlessness over extended periods of time; (2) up-to-date review articles to be presented by outstanding authorities in their subspecialties; and (3) brief reports on current investigations with special emphasis on the genesis of "vestibular" nystagmus when animal or human subjects are exposed to certain patterns of linear accelerations and the possibly related phenomenon of modulation of canalicular nystagmus by linear accelerative forces.

On the basis of experience, nearly as much time was allocated for discussion as for the presentation of papers. In editing the discussions—as a whole, it was even more apparent than in the meeting—that at times the dialog reached an unusually high order of excellence. Many ideas were presented and discussed which had not previously found their way into the scientific literature. Moreover, it became apparent that a number of participants, not listed as speakers, came prepared to make brief yet formal presentations. Although the relevance and importance of these presentations varied, the overall contribution was sufficiently great to suggest it should not be completely discouraged. In the future, an attempt will be made to allocate additional time for a limited number of such presentations.

The great majority of those in attendance could be divided into two groups; namely, those whose primary interest centered in the vestibular mechanisms, and those for whom an understanding of these mechanisms was essential yet only incidental to their main interests. The interaction between these groups was far from that of teacher and pupil. The "users" not only were articulate in describing man as an element in a closed-loop control system but also, implicitly at least, challenged the vestibular specialists to demonstrate the role played by these organs in real-life situations. This points up a great gap in our knowledge which Brodal emphasized in stating:

> As far as the vestibular mechanisms are concerned, it is probably justified to say that recent research has demonstrated structural peculiarities and differentiations in the

sense organs and their central connections which go far beyond what can at present be properly correlated with observations of function.

But this is by no means the only gap. Much of the investigative work on the vestibular organs has been carried out on animals where, in contrast to man, the vestibular system is more highly developed than the pyramidal system. This points up the need to conduct studies on man, where experimentation is notably difficult. This is illustrated by the fact that we still lack such a basic prerequisite as precise, valid tests of function of the canals and otolith organs.

The success of this symposium was insured by the enlightened self-interest of the Office of Advanced Research and Technology of the National Aeronautics and Space Administration, implemented by the resourcefulness of the National Academy of Sciences. The splendid efforts of the general chairman, Jorge Huertas, combined with the politeness and generosity of our hosts at the Ames Research Laboratory, were beyond what we had a right to expect. As on the occasion of the first symposium, the spirit displayed by the speakers, chairmen, and audience was evidenced in a manner and degree rarely observed in meetings of this nature.

ASHTON GRAYBIEL
U.S. Naval Aerospace Medical Institute
U.S. Naval Aviation Medical Center

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Chairman: **Henning E. von Gierke**
Cochairman: **William E. Collins**

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Chairman: **Charles F. Gell**
Cochairman: **William M. Helvey**

Welcome

WALTON L. JONES

In behalf of NASA Headquarters and specifically the Office of Advanced Research and Technology, I welcome you to "The Workshop on Orientation in the Exploration of Space." At this time, I would like to extend the regrets of Dr. Mac C. Adams, Associate Administrator of the Office of Advanced Research and Technology of NASA, for being unable to attend and greet you in person. Dr. Adams was eagerly looking forward to participating in these deliberations, as he realizes the necessity of solving the potential problems of man for future extended flights in space. He is also aware that your area of research, namely, orientation in space, is one of prime importance to the success of any future long-term space missions. We are delighted that CHABA of the National Academy selected Ames as the location for this workshop conference. We are proud of our new life-science facilities here, and hope that you take the opportunity to examine them during your stay.

As you all know, last year NASA sponsored a Vestibular Symposium at the U.S. Naval Aerospace Medical Institute at Pensacola, under Dr. Graybiel. As a result of that excellent meeting, it seemed advisable to hold a sequel and today's workshop conference materialized.

NASA is interested in vestibular problems in two categories; namely, the prevention of vestibular disturbances in weightlessness, and the possible need to generate an artificial gravity. There are other scientific aspects in which we are interested also.

The potentially disturbing symptoms which can be experienced in long exposure to weightlessness require much detailed study. I am sure that the new disciplines and techniques available to us will be a major factor in solving these problems. However, the experts in this most intricate and difficult field are our chief hope in providing solutions to these problems. In spite of the excellent ground-based research which has been carried on, we will not be absolutely sure of the solutions until we have conducted appropriate experiments in orbit under weightless conditions for a considerable period of time. I would like to stress the importance of flight experiments, an area which is discussed in Session VI. We are tailoring our research so that wherever weightlessness is a factor in a particular problem area, the solution will be absolutely proven in flight under weightless conditions. Some of the concrete results we are seeking from this conference are ideas for good flight projects required for the vestibular problem area.

The second problem which arises in this research area is the possibility that artificial gravity will be required. We would like to know what provisions should be made if this possibility becomes a necessity.

We understand that in order to solve the many problems in this area, a great deal of basic and applied research must be conducted. We are in favor of this and are backing this research with encouragement and available funds. We would also appreciate consideration of some of our additional needs. At certain points in time, we must provide our engineers with data in order for them to plan and fabricate spacecraft and equipment for flight. Thus, the need for engineering data should be considered in planning research. Ultimately, the practical significance of the results of research in this field will be realized in the form of good useful engineering data.

At this time, I would like to thank the CHABA Committee for the fine job they have done in organizing this conference. I am sure their efforts will be more than rewarded by the results of the meeting.

I wish you every success in your deliberations.

SESSION I

Chairman: **CHARLES A. BERRY**
Manned Spacecraft Center, NASA

Cochairman: **ALLEN B. THOMPSON**
General Electric Co.

Russian Experience of Problems in Vestibular Physiology Related to the Space Environment

JOHN BILLINGHAM

Ames Research Center, NASA

This brief review concerns the biomedical findings, in animals and man, obtained during Russian space flights and related aircraft and laboratory experiments. It concentrates on the problems of weightlessness and motion sickness and attempts a comparison of Russian with American experience, particularly with regard to man. It examines briefly the need for further biomedical experiments in space in this area and concludes with a discussion of requirements for selection and training of astronauts.

Work with animals by Russian investigators may be divided into two groups. The first group deals with experimental results from prolonged alterations of acceleration applied to the body. This includes exposure of animals to prolonged weightlessness in orbital flight and to prolonged accelerations of more than 1 g on the centrifuge. The second part refers to the short-term experiments carried out on board aircraft in parabolic flight.

With regard to the first group, it was established by Gyurdzhian (1964) that rats bred under conditions of prolonged acceleration on the centrifuge exhibit a significant reduction in the reaction of limb-extensor muscles, as measured by the electromyograph, to an oscillating stimulus with the axis parallel to the long axis of the body at a frequency of 0.6 cycle per second and an amplitude of $\pm 25°$. The recordings indicated a diminished intensity of the EMG and a delay in its appearance. In some cases the change in EMG signal after the onset of oscillation did not appear until two or three cycles had been completed. In contrast, the experiments on a guinea pig flown on the fourth experimental Russian vehicle indicated that there was an increase, after the flight, in spontaneous activity of the hind-limb antigravity muscles. Using vestibular stimulation similar to that used for the rats, the EMG records now showed an increased intensity of response and a decrease in the latent period of its appearance compared with controls which were kept at the launch site and not subjected to weightlessness. These experiments seem to indicate a clear increase in the sensitivity of the vestibular system after reduced g and a decrease in sensitivity after increased g. It is interesting that in the case of the guinea pig, the augmented extensor responses were maintained for a period of many days after exposure to weightlessness.

Many other observations have been made by Russian investigators on animals during the orbital-flight series preceding their first manned space flights. Much of this information is not relevant to vestibular physiology, but some of the observations agree with the results described above in that they tend to indicate an increased sensitivity of the animal's orientation mechanisms; for example, in the case of a dog, it was observed that the feet, when touching the floor of the vehicle, immediately produced a profound rigid extensor response in all four limbs, with the feet subsequently remaining firmly applied to the floor. This reaction is what one might expect, since in essence the condition is that fleetingly experienced by the dog which is dropped from a certain height so that it passes through a period of transient weightlessness in

a 1-g field at the same time as its paws hit the ground. In the space environment this dynamic response could be viewed as having been frozen.

A number of Russian investigators have reported effects on different types of animals of exposure to weightlessness in a parabolic flight trajectory in aircraft. Many of the results are similar to those obtained by American investigators using this procedure. In general, during the period of weightlessness the animals exhibited a mixture of chaotic rotation about various axes and sustained gliding-type movements. Rats and mice seemed to exhibit the worst disturbance. Cats varied considerably, while dogs appeared to be the most stable and adapted the most rapidly. It is possible that this sequence reflects an increase in the ability to control the vestibular system by a learning process in higher centers. Birds appeared initially to be trying to fly in an upward direction, but rapidly adapted and then appeared to be flying in a forward direction. Few details of these experiments were available and the results for birds should be interpreted with caution since this experiment must be extremely difficult to perform in a confined space.

Yuganov (1964) also examined the effects of exposing chronically labyrinthectomized mice to the weightless condition. He found that in all cases they exhibited a much more stable pattern of motor behavior than did normals. There was little of the chaotic rotation seen with normal animals, and the mice tended to glide from one position to another and to respond much more normally to contact with a wall when it occurred. During this series of experiments, Yuganov also set up an animal centrifuge on board the aircraft and examined the normal and labyrinthectomized mice on the centrifuge. He found that 0.3 g of radial acceleration on the centrifuge sufficed to bring the motor activity of the animal down to a level which was virtually the same as that of normal controls on the ground in a 1-g field. However, the labyrinthectomized animals were able to move about in a coordinated fashion at 0.1 g. This result might have been expected in view of the generally recognized improvement of proprioceptive, visual, and skin-receptor information with regard to position sense in labyrinthectomized animals.

In general, Russian experience with exposure of man to short periods of weightlessness during parabolic flight has been very similar to that obtained by American investigators. They divide their subjects into three categories according to the following gross reactions to the stimulus:

(1) A general lack of response to the stimulus;
(2) Development of specific illusions generally similar to those described in American literature as the oculogravic illusion;
(3) A category where there was an immediate induction of motion sickness.

This classification is very similar to that adopted by Gerathewohl, although detailed statistics, as in the case of many Russian reports, are few.

Yuganov (1964) has examined the characteristics of the reaction of subjects to vestibular stimulation during weightless flight in a paper entitled "The Problem of Functional Characteristics and Interaction of the Otolithic and Cupular Portions of the Vestibular Apparatus Under Altered Gravity." During parabolic flights he found that postrotatory nystagmus, occurring as a result of rotation during the period of weightlessness, was significantly less than under 1 g, and that the counterrotation illusion was also less. Yuganov concludes that an analysis of his data "indicated weightlessness does not lead to functional elimination but to specific vegetative interaction depending on the g-forces." For example, a lessening of the g-force retards the nystagmic reaction.

Turning to the Russian experience with their cosmonauts during orbital flight, it is very clear that they have now experienced the development of a form of motion sickness in three of the cosmonauts: Titov in the Vostok II, and Feoktistov and Yegorov in the three-man Voskhod flight.

The details of the Titov episode are now very well known. The significant findings were as follows: Immediately after launch-vehicle ac-

celeration ceased, surrounding objects appeared to float upward, and only after 1 or 2 minutes did they seem to return to their normal place. This is a straightforward example of the oculogravic illusion. A second significant feature was that Titov felt that he was in the head-down or inverted position. This illusion has been described by many cosmonauts and astronauts and clearly there is wide variation from person to person. It is probably dependent at least in part on the otolith mechanism and will be discussed in more detail below. However, the most significant finding during Titov's flight was the appearance during the fifth or sixth orbit of an unpleasant sensation resembling motion sickness, with giddiness and nausea, both of which were increased during sharp movements of the head. Appetite decreased, and the sensation was considerably limited by keeping the head still. It was reduced after a period of sleep and disappeared completely after the beginning of the reentry g-forces.

There was little question but that the symptoms were very similar to those of vestibular sickness, and the main question was whether the symptom complex had developed as a result of Coriolis forces induced by head movements in a spinning vehicle or by a conflict in the central nervous system, developing as a result of the abnormal input from the otolith during the weightless period. At the International Symposium on Basic Environmental Problems of Man in Space held in October 1962 in Paris, the reports of the discussions, following a paper by Yemelyanov et al. on "Problems Concerning the Interplay of Physiological Sensing Mechanisms During Space Flight," indicated that those attending the conference also had this question uppermost in their minds and were attempting to find out what the rate of rotation of the Vostok spaceship was. In answer to questions by Lovelace and Rose, Gurjian indicated categorically that the vehicle was stabilized. Subsequent evidence from other quarters indicates in fact there was probably a very slow rotation rate. In a paper by Lebedensky et al., entitled "Autonomic Nervous System Responses From the Stimulation of the Vestibular Analyzer and Their Possible Role in Complicating Space Flight Conditions," subjects were exposed in a slowly rotating chamber (5.3° per second) for 1 to 5 hours. The subjects experienced no vestibular-vegetative disorders and the authors conclude:

> This gives us the basis for supposing that possibly both the time characteristics of the stimulus and the absence of stimulation of the otolith by the Earth's gravitational pull under conditions of long-term weightlessness could have played a part in the occurrence of G. S. Titov's vestibular-vegetative disorder.

This report might indicate that the Vostok vehicle was in fact rotating but at a low angular velocity and that the Russian conclusion at this time was that they had indeed experienced vestibular sickness in weightless flight as a result of the altered inputs from the otolith.

Following the Titov flight, the Russians went to some length to obtain improved measures of physiological functions relating to the vestibular system during their orbital flights. They added a number of tests to the inflight biomedical test program and added physiological instrumentation such as the EOG and the EEG. Finger-nose tests, outstretched arms tests, writing tests, and the results of different types of body and head movement were examined. In the two subsequent flights by Bykovsky and Tereshkova, there were no vestibular abnormalities of comparable significance to those exhibited by Titov. In some cases, the measurements actually indicated a better performance, as in the case of some of the writing tests, than under a 1-g acceleration, although it is doubtful that this change is significantly related to the change in the force environment. At the same time, the Russians stepped up their cosmonaut training and instituted "a program of special vestibular training after the flight of G. S. Titov, who experienced autonomic maladjustment." The purpose of the training was to provide data for future criteria for selection or elimination of cosmonauts and to increase the resistance of the central nervous system to conflicting inputs from different sensory organs, particularly those of the vestibular system. The specific aim was "to reinforce the functional interaction of vestibular, visual, and kinesthetic analyzers to eliminate postural-spatial illusions in altered g and increase inhibition of the vestibular function."

It was also stated that this training would be custom tailored for individual weaknesses.

In the meantime, the American orbital flights of the Mercury series were completed with a complete absence of any symptoms of vestibular sickness in the astronauts.

On October 12, 1964, the U.S.S.R. launched the Voskhod spaceship with a crew of three. For the first time a scientist and a physician were carried on board a space vehicle during orbital flight. The crew consisted of Komarov, commander and pilot; Feoktistov, scientist; and Yugarov, physician. In most respects, the flight appeared to be normal and some of the achievements were as follows:

> A more comprehensive and reliable life-support system has been developed and tested in flight. It permitted the crewmembers to manage without any spacesuits and to work in light sport suits. The ship's soft-landing system guaranteeing safe return of the crew to the Earth has been created and tested.

With a physician on board, an increased number of medical tests were planned, including "investigations of the functional condition of the analyzers in weightlessness." Electroencephalograms, electro-oculograms, and dynamograms and indexes characterizing the coordination of movement were recorded during the flight. The report on the development of vestibular symptoms is worth quoting in full:

> With the placing into orbit the feeling of easiness appeared and the nervous and psychic distress decreased, at the same time self-observations of the cosmonauts confirmed that the complex of the flight factors specifically affects the state of the statokinetic analyzer. This was expressed in illusory conceptions on the spatial position of the body and in sensory and vegetative reactions appearing during sharp movements of the head (Yugarov and Feoktistov). They noted the illusion of the body position turned over, both when their eyes were opened and when they were closed in the whole period of weightlessness, up to the beginning of the effect of acceleration during the reentry. Alongside illusions, especially in the middle of the flight, an unpleasant sensation of slight short-time giddiness was observed when the head was turned sharply (Feoktistov and Yugarov). In this connection, while performing working operations, the space pilots tried to make motions more smoothly than under conventional ground conditions. Of great importance was this observation of the astronauts to the effect that the character and degree of illusions and giddiness were equally pronounced in free flight and during stabilization of the ship. After 1½ to 2 hours of the flight, Yugarov noted the first signs of vestibular-vegetative reactions expressed in a decrease of appetite and in unpleasant sensations in the pit of the stomach, which are regarded by him as the first symptoms of nausea. These phenomena were most pronounced on the fifth orbit of the flight. Feoktistov noted similar symptoms, but they were less pronounced. After sleep, the vestibular-vegetative syndrome vanished almost completely and the space pilots actively continued to fulfill their flight program. On the basis of these data, Yugarov considers weightlessness one of the unfavorable factors of space flight requiring serious study by physicians. Analysis of fulfilling the flight assignment and of individual elements of labor activity showed that Yugarov's performance was somewhat reduced during the orbital flight. To conduct active experimental work, Feoktistov was to spare much greater nervous and physical efforts than under ground conditions. During the whole flight Komarov's performance was at a high level.

With regard to physiological measurements:

> While conducting special vestibulometric investigations, no changes in the sensitivity thresholds of the otolith apparatus to galvanic current were observed. The results of demonstrative and graphic samples have shown a decrease in the accuracy of performing fine coordinated movements under weightlessness which apparently may be regarded partly as the consequence of the change in the functional state of the vestibular analyzer. Postflight investigations did not reveal any substantial changes in the function of the vestibular analyzer.

Postflight examination, insofar as it is reported, did not show any abnormalities with regard to the vestibular system. The conclusion of the Russian authors is—

> the data obtained in the course of the flight have confirmed that the weightless condition can be accompanied by unfavorable reactions which worsen the cosmonaut's general state of health and performance. At the same time, the fact has been confirmed once more that these reactions exhibit themselves in a different degree, depending on the individual features and training of man. Some specific features of the reaction of the cardiovascular system and of the respiratory system were recorded which complement the known data on the influence of the flight factors on an organism. Analysis of the totality of the data obtained in this flight has shown that the physiology of prolonged weightlessness alongside the problem of life support at present continue to be important problems which should be closely investigated.

Further details of physiological measurements made before and during the flight are reported by Yuganov et al., in Aerospace Medicine, July 1966.

It is fairly clear that the Russians have now experienced three cases of a syndrome resembling motion sickness during orbital flight in space vehicles. It is apparent that the program of special vestibular training introduced after Titov's flight has not succeeded in preventing the occurrence of this syndrome. It is of great interest to note that the symptoms appeared in the two crewmembers who were not experienced as pilots and not in the crewmembers who were experienced. Subsequent flights of American astronauts in the Gemini series have indicated a complete absence of the syndrome in all cases.

There seems to be no question but that the development of sickness in response to the abnormal combination of sensory inputs during weightlessness can occur in certain individuals. It is further very apparent, not only from the results of the orbital flights but also from work in various vestibular physiology laboratories, that there is a wide individual variation in the probability of the development of vestibular sickness when induced by different abnormal sensory cues in different individuals. Perhaps the most important question today is the nature of the attempt that should be made further to understand the complex central-nervous-system processing of information relating to man's position in space and the concomitant attempt to develop some way of predicting whether or not a given individual will develop the space vestibular-sickness syndrome during weightless flight. Graybiel has argued for a comprehensive clinical-physiological examination, including the answering of a questionnaire so that we may be in full possession of all available information on the vestibular systems of individual astronauts. In view of the Russian results described above and of the coming use of scientists as astronauts in the American space program, it may be prudent to incorporate such an examination into the routine medical examination of U.S. astronauts, not as a method of selection but as a means of obtaining physiological baseline data against which inflight and postflight measurements may subsequently be compared.

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DISCUSSION

MAYNE: We have developed a theory of otolith functions which seems to account for the disturbances experienced by astronauts in space. This theory will be covered in one of our reports to NASA. I am not prepared to discuss it here, but will say that it is related to the function normally performed by the vestibular system in separating acceleration from velocity when man is transferred without previous adaptation to an environment other than 1 g.

GUEDRY: I didn't understand something you said concerning the exaggerated acceleration the Russians experienced on reentry. Was it an experience of velocity or pressure?

BILLINGHAM: First of all, the unpleasant sensations experienced by those Russians who got syndromes resembling motion sickness disappeared when the reentry g load was imposed. Secondly, the subjective sensation of acceleration acting on the body seemed in our own astronauts to be considerably greater than they would have expected from such an acceleration, knowing what such an acceleration is.

GUEDRY: When they say that this acceleration experience is exaggerated, do they feel that the weight is greater than they remembered experiencing for comparable g-loads in centrifuges or do they feel a greater velocity during this changing acceleration than they anticipated?

BERRY: It is a sensation that has been described by the men on all the long-duration flights. They anticipate the total g load they are going to get, and still they feel they are at a higher g level initially; however, their total response to the peak g load is no different from that of their training periods.

BENSON: At the 1964 COSPAR meeting it was said that Tereshkova had nystagmus from the 38th to 45th orbit, and they produced an illustration which purported to show it. Have you any more information about this?

BILLINGHAM: None. I have seen this observation recorded. They dismissed it as being of no significance, and I have no more evidence or information about it.

GUALTIEROTTI: There is one point here which I think is very important. There is a difference in the reports from Russian and American astronauts. It seems that Russian astronauts occasionally had vestibular symptoms, while the American astronauts did not. I wonder if this is due to one of these two factors: Either the American astronauts who are jet pilots have been chosen on the basis of nonsusceptibility to seasickness, or it is a question of training. The Russians, especially, observed vestibular symptoms in personnel who were not trained. Titov is an exception, of course. The other two were a scientist and a physician who didn't have the qualifications of a jet pilot. Do you think the reason American astronauts have not suffered from vestibular symptoms is due to the fact they have been chosen particularly on the basis of immunity from motion sickness? For instance, they didn't appear to experience the Coriolis illusion during turning of the spacecraft. We know that is a common symptom. Is that because they had been selected as particularly stable against this, or is it a question of training? This is important in a way, because in the future we must think of a space flight made by nonpilot personnel, up to a point. I think it is very important that we have a way of determining whether persons who have not been trained for it can sustain space flight without excess vestibular symptoms.

BILLINGHAM: I think the point about the Coriolis forces is probably not too relevant here. The rates of rotation were sufficiently low so that it would have been improbable that anybody would have developed a Coriolis illusion. There does seem to be a point that a man who is not a trained jet pilot may be more susceptible to development of this type of sickness than a man who is trained.

GUALTIEROTTI: My main point is whether the jet pilot is specially chosen among those who are particularly resistant to vestibular symptoms. Is this aspect considered in the training or in the examination of the jet pilot or cosmonaut?

BILLINGHAM: I am not really qualified to answer that question.

BERRY: The astronauts we have at the present time have been given vestibular examinations which are of the standard type. We have done nothing magic to allow us to pick some who do not respond to vestibular influences. I believe our vestibular physiologists or ENT men will certainly agree with that. I don't know of anything particular we have done to select out, other than the fact that all American astronauts are jet pilots. Each one who, to date, has flown a space mission has been a jet test pilot. They are constantly flying aircraft, of course. I, too, would like to know why they are different, as there is indeed some difference. Training is not the whole answer because, again, let me say, they do not undergo any particular training. We have seen no reason whatsoever to do any sort of vestibular training of our men because we have never had that type of problem. The Russians, on the other hand, have spent a good deal of time training their cosmonauts and they still have a problem. I would be delighted to hear some answer.

GUALTIEROTTI: Except Titov. It might be just a casual thing, but the other two were not jet pilots.

BERRY: I do not know how much training the scientist and physician had. The Russians have exposed their men to swings and other similar apparatus, but we have not done so. I know that, in Titov's instance, he did not have the same number of flying hours in jet aircraft that our crews have had. I think that is interesting. In a talk last year (1965) in Athens with our Russian counterparts, Astronauts Conrad and Cooper and I learned the cosmonauts were mildly impressed with the fact that the vestibular aspect had gotten completely out of hand as far as their particular training was concerned. They felt Titov was the cause of this. Ever since he experienced "motion sickness" it seemed that undue emphasis was being placed in their training procedures. They thought this was all to naught. We did not question them about this; they volunteered the information. They said their entire medical orientations had been focused on the ear ever since the Titov experience. Of course, it was not helped, I imagine, by the first Voskhod flight either.

BILLINGHAM: The experience of Titov is the one argument, Dr. Gualtierotti, which is opposite to that which states persons who are not trained as jet pilots are particularly susceptible. It appears as if jet-pilot training may well serve as a much better prior experience criterion for selection than no jet-pilot training. I think this is about as much as one can say at the moment.

LOWENSTEIN: May I come back to the head-down illusion? The normal position and the head-down position are, so far as the utriculus is concerned, posi-

tions of sensory ambiguity; that is, the signal coming in from the periphery is very similar and in the head-down position extremely labile. Are there any observations of an oscillation of this illusion in time, as is quite usual with sensory illusions arising from sensory ambiguity? There is a 10-second illusion one way and a 10-second illusion another way; for example, the optical staircase phenomena. I wonder whether this might be a central nervous phenomenon.

BILLINGHAM: I have not seen any report of this, but it is an interesting question.

LI: I would like to return to the question of motion sickness. Did the Russian report point out clearly whether the two scientists were also subjected to motion sickness in other kinds of motion; on the ground, for instance? Are these correlated with their feeling of motion sickness in a weightless environment? In other words, "motion sickness" may require a little more qualification. What kind of motion sickness? Is it due to weightlessness or due to ordinary motion? In addition, there are many kinds of motion. Some people are sensitive to high-frequency motion and others to low-frequency motion. We also mentioned training. Does the training of jet pilots make them immune to a weightless environment or is it just a coincidence that some of them are? If we believe it is training, then we should know how to train them. Should they also be trained in weightlessness? As I remember, when Carpenter stepped out of his Mercury capsule bobbing in the sea, he felt a sort of nausea after he got out. Maybe that was related to a brief period of weightlessness or was merely the result of the bobbing on the sea.

BILLINGHAM: These are some very interesting questions. If it is true that jet pilots and test pilots are the ones least susceptible, I think it is probably the result of two factors: One is automatic selection, because flyers who do develop motion sickness during test flying are automatically eliminated; the second is the training factor. I think it is most unlikely that Feoktistov and Yegorov were subject to ordinary types of motion sickness. Contrary to Titov, they probably had been selected for their resistance to motion sickness. I don't know.

STROUD: There is one other possible factor that might come into the difference between the American and Russian sensitivity to travel sickness. I am referring to what they were actually doing on the space flight. Were the Russians moving around more or was there more vibration on the ship? Did the Russians have more motors and mechanisms on the ship which would cause more vibrations than the Americans were exposed to? Another point occurred to me. The inversion illusion may be due to the utricle, as Dr. Lowenstein mentioned, but a possible mechanism could be that in the normal upright position, the otoliths are pressing down on the utricle, but when the subject is exposed to weightlessness, this would not occur. A similar pattern of impulses from vibrations might possibly arise when the patient is upside down, and again the otoliths are not pressing down on the utricle.

BILLINGHAM: We don't have a complete time history of the complete force environment of all American astronauts and all Russian cosmonauts, but both groups have carried out extensive tests of one sort and another of head movements during weightless situations. I would have to say probably that there was not a great deal of difference except in the three Russian cases where there was a deliberate attempt on the part of the astronauts to limit the rates of movement of the head.

KELLOGG: How many of the cosmonauts experienced the inversion illusion? Do I understand correctly that most of them did?

BILLINGHAM: This is not clear. It was experienced, as I say, by a number in different intensities in each of the flights. They describe it very specifically for Titov and for the Voskhod crew, but in the other cases it is difficult to find out. The reports are in many cases incomplete.

COHEN: I wonder whether the differences in disorientation reported by Americans and by Russians might be somewhat exaggerated by difference in nomenclature usage. It is a rather simple and an obvious point, I think, but should be emphasized. We say that the Americans did not experience any motion sickness, but nonetheless we concede or freely describe the inversion illusion which they did experience. By many criteria, the inversion illusion *is* "disorientation." If by "motion sickness" we mean actual vomiting as a physical endpoint, then we are talking about something different from "disorientation." Maybe you have information as to whether this is also what the Russians meant when they said their cosmonauts experienced "motion sickness." Was there actual vomiting? If we use "disorientation" as a general term, it may well be that our astronauts showed disorientation and so did their cosmonauts. And the difference might not be so large as it first appears.

BILLINGHAM: I don't think there is any question that there is a difference, because of all the other symptoms that went along with whatever you want to call it, illusion or disorientation. The question of whether the inversion illusion should be classified as disorientation is interesting. You could argue for or against this.

GRAYBIEL: We have carried out motion-sickness-susceptibility tests on Navy flight personnel ranging from flight trainees all the way up to test pilots. Susceptibility was greatest in trainees and least in test pilots.

BERRY: That is an interesting point.

The Inversion Illusion in Parabolic Flight: Its Probable Dependence on Otolith Function

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SUMMARY

Observations were made on normal subjects and deaf persons with bilateral labyrinthine defects (L–D subjects) under three different conditions in parabolic flight: (1) free floating, (2) restrained in a Fiberglas mold, and (3) "standing" on the overhead during a modified parabola generating about −0.05-g unit. There were interindividual differences in the reactions among the normal but not among the L–D subjects. Some of the normal but none of the L–D subjects experienced a reversal of their personal orientation with regard to up-down under all three conditions. This "reversal" was considered to have its genesis in the vestibular organs, probably the otolith apparatus. Our findings are in accord with Russian reports describing feelings of inversion among cosmonauts in orbital flight. Attention is called to the necessity of distinguishing between information furnished by touch pressure, kinesthesis, and stereognosis under ordinary conditions and agravic touch pressure, agravic kinesthesis, and agravic stereagnosis.

INTRODUCTION

The twofold purpose of this report is to describe three brief experiments carried out in parabolic flight and to discuss the findings in the light of their possible implications for space flight. The stimulus to initiate these investigations was provided by Lt. B. C. Neider, Jr., USN, who experienced a curious illusion while free floating during parabolic maneuvers. His observations were made, incidentally, during a concurrent experiment in which normal subjects and persons with bilateral labyrinthine defects (L–D subjects) were being tested. Although there was only a limited opportunity to expand the scope of the primary undertaking, it seemed worthwhile to make some comparative observations on the perception of the upright in the normal and L–D subjects.

SUBJECTS AND AIRCRAFT

Four L–D and seven normal subjects participated. All were in excellent general health and perforce had gone through the medical and indoctrinational tests qualifying them for zero-g flights.

The significant clinical findings in the L–D subjects are summarized in table 1. The normal subjects ranged in age from 19 to 38 years. None had any symptoms referable to the organs of the inner ear. All had normal hearing and normal semicircular canal function as deter-

Table 1.—*Clinical Findings in 4 Deaf Subjects With Bilateral Labyrinthine Defects*

Subject	Age	Auricular defects		Hearing [1]		Nystagmus response, caloric test		C–R index [2]	
								Maximum tilt [3]	
		Etiology	Age onset	R	L	R	L	50°	75°
JO	34	Meningitis	7½	Nil	Nil	Nil	Nil	126	176
HA	29	Meningitis	13	Nil	Nil	Nil	Nil	47	53
PE	33	Meningitis	12	Nil	Nil	Nil	Nil	21	30
MY	25	Meningitis	8	Nil	Nil	Nil	Positive [4]	63	82

[1] Response to 160-dB white noise.
[2] One-half the sum of maximum roll right and left (minimum of arc).
[3] Angular displacement of body from vertical in frontal plane.
[4] Irrigation, 40 seconds; water temperature, 7.9° C.

mined by routine tests. Ocular counterrolling, a test of otolith function, was carried out in four of the seven and the findings were normal.

Although all of the subjects had experienced the parabolic maneuver, only three were highly sophisticated and, for convenience, the other eight are referred to as "unsophisticated."

Both the KC–135 (Boeing 707) and C–131B (Convair) planes were used. The flight profile for the KC–135, described elsewhere in detail (ref. 1), consisted essentially of a ballistic trajectory with a weightless period of about 25 to 30 seconds preceded and followed by a pullup, quickly generating about 2.0 g. In the C–131B the weightless period was 12 to 16 seconds, and the g load during pullups was about 2.5 g (fig. 1). It is essential to emphasize that the entire parabolic maneuver is measured in seconds and that 20 maneuvers might be flown on a single sortie. Moreover, accelerometer readings at the center of gravity of the aircraft varied by at least 0.02 g even under good weather conditions. Fore and aft of the center of gravity the g load was slightly less and greater, respectively, but the amount was "insignificant." When secured to the aircraft the subject experienced not only the variations in g load but also very small changes in angular velocity. While free floating in the padded afterportion of the aircraft, the subject was weightless except for the inertial forces generated by bodily movements (ref. 2).

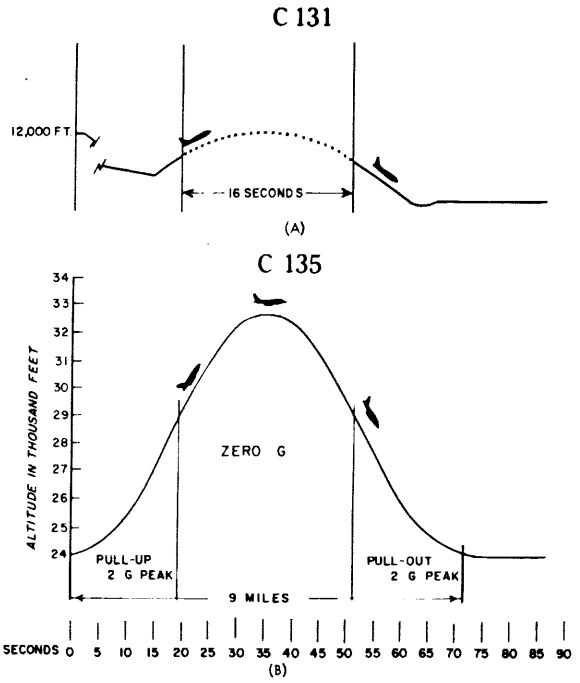

Figure 1.—*Flight profiles of zero-g maneuvers.*

EXPERIMENTS

I. Free Floating

The initial observations were made by Lieutenant Neider, an aviator serving as one of the normal subjects in the primary experiment but with no previous experience in zero-g flights. In an off-duty period, while free floating in the

afterportion of the aircraft, he experienced "a sudden reversal of up and down." The illusion lasted only a matter of seconds and had two related aspects: a bodily feeling of sudden reversal of the upright and a belief that the plane was flying upside down. He determined that at least two additional factors seemed to be essential for the perception of the illusion. The more important of the two was a head-lower-than-foot position with reference to the cabin; indeed, the more closely he assumed the inverted position, the more readily he experienced the illusion. The second condition was the necessity of facing the forward end of the cabin, the long axis of which had the "characteristics of a tunnel." He stated that these were the only occasions during which he had experienced disorientation in flight.

Three normal subjects, of whom two were sophisticated, were instructed to assume the position Neider had found most advantageous for experiencing the illusion. Upon entering weightlessness, the men, through their own efforts, assumed a head-down position with respect to the aircraft and faced the long axis of the cabin. This was usually accomplished within a period of 2–6 seconds. Although all reported that "down" was where their feet were, only the naive subject thought that the plane was upside-down. The four L–D subjects, who experienced a very brief period of free-floating in the "Neider position," in connection with the negative-g experiment, did not experience an illusion.

Comment.—Simons and Gardner (ref. 3) published verbatim accounts of subjects' perceptions while free floating in darkness with a single light source. One subject stated, "As soon as my feet were placed on the ceiling I regained my orientation with the ceiling as down" (ref. 3, p. 12). Another subject reported, "Now I have the sensation of moving forward, I am against [momentarily touched] the floor, now I feel upside down [free floating]" (ref. 3, p. 55). Several variants of these perceptions were reported also. Captain Simons (personal communication) has stated that, although it was a fairly common experience for subjects suddenly to feel reoriented with regard to up-down, he did not recall that they expressed the belief that the plane had inverted, and added, "Maybe they did not get that far in their thinking."

Kas'yan, Kolosov, Lebedev, and Yurov (ref. 4) have reviewed the experiences of Russian cosmonauts in parabolic flight and have written as follows:

> In the case of visual control of the position of the aircraft under conditions of weightlessness, no instances of spatial disorientation were observed in the cosmonauts. When eyes were closed, illusory sensations of the position of the aircraft and body in space were observed. None of them was able to determine the actual nature of evolutions effected by the aircraft.

No further details were given.

Neider's emphasis on the head-down orientation with reference to the cabin as a contributing factor is at least in line with Simons' (ref. 5) concept of "foot-down orientation"; that is to say, in weightlessness, one tends to regard where the feet are as "down." Although one might be inclined to ascribe the illusion to negative g, this was almost ruled out by the fact that the subjects were free floating. The possibility that a thrust by hand or foot against the fuselage generated an adequate stimulus was unlikely in view of the elapsed time of at least 2 seconds required to get into position. Many more observations under carefully controlled conditions will be required to determine both susceptibility to and the precise role of the several factors contributing to the illusion.

II. Negative g

In this experiment the subject's task was to "stand" on the overhead of the aircraft while exposed to small negative-g loadings in modified parabolic maneuvers (fig. 2). It was not part of the primary experiment, and we are greatly obliged to Capt. E. J. Hatzenbuchler, USAF, through whose efforts this was made possible.

All of the L–D and five of the normal subjects participated; three of the latter were sophisticated. In the C–131B aircraft, the overhead walkway was aft of the center of gravity. The parabolic trajectory was altered in order to generate small negative-g loadings lasting a matter of seconds. It is important to point out that

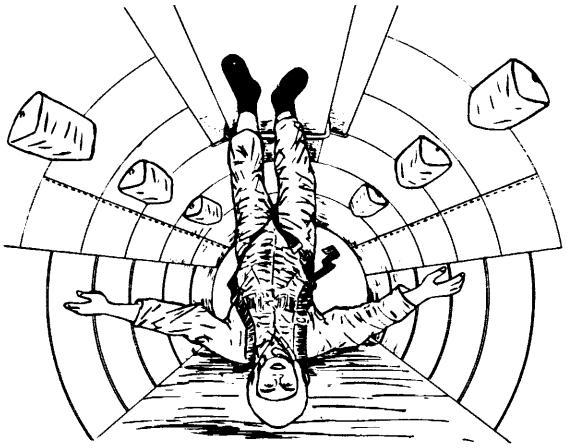

Figure 2.—*Subject standing on overhead of C-131B aircraft in modified parabolic maneuver.*

during these brief periods, the gravitoinertial *upright* was directed toward the floor approximately 180° from the visual upright.

The subjects were shown the procedure, and in some instances a familiarization trial was required. The procedure consisted of lying supine and, as the transition through the weightless period occurred, rising to a standing position in a matter of 2–3 seconds. The period during which the subject's feet were on the overhead was less than the total available time, but probably included the occurrence of the peak load in each instance. Table 2 summarizes the accelerometer readings obtained during the exposure of the L–D subjects; the normal subjects were exposed to at least two parabolic maneuvers under similar negative loadings.

Results.—Each of the L–D subjects expressed himself differently, but all felt upside-down with reference to the cabin. JO: "Feet upside down very definitely [with reference to the cabin]. No feeling of being upright. The pressure on my feet was too little to cause any postural feeling." HA felt he was upside-down: "Everything completely normal; i.e., I felt I was doing exactly what I *was* doing. Negative g only was not enough to give sensation of normal weight." PE: "Both times it was difficult to say whether I was upside down or the plane was. If not for the standard (visual) cues (seats, passages, etc.), I couldn't tell. If my eyes were closed I would feel upright." MY: "For the first few seconds (about 5 seconds) I felt that I was not upside down except for seeing that I was—after about 5 seconds, I began to feel blood coming to my head, and then I began to feel that I was upside-down."

Two of three sophisticated normal subjects regarded themselves simply as being upside-down in a right-side-up aircraft. In other words, their experience was similar to that of the L–D subjects. The remaining three subjects, one of whom was sophisticated, reported that they regarded themselves as right-side-up in an aircraft flying in an inverted position.

Table 2.—*Level and Duration of Negative g to Which 4 L–D Subjects Were Exposed in Modified Parabolic Flight*

Subject	Trial	Total period		Peak period	
		Duration, sec	g-level	Duration, sec	g-level
JO	1	6. 5	−0. 075	0. 5	−0. 10
	2	6. 5	−. 048	. 25	−. 072
	3	8. 0	−. 049	. 5	−. 09
HA	1	6. 5	−. 04	. 05	−. 058
	2	8. 5	−. 048	2. 8	−. 058
PE	1	9. 1	−. 049	. 5	−. 09
	2	7. 5	−. 048	. 25	−. 07
MY	1	7. 0	−. 048	. 1	−. 06
	2	7. 5	−. 049	1. 5	−. 06

Comment.—The analogy to "contact" flight may be pertinent. The tyro usually regards the aircraft rather than the Earth as the fixed frame of reference, but quickly learns to correct the error. He comes to regard conflicting visual and gravitational cues as normal inputs under the circumstances, although requiring special interpretation. The L–D and unsophisticated subjects behaved differently: The former described the circumstances correctly but accepted the apparent miracle of "standing" on the overhead; the normal subjects (and one sophisticated subject) correctly interpreted the gravitoinertial up-down and regarded the aircraft as inverted. The very short time involved and the need to maintain their balance prevented these subjects from giving much thought to the matter, and the influence of the otolith apparatus might explain the differences in their experience.

Simons (ref. 5) conducted tests in the identical C–131B aircraft that was used for the present experiments. For his experiments the subjects walked on the overhead, in the *weightless* phase of the parabolic maneuver, by means of "magnetic shoes." He wrote, "An apparently universal orientation phenomenon was noted by the four subjects participating in this experiment. All subjects reported an immediate spatial orientation of "down" being where their feet were. . . ." One subject reported that the foot-down orientation was strong with his eyes closed and his feet fixed to the walkway. The author, a pilot, had the weird visual experience of looking forward and seeing the pilots sitting upside-down! Two subjects walked "spider fashion "(between the floor and ceiling using arms and legs), and one reported that "the walkway became the floor"; the other subject reported a sense of oscillation between the floor and the walkway as being "down."

It would seem reasonable to conclude that if Simons' subjects in weightlessness regarded the overhead walkway as "down," our two subjects who regarded it as "up" were exceptional rather than the three who did not. Although these observations are too limited to draw definite conclusions with regard to the contribution of the vestibular (otolith) organs to perception of the upright under these circumstances, they do suggest at least that they may have played a role.

III. Restrained in the Weightless Phase of Parabolic Flight

Advantage was taken of an ongoing experiment to collect information on the subject's perception of the upright with reference to the cabin during the weightless phase of parabolic maneuvers. Two naive normal and three of the L–D subjects (HA excepted) participated.

In the primary experiment the subjects' task was to set a dim line of light in the dark to what they regarded as "horizontal" in the weightless phase of the parabola. The signal to make the setting was relayed from the pilot. The target device has been described elsewhere (ref. 6). It did not constitute an adequate visual cue to the visual upright; hence, in this experiment the subjects were in "darkness." They were encased in a Fiberglas mold and rigidly secured to a tilt device which in turn was secured to the aircraft about 15 feet aft of the center of gravity in the KC–135. Each subject was exposed to 5 parabolas while in 4 different positions with reference to the cabin: upright, and at 30°, 60°, and 90° tilt, making 20 trials in all.

Results.—After completion of all trials, each subject was asked whether he experienced any change in body position during the weightless phase of the parabola. The two normal subjects stated that they perceived a change in body position from "head-up" to "head-down" on entering weightlessness and a return to the head-up position on the pullout. This occurred in every parabolic maneuver regardless of body position in the tilt device. The L–D subjects did not experience a head-down feeling on any occasion.

Incidental Observations

Ample confirmation of these results was provided by Dr. Earl Miller in response to our inquiry. He participated in many flights in which ocular counterrolling was measured during parabolic maneuvers (ref. 7). The subjects were rigidly secured to a tilt device just aft of the center of gravity of the C–131B aircraft.

Photographs were obtained by a flash unit while the subject fixated a dim point of light. The positions of the subject with reference to the cabin were upright and at 25° or 50° tilt. He clearly recalled that subjects not infrequently volunteered the information that they felt upside down during the maneuver.

Comment.—The findings of this experiment strongly suggest that our normal subjects were responding to sensory information not available to the L–D subjects which must have had its origin in the vestibular apparatus inasmuch as these two groups were alike with respect to the physiologic deafferentation of nonotolithic gravireceptors. There are two reasons for ruling out the semicircular canals as the source. First, the changes in angular velocity were very small, and, second, the perception reported by the subject was that of up-down and not rotation.

Among 10 L–D subjects (ref. 8), JO had the highest and PE the lowest counterrolling values, with MY's values falling close to the mean. These differences in counterrolling index had no significance in terms of this particular behavioral experience in weightlessness. It raises the question at what counterrolling value, or subgravity level, the inversion illusion may be experienced.

GENERAL DISCUSSION

Although the American astronauts, in describing their experiences in Mercury and Gemini space flights, did not report a feeling of being upside-down, comments by Soviet authors on the experience of their cosmonauts during orbital flights are in accord with our experimental findings in parabolic flight. Gazenko (ref. 9) writes as follows: "In some of the cosmonauts (G. Titov, A. Nikolayev, P. Popovich) illusory feelings as to the wrong position of the body in space occurred at once, while in other cases, the illusion developed gradually (K. Feoktistov, B. Yegorov)." Vasil'yev and Volynkin (ref. 10) add the interesting note that Feoktistov's and Yegorov's illusion of being upside-down occurred throughout the period of weightlessness whether their eyes were open or closed. It disappeared only with the beginning of acceleration when the craft was being braked. Of considerable significance, too, is the cosmonauts' observation that the nature and intensity of the illusion and vertigo were the same in free flight as when the craft was stabilized. Gazenko adds:

> It was especially interesting to note that, when the cosmonauts gained a foothold on the chair by straining their muscles, the illusions diminished or even completely disappeared. This fact underscores the significance of cutaneous and muscle perception in restoring a correct analysis of the position of the body in space.

In weightlessness, with body (head) fixed and without visual cues, knowledge of the upright of the cabin must come from contact with objects whose relation to the cabin has been remembered. These cues, however, must not be confused with normal contact cues, although some of the same sensory receptors may be involved. In weightlessness these cues should be termed "agravic contact cues" to emphasize qualitative and quantitative differences in the information they furnish. They include agravic touch pressure, agravic kinesthesis, and their derivative agravic stereognosis. Bodily movements contribute additional information and, in the case of the vestibular organs, the stimulus to the semicircular canals on moving the head is normal, although the response may be slightly different from normal; the stimulus to the otolith apparatus resulting from body and head movements would, of course, be greatly different in the absence of gravity.

If it is assumed that conditions existed for Feoktistov and Yegorov in which these nonvisual agravic cues were inadequate for proper orientation to the spacecraft, important questions must be raised. Why should a person feel upside-down rather than simply a lack of awareness of the upright? And, if the contact cues did not vary, what precipitated the illusion on one occasion and not another? How are individual differences explained?

The feeling of being upside-down with eyes open, ascribed to Feoktistov and Yegorov, is even more difficult to explain if there were strong visual cues to the upright of the spacecraft. That it lasted for some time was suggested by Gazenko (ref. 9), as noted above. Although there are few reports of this inversion

illusion in weightlessness in the presence of visual cues, they indicate a tendency to its occurrence. This is extraordinary in view of the great influence of vision in the interaction between visual and gravitational cues, not only under normal terrestrial conditions (ref. 11) but also under conditions of moderately increased g loadings (ref. 12). It suggests that persons in weightlessness are vulnerable to influences which determine the feeling of up or down based on gravitational cues. This "vulnerability" exposed by weightlessness seems to consist of "up-downness" having the character of a qualitative phenomenon. Under the "influences" investigated in parabolic flight, it would appear that this vulnerability is greater in normal persons than in persons who have lost the function of the vestibular organs.

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DISCUSSION

JONES: I would like to make one remark here concerning this. Aviators are trained primarily to become more vision dominant and thereby to disregard all other cues. This training, or lack of it, may be a factor here. I think this may be why many people do not get this upside-down illusion. Perhaps those who do have not been so trained. Most of our aviators do not realize how much they rely on their peripheral cues. In some work in the armed services years ago, we closed off the sides of the cockpit, and the aviators were most uncomfortable. This clearly indicated the heavy dependence on peripheral cues. A lack of these peripheral cues (the inside of the test aircraft in this paper appeared to be a dull gray) may have contributed to this upside-down illusion.

THOMPSON: One of our engineers, a former pilot who was a skindiving fanatic, did some summer work on the underside of a ship. As he was working he discovered that he was having difficulty doing the work due to his free-floating condition. So he ballasted himself so that he would rise toward the surface at about the equivalent of 0.1 g and stood head-down on the bottom of the ship. He said he immediately had the illusion that the undersurface of the ship was the surface and that he was standing upright rather than head-down; he went on and performed the work with no problem at all. This might be an area for corollary investigations of the type you are doing in water tanks.

LI: I believe the semicircular canals in an engineer's terms are velocimeters, angular velocimeters, because they function as an overdamped system. But sensation about the vertical is generated by sensors acting as an accelerometer. In other words, you really feel the gravitational pull when the gravitational pull is applied. This type of system is not overdamped. For that reason, if you are subjected to a 2-g gravito-

inertial "up-push" when the airplane goes into a parabola, the pullup is 2 g. If there is a 2-g inertia step function and the plane comes down to zero g, there is an overshoot, and you wrongly sense a negative g. That means the sensor gives you an inverse negative g, and certainly the subject will feel that he is inverted.

VON GIERKE: I would like to raise a question as to whether this phenomenon should be called an "illusion." I think it is not the same situation as that we have with most other observations called illusions. Illusion is a perception which fails to give the correct impression of an objective situation or environment. In the aircraft environment under discussion, assuming that we have a true zero-g environment, what is objectively correct is undefined; i.e., whether the subject should feel upside-down or right-side-up. Should he give preference in his decision to the familiar environment of the aircraft and assume it to be in its "normal" flying position or should he give preference to the optical clue where he sees his feet? The optical cues justify an ambiguous interpretation and ambiguous, reversible perceptions. The answer might depend on training, background, instructions, etc. The decision as to whether the upright position or the inverted position (or neither of them) is "correct" is arbitrarily defined by the experimenter. In addition, the basic phenomenon is probably not restricted to the 180° "inversion" phenomenon described. If the subject were to walk on a surface inclined, for example, at 45° to the "normal" floor of the aircraft, his interpretation of his position would probably fluctuate according to the angle between the two planes; i.e., not a complete 180° "inversion." In summary, I don't think one should introduce the term "illusion" for this phenomenon.

GRAYBIEL: What would you like to call it?

VON GIERKE: A phenomenon.

GRAYBIEL: Inversion phenomenon?

VON GIERKE: Yes. Or perhaps one should call it ambiguous perception or reversible perception. I cannot see that the situation is similar to any of the other effects we call illusions because our perceptions are not in agreement with the objective, physical world.

GRAYBIEL: Then we should reopen the whole question of illusions. A lot of things we call illusions are not strictly illusions.

VON GIERKE: I don't think this is necessary. I think most other perceptions we call illusions are really illusions.

GRAYBIEL: Let me give you an illustration. Suppose you are subjected to a change in the gravitoinertial vertical with reference to yourself. Is this a basis for experiencing an illusion?

VON GIERKE: One must try to have a good definition of what one calls an illusion. If the phenomenon you described in the aircraft experiment were to be called an illusion, it would, in my opinion, require a broadening of the definition used in classifying other observed phenomena as illusions.

GUALTIEROTTI: Sometime ago I published results of a study of detection of the vertical under water. In the physiological laboratory in Milan we immersed a man while he was completely blindfolded into a tank and had him indicate the vertical on a dial. We found out that, after training, there was no difference between the accuracy of the indication of the vertical by this man under water and in air. This appears to indicate that when all the psychological effects such as unusual environment and a situation which is not familiar are taken away, the sense of the vertical is a function only of the vestibule. When a person is immersed and floating in water without touching anything, any information from skin or muscle or any other part of the body except the vestibular system is discontinued. As far as the lack of delay of the otolith is concerned, the gravitoreceptor responds very promptly to acceleration. There are two kinds of otolith receptors: one that shows relatively fast accommodation and one that does not show any accommodation at all. These are really statoreceptors; they are very prompt and very sensitive to any change of position of the head.

LANSBERG: I think the word "illusion" is absolutely correct since the word "sensation" is not correct, or I misunderstand what sensation exactly is. I think the man indeed feels he is upside-down, whereas the only correct sensation would be to have no sensation at all about whatever end is up. It is not relative to the airplane only; the sensation is indeed a sensation of gravity upside-down, and as this sensation is false, I think the word "illusion" is correct.

BERRY: I think we ought to make very clear what the two astronauts described on the last space flight (Gemini VII). I think we are getting the idea these people felt they were upside-down. I want to make it very clear they did not feel they were turned upside-down. We asked this question repeatedly in every way we could possibly ask to try to clear this up. That was the first impression I had on asking the question. The only way they could describe the physiologic sensation they had was that it was the same as if they were hanging on a parallel bar, or something of that sort. The feeling that they got in their head was that same type of feeling. This was the sensation that they had. They said that they knew perfectly well that they were not upside-down. There was never any doubt in their mind whether they were upside-down or not. They knew that. But they did have this feeling as if their head was ballooning in effect. Frank [Borman] was more vociferous about this than Jim [Lovell]. He said he actually felt his head was ballooning somewhat. He went back to a time when he had seen an experiment which was being run in the laboratory. There was a person on a table upside-down in an inverse tilt for a period of time. Frank investigated this to the point that he tried to determine whether he felt an increased pulse. We questioned this a great deal. Neither of the men felt an increased

pulse rate. They felt no pounding in their head; that remained normal.

They even looked in the mirror to see if their faces were red and if any increase could be determined. There was none. They could not see their faces were necessarily any more red. It didn't change. It didn't vary. It didn't come and go, in other words, during that first few hours. It is indeterminate exactly when it went away, but it is somewhere during that first day in flight up until the time they went to sleep. It wasn't important enough to them. They apparently adapted to it very readily. It wasn't important enough to them to mark exactly when it went away or anything. Jim McDivitt and Ed White also described this. When we requestioned them, they said they felt for awhile there was an increase in fullness in the head. Because of this description, we talked to them again. They also described this same sort of a thing. They didn't describe it in as marked terms probably as Frank and Jim did. I don't know if that helps or hinders, and I still have no explanation for it, but that is the description.

GRAYBIEL: This is precisely the type of information I hoped we would get from you. We needed to have a correct report regarding the Gemini VII astronauts.

I do think that sometimes we can manage to have, simultaneously, two different sorts of feelings, the visual impression that "yes, everything is right-side-up" and the inner sensibility that "I really am inverted." As Dr. Lowenstein said, in weightlessness a person is rather ambivalent with regard to up-down, and under the proper conditions the feeling of being up or down would fluctuate. One would expect visual cues to "take over." The fact some "feel" upside-down with reference to the visual upright suggests that it has a strong basis in our sensory experience involving other than gravireceptor mechanisms.

MAYNE: Whatever sensation an astronaut may experience in space is the result of a complex interpretation of sensory data in perception and depends on which data have priority. Furthermore, it is easily possible to have a discrepancy betwen sensory modalities without distinct illusion and only a feeling of discomfort. Or again, it may be a condition of "sometimes you see it and sometimes you don't," with a gradual shift of emphasis from one sensory modality to another, all the time knowing perfectly well on the intellectual level what the objective true situation may be. At the same time, the disturbance created by such a sensory discrepancy depends on the threat it involves. It can be expected that a trained pilot would not be highly disturbed by a vague feeling that he may be upside-down when he knows on the intellectual level what his precise situation may be. He has probably had similar experiences previously.

We find a good deal of evidence to the effect that the otolith organs combine two transducers in one: an overdamped integrating accelerometer and an underdamped ordinary accelerometer. A zero-g situation to a man adapted to an Earth situation can only signify 1-g acceleration directed toward the feet or an inertial force directed toward the head as in an upside-down position on the surface of the Earth.

GRAYBIEL: The experiment in which the subjects were in the Fiberglas mold and felt upside-down on transition into weightlessness might be more readily explained by your theory than in the circumstance where Lieutenant Neider was free floating in weightlessness.

GUALTIEROTTI: I think I can answer that question because I have been measuring single-otolith output during parabolic flights, with changes of acceleration from 1 g to 2 g, from 2 g to 1 g, from 2 g to zero g. What happens is very complicated. It is not only a question of overshooting or undershooting. What happens in the frog is this. First of all there is a very definite difference when you go from 2 g to 1 g, or when you go from 2 g to anything approaching zero g. In the latter case, let's consider a unit that responds to horizontal acceleration only. Consequently, there will be no direct effect on the unit excitation in zero g. Our results show an increased rate of spontaneous firing during the zero-g period. That means there is some sort of release phenomenon in the unit. We have an explanation for that, but I won't go into it here. Then, if at this point you apply the proper excitation in the horizontal direction, first there is a very large response, much larger than the one obtained during level flight immediately before, then can be seen a peculiar suppressory phenomenon, by which the unit is not stimulated any more. However, at the end of a certain period of time, the unit starts responding again. So, you see, it is very difficult to describe this in terms of pure mechanical factors. There is more active inhibitory action or some kind of suppression mechanism which takes over as soon as the firing rate increases too much. This suppression is not due to the rate of firing being absolutely too high, because in laboratory experiments, with a sudden tilt or sudden excitation, a rate of firing which is much higher than this can be obtained. But it has something to do with the influence of the other units which respond to the vertical acceleration.

THACH: A year and a half ago on our zero-g flights with squirrel monkeys, we observed what might be called a critical incident bearing on the generality of the inversion illusion. As part of the experiment, the monkeys rode through several parabolas in a small clear Plexiglas cage, 9 inches $\times$ 2 inches, in which they pressed a bar for a food reward. The normal, naive monkeys' initial response to the onset of zero-g was to turn upside-down, and then assume various postures and orientations with little regard to the walls and floor of the aircraft, despite being able to see out of the cage. They all seemed, therefore, to at least momentarily lose their orientation to "down" even though

visual reference continued unchanged, which probably means the monkeys were using vestibular reference to orientate themselves. This is obviously similar if not equivalent to the inversion illusion as experienced by humans. There are two qualifying factors. The first is the occurrence of small negative-g loadings of short duration as the transference from +1 g to zero g was made. Transition was seldom perfectly smooth. The second is the squirrel monkeys' probably higher tendency to use the vestibular reference over the visual one as a result of natural selection for an arboreal existence.

BERRY: I seriously wonder what value zero-g flights are for determining physiological inputs when this is such a complicated environment for such a short period of time. I think we are in grave danger in trying to determine conclusions from periods of 20 and 30 seconds of zero g and relate them in any manner whatsoever to the real zero-g environment. That is my opinion.

Problems of Man's Adaptation to the Lunar Environment

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INTRODUCTION

Man is provided with a number of sensory organs: namely, the eyes, the vestibular labyrinth, and the proprioceptive sensors which are interdependent in establishing man's equilibrium and orientation in the normal 1-g environment. These organs establish the postural vertical, and control and coordinate the position and motion of the various parts of the body. Ordinarily little attention is paid to this innate capability, and it is only when the cues from one or more of these organs are lost because of disease or as a result of a changed environment that man is caused to consider the mechanisms of equilibrium.

The lunar environment provides only one-sixth of the gravity that man is normally subjected to; as a result there will be a direct reduction in the stimulation to some of the organs of equilibrium. This paper will concern itself with the effects of this reduced stimulus on the general senses of equilibrium and therefore on man's ability to orient himself and move about the lunar surface.

GENERAL CONSIDERATIONS

It is first necessary to identify those organs whose function is subject to changes in gravity and to determine the degree to which the organ will be affected. In 1952 it was postulated in reference 1 that zero gravity would have little effect on visual perception; this hypothesis apparently has been borne out by the recent orbital flights. Also, orbital flights have provided little indication that reduced gravity adversely affects the control of eye movements. However, the apparent increase in acuity in orbital flights is considered, as pointed out in reference 2, to be caused by an increase in physiological nystagmus due to a reduction of frictional and damping forces of the eye in zero g.

Because of the lack of atmosphere it is expected that there will be an increase in contrast on the moon which should affect depth perception. This, however, would not very likely have any effect on the visual contribution to orientation. Vision is, of course, susceptible to contradictory cues from the other sense organs (refs. 3 and 4); however, for a person using self-locomotion on the moon, contradictory cues are not expected to be present. Therefore, it can be concluded that the eyes will be of primary importance for orientation in lunar operations. Eventually, manned lunar-surface vehicles will be employed and the possibility of vehicle-induced visual illusions will have to be considered.

As far as the vestibular system is concerned, it is anticipated that the semicircular canal function will not be affected by reduction in gravity, since the canals are considered to be essentially angular acceleration sensors. The

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canals can only be affected by linear acceleration if differences exist in the specific gravity of the cupula and the endolymph fluid.

The otolith organ which is the linear acceleration or gravity sensor and the proprioceptive mechanisms will, of course, be directly affected by reduced gravity. It can be expected that with reduced stimulation of these organs and in the absence of vision, man may have difficulty in judging the vertical. As reported in references 5, 6, and 7, this has been demonstrated, for the situation of reduced proprioceptive cues, in tilt tests which showed that padding of the tilt chair or tilting the subjects in water decreased their accuracy in indicating the vertical. In these situations the stimulus to the otolith was normal.

Very little data exist on the effects of reduced otolith stimulation on equilibrium, although many experiments to determine otolith threshold have been made. These threshold values are reported to be in a range from about 0.00034 g to 0.010 g, which is much less than the one-sixth lunar gravity. However, these threshold values must be maintained for a certain length of time before being perceived, and the minimum values of acceleration for maintaining undegraded postural equilibrium have not been established. Also not known is the level at which the proprioceptive cues become useless in orientation although, as mentioned, tilt tests in water indicate judgment is impaired, with the degree of impairment reduced by training.

In summary, it appears that the otolith and proprioceptive sensors will be directly affected by a reduction in gravity, while vision and the semicircular canals will be relatively unaffected. Complete loss of two of the general sensors probably makes it impossible for man to maintain his equilibrium, especially if vision is involved. However, there still remains some question as to the effects on equilibrium of reducing the stimulus to two of the sense organs to only one-sixth of the normal as would be the case in lunar conditions. Some insight into this problem may be gained from the following discussion of the self-locomotion experiments of reference 8, performed on the lunar-gravity simulator at Langley Research Center.

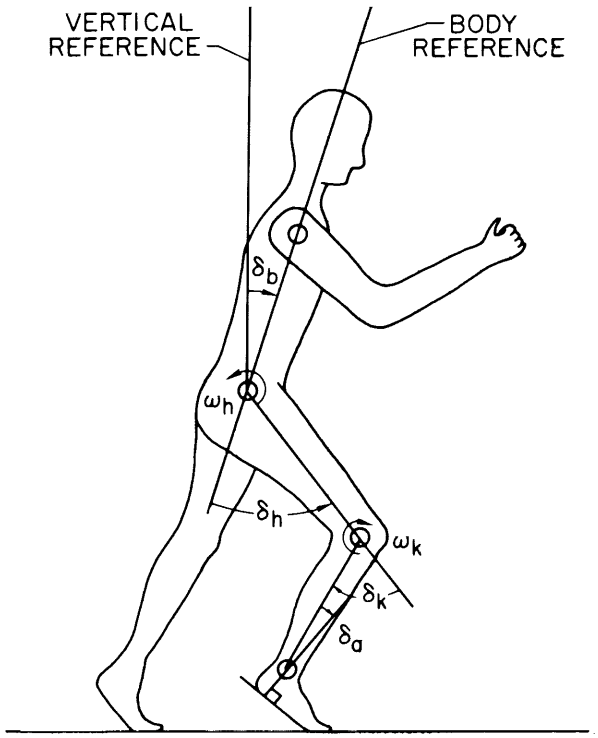

FIGURE 1.—*Definition of body angles. All angles are positive as shown.*

NOMENCLATURE

The following, together with figure 1, define the symbols used herein:

δ_b back angle, angular deflection of the reference line joining the hip and shoulder joints relative to the vertical, degrees

δ_h hip angle, angular deflection of upper leg (thigh) relative to the back reference line, degrees

δ_k knee angle, angular deflection of the lower leg relative to the upper leg, degrees

δ_a ankle angle, angular deflection of the foot relative to the down leg (calf), degrees

ω_h rate of change of hip angle, degrees per second

ω_k rate of change of knee angle, degrees per second

g gravitational unit, relative to acceleration produced by Earth's gravitational field

T time, seconds
V velocity, feet per second

Subscript:
max maximum value

DESCRIPTION OF REDUCED-GRAVITY SIMULATOR

A sketch of the simulator is shown in figure 2. The simulator supports a subject on his side, inclined about 9.5° from the horizontal, for lunar simulation, by means of a system of cables attached to the various body members and to an overhead trolley system. The trolley unit moves along an overhead track which is parallel to the walkway on which the subject is free to walk, run, and perform other self-locomotive tasks in essentially a normal manner though constrained to move in one plane. This constraint does not appear to be too serious if one considers the fact that the body members normally translate and rotate, fore and aft and up and down, essentially in parallel planes as a person walks, runs, and jumps in a normal manner. Figure 3 shows a subject in the simulator cable harness. As mentioned, the subject is inclined 9.5° from the horizontal, and the component of his weight normal to the walkway and supported by his feet is one-sixth that of his normal weight, as would be the case in lunar gravity. The ⅙-g component is therefore considered to be the one which is important for balance and locomotion in the plane normal to the walkway.

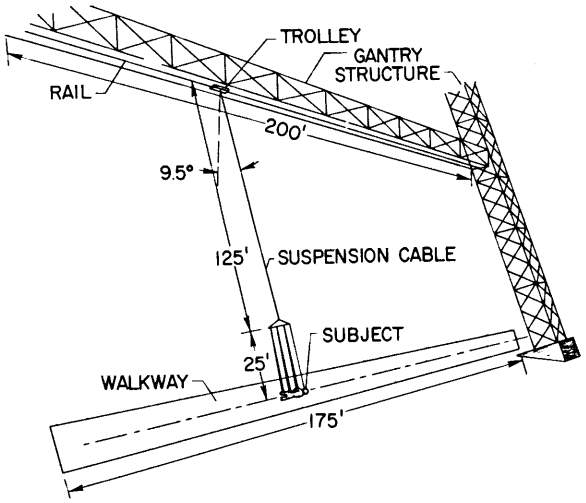

Figure 2.—*Illustration of the reduced-gravity simulator.*

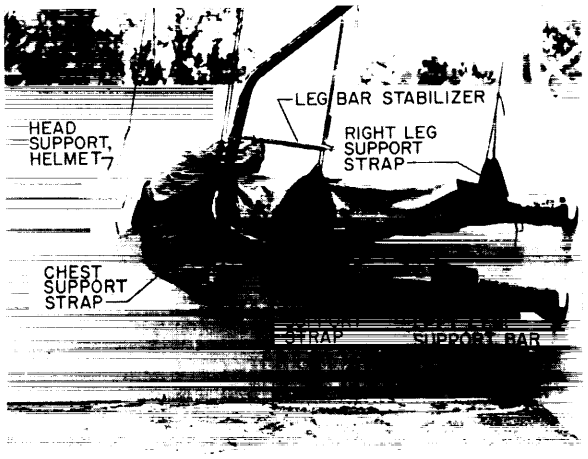

Figure 3.—*Body harness details.*

It is recognized, of course, that there remains a 1-g vector acting on the body. It should be pointed out, however, that this vector is essentially constant during normal simulator usage, and subjects readily adapt to their new orientation by recognizing the tilted walking board as the ground plane and relating their body motions to it rather than the customary ground reference. One of the limitations of the simulator is that the subject's motion is restricted to one plane and the device is not adaptable to studies requiring out-of-plane motions. Despite this limitation, the simulator is useful for studying man's equilibrium and motion capabilities in the sagittal plane under reduced gravity conditions. For the investigation discussed herein, the simulator was used with the subject's vision unrestricted and with the walkway displaced to provide the lunar level of stimulation to the otoliths and the kinesthetic sensors in the plane of activity.

MEASUREMENTS

All tests were recorded by means of motion-picture cameras operating at 24 and, at times, 48 frames per second. An observer using a stopwatch obtained the time required to travel the 100-foot distance in the middle portion of the walkway. This time was used to establish the average velocity for each test.

Positions and rates of movement for the various body members relative to each other and to the ground were obtained from measurements of the projected images of the motion-picture film. The accuracy of the angular measurements is considered to be about ±2°.

RESULTS AND DISCUSSION

A film supplement illustrating some of the results of this investigation is available on loan from Langley Research Center (L–894).

Some of the subjective results of the present investigation indicated that the subjects tested had, initially, some difficulty in sensing the vertical to the walkway and stood rocking to and fro, perhaps trying to increase the vestibular response. The subjects also ended up standing on tiptoe in an apparent attempt to increase the stimulus to the tactile and pressure sensors and thereby improving their balance. It is expected that this will also be the case on the lunar surface. Several subjects indicated a sensation of being inclined after leaving the device, indicating adaptation to the constant lateral tilt required when using the simulator. Adaptation to continued tilt was expected on the basis of the experiments of reference 9 conducted with animals. In those experiments, measurements of neural impulses indicated a vigorous initial response to tilt which diminished in about 20 or 30 seconds with the steady-state response to tilt relatively weak. In the present experiments it was found that with little practice, the subjects were able not only to maintain their static equilibrium but could walk, run, and perform other self-locomotive tasks, which indicates that man will find it relatively easy to adapt to the lunar conditions.

Some data were obtained for three subjects comparing the difference in posture and limb motion during locomotion for Earth and lunar gravity conditions, and the results are presented in figures 4 through 12. The discussion herein will be limited to those aspects which it is felt are important for equilibrium and which indicate the extent of control and coordination of the motion of various parts of the body. (For a discussion of the data relative to locomotion characteristics, see ref. 8.)

Figure 4, reconstructed from the film records, presents qualitatively the difference in posture and position of the body members for the subjects walking, loping, and sprinting in the Earth and simulated lunar gravity conditions. The figure is in the form of line diagrams (stickmen) presented at about 1/6-second intervals giving time histories of body-member positions for at least one step as denoted by the solid horizontal bars at the ground level. The comparison made for sprinting is for the maximum sprinting speed which turned out to be 19.8 ft/sec for Earth gravity and 13.1 ft/sec for simulated lunar gravity. The lower value of maximum sprinting speed in lunar gravity is attributed to the loss of traction in 1/6 g. From the figure it is readily seen that there are relatively large differences in locomotion characteristics for the two gravity conditions. This is also apparent in figures 5 through 7 in which data corresponding to that of figure 4 are presented in quantitative form. The symbols in these figures indicate the beginning and end of a step. From figures 5 through 7 it can be seen that there are large differences in amplitudes and rates of motion of the various body members for the different gravity conditions, as well as large differences in the body lean or back angle. There is also a large variation of back angle with rate of locomotion. This is more easily seen in figure 8 which is a plot of back angle versus locomotive rate. Figure 8 shows that the back angles increased at a much higher rate

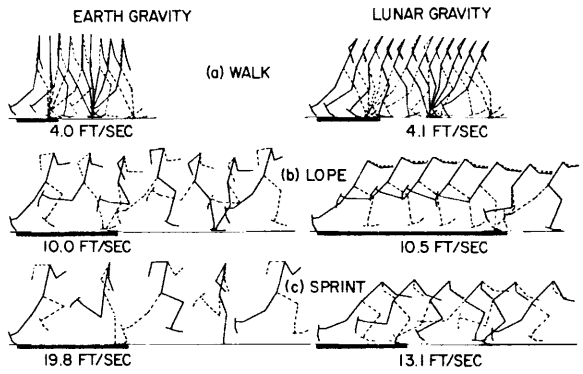

Figure 4.—*Stickman representation of a typical walk, lope, and sprint in Earth and lunar gravity. Length of bar at ground line denotes distance of one step. Dashed line denotes position of the left arm and leg. The time interval between each figure is 0.16 second.*

for simulated lunar gravity than for Earth gravity and attained values as high as 60°. These angles are three or four times greater than the maximum obtained in the 1-g environment. Figure 9 shows how the body lean or back angle affects the component of gravity along and perpendicular to the subject's body.

The components are simply sine and cosine functions of the body-lean angle as indicated

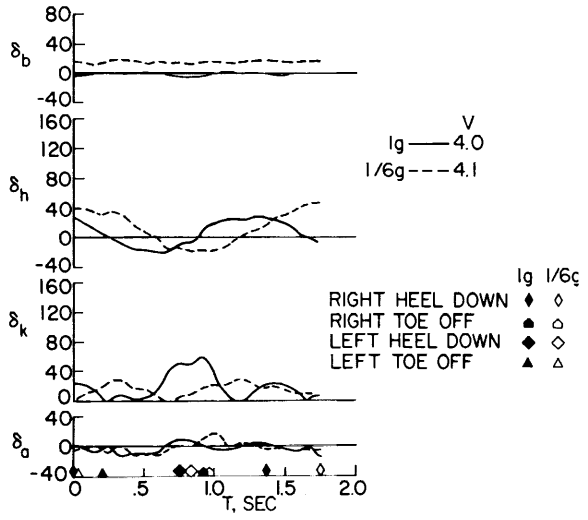

Figure 5.—*Time history of the relative motion of various body members while walking in Earth and lunar gravity.*

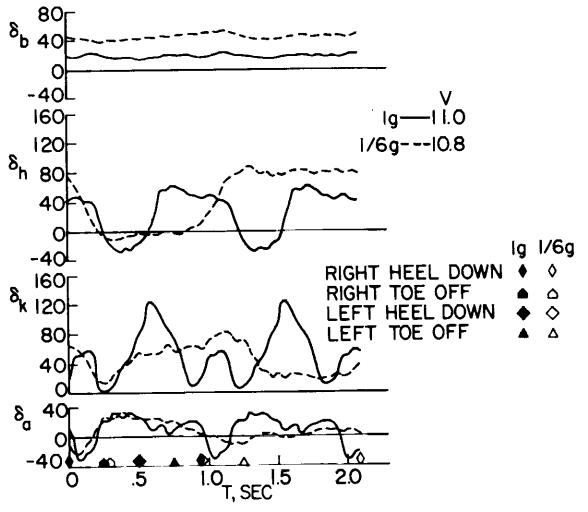

Figure 6.—*Time history of the relative motion of various body members while loping in Earth and lunar gravity.*

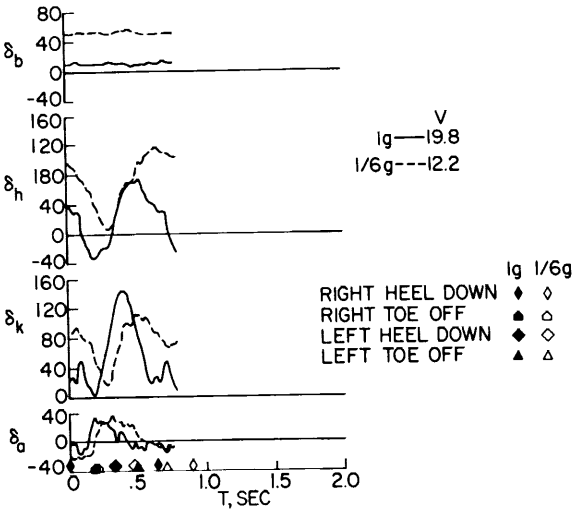

Figure 7.—*Time history of the relative motion of various body members while running at maximum velocity in Earth and lunar gravity.*

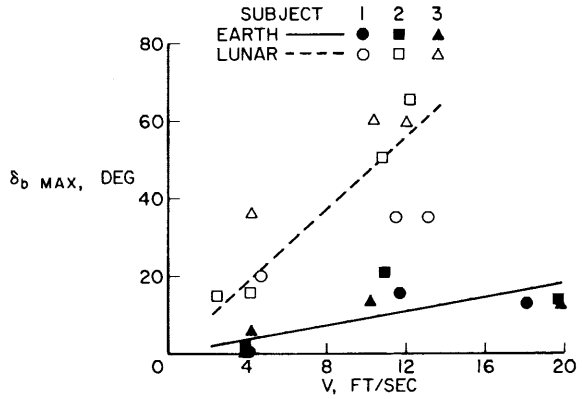

Figure 8.—*Maximum body lean or back angle versus locomotion rate at 1 g and ⅙ g.*

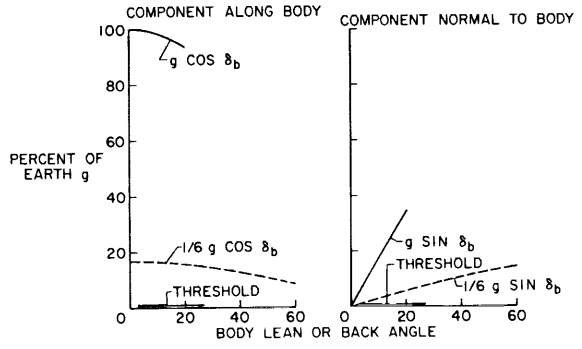

Figure 9.—*Gravity components versus body lean or back angle for 1 g and ⅙ g.*

in the figure. The data in the figure illustrate that even though the component along the body in simulated lunar gravity is decreased by 50 percent when leaning from 0° to 60°, it is still considerably greater than the threshold value indicated by the solid horizontal line. The component normal to the body increases with body lean, but the maximum obtained for simulated lunar gravity at 60° is much less than that obtained at the maximum body-lean angle of 20° used in Earth gravity. Figure 10 shows the variation with velocity of body-lean angles for one of the subjects carrying various loads in simulated lunar gravity. The data show that as the total weight, that is the weight of the subject plus weight of his load, approaches that of the man with no load in Earth gravity, the rate of increase of lean generally decreases and is more nearly like that for man with no backpack in Earth gravity. This would appear to indicate that the large body lean used by the subject in simulated lunar gravity is related more to the mechanics of locomotion rather than an attempt to modify the stimulus to the vestibular organs. Since the subject carried the weights in a frame mounted on his back, an initial upper body lean or back angle was required to keep the resultant center of gravity over his hip joint. This initial lean accounts for the large upper-body-lean angles used, even at low locomotion velocities, by the weight-carrying subject. It should be pointed out that despite these large body-lean angles, the subject had no trouble in maintaining his balance while walking or running on the simulator. An analysis of the restraints of the simulator is given in the appendix of reference 8 and indicates that only about 5° of the maximum lean angle is a result of the restraints considered.

The data of figures 11 and 12 summarize other differences in the relative motions of the various body members. First of all, as illustrated in figure 11 (left graph), the hip flexion angles are larger for the lunar condition than for Earth gravity, indicating that the legs were carried farther forward in the lunar gait than in Earth gaits. This is attributed to the fact that with the large body inclinations noted, the legs had to be carried farther forward to maintain balance. This, in turn, resulted in decreased knee flexion (fig. 11 (right graph)), and gave the subject an appearance of walking stiff legged for the lunar simulation. It appears likely that the normal knee action is not required for lunar activities, with the weight on the legs relatively low.

As shown in figure 12, there was also a difference in rates of limb motion between Earth walking and simulated lunar walking. The maximum angular rates for hip-and-knee motions for lunar walking were about one-half that for Earth walking.

The results of these experiments generally indicate that the subjects are able to adapt their limb motions to the decreased gravity conditions and are able to maintain equilibrium even

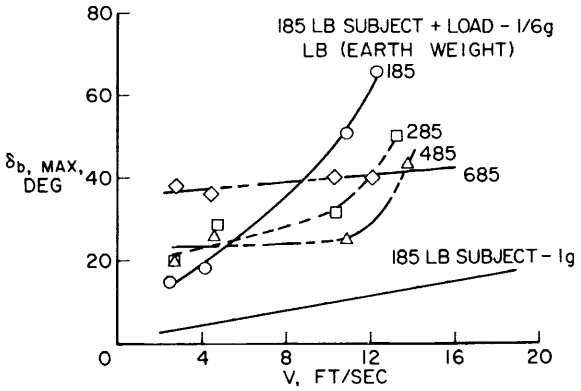

Figure 10.—*Maximum body lean or back angle versus locomotion rate with subject carrying various loads.*

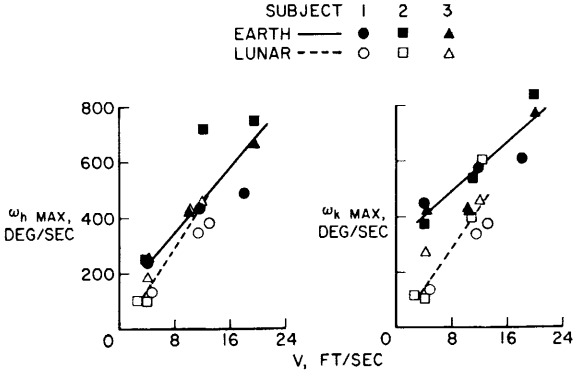

Figure 11.—*Maximum upper and lower leg rates, as indicated by* $\omega_{h_{max}}$ *and* $\omega_{k_{max}}$, *versus locomotion rate in 1 g and ⅙ g. Upper leg rate* (left graph), *lower leg rate* (right graph).

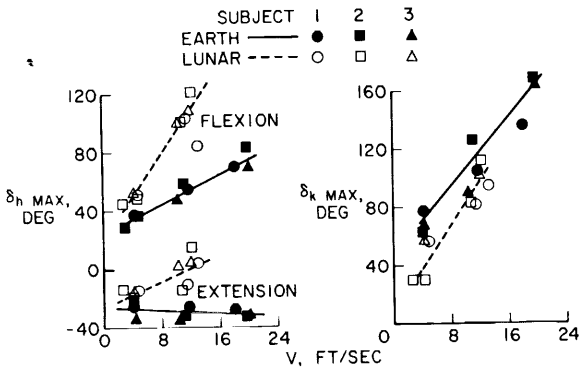

Figure 12.—*Maximum upper and lower leg displacements, as indicated by* $\delta_{h_{max}}$ *and* $\delta_{k_{max}}$, *versus locomotion rate in 1g and* $\frac{1}{6}g$. *Upper leg displacement (left graph), lower leg displacement (right graph).*

while running at about 13 ft/sec. Indeed, as the short film supplement shows, man can also jump and perform acrobatics using the simulator, indicating the ease with which man accommodates to the unusual environment. Some experiments performed with the subjects wearing a suit pressurized to 3.7 psi indicated that wearing a pressurized suit would not affect the results enough to alter the general conclusions reached herein pertaining to man's equilibrium. Of course, it is assumed that the suit would not severely restrict the subject's vision.

CONCLUDING REMARKS

On the basis of the observations and tests examining man's ability to perform under the reduced gravity conditions on the lunar surface, it appears reasonable to assume that, with some training, man will be able to maintain his equilibrium and orientation while moving on the lunar surface. It is suggested, however, that experiments in reduced-gravity simulators as well as in $\frac{1}{6}$-g parabolic flights be continued to obtain additional pertinent information.

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DISCUSSION

GUALTIEROTTI: There is an obvious observation to make here. This is not at all a simulation of the lunar situation. The vestibular apparatus in your experiment is at exactly 1 g, so you can't really compare your results with what would happen, as far as balance is concerned, on the lunar surface. Of course, we have some useful information about muscular strength applied to a different body weight, but we don't have any information about the behavior of the vestibule in this particular case. And the vestibule is the one that determines equilibrium, posture, and balance.

WHITCOMB: Have you investigated the possible virtue of using spikes or cleats under lunar sprint conditions?

SPADY: No, we haven't; primarily because we are using a wooden walkway. Some thought has been given to this, but as yet no tests have been conducted. It may well increase performance.

YOUNG: In a paper at the IAF in Warsaw in 1964, Margaria and Cavagna of Italy concluded, as I remember, that the resultant reduced friction forces on the lunar surface would lead to an entirely different sort of gait than either running, loping, or walking, some-

thing of the order of a skip. Do you have any comment on their work?

SPADY: This would be comparable, I think, to the second classification you made, the lunar lope, where a subject is using strides up to 30 feet in length; that is, from right-heel-down to right-heel-down again. We have measured strides up to 30 feet and consequently have found very great reduction in the stepping rate in the subject in these conditions. In fact, the subject can run or can move about almost as fast using a lope as he can using a maximum-effort sprint.

TILLER: I understand there is some evidence to show that the energy that is used in less than 1 g is more than you find within the 1-g environment. Did you make any measurements on this at all?

SPADY: Not really, other than personal observations. And here again they would be biased. But it is a lot easier to move about at a greater velocity under the lunar gravity condition than the similar case in Earth gravity, especially if you are using a lope. A walk under simulated lunar gravity is fairly monotonous and boring, whereas a lope is fairly exhilarating, and you can keep it up for a fairly long period of time.

TILLER: Did you do anything that would resemble maintenance?

SPADY: No.

LI: A few years ago, I did an experiment with the Army Quartermaster Research Lab in Natick, Mass., about the mechanism of walking. The idea we had in mind was the walking mechanism involving the swinging of the leg in a natural pendulous frequency pulling by gravity. The natural period is a little better than 1 cycle per second. If you use that natural frequency and swing along, it is very easy for walking. At that time we also thought that if one tuned up the leg with a pair of springs to increase the natural frequency of the leg, one might be able to walk faster, and indeed we tried this on a treadmill. I was the test subject and carried the analyzer on my back to walk at 5 mph on the treadmill. We found that with the spring to tune the leg up to a higher frequency, you can walk faster and with slightly less consumption of energy as shown by measuring the carbon dioxide production. According to your film demonstration of loping, you really depend on jumping; the action involved shows low frequency and long stride. But for running in your study, apparently the motion depends on the use of a short stride and the frequency of the swing of the leg. So if you run at higher speed, you must use high frequency. For that reason on the lunar surface you have just about one-half the frequency, and you cannot run very fast. This may explain the result of your experiment. Am I right?

SPADY: I am not sure I am really following you. I am sorry. Go ahead.

LI: In other words, for running, with your foot in contact with the surface to limit your stride, then the speed is proportional to the natural frequency of your leg. Since you have lower gravitational pull on the lunar surface the natural frequency of your leg is also lower. For that reason your speed is lower. If you use loping, you don't depend on frequency.

SPADY: That is right.

VON GIERKE: I wonder if a quantitative aspect of your data is not the change in body angle. Did you analyze the forces on the body for your simulator situation in detail so you know that the suspension does not introduce an artifact here? I think you have different lengths of suspension. Is that not influencing the body angle you observe?

SPADY: That is true. Such an analysis has been made and is referred to in reference 8 in the paper. A complete analysis of the system is given along with the effect of the cables and various restraints on the subject. For the conditions of carrying the maximum weight at a maximum velocity, the effect on body lean is something less than 5°.

VON GIERKE: Did you do it for different suspension lengths?

SPADY: We have done it for two: a 40-foot suspension length which was the original simulator, and the current simulator which has a 150-foot suspension cable.

VON GIERKE: Did you get a difference in body angles?

SPADY: No. The body angles used are essentially the same for both of them.

OGDEN: In Earth walking at level or slightly up or down hills, by far the greatest correlation with the total energy consumed is the aggregate up-and-down movement of the center of gravity. Do you have good records of changes in total up movements, aggregate up movements, of the center of gravity for a 100-foot walk on the apparatus as compared with Earth g.

SPADY: I have the information primarily because these were recorded using an overhead camera by looking in on the side of the subject. We have not completely analyzed all the data we have; accuracy for determining this is not really too good, but we do get some indication of the amount of movement. For the walking condition, there is very little rise of the hip; for the lope, it is a fair amount. For the run, again it shows to be very little rise in the hip joint.

OGDEN: When you say it is low, you mean it is lower in the low g than in regular-gravity walking?

SPADY: I haven't compared the two gravity conditions.

STONE: Metabolic rates have been measured, not by us at Langley but by a contractor who is investigating similar amounts of work. They have shown the rate to be less performing the same speed of locomotion at simulated lunar gravity than at Earth gravity. We have not performed other kinds of work, however. We agree, of course, there is 1 Earth g operating here on the subject and on the otolith system. However, the variations of the component of that gravity in the plane of the body as he leans are appreciably less than they are as he leans in 1 Earth g; so there is some relation, although we would have to agree this is not a total simulation of lunar gravity.

Anatomical Features of the Auricular Sensory Organs [1]

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SUMMARY

This paper contains a general survey of the structure of the vestibular sensory system—of the utricle, saccule, and semicircular ducts and crests. The major interest has been devoted to "gross" anatomical features, but a general description of the epithelial structure and also of nerve endings and blood vessels is included.

INTRODUCTION

The mammalian inner ear has two major subdivisions; the auditory end organ or cochlea and the nonauditory vestibular end organs which are important receptors for orientation and balance.

The cochlea in mammals is a helical structure (figs. 1–3) in which sensory cells and their supporting elements together form the organ of Corti, also called the acoustic papilla. The sensory cells are of two basic types—the inner and the outer hair cells—each group showing further internal differences with respect to details of structure and innervation. The organ of Corti is innervated by a large number of centripetal or afferent nerve fibers, varying from 25 000 to 50 000 in different species, and a much smaller number of centrifugal or efferent nerve fibers which vary from 500 to 600, depending on the species. The afferent fibers are peripheral processes (dendrites) of cells which compose the spiral ganglion. The efferent fibers are axons of cells located in several centers in the brain stem (refs. 1–4).

The vestibular portion of the inner ear is connected to the cochlea by a narrow canal, the ductus reuniens. This paper mainly concerns the vestibular end organs; however, certain key features of cochlear anatomy should be mentioned because they have an important bearing on the developing study of vestibular anatomy. Recent studies (refs. 5 and 6) have demonstrated a high degree of geometric precision in the structure of the mammalian organ of Corti (fig. 4). This organizational regularity makes it easy to describe and register in detail the location and extent of cellular damage or depletion in the organ of Corti of animals exposed to noxious agents such as noise or ototoxic antibiotics (ref. 7). Whether an equally regular order exists in the vestibular sensory epithelia is

[1] This study has been supported by the Office of Naval Research (Contract No. N 62558–4264) and by the Swedish Medical Research Council.

Figure 1.—*Human cochlea showing blood vessels injected with dye. Due to the injection the form of the cochlea is well visualized. (Preparation by Axelsson.)*

Figure 2.—*Human cochlea seen from the side showing the general form and vascular supply. (Preparation by Axelsson.)*

as yet uncertain; however, this problem is the subject of intensive study, and there is increasing evidence of a high degree of structural organization in these epithelia also. The demonstration of a comparably regular patterning would represent a great advance in the study of the normal and pathological vestibular end organs. A thorough knowledge of cellular density, frequency and distribution of sensory cell types, and of structural and functional orientation of the cells is essential for the evaluation of small changes in the vestibular sensory areas.

The vestibular labyrinth comprises a system of canals and sacs which are filled with endolymph and contain several patches of sensory epithelium, the specific receptor organs. The

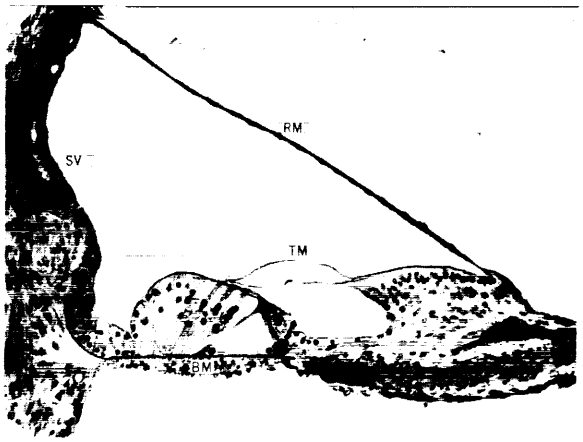

Figure 3.—*Organ of Corti and the cochlear duct from a squirrel monkey.* RM: *Reissner's membrane.* TM: *tectorial membrane.* BM: *basalar membrane.* SV: *scala vestibuli.*

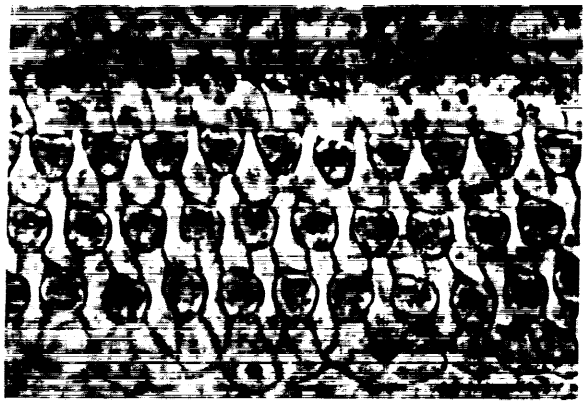

Figure 4.—*Surface preparation showing cellular pattern in the organ of Corti of a squirrel monkey. Phase-contrast photomicrograph.*

endolymphatic canals and sacs are surrounded by another fluid, the perilymph, which, in certain areas, is traversed by a delicate meshwork of cells, blood vessels, and fibrous strands anchoring the canals to the walls. The perilymphatic space is free of this meshwork in other regions, notably the cisterna perilymphatica just inside the stapedial footplate. Outside the perilymphatic space, the membranous labyrinth is surrounded by a shell of hard bone, the bony labyrinth.

In each labyrinth there are three semicircular canals and two sacs, the utricle and the saccule. Each semicircular canal forms a narrow duct which widens to a single ampulla in each canal. The ampullae of the upper vertical and of the horizontal canal are situated close together, while the posterior canal has its ampulla at the other end of the utricle, which forms a tubelike connection between all three semicircular canals (figs. 5–12). The canals are arranged in a three-dimensional system, the planes being almost orthogonal to each other. Their interrelated position can be recognized easily from figures 5 and 6. The horizontal canals of the right and the left ear are both in the same plane, while the upper vertical canals form an angle of about 90° to each other. Each thin, tubelike canal has in its widened ampulla a crista ampullaris containing the sensory cells. The sensory areas of the utricle and saccule are found in the macula utriculi and macula sacculi, respectively.

Figure 5.—*General view of the membranous labyrinth of a guinea pig. The cochlea* (C) *and the three semicircular canals* (S, P, H) *are easily recognized.*

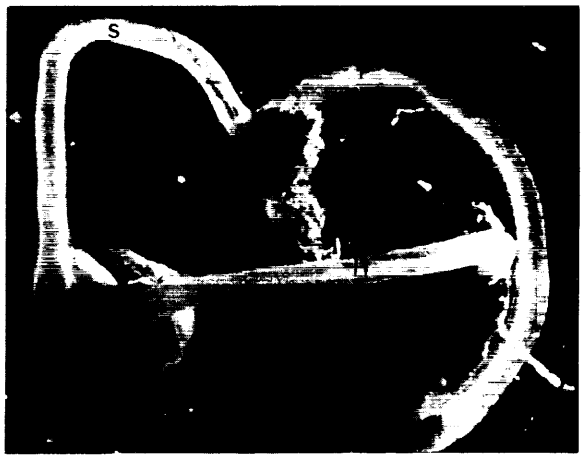

Figure 6.—*The three semicircular ducts of a guinea pig.* H=*horizontal,* S=*superior,* P=*posterior.*

Figure 7.—*This figure shows the narrow utriculosaccular duct* (arrow), *forming a connection between the utriculus* (U) *and the opening of the endolymphatic duct* (DE). (S=*sacculus.*)

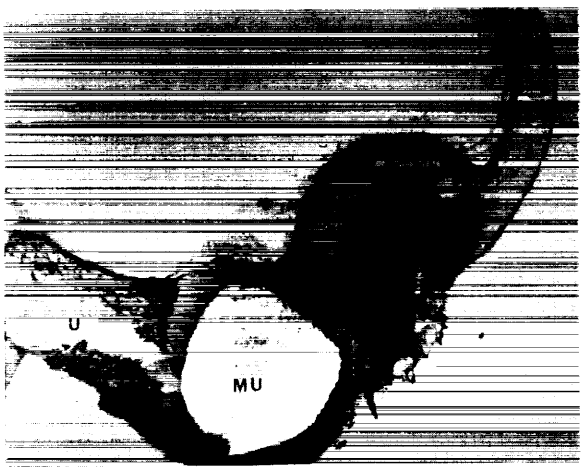

Figure 8.—*Utricle* (U) *of left ear showing macula utriculi* (MU) *covered by white statoconia. The horizontal semicircular duct* (SD) *can also be seen.*

Figure 9.—*Right ear of same animal as in figure 8; corresponding view showing the utricle* (U), *macula utriculi* (MU), *and the horizontal duct* (SD).

All the sensory areas of the inner ear conform to a general principle in that they are provided with sensory cells, bearing "hairs" on their surfaces and in direct contact with nerve fibers at their bases. Above the hairs the organ of Corti and the semicircular canals are provided with a jellylike structure, the tectorial membrane (fig. 3), and the cupula (fig. 13), respectively. This jellylike material has approximately the same density as the endolymph. Similar structures are found above the surface of the sensory areas of the utricle and saccule, differing from tectorial membrane and cupulae in that they contain large numbers of crystals called statoconia. In mammals the statoconia consist of calcite ($CaCO_3$), with a density of around 2.74. The composition of these crystals has been analyzed systematically in a representative series of animals (refs. 8 and 9).

During acceleration, deviations of the cupula or translatory movements of the statoconium layer relative to the macular surface result in

Figure 10.—*Higher magnification showing the macula utriculi of a guinea pig. In the guinea pig the following measurements have been made on the macula utriculi* (MU) :
Length: 0.95, 1.0, and 1.04 mm (three animals).
Width in center: 0.91, 0.90, and 1.04 mm (three animals).
Surface: ca 0.60 mm².
Total number of sensory cells: ca 9000.

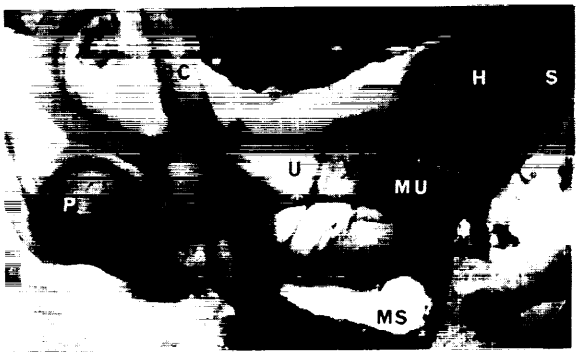

Figure 11.—*Utricle* (U) *and saccule with macula utriculi* (MU), *macula sacculi* (MS), *and the three ampullae of the semicircular ducts* (H=*horizontal*, S=*superior*, P=*posterior*). *The crus commune* (CC) *connects the superior and posterior ducts with the utricle.*

shearing movements at the level of attachment of the hairs. These movements stimulate the sensory cells, modifying nerve impulse formation in the vestibular nerve. This nerve consists of an upper portion which transmits impulses from the macula utriculi, the upper vertical and the horizontal canals, and also from the macula sacculi, and a lower portion which transmits impulses from the major part of the macula sacculi and from the posterior vertical canal.

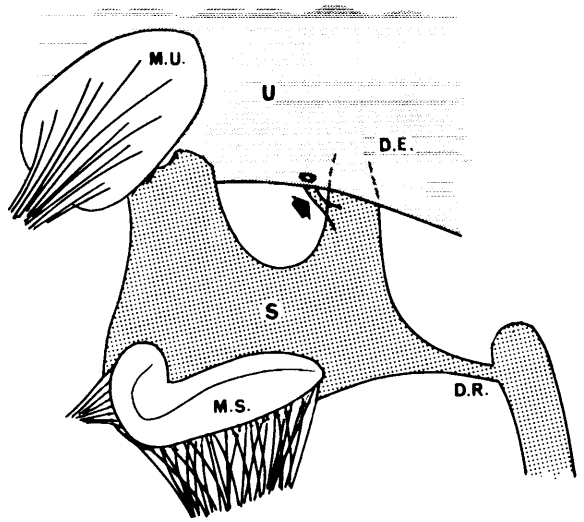

Figure 12.—*Schematic figure showing the form of the saccule and the relation between the saccule* (S) *and the utricle* (U). *The black arrow indicates the utriculosaccular duct;* D.E. *is the endolymphatic duct, and* D.R. *is ductus reuniens connecting the cochlear duct with the saccule.* M.U.=*macula utriculi.* M.S.=*macula sacculi.*

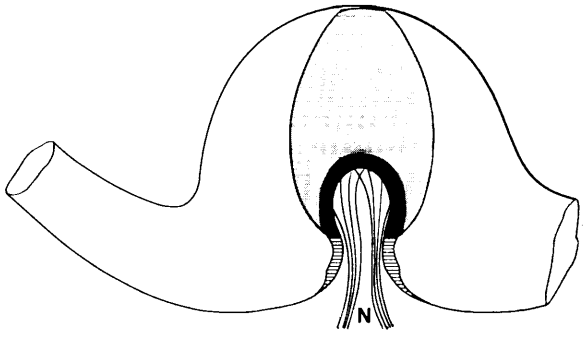

Figure 13.—*Schematic figure showing crista ampullaris covered by sensory epithelium (black) and cupula (gray). N represents the nerve fibers.*

In the following paragraphs a more detailed description is given of the form, size, and structure of the different parts of the vestibular apparatus. This is intended to form a basis for further discussion of the ultrastructure and functions of special regions of the vestibular sensory epithelia.

For our studies we have had available large numbers of specimens from guinea pig, squirrel monkey, man, and rabbit. There is considerable similarity in the structure of vestibular epithelia among these species; however, certain variations may be pointed out. The macula utriculi in man and squirrel monkey is larger than that of the guinea pig, and in man the cristae of the semicircular canals seem to be lower than in the other species. If we disregard these minor differences, there is a general resemblance among all the mammals studied, and the following description applies generally in all other respects.

The utricle is an irregular thin-walled tube with an oval cross section. In many guinea pigs the thin wall is heavily pigmented, but there is always a pigment-free zone in the upper anterior part, the recessus utriculi, which contains the macula. Close to this region the upper vertical and horizontal ampullae can be observed. At the lower, posterior end of the utricle, the sinus posterior, we find the ampulla of the posterior, vertical canal. The ampullar ends of the horizontal and upper vertical canals are situated in close relation to each other (figs. 5, 6, 11, 14, and 15). The nonampullar ends of the upper vertical and the posterior vertical canals, on the other hand, coalesce to form a common canal, the crus commune, which terminates in the superior sinus of the utricle. The nonampullar end of the horizontal canal terminates separately in a slightly widened part of the utricle. The utricle thus has five openings for the semicircular canals. A sixth and very small opening is that of the utriculosaccular duct, a structure which has been discussed by Bast and Anson (ref. 10). This duct is extremely narrow in most of the animals studied. It begins as a very small slit in the utricular wall and tapers off almost to nothing in many animals. Very few of the many guinea pigs

Figure 14.—*Horizontal* (H) *and upper vertical* (S) *ampullae with cristae* (arrows). *Utriculus* (U) *is seen at lower right.*

Figure 15.—*Ampullae with cristae, one seen from above and one from the side.*

studied have had more than a very narrow connection between the utricle and the endolymphatic duct, while many animals have displayed an almost capillary-sized lumen. It is most interesting to contrast the size difference of the utriculosaccular and the endolymphatic ducts. Even the ductus reuniens, connecting the cochlear duct with the sacculus, is much broader. The problem of interconnection between the fluid spaces in the labyrinth is now being studied by Lindeman at Göteborg.

The macula of the utricle is shaped like a spade with a broader base and a pointed tip (figs. 11 and 16). Not only is the base broader but it is also much thicker than the outer part because of the clustering at that region of nerve fibers from the whole macula to form the macular nerve. The nerve fibers and connective tissue cells intermingled with a rich plexus of blood vessels form a base for the macular sensory cells. The surface is not entirely flat, the basal portion being curved relative to the remainder to form a concave surface on which the statoconial membrane rests. This membrane consists of a jellylike matrix in which are imbedded large numbers of hexagonal prisms, the statoconia. The statoconia vary in size, the smallest being less than 1μ, the largest up to 25–30μ. The largest statoconia are regularly situated closest to the epithelium and also most marginal with respect to the macular surface (figs. 16–19).

The saccule forms an irregular sac of a shape similar to that of the goatskin water bags still in use in some countries (figs. 12 and 17). The ductus reuniens connects the saccule to the ductus cochlearis, and the endolymphatic duct to the endolymphatic sac. As in all the other regions the wall is very thin, consisting of epithelium lined with a slight, connective tissue reinforcement close to the oval window region. The walls of the utricle and saccule are actually in direct contact, adhering to each other in a region close to the macula utriculi.

The sensory cells are found in the macula sacculi, a hook-shaped area lying close to the wall of the bony labyrinth. Its form is very characteristic in all animals studied, though there is some variation in width of the macula in dif-

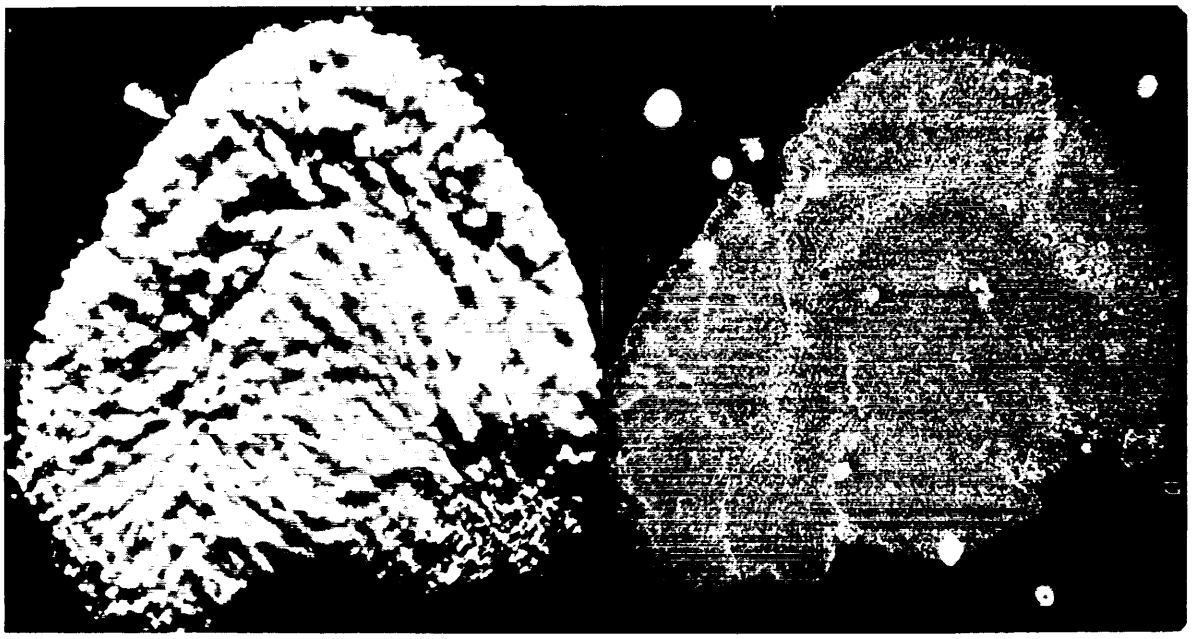

Figure 16.—*Two photomicrographs of the same macula utriculi.*

ferent species. The crystals at the surface form a distinct snowdrift-like configuration along the midline of the macula (fig. 17). On the utricular macula the statoliths also form a similar ridge in the shape of a horseshoe over the surface epithelium (the striola). These macular landmarks form a boundary of different morphological polarization of the cellular components. The macula sacculi is very closely attached to the bony wall and is therefore difficult to separate from the bone. In the subepithelial layer there is a rich plexus of nerve fibers and blood vessels.

The ampullar crests vary slightly in form in different species. From the base of the ampulla a septum containing nerve fibers and blood vessels reaches up to about one-third of the ampullar diameter (figs. 13 and 20). Over the septal surface, the epithelial lining forms a smooth, rounded crista ampullaris. The upper part contains the sensory epithelium which extends down the slopes of the crest to a border of junctional epithelium which merges gradually into the lining epithelium of the canal. On both sides of the crista, a special region, presumably of great importance for the fluid exchange of the labyrinth, is formed by the planum semilunatum, having specialized cells of two types. The nerve fibers inside the crista show a distinct tendency toward somatotopic organization. In

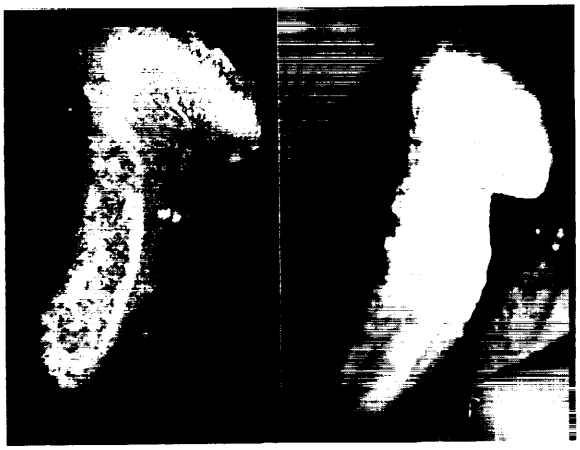

Figure 17.—*These two figures are made from the macula sacculi of a guinea pig. In the right figure the statoconia are in position over the macular surface. In the left figure the crystals have been lifted off from the macula, and the figure shows the crystal layer only. Magnification 68 ×. From the guinea pig the following measurements have been made: Length: 1.41 mm; width: Uppermost portion=0.61 mm; width: Lower portion=0.35 mm; surface area: 0.54 mm²; total number of sensory cells: 8100.*

Figure 18.—*Statoconia from a macula sacculi. The phase contrast micrograph shows a form and size of the crystals in this region. The crystals vary in size from 1 μ to 28 μ in our specimens.*

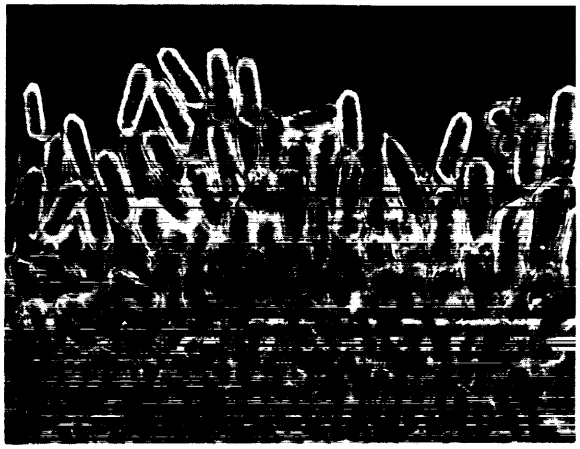

Figure 19.—*Phase-contrast micrograph showing the form and size of statoconia. Observe the small dots in the centers of the crystals.*

the guinea pig as well as in man, the nerve fibers from each half crista come together on either side of a core which is relatively poorer in nerve fibers but contains blood vessels (fig. 13). In the cat, the cristae are divided across the middle by a ridge devoid of sensory cells; hence, there is a tendency to quadrantic division of the ampullar nerves.

EPITHELIUM OF THE VESTIBULAR SENSORY REGIONS

All the vestibular sensory epithelia bear a close resemblance to each other, a general principle recognizable in all the mammals studied. Studies by Wersäll (ref. 11) showed that the ampullar cristae were provided with sensory epithelium of a characteristic type, and further studies by Smith (ref. 12), Engström and others (refs. 13–15), proved that the same principles could be recognized in the maculae as well. A considerable literature has now accumulated on this subject, and detailed studies presented at a previous meeting have been published (refs. 13 and 16). These and a series of related studies (refs. 11 and 17–19) have provided a basis for functional discussions, some of which will be found in subsequent papers.

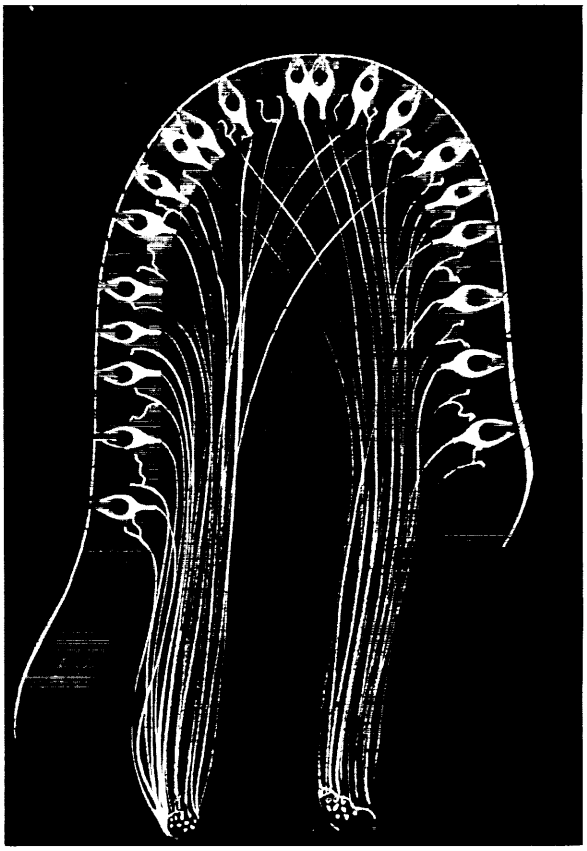

Figure 20.—*Figure corresponding to previously mentioned figure 13, showing detail of epithelium, with the two types of sensory cells and nerve endings in correct relations.*

In all the vestibular epithelia, two types of cells can be recognized. Of these, type I is a flask-shaped cell surrounded by a large nerve chalice, and type II is a more cylindrical cell with a rounded bottom. Transitional varieties between these types may be found as well (ref. 14). Type II is evidently the more primitive one, and type I the more differentiated. Their form and general arrangement can be seen in figures 20–22.

The cell surfaces are provided with hairs of two different kinds, each cell having 1 kinocilium and about 60 stereocilia arranged in a regular and characteristic pattern. In 1962, Engström, Ades, and Hawkins (ref. 20) demonstrated that the cilia had varying lengths, increasing toward the kinocilium, and that the macular surface had a morphological polarization over large areas. Löwenstein and Wersäll (ref. 19) had previously described a morphological polarization of individual cells which, together with increasing evidence that this polarization was of great functional significance, has stimulated further studies. The works of Flock (ref. 17), of Dijkgraaf (ref. 21), and of Spoendlin (ref. 16) have dealt extensively with the morphological polarization and its functional significance. From a functional point of view, the presence of two types of sensory cells is of great interest. Related to this is the question as to what extent lower animals, which display only one type of sensory cell, may be shown to differ functionally from mammals having two types of sensory cells.

Figure 21.—*Arrangement of the sensory cells along the surface of crista ampullaris of guinea pig. Nerve stain.*

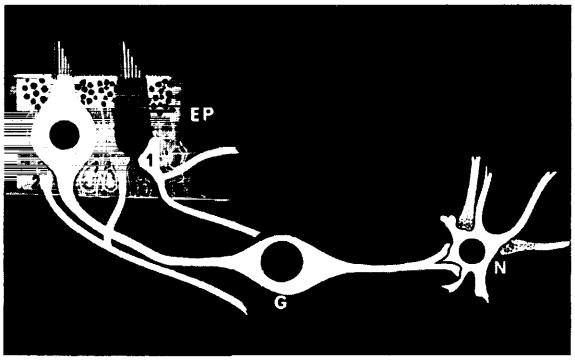

Figure 22.—*Schematic figure of the two types of sensory cells and their relations to nerve endings and nerve fibers.* EP=*epithelium.* G=*vestibular ganglion.* N=*vestibular nuclei.*

One of the major differences between the two types of sensory cells is the form of the neural terminations at the cell membrane. This innervation has been the subject of extensive study (refs. 11 and 13–15). It has been shown that there are two principal varieties of nerve endings at the type II cells (fig. 22). Of these, one variety, type 1, is little granulated and supposed to be of afferent or centripetal nature, while type 2 is richly granulated, presumably of efferent or centrifugal nature.

The sensory cell of type I is surrounded by a nerve chalice as described by Wersäll (ref. 11). It was earlier supposed that synaptic contact between the nerve chalice and the sensory cell took place only at the neck region. At a symposium in Budapest in 1963, it was demonstrated (ref. 15) that the sensory cells of type I had special areas well suited for synaptic contacts between the sensory cell and the nerve chalice. Over large areas the membranes of the two are separated by a space of more than 250 Å; however, this gap may be reduced almost to fusion at certain points, as shown in figures 2, 3 and 27 of reference 13. In describing the structure of these areas along the surface of the type I cell, we wrote, "It is possible that the synaptic contact between sensory cell and centripetal nerve ending signifies an electrical form of

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transmission, while the granulated endings act with a chemical transmitter." Sir John Eccles (ref. 22) made the following comment in discussion of the paper: "Engström's pictures have very clearly indicated to me that the transmission from receptor cell to afferent terminal is electrical." This problem is being investigated further by others.

Gacek (ref. 23) reported that the vestibular nerve in the cat contains about 12,000 fibers of afferent nature, while the count of efferent fibers is much smaller, numbering about 200 in the entire nerve. If we accept the theory that the granulated nerve endings in the vestibular epithelia are all of efferent nature and therefore must have their origin in the 200 fibers mentioned, each of these fibers must ramify very widely. An alternative hypothesis has been suggested that some of the apparently efferent fibers in the vestibular sense organs might actually originate as branches of afferent fibers feeding back from short distances proximal to the afferent endings. This problem has not been solved. The same problem is also of enormous interest in the organ of Corti where type II granulated nerve endings form such a large volume, especially in the basal turn.

The sensory areas in the vestibular labyrinth are provided with a rich capillary network. The proximal arteries leading to the vestibular regions form complicated coils, such as are well known in the plexus caroticus (ref. 24). Koburg (ref. 24) has shown, in experiments with isotopes, that there was a high metabolic rate among these coils. On the basis of these studies, it was concluded that the plexus regions were of great interest from a functional point of view. Studies by Engström (ref. 14) have shown that the arteries of these regions are provided with abundant perivascular cells (figs. 23–25) which are of irregular form and have long extensions which form a plexus around the arteries. It is quite evident that these cells are found in regions exactly corresponding to the plexus cochlearis and also along the contorted vessels supplying the vestibular areas.

Let us summarize, then, the structural features of the vestibular apparatus which appear to offer at least a part of the means, or suggest experimental approaches, for explaining the observed complexities of vestibular function.

(1) The mammalian vestibular epithelia all show two types of sensory cells which differ from each other in form and in the exact pattern of nerve endings.

(2) There are at least two morphologically distinguishable types of synaptic connections which may be also functionally distinguishable.

(3) The sensory cells have both afferent and efferent endings, the functional significance of the latter being as yet little known.

(4) The polarization and orientation of vestibular hair cells, as indicated by position of kinocilia, follow definite patterns for each of the cristae and maculae and have important implications bearing on directional sensitivity in the maculae especially.

Figure 23.—*Blood vessels in the modiolus of the cochlea* (arrows) *with perivascular cells.* G= *ganglion cells. Compare the two following figures also.*

Figure 24.—*Perivascular cells from modiolus of guinea pig cochlea.*

Figure 25.—*Perivascular cells from modiolus of guinea pig cochlea.*

(5) The arterial capillary network supplying the vestibular epithelia shows structural peculiarities (similar to the cochlea) which offer two adaptations for local control of blood supply, one being their tortuous course; the other, the abundant network of perivascular cells seen on these vessels.

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DISCUSSION

COHEN: Dr. Engström, I was interested particularly in any evidence you may have as to how close the lining of the endolymphatic fluid is to the bony container in which it fits under normal living conditions. Would there be any chance really for any shifting of the fluid because there may be some space in any portion, not only between the membrane and the bony canal of the semicircular canal, but in the otolith region, or even in the endolymphatic duct? Does the membrane always fit tightly right up against the bone or is there some space?

ENGSTRÖM: The semicircular duct is adherent at the outside very close to the bony wall. The macula of the utricle has its sensory epithelium in direct contact with the bone below. The utricle is situated almost free, adhering only proximally to the bony wall.

COHEN: The saccule?

ENGSTRÖM: The macula of the utricle sits at one end of the utricle close to the upper vertical and horizontal ampullae. The macula utriculi is rather stiff, anchored by its base to the bone and standing out like a tongue. There is a possibility of movement in the soft endolabyrinth. All these structures have great possibilities of movement, and it is easy to change the form of the utricle as we can see.

GILLINGHAM: We have a problem which has come to light recently that might possibly be explained by free-floating otoliths or free-floating fragments of otolithic membrane. Have you ever found, or do you often find, free-floating otoliths in the endolymph system?

ENGSTRÖM: At preparation we find them very often free, but it is very difficult to state if that is an artifact. A few small statoliths may be moving around, but the whole system is a rather stiff system. You can lift off the whole thing practically in one piece. But at the margin of the crystal layer on the maculae, there are regions where statoconia are scattered more or less around. If you rotate the animals, it is known that you can centrifuge off the statoconia. I believe the major parts of the statoconia membrane stand in place. Occasional statoconia, perhaps, move out, but I don't believe they could play any major role. But the whole system can be centrifuged off, as has been known for a long time.

GUALTIEROTTI: You mention briefly the importance of the blood-vessel structure. I would like to hear more about that. The second point is this: You mention the change in shape and dimension of the utricles. Would that happen normally and would that correspond to an angular shift of the position of the macula too?

ENGSTRÖM: I hope a thesis will come out in 1966 about the blood vessels of the labyrinth, containing very much of the information in this field. I think that this is a very important problem and one of the things that should be studied much more than we have done up to now. It is possible to make different kinds of stains, injections, et cetera. I know Dr. Hawkins is working along the same lines too, and is using a method for staining the blood vessel. It is also pos-

sible to open the labyrinth too and to look into it, into the circulation going on. Just below the macular epithelium, scattered blood vessels are found all the way along the endolabyrinth and along the bony wall outside, but nothing to compare with the density found under the sensory epithelium. The blood vessels often form loops going out to the sensory epithelium where they form a very rich plexus.

In the cochlea there is a certain distance between the blood vessels and the sensory epithelium. In the vestibular apparatus the blood vessels are very rich close to the basal part of the sensory epithelium. There is a glomerulus-like structure on the way to the vestibular apparatus formed by the major arteries going in. Around those loops we find special pericytes. I can't answer if there will be a movement of translation of crystals at the surface. I only know that the fluid in the semicircular canals can be graded in its movement without any kind of acceleration, just by moving the fluid (perilymph) outside the labyrinth. If you move that, the fluid will sink in the semicircular canals and get a resultant deviation of the cupula just as in the case of an acceleration stimulus.

GUALTIEROTTI: What about the utricle?

ENGSTRÖM: I can't answer that question. I don't know that. That depends, I believe, on the speed with which you modify the distention of the utricle.

MEHLER: Do you have any observations on the guinea pig relative to this status hypnoticus-like phenomena that appear in the guinea pig when it is laid in a supine position? More specifically, have you observed any differences in labyrinthectomized versus normal, unoperated guinea pigs?

ENGSTRÖM: No, I have not.

MAYNE: Dr. Engström, I have been very much interested in your comments on the mobility of the otoliths. We have suggested in one of our reports a mode of stimulation of these organs which requires such mobility. According to our suggestion, the otolith mass would have two modes of movement, a shear with respect to the epithelium and a movement of the whole organ, including the membranous sac supporting it, into the perilymph cavity. The first movement would be underdamped and therefore would be a measure of acceleration. The second would require a flow of the endolymph to or from the ductus endolymphaticus through a small canal and would be, therefore, overdamped. Both movements would react on the same hair cells, and the signal would contain data about both acceleration and velocity. The model accounts for the type of response of single fibers reported by Lowenstein et al. Does this mechanism appear plausible to you?

ENGSTRÖM: The possibility of two different systems of movement is what you are asking for in reality. I have difficulty in understanding how there could be two different kinds of movements that way. There is a division inside the macula in the sensory-cell components, so there is a gradation perhaps inside the cells. Could it not be instead that some cells are much more sensitive, others less sensitive, as there is a dualism among the cells in this region? Instead of a very damped system, couldn't you have a system which is just as much damped from a physical point of view but more sensitive from a neural point of view? Is that not possible in your system?

MAYNE: Yes; it would be possible to account for the same response in terms of cells of different sensitivity and rate of adaptation. But the theory of two transducers combined into one seems attractive to us. The morphological basis for this theory appears to exist; the hydromechanical principle is quite parallel to that of the semicircular canal; the mechanism simplifies the design by utilizing identical cells affected differently by the movements of the otolith mass. It is, however, only a hypothesis which calls for experimental check.

ENGSTRÖM: The neural components form different sets of systems in there. It is quite possible to imagine that there can be very pronounced gradation between these different types of cells. Nature has solved some of these problems by arranging cells in a special kind of pattern that has been studied by Wersäll, Flock, and Dijkgraaf. The cells can arrange themselves. Their neural components can be modified in that way. We could get a different form of transducer in the way they act.

GUALTIEROTTI: I think I have some suggestion about what you said. Certainly there are two different kinds of cell function. The neural supply is different. The flask-shaped cells are either provided with one, or a little more than one, private fiber, or two or three of these cells are combined in one private nervous fiber; whereas, the cylinder-shaped cell seems to be disposed in a sort of netlike system in series. If they are disposed differently with respect to the vertical, this of course will damp physiologically the information, as the responses in opposite direction will tend to cancel themselves out. Another point is that we have definite evidence that there are at least two kinds of cells as far as sensitivity is concerned. There are highly sensitive cells which respond to 0.01 g or even less. There are less-sensitive ones with a much larger range. These two kinds of sensors might have a completely different effect on the central integration.

ENGSTRÖM: Certain problems arise as to the population of sensory cells in more primitive animals because then the same type of population doesn't exist any more. There is a division even there.

MONEY: In your diagram of the crista, I thought there were hairs represented as being in length even greater than the height of the crista. I would like your assurance that there are no hairs that long. I wonder, also, if you could give me an estimate of what fraction of the endolymph ring is completed outside the utricle and what fraction is completed inside the utricle.

ENGSTRÖM: If the length of hairs at the crista has been depicted that way, then it is wrong, but I don't believe it has. From my point of view there are very long hairs at the crista. They run farther out than the diameter, the thickness of the epithelium, around 40 microns, perhaps, or even more. There are, maybe, very long hairs in these regions. I don't believe they go all the way out to the outer ends, as you suggested. As to the volume inside the utricle and in the semicircular canals, I have not calculated that, but that should be rather easy to do. It depends on where you start to weigh or calculate your mass because the utricle is a cylindrical structure and it forms the sinus superior, posterior sinus, and a common duct. If you ask me what part you want to know the volume of, I will tell you the volume of it later on.

Dimensional Study of the Vestibular End Organ Apparatus

MAKOTO IGARASHI

U.S. Naval Aerospace Medical Institute

SUMMARY

Micromeasurements were performed to determine the dimensions of the inner-ear apparatus in the human, the squirrel monkey, and the cat by using a series of horizontal sections. The membranous semicircular canal structure in the human is almost twice as large as that of the squirrel monkey and of the cat, although the sizes of the skulls are quite different. On the other hand, the size of the canal crista is not significantly different among these three species. In other words, the human has a considerably smaller crista.

The surface area of the human saccular macula is almost three times as large as that of the squirrel monkey and of the cat. Both the diameter of the cochlear base and the length of the basal membrane are less than twice as large in the human as in the other two species.

INTRODUCTION

The vestibular end organs which are located deep in the temporal bone are stimulated physiologically by gravitational and inertial forces. The macula-otolithic membrane system is considered to respond to linear gravitoinertial forces, and the crista-cupula system to angular accelerative forces. To better understand the relation of structure to function, certain critical measurements have been made mainly of the gross structure of the sensory organs of the inner ear in man, monkey, and cat. These are presented in summary form together with some descriptive remarks and comparative data.

PROCEDURE

Ten human temporal bones, 10 squirrel monkey temporal bones, and 5 cat temporal bones, all from adults with no known otological disorder nor any prior experimental procedure, were used for this study. The horizontal serial sections were prepared following the standard procedure: Heidenhain-Susa perfusion fixation, 5 percent trichloroacetic acid decalcification, neutralization with 5 percent sodium sulfate, dehydration with ethanol, celloidin embedding, sectioning, and staining in hematoxylin-eosin. For the purpose of comparing the tissue changes after the different procedures, some of the squirrel monkey bones were fixed by 10 percent formalin perfusion and decalcified with 20 percent DECAL or 10 percent EDTA.

The micromeasurements were performed to determine the following:

(1) Circle diameter of the horizontal semicircular canal
(2) Height of the horizontal semicircular canal ampulla (membranous labyrinth)
(3) Height of the cupula of the horizontal semicircular canal
(4) Thickness and height of the horizontal semicircular canal crista
(5) Long axis and short axis of the cross section of the posterior semicircular canal (bony canal)
(6) Long axis and short axis of the cross section of the posterior semicircular canal (membranous canal)

(7) Width of the posterior semicircular canal ampulla (membranous labyrinth)
(8) Width of the posterior semicircular canal crista
(9) Thickness of each zone of otolith end organ (cross section of the saccular macula)

In addition to the micromeasurements, the following measurements were performed for the purpose of comparison among the different species:

(1) Skull size
(2) Surface area of the saccular macula
(3) Cochlear reconstruction, which includes length of basal membrane, diameter of cochlear base, and number of cochlear turns

RESULTS AND DISCUSSION

When sectioned in the plane parallel to its long axis, the shape of the horizontal semicircular canal appears more nearly circular than elliptical in the human, squirrel monkey (fig. 1), and cat. In birds, the canals are not of circular or elliptical shape, but are like twisted ropes (refs. 1 and 2; Igarashi, personal data), especially the superior semicircular canal. Inasmuch as the plane of the horizontal semicircular canal is not in the same plane as the midmodiolar plane, this entire circle could not be observed in so-called horizontal serial sections in which the good midmodiolar sections of the cochlea, necessary for the cochlear reconstruction, are expected.

Figure 1.—*Horizontal semicircular canal of squirrel monkey, sectioned parallel to the plane of the canal. The membranous canal is circular, smooth, and of even width.*

Some measurements of canal structures have been previously done in lower animals by de-Burlet (ref. 3).

As is seen in figure 1, the membranous canal is rather smooth and the size of the membranous canal seems likely to be even all the way around in the squirrel monkey and in most cats. In the human, slightly uneven canals or bleb formations inside the canal were observed occasionally, but these were usually insignificant.

The canal opening to the ampulla (ostium tubulare) is usually located somewhat laterally to one side when the eminentia cruciata exists on the crista (refs. 4–6). Even without the eminentia, as in the horizontal canal of the squirrel monkey, the opening seems more likely to be located slightly laterally to one side of the ampulla.

The horizontal semicircular canal of the human is twice as large as that of the squirrel monkey, although the skull and the eye are about 10 times larger in the human. In comparison with the sizes of the skulls, the circular diameter of the horizontal semicircular canal is relatively larger in the squirrel monkey than in the human or in the cat (fig. 2). The squirrel monkey has very well developed mastoid air cells which may allow greater development of the semicircular canal.

The distance between the center of the foramen magnum (which is the location of the long axis of the primate body) and the center of the horizontal semicircular canal circle was 42–50 millimeters in the human, 11–13 millimeters in the squirrel monkey, and 13–15 millimeters in the cat. Thus, if these are compared between the human and the squirrel monkey, the diameter of the horizontal canal is found to be twice as large in the human; however, the canal is located roughly four times as far away from the center of the foramen magnum which is the axis of spontaneous head rotation (fig. 3).

Dimensions inside the horizontal semicircular canal ampulla were measured microscopically.

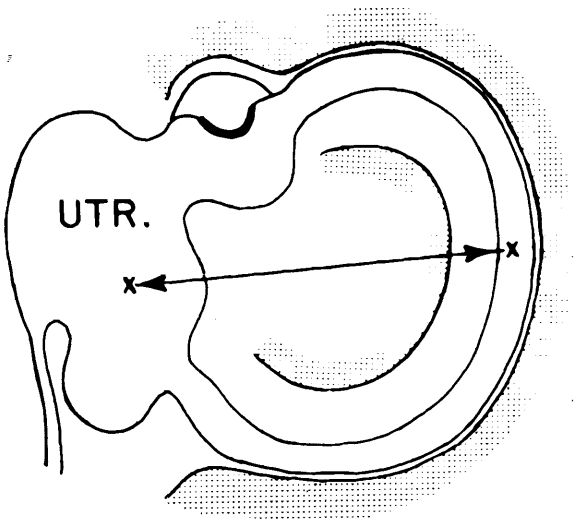


Figure 2.—*Comparison of circular diameters of the horizontal semicircular canals. Human, 6.4 mm; squirrel monkey, 3.6 mm; cat, 4.1 mm.*

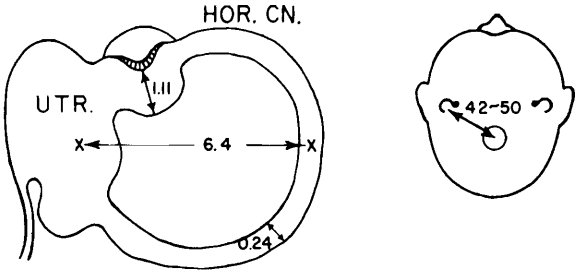


Figure 3.—*Location and dimensions of the membranous horizontal semicircular canal in the human.* UTR. =*utriculus.* HOR. CN.=*horizontal canal.*

Each specimen was cut at a slightly different angle, since it is impossible to cut all specimens in exactly the same plane. The measurements were made following the serial sections, and the largest value of A (height of membranous ampulla) in the series was recorded because this point was considered as the closest to the center of the ampulla. B (height of cupula), C (thickness of crista), and D (height of crista) were measured in the same slide (fig. 4).

The ratio between the sizes of membranous ampullae (A) among three species was almost the same as that of the circular diameter of the canal; the squirrel monkey and the cat are similar in size, and the human is slightly less than twice the squirrel monkey and cat.

However, the sizes of the cristae (C, D) were not so much different. In other words, the human has considerably smaller crista than have the other two species (fig. 5).

The shrinkage of the membranous semicircular canal is always a problem in histological investigation. In any sort of microscopic preparations, one never can be convinced that the measurement in the preparation is exactly the same as in the live state. Thus, the slight morphological changes after the preparation procedures cannot be overlooked. However, the difference among these groups was usually not more than an intraindividual difference after using different procedures, such as: Heidenhain-Susa fixation, 5 percent trichloroacetic acid decalcification; Heidenhain-Susa fixation, 20 percent DECAL decalcification; 10 percent formalin fixation, 5 percent trichloroacetic acid decalcification; and 10 percent formalin fixation, 20 percent DECAL decalcification. Therefore, as long as these are nonpathologic cases, the difference between different procedures is

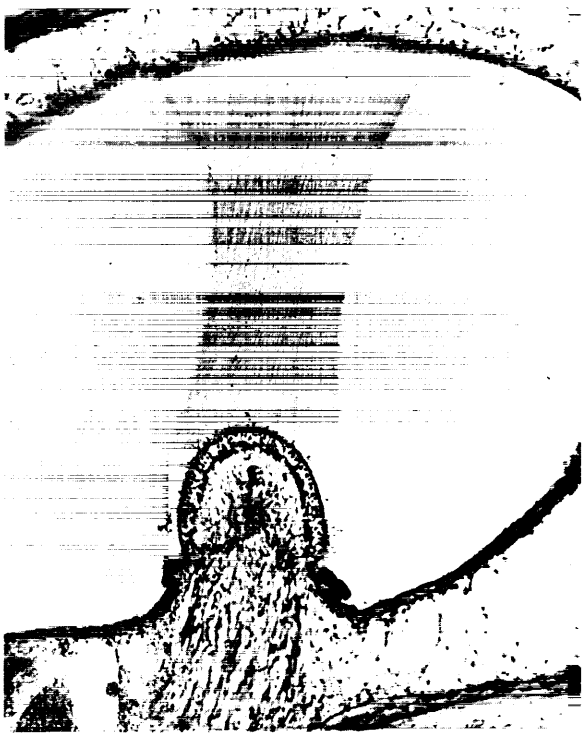
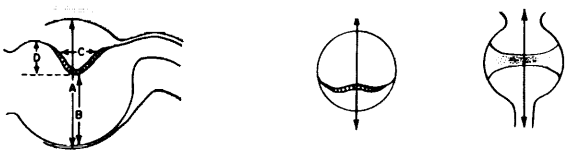


Figure 4.—*A view of crista-cupula system of the horizontal semicircular canal from a squirrel monkey.*



	Height of membranous ampulla, mm (A)	Height of cupula, mm (B)	Thickness of crista, mm (C)	Height of crista, (D)
Human	2.07	1.11	0.42	0.25
Squirrel monkey	1.28	.71	.23	.23
Cat	1.22	.67	.22	.22

Figure 5.—*Dimensional comparison of crista-cupula system of the horizontal semicircular system.*

negligible, though a great difference could be observed in cupulae and otolithic membrane among these groups (ref. 7).

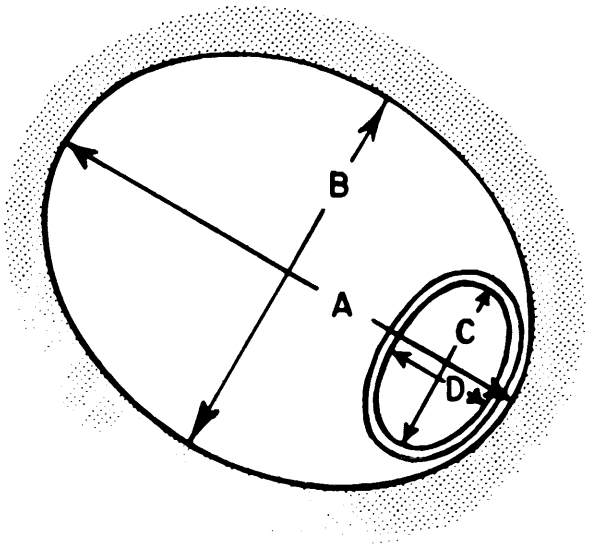
The measurements of cross sections of the posterior semicircular canal were made by using the horizontal series. The records were made by the smallest measurements from each series, which was actually the very cross section of the posterior semicircular canal crus (fig. 6).

The cross section of the bony canal crus is oval in the human, while it is almost circular in the squirrel monkey and in the cat. The long axis of the cross section was almost parallel to the long axis plane of the canal itself. The human canal crus is over twice as large as that of the squirrel monkey or of the cat.

The membranous canal crus has an almost circular cross section both in the squirrel monkey and in the cat, but it is again oval in the human. The long axis of this oval of the membranous canal crus is perpendicular to the long axis plane of the canal itself. The size of the cross section of the membranous canal in the human was slightly less than twice as large as that in the squirrel monkey and in the cat.

A comparison of the measurements of the membranous canal structure with the skull sizes among the three species is shown in table 1.

The width of both posterior semicircular canal ampulla (membranous labyrinth) (*A*) and of the crista (*B*) was measured by using the serial sections. However, in more than 70 percent of all the cases, the entire crista does



	Height of membranous ampulla, mm (A)	Height of cupula, mm (B)	Thickness of crista, mm (C)	Height of crista, (D)
Human	1.41	1.07	0.44	0.24
Squirrel monkey	.51	.50	.28	.20
Cat	.32	.31	.23	.27

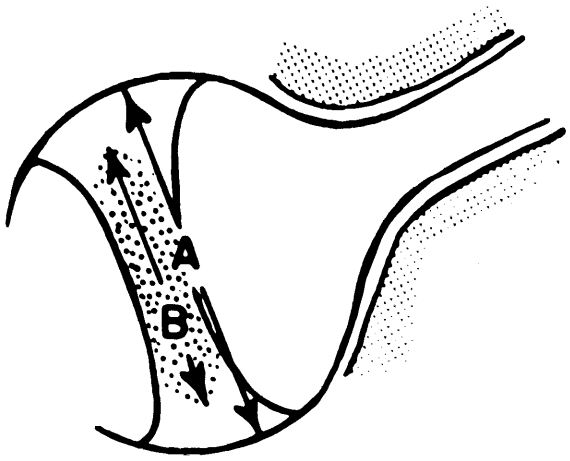
Figure 6.—*Dimensional comparison of cross sections of the posterior semicircular canals.*

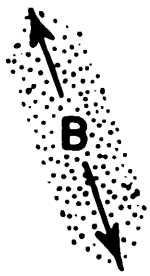
Table 1.—*Dimensional Comparison of the Semicircular Canals Among 3 Species, in mm*

	Skull size	Circle diameter	Cross section of canal	Height of ampulla	Height of cupula
Human	180 x 135	6.4	0.44 x 0.24	2.07	1.11
Squirrel monkey	47 x 35	3.6	0.28 x 0.20	1.28	.71
Cat	70 x 40	4.1	0.23 x 0.27	1.22	.67

not appear within the one section; therefore, in these cases, the measurement was based on estimations (fig. 7).

The width of the membranous ampulla in the human is slightly less than twice that of the other two species. Therefore, the membranous canal structure in the human is almost twice as large as in the squirrel monkey and in the cat.





	Height of membranous ampulla, mm (A)	Height of cupula, mm (B)
Human	1.84	1.28
Squirrel monkey	1.08	.82
Cat	1.15	.90

Figure 7.—*Dimensional comparison of the posterior semicircular canal ampullae and cristae.*

On the other hand, the width of the posterior canal crista (*B*) was not so wide when compared with those of the other two species. In other words, the size of the sensory crista is not significantly different among these three species, although the human has twice as large a membranous canal architecture.

According to Werner (ref. 8), the angle between superior canal and posterior canal was 90° in the human and 95° in the squirrel monkey, and the angle between the superior canal and the horizontal canal was 90° in human and 85° in the squirrel monkey. In about 80 percent of the cases, the planes of the horizontal semicircular canals were within a ±10° range of the Frankfurt horizontal plane.

Thickness of each zonal structure of the otolith end organ was measured microscopically, using the cross-horizontal section of the saccular macula in the squirrel monkey. The specimen was intravitally fixed with 10 percent formalin and decalcified with 10 percent EDTA. All the rest of the preparation procedures were exactly the same as in the others (fig. 8). The otolithic membrane structure was best preserved by this procedure (ref. 7). The crystal-shaped otoconia, not frequently seen in the slides of the squirrel monkey ear after routine procedure, was observed under the higher magnification, and the size of the otoconia was about 7–8 microns in this species (fig. 9). The thickness of each zone is slightly different among the different locations of the macula: periphery and center (ref. 8). The values of the present measurement are similar to those obtained by Werner in the rabbit (fig. 10).

The surface areas of saccular maculae in the human, the squirrel monkey, and the cat were calculated from the macular areal reconstructions based on the measurements of the horizontal serial sections. Some representative saccular surface area reconstructions are shown in figure 11, and the average is shown in table 2. The area of the human is almost three times as large as that of the squirrel monkey and of the cat; however, the ratio of the hair cell

Figure 8.—*A view of macula sacculi from a squirrel monkey. Zonal structure is clearly seen.*

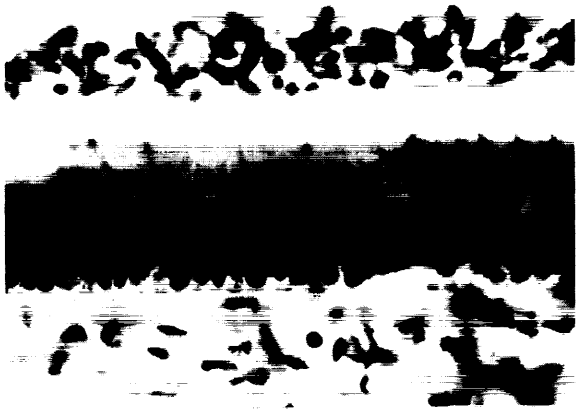


Figure 9.—*A high magnification view of macula sacculi from a squirrel monkey showing the crystal-shaped otoconia.*

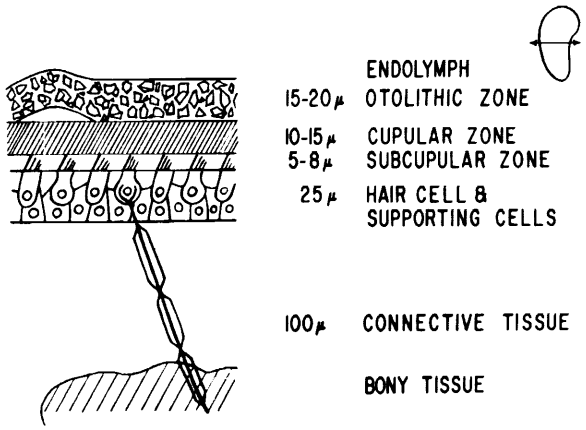


Figure 10.—*Schematic of the zonal structures of macula sacculi in the squirrel monkey.*

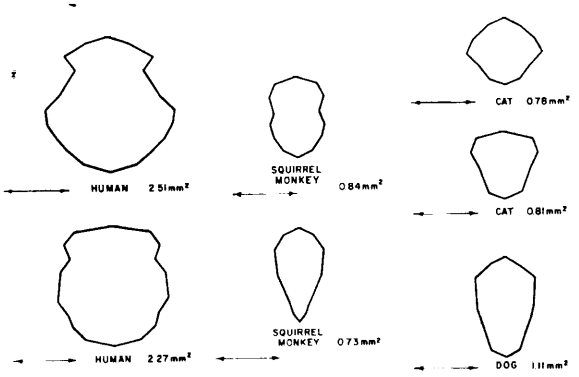


Figure 11.—*Schematic comparison of the surface area of the macula sacculi. This does not demonstrate the shape of the macular surface.*

population is not the same among these three species.

Both the diameter of the cochlear base and the length of the basal membrane are slightly less than twice as large in the human as in the other two species (table 3), and this ratio is almost the same as the one of the membranous canal structures.

Table 2.—*Comparison of Average Surface Area of Macula Sacculi, in mm²*

Human	2.08
Squirrel monkey	.73
Cat	.74

Table 3.—*Dimensional Comparison of Some of the Inner Ear Structures: Cochlea, Semicircular Canals, and Saccular Macula*

Species	Skull size, (mm, long axis × short axis)	Diameter of cochlea basal turn, mm	Length, basal membrane, mm	Turns in cochlea	Circle diameter of horizontal canal, mm	Cross section of membranous canal, mm	Cupula (height × width, mm)	Surface area, macula sacculi, mm²
Human	180 x 135	6.2	32	2½–2¾	6.4	0.44 x 0.24	1.11 x 1.84	2.08
Squirrel monkey	47 x 35	3.7	22	2¾–3	3.6	0.28 x 0.20	0.71 x 1.08	.73
Cat	70 x 40	3.8	22	2½–2¾	4.1	0.23 x 0.27	0.67 x 1.15	.74

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DISCUSSION

BENSON: Can either Dr. Igarashi or Dr. Engström state from their anatomical studies whether they would predict the differences in the dynamic responses of the horizontal and vertical canals purely from measurements of the radius of the duct, and duct diameter?

IGARASHI: I think the dimensional difference is quite minimal. However, the structure of the crista is quite different. As you know, in lower animals we have a structure, the so-called eminentia cruciata, which is a small bulge located on the crista itself, and that is located only in two vertical canals, superior and posterior. Especially in these two cristae we have the canal opening to the ampulla toward the one side of the ampulla.

MONEY: Could you tell me by what technique you preserve and stain the cupula which showed so beautifully in one of your slides?

IGARASHI: For the cupula, Heidenhain-Susa fixation and 3× DECAL was used for this particular specimen, but I still cannot succeed in 100 percent of the cases. On this particular specimen, I had previously operated on the utricle, and the specimen was simply immersed in a cool fixative, between 10° to 15° C for 6 days. For otolithic membrane, I have used cold 10 percent formalin, 2 weeks for the fixation, and after that, embedded it in celloidin. After embedding in celloidin, I used 10 percent EDTA to decalcify it.

ENGSTRÖM: A comment upon a few things about the technique. Have you ever made a measurement on the whole macula, which is very easy to take out as a whole? Then you can project it down and measure from that projection and compare it with the surface you got by reconstruction. That would give a rather exact measurement of the mean area the same you have from reconstruction. I think it would be much easier to make a measurement of the macula that way. It's also very important to give as exact measurements as possible. You said, for instance, that the crystals on the maculae had a size of 5 to 7 microns. I must tell you that in no specimen you have ever studied will you find that range of sizes, because there is a much larger variation of crystals. We find the smallest crystals below 1 micron, and then they increase in length, in all those specimens in cats, in squirrel monkeys, and man, up to a length of 30 microns. And the 30-micron crystals are found especially at the rampa. The easiest way is to look at the crystals directly in nondecalcified specimens and get different sizes without sectioning.

SESSION II

Chairman: **CATHERINE SMITH**
Washington University School of Medicine

Cochairman: **CÉSAR FERNANDEZ**
University of Chicago

Morphological Polarization of the Mechanoreceptors of the Vestibular and Acoustic Systems[1]

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Karolinska Sjukhuset
AND
PER-G. LUNDQUIST
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SUMMARY

The vestibular sensory cells in birds and mammals contain two types of sensory cells, whereas in frogs and fishes only one type is distinguished. All sensory cells in the labyrinth are morphologically polarized with regard to the centriole of the cell. One of the sensory hairs is identical in structure with a kinocilium protruding from the centriole. It is always found at the periphery of the sensory hair bundle.

Available functional and morphological data from the acousticolateralis system indicate that maximum stimulation of the hair cell occurs when the sensory hairs are displaced toward the centriole and kinocilium of the cell. A kinocilium is found also on cochlear hair cells during development, but disappears from the cochlear hair cells of mammals during late stages of differentiation. The asymmetry of the sensory cell is considered to reflect an asymmetrical organization of the hair-bearing end of the sensory cells at the molecular level, forming a direction-sensitive transducer mechanism. It is suggested that the main orientation of the sensory cells, within a certain area of the sensory epithelium, reflects the directional sensitivity of that area, which allows a charting of each part of the inner ear with regard to its function.

Each sensory cell in the inner ear is innervated by efferent and afferent nerve endings, forming typical synaptic structures of a sensory neuronal type, with the sensory cell. Areas of synaptic contact are found between the hair cell of type I and its innervating nerve chalice. Where the distance between the membranes of the synaptic junction is less than 100 Å, an electrotonic transmission mechanism in the nerve chalice may be indicated. On the other hand, scattered synaptic bars with synaptic vesicles are also found on the nerve chalice membrane, which suggests chemical transmission.

INTRODUCTION

The vertebrate labyrinth contains sensory areas responding to forces caused by angular acceleration, linear acceleration, and vibration. The vestibular and cochlear nerves convey coded messages from the labyrinth to the central nervous system about the location and nature of the stimulus. The central nervous system registers the localization of the receptor area responding to the stimulus, the intensity of the stimulus, and the direction in which various vectors of the stimulus forces act upon each sensory area responding to the stimulus.

[1] Supported by U.S. Public Health Service Grant No. NB 3956-03 and the Swedish Medical Research Council, Grant No. Y 427 and B 66-142.

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This complicated pattern of information is achieved only through the existence of highly specialized receptor sites within the labyrinth and of a close correlation between the spatial organization of the labyrinth and that of the corresponding areas of the central nervous system.

The aim of the present paper is to discuss some problems in connection with the transducer function of the cells in the labyrinth, with special reference to the directional sensitivity of the labyrinthine receptors and the sensory neuronal transmission.

MORPHOLOGY

Vestibular Sensory Cells

The vestibular sensory areas in vertebrate labyrinths are composed of mechanoreceptor cells, the so-called hair cells, maintained by a framework of supporting cells, and innervated by nerve endings from the bipolar vestibular ganglion cells (figs. 1 and 2).

The hair cells in fish and frog labyrinths are simple cylindrical cells innervated by myelinated nerve fibers forming bud-shaped nerve endings at the base of the hair cells (fig. 3). In mammals and birds there are two different types of hair cells (ref. 1). In type I, the hair cells are amphora shaped; i.e., each hair is shaped like a bottle with a narrow cylindrical neck, a rounded base, and a widened upper part, the hair-bearing end, from which a tuft of sensory hairs protrudes. In type II, the hair cells are

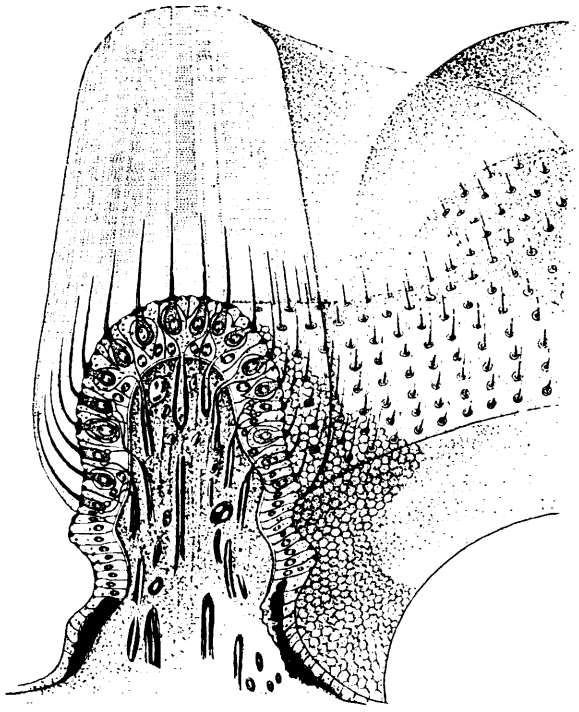

Figure 1.—*Schematic drawing of the crista ampullaris (from ref. 1).*

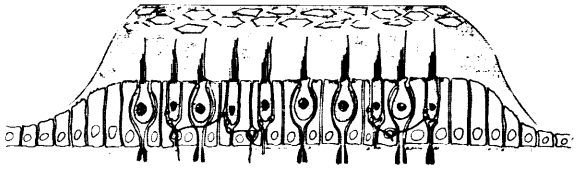

Figure 2.—*Schematic drawing of macular epithelium.*

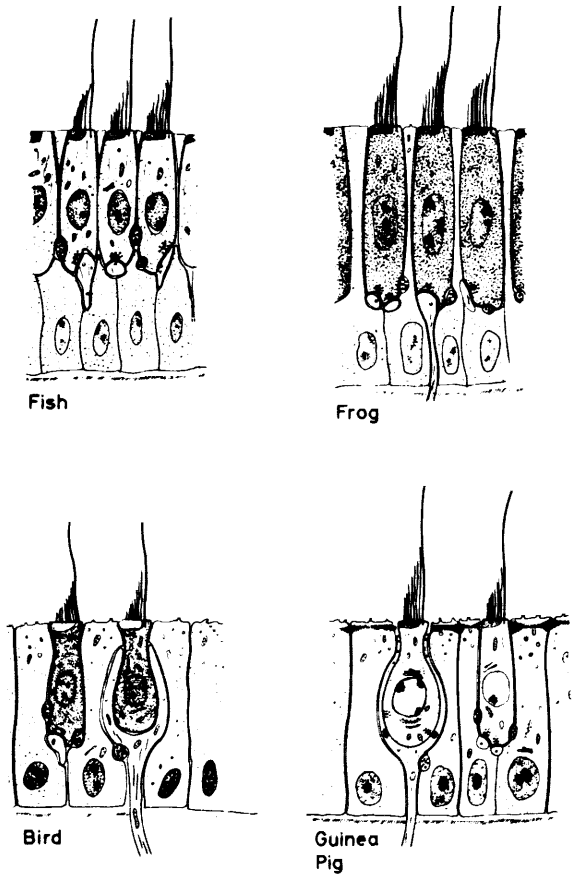

Figure 3.—*Schematic drawing illustrating the differences in structure between the vestibular sensory epithelia in fish and frogs, and in birds and mammals. (From Wersäll, Flock, and Lundquist, 1965. Courtesy of Cold Spring Harbor Symposia.)*

simple cylindrical cells like those in fish and frogs (figs. 3–5).

The hair-bearing end of each sensory cell contains a thin plate of densely packed granular substance, the cuticle, located immediately below the flat surface layer of the plasma membrane facing the endolymph. The bundle of sensory hairs consists of 1 kinocilium-like structure and approximately 100 stereocilia. In each bundle the stereocilia are longest toward the kinocilium and decrease in length, so that the shortest project only about 1 micron from the surface. The stereocilia have a central core of fibrils 70 Å thick, surrounded by a plasma membrane continuous with the plasma membrane of the cell

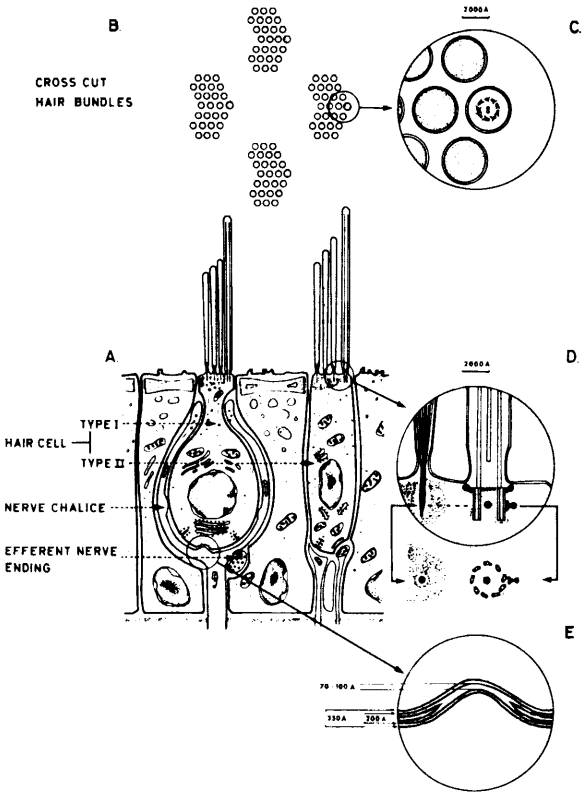

Figure 4.—*Schematic drawing of the types of sensory cells in mammalian vestibular sensory epithelium.*
A. *Survey.*
B–C. *Crosscut hair bundles illustrating the regular arrangement of the hairs with the oriented kinocilium of each bundle.*
D. *Detail of the centriole and the basal part of the kinocilium and a sensory hair.*
E. *Contact area between the plasma membranes of the nerve chalice and the sensory cell.*

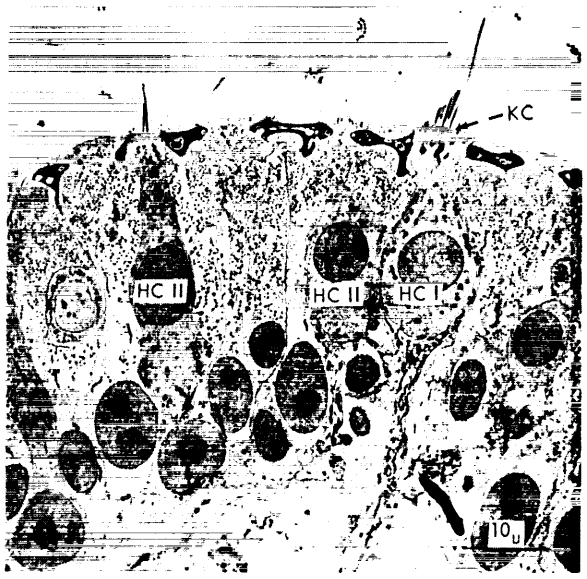

Figure 5.—*Survey of epithelium from the crista ampullaris with hair cells of type I* (HC I) *and hair cells of type II* (HC II). *Basal part of kinocilium* (KC).

(fig. 6). The fibrils of the axial core are basally compressed, forming a thin rootlet which passes through the cuticle and can be followed through the cuticle down to the subcuticular cytoplasm.

The kinocilium projects from one of the two centrioles of the cell (fig. 7) located on one side of the hair bundle in an opening in the cuticle immediately below the cell surface. The centriole is composed of nine triplet tubules forming a cylinder about 0.4 micron in length and 0.2 micron in width. The center of the cylinder contains two thin plates of electron-dense material (figs. 4 and 7). Nine electron-dense conical spokes radiate outward and obliquely upward from the triplet tubules. Each spoke terminates in a globular condensation (fig. 8). The triplet tubules are embedded in a thin coat of electron-dense material (fig. 9). On the side facing away from the cuticular plate, there is a more or less pronounced condensation of dense material similar to the basal foot in the basal body of motile cilia.

The kinocilium is formed by a bundle of nine peripheral double tubular filaments continuous with the triplet tubules of the basal body and by two central fibers which end above the cen-

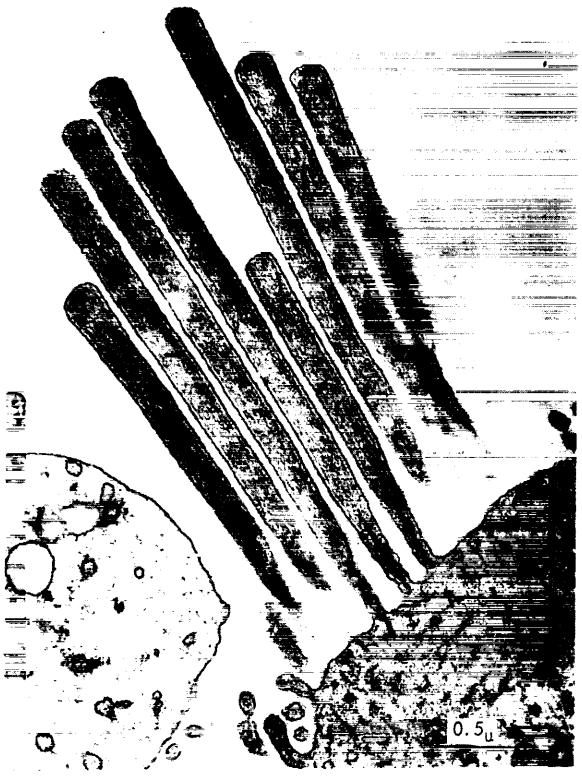

Figure 6.—*Sensory hairs showing the fibrillar structure of their axial core.*

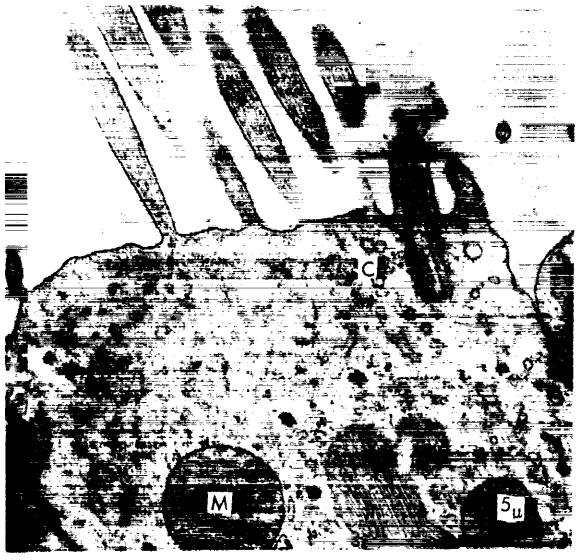

Figure 7.—*Hair-bearing end of a sensory cell with a centriole* (C) *and mitochondria* (M).

Figure 8.—*Cross section through the centriole, illustrating the triple tubules* (single arrow) *forming the cylinder of the centriole and the spokes* (double arrow).

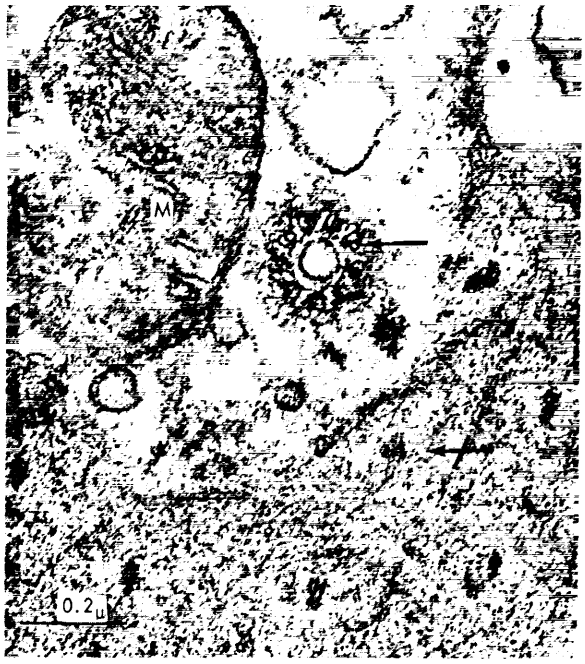

Figure 9.—*Cross section through the basal part of the centriole* (arrow) *and rootlets of the sensory hairs* (crossed arrow). *Mitochondria* (M).

triole close to the cell surface (fig. 10). Cross sections through the hair bundles of the various areas of the vestibular sense organ demonstrate that both the hair bundles and the hairs in each bundle are organized according to a regular pattern.

The kinocilium is always located in the periphery of each hair bundle (fig. 11). When large surface sections from the various sensory areas are studied, it is clearly seen that the cristae ampullares have the most regular organization of the sensory cells. The vast majority of the sensory cells are thus oriented in the same direction over the surface of each crista, with regard to the location of the kinocilium (fig. 12). Thus, on the crista of the horizontal canal the kinocilium is on the side of the cells which faces the utricle, whereas the hair cells of the vertical canals are oriented with their kinocilium toward the canal.

In the macula of the utricle, the main direction of the kinocilium is lateral, turning 180° at the periphery of the macula, and with a fan-shaped spread of the orientation over the surface (refs. 2 and 3). The saccular macula was not studied in mammals, but in the fish it has a rather complex orientation (ref. 4). The major pattern of organization was the same in frog, fish, chicken, guinea pig, and cat (ref. 5). On each crista a few cells were observed with two kinocilia pointing in the same direction, and a few cells where the location of the kinocilium was displaced in relation to the main orientation. This, however, did not disturb the general pattern of organization.

A second centriole is regularly situated below the first centriole. Its location in the cell is, however, rather irregular, although it is often found near the first centriole. When two kino-

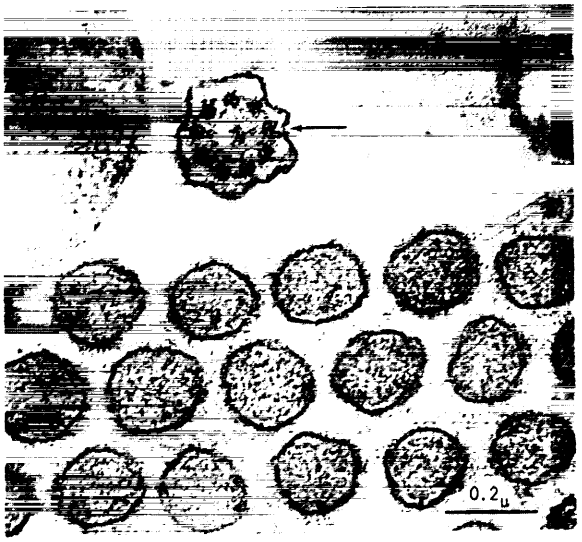

Figure 10.—*Cross section of part of a hair bundle with a kinocilium* (arrow) *demonstrating the regular arrangement of the nine peripheral double tubules.*

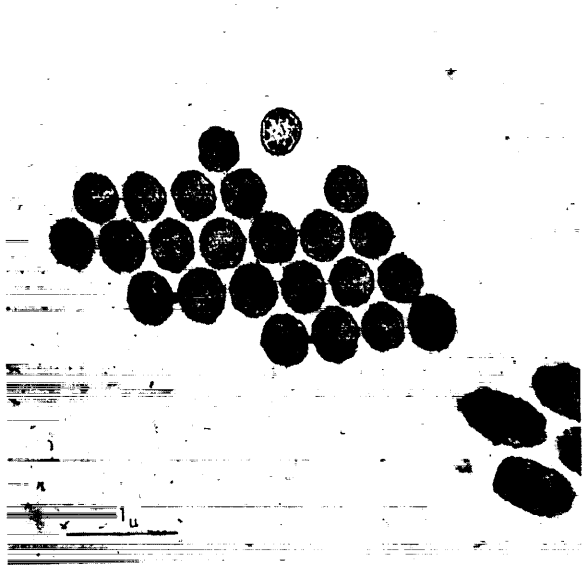

Figure 11.—*Cross section of a bundle of sensory hairs at some distance above the surface of the sensory cell.*

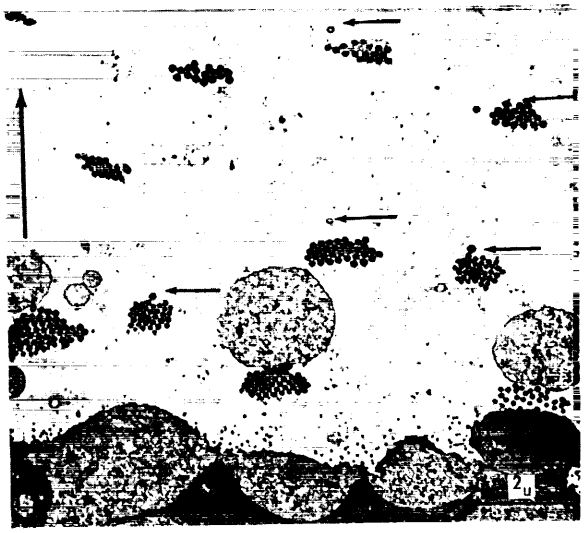

Figure 12.—*Cross section through a number of hair bundles from the horizontal crista ampullaris of a guinea pig, demonstrating the orientation of the hair bundles, indicated by the thick arrow to the left. The kinocilia* (thin arrow) *are observed on the utricular side of the hair bundles.*

cilia are observed, the second is presumably protruding from the above-mentioned second centriole.

Below the cuticle there are always a number of mitochondria and some thin filaments (fig. 13). In mammalian hair cells, of type I, these filaments or tubules form a bundle which practically fills the thinnest part of the neck region and continues down into the rest of the supranuclear part of the hair cells. Some small vesicles and granules are found also near these fibrils. The fibrils can be followed down to the region of the Golgi membranes, which cover a large area immediately above the nucleus.

A system of parallel flat spaces surrounded by a membrane appears regularly below the nucleus. A layer of ribosomes adheres to the outer surface of each membrane. Otherwise, ribosomes form clumps and rosette-like formations throughout the cell.

Figure 13.—*The hair-bearing end of a hair cell of type I. The rootlets* (arrow) *of the sensory hairs can be followed through the cuticular plate. They end in close contact with thin fibrils or microtubules leading into the deeper part of the cell* (crossed arrow).

Synaptic Structures and Nerve Endings

Each sensory cell of type II is innervated by several thin nerve-end branches forming synaptic contacts with the bottom of the sensory cells. Presynaptic, efferent, and postsynaptic, afferent, nerve endings with a characteristic structural organization are present in each cell (fig. 14). The presynaptic nerve terminal, which comprises an ending of a thin branch of an efferent nerve fiber, is filled with synaptic vesicles about 300 Å in diameter. Several patches of increased density and thickening of the synaptic membrane of each side of the synaptic cleft are regularly observed. In these areas the synaptic vesicles may be densely clumped together. On the hair-cell side of the efferent ending, there is always a flat space or synaptic sac surrounded by a membrane located close to the plasma membrane of the sensory cell. The postsynaptic, afferent, nerve ending is formed by the distal nerve-end branch of a dendritic

Figure 14.—*Bottom of a hair cell of type II* (HC II), *illustrating the synapses between the afferent* (Aff) *and efferent* (Eff) *nerve endings. Synaptic bars* (SB) *are observed close to the synaptic area of two afferent endings. Mitochondria* (M).

axon of a bipolar vestibular ganglion cell. These endings contain scattered vesicles, neurofilaments, and mitochondria. A slight thickening of the plasma membrane is seen in small areas of contact between this nerve ending and the hair cell. The plasma membrane on the hair-cell side of these endings has also thickened areas, some of which are in close contact with the distal end of a synaptic bar surrounded by vesicles.

The nerve fibers, innervating the hair cells of type I, form nerve chalices, each of which encloses the major part of a hair cell of type I, except for part of the neck region and the hair-bearing end of the cell which is in contact with the surrounding supporting cells and closely attached to these with junctional complexes (figs. 4 and 15).

The synaptic structure, between the hair cell of type I and its innervating postsynaptic, afferent nerve chalice, has a complicated pattern. The major part of the synaptic contact is made up of the plasma membrane which is 70 Å thick, and separated from the equally thick plasma membrane of the nerve chalice by an interspace 200 to 250 Å thick. Within this interspace

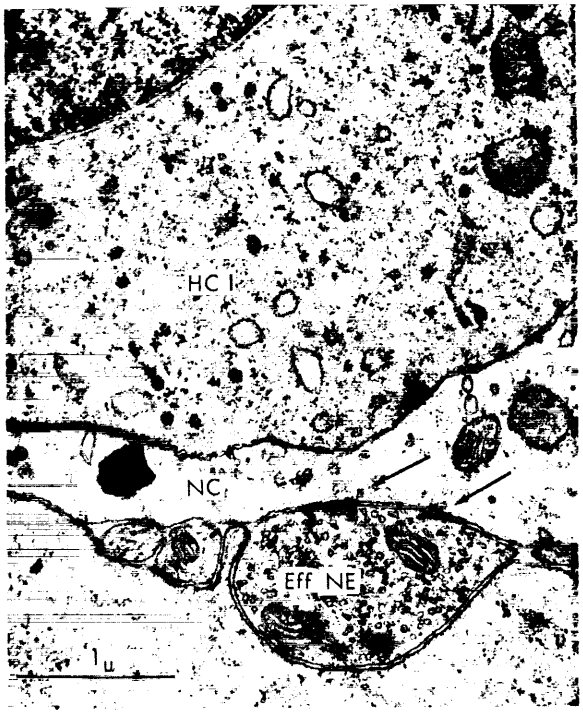

Figure 16.—*Basal part of a hair cell of type I* (HC I) *with nerve chalice* (NC), *and an efferent ending* (EffNE) *in synaptic contact with the nerve chalice* (arrows).

Figure 15.—*High magnification of a synaptic bar* (SB) *in a hair cell of type II. The plasma membrane of the nerve ending is thickened* (arrow).

there is a triple-layered structure composed of two layers of a somewhat irregular opaque material and a less dense interspace (figs. 4, 15, and 16). This material is less distinct than the membranous layers of the plasma membrane itself. Within areas of varying size, most of which, however, seem to have a diameter of about 0.2–0.4 μ, the synaptic space is reduced to less than 100 Å, and the substance between the two plasma membranes disappears (fig. 16). These areas are often observed at the bottom of shallow groves or pouches where the synaptic membranes bulge into the sensory cell.

Serial sections through the vestibular sensory cells of type I show that there are also a few synaptic bars, i.e., short dense rods, resting on the plasma membrane and surrounded by synaptic vesicles (fig. 17). Even within the neighboring area of such a synaptic rod, the synaptic space is reduced. A thickening of the plasma membrane of the nerve chalice is often observed also in these areas.

Figure 17.—*Synaptic contact* (arrow) *between the plasma membranes of the nerve chalice* (NC) *and a sensory cell. Mitochondria* (M).

Synaptic terminals, containing large numbers of densely packed synaptic vesicles, are regularly observed in contact with the base of the nerve chalice or with the nerve-end branch forming the nerve chalice (fig. 15). This nerve ending has the typical appearance of presynaptic nerve endings in other areas. The plasma membrane of the nerve ending is separated from the plasma membrane of the nerve chalice by an interspace of 200 Å within which there is often a more dense intermediate layer. Small areas of the plasma membrane, on both sides of the synaptic space, are covered with dense material forming synaptic junctions (fig. 18).

To summarize the morphological findings which are of special interest for the discussion on function: The vestibular sensory cells are of two types, both of which are provided with sensory hairs and nerve endings. With regard to the centriole and the kinocilium protruding from the centriole, all vestibular sensory cells are oriented in a characteristic pattern over the epithelial surface. Type I cells are innervated on the outside with nerve chalices, which are in contact with efferent nerve endings. The synaptic space, between the nerve chalice and the hair cell of type I, is reduced within certain

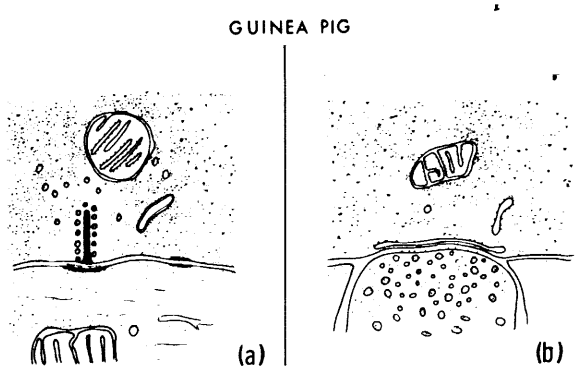

Figure 18.—*Schematic drawing of* (a) *afferent nerve ending with a synaptic bar. Compare with figure 4.* (b) *efferent nerve ending.*

areas to less than 100 Å. Type II cells have presynaptic, efferent, and postsynaptic, afferent, nerve endings contacting the base of the hair cell.

The Organ of Corti

The organ of Corti is the most highly developed epithelium in the labyrinth. It is composed of a framework of supporting cells or hair cells (figs. 19 and 20). The outer hair cells are cylindraceous. Their upper end is firmly fixed between the heads of the supporting cells, but the greater part of their surface is surrounded by intraepithelial fluid, Nuel's space. The bottle-shaped inner hair cells are surrounded by supporting cells resting on the innermost part of the basilar membrane and the spiral lamina.

Surface sections through the sensory hair bundles of the fully developed organ of Corti show that the sensory hair bundles protruding from the cell surface are regularly arranged

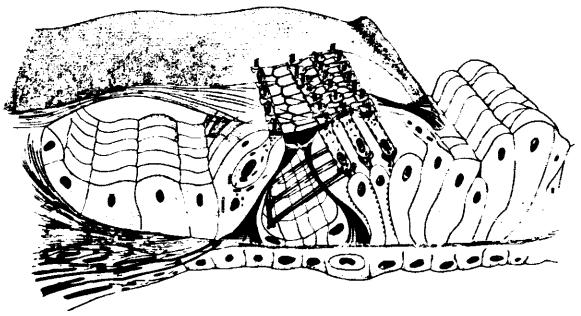

Figure 19.—*Schematic drawing of the organ of Corti.*

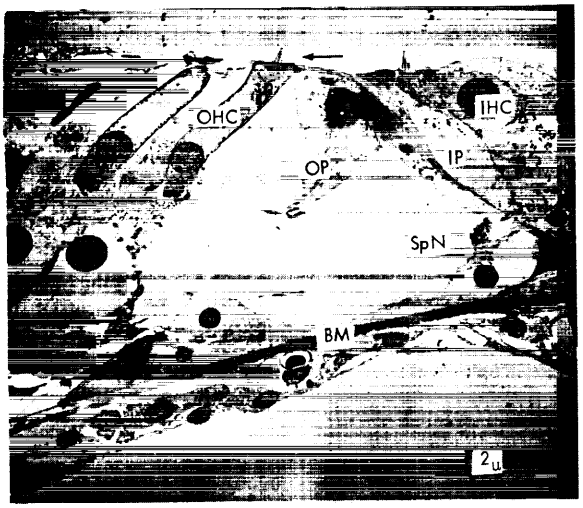

Figure 20.—*Survey of the organ of Corti with outer hair cells* (OHC) *and inner hair cell* (IHC) *separated by the outer* (OP) *and inner* (IP) *pillar cells. Spiral nerve fibers* (SpN) *are recognizable in the tunnel of Corti above the basilar membrane* (BM).

(fig. 21). The crosscut hair bundle of each outer hair cell forms a W (figs. 21 and 22), whereas the hair bundle of the internal hair cells forms almost straight rows, with only a slight wing formation (fig. 23). No kinocilium is observed, but when serial sections are continued through the cuticle, a centriole, similar in appearance to that in the vestibular epithelium, is noted. This centriole is always located in an opening in the cuticle at the top of the hair cells; and its location is invariably accentric on the side of the cuticle closest to the stria vascularis (fig. 24). The fine structures of the sensory hairs and the centriole differ only slightly from the fine structures of the vestibular cells, except that the basal foot is missing in the cochlear hair cells.

The inner hair cells have a simple cytoplasmic structure closely resembling that of the hair cells of type II. They are also innervated by thin nerve-end branches, forming afferent and efferent nerve endings in the base of the cell. Synaptic bars are observed in the contact area between the afferent nerve endings and the sensory cell. The outer hair cells have a more complicated cytoplasmic architecture.

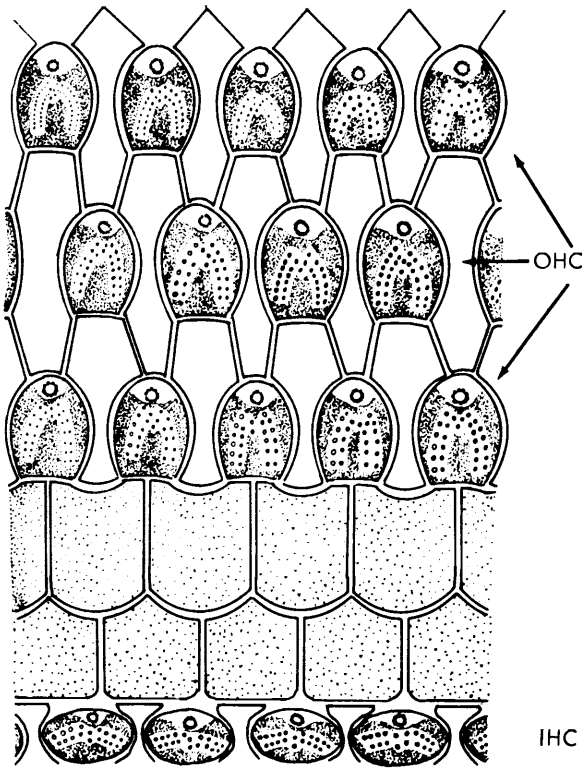

Figure 21.—*Schematic drawing of a cross section through the hair-bearing end of inner* (IHC) *and outer* (OHC) *hair cells, demonstrating the orientation of the cells with regard to the centrioles.*

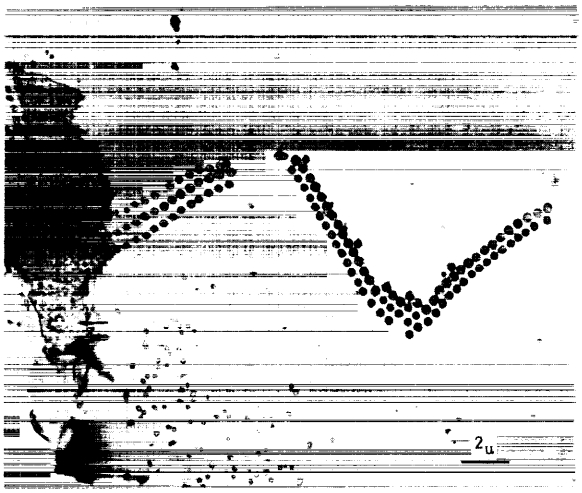

Figure 22.—*Cross section through the W-shaped hair bundles of two outer hair cells with a centriole* (arrow).

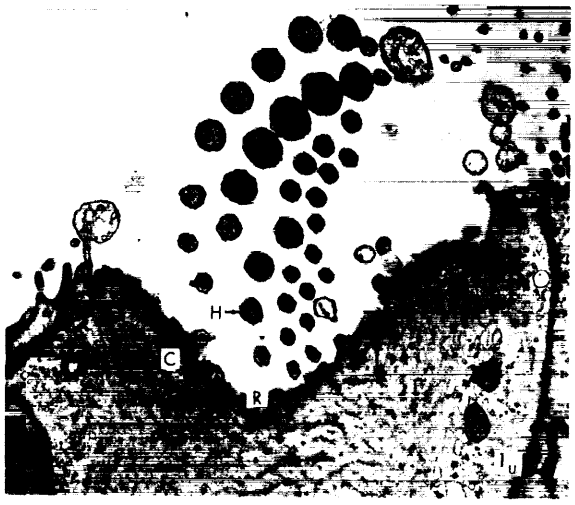

Figure 23.—*Cross section through part of the cuticle of an inner hair cell. Centriole* (C), *hair* (H) *and rootlet* (R).

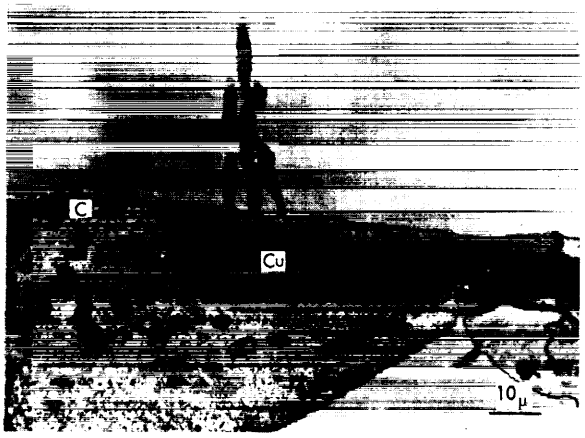

Figure 24.—*Top of an outer hair cell with cuticle* (Cu) *and a centriole* (C).

Along the straight vertical sides of the outer hair cells, there is a system of fenestrated lamellae, concentrically arranged on the inside of the plasma membrane. These lamellae are built up of two dense membranes separated by a less dense interspace. These membranes extend from the cuticular area down to the level of the nerve endings.

The nerve endings form a dense cluster at the bottom of the outer hair cells (fig. 25). Large efferent nerve endings and small afferent endings form a synaptic contact with the base of the outer hair cell. In the basal coil, all the hair cells have a large number of efferent nerve endings, but these decrease toward the apex of the cochlea in the two rows of outer hair cells closest to the stria.

The large efferent presynaptic endings have numerous synaptic vesicles concentrated in the part of the ending closest to the hair-cell surface. The outer part of the ending is filled with densely packed rod-shaped mitochondria. The plasma membrane of the nerve ending and that of the sensory cell are separated by a synaptic space of 200 Å. A flat synaptic sac adheres closely to the plasma membrane of the sensory cell along the whole extent of the synaptic surface. This synaptic sac is, in turn, closely connected peripherally to the end of the lamellae along the sides of the sensory cell (fig. 26).

The afferent nerve endings are small compared with the efferent endings. The afferent nerve endings contain some large vesicles, a few mitochondria and neurofibrils. The plasma membrane of these nerve endings is separated from that of the hair cell by a synaptic space, in which there is often an intermediate layer.

DISCUSSION

The sensory hairs of the mechanoreceptor cells in the labyrinthine receptor areas are closely related to auxiliary structures: the cupula of the cristae ampullares, the otolith membrane of the utricular and saccular maculae, and the tectorial membrane of the organ

Figure 25.—*Bottom of outer hair cell with afferent* (Aff) *and efferent* (Eff) *nerve endings.*

Figure 26.—*The synaptic area between afferent* (Aff) *and efferent* (Eff) *nerve endings and an outer hair cell.*

of Corti. Forces acting upon the cupula or the otolith membrane will cause these structures to have a sliding motion over the surface of the epithelium. The sensory hairs, which protrude into the gelatinous substance forming the cupula or the otolith membrane, act as levers on the hair-bearing end of the sensory cells.

Apart from the distal flexible portions of the kinocilium and the longest hairs closest to the cilium, the sensory hairs are rigid rods, bending only in their most basal portion. Thus the sensory hairs function as levers, transferring the mechanical energy of the cupular movement into the area of the rootlet of the hair and into the cuticle.

Van der Stricht (ref. 6) described the sensory hair bundle of the vestibular sensory cells as a number of fine fibrils held together by an intermediate substance and forming the sensory hair proper. Separated from this bundle, and situated more toward the periphery, there was a flagellum united with the centriole of the cell. Held (ref. 7) maintained that all epithelial cells covering the labyrinthine ducts had a flagellum proceeding from the centriole of each cell. These observations were confirmed by Kolmer (ref. 8). Wersäll (ref. 1) was able to verify the existence of a kinocilium-like structure located on one side of the hair bundle, and to demonstrate also the structural similarity between this cilium and the moving cilia of the respiratory pathways. Although Ecker (ref. 9) and Bowen (ref. 10) had described moving cilia on the sensory cells of the cristae, no such movements were noted by Wersäll (ref. 1) on direct observation of isolated sensory cells. It is very unlikely that an actual movement would occur in the vestibular sensory epithelium, as this would disturb the sensitive receptor mechanism. Lowenstein and Wersäll (ref. 11) were the first to demonstrate the morphological polarization of the vestibular epithelia in the crista ampullaris of the ray. They found that the orientation of sensory cells of the crista ampullaris coincided with the direction of stimulation in the epithelium, with regard to the location of the kinocilium. This is the direction of cupular displacement, which increases the activity in single fibers of the vestibular nerve, as shown by Lowenstein and Sand (ref. 12). Displacement of the sensory hair of the vestibular sensory cell toward the centriole and kinocilium would thus increase the discharge rate of the innervating nerve fibers; and displacement in the opposite direction would decrease the discharge rate. Lowenstein and Wersäll (ref. 11) pointed out that the kinocilium might possibly serve as a cilium in reverse, transforming the mechanical energy of the moving cupula into sensory cell activity. The importance of the kinocilium for the stimulation of the sensory cell has not been established. It is interesting to note, however, that the basal foot of the kinocilium, as described by Lowenstein and Osborne (ref. 13), Flock (ref. 2), and by Flock and Duvall (ref. 14), is always located in the direction of the stimulation of the cell. The same structure in the moving cilia is invariably on the side of the fast beat in the cilium (fig. 27).

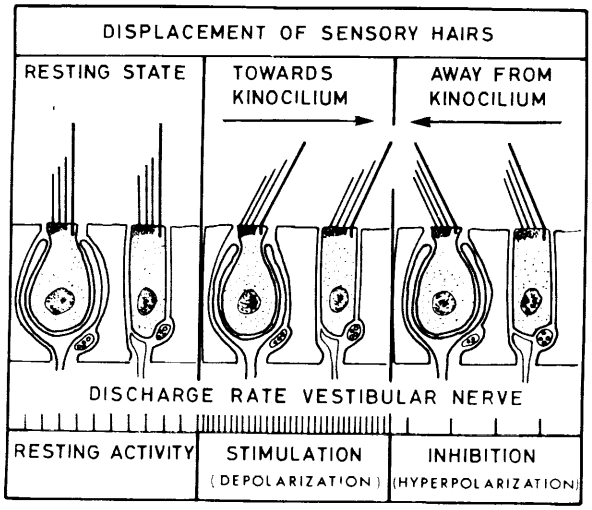

Figure 27.—*Electrical discharge rate of the hair cells as a function of displacement of the sensory hairs.*

The sensory hair rootlets end immediately below the cuticle in the vestibular receptors. In the hair cells of type I, where the cytoplasm below the hair-bearing end is compressed into a thin neck, there is, however, a large bundle of filaments or microtubules, which start in the region of the ending of the rootlets and continue down to the lower parts of the sensory cell. In the sensory cells of the crista in the labyrinth of the ammocoete larva, Lowenstein and Osborne (ref. 13) observed a striated structure extending from the end of the sensory hair rootlets to the nerve endings. As was pointed out by these authors, structures like the fibrils in type I cells, and the striated structure in the ammocoete labyrinth cells, might function in relaying the excitation, caused by the mechanical deformation of the hair bundles and the cuticular plate, to the synapses and nerve endings of the cell.

The correlation between the morphological polarization of the sensory cells and the directional sensitivity of the sensory cells in the vestibular end organs has been further studied, in the lateral line organ of the fish, *Lota vulgaris* by Flock and Wersäll (ref. 15), and in the toad, *Xenopus laevis* by Dijkgraaf (ref. 16) and by Görner (ref. 17). These studies clearly demonstrated that the sensory cells in the lateral line canal organs of fish and *Xenopus* are oriented with their kinocilia pointing along the canal. Two groups of cells were observed: In one, the cilia pointed toward the head, and in the other, toward the tail of the fish. Dijkgraaf (ref. 18), Görner (ref. 19), and Sand (ref. 20) found that the lateral line canal organ contains some nerve fibers responding to head-tail displacement of the cupula, and other fibers responding to tail-head displacement. According to Flock and Wersäll (ref. 15) and Dijkgraaf (ref. 16), each group of fibers could be derived from hair cells oriented with their cilia toward the tail and the head, respectively, as was practically demonstrated by Görner (ref. 17).

The orientation of the cells has been related also to the electrical potential phenomena recorded in the neighborhood of the epithelia during mechanical stimulation. The frequency of the microphonic potential, recorded between an electrode in the endolymph close to the crista ampullaris and another outside the ampulla, follows the frequency of a given stimulus (refs. 21 and 22), whereas the frequency of the microphonic potential of the lateral line canal organ is twice that of the stimulus (refs. 23–25). This difference in response can be explained by the fact that all hair cells on the same cristae were oriented in the same way, whereas the lateral line organs contain cells oriented in opposite directions. A displacement of the cupula in one way in the crista will cause a change in potential over the cuticular surface of the sensory cells, with a potential drop in the intra endolymphatic electrode in relation to the ground, whereas a movement of the cupula in the other direction will cause a rise in the potential at the same electrode. An alternating stimulus will produce an alternating-current flow between the two electrodes, with the same frequency as the stimulus. Flock and Wersäll (ref. 15) advanced a theory, further analyzed by Flock (ref. 25), according to which the variation is dependent upon a directional sensitivity of the individual sensory cell. The potential recorded is the sum of potential changes over the cuticle of all sensory cells in the area. In the lateral line canal organ, where the cells face alternately toward the head and toward the tail of the fish, a one-way displacement would cause hyperpolarization of one group of cells, and depolarization of another

group of cells. If depolarization were equal to hyperpolarization, no potential would be recorded. However, such a potential is recorded with twice the frequency of the stimulus. This implies that the simultaneously recorded potentials from the two groups are not equally large. Flock (ref. 25) showed that a transient displacement in any direction always causes a decrease in the recorded potential. Thus, the depolarization must be larger than the hyperpolarization; and hence the theory indicates nonlinearity of the receptor function, at any rate, in the lateral line canal organ.

The fact that an orientation of the hair bundles, with regard to the centriole, occurs even in the cochlea indicates a directional sensitivity also in the cochlear hair cells (refs. 5 and 26). Although as a directional sensitivity it might seem to be of less importance in the organ of Corti than in the other receptor systems of the acoustic lateralis system, it is possible that it might increase the sensitivity of the sensory cells at threshold. The Brownian movement and other irregular molecular movements may possibly disturb the function of the organ of Corti during stimulation at threshold. These movements, however, would strike the hairs at random, and cause irregular movements in them which would be far less effective than those due to a directed stimulus caused by the regular shearing movements of the tectorial membrane in relation to the surface of the epithelium, which results in stimulation of a large group of hair cells in the direction of maximal sensitivity.

Wersäll (ref. 1) demonstrated that two types of nerve endings can be observed within the vestibular sensory epithelia. Engström suggested (ref. 27) that all sensory cells in the inner ear are of two types: one, with efferent fibers going from the central nervous system to the sensory cells, and the other, with afferent fibers coming from the sensory cells. This suggestion was supported by the fact that nerve endings of presynaptic type and postsynaptic type were found to be present in all sensory areas and that efferent nerve fibers had been traced as far as the organ of Corti by Rasmussen (ref. 28).

Sectioning of the efferent nerve bundles to the cochlea has also demonstrated that the large nerve endings, filled with synaptic vesicles, are efferent in character, whereas the small nerve endings are afferent (refs. 29–31). Although a complete sectioning of the vestibular efferents, with degeneration of the efferent endings, has not yet been done, morphological data strongly indicate that the vesicle-filled endings in the vestibular sensory epithelia are efferent.

The question of whether electronic or chemical transmission is responsible for the transmission between sensory cells and nerve endings has not been clarified so far. The structural analyses of the nerve ending on the cochlear hair cells and all efferent endings suggest a chemical transmission between these endings and the hair cells; however, this is not so evident in the case of the nerve chalice. The major part of the plasma membrane of the nerve chalice is separated from the plasma membrane of the sensory cell by a space more than 200 Å in width, but in some areas there is close contact between these two membranes, and the distance is less than 100 Å. Although there is no actual fusion between the two membranes, such as occurs in the electrotonic junctions between teleost spinal neurons, as demonstrated by Bennett et al. (ref. 32), these areas of close apposition of the membranes could be signs of electrical transmission between the nerve chalice and sensory cell type I. This was recently discussed by Engström and colleagues (ref. 33) and by Eccles (ref. 34). At the Cold Spring Harbor symposium, 1965, Dr. Bennett and Dr. Lowenstein inquired about this possibility. At that time we did not have sufficient data to answer their questions about the occurrence of areas with spaces less than 100 Å in this synapse, but several serial sections through the nerve chalice have clearly demonstrated the occurrence of such areas. However, synaptic bars are often found also on the nerve chalice with connected synaptic vesicles; and it is possible that both chemical transmitters and electrotonic spread are important for the sensory neuronal transmission between type I sensory cell in the vestibular sensory epithelia and the innervating nerve chalice.

CONCLUSION

The way in which mechanical energy of hair displacement is transformed into nerve impulses is not known. The directional sensitivity of the mechanoreceptor cells is, however, closely related to the asymmetric structure of the sensory cells, which is indicated by the location and structure of the centriole and by the asymmetry in length of the sensory hairs. We had already earlier concluded that this gross asymmetry of the cell reflected an asymmetry of the molecular organization of the area of the hair-bearing end which is responsible for the transformation of the energy derived from the hairs into electrochemical energy in the cell (ref. 35). Although Engström et al. (ref. 36) consider that the centriole is the important structure for this transformation, we would rather seek for this active transducer in the area of the hair roots and the centriole. In view of how little is known about the functioning of this transducing mechanism, we will refrain from drawing further conclusions, from our findings, on the appearance of such a mechanism. Perhaps the solution of the problems concerning the movement of vibratile cilia will afford also a background for the further understanding of the mechanoreceptor transducing function.

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DISCUSSION

SMITH: I would like to ask you a question about the nerve fibers, those which I believe you called the granulated nerve fibers and which you thought possibly could be efferents. Are these bulbous structures truly endings or could they be "en passant" synapses; that is, just enlargements on nerve fibers? If the latter, they could contact a large number of hair cells rather than perhaps just terminating on one hair cell.

WERSÄLL: Although en passant synapses occur, serial sections demonstrate that most of the thin efferent nerve fibers branch and form true nerve endings.

SMITH: I have brought the question up because, as you well know, the efferent fibers in the cochlea do have this sort of pattern; that is, they do make extensive axodendritic contacts.

WERSÄLL: That is true in the bundles, but there you also have the same type of enlarged ending at the end of the peripheral branches to the outer hair cells.

SPOENDLIN: I would like to make a short comment on that. Recently Iurato has published a report about the distribution of the vesiculated, presumably efferent, nerve elements in the vestibular epithelia. According to him, one fiber forms numerous vesicle-filled enlargements and is in synaptic contact with many afferent dendrites. We made very similar observations in our material.

Now, another question regarding the polarization of the cochlear hair cells. As you have very nicely shown in your pictures, the kinocilium of the internal hair cell sits always at the distal pole of the hair-cell surface away from the modiolus. How does this conform with the findings of von Békésy, according to which the internal hair cells present the greatest sensitivity to shearing motions in a direction longitudinal to the cochlea?

WERSÄLL: The centriole is found on the outer side of the hair bundle of all hair cells in the organ of Corti. A vibrating electrode put on the tectorial membrane over the inner hair cells gave maximum output of microphonics when vibrated in the tangential direction, according to Békésy. We can, however, not be sure of the direction of displacement of the sensory hairs in that experiment. An up-and-down movement which could be caused by the vibrating electrode would

always give a radial displacement of the electrode. Further experiments in order to solve this problem are underway in our laboratory.

GUALTIEROTTI: From your study of the efferent junction on these cells, do you have the impression that we are dealing with a diffused system controlling the cells of the entire organ, or are there specific pathways to certain parts of the organ? Can you tell us something about that?

WERSÄLL: Well, right now we are going through whole cristae; it is a lot of work.

Our material does not yet clarify whether a true fusion exists or not. Although the distance between the membranes might be less than 70 Å, there always seem to be four layers. The areas of close approximation of the membranes are, however, small in the guinea pig and further studies have to be performed to make this clear.

ENGSTRÖM: We are studying the same things in so many labs nowadays that someone must find it soon. In our laboratory we are using a nerve stain which has a rather specific affinity to the efferent fibers and are trying to follow these not only by electron microscopy but by light microscopy first of all. As the stain is specific also for electron microscopy, we can make rather thick sections and follow the fibers. They are branching a lot; even the afferent fibers are spreading much more than I believed before. There are only around 200 fibers, according to Gacek, going to the whole vestibular system and they have to reach many thousands of cells; whereas there are around 12 000 afferent fibers, so the efferent fibers have to spread immensely inside. I think it would be very difficult to get the pure pattern of innervation.

FERNANDEZ: I would like to make a small commentary on the morphology of the electrical synapse found in the cell of type I. Do you think that a cleft of 70 Å is large for an electrical synapse? It is my understanding that morphologically an electrical synapse is characterized by an occlusion of the cleft so that only three membranes can be differentiated. At any rate, it is very interesting to find this type of synapse in the vestibular system. Electrical synapses have been demonstrated by Dr. Hinojosa between vestibular fibers and neurons located in the tangential nucleus of the chick. Furthermore, the Mauthner's cell of the goldfish also has electrical synapse with the eighth nerve. In these two cases there is occlusion of the cleft. I think that a synapse with a cleft of 70 Å cannot be considered as electrical.

SMITH: I would like to add that I have been looking for fusions in the same place for the past year and haven't been able to find any evidence for a fused membrane. There has always been a gap in the material that I have examined.

WERSÄLL: But have you seen this area as a whole?

SMITH: Oh, yes.

IGARASHI: I am afraid that I might have missed one important point from your presentation, Dr. Wersäll. Is the tip of the kinocilium embedded in the cupula or just attached to the cupula?

WERSÄLL: The kinocilium is often very long. The stereocilia form a very close bundle with some sort of substance connecting the hairs, whereas the kinocilium is located in the periphery of the bundle. There are true canals in the cupula. The kinocilium is always in the canal; it is always longer than the shortest hairs in the bundle. Some of the shorter hairs will, however, not be found in the canals of the cupula but resting on the bottom of the cupula or even in the subcupular area.

IGARASHI: If the motion of the cupula is not so simple, just moving forward and backward, and if the cupula is twisted or in push-pull action, then actually what happens?

WERSÄLL: That is a very good question. Some of you are, I am sure, familiar with studies of the microphonic output of the semicircular canals done by DeVries and others where they demonstrated that normally the microphonic potential of the crista follows very nicely the frequency of the mechanical stimulus, provided you stimulate only one canal and provided you stimulate this canal strictly in one direction. By direct stimulation of the crista, the twisting effect is easily studied. We use a Goodman vibrator with a small plastic tubule which is fitted over the cupula. We open the ampulla, put some zinc oxide on the surface of the cupula to show the shape of the cupula, and push the tubule, which is a little horseshoe shaped, over the cupula. The vibrator will then move the cupula and the hairs, and the microphonic output can be recorded from the crista. If the tube is put over the cupula without pushing or twisting, the potential recorded from the ampulla will follow the stimulus. Exactly as soon as the cupula is compressed or twisted, a double microphonic is recorded with a frequency twice that of the stimulus. This must depend on the fact that some of the hair cells will be depolarized and others hyperpolarized at the same time during a twisting movement. Normally all cells in the crista will depolarize or hyperpolarize at the same time. I think that this proves very nicely the fact that you have a system here of transducers which depolarize in one direction, hyperpolarize in the other direction, but the depolarization is always larger than the hyperpolarization. It is very easy to get this twisting, and you can also find that if you get stimulation of the horizontal canal and the anterior vertical canal at the same time, you will get the same picture because then you will get some cells hyperpolarizing and some cells depolarizing at the same time.

The Functional Significance of the Ultrastructure of the Vestibular End Organs

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SUMMARY

The vertebrate labyrinth is introduced as an organ involved in the control of posture and movement and is seen in this role to be superimposed upon the kinesthetic and myotactic organs associated with skin, joints, and muscles. The most important features of the comparative anatomy of the labyrinth are briefly reviewed. The cupula-endolymph system of the semicircular canals and the otolith organs are described as accelerometers sensitive to angular and linear acceleration. The signals generated by them in response to such stimuli are described with special reference to coding, and differential equations covering the transfer of information are introduced. The analysis of vestibular responses to supraoptimal stimulation in space-flight simulators is briefly mentioned.

The ultrastructure of the sensory hair cell is then discussed in the light of our knowledge of its mode of functioning. The double innervation of the hair cell and the composition and topographic orientation of the sensory hair processes of the sensory cells in the cristae and maculae are described in this context. Finally, unsolved questions of mechano-electric transduction are discussed.

INTRODUCTION

Posture and movement of the vertebrates are controlled by diverse but hierarchically interlocking mechanisms. When Sherrington began to operate with the concept of proprioception, he understood by this any sensory mechanism that related to adjustment of the spatial relationships between parts of the body in posture and movement. In this sense the term may still be considered a meaningful one. However, its range of application is then so wide that its discriminatory usefulness is minimal. Moreover, when it became customary to make proprioception antithetic to exteroception, the meaningfulness of the concept became seriously impaired by the fact that the majority, if not all, so-called proprioceptive sensory mechanisms are exposed to gravity. So long as terrestrial life forms remain earthbound, this is of doubtful and rather sophistic significance. The situation has now changed. A number of people have actually experienced the zero-gravity condition for protracted periods. Some of the postural control mechanisms are, for once, truly confined to stimuli generated within the body proper in consequence of muscular activity and the internal stresses it causes. Optical and integumental thigmotactic orientations presumably play the principal part in spatial orientation in a weightless environment. Eyes and skin have been called proprioceptors in the widest sense of the term and they make up two members of a trio, of which the third member is the labyrinth. Like those of the eye and of the integumental and visceral touch and pressure receptors, the control circuits governed by vestibular

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receptors are superimposed upon those associated with kinesthetic and myotactic organs in the joints and in the so-called antigravity musculature of limbs, neck, and trunk.

The vestibular receptors in the cristae of the semicircular canals and in the maculae of the otolith organs are, physically speaking, accelerometers monitoring angular and linear accelerations of the head in space during movements, both passive and active. The gross anatomical features of the vestibular sense organs are described in the preceding paper by Hans Engström, but it may be useful to consider a few facts of comparative anatomy. From an evolutionary viewpoint the vestibular organ is a fairly conservative structure, always situated in the head, and associated with the eighth cranial nerve. It is found in all known vertebrates. When, where, and how it emerged in the ancestors of the vertebrates are not known. It is anatomically least complex in the jawless cyclostome fishes *Myxine*, *Petromyzon*, and *Lampetra*. In *Myxine* there are only two ampullae joined by a single semicircular tube, and attached to a vestibular sac equipped with an otolith-bearing single macula. In *Petromyzon* and in *Lampetra* (the fresh-water lamprey), there are still two ampullae, but the single connecting tube shows signs of subdivision into anterior and posterior canals anatomically homologous with the canals of the same name found in the gnathostome or jawed vertebrates. Here the vestibular sac is subdivided into a number of recesses with a corresponding subdivision of the statolith-bearing macula. This foreshadows the emergence of the three otolith organs—utriculus, sacculus, and lagena—found in the true fishes, amphibians, reptiles, and birds, in all of which a third semicircular canal, the external or horizontal canal, is added to the anterior and posterior vertical canals. The ampulla of the horizontal canal lies near that of the anterior one.

In the birds and mammals, the auditory cochlea forms a posterior outgrowth of the vestibular sac. The lagena, the posterior of the three otolith organs, persists in birds where it is found at the tip of the curved cochlea. It is absent in the mammals which have only two otolith organs; viz, the utriculus and the sacculus. In the lampreys the labyrinth contains a unique formation absent in *Myxine* as well as in all gnathostome vertebrates. This is a very conspicuous part of the labyrinth consisting of two large chambers in the middle of the vestibular sac. Like the rest of the labyrinth, they contain endolymph, but here the endolymph is continuously agitated by giant cilia. Their rhythmically synchronized beat makes the fluid rotate in a dorsoventral plane. The chambers are in open communication with the lumen of the anterior and posterior ampullae, respectively. Mygind (ref. 1), who described the ciliated chambers in detail for the first time, believed that a possible gyroscopic action of these fluid vortices may serve as a substitute for the missing horizontal semicircular canal. This hypothesis is now under experimental scrutiny in the author's laboratory, but there are as yet no significant results to report.

To return to the situation found in gnathostome vertebrates, the information contained in the present paper is derived from a study of the labyrinth of elasmobranch fishes such as sharks and rays. These belong to a group of fish characterized by the absence of bone in their skeleton which, including the skull, consists to a large extent of hyaline cartilage. This single anatomical feature is one of the important reasons for the fact that we know more about the function of the various labyrinth sense endings of this class than of any other vertebrates, including man.

The elasmobranch labyrinth contains the following sense endings: In each of the ampullae of the three semicircular canals, a crestlike prominence or crista extends across the floor of its cavity. This carries the sensory epithelium consisting of supporting cells and sensory hair cells. The hair processes are ensheathed in a jellylike transparent cupula of approximately the same refractive index as the surrounding endolymph. The transparency of the cupula was responsible for the rather belated demonstration of its existence and shape by Steinhausen (ref. 2) and by Dohlman (ref. 3). The cupula reaches the roof of the ampulla and makes close-enough contact with it to prevent,

normally, any significant leakage of endolymph across its top, when it is made to deviate from its resting position by endolymph movement. The cupula and the endolymph on either side of it thus form a rigidly coupled system.

Cupula-endolymph movements are brought about by inertia whenever the vestibular organ is subjected to angular acceleration at the onset and end of rotatory head movement in space. The semicircular canal acts thus as an angular accelerometer. That it does not normally respond to linear acceleration of the head, due either to linear progression of the head or to gravitational pull or linear oscillations within the frequency range of acoustic stimuli, is entirely due to the spatial arrangement and dimensional properties of the semicircular canals. It has been found that each semicircular canal is maximally sensitive to angular accelerations in its own plane, the horizontal canal having the most restricted range of sensitivity of the three.

The three recesses of the membranous vestibular sac contain the maculae of the utriculus, the sacculus, and the lagena. The sensory epithelium of each macula again consists of supporting cells and sensory hair cells, whose hair processes are ensheathed in a so-called otolith membrane. This covering structure is of a consistency similar to the cupulae of the semicircular canals, but it is weighted by a stiff paste of so-called otoconia. The nature of the otoconia varies in different elasmobranch species. In some, such as *Raja clavata*, the thornback ray, on which most of the author's experiments were carried out, the otolith consists of spherites of calcium carbonate which range widely in diameter and form a whitish mass resembling toothpaste. The specific gravity of the otolith mass is roughly twice that of the endolymph and vestibular tissues in general. In other elasmobranch fishes, the otolith consists of a mass of minute sand grains taken up from the outside world through an open endolymphatic duct.

In the normal position of the head in space, the main portion of the macula of the utriculus lies approximately horizontal, or tilted down slightly backward and inward. This part is coverd by a weighted otolith membrane. A tonguelike projection of the main macula extends posteriorly upward toward the roof of the utricular recess. This is the lacinia utriculi. Its covering membrane is not encrusted with lime and it is innervated by a separate branch of the utricular nerve.

In the ray the sacculus is the largest of the three recesses. Its macula is elongated and, unlike that of the utriculus, lies approximately vertical in the normal spatial position of the head, tilting, if anything, slightly inward. Macula and overlying otolith mass are undivided over their whole length. On the inner wall of the sacculus recess, where it communicates with the almost fully circular posterior canal, lies a sensory epithelium known as macula neglecta. This is a small sensory end organ innervated by a separate branch of the saccular nerve. Its covering structure is unweighted. Finally, communicating with the posterior end of the saccular recess is the recess of the lagena. Its sensory macula lies approximately vertical in the normal spatial head position and is covered by a lime-encrusted otolith membrane. The lagenar nerve joins the saccular nerve to form, together with the nerve of the posterior vertical canal, the posterior or inferior branch of the eighth nerve, the anterior branch being made up of the nerves innervating the anterior vertical and horizontal ampullae and the two portions of the utriculus macula. As already mentioned, there is an endolymphatic duct extending dorsally from the center of the vestibular sac alongside the common limb of the two vertical canals. This duct, which in most vertebrates ends blindly in a so-called saccus endolymphaticus, in the elasmobranch fishes opens onto the outside world through a pore at the top of the skull.

Two factors have made it possible in the past to gain access to all individual nerve branches innervating the various sense endings of the ray labyrinth and to obtain in many cases single-unit records of the electrical activity of the sensory cells. One of these is the already-mentioned nature of the skull. Its cartilaginous walls can be sliced away in thin layers and the hyaline transparency of the cartilage allows this

to be done under normal control. The second is the remarkable duration for which the isolated labyrinth remains functional and responsive to adequate stimulation by rotation and tilting after removal from the animal. In fact, a useful preparation of the labyrinth consists of the isolated braincase radically trimmed to the size of a large matchbox, after removal of eyes and brain with exposure of the nerve branch to be recorded from by means of a silverplated forceps electrode. This preparation can be mounted on a turntable, torsion swing, or tilting device and connected by suitably arranged leads to a high-gain biological amplifier and through it to a magnetic tape recorder, oscilloscope with camera, and impulse counter or ratemeter.

In this way, information on the quantitative stimulus response relationships of all vestibular end organs has been obtained (refs. 4–8).

A résumé of the relevant electrophysiological findings will make it possible to relate the various functional features with the ultrastructure, mode of innervation, and topographic disposition of the hair cells in the various sensory epithelia.

We may start with the horizontal semicircular canal. It has previously been demonstrated that a single horizontal semicircular canal responds to angular acceleration in the horizontal plane in two opposite directions. It was shown in the pike (*Esox lucius*) that unilateral interruption of the nerve supply to a single horizontal canal affects the tonus distribution to the horizontal eye muscles, but does not abolish the compensatory eye movements in response to either clockwise or counterclockwise angular acceleration in the horizontal plane. That is to say, it was established that a semicircular canal is a bidirectional accelerometer (ref. 9). This means that the cupula movements, directly observed by Steinhausen and Dohlman to take place in two opposite directions from a resting position, must effectively transduce mechanical deformation into direction-specific nervous information. How this information is coded was shown in the experiments on the ray labyrinth.

For a single-unit preparation of the right horizontal canal mounted on a turntable, the following observations may be made:

(1) At rest, the nerve fiber innervating a hair cell in the crista of the horizontal canal carries an afferent low-frequency resting discharge (about 6–10 impulses per second). This discharge frequency is remarkably steady, varying by not more than 4 percent (ref. 4).

(2) Angular acceleration of the preparation by clockwise (ipsilateral) rotation produces an increase in the impulse frequency which is, over a considerable range of accelerations, a linear function of the acceleration. Under these conditions the ampulla is known to become deflected toward the utriculus and away from the canal end of the ampulla (utriculopetal cupula deflection).

(3) As soon as the turntable has reached a a constant angular speed, the discharge frequency drops, returning to the resting level during continued constant-speed rotation. We know that under these conditions the cupula returns to its resting position under the influence of a restoring force derived from its own elasticity and perhaps from elastic forces generated by the hair bundle.

(4) The turntable is now abruptly stopped. This results in an immediate reduction and probably a complete inhibition of the resting discharge. The slope of the reduction in impulse frequency is a linear function of the deceleration over a wide range of decelerations. We know that under these conditions the cupula swings to the opposite side; viz, away from the utriculus and toward the canal end of the ampulla (utriculofugal ampulla deflection). The resting discharge is then gradually reestablished, while the cupula once more returns to its resting position.

(5) Counterclockwise (contralateral) rotation of the turntable produces the opposite cupula movements and corresponding changes in impulse frequency. Acceleration is followed by impulse inhibition, with a reestablishment of the resting activity during constant-speed

turntable rotation. Sudden arrest of the turntable now evokes an outburst of discharge activity, the increase in impulse frequency being a linear function of the deceleration. This activity then gradually returns to the resting level.

In experiments in which preparations were rotated in the planes of the two vertical canals, it was shown that, in accordance with early reflex data, these canals show a diametrically opposite response picture; viz, excitation on utriculofugal and inhibition on utriculopetal cupula deflection.

The cupula endolymph system behaves like a highly damped torsion pendulum and it was shown that its behavior can, in the absence of external forces, be represented by the equation

$$\Theta\ddot{X}+\Pi\dot{X}+\Delta X=0 \qquad (1)$$

or

$$\ddot{X}+\frac{\Pi}{\Theta}\dot{X}+\frac{\Delta}{\Theta}=0 \qquad (2)$$

where $\Theta\ddot{X}$ is the inertia, $\Pi\dot{X}$ the frictional resistance, and ΔX the restoring force of the system, Θ is the moment of inertia of the cupula-endolymph system, Π the moment of friction at unit angular velocity, and Δ the cupula restoring couple at unit angle (ref. 7). Groen, Lowenstein, and Vendrik established the fundamental linearity of the system by recording the impulse frequencies from single and multiunit preparations of the horizontal semicircular canal during sinusoidal movement of the preparation on a torsion swing at various circle frequencies (fig. 1). These experiments yielded

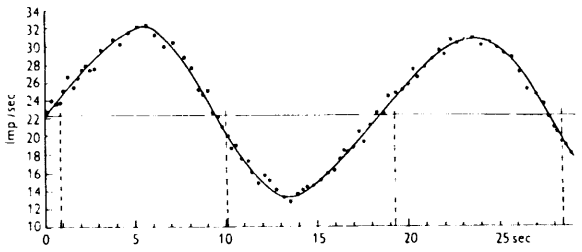

Figure 1.—*The frequency of nerve impulses as a function of time during a sinusoidal movement of the horizontal semicircular canal in the isolated labyrinth of the ray* (Raja clavata). (*From ref. 7.*)

phase lags and phase advances between the plot of impulse frequency and swing movement. From these phase shifts the following average values were calculated: natural circle frequency of the cupula-endolymph system=

$$\omega_0=1\cdot3\ \text{sec}^{-1}; \qquad \frac{\Pi}{\Theta}=36\ \text{sec}^{-1};$$

$$\frac{\Delta}{\Theta}=1\cdot64\ \text{sec}^{-2}; \qquad \text{thus: } \frac{\Pi}{\Delta}=22\ \text{sec}.$$

Experiments on the turntable of the kind described above yielded decay curves of impulse frequency after sudden stopping of rotation at various angular speeds. The logarithm of increase or decrease in the discharge frequency plotted against time normally yielded a straight line, the slope of which represents the quotient Π/Δ (fig. 2). In this case the slope was 40 seconds as compared with the value of 22 seconds resulting indirectly from the torsion swing experiments.

Finally, a theoretical value calculated for the quotient Π/Θ on the basis of the viscosity and density of the endolymph and the cross section of the canal was 35 sec^{-1}. This agrees well with the value 36 sec^{-1} obtained on the torsion swing.

A careful consideration of systematic errors in the experimental measurements suggested the following approximate average values:

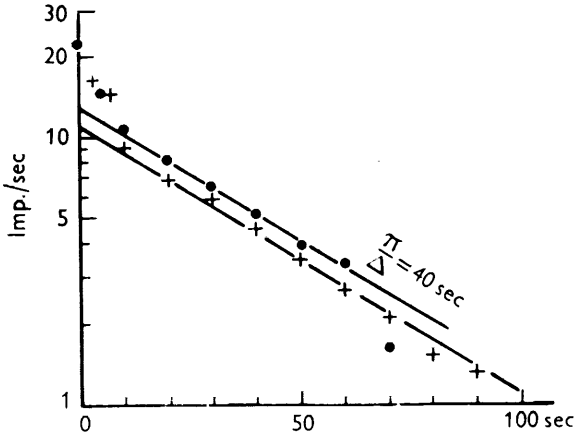

Figure 2.—*The logarithm of the increase or decrease of the impulse discharge from the horizontal semicircular canal of the ray* (Raja clavata) *plotted against the time after a sudden change in angular velocity.* (*From ref. 7.*)

$\Pi/\Theta=35$ sec^{-1}, $\Delta/\Theta=1.0$ sec^{-2}. Consequently, the following equation emerged for the behavior of the cupula-endolpmph system:

$$\ddot{X}+35\dot{X}+X=0 \qquad (3)$$

Based on the sensations of human subjects tested on turnchair and torsion swing, Van Egmond, Groen, and Jongkees (ref. 10) had arrived at a differential equation in which Π/Δ was put at 10 seconds with a possible margin of error of 30 percent and Δ/Θ at 1.0 sec^{-2} (ref. 10).

Despite the relative inaccuracy of such results, there is sufficient reason to accept them as demonstrating the correctness of the interpretation of the cupula-endolymph system in terms of an overcritically damped elastic pendulum.

Niven and Hixson (ref. 11) carried the matter forward to a study of the relationships between eye-muscle response and high-level sinusoidal angular rotations. Introducing the concept of the "system transfer function," they went beyond the limits of naturally occurring "physiological" accelerations and concerned themselves with the consequences of such high-level vestibular stimulation applied simultaneously to more than one canal pair under conditions that may be expected to arise in and outside the confines of a space vehicle in orbit. Peak angular accelerations were as high as 40°/sec^2. In experiments on the horizontal torsion swing, the nystagmic eye response to sinusoidal acceleration showed characteristic phase shifts which could be plotted against frequency of the swing.

Despite the high-level accelerations used, the various subjects yielded consistent results. Only exceptionally did responses to left and right swings differ, and those differences were consistent and characteristic for the subject in question.

More remarkably, the results based on high-level accelerations yielded coefficients in good agreement with those obtained by Van Egmond, Groen, and Jongkees, based on low-level stimulation. The average Π/Δ found was 12 sec as against 10 sec, and $\Delta/\Theta=1.2$ sec^{-2} as against 1.0 sec^{-2} (ref. 10). The cupula system appears therefore to be more robust than had hitherto been assumed.

It can be seen from the plot of impulse frequencies in the elasmobranch preparation (fig. 1) that at any moment during an angular acceleration or deceleration, the impulse frequency is a function of the angular velocity attained. Translated into terms of human subjective experience, this supports the observation that during acceleration, the sensation of angular velocity at any given moment likewise corresponds to the speed of rotation attained at that moment (ref. 10).

The range of stimulus intensities over which the impulse response of a semicircular canal is a linear function of acceleration or deceleration is shown in the bottom part of figure 3 (solid line) based on the combined maximum or minimum impulse frequencies from three sensory units recorded immediately after abrupt stoppage of the turntable rotating at a range of constant velocities. The stimulus here may be described as a "mechanical impulse" applied to the cupula-endolymph system at rest during contant speed rotation. It will be seen that the curve obtained resembles the characteristic curve of a radio valve with the zero working point in the middle of the straight part. It should be noted that "physiological" accelerations are well confined to the linear part of the curve. Ledoux (ref. 12) found a similar but steeper curve in experiments on the frog (bottom part of fig. 3, broken line).

Sensory units are found in semicircular canals whose working point appears to be "biased" up or down on this curve. Some are spontaneously silent and respond only to utriculopetal excitatory cupula deflection. Others have a relatively high-frequency resting discharge, with very little response to cupula deflection in either direction. In fact, it has been possible, by means of polarizing currents routed through the sensory nerve, to shift the working point of linearly responding units up or down the characteristic and in this way to transform a linear bidirectional unit into a unidirectional or into a relatively unresponsive one. The first type can be observed to become recruited into the response picture on rapid change of rotational

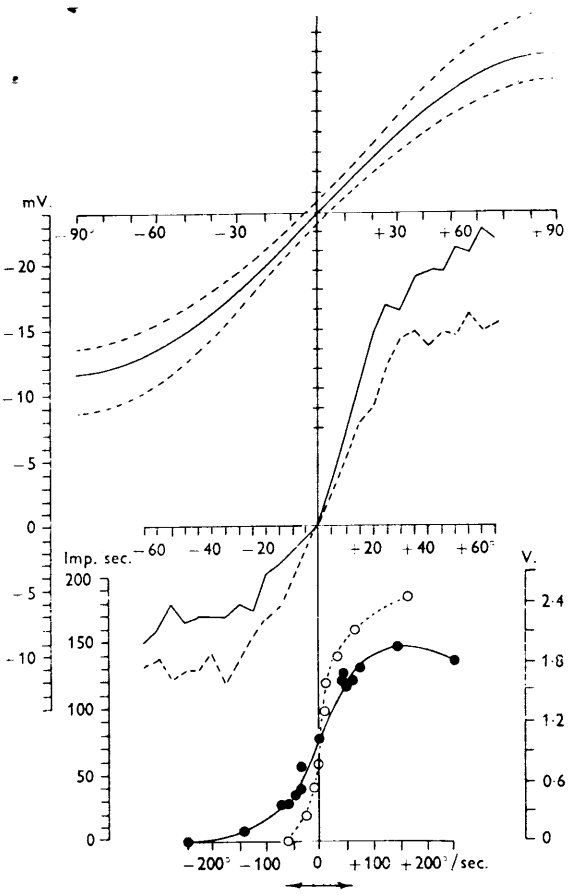

Figure 3.—*Characteristic curves of the semicircular canal receptor system.* Above: *Tangential component of shearing force acting on the sensory epithelium of the crista.* Central: *Changes in dc potential in millivolts inside the hair-cell layer of the crista of the horizontal canal in the guinea pig. Utriculopetal cupula deflection produces negative and utriculofugal deflection positive change from zero resting level.* Below: *The relation of impulse frequency to mechanical impulse on a sudden arrest of constant-speed rotation at various angular velocities.* Continuous line: *Horizontal canal of ray. (From ref. 7.)* Broken line: *Horizontal canal of frog. (From ref. 12.)* *(From ref. 16.)*

velocity; the second may be thought to furnish the fairly constant background of "tonic discharge" emanating from the semicircular canals, the existence of which has been postulated from the results of unilateral elimination of single canal (refs. 8 and 9).

Let us now turn to the otolith organs. They, too, are accelerometers but, by reason of their physical properties and anatomical arrangement, they are fundamentally capable of responding to both angular and linear accelerations as well as to rotation at constant speed. In fact their greater inertia may make them hardly capable of competing with the cupula organ of the semicircular canals in the monitoring of angular acceleration. They have, therefore, been described as tonic or static in function in comparison with the dynamic function of the semicircular canals. Whether this is in fact appropriate is questionable in view of the fact that otolith organs have been shown to be highly sensitive vibration detectors covering a considerable range of frequencies (refs. 6 and 13).

The principle underlying the design of an otolith organ is as old as cellular organisms. In the *Protista* and in tissue cells of plants, the direction of gravity is monitored by means of dense cell inclusions such as food vacuoles or starch grains (refs. 14 and 15). Their mechanical action on the surrounding cytoplasm brings about directional changes in the development of the cell, be it through compensatory movement or differential growth. Statocysts are ubiquitous in the animal kingdom. The principle of design invariably centers around a body denser than the surrounding tissues, enclosed in a fluid-filled sac, the wall of which incorporates mechanoreceptors. In the case of the *Arthropoda*, the receptors may be chitinous hair sensilla in contact with a "statolith." In the vertebrate labyrinth the receptors may be hair cells incorporated in the sensory epithelium of a macula. It has been shown conclusively that the adequate stimulus for the hair cell in a macula is the tangential shearing force applied to it on displacement of the otolith in one or other direction. This displacement follows any deviation of the head from its normal spatial position. The shearing obeys the relationship $f=g \sin \alpha$, where g is the gravitational force and α the tilting angle from the normal (refs. 16 and 17).

It has been mentioned that otolith organs potentially respond to vibrational stimuli (ref. 6). The following steady-state equation for harmonic oscillation thus applies:

$$(1+1/\rho)m\ddot{x}+r\dot{x}+kx=m\ddot{y}(1-1/\rho) \qquad (4)$$

The factors $1+1/\rho$ and $1-1/\rho$ account for the additional inertia of the endolymph displaced during the displacement of the otolith and to the buoyancy of the otolith, respectively. Gravity only is considered as the external force. The internal resistances of the otolith system are the frictional resistance (r) and the elastic resistance due to the restoring force (k) inherent in the otolith-macula system (ref. 17).

Single-unit recordings from the maculae of all three otolith organs of the isolated labyrinth of the ray have demonstrated that, whether a given otolith organ responds exclusively to gravitational stimuli and to linear translation, on the one hand, or to vibrational stimuli, on the other, seems to depend on the anatomical disposition of the organ within the skull and the consequent preferential exposure to one or the other of these stimulus modalities. In the ray, the lacinia utriculi, the anterior two-thirds of the sacculus macula, and the macula neglecta were shown to be highly sensitive to vibrations, yielding frequency-synchronized vestibular microphonics over a wide frequency band and phase-bunched spike discharges up to 120 cps. The gravitation receptors are thus confined to the otolith-covered part of the macula utriculi, to the posterior end of the sacculus macula, and to the lagena. Utriculus and sacculus responses are roughly synergistic, whereas the lagena responds antagonistically to both. The utriculus responses monitor tilts about all horizontal body axes: longitudinal, transverse, or diagonal. Discharge frequencies from single units are minimal in the normal position with maxima near the 90° head-up or -down and side-up position in space. The impulse frequency changes linearly during the tilt as a function of the sine of the tilting angle. Maintained deviations from the normal are characterized by specific impulse activity. There exists within the population of units, however, a spectrum of maxima corresponding to various inclinations on either side of the normal so that sensory units function in relays over the whole range of tilting. In the ray, however, very few, if any, preparations were found to respond to lateral downtilt. This was the more astounding, as all previous theoretical considerations have been based on the assumption of a high sensitivity of the utriculus to side-down tilting (refs. 5 and 6). In recordings from the vestibular nuclei made by Schoen (ref. 18) on fish and by Adrian (ref. 19) on cats, static neuron responses to tilts around the longitudinal axis were registered both on side-up and side-down tilting. However, in these cases the peripheral origin of the responses could not be ascertained. There is no reason for the utriculus macula to be devoid of sensory units responding to down tilting, and ultrastructural findings in the labyrinth of the ray and the lamprey (refs. 20 and 21) and in *Lota vulgaris* (ref. 22) encourage this postulate.

Figure 4 shows a single-unit response from the utriculus to a continuous, very slow constant-speed 360° tilt about the longitudinal axis. It shows a maximum near the side-up position. Arrest of the tilting movement at any point of a full circle would usually be followed by an adapting change in the discharge frequency toward the level of discharge-frequency characteristic of the normal spatial position. However, this adaptation, which may be mechanical to a large extent, is limited and settles

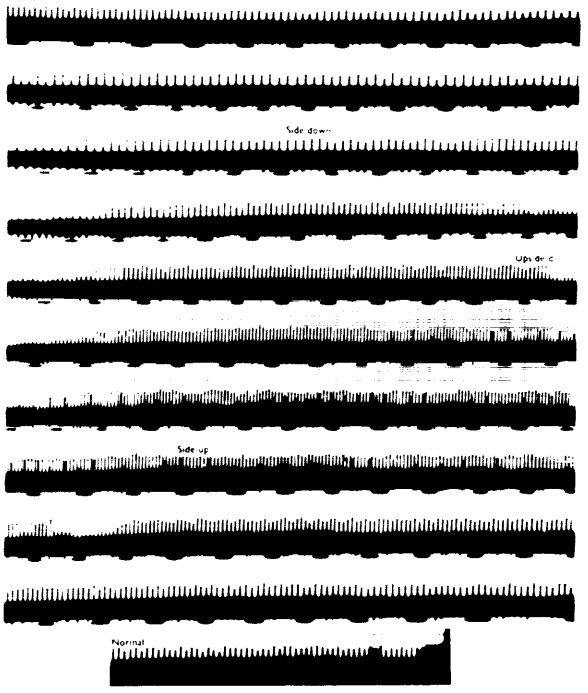

Figure 4.—*Single-unit response from the utriculus of a ray* (Raja clavata) *to a 360° lateral tilt of constant low velocity.* (*From ref. 5.*)

down to a frequency linearly related to the angle of maintained tilt. Units of the lagena show a relatively high activity in the normal position which declines during tilts from the normal in all directions. There is evidence from some lagena preparations of a second maximum of activity in the upside-down position of the head. Some units in the various maculae, although reacting sensitively to tilting, are not all capable of signaling maintained displacements of the head from the normal. Their discharge activity returns to that characteristic for the normal position, whenever and wherever a tilting movement is stopped. They have been described as out-of-position receptors and may perhaps be thought to occupy marginal positions on the macula with respect to the otolith mass.

In any argument involving consideration of the significance of positioning of receptors within the sensory epithelia, the question arises whether the receptor cells themselves have directional sensitivity. This is where the ultrastructure of the sensory hair cell becomes significant. The hair cell is a secondary sensory cell. It is a cell of epithelial origin and in the cristae and maculae it forms, together with supporting and glandular cells, part of a true two- to three-layered epithelium resting on a typical basement membrane. The fundamental cytological features of the vestibular hair cell have already been dealt with in preceding papers by Wersäll and Spoendlin. So far as the fish labyrinth is concerned, it may be sufficient to point out that it contains type II cells only (ref. 20). They are roughly cylindrical cells with a diffuse innervation near the base; the flask-shaped type I cells of the mammalian labyrinth with their envelopes of nerve calices are absent. The type II cell appears therefore to be the more generalized of the two (fig. 5). In the lamprey (ref. 21) the hair cells vary from cylindrical to completely spherical.

A description of the ultrastructure of the various cytoplasmic inclusions is not relevant here, the important features of interest being the composition and arrangement of the hair processes and the innervation. The hair cells are innervated by the dendritic branches of first-order sensory neurons, the cell bodies of which can be found in the course of a given

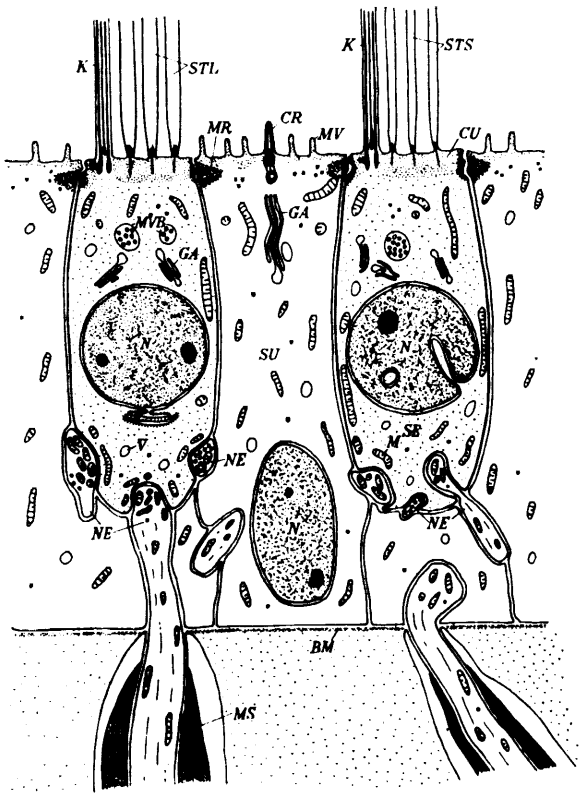

Figure 5.—*Diagrammatic view of the combined ultrastructural features of two sensory hair cells and one supporting cell from the crista of the horizontal canal of the ray* (Raja clavata). BM, *basal membrane;* CR, *ciliary rod;* CU, *cuticular plate;* GA, *Golgi apparatus;* K, *kinocilium;* MR, *membrana reticularis;* MV, *microvillus;* N, *nucleus;* NE, *nerve endings;* MS, *myelin sheath;* SE, *sensory cell;* STL *and* STS, *stereocilia;* SU, *supporting cell;* V, *vesicle. Note the basal prominence on the left side of the kinocilial root.* (*From ref. 20.*)

branch of the eighth nerve. They can be dispersed or can form peripheral ganglionic assemblies corresponding to the spiral ganglion of the cochlea. The nerve endings contain transparent vesicles and are well supplied with mitochondria. They often penetrate deep into the hair-cell body, but are always separated from its cytoplasm by the double membranes of the dendrite and the cell body. Synapses are found between hair cell and dendrite, the arrangement of which clearly shows that synaptic conduction takes place in the direction from hair cell to dendrite. In the lamprey (fig. 6), typical synaptic bars with their characteristic halo of electron-transparent vesicles are found

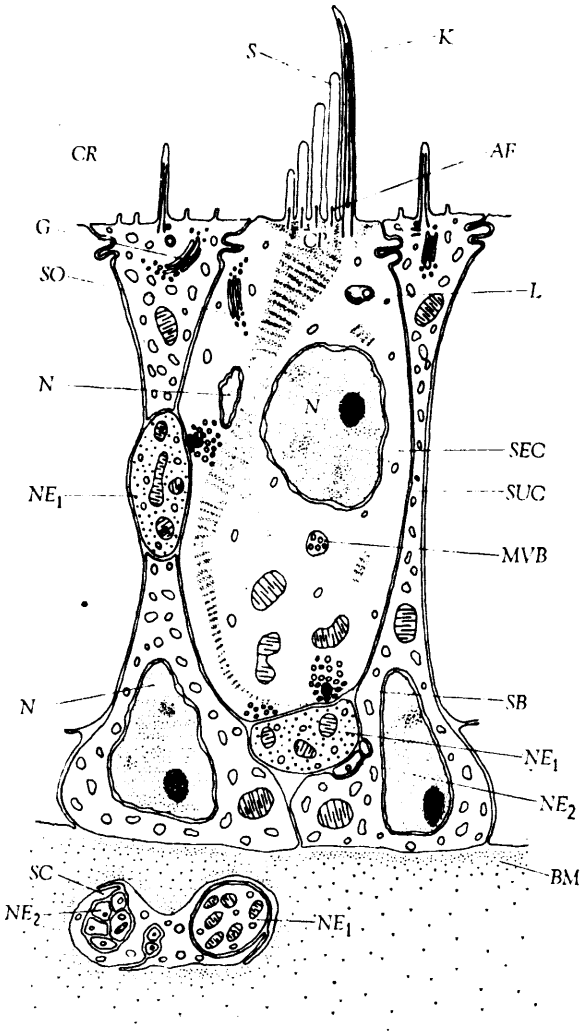

Figure 6.—*Diagrammatic view of a sensory hair cell and two supporting cells from a canal crista of the labyrinth of the larva of the lamprey* (Lampetra fluviatilis). AF, *axial fiber;* BM, *basement membrane;* CR, *ciliary rod;* CP, *cuticular plate;* G, *Golgi apparatus;* K, *kinocilium;* L, *lysosome;* MVB, *multivesicular body;* N, *nucleus;* NE_1, *large nerve endings;* NE_2, *small nerve endings;* S, *stereocilia;* SB, *synaptic bar;* SC, *Schwann cell;* SEC, *sensory cell;* SO, *striated organelle;* SUC, *supporting cell.* (*From ref. 21.*)

within the hair-cell body lying close to the area of contact between the cell and dendrite. Synaptic membrane configurations were found in the sensory cells in the ray and confirmed in the lamprey by means of better fixation and special staining.

The hair cell with its synaptic apparatus has thus clearly neuronal properties and bridges the gap between ciliated epithelial cell and sensory neuron, structurally as well as functionally.

A second type of dendrite is associated with the hair cell. It often contains more electron-dense vesicles. The ultrastructural appearance of its synapses with the hair-cell body clearly points to conduction from dendrite to hair cell, belonging to an efferent and probably inhibitory control system.

We are now beginning to understand the role played by efferent loops to peripheral sense organs, especially in the retina and also in the cochlea of the ear. The sharpening of contrast by a contour of inhibition around a field of excitation is an important method of peripheral stimulus processing. One of the possible functions of efferent control might be the setting of the working point of a given sensory unit on a certain part of its characteristic as a means either of controlling its linearity or of biasing it down or up toward unilateral or tonic functioning, respectively (fig. 3). Be this as it may, the ubiquity of efferent endings points to an important participation of central control in the receptive mechanism.

The sensory unit may consist of one hair cell and associated sensory neuron, but there is evidence for convergent innervation of assemblies of hair cells by candelabra-like dendritic trees belonging to a single thick stem fiber and neuron. This type of innervation is beautifully shown in the crista of a semicircular canal of the lamprey. The sensory field served by a single efferent element can be very wide indeed and may extend over a considerable area of a crista or macula. We shall come back to this type of linkup when we consider the topographic arrangement of hair cells within the various vestibular end organs. Whether the reverse arrangement of one hair cell synapsing with the dendrites of a number of sensory neurons also obtains is an open question that would call for very thorough histological reconstructions.

We now turn to the hair processes. They are compound structures consisting of two kinds of

elongated hairlike outgrowths from the top of the sensory cell; viz, a varying number of very elongated microcilli, the so-called stereocilia, and a single nonmobile flagellum paradoxically known as kinocilium. The stereocilia are arranged in interspaced straight rows and inserted in an electron-dense cuticular plate through which they send their long roots far down into a cytoplasm of the hair cell.

Figure 6 also shows a striated organelle which is clearly associated with the cuticular plate and which takes its course past the nucleus to end in the vicinity of a synaptic locus. The kinocilium shows the typical "nine plus two" filamental structure of a mobile cilium or flagellum (hence the name "kinocilium") and also a characteristic root. There is a single lateral excrescence from the kinocilial root projecting in a direction perpendicular to a line connecting the two central filaments. Such basal structures are known to exist in the mobile cilia of the gill of mollusks, and it has been shown that they point in the direction of the working stroke of the cilium (ref. 23).

The kinocilium stands in a recess in the assembly of stereocilia, and its root lies outside the cuticular plate in which the latter are inserted. It will therefore be independent of any passive movement of the stereocilial bundle, although, of course, coupled to it by being sheathed in the same cupula. Ultrastructurally we know very little of the cupula. It is very delicate and shrinks under fixation. However, under phase contrast, it can be seen to consist of a ground mass of jelly in which longitudinal channels are spaced out. These house the individual hair process which can apparently slide along them lubricated, so to speak, by a coating which has been shown to be acid mucopolysaccharide.

In specifically oriented ultramicroscopic sections through a crista or macula, the orientation of the cilia on to the sensory cell can be mapped, and it emerged, in the case of a labyrinth of the ray (ref. 20), that the hair bundles in the cristae of the semicircular canals exhibit a uniform orientation with respect to the topography of the ampulla. In the horizontal canal the notches in the stereocilial assembly, and within them the kinocilium, all point in the same direction transversely up and down the crista toward the utriculus and away from the canal end of the ampulla. In the vertical canal the orientation of the hair bundles is the reverse; i.e., the kinocilia face the canal end and point away from the utricular end of the ampulla.

We know that cupula displacement toward the utriculus increases the frequency of impulse discharge from the crista of the horizontal semicircular canal and that utriculofugal displacement is inhibitory (refs. 4 and 12). Seeing the topographic arrangement of the hair bundles in this context, we realize that a shearing force which leads to a displacement of the whole hair bundle toward its kinocilial aspect is excitatory and displacement in the opposite direction inhibitory. In the excitatory displacement, the kinocilium moves perpendicularly to the plane through its two central filaments in the direction of the basal excrescence (see above). This corresponds to the direction of the working stroke in the case of a mobile cilium (ref. 23). It has thus been suggested that the kinocilium may play an important role in the process of mechanoelectric transduction (ref. 20).

Before we deal further with the problems of stimulus transduction, let us have a look at the pattern of hair-cell development in the otolith maculae. This has been mapped in all otolith organs of the ray (ref. 20) and in the utriculus of *Lota vulgaris* (ref. 22). In the utriculus of the ray (fig. 7), the sensory cells are not uniformly oriented as those of the canal cristae. In fact, cells whose kinocilia point in opposite directions are found side by side in a given area of the macula. The hypothesis of a uniformly directed receptivity of the utriculus macula, as formulated by Von Holst (ref. 24), is therefore untenable. A movement of the overlying otolith must excite some and inhibit other members of the hair-cell population, leaving those unaffected whose kinocilial axis lies at right angles to the direction of the shearing force. A composite and complex signal will thus reach the vestibular nucleus. In view of the various response types found in the electrophysiological study of the utriculus of the ray, it may well be that a

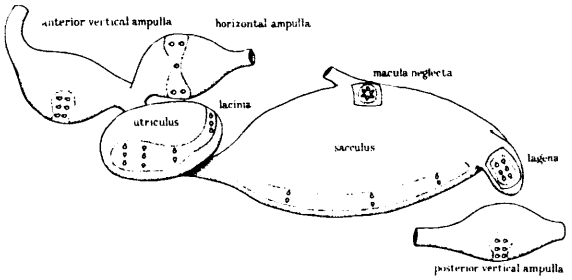

Figure 7.—*Diagrammatic representation of the polarity of sensory hair bundles found in the cristae and maculae of the left labyrinth of the ray* (Raja clavata). *The kinocilium is symbolized by a dot, the stereocilial bundle by a circle.* NOTE.—*The rows of hair cells in the utriculus may in reality be assumed to run perpendicular to the outer margin of the macula.* (*From ref. 20.*)

still more minute analysis of the directional arrangement of the hair cells would show preponderance of one or the other orientation in specific regions of the macula. At least two functionally different types of sense endings were found. The one responds with an increase in the discharge frequency to side-up and nose-up, and the other to side-up and nose-down tilting of the head. Nerve fibers conducting these two different responses were found side by side in the same nerve twig. The units responding with excitation both to side-up and nose-down tilt must have been hair cells whose kinocilial axis pointed forward-inward; those responding to side-up and nose-up must have pointed diagonally backward-inward, respectively.

There is no reason why the distribution of hair-cell axes within the utriculus macula of the ray (fig. 7) should not be similar to that found in *Lota* (fig. 8) as described by Flock (ref. 22). In this case both these receptor types are represented in the macula. In *Lota* they are confined to the margin of the macula, whereas they are more widely distributed in the ray. The curious fact remains that Lowenstein and Roberts (ref. 5) failed to find preparations responding by excitation to lateral downward tilt. This means that they were unable to record from cells whose kinocilial axis pointed either outward-forward or outward-backward. Such cells certainly exist (ref. 20), but may either be numerically in the minority in contrast to the situation in *Lota*, or their stem fibers may be less accessible.

The arrangement of hair cells in the sacculus of the ray is less complex. Here the macula is subdivided longitudinally into two fields with hair cells pointing uniformly upward or downward, respectively (fig. 7). As the greater part of this macula yields vibration responses, the situation here is of little importance for our argument. The lagena, however, is involved in gravitational responses exclusively, and here again we find oppositely orientated hair cells side by side (fig. 7). Two maxima of activity were found in lagena units, one in the normal position and the other with the head upside-down. In both positions the macula lies almost vertical and either the upward- or the downward-pointing hair cells are subject to a maximum of tangential shearing force.

So much then may be said about the importance of the topographic distribution of directionally specific hair cells in crista and macula. We now turn our attention to the question of stimulus transduction.

Following the assumption, now generally accepted, that shearing of the hair processes of the sensory cell is the adequate mechanical

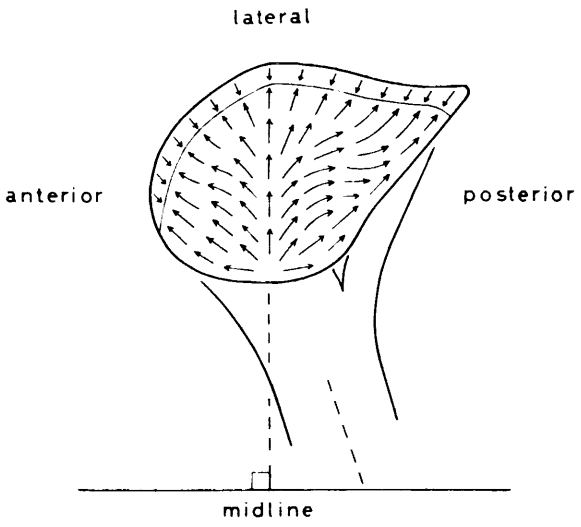

Figure 8.—*Diagrammatic illustration of the pattern of morphological polarization of the sensory cells in the macula utriculi of* Lota vulgaris. *The arrowheads indicate the direction toward which the kinocilium points in the various regions of the macula.* (*From ref. 22.*)

stimulus, the first problem we have to investigate is the locus of depolarization on the membrane system of the sensory cell. The existence of typical synapses between the hair cell and the dendritic terminals of a sensory neuron makes it virtually certain that chemical transmission takes place from hair cell to dendrite, and the fact that there is typically a level of basic activity in the afferent nerve points to a "leaky" synaptic system poised delicately on the verge of full-scale firing. The exceptional regularity of the "resting discharge" makes it unlikely that this is just "noise." It has very likely the important function of contributing to vestibular tonus and making the sensory input virtually thresholdless. What then is responsible for the modulation upward or downward of the quantity of neurohumor secreted across the synaptic gap from cell to dendrite? A generator process will have to be found which couples the shearing of the hair bundle to the synaptic process.

The cell membrane of the hair cell is continuous over all hair processes (stereocilia and kinocilium), and the only part of the hair-cell membrane mechanically disturbed by cupula or otolith action is the membrane of the hair processes. Might this then be considered to be mechanically excitable membrane and likened to the membrane of a terminal axoplasmic filament of a tactile organ or a stretch receptor? In this case it ought to harbor the battery connected with the ionic sodium-potassium transfer mechanism. That this is rather unlikely, if not impossible, has been pointed out by Trincker (ref. 16). The results of the chemical analyses of the endolymph of the vestibular organs of a number of vertebrates in which it was established that it has as high a potassium and as low a sodium content as the interior of a cell or a nerve fiber (refs. 25–27) exclude this possibility. Thus it may be concluded that there exists no possibility of a potassium battery across any part of the hair-cell membrane bathed in endolymph; i.e., at the hair-bearing apex of the sensory cell. The trunk and base of the hair cell, however, are bathed in perilymph or intercellular fluids which have roughly the chemical composition of cerebrospinal fluid or blood, respectively.

The immediate cover of the hair processes consists, according to Dohlman (refs. 28 and 29), of potassium salts of acid mucopolysaccharides which react to mechanical deformation by "static" electric change. The possibility of a capacity-governed electric change across the membrane of the hair processes was envisaged by Dohlman et al. (refs. 28 and 29) and discussed theoretically by Christiansen (ref. 30), and was held responsible for the generation of microphonic analog potentials which have been found in all parts of the ear and also in the lateral line (refs. 31 and 32) and may possibly form an essential part of the transducer mechanism. Dohlman (ref. 28) points out that the total surface of the hair processes coated with mucopolysaccharide is 200 times that of the rest of the hair cell. This, of course, includes the surface of stereocilia.

The endolymph in a canal ampulla is strongly postive to the perilymph, and Trincker (ref. 33) has been able in the guinea pig to record ordered changes in endolymph potential during cupula displacement (fig. 3). Trincker found potential changes on utriculopetal and utriculofugal cupula displacement which run exactly parallel to the trend in impulse activity in the ampullary nerve, as recorded by Lowenstein and Sand (ref. 4) and by Ledoux (ref. 12) in elasmobranches and in the frog, respectively. Trincker also found especially high positivity in the intracupular spaces which harbor the hair processes of the sensory cells.

It has been taken almost for granted that any transducer role of the sensory hair processes should be associated with the kinocilium, and it has been suggested that, in this, the kinocilium acts as a mobile organelle in reverse. That is to say, it responds to mechanical deformation by electric change transferred to the hair cell by its internal fibrillae and root structures, instead of being thrown into mechanical deformation by an imposed depolarization of its center of excitability, as is presumably the case in mobile cilia and flagella (ref. 20). However, the ultrastructure of the cochlear hair cell presents a serious difficulty. It has no fully developed kinocilium. This is represented by its basal body only, which is found in the niche

between the rows of stereocilia. This basal body apparently governs the symmetry and orientation of the hair bundle, but no hair process emerges from it. There are also some recent findings in the vestibular hair cells of the lamprey (ref. 21) which draw attention to a possible involvement of the stereocilia in the transducer process. It has been mentioned that the sterocilia alone are associated with the cuticular plate at the apex of the hair cell. This plate has now been shown to show a periodic striation and that this periodicity is continued into a striated organelle, branches of which penetrate through the whole length of the hair cell to end near the synaptic loci. Here we have at least a morphological intracellular pathway following from hair process to synapse. Could this be the essential link in mechanoelectric transduction? Could, therefore, the stereocilia have a role in the transduction process more important than being a source of elastic restoring force? Their exclusive presence in the cochlear hair cells points in this direction. However, it has recently been shown that the primary sensory hair cells in the cristae of the statocyst of the octopus have hair processes practically identical with the vertebrate kinocilia, each hair cell having a large assembly of these and no stereocilia (ref. 34). Here, then, the problem of mechanoelectric transduction is wide open, and we shall have to leave it so for the time being.

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DISCUSSION

GUALTIEROTTI: Dr. Lowenstein, can the beautiful results on the isolated labyrinth, in your opinion, be applied as a mechanism of information transfer to the intact animal? The reason I am asking is that our experiment on the frog and other experiments performed on the cat by Bizzi and Pompeiano, for instance, seem to show that the variability of the rate of firing at any given steady state is relatively large. An information mechanism based only on a flat change of frequency can't possibly be applied in this case. I am speaking about the otolith organ and not the semicircular canals. It seems the variability of the rate of firing is so large that some imprecision must finally appear in the central analyzer, if this relies only on a flat rate of firing.

LOWENSTEIN: First of all, speaking about the reliability of the single-unit experiments in surviving labyrinths, we were encouraged to go on to surviving labyrinths only after we had found the same response picture in spinal dogfish which survived the recording from their semicircular canals. Furthermore, when you have a linear response like the one I showed you on the torsion swing, and when you find that the response picture remains the same for an hour or two after the death of the fish, you begin to get confidence in the repeatability of these experiments. The firing rate isn't all that high. There is a 6-per-second to 10-per-second resting discharge in some units; it may rise to 40 per second. There is, of course, the population of cells which fire at different frequency ranges. It would be ideal if we could telemeter the function of these various sense endings. One should never say it cannot be done. I'm fairly sure I shan't do it.

CRAMPTON: I have previously communicated with several people here about a particular topic, and I think the question is of sufficient interest to raise it more publicly at this time. It concerns the classes of responses one obtains from single units during angular acceleration. If one records from single cells in the vestibular nuclei of the cat, one finds several well-defined kinds of responses. The most frequent, type I by Gernandt's classification, is the classical type in which there is an increase in the discharge rate with movement of the cupula toward the utricle. Gernandt described a type II in which there is an increase in discharge rate with acceleration in either direction. I have not seen such a unit, but others have. Gernandt also reported a type III in which the resting discharge is inhibited with acceleration in either direction. I have found and thoroughly tested one type III.

A fourth type is now being reported frequently and is best described as a "backward" cell, if you will. It responds with an increase in the discharge rate with movement of the cupula away from the utricle (assuming that the activity is originating in the lateral canal). On days which I characterize as bad days, perhaps as

many as one-third of the units will perform in this nonclassical manner.

Now, as tradition has apparently worn away, there are more reports of this class: Duensing and Schaefer, Eckel, and Shimazu and Precht. Even I am getting bolder. The source of these cells is in question. There are several possibilities, and the most likely one, and the one which many of you favor, is that activity for such a unit is originating from one of the vertical canals. It is, indeed, the likely interpretation. The units are obtained after section of the contralateral VIIIth nerve and after removal of the cerebellum. But these units are so commonly found that other interpretations might well be examined. The question that I wish to ask those of you who deal with the electron microscope is: What is the frequency of aberrant or turned-around hair cells or kinocilia within the cupula?

LOWENSTEIN: First of all, when you record from a nucleus, you might get anything. You have excluded the opposite labyrinths by elimination, but what you did not exclude, and you quite rightly say this is a possibility, are quite numerous units to be derived from the vertical ampulla.

Alex Sand and I reported long ago that of the three semicircular canals, the horizontal is the only one that is hidebound so far as plane of rotation is concerned. It has a very narrow range of rotational planes, beyond which it will not respond, whereas a vertical canal will in fact respond to accelerations going on in any plane, including the horizontal plane. Some years ago we were at pains to build this response of the vertical canal to rotation in the horizontal plane into a reflex schema or flow diagram in which we had to make certain assumptions. You know that anything can be proved by such a flow diagram, and we proved a very valuable function for this response of vertical canals to rotation in a horizontal plane.

If we look at eye nystagmus as such, assuming a certain wiring diagram from the vertical ampullae to eye muscles, we find that when all four vertical ampullae respond to a rotation in a horizontal plane, they will make the antagonistic diagonal and vertical eye muscles contract simultaneously. What better pivot could you have for the horizontal nystagmus than such a synergistic contraction of all other eye muscles? It was Lorente de Nó who, by kymographic experiments in the rabbit, showed that the vertical eye muscles and the diagonal eye muscles react in this way when the preparation is rotated on the turntable. While the horizontal eye muscles carry out their nystagmic swing, all the other eye muscles contract simultaneously, and the response from the vertical canal would now be a necessity rather than a nuisance.

ENGSTROM: In your lecture you mentioned the macula of the saccule. I have been asked on many occasions, even today, something that I ask you to settle for good. The macula of the saccule has a different function according to what you told us now. The small hook-shaped part of the macula has been said to react in a different way from the rest. The nerve fibers from the hook-shaped part lead in a different way from the fibers of the main portion. I wonder if recording has been made from the small nerve belonging to the upper part of the vestibular nerve or from the nerve that goes in the interior vestibular nerve.

LOWENSTEIN: In the ray, on which we worked, the sacculus macula is straight; it hasn't got a bend. You mustn't forget the ray has the luxury of having a lagena; this stands vertical. The sacculus macula is straight, undivided, tilting slightly inward, and having a certain pleated-up rim on this side, the otolith resting in this.

There is one interesting thing which has puzzled me quite a lot which we would have to explain sooner or later. If you look at the lagena macula in a ray, you can sometimes find that the otolith from the sacculus has run over and is continuous with the lagena otolith. That means that the soft pastelike otolith here cannot be as uniform in its function as one might assume.

ENGSTRÖM: You said that the sensory cells in the cochlea have no kinocilia. There is a modified kinocilium present on every cell. As far as we know the modified kinocilium could be working just as the kinocilium because in some of the primitive animals which have the aciliate kinetosomes around their mouths, they react to them as triggers. The moment you touch them, they bite.

LOWENSTEIN: Now, what is the modified kinocilium like?

ENGSTRÖM: It is the basal body of a kinocilium.

LOWENSTEIN: But it doesn't project into the cupula; therefore, its mechanical function is a bit difficult to see.

ENGSTRÖM: The cuticle is extremely thin at the basal body so that's a very mobile structure, just as mobile as the kinocilium itself.

LOWENSTEIN: So in this case the kinocilium root lies in the particular plane?

ENGSTRÖM: No; not quite.

LOWENSTEIN: Very close to it?

ENGSTRÖM: Very close to it; yes.

GUEDRY: In connection with the different firing in the saccule, was that true for all orientations to the saccule relative to gravity? In other words, did you try this in different positions? What I am suggesting is the possibility that the saccule was actually tilted slightly relative to gravity and therefore the mechanical characteristics of one portion of the saccule would be different from another portion.

LOWENSTEIN: Insofar as we could, we put the sacculus in a perfectly horizontal position. But that is not very easy in an organ which naturally has a tilt. And it may well be, as we assume, that anteriorly the saccular otolith is free to vibrate, whereas it is wedged in at the posterior end. Why there should be no gravitational response here, however, has puzzled us quite a lot.

GUEDRY: Another question concerns the snowdrift that several people have described in the utricle and also the saccule. I have heard this from Dr. Engström some time back and from Dr. Spoendlin. I wonder if the orientation of the kinocilia bears a particular relationship to this snowdrift?

LOWENSTEIN: By "snowdrift," you mean the Flock pattern?

GUEDRY: That's right.

LOWENSTEIN: Yes; definitely yes. In the elasmobranch we assume that. In the utriculus we found it easiest to record from the sector which looks diagonally forward outward, and we assumed that the cells which we found electron microscopically might, in fact, lie radially just as Flock states. We have no evidence that they should be directly transversely outward, so we have the same snowdrift. Therefore, in view of the diagonal orientation of the longitudinal axis of the overall utriculus macula, we have in fact all necessary directions of hair cells represented in the macula.

SPOENDLIN: In this connection I would like to show you the polarization pattern of the kinocilia of the vestibular sensory cells in the saccular and utricular macula in guinea pigs. Those reconstructions of the macular surface were based on phase-contrast microscope studies (figs. D1 and D2). The arrows indicate the direction of polarization. The saccular macula appears to be divided into two parts with divergent polarization. The reversal of the kinociliar polarization follows a definite line through the entire length of the macula (fig. D1), which seems to be coincident with the snowdrift of the otoliths.

Similar conditions are found in the utricular macula where the polarization spreads fanlike from medial to lateral up to a curved reversal line beyond which the polarization is reversed (fig. D2). Here, however, the polarization is convergent along the reversal line in contrast to the divergent pattern in the saccular macula.

LOWENSTEIN: All during my flight from Britain here I have been looking forward to this figure, but is there a possibility of a slight overlap here along this line?

SPOENDLIN: There is certainly a slight overlap along this line.

LOWENSTEIN: So, in a section in a certain direction you may in fact have face-to-face and back?

SPOENDLIN: In large electron-microscopic sections, through the surface of the macula the kinociliar polarization of each hair cell or associate hair bundles can be evaluated. In every section we find a clear predominance of polarization in one direction, but there is always a certain number of cells with a divergent polarization. Such divergent-polarized units have been found in the maculae as well as in the cristae.

LOWENSTEIN: But would you say that your divergences were in the marginal areas of the crista?

SPOENDLIN: They were perhaps more pronounced in the marginal areas.

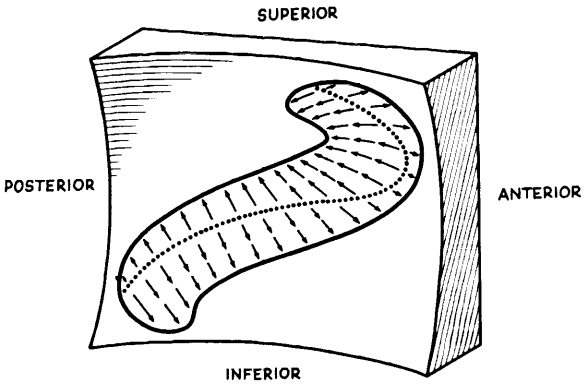

Figure D1.—*Reconstruction of the saccular macula based on phase-contrast microscopy. Arrows indicate direction of polarization.*

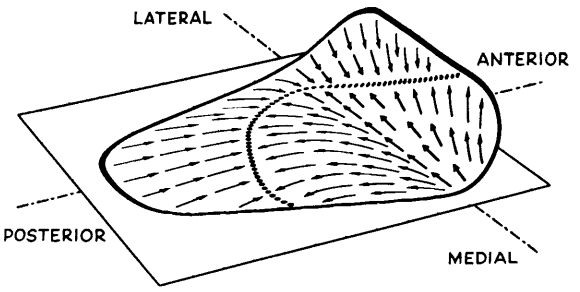

Figure D2.—*Reconstruction of the utricular macula. Arrows indicate direction of polarization.*

LOWENSTEIN: This is what we find in the lamprey now.

SPOENDLIN: Do you have any idea of the kind of transmitter which acts on the synapses between sensory cell and nerve ending?

LOWENSTEIN: This is, of course, an unfair question. If I say it is acetylcholine, you will laugh, and if I say I don't know if it is, you might also laugh. But I think the afferent system may be cholinergic.

Let's assume for a moment that the kinocilium has nothing to do directly with the mechanoelectric transduction process: would it be feasible to make it responsible for the orientation and for the functional health of the hair cell, especially in the early stages of the establishment of the innervation, et cetera; would it be a center of organization?

SPOENDLIN: Yes; this is exactly the opinion I expressed at last year's symposium in Pensacola.

LOWENSTEIN: You would say yes? This is a possibility, so we have one more pawn on our chessboard.

WERSÄLL: There is some indication that the fibers forming the bundle in the stereocilium have actually come from the area of the centriole. We know that the centriole can form contractile and very specifically active protein fibers, and it seems possible from what

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we are getting out now that actually fibers grow out from the centriole into microvilli and form the stereocilia.

COHEN: I have two questions, Dr. Lowenstein, both bearing on the specialization of function that has been alleged since the 1800's between the cupulae and the otoliths. Have you recorded the response from any one receptor to both linear and angular acceleration without moving your recording setup in any way?

LOWENSTEIN: I have recorded responses to linear acceleration from the otolith organs, and they go the expected way.

COHEN: But I mean have you combined stimulations? For example, without moving from the otolith, did you see if there was any response to angular acceleration?

LOWENSTEIN: In the impatience of my experimentation I have subjected otolith organs to angular accelerations. I showed you the picture of the rotation at constant speed. This was meticulously planned, but very often one tilts and in the initial stages one accelerates angularly and, of course, you get a response. There is no reason why an otolith organ should not respond to angular acceleration.

COHEN: I agree.

LOWENSTEIN: And it is in fact capable of responding to angular acceleration, to rotating with constant speeds and, of course, to all types of linear accelerations including the to and fro of vibration.

COHEN: Have you recorded from the cupular receptors while stimulating with linear acceleration?

LOWENSTEIN: You know how difficult it is to subject something to linear acceleration. I have tried it in the ampullae and have convinced myself that the old tale which I was told when I was an apprentice, that the semicircular canals do not respond to linear acceleration, still holds. I think this is a topological necessity. However, you know that they do respond to vibration, i.e., linear acceleration, when they are exposed, say when the padding which fills out the space between the bony canal and the semicircular canal is removed by fenestration operations. Then immediately sound produces vertigo but not in the intact canal.

Specific Gravity and Viscosity of Endolymph and Perilymph [1]

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SUMMARY

Samples of endolymph and perilymph of between 0.002 and 0.003 milliliter were obtained from single ears of living pigeons. Measurements of specific gravity were made in density gradient column, and it was shown that at the pigeon's body temperature (approximately 40° C), the specific gravity (referred to water at 4° C) of endolymph is 1.0033 and that of perilymph is 1.0022. Preliminary measurements indicate that at 40° C, the viscosity of endolymph is 1.15 centipoise and the viscosity of perilymph is 0.78 centipoise. The unusual high potassium concentration and low sodium concentration of endolymph reported for the cat and the guinea pig were confirmed for the pigeon.

INTRODUCTION

A semicircular canal senses angular accelerations by means of inertial endolymph movements inside the membranous canal (refs. 1–5). Specific gravity and viscosity are therefore fundamental properties of endolymph (refs. 2 and 6–8).

The occurrence of positional nystagmus, and nystagmus in response to changing linear accelerations (refs. 9–14) suggests the possibility that in some instances a semicircular canal responds to linear accelerations or gravity. It is therefore of interest to know whether the specific gravity of endolymph differs from that of perilymph. The number of original reports of the physical characteristics of endolymph and perilymph (refs. 15 and 16) is apparently even smaller than the number of original reports of the chemical characteristics (refs. 15 and 17–21). The specific gravity of endolymph and perilymph taken from freshly killed sharks (ref. 15) was reported as 1.0204 and 1.0200, respectively. The respective viscosities of these fluids taken from pigeons have been reported as 2.9 and 1.7 times the viscosity of water (ref. 16) at 18° to 20° C.

In the present study, measurements of specific gravity and viscosity were made on the labyrinthine fluids of the pigeon, *Columba livia*, a species in which it was found that relatively large samples of endolymph can be taken from the living animal.

METHODS

Surgical Procedure

After the feathers were clipped from the skin behind the external auditory meatus, the bird was anesthetized (with ether for specific grav-

[1] DRML Research Paper No. 629.

ity determinations, with sodium pentobarbital for viscosity determinations), a tracheotomy tube was inserted, and a headholder was applied. The skin over the periotic capsule was incised and retracted, and the lateral wall of the capsule was removed under an operating microscope. The bony spicules were removed from the semicircular canals. If the samples were to be used for specific gravity determination, the ether administration was discontinued at this point and the samples were taken 45 minutes later; if the samples were to be used for viscosity determination, they were taken without delay.

Perilymph samples were taken most often from the semicircular canal ducts. A hole in a bony canal duct was cut with a scalpel, and if the perilymph appeared free of blood it was drawn by gentle suction into a glass capillary tube. Occasionally perilymph samples were taken from the vestibule through a hole made with a needle between the anterior and horizontal ampullae. The capillary tubes were pulled by machine from tubing 2.2 millimeters in diameter and the tips were cut and fire polished at a diameter of approximately 0.05 millimeter.

Endolymph samples were obtained from the duct of the anterior (superior) canal. A hole was cut in the anterior bony canal and a small hook was inserted and used to open the bony canal along half its length (fig. 1), exposing the membranous canal. The membranous canal was seized near the ampulla with special pointed forceps and it was pulled out and cut across on the ampullary side of the forceps. It was then bent out of the bony canal, and the part squeezed by the forceps was cut off with scissors. The

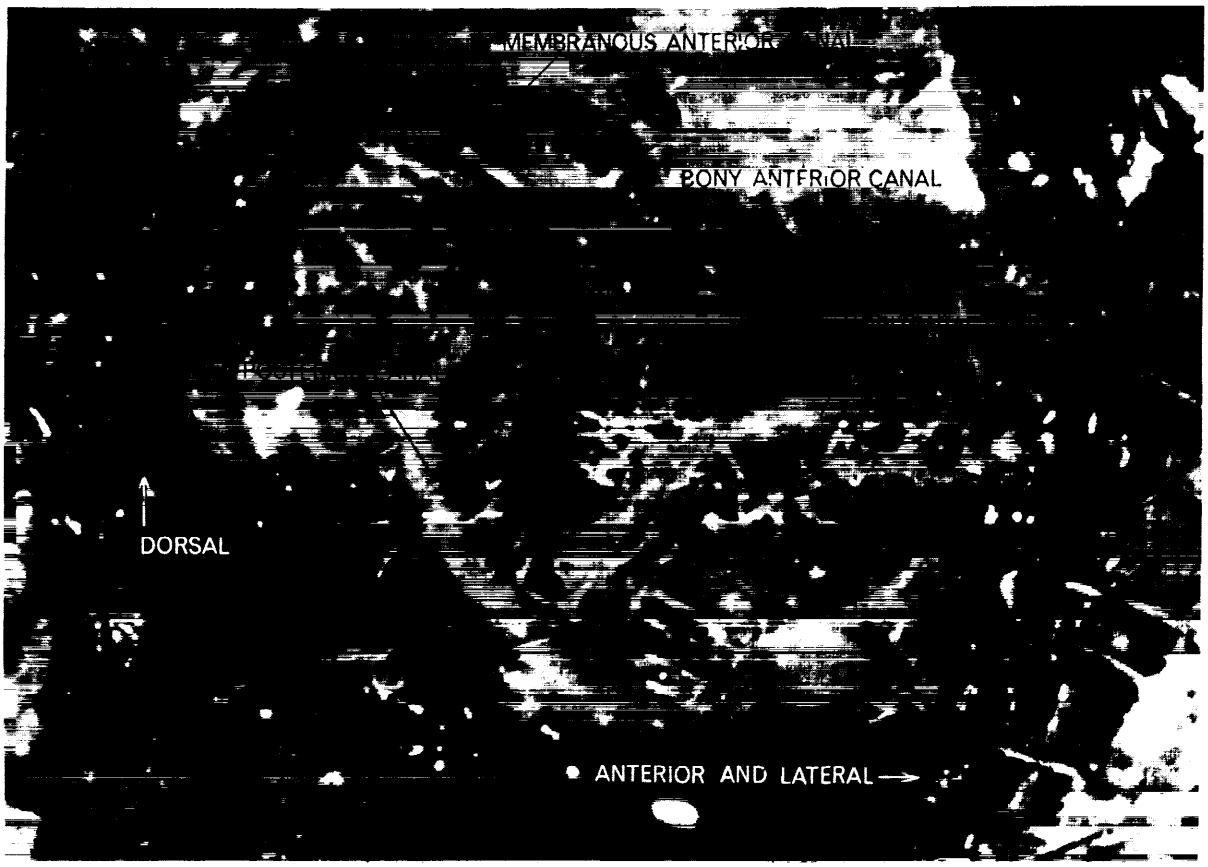

Figure 1.—*Right ear of pigeon. Bony anterior canal has been opened and the membranous canal can be seen lying inside.* × *17*

scissors usually sealed the canal by pinching while they cut across it, but the capillary tip was easily inserted through this seal and endolymph was collected by gentle suction (figs. 2(a) and 2(b)).

Before samples of endolymph or perilymph were used for any determination, they were examined against both dark and light backgrounds with the 40 × power of the operating microscope. If any evidence of blood contamination was detected, the sample was discarded. Pigeon blood diluted 1 part to 5000 in Ringer's solution was readily visible when examined in this way.

Measurement of Specific Gravity

The sample was transferred in the capillary tube directly from the animal to a density gradient column (fig. 3). The column was made with two silicones (Dow Corning 702 fluid and 200 fluid) mixed so that the density varied continuously from 1.0115 g/cc at the bottom of the cylinder to 1.0050 at the top at 23.0° C. The two fluids were washed four times with distilled water before use, and although they were the least miscible with water of many fluids tried, it was found necessary to fix a strip of Webril bandage soaked with water inside the cylinder to keep the column saturated with water. The column was 20 centimeters high in a 100-milliliter graduated cylinder. It was kept in a water jacket containing a saturated solution of copper sulfate (to reduce temperature changes due to radiation) and the jacket in turn was immersed in a constant temperature bath maintained at 23.0° C. The temperature in the water jacket varied by only 0.05° C, and presumably the temperature of the column itself varied less. The column was made in 60 layers and it was kept for 1 week before use to allow saturation with water and diffusion of the layers.

The sample was released in the column by gentle air pressure and by abrupt upward movement of the capillary, and its position in the column was recorded periodically until it had stopped falling. The column was calibrated

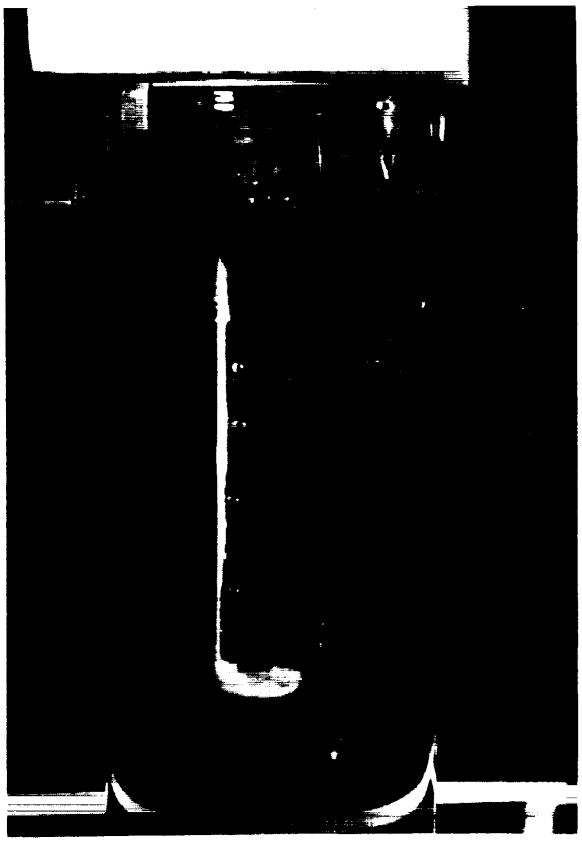

Figure 3.—*Density gradient column in water jacket and constant temperature bath. Four calibrating glass spheres and two saline droplets can be seen in column. The bandage at bottom and side of column hold water to keep the column saturated. Bottom of column had specific gravity of 1.0115 and top of 1.0050.*

Figure 2.—*Glass capillary tube in membranous anterior canal of pigeon's right ear* (a). *In* (b), *drawing assists orientation in photograph.*

with glass spheres of known specific gravity and also with droplets of saline solution of measured density.

Measurement of Viscosity

Viscosities were measured with the rolling-sphere viscometer of Flowers (ref. 22). Approximately 0.012 milliliter of pooled fluid from several animals was placed in a separate piece of capillary in which 0.01 milliliter occupied a length of 1.5 centimeters. The pipet sections were kept in enclosures of high ambient humidity to reduce evaporation of water. A chrome-steel-bearing ball of density 7.83 g/cc and $\frac{1}{32}$ inch (0.79 millimeter) in diameter was put into the pipet which was then fixed at an angle of 10° to the horizontal in a constant temperature enclosure. After allowing the time (approximately 15 minutes) required for a glass thermometer to attain the constant temperature of 40° C in the enclosure, the time required for the ball to roll through the fluid between marks on the pipet 1.5 centimeters apart was determined. A magnet was used to raise the ball for repeated tests. A given pipet was always used in exactly the same position and orientation and with the same ball, and calibration tests were done with distilled water and with ethyl alcohol. The collection of fluid and the measurements were completed within a 10-hour period.

The concentrations of sodium and potassium in the pooled endolymph and perilymph samples were determined with a flame photometer.

RESULTS

Surgical Procedure

Approximately three of every four perilymph samples and two of every three endolymph samples were discarded because of contamination with blood. The uncontaminated samples obtained were usually between 0.002 and 0.003 milliliter from one ear for both endolymph and perilymph.

Measurement of Specific Gravity

The droplets fell for approximately 5 hours in the column, but remained indefinitely at their equilibrium positions (where density of column equals density of droplet) once these were reached. The columns were replaced when they became too crowded.

Table 1.—*Specific Gravity of Labyrinthine Fluids of Pigeon at 23.0° C (referred to water at 4° C)*

[In top 6 lines of table, 2 values on same line represent endolymph and perilymph from same bird; in lower part of table, 2 values on same line represent endolymph and perilymph from different birds]

Endolymph	Perilymph	Endolymph	Perilymph
1. 0084	1. 0078	1. 0088	1. 0073
1. 0087	1. 0087	1. 0085	1. 0071
1. 0084	1. 0082	1. 0084	1. 0068
1. 0093	1. 0086	1. 0088	
1. 0084	1. 0077	1. 0102	
1. 0093	1. 0071	1. 0085	
—	—	1. 0085	
1. 0086	1. 0074	1. 0085	
1. 0079	1. 0071		
1. 0085	1. 0077	MEAN:	
1. 0085	1. 0078	1. 0087	1. 0076
1. 0087	1. 0071		

The values obtained are shown in table 1. At 23.0° C the mean value of the specific gravity of endolymph was 1.0087 and of perilymph was 1.0076 (referred to water at 4° C). The difference was significant at the 0.001 level. Assuming that the temperature of the pigeon's ear is 40.0° C and assuming that the densities of these fluids vary with temperature in the same way as that of pure water, the specific gravities in the ear of the living bird are 1.0033 for endolymph and 1.0022 for perilymph. Essentially the same values were obtained by assuming that the densities of endolymph and perilymph vary with temperature in the same way as sea water.

Measurement of Viscosity

Viscosity was calculated from the formula

$$\eta = K(\sigma - \rho)T$$

where η is the dynamic viscosity in centipoise, σ is the density of the sphere in g/cc, ρ is the density of the liquid in g/cc, T is the time in seconds for the sphere to roll across the

standard course, and K is the instrument constant determined by calibration of the instrument with liquids of known density and viscosity (refs. 22 and 23). Calibration and testing was at 40° C.

Using the endolymph viscometer with water ($\rho=0.995$, $\sigma=7.83$, $\eta=0.6560$, $T=2.38$), it was found that $K=0.0403$; with ethyl alcohol ($\rho=0.772$, $\eta=0.834$, $T=3.21$), it was found that $K=0.0369$. The mean instrument constant was therefore 0.0386. The values of K for the perilymph viscometer were 0.0377 ($T=2.55$ seconds using water) and 0.0399 ($T=2.96$ seconds using alcohol) yielding a mean instrument constant of 0.0388.

Values of T for endolymph and perilymph at 40° C are recorded in table 2. For endolymph ($\rho=1.0033$), $T=4.36$ seconds and therefore $\eta=1.15$ centipoises. For perilymph ($\rho=1.0022$), $T=2.95$ seconds and therefore $\eta=0.78$ centipoise. These values of viscosity are regarded only as preliminary estimates because of the limited accuracy of the time measurements, because of the variation in instrument "constants," and because only one pooled sample of endolymph and one pooled sample of perilymph were measured.

Table 2.—*Times in Seconds for Sphere To Roll Down Standard Course Through Labyrinthine Fluids of Pigeon at 40° C*

[Only 1 pooled sample of endolymph and 1 pooled sample of perilymph were obtained; values represent repeated tests of the same 2 samples]

	Endolymph viscometer $K=0.0386$	Perilymph viscometer, $K=0.0388$
	4.6	3.0
	4.4	2.9
	4.4	3.0
	4.3	3.0
	4.2	3.0
	4.4	2.9
	4.5	3.1
	4.4	2.9
	4.2	2.9
	4.2	2.8
MEAN:	4.36	2.95

DISCUSSION

With knowledge of the specific gravity and viscosity of endolymph, the moment of inertia I and the frictional torque per unit angular velocity Π of the endolymph ring can be calculated without any quantitative assumptions concerning the crista's response to endolymph movement. As an approximation, $I=2\rho\pi^2r^2R^3$ (ref. 7), where ρ is the density of the endolymph, r is the radius of the endolymph tube, and R is the radius of the circle of the canal.

The anterior canal of the pigeon is 15 millimeters long, and although its shape resembles a question mark more than a circle, dividing its length by 2Π gives $R=0.25$ centimeter; $\rho=1.0033$ gm-cc^{-1}, and $r=0.022$ centimeter, so that $I=1.5\times10^{-4}$ g-cm^2. In the horizontal canal of the pigeon, $R=0.16$ centimeter, $r=0.016$ centimeter, and $I=2.1\times10^{-5}$ g-cm^2.

The frictional torque at unit angular velocity of the endolymph ring, Π, is $8\eta\Pi^2R^3$ in cases where the canal length is half the circumference of the circle (ref. 7). In the anterior canal of the pigeon, almost all the circle is completed outside the utricle. The canal length is therefore roughly equal to the whole circumference of the circle, so that for this canal $\Pi=16\eta\Pi^2R^3$, and since $\eta=0.0115$ poise and $R=0.25$ centimeter, $\Pi=0.028$ g-cm^2-sec^{-1}. The length of the horizontal canal of the pigeon is also roughly equal to the circumference of its circle, and since $R=0.16$ centimeter, $\Pi=0.0075$ g-cm^2-sec^{-1}.

The ratio $\frac{\Pi}{I}$, which is $\frac{8\eta}{\rho r^2}$ for the pigeon (ref. 7), is 190 sec^{-1} for the anterior canal and 360 sec^{-1} for the horizontal canal, estimates which are much larger than those calculated previously for other species (refs. 2 and 7). These estimates are larger, partly because of species differences (smaller r in the pigeon and smaller fraction of the endolymph tube in the utricle) and partly because of the use of a measured value for the viscosity of endolymph instead of using the viscosity of water.

At 40° C, the measured viscosity of endolymph (1.15 centipoise) is 1.8 times that of water (0.656 centipoise), and the measured viscosity of perilymph (0.78 centipoise) is 1.2 times that of water. Rossi (ref. 16) reports that at

18° to 20° C, the relative viscosities of these fluids are 2.9 and 1.7, respectively. Aside from the difference in temperature, which is undoubtedly important, the discrepancies might also be the result of different methods of obtaining the fluids. Rossi decapitated the pigeon before collecting the fluids, which would introduce postmortem changes, and by carefully removing perilymph with strips of blotting paper before collecting endolymph, he might have lost considerable amounts of water from the endolymph.

The finding that endolymph is denser than perilymph by one-tenth of 1 percent supports the suggestion that the semicircular canals can respond to linear accelerations and gravity (ref. 24). When lengths of the membranous canals filled with endolymph were put into the density gradient column, they moved past the level of the density of perilymph at impressive speed and fell quickly to the bottom of the column. A much denser column will be required to measure the specific gravity of the membranous duct filled with endolymph.

Flame photometry of the pooled endolymph and perilymph used for viscosity determinations confirmed in the pigeon the unusual high potassium concentration and low sodium concentration of endolymph (and high sodium, low potassium composition of perilymph) reported in the cat and guinea pig (refs. 17 and 20). Chemical analysis of endolymph and perilymph of the pigeon will be the subject of a separate study.

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DISCUSSION

MAYNE: There is a longstanding need for reliable values of the specific gravity and viscosity of the endolymph. Dr. Money's paper is a welcome contribution in this regard. I would be inclined to believe, without too much familiarity with the literature in this regard, that the physical characteristics of the endolymph are similar for various species because of the close correlation shown by Jones et al. between canal dimensions and weight of species. An immediate application of these values is in the calculation of one of the constants of the semicircular-canal differential equation. This is particularly significant because this constant is difficult to obtain experimentally. The constant can be computed by a formula derived by Schmaltz when the internal radius of the canal, the specific gravity, and the viscosity of the endolymph are known. Schmaltz used a value of 0.01 g/cm-sec for viscosity and 1.0 g/cm^3 for specific gravity, and obtained a value of 200 for the constant of the canals of man. We have shown in one of our reports that there were two compensating errors in the use of the formula outside of the mistaken value of viscosity as compared to that given in Dr. Money's paper. The mass of the fluid should be increased by 2 to account for the parabolic distribution of velocity, and the radius squared should be reduced by 2 to correspond to measurements given in recent references. If we correct the computations for the new value of viscosity, the constant becomes 320 and the equation for the canal

$$\ddot{\theta}+320\ \dot{\theta}+320=f(t)$$

assuming a time constant of 10.

MONEY: These numbers are larger only partly because of the larger value for viscosity which we used. They are also larger because of the smaller radius of the canal duct in the pigeon, and, as you know, the larger fraction of the endolymph ring outside the utricle.

BERGSTEDT: I believe there is a small difference in concentration, density, and viscosity. How could this difference influence our different tests, for example, the positional tests or centrifuge tests? You mentioned your study as a clue for the explanation of positional nystagmus. As you know, we find a very weak positional nystagmus in about 10 or 20 percent of normals. If this difference in physical qualities existed normally, you should have positional nystagmus as a normal finding. Even if you have this difference in specific gravity and density, how could it reasonably affect and stimulate the sensory receptors? If you have a ring of fluid that is heavier than the surrounding fluid and if you have a g pull, this ring is moved against the Earth or from the centrifuge. If this is your picture of the soft labyrinth, which receptor is affected? Is it the cupula or is it the utricle?

MONEY: I would say that your second question answers your first, and that in fact there is no gross positional nystagmus because normally this difference in specific gravity doesn't cause nystagmus. Under certain other abnormal circumstances it is possible for this difference to become important and cause nystagmus. The abnormal is continuing nystagmus with constant angular velocity rotation about a horizontal axis, for example. This is one explanation, according to Benson's theory, which the difference in specific gravity bears out.

BERGSTEDT: Shouldn't you find it in many other tests too where this difference in specific gravity is still more pronounced? It should reasonably stand in relation to the pull. If you pull higher g, you should have a better chance of getting a difference or change in positional nystagmus. But you did not find this.

MONEY: This is very true. I will grant you that with a straight linear acceleration, there is no positional nystagmus in most people. The difference in specific gravity is nevertheless there in pigeons and, as I said, significant to the 0.001 level. It seems to be perfectly consistent in pigeons.

BERGSTEDT: I think the figures are very interesting concerning the difference between endolymph and perilymph in the results we got. But I don't believe it explains positional nystagmus or other mysterious vestibular findings.

STEER: Have you extrapolated these values, these numbers plugged into the physical dimensions of the human system, to evaluate the time constants of that and compare it to the time constants evaluated by the various experiments?

MONEY: No; I haven't done this in the human, only in the pigeon. The specific gravities might be quite different in a human, but it could be done with no trouble at all if you want to assume that the specific gravities are the same; the dimensions are known. It wouldn't be difficult.

STEER: In the evaluation of the viscous-drag term, all the classical derivations which arrive at a formula

like the one you have, as far as I have been able to tell, do not consider the fact that this fluid is almost entirely in a viscous boundary layer and in reality only a very small portion of it may be flowing. Mr. Mayne said essentially the same thing in that it is fundamentally a parabolic-distribution flow. I was wondering if this was considered in your derivation or if it was in the derivation that arrived at that formula?

MONEY: No; this wasn't considered. We just accepted from van Egmond, Groen, and Jongkees the formula with the change for our particular dimensions. We checked mathematics and found that, according to what he considered, he was correct.

GUEDRY: In connection with Dr. Bergstedt's comments—according to Dr. Benson's notion, which Dr. Money has referred to—if we have a flow of endolymph attributable to the difference in specific gravity between perilymph and endolymph, a continuous cupula deflection should not be maintained by an increased g field unless there was reorientation relative to the g field.

LOWENSTEIN: Was 10 the value of π/Δ found by the Pensacola investigators?

MONEY: I believe it was 12.

LOWENSTEIN: We found a value of 35.

MONEY: My numbers are open to criticism because I measured viscosity outside of its normal position for one thing. The results of other workers are open to criticism because they were really dependent upon an assumption regarding the quantitative response of the crista's transducers to endolymph movements.

LOWENSTEIN: The figure which was derived by Groen was worked out in a vacuum, so to speak; therefore, I wouldn't put too much trust in his value. He tried to carry out a dimensional analysis, making assumptions about the viscosity, but you have all the aces.

MONEY: I recall that he used the viscosity of water at body temperature for humans. I believe it was around 20° for a fish. This, of course, accounts for a good deal of the difference. My viscosity figures were 1.8 times the viscosity of water.

BENSON: I would like to thank Dr. Money for at least supporting this difference in density between endolymph and perilymph which was put forth as a possible explanation for the sustained per-rotational nystagmus which Dr. Guedry and I have both seen during horizontal rotation. But this is not the total story by any means because although there may be a difference in density, there must also be relative movements of endolymph in the duct within the canal itself. This assumption and the whole problem of the role of otolithic signals have to be considered in any discussion of the mechanism by which nystagmus of this type is brought about. However, it would give a sustained nystagmus during rotation and it might give a *position-changing* nystagmus, but not a sustained *positional* nystagmus.

Regarding positional nystagmus, did you extend your observations of density to those of the cupula itself?

MONEY: We planned this, but we haven't been able to find the cupula yet. The density gradient column technique is capable of measuring the specific gravity of something that small. It is simply a matter of getting it out uncontaminated and getting it into the column. We are attempting this but haven't succeeded yet.

BENSON: Then we come to the problem of positional alcohol nystagmus in which I know you are interested. Have you looked at this yet and are you prepared to say anything about the effects of alcohol on the density of perilymph and endolymph?

MONEY: This hasn't been completed. According to the theory we are working on, after alcohol intake the density of endolymph should increase. But, in fact, we found that after alcohol, the density of endolymph decreased by quite a lot. We are not very pleased with that result, but we are going ahead to establish it one way or the other, and it looks as if it is lighter.

BENSON: At least you got a change.

CAPPEL: We checked the ratio of damping to moment of inertia on the basis of data provided by Prof. Melvill Jones, and we found the value to be of the order of 200, just as you predicted here. This is on the basis of a 0.14-mm internal radius of the canal and of the density and viscosity of the fluid equal to that of water, which isn't too far off. It still requires an explanation as to why the actual experimental results do not bear out this number.

In reply to Mr. Steer, I believe that the Hagen-Poiseuille equation for motion of the viscous fluid through a tube is correct whatever the dimensions are because in this equation it is assumed that the fluid is stationary next to the wall and shaped parabolically across the cross section. The difference between tubes of varying diameters is only the steepness, if you will, of the parabola. I do not think there is a qualitative difference in the flow. It is a quantitative difference only.

MAYNE: We checked the formula in the computation of the constant of viscosity over moment of inertia of the canals of the pike and obtained a value of 35, corresponding exactly to that determined experimentally by Lowenstein et al.

Some Morphofunctional and Pathological Aspects of the Vestibular Sensory Epithelia

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NEURONAL CONNECTIONS IN THE SENSORY EPITHELIA

The junction between nerve endings and sensory cells is morphologically variable. The hair cells of type I and type II are distinguished mainly by their different modes of innervation. The type I hair cells, with the exception of the hair-bearing receptor pole, are entirely surrounded by a single nerve chalice which constitutes the ending of a single large nerve fiber. The type II hair cells, on the other hand, are innervated by a great number of independent small nerve endings, originating in most cases from different nerve fibers (figs. 1–4).

The adjacent plasma membranes of nerve chalices and sensory cells usually run more or less parallel at a fairly regular distance of about 200 Å. The intercellular space is filled with a relatively dense material (fig. 5). At many places the intercellular gap is further enlarged, an effect which is possibly due to tissue-preparation artifacts.

At certain spots, however, the junctional membranes exhibit further differentiations, characteristic of synapses elsewhere. In such restricted areas the presynaptic and postsynaptic membranes are thickened and run absolutely parallel. Marked accessory synaptic structures are usually found in the cytoplasm of the sensory cells in such places. All these accessory synaptic structures show basically the same architecture. They consist of a very dense core of finely granulated material surrounded by vesicles resembling any other synaptic vesicles (fig. 6). The core exhibits all possible forms from a simple bar, such as the synaptic bar described by Smith and Sjöstrand (ref. 1), to long, bent or straight laminae, single or multiple circles, or quadrangles of considerable size, frequently penetrating deep into the sensory-cell cytoplasm (ref. 2). Similar to synaptic structures in other tissues, such structural differentiations can certainly be accepted as evidence of synapses. According to a generally accepted

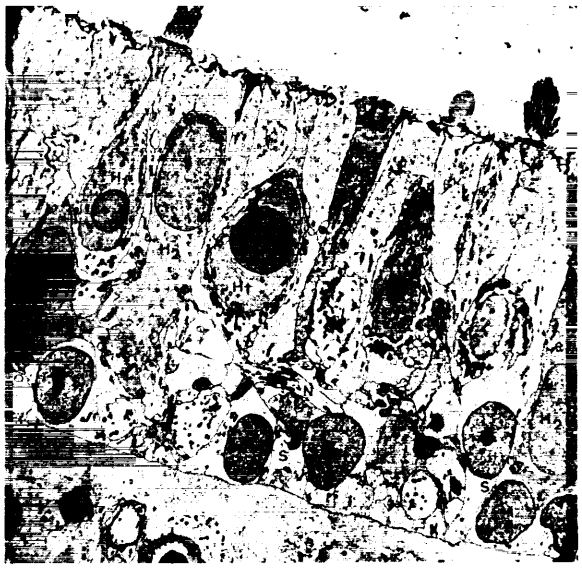

Figure 1.—*View of part of the sensory epithelium of macula utriculi with the type I hair cells* (H_1) *and the type II hair cells* (H_2) *which frequently have their nuclei close to the basally located supporting cells* (S). *Between the sensory and supporting cells, there is an extensive network of unmyelinated nerve fibers* (N) *of varying caliber.*

Figure 2.—*Lower portion of a type II hair cells* (H). *It is connected with a great number of individual nerve endings* (N). *At several places accessory synaptic structures* (s) *are seen.*

scheme, a transmitter substance is contained in the small vesicles and released upon stimulation. As a consequence of the chemical process which is necessary for transmission, such synapses have a certain latency, and the transmitter is released stepwise in quanta (ref. 3).

At other places along the junction of the nerve chalice and the hair cell, distinctive invaginations of the nerve chalice into the sensory cell are observed without any other specific accessory structures adjacent. The question is whether those invaginations also play a role in the transmission from sensory cell to nerve chalice. A close look at them reveals an extremely close connection between the plasma membrane of nerve chalice and sensory cell (fig. 7).

As first determined by Robertson (ref. 4), all plasma membranes consist of three different layers; that is, outer and inner dense leaflets separated by a lighter zone of about 20-Å thickness. With appropriate fixation and stain, the so-called unit membrane can be seen with somewhat varying dimensions in almost all cellular membranes. Normally, there is a gap of about 200 Å between the unit membranes of two adjacent cells. At certain places, however, the intercellular gap disappears and the outer leaflets of both unit membranes lie directly against each other and apparently fuse to one single dark layer, the fusion line. This type of membrane junction, which is found in many tissues, is called an external compound membrane by Robertson.

This is exactly what is found in the area of those invaginations where the plasma membrane of the nerve chalice and sensory cell form an external compound membrane. The so-called fusion line, formed by the fusion of the two outer leaflets of unit membranes, is clearly visible. There is no extracellular space left between the sensory cell and the nerve ending (fig. 8 *A* and *B*).

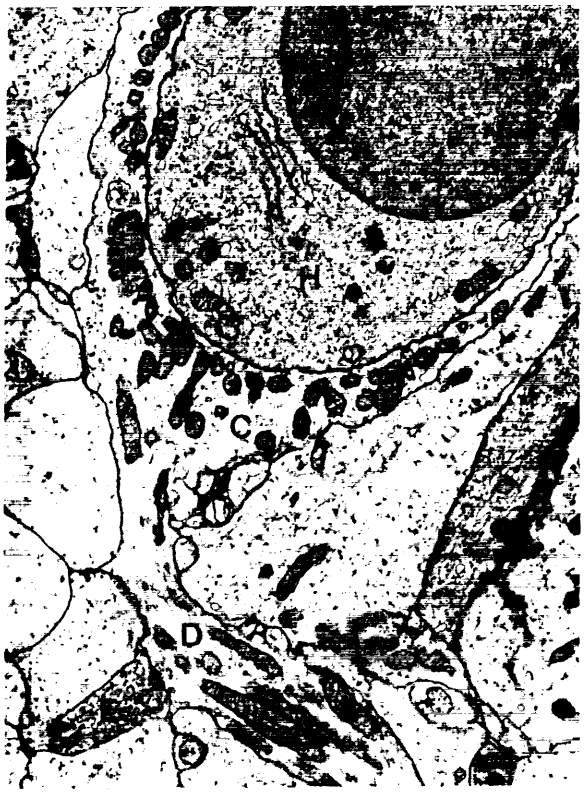

Figure 3.—*Lower part of a type I hair cell* (H). It *is surrounded by one single nerve chalice* (C) *which leads to a dendrite* (D). *A vesiculated efferent nerve ending* (E) *is seen in synaptic contact with the dendrite.*

Figure 4.—*Enlarged detail of figure 3 showing the vesiculated efferent nerve ending* (E) *in synaptic contact with the dendrite* (D). *The synaptic area* (S) *is characterized by a thickening of the pre- and postsynaptic membrane as well as an accumulation of vesicles close to the presynaptic membrane.*

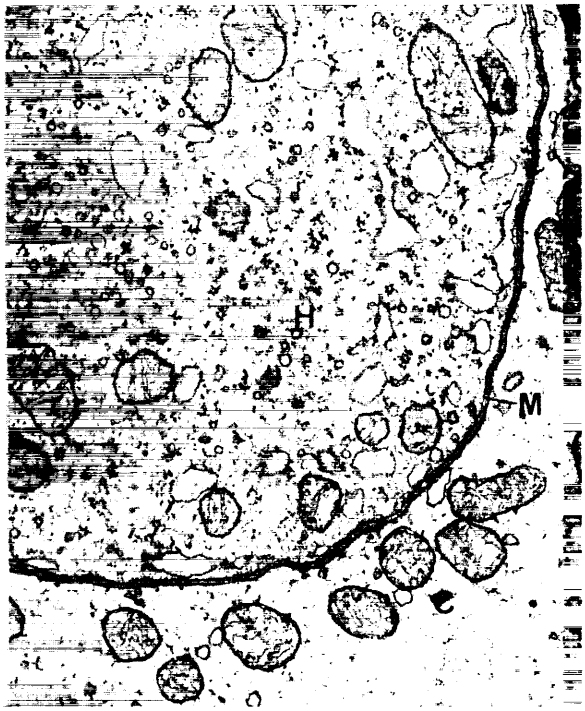

Figure 5.—*At the junction of the type I hair cell* (H) *and the nerve chalice* (C), *the adjacent plasma membranes of the two cellular elements* (M) *usually run parallel at a distance of about 200 Å. The intercellular space is filled with relatively dense material.*

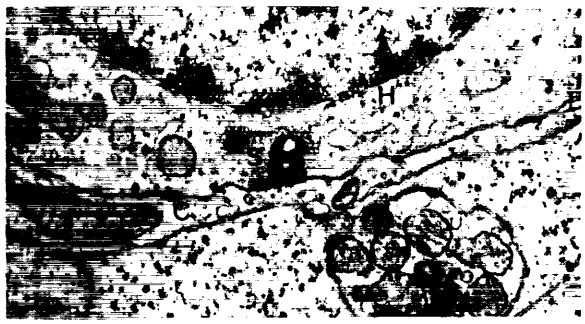

Figure 6.—*Synaptic differentiations* (S) *are frequently found between a type I hair cell* (H) *and its nerve chalice* (C). *Beside the typical thickening of the presynaptic and postsynaptic membranes in those places, there are always accessory synaptic structures with a dense core of varying forms surrounded by numerous small vesicles.*

This brings up the question of electrical transmission. Furshpan and Potter (ref. 5) provided very convincing evidence of electrical transmission in crayfish giant-fiber synapses. This has been correlated by Robertson (ref. 6) with an intimate connection of presynaptic and postsynaptic membranes in the sense of external compound membranes found in those crayfish median-giant-fiber synaptic areas. Similar external compound membranes have been found by Robertson et al. (ref. 7) in Mauthner cell club endings of goldfish brains where Furshpan and Potter apparently also found electrophysiological evidence for electrical transmission. According to these authors, the presence of external compound membranes in synaptic areas at least suggests electrical transmissions.

Although today it is generally believed that neurohumoral transmission prevails in the vertebrate nervous system (ref. 8), there exist incontestable areas of external compound membranes between nerve chalice and type I hair cell in cristae and maculae very similar to the synaptic disks described in crayfish giant fibers and goldfish Mauthner cells. Whether this really indicates that we are dealing with partly

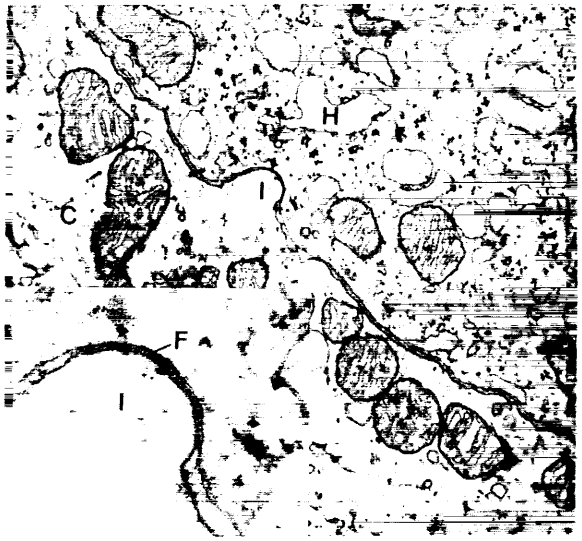

Figure 7.—*Frequently, invaginations* (I) *of a nerve chalice* (C) *into the type I hair cell* (H) *are found. At those places the plasma membrane of hair cell and of nerve chalice are very closely approximated. As seen in the insert, the intercellular space between nerve chalices and hair cell at those places disappears completely, and the outer leaflets of the unit membranes form a single line, the so-called fusion line* (F).

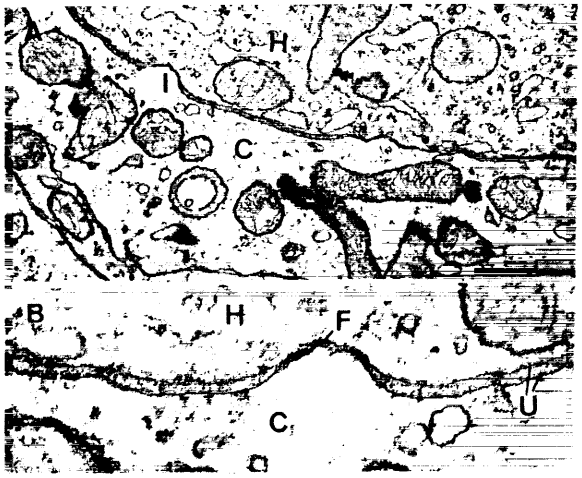

Figure 8.—A. *An invagination* (I) *of the nerve chalice into the type I hair cell* (H) *is shown. The intercellular gap in the area of this invagination is clearly reduced.*

B. *Detail of the invagination in* A, *showing clearly the pattern of an external compound membrane where the outer leaflets of the unit membranes* (U) *of the hair cell plasma membrane and the nerve chalice plasma membrane fuse to form the so-called fusion line* (F).

electrical or, in other words, ephaptic (ref. 9) transmission between type I hair cell and nerve chalices cannot be decided before electrophysiological evidence also can be produced. In any case, this would be of considerable importance toward the understanding of the physiology of the vestibular receptors.

So far we have not been able to demonstrate external compound membrane connections between nerve endings and type II hair cells. There are only structural membrane differentiations suggestive of regular neurohumoral synapses of the same varying appearance as in type I hair cells.

When the totality of synaptic or ephaptic connections between sensory cells and nerve endings is taken into consideration, the great variation in their number per sensory cell is striking. This was evaluated mainly in serial sections on type I hair cells. There are cells with very few and small synapses or ephapses and others with a great number of them.

There might be a correlation between those morphological findings and electrophysiological observations. Lowenstein and his group (refs. 10 and 11) found, for instance, different sensory units in cristae and maculae of the thornback ray. Two main groups of units were detected: one with marked spontaneous activity and the other without such activity. Between those two extremes a smaller number of more or less spontaneously active fibers were described. Similar differences among the vestibular sensory units were also found by Gernandt (ref. 12) in guinea pigs. Gualtierotti (personal communication) found some sensory units with a very high and others with a low sensitivity.

An attribution of those units to the type I and type II hair cell in the sensory epithelia was ruled out by the observation of Lowenstein, Osborne, and Wersäll (ref. 11) that the thornback ray labyrinth contains only type II hair cells. It seems, however, most likely that those electrophysiologically different sensory units may also exhibit different ultrastructural features. The difference in functional behavior of the spontaneously silent and spontaneously active sensory units might be related to the number and state of synapses in the different sensory

cells. The spontaneously silent receptors which are considered to be less sensitive would correspond to the sensory cells with few functional synapses.

If electrical transmission from the sensory cell to the nerve endings really does exist at the sites of external compound membrane junctions, it might be just those connections which contribute essentially to the spontaneous activity or high sensitivity of the sensory units. Electrical transmission means that there is a current flow whenever and wherever there is a potential difference between the junctional elements, in the present case between sensory cell and nerve endings. Very little is known about the neurohumoral transmission from sensory cells to nerve endings. If we assume similar mechanisms, as in interneural synapses, transmission will occur only if there are potential changes in the presynaptic cell. However, where extensive electrical connections are present, a steady current will flow between the sensory cell and the nerve ending as long as there is a difference between the receptor potential in the sensory cell and the resting potential in the nerve ending. In this way there will be constantly renewed generator potential within the peripheral nerve fiber branches, giving rise to action potentials at the initial segment. The synaptic neurohumoral junction between sensory cell and nerve endings, on the other hand, will come into action only when there is a change in the receptor potential in the sensory cells undergoing active stimulation.

Such considerations are, however, still hypothetical until more information on electrical transmission in the vestibular sensory receptors is available. But such a dual (electrical and neurohumoral) transmission system between sensory cells and nerve endings could help to explain many functional features of the vestibular sensory epithelia.

A very primitive scheme might illustrate this point. As already suggested by Lowenstein (ref. 10), we might compare the sensory-cell nerve ending unit with a triode (fig. 9). The anode current depends basically on the grid voltage and on the number and size of the grid holes. The sensory cells would be the cathode,

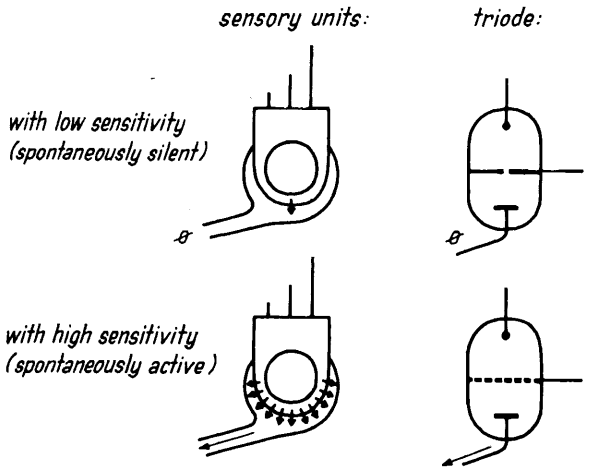

Figure 9.—*The sensory units (left) are compared with electronic tube triodes (right). Upper line: sensory cell with few synaptic connections with the nerve chalice corresponds to a triode with a very small number of grid holes. If there is a potential difference between sensory cell and nerve ending (cathode and anode), there is very little current flowing from the sensory cell to the nerve ending (cathode to anode). Such a sensory unit would be considered of low sensitivity. Lower line: sensory cells with a great number of synaptic and electric contacts with the nerve ending would correspond to a triode with a great number of grid holes. In this case the current flow from sensory cell to nerve ending (cathode to anode) would be much easier and greater if there is a potential difference between sensory cell and nerve ending (cathode and anode). Such a sensory unit would be considered to be of high sensitivity.*

the nerve ending the anode, and the junctional membrane the grid, whereas the synapses and especially the electrical contact areas would correspond to the grid holes. That the behavior of the sensory units can be changed by polarizing dc currents has been demonstrated by Lowenstein. We wonder, however, if the size and number of synapses or electrical contacts could also influence the behavior of the sensory units. The more or larger holes in the grid of a triode at a given grid voltage, the easier is the current flow from cathode to anode. For the sensory cell, it would mean that a great number of synapses or electrical contacts would allow an easy current flow from the sensory cell to the nerve endings and therefore induce a spontaneous activity or facilitate the transmission from the sensory cell to the nerve endings,

in general increasing the sensitivity of such sensory units.

The ramifications and connections of the unmyelinated nerve fibers in the vestibular sensory epithelia are doubtless of primary importance for the integration and coding of the neuronal message in the vestibular nerve. The number of nerve endings and synaptic or ephaptic contact of a particular nerve fiber of the vestibular nerve and the sensory cells is most probably related to the threshold of that fiber. All synaptic and ephaptic transmissions in the ramifications of one sensory unit will comprise the final generator potential which will then trigger off the action potentials at the initial segment of the nerve.

At this level the efferent innervation of the vestibular sensory epithelia becomes involved. The existence of efferent vestibular fibers in the periphery has been shown by Gacek and Rasmussen (ref. 13). Nomura et al. (ref. 14) and others were able to demonstrate acetylcholinesterase activity within the vestibular sensory epithelia.

In electron-microscope investigations, vesiculated nerve endings have been found in the vestibular sensory epithelia of all animals and humans examined to date. Their structural similarity to the known cochlear efferent nerve endings makes it most likely that they really represent the peripheral terminations of the vestibular efferent fibers, although the direct proof is still lacking (fig. 10).

We studied the vestibular sensory epithelia in two cats in which, more than 1 year previously, the entire VIIIth nerve had been cut, doubtless including the vestibular efferent fibers. The vesiculated nerve endings in the vestibular sensory epithelia were still present, however, unlike those in the organ of Corti where they had completely disappeared. Any origin of those vesiculated nervous structures other than through the VIIIth nerve is hardly conceivable. We must, therefore, assume that the efferent terminations in the vestibular sensory epithelia do not degenerate after transection of their axons in the internal acoustic meatus. This is perhaps not so unbelievable as it might seem, since we found a similar survival of the

Figure 10.—*A group of nerve endings at a type II hair cell* (H). *An efferent nerve ending is seen at* E *filled with synaptic vesicles which show a particular concentration close to the presynaptic membrane. Adjacent to this efferent ending, two afferent nerve endings* (A) *are present.*

peripheral *afferent* dendrites in the organ of Corti after transection of the cochlear nerve.

Nevertheless, the structural characteristics allow us to classify these vesiculated endings as efferent. However, as mentioned earlier, the closeness of vesiculated elements to other nerve fibers or sensory cells cannot be taken as evidence of their functional interaction. Only presynaptic agglomerations or condensations of vesicles eventually associated with a thickening of the postsynaptic membrane and some dense material seen under the electron microscope in the synaptic cleft provide direct evidence of synaptic contact. Efferent endings are found in great numbers in such a synaptic contact with the type II hair cells and also to a lesser extent with the nerve chalice of afferent nerve fibers from type I hair cells (figs. 3 and 4). There seem to be considerable differences among different animals. Whereas synaptic contacts be-

tween efferent endings and afferent nerve fibers are quite common in cats, they are extremely rare in monkeys where the great majority of the efferent endings are found in connection with the type II hair cells. As compared to the internal hair cells of the organ of Corti, the efferent terminal branches enter in synaptic contacts with several different sensory cells or nerve fibers as was observed by Iurato and Taidelli (ref. 15) in the rat.

Whereas the efferent nerve endings have direct connection with type II hair cells, they never enter in direct contact with the type I hair cells. Here they show only synaptic connections with the nerve chalice or afferent dendrites (fig. 10). From this structural point of view it would seem that they have a direct influence on the type II hair cell in the sensor of presynaptic inhibition, whereas in the case of type I hair cell they exert their action only on the nerve chalice or dendrites in the sense of a postsynaptic inhibition. In this way undoubtedly the efferent fibers influence considerably the formation of the generator potential in the afferent dendrites. All electrical activities of the dendrites induced at the synaptic or electric junctions with the sensory cells will be added to and integrated with the inhibitory efferent impulses, resulting in the final generator potential which triggers off an action potential at the initial segments of the neurons if the threshold is reached.

A final modification of the neuronal message in the vestibular nerve might come from the adrenergic innervation of the vestibular sensory epithelia which we were able to demonstrate histochemically, using the method of Falk and Hillarp, and which appeared to be independent of the blood vessels (ref. 16).

PATHOLOGICAL ASPECTS OF THE VESTIBULAR EPITHELIA

With the intention of finding out whether the ultrastructural organization of the gravity receptors becomes altered under exposure to high levels of gravitoinertial force comparable to what could be experienced by astronauts during launch and reentry, we exposed 11 squirrel monkeys to 5.4 g or 10.9 g on the centrifuge in Pensacola for up to 10 minutes in different head positions (ref. 2). Although some of the monkeys manifested marked ataxia for hours following exposure, the ultrastructure of the maculae was not altered in any of the animals. The poststimulatory ataxia thus cannot be attributed to end organ changes. It might have its origin in disturbances of the central nervous system. The fact that exposure to 10 g did not affect the ultrastructural appearance of the vestibular sensory epithelia is actually not too surprising when we consider the enormous increase of acoustic energy needed to produce the first visible changes in the cochlear receptor. Whereas in our present experiment we were dealing with a tenfold increase in the normal stimulation energy, we need about a 1-million-fold increase to produce a stimulation damage in the cochlear receptor.

However, in a report by Vinnikov and Titova (ref. 17) they describe ultrastructural changes in the vestibular sensory epithelia after repeated exposure to high g. This of course would be of utmost significance in future space flights.

At the ultrastructural level it is always an extremely difficult task to decide what changes are significant and specifically due to the experimental conditions used. Very little is known in general about the ultrastructural pathology of the vestibular sensory epithelia. In the present study we tried, therefore, to analyze possible pathological aspects of the vestibular sensory epithelia under normal and experimental conditions.

In the vestibular sensory epithelia of apparently normal animals, a certain number of structural variations can be found which could be taken at first sight as pathological changes.

First of all, the cytoplasm of the sensory cells shows a great variation in density. In the cat it is usually denser than that of the supporting cells and always contains a large number of ribosomes, a well-developed endoplasmic reticulum, and infranuclear rough ergastoplasma membranes (figs. 11 and 12). In certain cells, however, it appears extremely dense, frequently associated with an extensive vacuolization (fig. 13). Between vacuoles and mitochondria the cytoplasm is packed with ribosomes and larger granules which probably are condensations of

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Figure 11.—*Detail from a hair cell with a very dense cytoplasm* (C) *which contains a great number of ribosomes and some larger dark granules which might be agglomerations of ribosomes. Large vacuoles* (V) *are usually found in the cytoplasm of those cells next to the mitochondria* (M) *and endoplasmic reticulum. Nucleus,* (N).

ribosomes (fig. 11). This signifies either that we are in fact dealing with many different kinds of sensory cells, or what is more likely, that the sensory cells present different functional states.

Cytoplasmic protrusions from the surface of the sensory cells are regularly observed but they are of moderate size. It is noteworthy that the nuclei of normal sensory cells always present a rather evenly distributed chromatin without a pronounced nucleolus. The latter is much more pronounced in the supporting cells.

In contrast to the sensory cell cytoplasm, there are no ribosomes in the nerve chalices or other nerve endings (fig. 12).

The mitochondria in the nerve chalices appear in different forms. Among a majority of normally appearing mitochondria with a fairly dense proper substance, some mitochondria are blown up like balloons with a very light substance around the tubular-shaped cristae (fig. 12). Such regularly observed variance in the appearance of the mitochondria might also be an expression of different functional states of the mitochondria. For instance, Packer (ref. 18) described a partly reversible mechanism of swelling and shrinking of mitochondria in correlation with oxidative phosphorylation. It is striking, however, that swollen mitochondria are seen exclusively in nerve endings and fibers. The reason for this might be the lack of ribosomes in the nervous structure which means that here no protein synthesis is taking place. Whether such swelling always corresponds to a reversible functional state or whether such mitochondria are worn out and doomed to disappear remains an open question. Myelin figures such as in figure 12 certainly suggest that some of such swollen mitochondria are going to be destroyed.

Figure 12.—*Type I hair cell* (H) *with infranuclear ergastoplasma membranes* (E). *In the nerve chalice* (C) *there are large blown-up mitochondria* (M) *with a very light ground substance next to normally appearing dense mitochondria* (m). *Myelin figures like the one seen at* (X) *might indicate that some mitochondria degenerate under normal conditions. It is also interesting to note that there are almost no ribosomes in the nerve chalice, but a great number of them are present within the cytoplasm of the hair cell.*

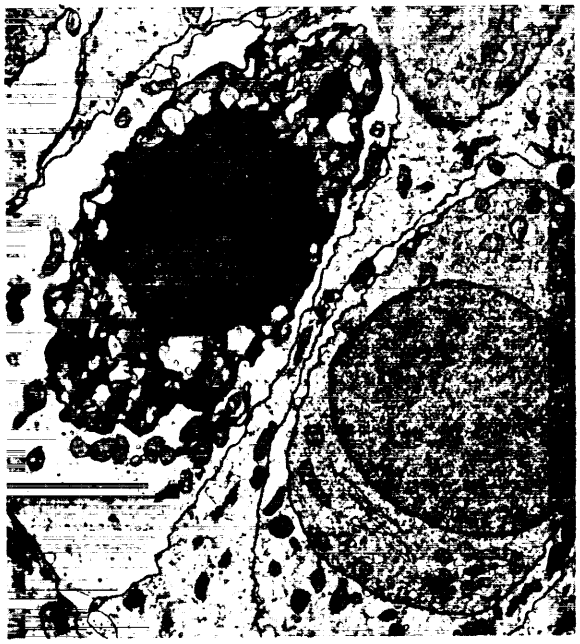

Figure 13.—*Two type I hair cells of the macula of a normal cat. The right cell has a normal appearance, whereas the left one shows an extremely dense cytoplasm with extensive vacuolization and chromatin changes in the nucleus.*

To elucidate further the question of possible pathological changes in the vestibular sensory epithelia, we tried to create reproducible pathological changes under well-defined simple experimental conditions in cats: In all cases the vestibular sensory epithelia were fixed and prepared under identical conditions.

Three different experiments were carried out:

(1) Under general anesthesia the trachea from each of two cats was clamped and the vestibular epithelia were taken out in the usual way after death of the animal.

(2) In another two cats the vestibulum was exposed and the utricle or the lateral ampulla was carefully opened. A physiological solution of sodium chloride was repeatedly injected with a fine needle into the endolymphatic space for 15 minutes. Thereafter the animals were sacrificed and the vestibular sensory epithelia fixed.

(3) In a third group, three cats received one-half gram of streptomycin sulfate injected into the bulla. After a few hours they showed the first signs of vestibular imbalance and nystagmus to the opposite side. In two cats those symptoms were rather mild, but the third presented a violent vestibular reaction. The reason for this unusually strong reaction turned out to be a small defect in the round window membrane, which had been made incidentally with the injecting needle, so that the streptomycin could freely diffuse into the labyrinth.

The results were as follows: As a consequence of acute asphyxia no really significant changes could be found in the vestibular sensory epithelia. The normally observed structural changes, such as a vacuolization of the cytoplasm in certain sensory cells, were perhaps somewhat more pronounced, especially in the lateral crista. In all cases the latter showed the most pronounced pathological alterations of all the vestibular sensory epithelia.

The injection of physiological sodium chloride solution into the endolymphatic space caused peculiar pathological changes, however. The normal regular row of the nuclei of the supporting cells is disrupted mainly in the basal portion of the vestibular sensory epithelia. The extracellular space is greatly extended, separating the supporting cells and nerve fibers from each other (fig. 14). In some places the plasma membranes of the supporting cells are disrupted. The nuclei are sometimes pyknotic or disintegrated. On the other hand, the sensory cells with their nerve endings appear quite normal in most instances. Their nuclei show the normal, even distribution of chromatin. In some places, where the extended intercellular spaces are very marked and reach high up, they might be deformed and pushed aside or a nerve chalice might be directly affected by the swelling (fig. 15). At such places the protoplasmic protrusions from the sensory cell surface are larger than normal, frequently incorporating the kinocilium whose circularly arranged peripheral filaments are clearly visible at several places within the protrusions

Figure 14.—*Part of a macula utriculi of a cat injected with physiological sodium chloride solution into the endolymphatic space. The great extension of the extracellular space* (E) *in the basal portion of the sensory epithelium is striking. The supporting cells* (S) *are irregularly arranged and pushed aside by the greatly extended extracellular spaces. Some of them show pyknotic nuclei* (P). *The hair cells* (H), *on the other hand, have an entirely normal appearance. At* (C) *is a capillary in the subepithelial tissue.*

(fig. 16). The basement membrane of the sensory epithelium, however, remains intact in all instances (fig. 17).

The pathological condition produced by injection of the physiological sodium chloride solution into the endolymphatic space is best characterized as an interstitial basal edema of the vestibular sensory epithelia. The change in the endolymph might not be the only cause for the pathological alteration in the sensory epithelium, however. The mechanical stress imposed during injection of the fluid into the utricle or the ampulla might also have been a contributing factor. In any case, such changes are certainly of interest in Ménière's disease. According to Schuknecht, the attacks of vertigo are caused by rupture of the endolymphatic membranous labyrinth, a condition similar to that produced in our experiments.

From the acute streptomycin intoxication produced by injection of the drug into the bulla, we intended to learn something about the initial changes produced by this ototoxic agent. In chronic intoxication experiments it is very difficult, if not impossible, to decide whether the observed pathological changes are primary or secondary effects of the toxic agent (ref. 19). By injecting the drug into the bulla we expected to get higher concentrations of streptomycin in the labyrinth without general intoxication of the animal. Nonspecific reactions of the labyrinth to this procedure must also be taken into consideration.

In our animals that showed a mild vestibular reaction, the crista epithelium appeared at first sight under phase-contrast microscopy to be normal, with a normal population of regularly arranged sensory and supporting cells. A closer look reveals, however, somewhat irregular nuclei in a number of sensory cells (fig. 18). The electron-microscope picture confirms this suspicion. The type I hair cells mostly show

Figure 15.—*Part of a crista in a cat injected with physiological sodium chloride solution in the endolymphatic space. In this case the damage to the sensory epithelium is more pronounced. The great extension of the extracellular space* (E) *is again very obvious and also some nerve chalices* (C) *appear to be greatly swollen. The major pathology here lies predominantly in the basal portion of the sensory epithelium. The hair cells* (H) *are grossly deformed by the extension of the extracellular spaces, but otherwise they show no typical alterations.*

very distinguishable pathological changes of their nuclei and cytoplasm, whereas the type II hair cells are clearly less affected, and the nerve endings and supporting cells appear to be entirely normal (fig. 19). It is just the opposite picture in the cristae where sodium chloride was injected in the endolymphatic space and the supporting cell primarily affected. The most outstanding pathological feature in the sensory cell is what is classically called a pyknotic nucleus. The chromatin is very unevenly distributed, being concentrated into dense clumps in certain spots and almost entirely lacking in others. The nuclear membrane is still intact in spite of the fact that some chromatin masses are found within the cytoplasm of the cell, expulsed from the nucleus (fig. 20). The cytoplasm itself exhibits a marked vacuolization which in a restricted number of cells could not be considered to be specifically pathological. However, since it appears in all type I hair cells of the cristae in acute streptomycin intoxication, it can be considered as a consequence of the experimental intoxication. The same thing is true for the mitochondria which show a spotty clearing of their substance.

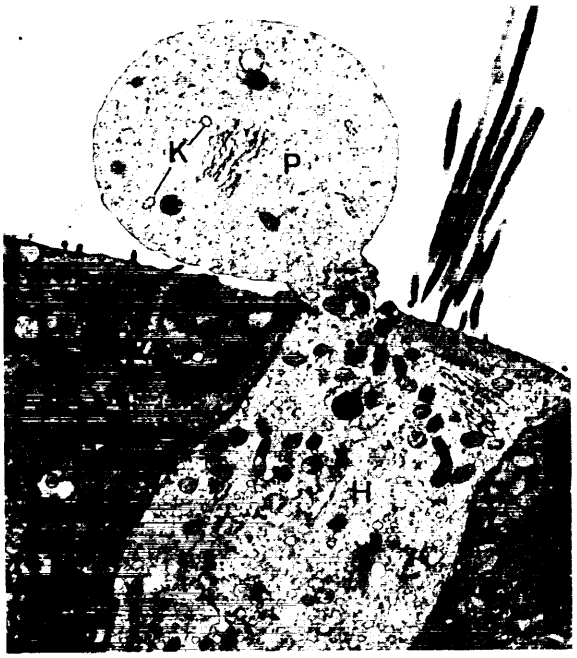

Figure 16.—*An apical protoplasm protrusion* (P) *from the surface of a hair cell* (H) *following NaCl injections into the endolymph. The protoplasm protrusion seems to include some kinociliar structures* (K) *clearly visible as the typical arrangement of nine peripheral filaments.*

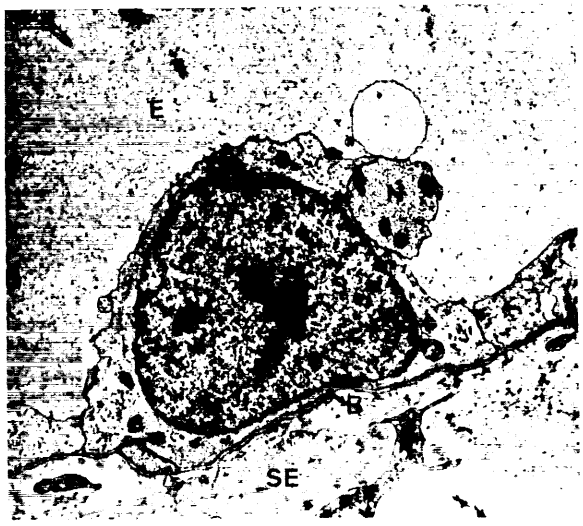

Figure 17.—*The basal portion of a macula from an animal injected with NaCl into the endolymph. The basement membrane of the sensory epithelium* (B) *remains intact in spite of the great extensions* (E) *of the extracellular space in the sensory epithelium. The supporting cells* (S) *are almost entirely disconnected from their surroundings and also the nerve fibers* (S) *run partly free through the extracellular space. Underneath the basement membrane is the subepithelial space* (SE).

Although one gets the impression that the nuclear changes are especially marked, the pathological alterations are already too fully developed to distinguish between the primary and secondary effects of the streptomycin on the cell.

The observation already made by Wersäll and Hawkins (ref. 19) and by Igarashi et al. (ref. 20) that macular sensory epithelia are more resistant to streptomycin intoxication proved to be true also in our cases. The sensory epithelium of the macula utriculi of our same animals presented much milder changes which might bring us closer to locating the initial points of attack by streptomycin on the sensory cells. An inexperienced observer would not find much wrong with these sensory epithelia where all cells, nerve fibers, and supporting cells seem to be in good shape. However, if we compare this epithelium with a normal one where the nuclei

Figure 18.—*Phase-contrast picture of part of a crista epithelium in a cat which had received one-half gram of streptomycin sulfate into the bulla 24 hours previously. The sensory epithelium has a normal appearance with the exception of some irregular nuclei* (N) *of some sensory cells. The supporting cells* (S) *are regularly arranged and appear to be normal.*

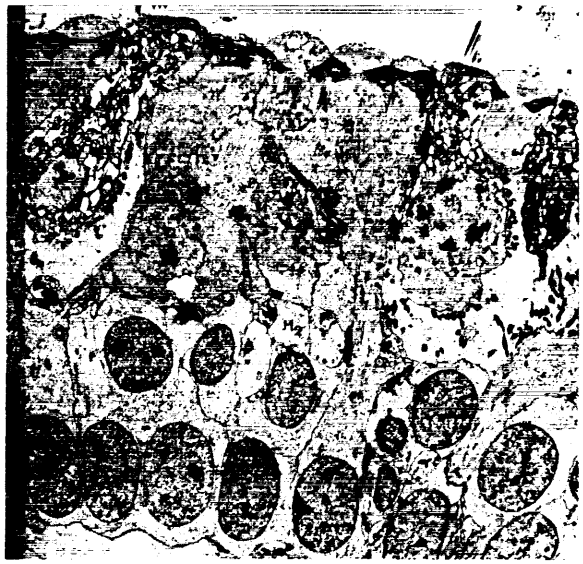

Figure 19.—*Electron-microscopic picture of the same crista as seen in figure 18. Mainly, the type I hair cells* (H_1) *show pronounced changes in form of a pyknotic nucleus and a great vacuolization of the cytoplasm. Type II hair cells* (H_2) *are much less affected, and the supporting cells* (S) *as well as the nerve endings appear to be normal.*

of all sensory cells show a very even chromatin distribution and where the cytoplasm is usually somewhat denser than the surrounding cells, we recognize immediately the alteration in the streptomycin-treated animals. In these animals the nuclei of type I hair cells show a clear clodding of their chromatin as opposed to the nuclei of type II hair cells and the supporting cells which appear to be entirely normal. The cytoplasm of the type I hair cells, on the other hand, no longer contains any ergastoplasmic membranes and is very light but otherwise exhibits no striking pathological features (figs. 21 and 22). At higher magnification it becomes obvious that there is a clear reduction in number and visibility of the ribosomes in the cytoplasm of those cells as compared to the great number of them in normal type I hair cells (fig. 23 *A* and *B*). In the type II hair cells, however, no reduction of ribosomes can be observed (fig. 24). At this early stage in streptomycin intoxication, no other significant changes than the alteration of nuclei and ribosomes in type I hair cells can

Figure 20.—*Detail of a type I hair cell* (H) *from the same crista as represented in figures 18 and 19. The nucleus* (N) *shows a very uneven chromatin distribution. Some of this chromatin seems to be extruded from the nucleus into the cytoplasm of the cell* (X). *The cytoplasm is greatly vacuolized, and the mitochondria show a spotty clearing of their substance. The nerve chalice* (C) *presents no major changes.*

indeed be found in the macular sensory epithelium.

Much more dramatic are findings in the vestibular epithelia of the animals where the streptomycin could directly penetrate the labyrinth through small defects in the round window membrane (fig. 25). In such massive intoxication, most of the sensory cells are completely degenerated, and little protection is given to type II hair cells. The nuclei of the supporting cells then are affected also, but to a lesser degree. However, the nerve endings and fibers still look fairly good in spite of the fact that their associated sensory cells are in a state of disintegration.

The present study provides us with the following information: streptomycin, which approaches the sensory epithelia from the perilymphatic spaces, affects primarily the type I hair cells. Type II hair cells and especially the supporting cells are more resistant. This confirms similar observations of Wersäll and Hawkins (ref. 19), who found that only a few type II hair cells survived after chronic intoxication and that the type I hair cells had completely disappeared from the crista epithelium. The supporting cells were not affected at all.

A massive acute intoxication, however, appears to affect also the type II hair cells and to a certain extent the supporting cells.

The structures primarily damaged appear to be the nuclei, the ribosomes, and the rough endoplasmic reticulum, all structures involved in the protein metabolism of the cell. Other changes, such as a vacuolization of the cytoplasm and mitochondrial damage, are most likely to be secondary and nonspecific.

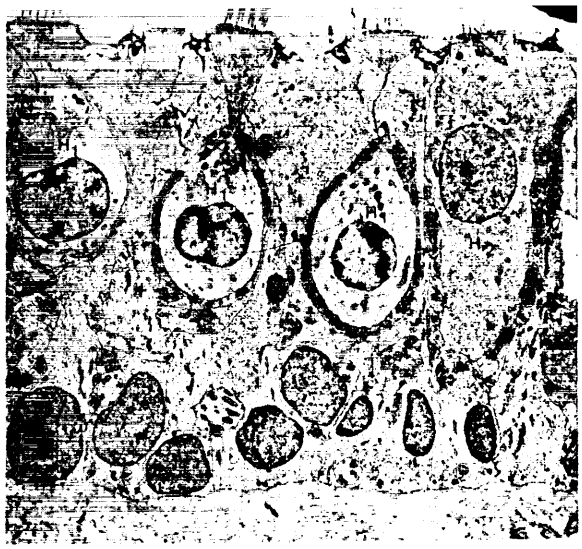

Figure 21.—*View of a macula of a cat which had received one-half gram of streptomycin into the bulla 24 hours previously. At first sight the sensory epithelium has a normal appearance. There is, however, a clear clodding of the chromatin of the nuclei of hair cells type I* (H_1), *whereas the nucleus of the type II hair cells* (H_2) *has normal chromatin distribution. In addition, the cytoplasm of the type I hair cells appears unusually light as compared with the surrounding supporting cells.*

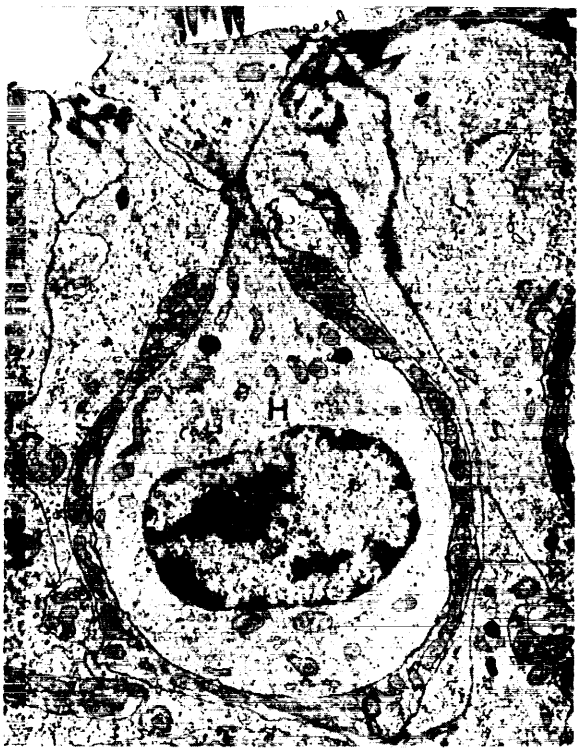

Figure 22.—*One hair cell of type I* (H) *of the same macula as in figure 21. Clear clodding of the nucleus and very light cytoplasm which contains very few granules as compared with the surrounding supporting cells. No infranuclear ergastoplasma membrane is found.*

The antibacterial action of streptomycin is fairly well known from microbiological studies (refs. 21 and 22). In the bacterial cell, two things seem to be affected by streptomycin: (1) the ribosome functions, in other words the protein synthesis; and (2) the membrane permeability, the alteration of which could be caused by this effect on protein synthesis.

Thus, the resistance to the antibiotic can be due either to the impermeability of the cell membrane to streptomycin or an inherent resistance in the ribosomes against streptomycin. Experimental evidence for both mechanisms could be found. Brock (ref. 22) presents the hypothesis that in the cell membrane and in the ribosomes, the same protein component is present which is responsible for resistance or sensitivity to streptomycin.

On a basic working hypothesis, there is no reason to believe that streptomycin affects the mammalian cell in any way basically different from the way it affects the bacterial cells. However, much higher concentrations are needed. In our acute intoxication experiments we have provided evidence that, like the bacterial cell and the type I hair cells of the vestibular sensory epithelia, the nuclear-ribosomal system primarily is affected by streptomycin. The penetration of streptomycin into the cell is, of course, a prerequisite, although we could not find ultrastructural changes in the cell wall. In the initial stage of intoxication the overall permeability of the cell wall seems not to be changed very much, however, since such membrane alterations would certainly lead to much more dramatic changes within the sensory cells. In the later, secondary phase of intoxication, such permeability changes certainly occur and lead to the disintegration of the cell.

The relatively higher resistance of mammalian cells in general to streptomycin intoxication seems to be due to the impermeability of their plasma membrane. Streptomycin-sensitive micro-organisms are not affected when they are intracellular. The specific sensitivity of the type I hair cells to streptomycin intoxication might, therefore, be due to a greater permeability of their plasma membrane to streptomycin.

The ototoxicity of streptomycin does not result only from its high concentration in the endolymph when it is administered intramuscularly, as was found by Rauch et al. (personal communication). There seems to be a selective sensitivity of the vestibular sensory cells.

The experimental pathology of the inner-ear sensory epithelia is still in its initial state. Well-defined experimental conditions with consecutively reproducible pathological changes might give us valuable information about the behavior of the vestibular sensory epithelia. They might aid in providing us with a basis for understanding the effects of more complex situations such as unusually strong accelerations or prolonged weightlessness.

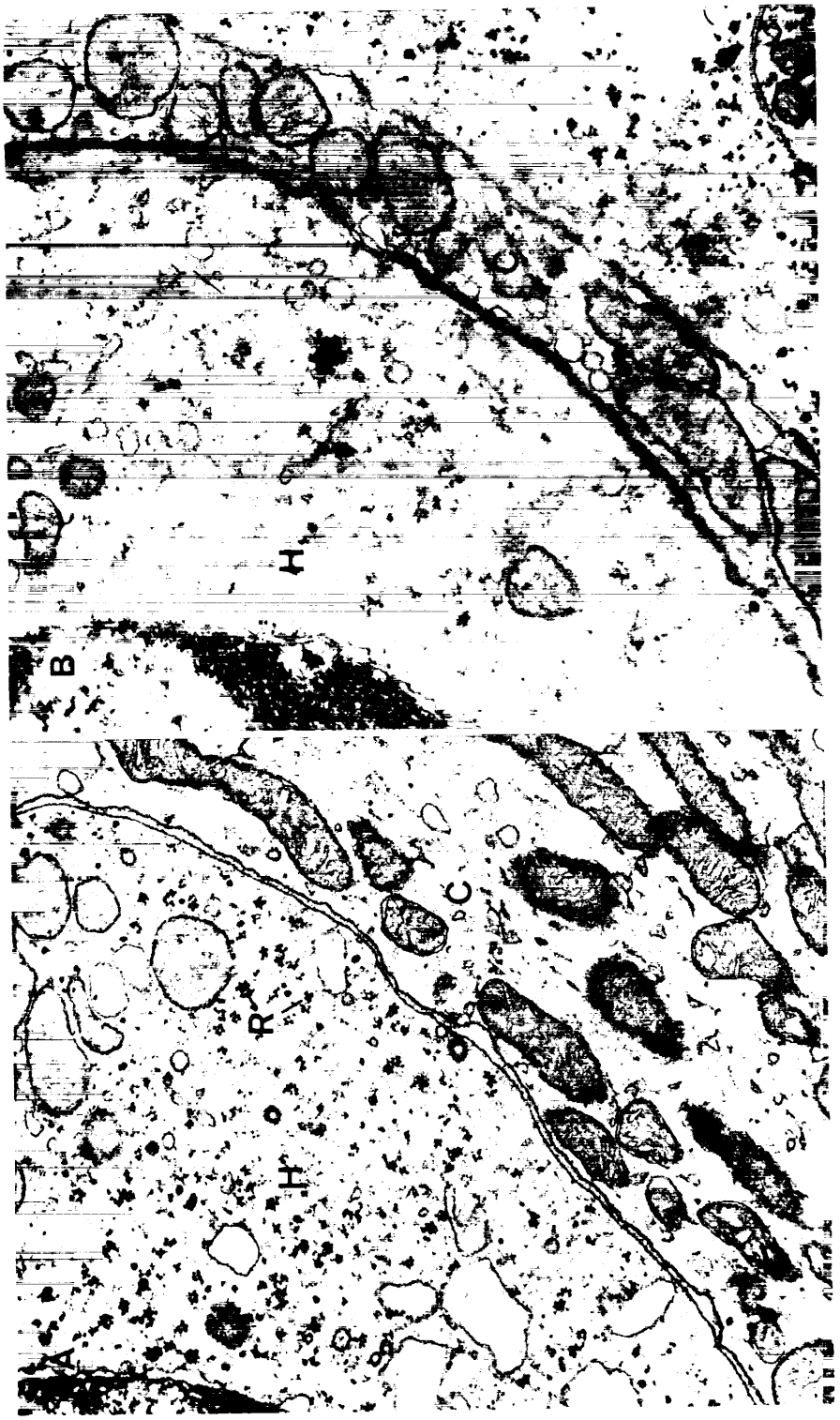

Figure 22.—*A. Cytoplasm of a normal type I hair cell* (H) *with numerous ribosomes* (R) *which are not present in the nerve chalice* (C).
B. Hair cell of type I (H) *from a macula utriculi of an animal which received streptomycin in the bulla. There is a clear reduction of ribosomes in the cytoplasm of this hair cell as compared with the normal ones.*

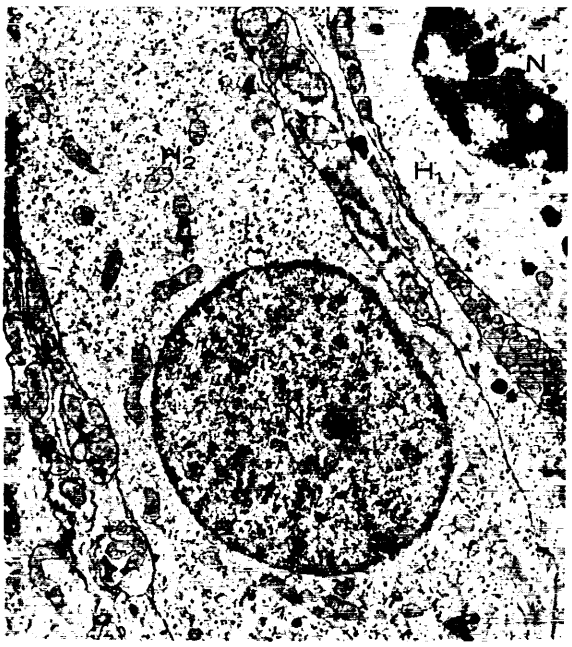

Figure 24.—*Part of a hair cell type II* (H_2) *of an animal which received streptomycin into the bulla. The nucleus* (N) *shows a normal, even chromatin distribution, and there is a great number of ribosomes in the cytoplasm of those cells. The difference from type I hair cells is clearly visible at the upper right corner where a part of a type I hair cell* (H_1) *is seen with some of its nucleus.*

Figure 25.—*Part of the macula utriculi of a cat in which free diffusion of streptomycin into the perilymphatic spaces had occurred. Great cellular damage is seen in both hair-cell types I and II* (H_1 and H_2). *The nucleus seems to be entirely disintegrated in a number of hair cells, and the cytoplasm is greatly damaged. Not much difference is visible between hair cells of type I and hair cells of type II. Even the nuclei of the supporting cells show clear alterations in the sense of clodding of their chromatin. Only the nerve endings and nerve fibers appear to be fairly normal.*

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DISCUSSION

LOWENSTEIN: I was fascinated by your hypothesis about the function of the ephaptic structures which you find in the type I hair cells, and if I understood you right, you think that the leakiness of the membrane which is responsible for the spontaneous basic activity may be due to these "ephaptic" synapses. Hitherto, of course, one blamed chemical synapses for this leakiness. We are told that parcels of acetylcholine, or whatever transducer, go out into the nerve dendrite. When I saw your pictures, however—this is not a criticism—it struck me that if these things are ephaptic, could they not play the role of recurrent collaterals? That is to say, conduct from the calyx into the cells in accordance to the excitation running down the calyx membrane. You see, this is an alternative explanation which would also have its parallels in other systems.

SPOENDLIN: This question should actually be answered by electrophysiologists. The ultrastructure so far does not tell us in which direction electrical transmission does occur. In lower animals some of the electrical synapses have been found to be unpolarized, conducting both ways, and others polarized allowing only one-way transmission. At the present time such electrophysiological information is not available for the vestibular sensory epithelium.

WERSÄLL: Even after seeing Dr. Spoendlin's beautiful pictures of the junction areas in the nerve chalice, it seems clear that we have to study these areas with various staining solutions and embedding media to make it clear whether or not there exists a true junction of the opposing layers of the cell membranes.

The studies on the effect of streptomycin injected intramuscularly or subcutaneously on vestibular sensory cells by Dr. Hawkins and me and Dr. Duvall and myself did not give the same results as those presented here by Dr. Spoendlin. According to his findings, streptomycin inhibits the synthesis of the stable factor of the cell membrane. This results in merging of the sensory hairs, disappearing of the hairs and bulging of the cell surface into the endolymph. The cell finally is pushed into the endolymph and disappears. Kanamycin, on the other hand, will, in guinea pig, cause an early degeneration of ribosomes and nucleus in the hair cells of the organ of Corti. A similar difference between locally injected and parenterally administered antibiotics has also recently been found in studies by Dr. Arstila and myself on the effect of neomycin on the olfactory epithelium. These will be published soon.

SPOENDLIN: Relying on what is known today, it might be important to know whether we deal with true fusions of the presynaptic and postsynaptic membranes. The only known morphological evidence so far for electrical transmission has been true fusion of the junctional membranes in the sense of external compound membranes. Different fixation or staining techniques bring out the unit membranes differently. When we see the outer leaflets of the unit membranes merge to one single layer, the so-called fusion line, we consider such places as external compound membranes. The overall thickness of one unit membrane in osmium-fixed, uranyl acetate-stained material appears to be between 50 and 70 Å. At areas with external compound membranes, the overall thickness of the fused membranes of sensory cell and nerve chalice is between 100 and 120 Å, which indicates that even when the outer leaflets of the unit membranes and the fusion line are not clearly visible, we would deal with a real

fusion of the two membranes. However, we cannot exclude for sure that such membrane fusions are some sort of artifact.

As far as the streptomycin effect is concerned, it was our aim to study the acute intoxication of this drug. Recent microbiological studies show that many toxic effects might be secondary to the primary point of attack which appears to be at certain steps in the protein synthesis of the cell. With acute massive intoxication we hope to find the most likely primary effects of streptomycin on the cell. Since the animals (guinea pigs and cats) do not tolerate large doses of streptomycin parenterally, we resorted to the application in the bulla. It might very well be that this local application provides different results from general intoxication by subcutaneous or intramuscular application of the drug.

SMITH: I would like to add a word to what Dr. Wersäll has said about the membrane fusion of the synapse because of what I had said in a prior discussion. Perhaps there are membrane fusions there. They may well be. But if they are present, I think one will find that there is some difference between these and membrane fusions elsewhere, because one can regularly demonstrate membrane fusions between supporting cells in the sensory areas. But, even in the same section, there is no evidence for fusion at the synapse.

SPOENDLIN: I certainly agree that similar membrane fusions are found among almost all epithelial cells. Such places are known as zonulae occludentes. So far we have not been able to find significant distinctive features between those zonulae occludentes and the external compound membranes of hair cells and nerve chalices in the vestibular sensory epithelia.

SMITH: It may be something that will only be brought out by certain kinds of fixatives.

SPOENDLIN: That's correct. As you know, Robertson studied his external compound membrane mainly with a special stain and a special potassium manganate fixative. He was also able, however, to demonstrate it less clearly with osmium fixation. He found a characteristic pattern in those junctional membranes, possibly related to electrical transmission.

HAWKINS: I have always been beset by a lingering doubt about the streptomycin experiments in which one instills the solution into the bulla, and that is whether we are really observing the same ototoxic effect that one gets when one administers it parenterally. It has long been known that hypertonic salt solutions instilled into the bulla will reduce the microphonic activity of the inner ear simply by their hypertonic effect, and it has also long been known that drugs of the streptomycin group have great difficulty in crossing certain biological membranes, which is presumably why they are not absorbed from the gut. I wonder whether this is simply a hypertonic effect or whether it is true streptomycin effect. Can Dr. Spoendlin reassure me on this point?

SPOENDLIN: This, of course, is an important point. We based our experiments mainly on earlier similar studies of different authors on the light-microscopic level.

HAWKINS: The question is whether we get the same effect with the hypertonic salt solution. I wonder if you had a control for that?

SPOENDLIN: The only control consisted of the injection of 50 percent sucrose or 25 percent mannitol in the bulla which showed no effect on the ultrastructure of the vestibular sensory epithelia.

SESSION III

Chairman: **ERIC OGDEN**
Ames Research Center, NASA

Cochairman: **G. RICHARD WENDT**
The University of Rochester

Anatomical Aspects on Functional Organization of the Vestibular Nuclei

A. BRODAL
University of Oslo, Norway

SUMMARY

A review is given of some of the many features in the anatomical organization of the vestibular nuclear complex of the cat, including some new, hitherto unpublished, observations on synaptic arrangements within the nucleus of Deiters.

Histological and topographical analyses of the vestibular nuclei show that in addition to the four classical vestibular nuclei (superior, lateral, medial, descending or inferior), the complex includes a number of minor cell groups. Within each of the four main nuclei there are regional differences in cytoarchitecture. These morphological differences suggest that there are functional differences between parts of nuclei and between cell groups. This suggestion is strongly supported by analyses of the efferent and afferent fiber connections of the vestibular nuclei. These connections have been studied experimentally by means of the modified Gudden method for mapping of the origin of fibers, with the Nauta method for the determination of sites and modes of endings of fibers.

The various contingents of afferents all have their restricted distribution within the vestibular nuclei. Thus in the lateral nucleus of Deiters, primary vestibular fibers end in the rostroventral part only. In a corresponding manner the sites of origin of the various contingents of efferent fibers are more or less circumscribed. For example, fibers to the spinal cord come only from the lateral and medial nuclei. The nucleus of Deiters, its projection to the spinal cord, and the pathways from the cerebellar vermis to the nucleus show a somatotopical pattern. The primary vestibulocerebellar fibers have a wider distribution within the cerebellum than known previously. They end as mossy fibers, which appear to differ in some respects from the classical type of mossy fibers.

In the lateral vestibular nucleus of Deiters, afferents from various sources differ with regard to their relation to the nerve cells. In Nauta sections it is found that some fibers, for example, the primary vestibular afferents, end on small cells, while others, for example, those from the cerebellar cortex, contact chiefly large cells. Most afferents end on somata and dendrites. In Golgi sections it is seen that fibers on the surface of a cell may climb for a considerable distance along a dendrite. Electron micrographs show that synaptical contacts are established between such fibers and the dendrite. Synapses are also formed with the spines of the dendrites. Degenerating boutons can be seen in electron micrographs following experimental lesions of afferent fiber systems. In this way it has been established that the afferents from the anterior vermis establish synaptic contacts with somata and smooth parts of dendrites, as well as with very thin dendrites and with spines. It appears from Golgi sections that cells which may be considered as internuncials are present only in the medial and descending nucleus.

The anatomical organization of the vestibular nuclei, just as of the receptor organs, turns out to be extremely complex. Much still remains to be investigated. Even if there is complete agreement on several points between anatomical data and results of physiological experiments, the anatomical data available at present indicate functional differentiations between cell groups and parts of nuclei which go beyond what has so far been clarified in physiological studies. The importance of considering the anatomical differentiations in future functional studies cannot be too strongly emphasized.

INTRODUCTION

The ultimate goal of anatomical studies of an organ or part of an organ is to contribute to an understanding of its function. Structural features may often give valuable hints concerning function, and a proper functional analysis is only possible when the structures involved are known. As far as the vestibular mechanisms are concerned, it is probably justified to say that recent research has demonstrated structural peculiarities and differentiations in the sense organs and their central connections which go far beyond what can at present be properly correlated with observations of function.

The following account will be centered around the anatomical organization of the vestibular nuclei, with emphasis on their fiber connections. Attempts will be made to relate the anatomical data to functional observations. The results to be presented are based largely on experimental studies in the cat, performed in collaboration with colleagues in the Anatomical Institute, University of Oslo. (The subject has been treated more fully in a monograph by Brodal, Pompeiano, and Walberg (ref. 1), and in previous reviews (refs. 2–4).) A brief introductory comment on the experimental methods employed is appropriate.

METHODS OF STUDY

The Vestibular Nuclei

The sites of origin of fibers have been determined by analyzing the occurrence of retrograde cellular changes in cells whose axons have been cut. The modified Gudden method (ref. 5), in which very young animals are employed, has turned out to be advantageous, since the cells in such animals are very susceptible to transection of their axons and react rapidly. Large cells and also small ones can be identified as effected when they present a clear-cut tigrolysis, a peripherally displaced and usually flattened nucleus. Figure 1 shows some examples of altered small cells in the medial and descending vestibular nuclei following a cerebellar lesion. It is important in such cases to record as positive only these cells which present all the criteria, since there are considerable variations between

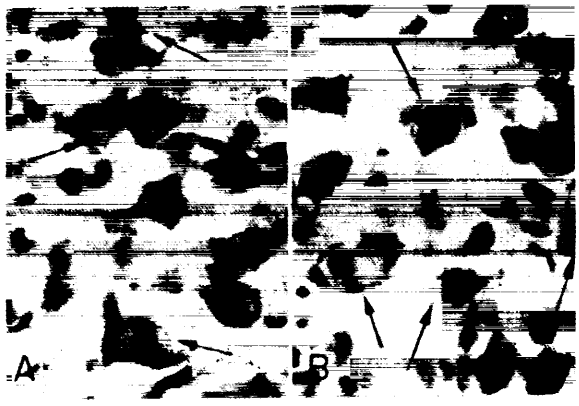

Figure 1.—*Photomicrograph of small nerve cells in the medial* (A) *and descending* (B) *vestibular nucleus of a kitten showing retrograde cellular changes* (arrows) *following a midcerebellar lesion involving the nodulus and fastigial nuclei.* (*From ref. 6.*)

normal cells in their content and distribution of chromatin material. Furthermore, absence of changed cells in a region is no proof that the cells of this region do not send fibers into the transected bundle, since for various reasons (inadvertent survival periods, preservation of collaterals, and other factors) changes may either not occur or may be missed. However, when used critically, the method usually makes it possible to determine the region from which a fiber bundle takes origin, whether the bundle is derived from all the cells in the region, and whether cells of different types, if they occur, contribute.

The course and termination of fibers have been studied by silver methods which impregnate degenerating axons and terminals, chiefly the method of Nauta (ref. 7), and to some extent the method of Glees (ref. 8). With the former, the terminal area can often be clearly seen even with low power as in figure 2*A*, which shows a small cell group x of the vestibular nuclei (fig. 3) following a lesion of the fastigial nucleus. With higher magnifications, fine degenerating particles can be identified as fragments of fibers, and often figures suggesting terminal boutons can be seen on the surface of the cells (fig. 2*B*). So-called "pericellular arborizations" can generally be taken to indicate that fibers encircling a cell make contact with its surface. Degenerating fine fibers can often

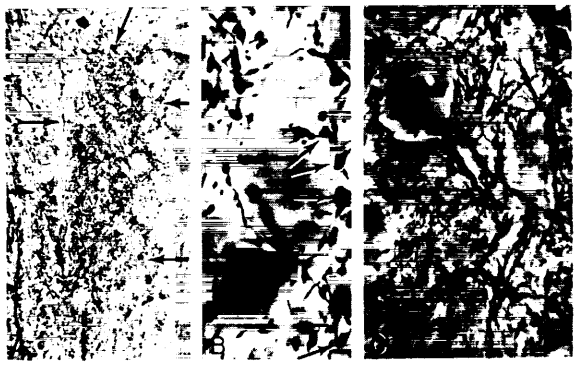

Figure 2.—*Photomicrographs showing degenerating fibers in silver impregnated sections (Nauta method).* A: *Low power view of the group* x *of the vestibular nuclear complex in the cat following a lesion of the contralateral fastigial nucleus. The whole group is filled with degenerating fibers and stands out against the surroundings. To the left, some degeneration belonging to the descending nucleus.* B: *High power view of the same section, showing numerous degenerating particles, some of them suggesting degenerating boutons (some marked with arrows), in contact with nerve cells.* C: *Two cells in the dorsal part of the lateral vestibular nucleus in a cat following a lesion of the ipsilateral fastigial nucleus. Degenerating fibers along the surface of soma and dendrites.* (A *and* B *from ref. 9.*)

be followed along the surface of the soma as well as of dendrites of the cells (fig. 2*C*). Degenerating terminal boutons can be identified with greater reliability with the Glees method, but owing to the lack of contrast in color between the normal and degenerating structures, the search for degenerating particles is far more laborious and time consuming than in Nauta sections. Therefore, the Glees method is chiefly used to check and extend the observations made with the Nauta method.[1] Care must, however, be exerted in deciding that a bouton has degenerated, since there are wide variations in the appearance of normal boutons in silver-impregnated sections. (See, for example, refs. 10 and 11.)

By using these methods it is possible to determine where transected fibers end, whether their terminal ramifications contact cells of different types, and whether they end on soma or cell processes, or both. However, the presence of a contact between a degenerating fragment and the surface of a cell is in itself no decisive proof that this represents a synapse. The final answer can only be obtained in electron microscopical studies. In electron micrographs, degenerating boutons and also details of the types and ultrastructure of synapses may be studied.

Observations made in animals can obviously not be directly transferred to the human brain. However, in some instances corresponding findings have been made in human material, and since the vestibular system is a phylogenetically old one, there is reason to believe that the main principles in the organization of the vestibular nuclei are the same in all mammals including man.

The vestibular nuclear complex is usually considered as being composed of the four classical nuclei: the superior (Bechterew), medial (Schwalbe), lateral (Deiters), and the descending (spinal or inferior) nucleus. However, a close analysis of the cytoarchitecture shows that there are in addition a number of minor cell groups and that various subdivisions may be distinguished within the main nuclei. Figure 3 shows our cytoarchitectonic map of these nuclei in the cat (ref. 12). The small groups are labeled $f, g, l, x, y, z, Sv.$, and finally there is the interstitial nucleus of the vestibular nerve. As to the main nuclei, it will be seen that, for example, the superior nucleus of Bechterew (drawings 3–9 in fig. 3) has larger cells in its center than in its periphery. In the lateral nucleus of Deiters (drawings 7–11), the giant cells are somewhat larger and relatively more numerous dorsocaudally than rostroventrally. The rostral part of the descending (inferior) nucleus contains some fairly large cells (drawings 11–13). In the medial nucleus the cells are generally larger medially than laterally (drawings 13–17). These architectonic features must be assumed to be related to functional differences. They make us suspect that the vestibular nuclear complex is an aggregation of several minor units. This suspicion is strengthened by the observation that the architectonic differences are often paralleled by differences in fiber connections.

[1] It appears that in some instances one of the methods may give results while the other fails.

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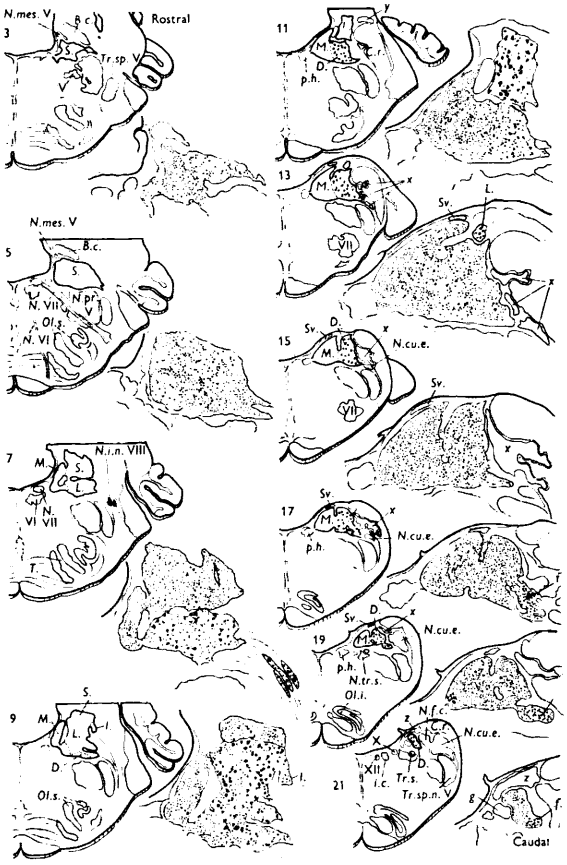

Figure 3.—*A series of equally spaced camera lucida drawings of transverse sections through the brain stem of the cat, to show the topography and (below to the right of each drawing) the chief cytoarchitectural features of the vestibular nuclei. The rings in the descending nucleus represent the longitudinally running fiber bundles within this. Note differences in cytoarchitecture within each of the four principal nuclei and several minor cell groups. (From ref. 12.)*

PRIMARY VESTIBULAR FIBERS

The course of the primary vestibular fibers within the brain has been described in great detail by Cajal and other workers on the basis of Golgi preparations, and some authors have traced the fibers in Marchi preparations. However, when we undertook an experimental analysis with silver-impregnation methods, further details could be established in the distribution of primary vestibular fibers within the vestibular nuclei (ref. 13) as well as in the cerebellum (ref. 14). It turned out that the primary vestibular fibers do not supply the entire territory of the vestibular nuclei. Within each of the four main nuclei there are parts which receive such fibers and others in which no terminations could be found. This is seen from figure 4, where the sites of termination are indicated by dots. The superior nucleus is supplied with primary vestibular fibers chiefly in its central region, the medial and descending chiefly in their lateral and medial areas, respectively. The lateral nucleus of Deiters receives vestibular afferents in its rostroventral area, while the dorsocaudal region is free. The restricted distribution is shown in the photomicrograph of figure 5. (Leidler, ref. 15, in his Marchi studies in the rabbit appears to have noted this in the nucleus of Deiters.) It is, furthermore, of interest that the primary vestibular fibers establish contact, at least largely, with the small cells of the nucleus of Deiters,

Table 1.—*List of Abbreviations Used in Figures 3, 4, 6, 8, 9, and 10*

B.c., Brachium conjunctivum
C.r., Corpus restiforme
D, Descending (spinal) vestibular nucleus
f, Cell group f in descending vestibular nucleus
Flocc., Flocculus
g, Group rich in neuroglia cells, caudal to the caudal end of the medial vestibular nucleus
i.c., Nucleus intercalatus (Staderini)
L, Lateral vestibular nucleus (Deiters)
l., Small-celled lateral group of lateral nucleus
M, Medial (triangular or dorsal) vestibular nucleus
N. cu. e., Nucleus cuneatus externus
N.d., Nucleus dentatus
N.f., Nucleus fastigii
N.f.c., Nucleus funiculi cuneati
N.f.g., Nucleus funiculi gracilis
N.i., Nucleus interpositus cerebelli
N.i.a., Nucleus interpositus anterior
N.i.n. VIII, Nucleus interstitialis nervi vestibuli
N.l., Nucleus lateralis (dentatus) cerebelli
N.m., Nucleus medialis (fastigii) cerebelli
N. mes. V, Nucleus mesencephalicus n.V
Nod., Nodulus
N. pr. V, Nucleus sensibilis principalis n.V.
N.p.s., Nucleus parasolitarius
N. tr. s., Nucleus tractus solitarii
N. tr. sp. V, Nucleus tractus spinalis nervi V
N. VI, VII, VIII, Cranial nerves VI, VII, and VIII
Ol. i., Oliva inferior
Ol. s., Oliva superior
p, Small-celled part of lateral cerebellar nucleus
Pfl. d. and Pfl. v., Dorsal and ventral paraflocculus, respectively
p.h., Nucleus praepositus hypoglossi
S, Superior vestibular nucleus (Bechterew)
Sv., Cell group probably representing the nucleus supravestibularis
Tr. s., Tractus solitarius
T. sp.n.V, Tractus spinalis n.V.
Uv., Uvula
V, VI, VII, XII, Cranial motor nerve nuclei
X, Dorsal motor (parasympathetic) vagus nucleus
x, Small-celled group x, lateral to the descending vestibular nucleus
y, Small-celled group y, lateral to the lateral vestibular nucleus (Deiters)
z, Cell group z, dorsal to the caudal part of the descending vestibular nucleus

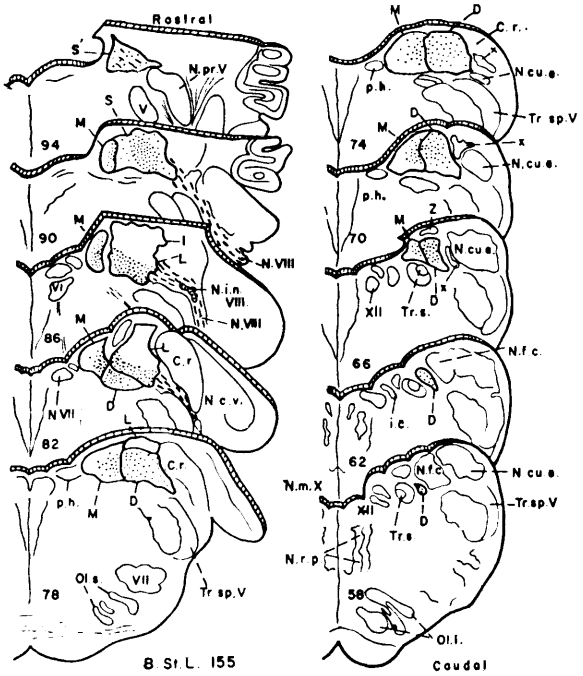

Figure 4.—*Diagrammatic representation of the findings in a cat in which the vestibular nerve had been completely destroyed 10 days before sacrifice. The ensuing degeneration is indicated in drawings of a series of transverse sections through the vestibular nuclei. Degenerating fibers of passage are shown as wavy lines, terminal degeneration as dots. (Abbreviations as in legend to fig. 3.) (From ref. 13.)*

while they appear to avoid the large ones (ref. 13).

As to the primary vestibular fibers to the cerebellum, we have found (ref. 14) with the Nauta method that their distribution within the cerebellum is more extensive than appears from Marchi studies (refs. 16 and 17). Not only the flocculus, the nodulus, and the ventral part of the uvula but also the ventral paraflocculus and to some extent the dorsal paraflocculus receive primary vestibular fibers[2] (fig. 6). We could not convince ourselves of the presence of fibers to the fastigial nucleus as advocated by some authors (refs. 16, 17, and 20). However, some fibers end in the parvicellular part of the lateral (dentate) nucleus (*p* in fig. 6). It follows from this that vestibular impulses reach regions of the cerebellum extending beyond the borders of the flocculonodular lobe, making up the classical "vestibulocerebellum."

The primary vestibular fibers end as mossy fibers (ref. 14), as suggested on the basis of indirect evidence by Snider (ref. 21). Their areas of distribution coincide with those regions of the cerebellar cortex in which the majority of the mossy fibers differ in certain respects from the classical ones (ref. 22). This suggests that the primary vestibulocerebellar fibers end as mossy fibers of a particular type, and probably differ functionally from the usual mossy fibers. Some primary vestibular fibers have been traced to the reticular formation (ref. 20). In our experimental material, only very few such fibers have been found.

Even if the total distribution of vestibular fibers within the vestibular nuclei and the cerebellum is known, a number of questions remain to be answered. In the first place: Are fibers from the cristae and the maculae distributed uniformly within the total terminal areas? As

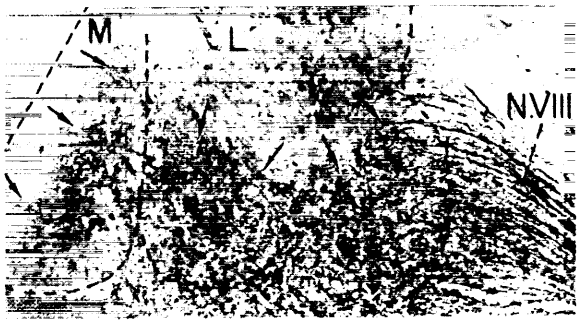

Figure 5.—*A photomicrograph of a transverse section through the brain stem of a cat following complete destruction of the vestibular nerve (see fig. 4, Nauta method), showing distribution of degeneration in vestibular nuclei. Borders of nuclei are indicated by broken lines. In the medial vestibular nucleus* (M), *degeneration at the level shown is restricted to the medial regions. In the lateral nucleus* (L), *a relatively sharp border* (arrows) *is seen between the ventral regions, showing degeneration, and the dorsal regions. Particularly in the latter, some perikarya of Deiters' cells are visible. To the right, degenerating fibers entering in the vestibular nerve* (N. VIII). (*From ref. 13.*)

[2] Evoked potentials have recently been recorded in the paraflocculus of the cat following rotation of the animal (ref. 18). For physiological reasons the vestibulocerebellar impulses were, however, assumed to be mediated via the reticular formation and not via the primary vestibulocerebellar fibers.

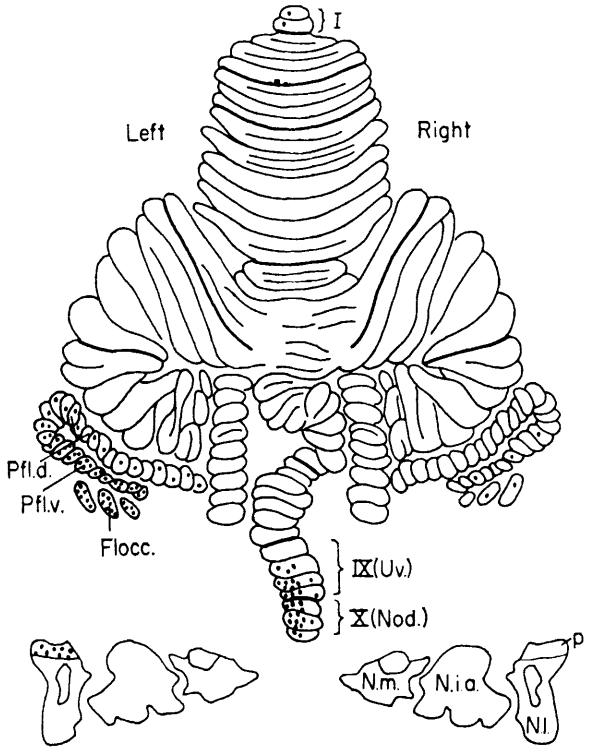

Figure 6.—*Diagram of the cerebellar surface of the cat (imagined unfolded) above, and of the intracerebellar nuclei (adapted from Flood and Jansen, ref. 19) below, showing the sites of termination (dotted) of primary vestibular fibers. (Abbreviations as in legend to fig. 3.) (From ref. 14.)*

discussed in some detail by Brodal, Pompeiano, and Walberg (ref. 1, p. 20 ff.), the studies of Lorente de Nó (refs. 23–25) indicate that there is at least some measure of a differential distribution. For example, it appears that the superior nucleus is supplied only by fibers from the cristae, while fibers to the lateral nucleus of Deiters (its ventral region) are derived from the utricular macula. Experimental studies, admittedly technically difficult, are needed to determine the precise central distribution of afferents from the various parts of the receptor organ.

In this connection the branching of the primary vestibular fibers is of relevance. Thus, most of the primary vestibular fibers to the cerebellum pass through or above the superior nucleus (see ref. 14), and many of them give off collaterals to it (ref. 26). If these fibers come from the cristae (see above), it would follow that at least many of the vestibular impulses reaching the cerebellum are derived from the cristae.

The recent findings on the fine morphology of the receptor organs (see, for example, refs. 27–32) indicate the existence of a fargoing functional differentiation within each of them, and the question may be raised whether the structural differentiations in the receptors are reflected centrally. For example, do the thick fibers, innervating the bottle-shaped receptor cells of type I, which are found chiefly in the central parts of the sensory epithelia, differ in their central distribution or mode of ending from the medium-sized fibers which supply the cylindrical cells of type II, most numerous in the peripheral parts of the epithelia? Furthermore, does the polarization of the receptors within the sensory epithelia (ref. 32) have a central counterpart as concerns the distribution of the fibers from various regions of the epithelia?

In view of the specific reactions that can be elicited from the labyrinth, it seems indeed very likely that the specificity found in the peripheral apparatus must be maintained in some way or other in the central connections. At present, however, practically nothing is known concerning this problem. Experimental studies as those performed in our laboratory give only little information. The original caliber of a degenerating fiber can only be judged approximately. However, it appears that all the vestibular nuclei are supplied by thick as well as medium-sized primary vestibular fibers. A fair number of the fibers to the cerebellum appear to be thick, but the small-celled part of the lateral nucleus (*p* in fig. 6) is supplied chiefly by relatively thin fibers.

A number of details on the fine anatomy of the primary vestibular fibers remain to be clarified. This is also true of the central connections of the vestibular nuclei, even though recent research has made clear certain major features. Some of these will be considered in the following, but a number of interesting details will have to be left out. It will be appropriate to start with the efferent fibers.

EFFERENT CONNECTIONS OF THE VESTIBULAR NUCLEI

The vestibular nuclei may act directly on various other parts of the brain by way of their efferent fibers. Rather schematically, the receiving parts may be listed as follows: spinal cord, cerebellum, nuclei of the ocular nerves and other nuclei in the upper brain stem, reticular formation. However, there is a considerable degree of specificity as to the parts of the nuclear complex which give off efferent fibers to the various destinations, another token of the existing differentiation within the vestibular mechanisms.

Connections From the Vestibular Nuclei to the Spinal Cord

Recent anatomical studies have cleared up some hitherto controversial questions and brought forward some new data. There are two main routes from the vestibular nuclei to the cord: one from the nucleus of Deiters, forming the well-known vestibulospinal tract; the other from the medial vestibular nucleus passing in the descending medial longitudinal fasciculus. Nyberg-Hansen (ref. 33) suggests that the latter should be called the medial vestibulospinal tract, and the former the lateral.

The lateral vestibulospinal tract has been known since the works of the early neuroanatomists to be ipsilateral and to descend in the ventrolateral funiculus throughout the cord. (For references, see refs. 1, 3, and 4.) Its site of origin is restricted to the nucleus of Deiters.[3] Only lesions which involve this nucleus give rise to degeneration in the vestibulospinal tract (ref. 34), and only this nucleus shows cells with retrograde changes following transection of the tract (ref. 38). Two additional features of interest were disclosed in our study (ref. 38). In the first place, small as well as large cells give rise to vestibulospinal fibers. Secondly, there is a clear somatotopical pattern within the nucleus, as seen from figure 7. The rostroventral part sends its fibers to the cervical cord and is thus a "neck and forelimb region"; the dorsocaudal part gives off fibers to the lumbosacral cord and represents a "hindlimb region." In between there is a "trunk region." This localization has been confirmed physiologically (ref. 39). The fact that small as well as large (giant) cells give rise to fibers in the tract agrees with the observations that the fibers are of different calibers, and with the varying conduction velocities recorded (refs. 40 and 41).

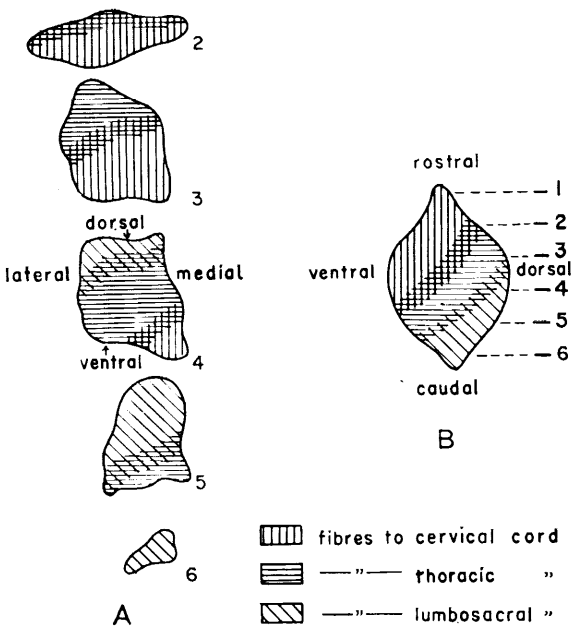

Figure 7.—*Diagram to show the somatotopic arrangement of the origin within the lateral vestibular nucleus of fibers passing to different levels of the cord. To the left* (A), *the pattern is shown as seen in transverse sections; to the right* (B), *as it appears when projected on a sagittal reconstruction of the lateral vestibular nucleus. (From ref. 38.)*

According to recent physiological findings, the vestibulospinal tract and the nucleus of Deiters exert an excitatory effect on extensor motoneurons (refs. 42 and 43) and are essential for the maintenance of postural tonus. The suggestive evidence that the nucleus of Deiters appears to receive fibers from the utricular

[3] It is necessary to emphasize that in this presentation the lateral vestibular nucleus of Deiters is taken (see ref. 12) to comprise that part of the nuclear complex in which the giant cells of Deiters form a characteristic element. This is in agreement with the delimitation used, among others, by Cajal (ref. 35) and Kappers, Huber, and Crosby (ref. 36). Several authors, most recently Voogd (ref. 37), use other delimitations. In some physiological papers the term "nucleus of Deiters" is used to denote almost the entire vestibular complex.

macula (as referred to above) is of interest in this connection. The nucleus influences alpha as well as gamma motoneurons (refs. 44 and 45), and it is tempting to speculate upon whether the two types of cells in the nucleus may be related each to one of the two types of motoneurons.

It has been concluded from physiological studies (ref. 43) that the vestibulospinal fibers influence the alpha motoneurons monosynaptically. A study of the sites of termination of the vestibulospinal fibers with silver-impregnation methods (ref. 34) shows that these fibers end in Rexed's laminae VIII and neighboring part of VII (fig. 8), while terminations are not found in lamina IX (except for a few in the thoracic cord) which harbors the perikarya of the alpha as well as the gamma motoneurons (refs. 46 and 47). The majority of the fibers end on dendrites. Since dendrites of motoneurons may extend rather far dorsally (refs. 48 and 49) into laminae VIII and VII (ref. 49), the anatomical and physiological findings are not necessarily in contradiction.

The medial vestibulospinal tract.—From a critical review of the literature (ref. 38), it appears that the vestibular fibers descending in the medial longitudinal fasciculus arise only from the medial vestibular nucleus. This has recently been demonstrated experimentally by Nyberg-Hansen (ref. 50) in a study of the degeneration as seen in silver-impregnated sections following isolated lesions of the various vestibular nuclei. No descending fibers were found from the descending nucleus, in agreement with Carpenter, Alling, and Bard (ref. 51). The fibers descend bilaterally, chiefly uncrossed, in the area of the medial longitudinal fasciculus (fig. 9), but can be traced only to midthoracic levels. They end largely in the same regions as the fibers of the lateral vestibulospinal tract, and like these appear to contact somata as well as dendrites.

In spite of largely corresponding zones of termination within the gray matter of the cord, the medial and lateral vestibulospinal tracts differ considerably in their anatomical organization. This difference is further emphasized when the primary vestibular fibers to the medial

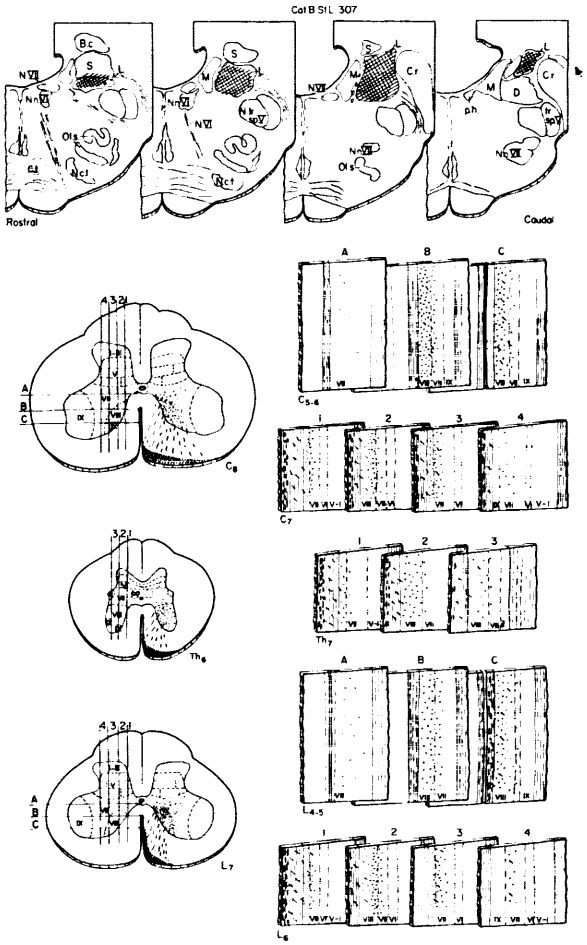

Figure 8.—*Diagrammatic representation of the course and site of termination (dots) of vestibulospinal fibers in the cat as determined experimentally. Above, a diagram of the lesion, confined to the lateral vestibular nucleus. Roman numerals in the spinal cord refer to Rexed's zones. Note absence of termination in lamina IX, harboring the motoneurons. (Abbreviations as in legend to fig. 3.) (From ref. 34.)*

and the lateral vestibular nucleus are considered. From the studies of Lorente de Nó (see ref. 1, p. 20 ff.) it appears that the medial nucleus is supplied chiefly by fibers from the cristae, and units responding to horizontal rotation of the head have been found in the medial vestibular nucleus (ref. 52). The suggestion may be ventured that the medial vestibulospinal tract is primarily concerned in the movements of the head occurring simultaneously with conjugate deviation of the eyes (ref. 50), the more

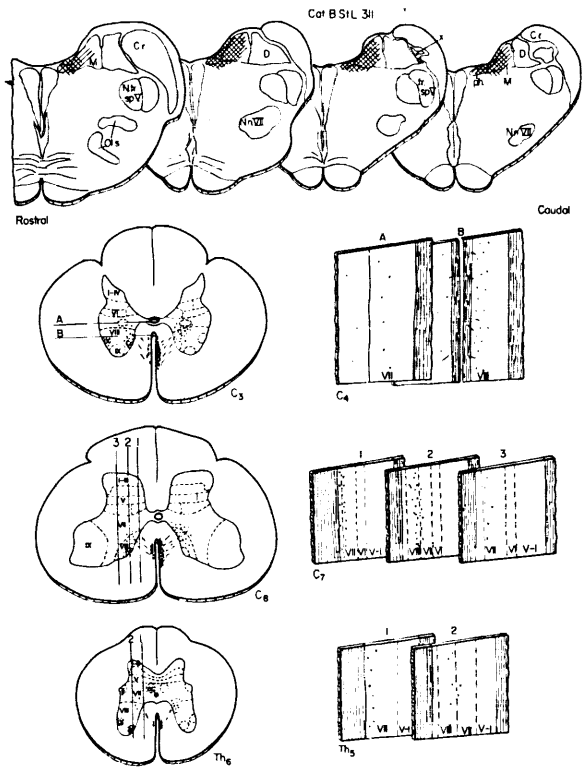

Figure 9.—*Diagrammatic representation of the findings in a case with a lesion restricted to the medial vestibular nucleus, showing the course and sites of termination of descending fibers in the medial longitudinal fasciculus in the cat. Roman numerals in the spinal cord refer to Rexed's zones. (Abbreviations as in legend to fig. 3). (From ref. 50.)*

so since the medial nucleus gives off ascending fibers as well, and there are fibers from the nucleus which dichotomize into an ascending and a descending branch (ref. 35).

Connections From the Vestibular Nuclei to the Cerebellum

Secondary vestibulocerebellar fibers have been demonstrated in normal material as well as experimentally (for references, see refs. 6 and 53). In Marchi studies they have been traced to the flocculus, nodulus, part of the uvula, and the fastigial nucleus. It remains to be seen whether silver-impregnation studies will reveal a more extensive distribution, corresponding to that of the primary vestibulocerebellar fibers (fig. 6). The exact site of origin of the secondary vestibulocerebellar fibers has been a matter of dispute. By taking advantage of the modified Gudden method (ref. 5), fibers to the cerebellum were shown to originate in the descending and the medial vestibular nuclei (ref. 6), chiefly ipsilaterally, as well as in the group *x* (fig. 10). Cells in these nuclei present retrograde cellular changes (fig. 1) following cerebellar lesions involving the areas of termination of the fibers. Corresponding observations were made by Carpenter, Bard, and Alling (ref. 54). However, the changes do not cover the entire nuclei. The majority of the fibers come from the ventrolateral parts of the descending nucleus (fig. 10). However our study does not entirely exclude the possibility that cerebellar fibers may arise in other parts of the nuclear complex as well, since negative findings with the method of retrograde chromatolysis are not decisive.

The mode of termination of the secondary vestibular fibers is not quite settled, but it ap-

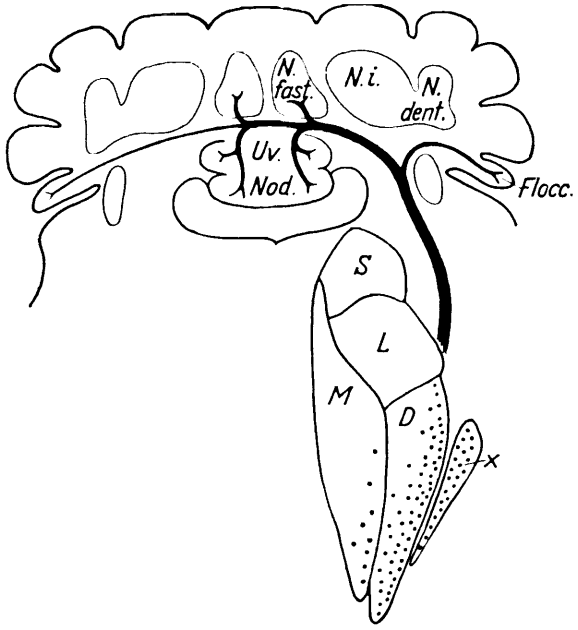

Figure 10.—*A summarizing diagram of the secondary vestibulocerebellar projection in the cat. The fibers come from the regions dotted in the diagram of a horizontal section through the vestibular nuclei; namely, the ventrolateral part of the descending nucleus (including groups* f, *not indicated), the caudal part of the medial vestibular nucleus, and group* x. *The course of the fibers and their sites of termination are indicated according to Dow (ref. 17). (From ref. 6.)*

pears likely from indirect evidence that they end as mossy fibers (ref. 55).

Efferent Connections From the Vestibular Nuclei to Higher Levels of the Brain

Because of the interest of clinicians in the various types of nystagmus, this component of the vestibular fiber connections has been the subject of a number of anatomical studies. The relevant literature is, however, rather confusing, in part because the lesions in experimental studies have often not been restricted to particular subdivisions of the nuclear complex and because of inconsistencies in the nomenclature. There is, however, agreement that at least most of these ascending fibers course in the medial longitudinal fasciculus (MLF). On the basis of experimental studies by means of the modified Gudden method (ref. 5), we concluded (ref. 56) that all four classical nuclei (as well as group x and the interstitial nucleus of the vestibular nerve) contribute fibers to the ascending MLF. This agrees with the results of experimental and Golgi studies of other authors.

As to the proportion of crossed and uncrossed ascending fibers, there is no unanimity in the literature (for a review, see ref. 56). Most authors agree that the fibers terminate in the nuclei of the nerves to the extrinsic ocular muscles, the interstitial nucleus of Cajal, the nucleus of the posterior commissure, and the nucleus of Darkschewitsch (for an account of these nuclei, see ref. 57). Other sites of terminations, such as the colliculi, the medial geniculate body, the red nucleus, and the thalamus, have been described as well (see refs. 1 and 56 for some references). Recently Carpenter and Strominger (ref. 58) traced the thalamic fibers to parts of the ventral posterior inferior and ventral posterior medial nuclei, and to the parafascicular, centromedian, and reticular nuclei.

In view of the clear correlation between stimulation of the various semicircular ducts and movements of the eyes in particular directions, it may be surmised that there are anatomically precise relations between a particular receptor (superior cristae, etc.) and specific groups of motoneurons to the extrinsic muscles of the eyes (see, for example, refs. 59–62). Physiological observations indicate that there may be a topical representation of the various vestibular nuclei (ref. 63) or of particular receptors in the cerebral cortex (ref. 64). However, we are only at the beginning of a clarification of the intricate anatomical connections which form the basis of these phenomena (see refs. 20, 58, and 65–67 for some recent data).

Efferent Connections to the Reticular Formation

Although it has been shown in Golgi studies (refs. 68 and 69) and in experimental studies (refs. 59, 65, and 67) that some fibers from the vestibular nuclei pass to the reticular formation, or that ascending and descending vestibular fibers give off collaterals to the reticular formation, a detailed mapping of these connections remains to be done. (For some further data from the literature, see ref. 1.)

Centrifugal Fibers in the Vestibular Nerve

These have been advocated by some early authors and have recently been investigated by Rasmussen and Gacek (ref. 70) and Gacek (ref. 71). They have been traced to all subdivisions of the labyrinth, and the suggestion of Gacek (ref. 71) that they are derived from the nucleus of Deiters has recently been confirmed (in the guinea pig) by Rossi and Cortesina (ref. 72), who in addition advocate the origin of some such fibers from a particular small cell group. The occurrence of cholinesterase in relation to the vestibular receptor cells (ref. 73) and the presence of "synaptic" vesicles in the small knob-shaped terminals on vestibular sensory cells (refs. 27 and 30) fit in with the existence of efferent vestibular fibers.

AFFERENT CONNECTIONS OF THE VESTIBULAR NUCLEI

Generally speaking, the vestibular nuclei receive afferents from the same regions to which they give off fibers. Studies of the afferent connections bring further evidence of the existing complexity in the organization of the nuclei: The various contingents of afferents do not end diffusely and indiscriminately all over the nuclear complex, but have their particular sites of

termination. This is seen from the diagram of figure 11 where the sites of terminations of the main contingents of afferents are indicated by different symbols. Some contingents are scanty and have a very restricted termination. This is the case particularly with the descending afferents in the medial longitudinal fasciculus. They are derived from the interstitial nucleus of Cajal in the mesencephalon (ref. 57) and supply only part of the medial vestibular nucleus (fig. 11). The cerebellar afferents to the vestibular nuclei, on the other hand, are particularly abundant.

The principle of a different distribution of afferents is most marked in the lateral nucleus, which will be selected here for a closer consideration. This is justified also, because this is the nucleus whose function is at present best known. As mentioned previously, the primary

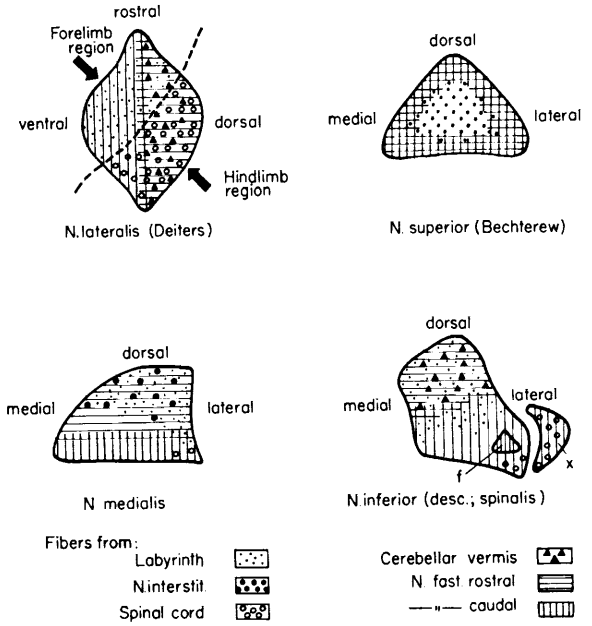

Figure 11.—*Simplified and diagrammatic representation of the distribution within the four main vestibular nuclei of afferent fibers from different sources. Only the main contingents of afferents are included (see key below). Varying densities of terminations are not shown. The nucleus of Deiters is represented in the sagittal plane, the three other nuclei as seen in transverse sections. Note differential distribution of the various contingents within each of the nuclei. (A similar diagram, including also the main efferent connections, is found in ref. 1, p. 93.)*

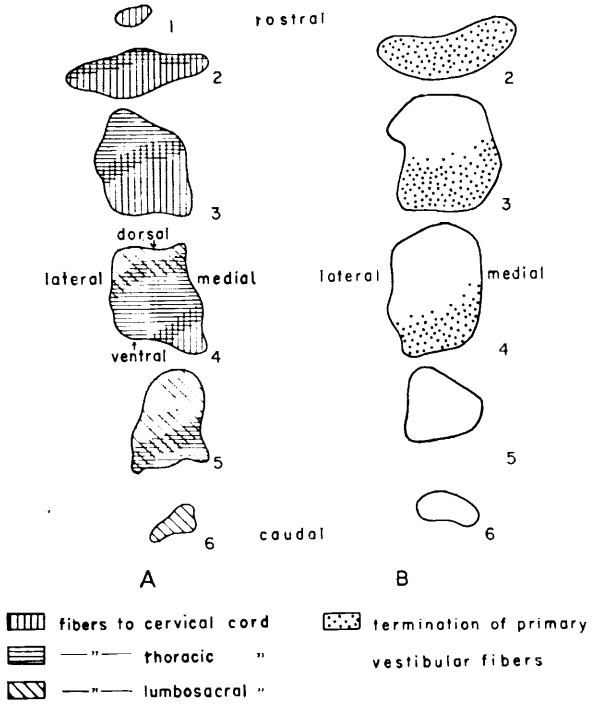

Figure 12.—*Diagram showing* (B) *the sites of termination of primary vestibular fibers (dots) in the lateral vestibular nucleus as seen in a series of transverse sections corresponding approximately to those seen to the left* (A), *showing the somatotopic pattern in the nucleus. Note restriction of vestibular afferents to the forelimb region. (From ref. 13.)*

vestibular fibers end in certain parts of the vestibular nuclei only, in the nucleus of Deiters in its rostroventral part (figs. 4 and 5). If their site of termination is entered in the map of the somatotopical projection of the nucleus of Deiters onto the cord (ref. 38), it is seen that their distribution coincides approximately with the forelimb and neck region of the nucleus (fig. 12). The fibers to the nucleus of Deiters coming from the spinal cord (ref. 74), on the other hand, are distributed to the dorsocaudal part, the hindlimb region only (fig. 11), which is devoid of vestibular afferents. This is the case in the cat (ref. 74) as well as in the monkey (ref. 75) and in man (ref. 76).

The cerebellar afferent input to the vestibular nuclei comes in part from the flocculonodular lobe, but chiefly from the anterior and posterior vermis. The flocculus and the nodulus both send fibers to the nucleus of Deiters as shown

in Marchi studies by Dow (refs. 17 and 77). The cerebellar vermis disposes of two routes by which it may influence the vestibular nuclei. There is a direct pathway and an indirect one, which involves a synapse in the fastigial nucleus. Both fiber systems show a high degree of differentiation. Our findings on these two systems, as far as the lateral vestibular nucleus of Deiters is concerned, are summarized in figure 13, which is diagrammatic and does not include all details.

The direct cerebellovestibular pathway to Deiters' nucleus (fig. 13, to the left) arises mainly in the vermis proper of the anterior lobe (lobuli II–V of Larsell), to a lesser extent in the posterior vermis, and its fibers end in the dorsal half of the ipsilateral nucleus (ref. 78). In their experimental study, Walberg and Jansen (ref. 78) brought forward evidence that the projection is somatotopically organized: Fibers from the hindlimb region of the anterior lobe end in the hindlimb region of the nucleus of Deiters; fibers from the forelimb region in the anterior vermis end in the corresponding part of Deiters' nucleus. Whether the projection from the posterior vermis as well is somatopically organized has so far not been decided anatomically.

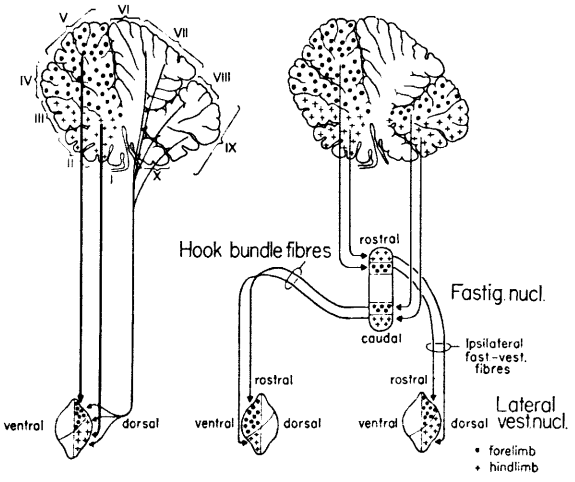

Figure 13.—*Diagram illustrating major features in the projections from the cerebellar cortex onto the nucleus of Deiters (to the left) and (to the right) in the projections from the cerebellar cortex onto the fastigial nucleus and from this to the lateral vestibular nuclei. Note that the direct cerebellovestibular fibers and the projection from the rostral part of the fastigial nucleus end in the dorsal half of the ipsilateral lateral vestibular nucleus, while the fibers from the caudal part of the fastigial nucleus via the hook bundle supply the ventral half of the contralateral lateral vestibular nucleus. Within each of these projections there is a somatotopic localization. Compare text. (From ref. 1.)*

The other cerebellovestibular pathway, passing via the fastigial nucleus, is more complex. The diagram in figure 13 (to the right) shows that part of it which is related to the nucleus of Deiters. (Fibers from the fastigial nucleus end in all vestibular nuclei, see fig. 11.) The diagram shows that there is an orderly arrangement in the projection from the anterior and posterior vermis onto the rostral and caudal parts, respectively, of the fastigial nucleus. This was inferred from findings made in Marchi studies (refs. 79 and 80), and even if later studies with silver-impregnation methods (refs. 81 and 82) have shown that the localization is not so sharp as originally supposed, the main principle appears to be valid. The projections from the two parts of the fastigial nucleus onto the vestibular nuclei, however, show marked differences. Those from the rostral part, receiving their cerebellar input from the anterior vermis, end in the dorsal half of the ipsilateral nucleus of Deiters; i.e., in the same region where the direct fibers end. The fibers from the caudal part of the fastigial nucleus, being influenced from the caudal vermis, behave in a different way. They cross in the hook bundle and enter the contralateral nucleus of Deiters. Furthermore, their termination is restricted to the ventral half of the nucleus; i.e., that part which is not supplied by the other contingents of fibers from the cerebellum. However, as seen from figure 13, both fastigiovestibular projections onto the nucleus of Deiters show a somatotopic organization. It is to be noted that the border between the regions which differ with regard to their cerebellar afferents crosses the border between fore- and hindlimb regions.

Physiological studies have been made, particularly by Pompeiano and his collaborators (see refs. 1 and 83), which can be correlated with these features of the anatomical organization of the cerebellovestibular connections.

Thus, physiologically a clear-cut somatotopical arrangement in the cerebellovestibular projections from the anterior lobe onto the nucleus of Deiters has been demonstrated (ref. 84). As referred to in a preceding section, the somatotopical pattern in the vestibulospinal projection has been demonstrated anatomically (refs. 34 and 38) as well as physiologically (ref. 39). Taken together, these findings explain why the postural responses to stimulation or ablation of parts of the anterior-lobe vermis of the cerebellum are somatotopically organized. (For further discussions of this subject, see refs. 83 and 85.) The data concerning the posterior vermis are yet not quite complete, but there is strong suggestive evidence that conditions are similar (see ref. 83).

The mechanisms of the cerebellar actions on the nucleus of Deiters are rather complex, as reviewed recently by Pompeiano (ref. 83). The chief action of the anterior lobe vermis appears to be a tonic inhibitory influence on neurons in the ipsilateral nucleus of Deiters, tending to reduce the extensor tonus. The caudal part of the fastigial nucleus exerts an excitatory influence on the extensor motoneurons of the contralateral side (ref. 86). However, there are further specifications. For example, the rostrolateral and rostromedial parts of the fastigial nucleus differ functionally (ref. 87). This indicates that there are details in the anatomical organization of the pathways involved which are so far not known.

Functional differences may also bear some relation to synaptic relationships, for example, to the fact that the direct cerebellovestibular fibers end chiefly on large neurons (ref. 78), while the fastigiovestibular fibers end, at least chiefly, on small cells in the nucleus of Deiters (ref. 9).

In addition to the major contingents of afferents discussed above, there are also others. Some axons of cells in the reticular formation, chiefly at least in its pontomedullary part, enter the vestibular nuclei, as do collaterals of reticulospinal and other tracts. A certain number of afferents from other sources have likewise been described (inferior olive, upper cervical dorsal roots, mandibular, and glossopharyngeal nerves). On the whole, the latter connections appear to be scanty, and little is known of their precise sites of termination. (For a review of the literature, see ref. 1.)

INTRINSIC ORGANIZATION OF THE VESTIBULAR NUCLEI AND SYNAPTIC ARRANGEMENTS

Cytoarchitectonic studies and experimental studies of fiber connections leave us with a picture of the vestibular nuclei as being composed of a number of minor units which differ with regard to their afferent and efferent connections. In fact, even within the individual nuclei there are subregions which have dissimilar connections. It must be assumed that these anatomical features have functional implications, and physiological evidence is accumulating which shows that this is indeed so.

An objection against these anatomical observations may be raised, however, that they do not give a picture which is valid as a basis for functional interpretations, among other things, because the methods employed do not give information about dendrites. If dendrites of cells in a region which does not receive, for example, primary vestibular fibers, extend far into the territory of a neighboring region, which does receive such fibers, the anatomical demonstration of a restricted termination of an afferent system would have little functional meaning. Recurrent collaterals and internuncial cells may likewise contribute to complicate interpretations. Only the Golgi method is at present available for a study of these problems. The classical studies of Cajal and Lorente de Nó have clarified many points in the finer anatomy of the vestibular nuclei, but it is not always possible to correlate their data with the observations made in experimental studies in the cat. Dr. Hauglie-Hanssen in our laboratory, therefore, has recently undertaken a Golgi study of the vestibular complex (ref. 88) in an attempt to answer some of the problems referred to above. It appears from his studies that there are in all places dendrites which extend from one vestibular nucleus to the neighboring ones. There are, however, considerable regional variations. Dendrites may also be traced into other adjacent nuclei and fiber bun-

dles. However, the vast number of dendrites is contained within the particular nuclear region (see ref. 2, fig. 14). The same appears from Mannen's (ref. 89) studies of the dendritic patterns, although this author does not consider the particular subdivisions specially.

Even if some dendrites do extend into a neighboring nucleus, their number appears to be relatively modest. It appears very doubtful, therefore, that their presence is of great functional importance or will seriously contradict the conclusions made on the basis of experimental anatomical studies.[4]

The possible presence of internuncial cells in the vestibular nuclei is another relevant point. Studies of the retrograde cellular changes in the lateral vestibular nucleus following sections of the lateral vestibulospinal tract suggest that this nucleus probably does not contain internuncial cells (ref. 38). Like Cajal (ref. 35), Hauglie-Hanssen (ref. 88) found only in the descending and medial nuclei small cells which have a number of branches close to the soma, and which may possibly be internuncials (fig. 14). As to recurrent collaterals, the axons of the lateral vestibulospinal tract appear to give off such collaterals to the descending and medial vestibular nuclei (refs. 35 and 91), but a detailed mapping has apparently so far not been done.

Since the Golgi method brings out only random samples of the cells present, it does not permit quantitative estimates. However, when contrasted with the clearer indications given by

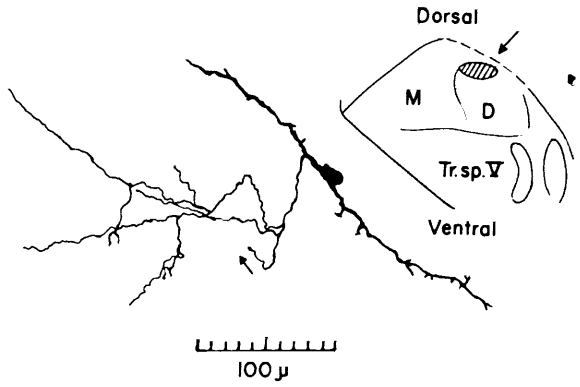

Figure 14.—*Drawing of a small nerve cell, presumably internuncial, in the descending vestibular nucleus (inset shows position). The axon* (arrow) *gives off a number of collateral branches which ramify close to the perikaryon. Golgi rapid preparation from a kitten. (Courtesy of Dr. E. Hauglie-Hanssen.)*

studies of architectonics and fiber and cell degenerations, the observations made in Golgi studies do not overthrow the conclusions based on such findings, but supplement them. On many points, moreover, the Golgi findings agree completely with observations made in experimental studies. For example, the primary vestibular fibers are clearly seen to be distributed to the rostroventral part only of the lateral vestibular nucleus (refs. 25, 68, and 88). Furthermore, a single fiber may give off a number of collaterals to one cell (fig. 15), as one would assume from the dense accumulation of argy-

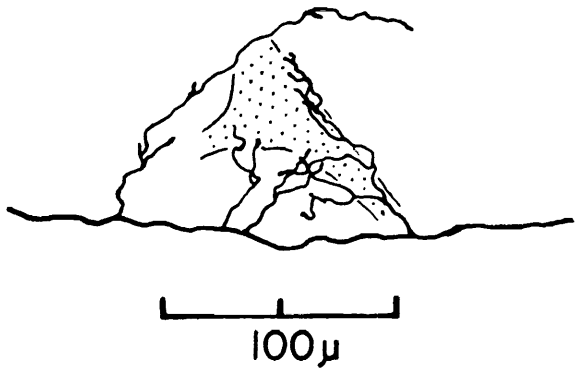

Figure 15.—*Drawing from a Golgi rapid preparation, showing how several branches of an axon establish contact with a nerve cell in the dorsal half of the lateral vestibular nucleus of the kitten. (Courtesy of Dr. E. Hauglie-Hanssen.)*

[4] The problem of the extension of dendrites is of special interest in the nucleus of Deiters. As referred to above, primary vestibular fibers are restricted to its forelimb region. However, it is well known that stimulation of vestibular receptors (natural or artificial) usually (see ref. 90, for an exception) gives rise to changes in postural tonus in the hindlimbs as well as the forelimbs. Since the medial vestibulospinal tract from the medial vestibular nucleus does not descend below midthoracic levels, this effect most likely is mediated via the lateral vestibulospinal tract from the nucleus of Deiters. Since only a modest number of dendrites of cells in the hindlimb region extend into the forelimb region, and no evidence has been found that axons of cells in the forelimb region extend into the hindlimb region, the vestibular impulses may, therefore, be assumed to influence the latter region via a circuitous route, most likely the cerebellum. On an anatomical basis, an influence via the reticular formation appears less probable.

rophilic particles on the surfaces of cells following interruption of afferent fibers (fig. 2(b) and 2(c)).

In addition, Golgi studies give information on synaptic relations which supplement those made with degeneration methods. In our studies on afferent fibers to the vestibular nuclei (refs. 9, 13, 56, 57, and 78), it has been a regular finding that degenerating particles are found along the surfaces of the somata of the cells as well as of the proximal dendrites (figs. 2(c) and 16). It is not possible, however, to decide whether the same fiber makes contact with both structures. In Golgi preparations a single axon may sometimes be followed for some distance, and it is often seen that it courses along the soma and a dendrite of the same cell. Sometimes a fiber may be seen "climbing" along a dendrite for a considerable distance.

With the refinement of neurophysiological techniques, a knowledge of the synaptic relationships in the various nuclei becomes of increasing importance. Some information on this subject may be obtained from silver-impregnation studies. As already mentioned, afferents from different sources may differ with regard to the types of cells which they contact. In the nucleus of Deiters, for example, primary vestibular fibers end, at least chiefly, on its small cells, while they seem to avoid the large ones

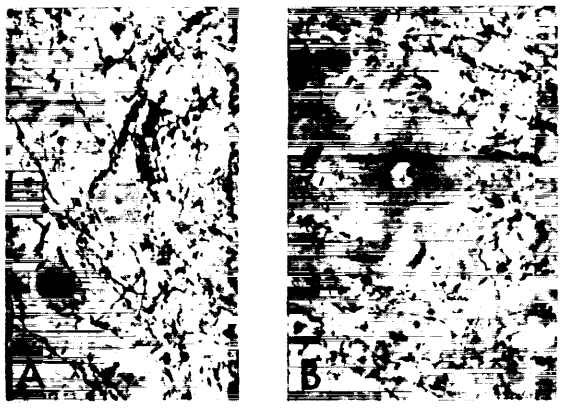

Figure 16.—*Photomicrographs from a Nauta-impregnated section through the lateral vestibular nucleus of the cat 10 days following interruption of the vestibular nerve. Degenerating fibers end abundantly on small cells* (A) *while they appear to avoid the large cell* (B). (*From ref. 13.*)

(ref. 13) as seen in figure 16. This is seen also in Golgi studies (ref. 88). The spinal afferents, on the other hand, end chiefly on giant cells (ref. 56). This is the case also for the fibers from the cerebellar cortex (ref. 78), while those from the fastigial nucleus (ref. 9) end largely on small cells.

However, there are questions on which such studies give no information. Thus it is virtually impossible to decide whether afferent fibers end on the fine peripheral parts of the dendrites. Furthermore, a contact between a degenerating particle and a cell body or dendrite does not necessarily mean that it represents a synapse. Structures such as a glial sheath, which cannot be identified under the light microscope, may well be interposed between the two elements. The final answer to these and other problems has to be obtained in electron-microscope studies. It is fortunate that such studies can be made also on experimental material (refs. 91 and 92). Technical directions for such studies have recently been published (ref. 93). Studies in our laboratory by Drs. Mugnaini and Walberg have brought forward some data of interest. So far they have been restricted to the nucleus of Deiters.

In Golgi sections, it can be seen that the proximal parts of the dendrites of cells in the Deiters nucleus are smooth, while the peripheral thinner branches are provided with spines (fig. 17). A question of some interest is whether the fibers running along the proximal parts of dendrites establish synaptic contacts with these, as appears to be the case from degeneration studies. In electron micrographs, thin fibers can be seen to run along and to be closely attached to the smooth dendritic surfaces (fig. 18), and there are a number of immediate contacts between the two structures which must be interpreted as synapses (single arrows in fig. 18). The situation is very similar to the relation between the climbing fibers in the cerebellum and the proximal parts of the Purkinje cell dendrites as described recently by Hámori and Szentágothai (ref. 94). Synaptic contacts with the spiny parts of dendrites are likewise present (fig. 19) and show several varieties.

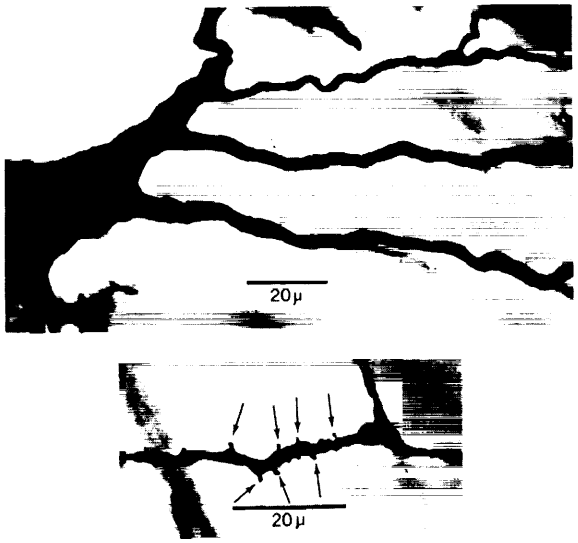

Figure 17.—*Photomicrograph of a Golgi-Kopsch section from the kitten showing smooth, proximal parts of the dendrites of a nerve cell in the lateral vestibular nucleus (above) and part of a peripheral dendritic branch, beset with spines* (arrows). *(Courtesy of E. Hauglie-Hanssen.)*

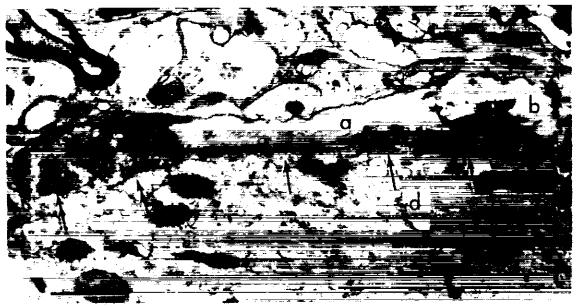

Figure 18.—*Electronmicrograph from the lateral vestibular nucleus in the cat. A thin axon* (a) *runs along the surface of a thick dendrite* (d) *and establishes synaptic contacts* (single arrows). *In addition, two boutons* (b) *belonging to the axon have synaptic contacts* (double arrows). *Scale line, 1μ. (Courtesy of E. Mugnaini and F. Walberg.)*

Fibers and terminal boutons can be identified in the electron microscope also when they degenerate. The early changes consist of shrinking of the bouton, denser packing of synaptic vesicles, and beginning disintegration of the mitochondria. The ground substance of the bouton becomes finely granular, and the whole structure becomes denser electron optically. These changes are seen from the second to the fifth day. This provides us with a unique possibility of extending the light-microscope studies and settling several questions. Some recent observations will illustrate the potentialities of the method. Three and one-half days following a lesion of the vermis of the anterior lobe, degenerating boutons can be identified in the dorsal part of the nucleus of Deiters, which on the basis of silver-impregnation studies (ref. 78) is known to receive such fibers (fig. 11). The presence of axosomatic synapses (fig. 20) agrees with the light microscopic studies and shows that the contacts observed in our sections are truly sites of synapses.

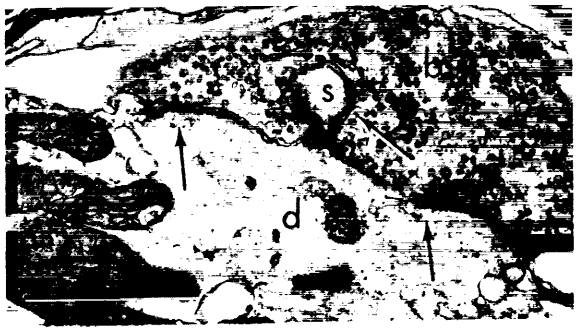

Figure 19.—*Electronmicrograph from the lateral vestibular nucleus in the cat. A dendrite* (d) *is provided with a spine* (s). *A terminal bouton* (b) *has synaptic contacts* (arrows) *with the spine* (s) *and with the dendritic trunk. Scale line, 1μ. (Courtesy of E. Mugnaini and F. Walberg.)*

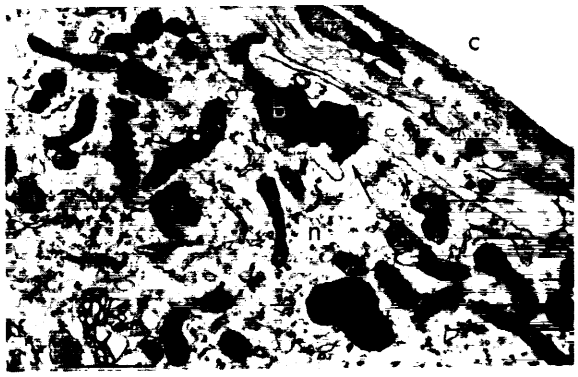

Figure 20.—*Electronmicrograph from the lateral vestibular nucleus in the cat, 3½ days following a lesion of the vermis of the anterior lobe. A degenerating bouton* (b) *contacts the surface of a large nerve cell* (n). *A synapse* (arrow) *is seen.* c *is a capillary lumen. Scale line, 1μ. (Courtesy of E. Mugnaini and F. Walberg.)*

The electron micrographs likewise confirm the presence of axodendritic synapses. These are present on large dendrites (fig. 21) and on spines. It is learned, furthermore, what could not be decided from the silver-impregnated sections, that synaptic contacts are made also with very thin dendrites. Figure 22 shows a degenerating bouton contacting a dendrite of very small caliber. This is of particular interest. According to Ito and Yoshida (ref. 95), stimulation of appropriate parts of the cerebellar cortex produces (monosynaptically) predominantly inhibitory postsynaptic potentials in the cells of the nucleus of Deiters. It seems currently to be believed that inhibitory synapses are situated on somata and proximal dendrites. The presence of endings of fibers from the cerebellar cortex on the distal dendrites of these cells, therefore, suggests that conditions are probably not so schematic as one might think.

The possibility of using electron microscopy in the experimental study of fiber connections and synaptic relationships opens up a vast field for further research and gives promise that a number of morphological details may be found which will be of immediate interest for functional interpretations and anatomicophysiological correlations. A necessary prerequisite for such studies is, however, that preliminary studies be made with light microscopical methods, because one has to know precisely where to look for possible changes.

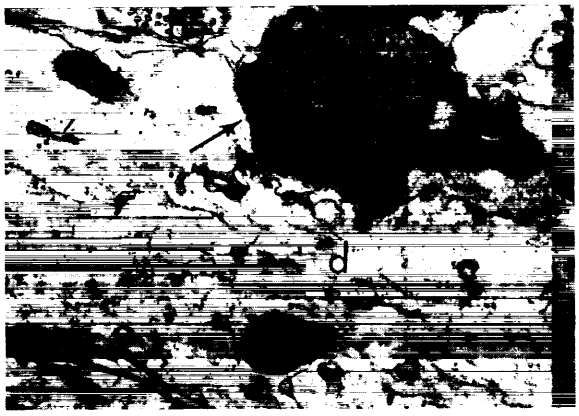

Figure 21.—*Electronmicrograph from the lateral vestibular nucleus in the cat, 3½ days following a lesion of the vermis of the anterior lobe. A degenerating bouton* (b_1) *contacts a large dendrite* (d). *A synapse* (arrow) *can be seen. To the left, part of a normal bouton* (b_2) *with synaptic vesicles. Scale line, 1μ. (Courtesy of E. Mugnaini and F. Walberg.)*

Figure 22.—*Electronmicrograph from the lateral vestibular nucleus in the cat, 3½ days following a lesion of the vermis of the anterior lobe. A degenerating bouton* (b) *is seen in contact with a very thin dendrite* (d). *Axodendritic synapses are present at arrows. Scale line, 1μ. (Courtesy of E. Mugnaini and F. Walberg.)*

CONCLUSION

It will appear from this survey that our knowledge of the anatomy of the vestibular nuclei is still far from complete. However, there is no doubt that the vestibular nuclei must be considered as being a mosaic of many minor parts, which differ in architecture as well as in their connections with other parts of the nervous system. Their finer organization is extremely complex and not the same in the various groups or subdivisions. These anatomical data strongly suggest that the various units (cell groups or parts of nuclei) are not functionally similar. To some extent this has been demonstrated physiologically, but much remains to be done in this field as well. In future functional studies it will be essential to be extremely precise as to which part of the entire complex one is dealing with and to take into account the anatomically known details. Otherwise, confusion will result.

For the morphologists there are still many tasks to be completed. For example, we do not yet have an adequate mapping of the vascular

and glial architecture of the vestibular nuclear complex. The interesting metabolic relations between the nerve cells and the oligoglia, in the nucleus of Deiters, reported by Hydén and his collaborators (see, for example, ref. 96) as well as other recent data bear witness to the functional importance of the glia. An extension of such studies, as well as histochemical studies which take into consideration the morphological differentiation of the vestibular nuclear complex, might contribute to a better understanding. Not least is there reason to expect that electron-microscopical studies of synapses in normal and experimental material will be of interest and serve for fruitful correlations with micro-electrode recordings.

In our striving to reveal the minutest patterns in the morphological and functional organization of the vestibular nuclei, we should, however, always keep in mind that these cell groups have connections with numerous other parts of the nervous system and, therefore, collaborate with them. This is not least important when one tries to analyze vestibular disturbances in clinical cases. It is common experience that an increased insight into basic problems in the beginning often tends to make clinical analyses more difficult. However, it is to be hoped that the perplexing multitude of new data on the vestibular nuclei and receptors will not discourage those concerned in problems related to vestibular function in man, when he is ill or when exposed to unusual circumstances such as space flights.

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DISCUSSION

GUALTIEROTTI: Considering the relatively recent view about the tonic inhibitory influence of the cerebellum on the vestibular nuclei, I'd like to know if there is any histological or anatomical evidence to show whether this inhibitory influence is applied discretely on different groups of neurons, or if we are dealing with a general system by changing the sensitivity of the cell population as a whole?

BRODAL: I would like to emphasize that I am not a neurophysiologist. On these matters I speak as an amateur. As far as I know, the inhibitory action is general; that is, the different parts of the anterior lobe all have an inhibitory action in certain instances. However, within the cerebellovestibular projections there is a localization. I think there is sufficient anatomical evidence to explain that by stimulation of the anterior lobe of the cerebellum, you can have inhibition acting, for example, on the foreleg only, not on the hindleg, if that is what you were thinking of.

GUALTIEROTTI: Do we have a point-to-point relationship between the cerebellar cortex and the nucleus as far as the major pathways are concerned, or do we have a diffuse system?

BRODAL: A point-to-point relation is a very strict one. I don't think we have this anatomically, but I would like to mention that in physiological studies, it is possible to outline a localization much more precisely than it is anatomically, because in anatomical studies we always have to trace a number of fibers which inevitably will spread a little, while the physiologist can pick up the maximum of any spread action.

MEHLER: Somewhat in answer to Dr. Gualtierotti's question to Dr. Brodal are the experimental observations in the frog reported by Don Goodman at the University of Florida at Gainesville. In cases of labyrinthectomized frogs, Goodman demonstrated that stimulation of the cerebellum completely reverses the frog's existing eighth-nerve-damage posture, suggesting in this case that even the primordial cerebellum in the frog is quite effective in overcoming existing postural changes elicited by the destruction of the eighth nerve. Whether the action is "inhibitory" or "facilitatory" is unknown. In respect to remarks that Dr. Lowenstein made yesterday, about putting all our eggs in one basket or "findings in one species," figure D1 is shown in confirmation of much of Dr. Brodal's work. The figure shows corroborative evidence of some of the detailed models of neural organization of the vestibular complex that Dr. Brodal and coworkers have presented to the anatomists and physiologists.

The figure presents studies of cerebellofugal fiber degeneration patterns, in cases of cerebellar lesions in the monkey and the rat, which essentially confirm the topical and somatotopical distributions reported in great detail by Dr. Brodal in respect to cerebellovestibular connections distributing throughout the vestibular nuclear complex in the cat. In regard to other findings, criticized by some neurophysiologists, is the somatotopic arrangement reported by Brodal et al., of the foreleg-hindleg regions in the lateral vestibular nucleus. Examination of material from previous studies of spinal pathways in a number of species, in the phylogenetic scale of mammals from the opossum to man, reveals that the "hindleg" region in the lateral vestibular nucleus in the monkey and chimpanzee equates quite well, analogously if not actually homologously, with the findings in the cat of Pompeiano and Brodal. Current studies in the monkey (at this laboratory) of primary vestibular fiber distribution demonstrate that the region of the lateral vestibular nucleus, from which the vestibulospinal projections to the lower thoracic and lumbosacral levels originate, apparently does not receive primary vestibular connections. These data appear to confirm and extend Walberg, Bowsher, and Brodal's observations in the cat. Thus, recent task efforts have been alined toward the examination of possible neuroanatomical species differences in the organization of the vestibular nuclear complex such as Dr. Lowenstein warned us about yesterday. To make our experimental series more truly phylogenetic, comparable studies have been initiated in frogs.

DAVEY: Dr. Brodal, in your presentation I had the impression that the vestibular nuclei seemed to be functioning all in a unilateral fashion. Are there any connections across the midline to the opposite vestibular nuclei, either in the brain stem or through some other circuitous pathway?

BRODAL: We have not studied this particular problem ourselves, but I think there is good evidence from the literature that there are fibers from the vestibular nuclei to the contralateral nuclei. However, in studies that we did, we have not found *primary* vestibular fibers crossing the midline. There are certainly quite a number of anatomical possibilities for an impulse in one vestibular nucleus to get across to the other side; for example, via the reticular formation. It would be very interesting to know these pathways. It is extremely likely that the interconnections between the vestibular nuclei may be very specific.

DOLOWITZ: Dr. Brodal, you have mentioned tract connections from the vestibule to the various extraocular nuclei. It seems incomprehensible to me that one can focus an eye without vision. Further, if we examine patients with induced nystagmus in the dark, by turning on a pinpoint light which is not consciously perceived, we can decrease the slow component speed of the nystagmus. Have any connections been found with the visual tracts?

BRODAL: From the vesticular nuclei?

DOLOWITZ: Yes.

BRODAL: Not as far as I know.

HIEBERT: Recently Dr. Fernandez and I have found some evidence for functional distribution within Deiters' nucleus. We were testing for responses to tilt, but noticed that there was a distinct difference in the resting discharge patterns between dorsally and ventrally located units within the nucleus. This is the only evidence that we could find for a functional distribution.

However, one of the things I think that came to us quite clearly was that when we repeated the tilt test, holding on to a single unit, the frequency responses did not repeat exactly, and this seemed to us quite important. The frequency changes were almost capricious—sometimes it seemed that there was a one-to-one relationship and again there wasn't—which pointed out that one cannot perhaps make a firm conclusion that you have a certain type of nuclear cell reacting to a certain type of end-organ stimulation. I think that one has to be careful in drawing conclusions about the function of the end organs from nuclear responses, since you may be dealing with a cell that is being modulated by other cells within the nuclei.

SMITH: There has been a recent report from Japan, Dr. Brodal, by Vchizono who thinks he can differentiate between inhibitory and activating nerve endings in the central nervous system. He believes the activating nerve endings contain round vesicles, whereas the inhibitory endings contain more oval vesicles. I noticed in some of your micrographs that some of the endings on the dendrites seem to have some oval vesicles in

Figure D1.—*Charting of anterograde cerebellofugal fiber degeneration (coarse dots) and terminal patterns (finer stipple) subsequent to cerebellar lesions (solid black) in monkey and rat. Except for the ipsilateral projections to the parvocellular reticular formation* (pc) *in the rat (not observed in the monkey or cat), the patterns of distribution to the vestibular complex and medullary reticular formation appear essentially homologous to those reported in the cat by Brodal et al. Essential abbreviations:* BCde, *descending brachium;* Gc, *nuc. gigantocellularis;* Lr, *nuc. lateralis reticularis;* nPas, *nuc. parasolitarius (Brodal);* pc, *nuc. parvocellularis. Vestibular nuclei:* Vl, *lateralis;* Vm, *medialis;* Vde, *descending;* X, *nuc. of Brodal;* sV, *pars subtrigeminalis of* Lr.

them, and I wondered whether you have made any observations on different kinds of nerve endings along the dendrites and whether you were able to find the differences described by Vchizono within them?

BRODAL: Personally I have not studied them, but Walberg in our department has observed these oval vesicles. Of course, when you come across them you think, "A-ha, this is something special." However, a closer study led Walberg to the conclusion that these oval vesicles are a result of fixation of the tissue with aldehydes. May I add as my personal belief that I do not think that, even if there were and are differences in the shape of synaptic vesicles, they would be correlated with different transmitters.

The Relationship Between the Unit Activity of the Utricle-Saccule of the Frog and Information Transfer

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SUMMARY

The problem discussed is the coding system of the otolith organ in the inner ear. The spontaneous firing and the evoked responses of single otolith units show such a marked irregularity that the mechanism of information transmission cannot be based on instantaneous frequency modulation. Averaging of a single-unit discharge requires a long period of time to obtain enough accuracy, whereas the reflex mechanisms of balance act on a split-second basis. Evoked responses from single units bear a logarithmic relation to the stimulus both during transients and during steady states. Therefore, a theory of information based on "edges" is not completely satisfactory inasmuch as a graded response is not required.

A new theory of information transmission is therefore presented, based on instantaneous averaging of the activity of a number of single otolith units through a time gate similar to that proposed for the auditory pathways.

INTRODUCTION

The basic problem discussed in this paper is: by which coding system is information conveyed from the peripheral gravitoceptors to the central analyzers in the frog, and presumably in other animals? In fact, given the characteristics of the activity of single otolith units in the intact animal, such transfer of information is not easily understood.

One of the cardinal functions of the gravitoceptor organs is to maintain posture, induced mainly by a shift in the body's center of gravity. To accomplish this, information coming from the sensory cells must be: (1) prompt, because to correct a tendency to fall in any direction, action must be taken within a fraction of a second; (2) sensitive, since it is known that a deviation of only 2° to 4° from the vertical can be detected (ref. 1); (3) precise, with continuous monitoring, generating self-correcting movements.

To study the information mechanism, the activity of more than 400 utriculosaccular units has been directly recorded for long periods of time with chronically implanted microelectrodes. Three parameters have been studied: (1) spontaneous firing in the absence of any stimulus, in some instances even excluding the stimulus of gravity; (2) response to continuously increasing or decreasing acceleration; (3) response to constant acceleration (gravity component).

METHOD

The activity of single otolith units has been recorded with chronic microelectrodes inserted in the VIIIth nerve. Stimulation was applied in laboratory experiments by means of a tilting table. Parabolic flight maneuvers were used in generating weightlessness and both positive and negative subgravity states with reference to the animal preparation. Details of this technique have been described elsewhere (refs. 2–4). Suffice it here to mention that the inflight preparation was immersed in water. The accelerations applied were measured directly by appropriate accelerometers. Data were analyzed by means of a CAT 400 and ancillary equipment to obtain histograms of intervals, to measure consecutive intervals and acceleration as a function of time, and to measure the intervals as a function of the stimulus applied.

RESULTS

Spontaneous Firing

All units showed spontaneous firing, the main characteristic of which was a marked irregularity. This resulted from two components: a basic slow rhythm which is quite variable and occasional bursts of shorter intervals in groups of from two to six spikes. These characteristics are shown in figures 2, 3, and 4 which are from ground-based experiments, and in figure 1, obtained during a prolonged period of level flight at 1 g. It will be noticed that in the latter, the overall rate of firing is lower, due to immersion in water, but the characteristics of the otolith activity remain generally the same.

The two rhythms, the slow basic one and the bursts, give a bimodal distribution of the interval values as shown in figures 3 and 4. The first peak corresponds to the bursts, the second one to the slow rhythm. During the first 2 hours after the implantation of the electrode, the first peak decreases progressively, and reaches a steady state, with minor fluctuations. This seems to indicate that the bursts are related to the initial handling of the preparation, but they do not result solely from this cause, since they are maintained throughout the experiment, even after 48 hours (fig. 4).

The possibility arises here that there might be more than a single unit in the recording. Irregularity might be a result of the summation of the two rhythms from units firing at relatively constant but differing rates. To eliminate such a possibility, the total interval data of the part of the experiment being analyzed are

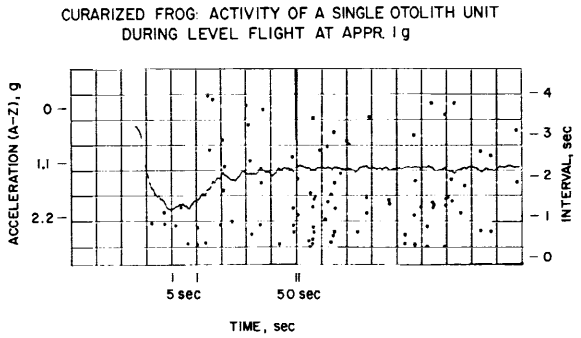

Figure 1.—*Otolith unit responding to vertical acceleration only. Computer analysis of intervals and acceleration as a function of time during a period of level flight. Consecutive intervals are measured as the distance from the "0" base line and each black dot (ordinate on right). Note that a minimum interval exists through the record, indicated by the distance between the base line and the nearest dot. Acceleration is indicated by the continuous line. "0" corresponds to weightlessness during parabolic flight. At the extreme left the plane is emerging from a parabola. Note the appearance of longer intervals as soon as the acceleration goes below 1.1 g from 2.2 g; i.e., below the threshold for this particular unit. A period of spontaneous activity follows.*

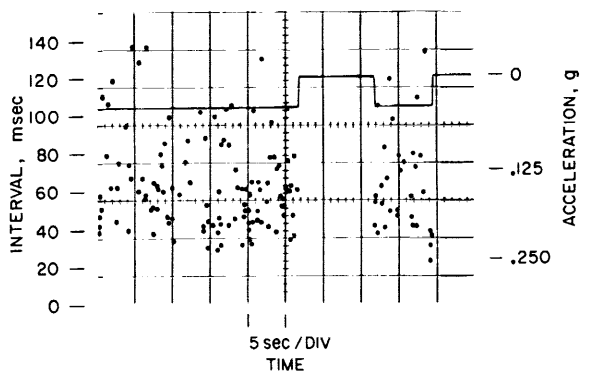

Figure 2.—*Computer analysis of the activity of an otolith unit during a spontaneous discharge at constant acceleration and during subthreshold rapid changes of acceleration. Intervals are measured as in figure 1. "0" g is set at an arbitrary value. Note the pause in spontaneous firing during fast back tilt.*

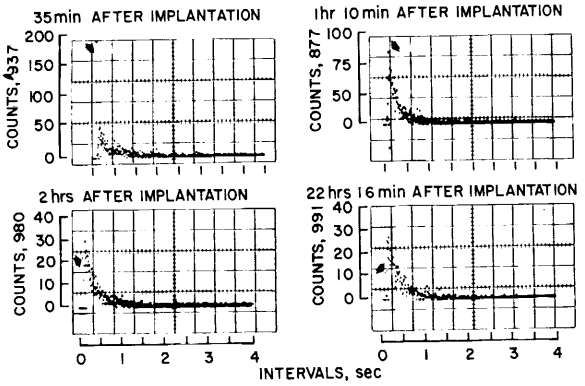

Figure 3.—*Histograms of intervals during spontaneous firing of the same unit over a 48-hour period. Results of the first 24 hours. Note bimodal distribution; first peak arrowed.*

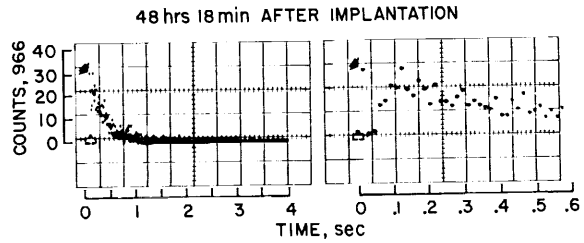

Figure 4.—*The same as figure 3 after 48 hours. On the right, same histogram with an expanded time base for better demonstration of the bimodal distribution. First peak arrowed.*

checked by means of a histogram with a short time base (25 msec) to demonstrate the existence of a minimum interval value (fig. 5). The existence of a minimum interval of some milliseconds will exclude the presence of a second unit, since over a large number of counts even two units firing with different rhythms are bound to show a range of intervals ending in complete synchrony. A range of minimum intervals of from 3 to 7 msec has been found in the various units regardless of their level of excitation.

As far as information is concerned, the spontaneous activity might be considered insignificant; i.e., related to the general life mechanisms of the receptor cells but not to their specific function. That this is not the case seems to be indicated by the prolonged suppressor effect observed when a sudden decrease in stimulation occurs (fig. 2). As a result, spontaneous firing is stopped; it can be restored by increasing the

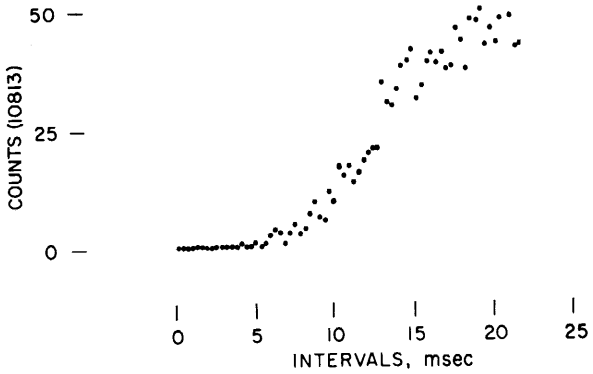

Figure 5.—*Histogram of a prolonged firing of the same unit: only the lower end of the histogram is shown to indicate the existence of a minimum interval of 4.5 msec. This proves that the activity of only one unit is recorded.*

stimulus process. It is also resumed after a certain time without applying below-threshold acceleration. The described phenomenon shows that in certain conditions, spontaneous firing becomes altered, thus indicating a change of position of the head. The importance of this phenomenon will be discussed later.

Response to Continuously Increasing and Decreasing Acceleration

A proportionally equal variability in the rate of firing with change in stimulus is shown during excitation. With an increasing stimulus (tilting with the appropriate direction and angle, or increasing the linear acceleration during level flights), the rate of firing increases. But a large variability in the resulting interval values still persists (fig. 6). As shown, each value of acceleration corresponds to a large range of intervals. This is shown better in figure 7, in which intervals are plotted as a function of acceleration. What really happens here is a progressive disappearance of the longest intervals; the envelope of the two-dimensional figure closely approximates a logarithmic curve.

This effect has been observed in the response of all the receptors studied. They differ greatly in the sensitivity range, from the ones reaching saturation at 0.2 g to the ones in which the entire change in interval value takes place for a change in acceleration of 0.005 g (fig. 8).

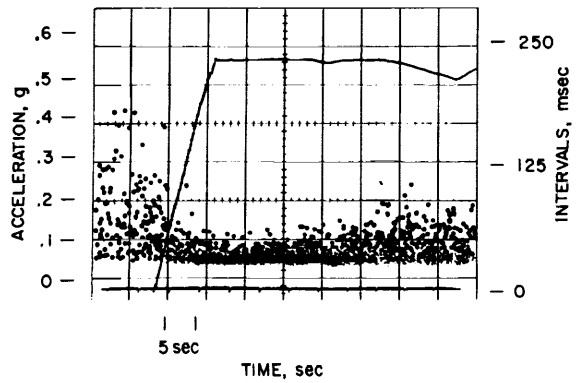

Figure 6.—*Computer analysis, same as figure 2. Acceleration is indicated by the continuous line. An arbitrary "0" has been established at the origin of the ordinate (on left) below the threshold for the otolith unit.*

Increasing the acceleration above threshold causes a progressive elimination of the longest intervals. When the acceleration is decreased (extreme right of figure) longer intervals start appearing again. Note the nearly complete lack of accommodation.

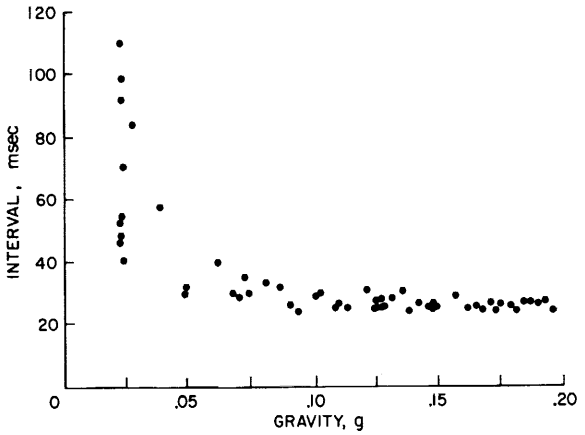

Figure 7.—*Computer analysis of interval changes as a function of the acceleration applied. Intervals measured as in figure 6 (ordinate). Acceleration on the abscissa. The progressive disappearance of the longest intervals approximates a logarithmic ratio with the increasing accelerator stimulus.*

However, the general pattern of the response is the same.

Decrease of the stimulus always produces a peculiar effect. The rate of firing slows abruptly, independently of the absolute value of the stimulus; this is followed by a complete suppression of firing for some seconds; then normal spontaneous activity is restored (fig. 9).

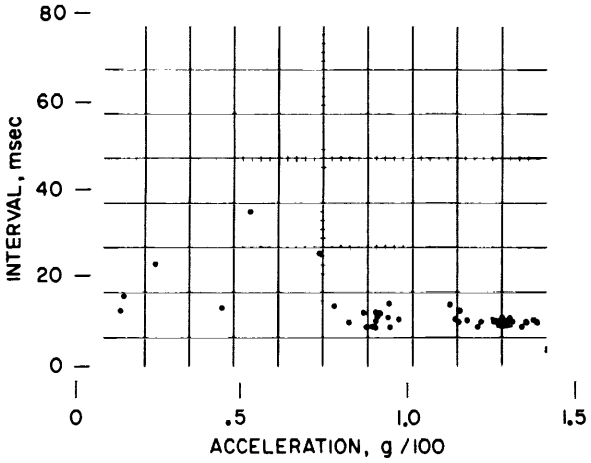

Figure 8.—*Computer analysis, same as in figure 7, but for a highly sensitive otolith unit. The entire range of this unit corresponds to less than 5/1000 of 1 g.*

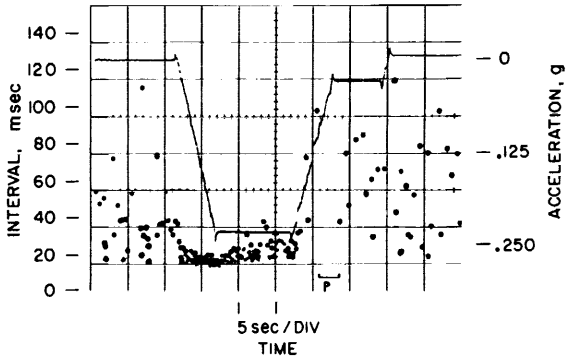

Figure 9.—*Computer analysis of the activity of an otolith unit, performed as in figure 2. During constant stimulation this unit shows accommodation. Note the pause corresponding to the onset of the decrease of acceleration (underlined and marked "P").*

Response to Constant Acceleration

Two kinds of gravitoceptors have been found: one with relatively rapid accommodation (fig. 9), and one showing almost no accommodation (fig. 10). For the latter group it is possible, by the use of histograms, to compare the time-

interval distribution, at different levels of steady-state excitation, with the distribution for spontaneous activity (fig. 11). The results indicate that while the peaks do not shift significantly, two major changes nevertheless occur: the tails shorten and there is a large concentration of data in a narrower range. These two changes are significant. These responses seem to bear a logarithmic relation to the stimulus and might provide the basic code of sensory information.

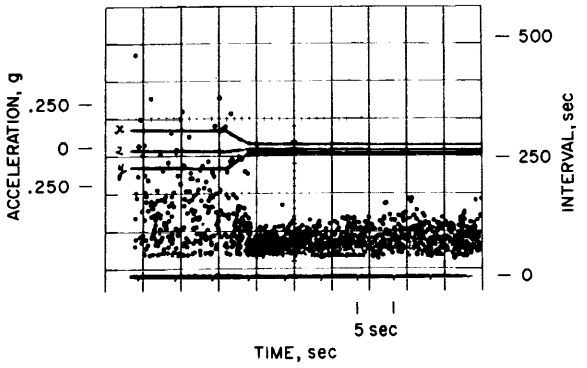

Figure 10.—*Computer analysis, as in figure 2. This unit does not show any accommodation at constant stimulation. Note that the acceleration has to be applied to this unit in a direction halfway between the x and y axes.*

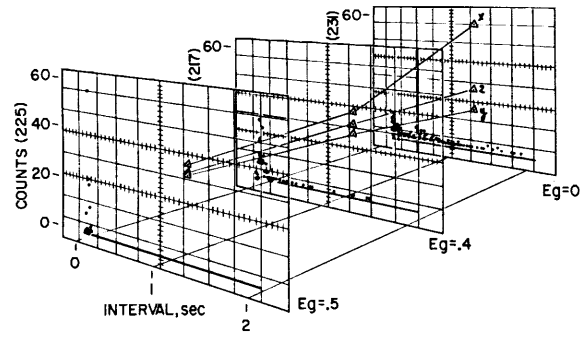

Figure 11.—*Histograms of intervals during the activity of the same unit during spontaneous firing* (Eg=*0*), *moderate accelerator stimulation* (Eg=*0.4*) *and supramaximal stimulation* (Eg=*0.5*). *On the abscissa: interval value in seconds. On the ordinate: number of intervals. Total count for each histogram is given in parentheses. The three acceleration changes are shown.*

DISCUSSION

The irregularity of both spontaneous firing and the response of the vestibular unit in animal preparations has been observed by several authors starting with Ross (ref. 5), who recorded the activity of the single isolated vestibular fiber in the severed frog head, and later by Adrian (ref. 6) in his classic work on the decerebrate cat. Records from papers by Gernandt (refs. 7 and 8) and by Rupert et al. (ref. 9) show the same variability in the discharge as described above. All these authors have used decerebrate animals, which might explain the large variability observed.

However, Bizzi et al. (ref. 10) recorded from the vestibular nuclei of an intact cat, free to move, and reported more or less irregular discharges from the various vestibular nuclei. In this case the objection may be raised that the irregularity is characteristic of the activity of higher order neurons but not of the receptors proper. The results reported here, using chronically implanted electrodes and prolonged recording (up to 48 hours) in the uncurarized and unanesthetized animal, are not subject to this objection and confirm that the results obtained with isolated preparations and decerebrate animals correspond to the true physiological firing characteristics. This being the case, the following remarks may be made.

(1) Both during spontaneous activity and during excitation, the rate of firing varies over such a large range that the mechanism of information transmission cannot be based on instantaneous frequency modulation. In fact, while intervals greater than a critical value are associated only with a low level of excitation, in the case of shorter intervals there is no way to determine to which specific value of excitation a given interval belongs.

(2) A mechanism based on time averaging of the output from a single unit could not work. Indeed, to provide enough precision the analyzers should average a large number of intervals. This process would require some seconds and would not allow a fast balance mechanism, the need for which has been previously pointed out.

(3) The results seem to agree, up to a point, with the theory of "edges" which was recently discussed by Whitfield (ref. 11). The difference in activity is particularly sharp between—

(*a*) units that are stimulated at an increasing or constant level; and
(*b*) units subjected to a decrease in stimulation.

The main rate of firing increases in (*a*), while in (*b*) firing is temporarily arrested. In this way contrast during any given movement of the head is sharpened. Fast movements suppress spontaneous firing of quiescent units. Thus, contrast is especially pronounced.

There are some elements which do not completely fit into the theory of "edges." Taking into account the reciprocal of the envelope of the two-dimensional figure, plotting interval changes against acceleration (fig. 7), a frequency curve results, indicating that the response bears a logarithmic relation to the stimulus both for transients and for the steady state. There is, therefore, a graded response which is not required for the "edge" theory. In that case only two steplike kinds of activity are necessary, easily distinguishable one from the other. In fact a graded response is a confusing factor as the edges' limit will not be as sharp as required.

It may then be tentatively suggested that the information coming from the otolith organ is analyzed centrally on a statistical basis but, to prevent delay, not using time as the abscissa. By what physical mechanism, however, such an analysis is made, remains to be discussed. The following hypothesis is proposed.

Let us imagine (fig. 12) a time gate, at the level immediately above the peripheral organs, on which a number of otolith units converge. Let us suppose that such a gate opens, allowing the information to go through, only when two or more spikes reach it within a given time. Naturally the probability of transfer in this system will be a function of the density of the spikes in each channel as shown in this figure for two units only.

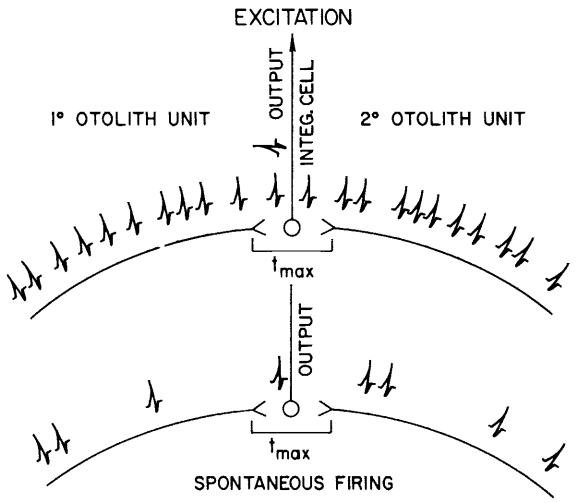

Figure 12.—*Schematic of a possible mechanism for central analysis of the information incoming from the otolith unit. Two conditions are shown, one during spontaneous activity with long intervals and high variability (lower sketch), and one during excitation. The long intervals have disappeared and although there is still some variability, all intervals are shorter than the maximum time for the gate to open.*
t_{max}: *maximum time at the gate that allows the spikes coming along the otolith nerve fiber and converging on the analyzer (integrative cell) to trigger the analyzer to activity. This happens when the spikes coming from the two pathways reach the integrative cell within* t_{max}. *For a full discussion, see text.*

Desmedt (ref. 12) has postulated a "gate control system" for the auditory system, and Wall and Melzack (ref. 13) for the general sensory apparatus. Indeed, this mechanism would also explain the progressive decrease of the rate of firing in the higher levels, which has been described for all sensory networks.

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DISCUSSION

LOWENSTEIN: We have to record information from the intact animal to get any idea how the sensory system works. If Nature had wanted to use single units for information transfer, it would have done so. It has chosen to work with populations.

I think it would be interpretively unproductive if one were to ask whether, in any single instance, the fibers recorded from were single- or two-fiber or three-fiber systems. I am fully prepared to assume that all the fibers which we have been hearing about this morning were in fact single units. The interesting situation arises that these results have borne out a number of facts which could be predicted in a way from the single-unit recordings reported on in my preceding paper.

Regarding the multiplicity of types of units, you will remember that we have in the elasmobranch utriculus, units which accommodate completely to a resting state in a very short time. We call those the out-of-position units, and they are usually extremely sensitive units. There are units with wide peaks, and units with very narrow peaks. There is a range fractionation around the whole circle of tilt, and so on. So, we have a population of units which differ qualitatively and quantitatively in their response characteristics.

The great value of experiments on the intact animal is that for once we see what the central nervous system does with its peripheral units. When we recorded from peripheral units, we had isolated them rigidly from the central nervous system. There was no interference from the center at all. The only suspicion of sensory interference which we could have were those from galvanic polarization experiments which showed that behavior of the units can be influenced.

The interpretation of how the central nervous system makes sense of the varied inflow from the periphery, I think is credible, and I would accept the hypothesis. On the basis of parallel considerations from various sensory fields with the hypothesis of the "edge," I accept that the "edge" is not the whole explanation. I think a gating mechanism such as was shown will very likely be the final answer. How one is to record simultaneously from a number of units is, of course, a different question.

Were these tilts very rapid ones?

GUALTIEROTTI: No. As you can see, the time marker is about half a second; so the tilt would be completed in 10 seconds or more. Rapid tilts would give a different response; however, I did not go into that.

LOWENSTEIN: You remember the experiments in which rapid tilts in fact give paradoxical results?

GUALTIEROTTI: I made a study of the response of the single unit to the same amount of stimulation, but varying the speed of stimulation. What happened was that going from just threshold to saturation, we got a response such as I have shown. If we perform a given tilt slowly enough, say 6 degrees in 1 second, we get a logarithmic curve describing the stimulation response ratio. By increasing the speed of tilting, for example, 6° in 0.7 or 0.1 second, there is an increasingly pronounced peak.

Spinal Reflexes During Increased Gravity

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FRANCESCO BRACCHI,
AND
BENJAMIN EPSTEIN
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SUMMARY

1. A method is described for preparing an experimental animal (cat or monkey) to obtain monosynaptic responses in a chronic preparation.
2. The spinal reflexes are facilitated during acceleration.
3. It is postulated that a vestibular mechanism may influence this phenomenon.

INTRODUCTION

The effects of increased gravity upon the body, first noted by military pilots and then by astronauts during takeoff and reentry maneuvers, have been studied from various points of view: psychological, behavioral, cardiovascular, respiratory, visual, and so on. While the effects of increased gravity on the spinal reflexes have not as yet been described, the present knowledge of spinal reflexes suggests that they can yield much information on the effects of increased gravity on the central nervous system.

The obvious difficulties which would be encountered in the study of spinal reflexes in man has led us to select appropriate laboratory animals for initial studies. We have chosen the cat and the monkey because the parameters of the spinal reflexes in the cat are well defined in the literature, and the sitting position of the monkey resembles the standing position of man.

Among the spinal reflexes the monosynaptic reflex is the simplest mechanism between input and output of the central nervous system. Therefore, it has been selected as the basis of our studies.

It has been demonstrated in the decerebrate cat that the monosynaptic reflexes are altered by angular acceleration (ref. 1). However, the effect of linear acceleration on the monosynaptic reflexes has not been studied and, further, no studies have been performed on the awake and intact animal.

Chronic preparations offer some advantage over acute preparations. One can perform the same experiment on the same animal under the same conditions many times, and errors in the interpretation of results due to different levels of anesthesia, experimental shock, or hormonal imbalances are avoided (refs. 2 and 3).

MATERIALS AND METHODS

We have studied seven cats and two monkeys (*Macaca mulatta*). Stimulating electrodes were implanted in both species, elaborating on a technique first used by Baldissera (personal communication) on cats. This technique is similar for both cats and monkeys.

Under general anesthesia (Nembutal, 40 mg per kilogram) the laminae of the last five lumbar vertebrae were exposed bilaterally, and the corresponding portions of the erector spinae were removed bilaterally. A hemilaminectomy was performed on the side in which the electrodes were to be implanted (usually the left side). If L6 were selected for stimulation, the left laminae of L5 and L6 were removed (fig. 1).

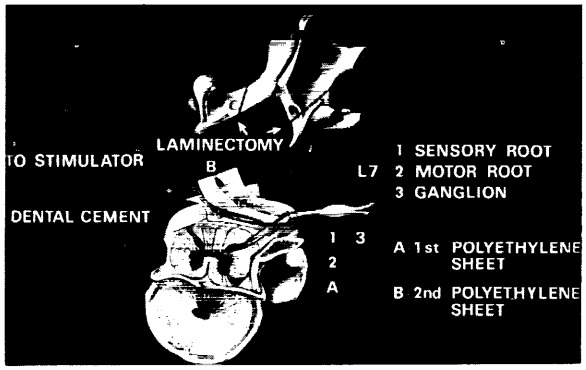

Figure 1.—*Chronic electrode implant on cat sensory root.*

The dura was exposed and the preselected root was inspected at the point of emergence into the spinal canal. A small dental-cement bridge was made to fuse the right laminae of L5 and L6. Two short segments of polyethylene tubing (18 gage) were embedded in the medial edge of this bridge. These tubes held a small strip of plastic tissue, about 2 centimeters in length by 8 millimeters in width, at their spinal end by means of flaring. The long axis of the strip was perpendicular to the long axis of the animal. The dura was then opened and, under a microscope, the sensory part of the root was dissected in a pool of normal saline solution at body temperature. Once the sensory root was isolated, another piece of polyethylene tissue was placed between the root and the rest of the spinal cord, wrapping all of the exposed surface of the latter. Two sections of 36-gage silver or gold wire, insulated by 30-gage polyethylene tubing, were pushed through the fixed polyethylene sleeve in the dental cement. The ends of the wires were wrapped around the sensory root at a distance of 6 millimeters apart. To prevent infiltration of physiological fluids into the preparation, which could cause electrical short circuits, Gelfoam® soaked in mineral oil was applied at both ends of the polyethylene tissue. The long strip of polyethylene tissue was then wrapped around the root and brought back into contact with the other end of the same tissue. At this point the distal ends of the stimulating electrodes were soldered to a connector, and stimulation was performed to test the preparation. If the system proved satisfactory, it was embedded in dental cement and the animal was allowed to recuperate for a few days. These preparations were used for 15–20 days with consistent results.

Stimulation of the root was accomplished by square waves of 100 microseconds' duration and an intensity ranging from 400 millivolts to 1 volt according to the preparation. Stimuli were delivered every 3 seconds.

Recordings were obtained from the hamstring muscles by means of chloridated silver wire electrodes. The active electrode was within the hamstrings and the indifferent electrode was under the skin in the area of the knee. During baseline recording and centrifugation, stimulation was performed at intensities supraliminal for the monosynaptic reflex, but with no signs of discomfort to the animals. However, in order to ascertain that there was no spread of stimulation to other muscles, the stimulus was increased to levels producing signs of discomfort; however, no spread of muscle contraction was noticed.

In two cats recordings were made from the intact sciatic nerve by placing two silver electrodes around the nerve. These electrodes were wrapped and isolated from the surrounding tissues with a thin polyethylene sheet. The end wires of these recording electrodes were tunneled under the skin to the same connector embedded in the dental cement of the spinal preparation described above.

The package in which the animal was placed to be centrifuged consisted of a frame to which the electronic equipment, the stimulator, and amplifiers were fixed (fig. 2).

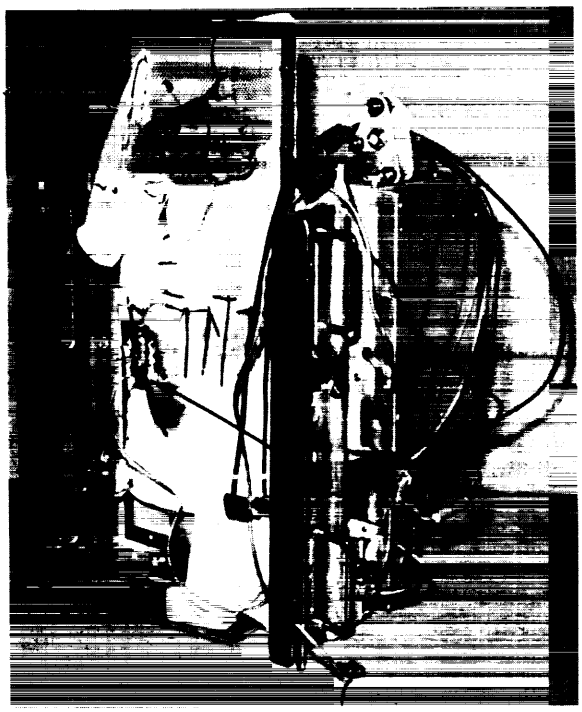

Figure 2.—*Cat on restraint ready to be placed in the centrifuge. Arrow points to electrical connector attached to animal.*

The cat rested on its abdomen in a nylon hammock in which perforations for the extremities had been made. The cat was gently but firmly fixed into this position. For the monkey, a contour chair was used.

The transistorized stimulator was of the constant-voltage type. It was battery operated and electrically floating from ground, which prevents stimulus artifacts. It was developed at Ames Research Center by Rodger Hayes. The bioamplifiers, also developed at Ames, are described elsewhere (ref. 4).

The centrifuge used in our experiments has an arm length of 8.2 meters. It has speed controls which permit an operator or an automatic programer to vary the angular acceleration and velocity at will. This allows any linear acceleration in the cabin, ranging from 1 g to 20 g. The cabin measures 1.5 x 1.5 x 2.0 meters, and is completely enclosed so that no visual clues to motion are allowed. A television camera is placed inside the cabin to observe the animal's activity. Vibration of the centrifuge is negligible. The animals were placed in the centrifuge in such a position that the acceleration would be directed in the transverse PAG or prone g direction.

To adapt the animals to the experimental package, they were placed in it about 3 hours before centrifugation. The spinal reflexes under study were continuously tested during this period; the animals accepted food and water, and no signs of discomfort were noted. The animal was then taken to the centrifuge, placed inside the cabin, and isolated for 1 hour to allow for further adaptation. The baseline was then recorded.

All data were simultaneously recorded on magnetic tape (Ampex 1300), recorded on an ink-writing oscillograph (Brush Mark 200), and displayed on an oscilloscope for monitoring purposes. The animals were subjected to randomly selected linear accelerations, which ranged from ½ to 2 accelerational g's over normal environmental gravity. In this paper, therefore, 1 accelerational g is used to denote environmental gravity, plus 1 g of rotational acceleration. The processing of the data was done using a Mnemotron CAT 400B computer with accessories developed at Ames. The analog output was displayed and photographed on an oscilloscope. The digital output was printed for further statistical analysis.

RESULTS

Although our studies have covered a wider range, this paper is limited to the effect of 1 accelerational g over environmental gravity applied for a period of 2 to 5 minutes.

In the awake animal, under stimulation with constant parameters, the monosynaptic reflex shows a spontaneous variation in amplitude from one response to another. At the onset of 1 g of accelerational gravity (fig. 3) and for several seconds after, this spontaneous variation disappears, and the amplitude of the monosynaptic responses is increased. After acceleration, the return to the initial baseline values took place in a few seconds in all animals. Only after repeated accelerations and particularly after higher accelerational levels (2 g) was a fall in the amplitude of the reflexes observed.

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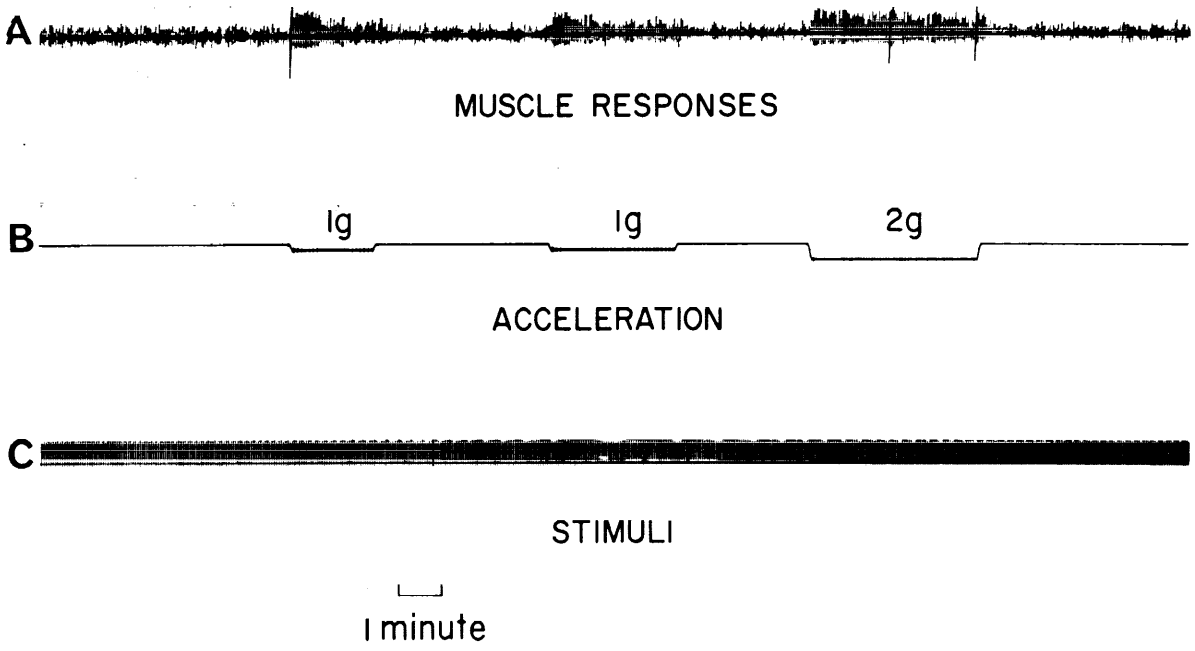

Figure 3.—(A) *Reflex responses recorded from the hamstring muscles.* (B) *Accelerometer readings.* (C) *Stimuli.*

The peak amplitude distribution, cumulative distribution, and averaging of the monosynaptic responses were used in treating the data, both for the determination of the baseline and for the study of the reflexes during centrifugation.

The peak amplitudes of the reflex discharge appear to follow a normal distribution (fig. 4), provided that the animal remains in a steady state; i.e., not disturbed nor asleep. If any disturbance occurs, particularly when the animal is directly involved, such as manipulation of the animal, a marked decrease of the amplitudes of the reflex responses occurs, and the distribution curve ceases to be normal because of an increase in the proportion of these small amplitudes.

When the animal is subject to 1 acceleration g, the distribution is no longer normal, but is skewed to the right because of an increase in the proportion of high amplitudes.

We have observed that if the average value of a number of discharges is computed during a steady state, the peak-to-peak values for two different samples taken from 10 to 45 minutes apart differ by less than 5 percent (fig. 5).

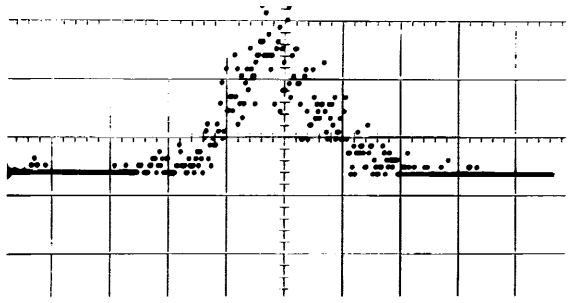

Figure 4.—*Histogram of peak amplitudes during steady state. Abscissa, amplitude in volts. Ordinate, number of events.*

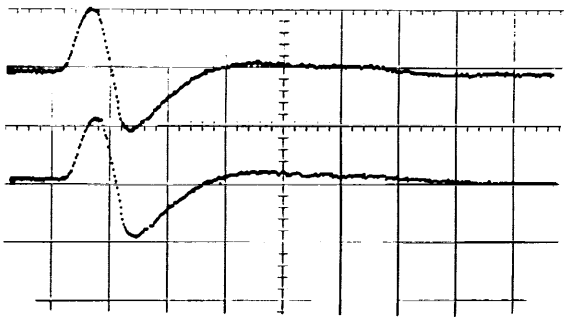

Figure 5.—*Steady-state 100-sample averages, taken 30 minutes apart.*

Again the average values of samples of the same number of responses are altered when the animal is subjected to acceleration, and the peak-to-peak values become significantly larger (fig. 6).

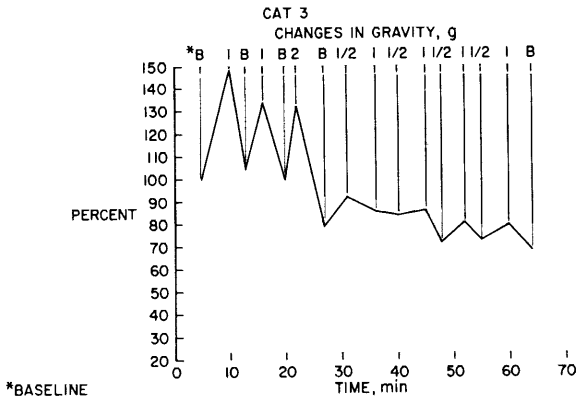

Figure 6.—*Plot of the 50-sample average values of monosynaptic response during increased gravity. Ordinate represents the values as a percent of value of the first sample (100 percent) taken during steady state.*

The baseline values in figure 6 are each an average of the amplitudes of 50 steady-state reflex responses. For further and more complete analysis of data, statistical analyses based on the cumulative distribution were performed. The cumulative distribution of this sample of 50 values appears to fall along the same straight line when plotted on normal probability paper. This is represented by line B in figure 7 (the statistical meaning of such a straight line is that the amplitude distribution of the reflex responses taken during steady state is normal). When the animal is subjected to 1 accelerational g the cumulative distribution is no longer linear when plotted on probability paper (fig. 7). The reason for this appears to be the larger proportion of high amplitudes. Both in figure 6 and in figure 7, it appears that after repeated centrifugation, the facilitatory response is decreased progressively.

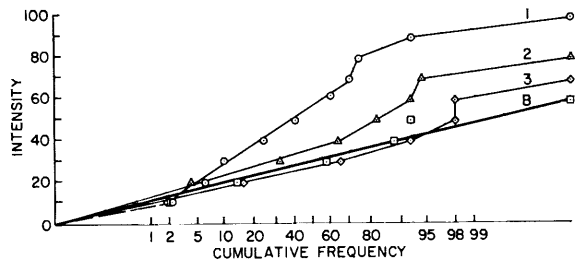

Figure 7.—*Cumulative distribution of data in figure 6. The straight line* B *represents the first three baselines* (B) *shown in figure 6. Lines 1, 2, and 3 represent the distribution of data taken during the first, second, and third accelerations.*

DISCUSSION

The modifications observed in the spinal reflexes can be due to any of the following factors.

A. Changes in the blood supply to the central nervous system.
B. Stretching of the muscles being tested.
C. Changes in the state of consciousness.
D. Modifications of the psychological bias of the animal.
E. Vestibular influences.

A. Changes in the Blood Supply

Our animals were accelerated along the PAG or transverse prone g-axis to eliminate accelerational forces parallel to the axis of the large vessels. Furthermore, the instantaneous increase in the amplitude of the responses with the onset of the accelerational g suggests that the spinal circulation is not a primary factor in the change of these reflexes.

B. Stretching of Muscles Involved

During experimentation, the animals were fully supported by a nylon net which prevented displacement of the trunk, and the legs were not receiving the weight of the animal. In addition, the limbs were firmly fixed to the supporting frame; this arrangement eliminated displacement of the joints and the stretching or shortening of the muscle under investigation. Therefore, the changes observed were not muscular in origin.

C. Changes in the State of Consciousness

Adequate description of the control of consciousness is a difficult task. However, previous studies in our own laboratories (ref. 5), by Baldissera et al. (ref. 6), and by Giaquinto et al.

(ref. 7) have shown that the spinal reflexes are changed only during the slow-wave and paradoxical phases of sleep, and that the change is expressed as an inhibition of the reflexes. This is not the case in the present experiments, since the animal was constantly alert.

D. Modifications in the Psychological Bias

This also is hard to quantify, but the following observations suggest a rather constant psychological bias: The results are repeatable in the same animal on different occasions and on different days; there is no increase in the spontaneous contractions of the muscles or signs of discomfort observed during the experiment; the animal continues to accept food and water. Moreover, manipulation of the animal produced a marked inhibition of the reflexes. Before the animal accepts the package as his temporary habitat, he struggles and this immediately inhibits the reflexes. These observations indicate that the facilitation observed in our experiments is not due to psychological factors.

E. Vestibular Influences

A vestibular effect facilitating the spinal reflexes during angular acceleration has been described by Gernandt et al. (ref. 1) on the decerebrate cat. Preliminary experiments performed in our laboratory with cats show that caloric tests produce a marked and prolonged facilitation of the monosynaptic reflexes which outlasts the nystagmus phase and continues for as long as 1 hour. The above factors indicate that the vestibular apparatus can produce facilitation of the monosynaptic reflexes. The vestibular apparatus is the primary sensor of gravity. The evidence presented here points toward a possible vestibular mechanism producing facilitation during increased gravity. We propose to continue our studies, including sectioning of both vestibular nerves, in order to determine whether or not the vestibular system is involved in these changes. The mechanism responsible for shorter and less intense facilitatory responses of the monosynaptic reflexes after each consecutive acceleration also remains to be found.

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DISCUSSION

RALSTON: Did I hear you say that conscious states do not produce changes in the spinal reflexes except in the way of diminution during sleep?

HUERTAS: Changes in the state of consciousness may be classified as follows: awake and asleep. If the animal is awake, there are changes of one type; if asleep, the changes produced are those of inhibition of the reflex. If the animal is awake and struggles, it shows an immediate facilitation or reflex. In our case we manipulated and placed the animal in such a state that it was neither asleep nor excited.

RALSTON: There are very marked effects of rela-

tively minor changes in conscious states in man on reflex excitability. We have been doing a lot of this in our laboratory and it is really extraordinary, the changes in the EMG which occur, let's say, in the knee jerk, as a result of relatively minor changes in conscious states.

HUERTAS: What do you call minor?

RALSTON: Such things as slight voluntary efforts, doing mental arithmetic, etc., will produce changes in the EMG response from the quadriceps.

HUERTAS: Minor marginal efforts in the cat are hard to detect. We monitored the EMG constantly, and any radical change in its pattern resulted in the exclusion of the data obtained during such period.

RALSTON: I am not denying that. This may very well be primarily a vestibular thing, but I would say that changes in the conscious state of a relatively minor sort in man certainly produce large changes.

HUERTAS: I am not debating that. I only said that we had a steady state, which I think is very acceptable. We couldn't do certain manipulations such as a cat doing arithmetic.

FERNANDEZ: I noticed that in your animals the head is free and I wonder whether the neck reflexes may have some influence upon the results. It is known that neck reflexes are effective upon posture. A second point is whether the proprioceptors also have some influence in your results.

HUERTAS: Those are two very important questions and two very important criticisms. I am very well aware of the first. The head was loose because I wanted to find out first whether there existed a change in the reflexes. Fixing the head would have limited our task, ruling out the neck reflexes as a possible contributory factor. We plan to fix the head of the animal in the future and see what kind of result we are going to obtain.

In answer to your second question regarding the effect of gravity acting upon muscles, that is why we restricted our experiment to 1 g. The gravity perceived by the stretching of the muscles when they are fixed and under 1-g conditions cannot stretch the muscle.

BENSON: In experiments in man we have shown that angular accelerations produce a much greater change in the tendon reflex than in the electrically elicited H reflex. This we interpreted as a manifestation of increased activity in the gamma motoneuron system rather than a change in alpha motoneuron excitability.

Now, in your experiments you were primarily stimulating alpha motoneurons?

HUERTAS: Yes.

BENSON: But alpha motoneuron excitability can, through the reflex arc, be influenced by a change in gamma motoneuron activity. Would you be prepared to say whether the changes in reflex activity seen in your experiments were due solely to vestibular and other sensory afferents acting on alpha motoneurons or were gamma motoneurons also activated?

HUERTAS: The electrical stimulation in our series certainly did not involve gamma motoneurons. In the way that this experiment was performed, their action can be disregarded.

BRACCHI: In reference to Dr. Ralston's question concerning reflexes in man, I believe that you tested the knee jerk probably with a mechanical stimulus. I have done some experiments on spinal reflexes in man, using electrical stimulation, stimulating the sciatic nerve at the popliteal fossa, and I have found that during the steady state (where the psychological and conscious state of the subject examined was normal), there was very little variability in amplitude of the reflex responses recorded from the gastrocnemius. What is more, I did not observe appreciable differences in amplitudes in greatly modified psychological states, such as stressing hypnotic suggestions. The modulation of amplitude of the spinal reflexes elicited with mechanical stimulation, such as the knee jerk, seems to be due to the status of the neuromuscular spindles. In man, spinal reflexes varied during changes of consciousness; e.g., in sleep where the amplitude of the reflex is decreased during slow-wave sleep and falls to zero during paradoxical sleep. In the cat, it is quite different. With electrical stimulation of a sensory root, there is a big spontaneous modulation of amplitudes during the waking state; during slow-wave sleep, there is a tendency to stabilization with inhibition compared to average of the waking state, while during paradoxical sleep the reflexes are completely inhibited.

About Dr. Fernandez' point, the neck muscles can play a part, and if after cutting the vestibular nerve, we will have the same findings; of course, this will be a fact to be taken into account, but there are experiments reported on decerebrate cats studying the influence of neck muscles on spinal activity induced with strychnine that seem to indicate that the neck receptors alone do not play a big role in the modulation of the spinal activity.

WENDT: Some years ago I did experiments on restraint of animals. Under restrained conditions, while they don't go to sleep, they frequently go into an unalert state in which the eyes sometimes don't track together and in which the lids tend to droop. Did your animals at any time show this picture?

HUERTAS: Our experience with restrained animals has been quite extensive with monkeys in chairs. They tend to go to sleep, and there is no doubt of their sleep. If they are quiet, and their eyes are closed, the electroencephalographic changes show that the animal is asleep. Experiments on the cats were performed during working hours which coincided with waking hours of the cats. We observed them constantly, and they were always alert. We didn't observe the animal that you are describing.

WENDT: I did another experiment some years ago on tonic neck reflexes in normal men and the effect of twisting the neck on the knee jerk. This does allow you to facilitate the knee jerk on the side toward which the neck is twisted, but inhibitory phenomena cannot be demonstrated if you twist in the other direction. It is merely a facilitatory thing.

HUERTAS: The conditions of our experiment don't resemble experimentation in humans in this respect; therefore the question is very difficult to answer.

Influence of Contact Cues on the Perception of the Oculogravic Illusion

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SUMMARY

The purpose of this experiment was to study the influence of otolith and nonotolith information in the perception of the visual horizontal during rotation. Five normal men and five men with defective labyrinthine function acted as observers. All measurements were made in a room which could be rotated. Initial, static measurements were made while the men stood erect in the stationary room. Similar measurements were made during rotation while the observer stood on a platform set to the resultant horizontal with head and body alined with the resultant force. Data were also obtained with three other combinations of head and body position. This procedure was designed to produce two situations for the normal men in which otolith and nonotolith information were synergistic and three others in which they were antagonistic. The results showed that the perception of the visual horizontal during rotation in this situation is quite different from that found when the observer is rigidly supported in a chair during rotaton. Settings to the visual horizontal during rotation were not systematically related to differences in head and body position, nor were there significant differences between the normal and labyrinthine-defective men. The results show that nonotolith information predominates in this experimental situation. Furthermore, the data suggest that the spatial orientation of a pilot strapped in a cockpit may be somewhat different from his spatial orientation when he is standing on a rotating space platform.

INTRODUCTION

The purpose of this study was to investigate the role of otolith and nonotolith gravireceptors in the perception of the visual horizontal in darkness when observers *stood* on a rotating platform. It was hoped that the results would shed some light on the contribution of the nonotolith gravireceptors in the perception of the visual horizontal in normal and labyrinthine defective (L–D) men. It is well known that normal and L–D men show significant differences in such phenomena as counterrolling (ref. 1), the oculogravic illusion (refs. 2 and 3), and the perception of motion on a parallel swing (ref. 4). At the same time it is also well known that L–D men can compensate for the loss of vestibular function in certain situations. For example, Clark and Graybiel (ref. 5) have shown that in a series of 30 successive settings to the *postural* vertical, both normal and L–D men made systematic improvement. The normal men showed smaller average errors, but the differences were small, particularly after 15 trials, and were not statistically significant. Spe-

cifically, the present study compares the performance of normal and L–D men with various head and body positions to determine their influence on the perception of the visual horizontal.

PROCEDURE

Subjects

Five normal and five deaf L–D observers were studied. The normal men were medical students who showed normal responses to caloric stimulation (ref. 6) and to an ataxia test (ref. 7). The L–D observers had acquired their bilateral deafness in childhood as a sequela of meningitis and showed abnormal responses to the caloric and ataxia tests. All of the men had had experience in making observations in rotating devices and with the goggle device used to measure the perception of the visual horizontal.

Apparatus

The experiment was conducted on the Coriolis Acceleration Platform, a slow-rotation room in which it is possible to rotate observers for prolonged periods. The room is a circular, windowless room 20 feet in diameter and 10 feet high without central supporting members. It has a direct motor drive and the capability of controlled angular accelerations at rates up to 15° per second either in a clockwise or counterclockwise direction, although in this experiment it rotated only counterclockwise. Angular velocities up to 35 rpm may be maintained with an accuracy of plus or minus 1 percent. It is capable of carrying a payload of about 9000 pounds, and up to 10 persons may participate in an onboard experiment. It is well instrumented and has provision for a wide variety of laboratory equipment and living facilities. The operations required in this experiment were well within the limits of the device.

All of the observations were made with the observer's head 7.5 feet from the center of rotation of the room and at a velocity of approximately 11.9 rpm counterclockwise. This produced a change in the direction of resultant force of 20° at the observer's head. He stood facing the direction of rotation (and for a second series opposite the direction of rotation) on a platform tilted upward 20° from the floor on the outboard side of the room. As a result, when he stood erect he encountered no difficulty in standing, and the resultant force acted directly from head to foot. Thus, during rotation, he stood comfortably erect on the platform with his body weight slightly greater than normal.

The observer's task was to set a collimated, red, luminous line to the perceived horizontal. He viewed the luminous line in a self-contained apparatus mounted in a goggle which he held snugly in position before his eyes. The apparatus consisted essentially of a luminous line which was viewed by the right eye only while the left eye was in complete darkness. The luminous line could be rotated either clockwise or counterclockwise by means of a knurled knob which was easily reached by either the observer or the experimenter. The digital readout was in degrees deviation from the horizontal axis of the device itself. The goggle was easily held in place by the observer and a flexible rubber fitting prevented light leaks under the operating conditions used. Three levels were used to monitor the alinement of the goggle apparatus, the observer's head and his body. The first level was located on the goggle itself, the second on a band over his head, and the third on his back.

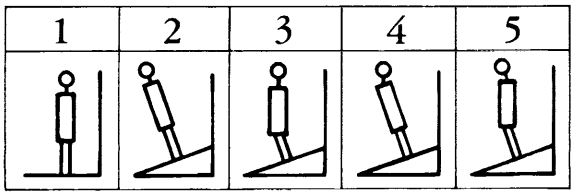
Method

All measurements were made with observer standing with his head and body in one of five different positions with respect to gravity. After the room had been maintained at a constant velocity for 1 minute, the position of his head and body and the goggle were set by means of the levels. Each trial was begun by an experimenter who offset the line from the horizontal, and observer's task was merely to set it to the *gravitational horizontal.* Three experimenters were required for every trial. One observed the level on the observer's back to monitor his body position; a second experimenter monitored the level on the head and on the goggle and offset the luminous line before each setting; and the third made and recorded the readings. No setting was recorded unless both monitors were satisfied that the proper head and body positions were maintained within a half degree.

An attempt was made to make the readings promptly in all trials because three of the positions were somewhat uncomfortable to maintain for prolonged periods. Nevertheless, the observer was permitted to take as much time as he felt he needed to make an accurate setting. The light was turned off while the line was offset. The observer made five successive settings to the horizontal for each of five conditions facing forward and then five additional settings facing backward, with an interval of several hours between the two series. The five conditions of head and body position were:

(1) Static settings made with observer standing on the floor with head and body erect and with the room stationary.
(2) Observer made settings to the visual horizontal with both head and body alined with resultant force (RF) while he stood on the platform set at 20° and with the room rotating to produce a change of direction of RF from gravity of 20°.
(3) The same as (2), except that the head and body were alined with the force of gravity.
(4) The same as (2), but the body was alined with RF while the head was alined with gravity.
(5) The same as (2), except that the body was alined with gravity while the head was alined with RF.

These five conditions are shown as follows:



RESULTS

For the purpose of analysis, all of the data during rotation were computed as deviations from the mean of each observer's settings to gravitational horizontal under static conditions (condition (1)). The mean of these static observations was considered to be his point-of-subjective-horizontal for this particular experimental situation, although the deviation of the point-of-subjective-horizontal from the gravitational horizontal in each case was very small (table 1). Therefore, all of the deviations in conditions (2) to (5) are deviations from observer's subjective horizontal using the goggle device rather than from gravity or resultant force.

An initial analysis of the data was made to determine whether the mean deviations of the settings while the observer faced forward were significantly different from those when he faced backward. Comparisons of these observations for all five conditions and for both groups of observers revealed no significant difference ($p>0.05$ or greater for all comparisons) between these two sets of observations. Consequently, the analysis of the data (tables 1 and 2) is made completely on the basis of the mean of the observations while observer faced forward and backward.

The combined data for the normal and L–D men and the five conditions (table 1) were subjected to a two-way analysis of variance (ref. 8) for repeated measures on the same elements, and the results are summarized in table 2. The analysis revealed no significant variation between the normal and the L–D observers, but the F was significant for the five conditions. The interaction between the two conditions was not significant, indicating that the profiles for the two groups have the same shape.

Perception of the Visual Horizontal Under Static Conditions

By the combined data for the first and second series of observations (condition 1), the normal men showed a mean deviation of 0.7° counterclockwise from the gravitational horizontal while the L–D's had a mean deviation of only 0.2° clockwise. Neither of these differed significantly from zero ($p>0.10$ in each case). Thus, both the normals and L–D's can be said to set the line to the gravitational horizontal with this goggle device under static conditions with a very small, insignificant error.

Table 1.—*Estimate of the Gravitational (Condition (1)) or Gravitoinertial ((2)-(5)) Horizontal, Made by Setting a Luminous Line in the Dark*

[Deviation from gravity or resultant force in degrees]

		(1)	(2)	(3)	(4)	(5)
Normal men ($N=5$)	Mean	0.7	4.4	6.3	4.4	4.0
	S.D	2.1	1.3	2.2	2.2	1.6
L–D men ($N=5$)	Mean	+.2	6.6	6.8	8.5	3.8
	S.D	1.6	3.2	2.8	2.8	2.9

Table 2.—*Analysis of Variance Summary Table*

Source of variation	Sum of squares	df	Mean square	*F*
Between subjects	90.9	9		
A. Normal—L–D	8.6	1	8.6	[a] 109
Subjects within groups	62.4	8	7.8	
Within groups	561.5	40		
B. Body position	317.8	4	79.5	[b] 11.9
A–B	28.1	4	7.0	[a] 1.0
Subjects within groups	215.6	32	6.7	

[a] $p>0.25$.
[b] $p<0.01$.

Perception of the Visual Horizontal During Rotation

During rotation the mean settings to the perceived visual horizontal deviated systematically from the resultant horizontal for conditions 2 to 5. In each case (table 1) this mean setting, which varied from 4.0° to 8.5°, was between the resultant horizontal and gravitational horizontal but much closer to the former. This means that the outboard segment of the line was set below the resultant horizontal. Specifically, both the normal and the L–D observers set the luminous line clockwise from the resultant horizontal when they faced forward and counterclockwise when they faced backward. All of these deviations were statistically significant from zero (for the normals $p<0.001$ and for the L–D's $p<0.01$ for each comparison). It should also be noted that the L–D men showed a slightly greater variance (table 1).

An additional analysis of the significance of the difference among the various combinations of head and body position *during rotation* revealed that for the normal men (table 1) there were no significant differences among conditions (2) to (5) ($p>0.05$ for all comparisons). All of these settings deviated significantly from the static settings, but head and body position did not appear to be determining factors within the limits of this experiment. It should be noted in particular that the setting of the luminous line with head and body alined with resultant force (condition 2) was no different from the setting when the head and body were alined with the force of gravity in condition (3).

Similar results were found for the L–D men with two exceptions. There were no significant differences between condition (2) and conditions (3) to (5), nor between condition (3) and (4) ($p>0.10$ in every case). There were, however, significant differences between condition (5) and conditions (2) and (4) ($p<0.01$ in each case.) It should also be noted that the low

mean performance of the L–D men was predominantly a result of the settings of one observer who set the line in the opposite sense from the others throughout his trials while he faced forward. It should also be noted that he had considerable difficulty maintaining the appropriate body and head positions, except when he stood with head and body alined with resultant force (condition 2).

DISCUSSION

The results of the static observations are well known (refs. 2, 3, and 9). Both normal and L–D men made very small errors which did not differ significantly from the gravitational horizontal. It is of interest to note that the L–D's actually showed a smaller constant error and a smaller variance than the normals. The static data also show that observations with the goggle device produce results which are similar to those found with other devices used to determine the accuracy of the perception of the visual horizontal.

The results during rotation are clear cut in showing no significant differences between normals and L–D men in setting a luminous line to the horizontal under the conditions of this experiment. The data suggest that contact information from the feet and kinesthetic information from the legs and body were adequate for the L–D observers to make the settings accurately; i.e., they were able to use the complex information available in this dynamic situation where they were required to stand erect (refs. 10 and 11). In the case of the normal observers, otolith information from the two head positions was integrated to produce a setting close to the resultant force. The particular role played by each sensory process is not made clear by these data. It is suggested, however, that kinesthetic cues are probably of special importance. This notion is supported by a study of the E-phenomenon under conditions of supported and unsupported tilt (ref. 12). By interpolation from the data, it was indicated that the E-phenomenon was about 3.5° for 20° tilts with the observer supported and that this increased to about 5.6° when he was required to maintain his own body position.

It should be emphasized that the differences between this experiment and experiments in which normal and L–D men show differences in the perception of the visual horizontal are related to the following importances in methodology:

(1) In this experiment the observer actively tilted his body from the waist and his head from the shoulders rather than being passively tilted.

(2) In the present experiment the observer was not supported in any way instead of being firmly supported in position.

(3) The observer's feet were firmly planted on the floor which was set at the resultant horizontal rather than sitting on a seat which was set at the gravitational horizontal.

(4) In the current experiment, the observer viewed a collimated, luminous line of light, but he was required to hold it in his hands rather than having the device supported independently.

(5) Observer perceived his body as being tilted away from the horizontal floor of the room by his own effort, whereas in the typical experiment on the oculogravic illusion, he perceived the chair, floor, and his body to be tilted outboard.

(6) In this study there was no pressure against the outboard side of his body as in the case of the supported, passive, apparent tilt.

The results of this experiment may be understood as a function of the complex, dynamic interaction of the many inputs from tactual receptors of the feet, kinesthetic receptors stimulated by the maintenance of bodily posture, and perhaps from other proprioceptors. In condition (3) for normals, otolith information and the nonotolith information from the head and trunk were the same as in the typical experiment in which the oculogravic illusion is observed. On the other hand, for both groups kinesthetic information in maintaining bodily posture was present as were tactile cues from the feet. Transient information was available from the semicircular canals at the time the observer tilted his head or body. Whereas in the situa-

tion in which the oculogravic illusion is observed, there is apparent tilt of the observer, the seat, and the floor, in the present experiment the information is merely that observer has tilted his body. The frame of reference for the L–D men in this experiment was, therefore, quite different with a resulting difference in the perception of the visual horizontal. It is particularly worth noting that in condition (2) where the head and the body were alined with resultant force, the point-of-subjective-horizontal was also rotated with the outboard segment downward. It is suggested that this may be explained by the fact that in this ambiguous situation, the outboard shoulder had a somewhat greater weight which was disparate with respect to the other information regarding the horizontal.

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DISCUSSION

YOUNG: With Dr. Graybiel we participated in a related set of experiments, and I would like to pose a methodological question which has puzzled us. In an attempt to produce a series of experiments testing the role of the otolith organs in perception of the vertical, for example, we used normal and L–D subjects. Do you see any way of determining whether the ability of the L–D subject to make astonishingly correct judgments is through kinesthetic and tactual cues, which the normals possess, or whether it is a compensatory mechanism which is built up through their years of having to get along without a functioning labyrinth?

CLARK: It seems to me somehow that these two things go together. I don't know how you can really separate them. Surely there are compensatory mechanisms, but they do involve other sensory processes. I think the point of this paper is really that although the otolith mechanism is very important in certain situations, when it is taken away, other mechanisms do indeed take over. Some of the repeated trials that we have done would certainly suggest that this is learned even on a short-term basis. I don't think I answered your question, but that is the best I can do.

LOWENSTEIN: I don't know how this works in man, but I think these acquired compensatory mechanisms can be disturbed by certain types of interferences or stimulations, such as noise, et cetera, momentarily. It would be quite useful to see whether the labyrinth-defective people who have learned to use other sensory cues could effectively be interrupted in doing so for short periods of time during rather cataclysmic incidents of other stimulation.

THACH: I was wondering about the lack of a statistically significant difference between the L–D and normal subjects. Your data suggest a difference, since the means were in the direction of larger numbers for L–D than for normal subjects. As you know, lack of a difference does not prove no difference; rather, it may mean that the difference is small relative to the variability, or the statistics may not be entirely appropriate. One possibility here is your stringent 0.01 confidence level. If you relaxed the requirement to a

normally quite acceptable 0.05 level, perhaps your conclusions would be reversed. A second possibility is the result of your small N of 5 subjects, small; that is, for analysis of variance techniques which customarily are applied to much larger N's. From the direction your data were moving, it seems highly likely that a larger N would have shown significance.

CLARK: This, it would seem to me, is possible. However, I think the difference is, in any event, quite small.

GUALTIEROTTI: I'd like to mention again, responding to the gentleman from MIT, the same experiment I mentioned yesterday. With a man blindfolded, floating under water, and without any movement of his joints, excluding any other input except the otolith, we find that there is no difference as far as the detection of the vertical between the same individual in this condition and in normal conditions. This means that the otolith organ (namely the gravitoceptors) is perfectly capable of working out the vertical without any further supply of information. That doesn't mean, of course, that when the vestibular organ is not there anymore, like in these people without labyrinthine input, some other systems cannot take over and substitute, as happens for any kind of sensory input. Have these means been compared statistically? I mean, did you do any test to find out how significant, if they are significant, they are?

CLARK: I'm sorry, I didn't get the first part of your question.

GUALTIEROTTI: You say that these means are not significant?

CLARK: Right.

GUALTIEROTTI: Are they proved not significant by a statistical test, or what?

CLARK: Yes; an F-test, analysis of variance.

GUALTIEROTTI: You don't compare the means?

CLARK: I thought that is what I was doing?

MAYNE: Dr. Clark, I wonder whether you made any attempt to control the time given your subjects in the detection of the vertical. A sensor could make up the poor quality of its information by averaging over a longer period.

CLARK: Yes; I might point out that these measurements were all made quite promptly. The reason for that was, as you may well imagine, when people are over and around, they don't like it particularly well; so our tendency was to make them respond as promptly as possible. That is why we had three experimenters. I would say that the judgments in every case were made quite quickly. It was a matter of a few seconds, 2 or 3, I would say. No; they could hardly set the line that quickly but within 5 or 6 seconds, perhaps.

MAYNE: I would like to confirm next that there is a a significant difference between I–D and normal subjects when they are seated.

CLARK: That is right.

FERNANDEZ: Dr. Gualtierotti has mentioned twice the same experiment. I'd like to ask him if it's true that when the subject is blindfolded and is drawn very slowly under water, and has no conception of the vertical, he is disoriented, but as soon as the head is moved, then the vertical is present.

GUALTIEROTTI: No; that is not what we found. It's a question of habituation. If you put a subject through the routine for a number of times on succeeding days, after awhile he can find his vertical quite well. That is what we found in a number of subjects. It takes at least a couple of weeks to habituate the subject in this new environment.

EPSTEIN: A number of statistical questions have been raised, and since statistics is my specialty, I would like to say a few words. It was evident from one of the questions that the term "analysis of variance" is causing some confusion. Analysis of variance is a standard statistical technique for detecting differences among means. This is done by comparing the variance from group mean to group mean, suitably normalized, with the variance among observations within groups. As a matter of fact, the widely used Student's t-test, which involves comparing two groups, can be readily formulated as an analysis-of-variance problem. What analysis of variance does is extend the capability of making comparisons to the case where there are more than two groups. I also wondered about the question of sample size, which has already been raised by one of the other gentlemen. There are two other technical questions that I would like to ask: What do you consider to be a significant difference? There is a difference between statistical significance and practical significance.

CLARK: Certainly.

EPSTEIN: What would you call practically significant? In other words, what would be the least significant difference that you would like to detect? Secondly, was more than one observation taken on a given individual?

CLARK: Five.

EPSTEIN: There were five observations on each individual?

CLARK: Each subject was represented by five observations for each condition. The level of significance that I used was the 0.01 level. When I said that there was a significant difference, it was at least at this level and usually greater. The others where they were not significant, interestingly enough, were far above this; so, I think that just on the face of the statistical data, that they are not significant.

Now, to the matter of practical significance. I think that this is a very important point, and I tried to get at this just in passing in connection with the subject's response. Subjects can set the line to within a degree. I would say a degree or two would be a range of some importance.

EPSTEIN: There were some differences that appeared to be larger.

CLARK: That is correct. But of course, as you, I am sure, would realize, the variance, particularly for the

labyrinthine-defective men, was rather substantial. The standard deviations that were also shown there showed this. In this case, in one particular case, one of the men had some difficulty in standing erect in one condition which increased the variance quite a bit.

GRAYBIEL: I can answer Mr. Mayne, I think, by an experiment Dr. Clark carried out some time ago. He had these same subjects on the centrifuge. Here they were seated passively, and the gravitoinertial vertical was changed with respect to them. Under these conditions, a normal subject will tend to aline the target with the vertical or horizontal, and he will do it with a fair degree of accuracy at an angle ϕ in the range of 20° to 30°. Using the same procedure, the L–D subjects are more erratic and may only indicate a very small change. However, we exposed them for an hour, and at the end of an hour they estimated the gravitoinertial vertical with almost the same accuracy as the normal subjects.

A crucial experiment was carried out in San Diego using the same procedures with two differences, both normal and L–D subjects wore individually fitted Fiberglas molds, thus insuring good contact with the immediate outer environment. They were exposed to changes with respect to the gravitoinertial vertical once under "dry" conditions and again when submerged in water to the neck. Under dry conditions the normal subjects again alined the target with the gravitoinertial horizontal, but the L–D subjects made an estimate somewhere between the gravitoinertial and the gravitational horizontal. When exposed submerged, the normal subjects set the line to the gravitoinertial horizontal as before, whereas in the case of the L–D subjects they set it very close to the gravitational horizontal. In other words, with loss of "contact" cues and in the absence of otoliths, the oculogravic illusion was not perceived.

We have been accepting as a challenge for a long time the attempt to demonstrate the usefulness, if you will, of the otolithic organs in man, and this is not an easy thing to demonstrate. The more we have wormed our way into this, the more it appears that there are only a few things, as Dr. Clark mentioned in the beginning, where it is easy to demonstrate differences between normal and L–D subjects. This type of investigation should be considerably expanded.

We have used as a measure of normal function of the otolith organs a counterrolling index value based on 100 normal, healthy subjects with normal hearing and normal response to irrigation of the ear. Persons with values within this range, we have said, have normal otolith function. We have found, however, that some persons with values within the "normal" range respond as if they had partial loss of otolith function.

YOUNG: In response to Dr. Gualtierotti's comment. The submerged experiments show the sufficiency of the otolith to orientation but do not indicate anything about the necessity of the otolith. In fact, it may bring up the same methodological question in which you may hypothesize that the submerged experiment in which the man must use otolithic cues is again a situation of his compensating for the lack of the tactile and kinesthetic cues. In our normal, everyday lives, we have the parallel cues coming in, and the question, I think, that Dr. Graybiel presented is, "In this everyday situation, how do we process the parallel information?" Going back to Dr. Wendt's experiment of some years ago, how does one combine the visual, the tactual, the otolithic, and other cues in a dynamic situation?

DAVEY: I just want to make an observation from the Submarine Service, and that is that men escaping at a depth from a submarine who have no visual or other cues, can't tell whether they are swimming upward or downward, and some of them do think they are going upward when they are actually going downward. It's my understanding that the new system of buoyant ascent was devised to help in forcing orientation upward simply by providing a buoyant jacket.

GUALTIEROTTI: That is quite all right. As Dr. Lowenstein showed yesterday very well, the otolith organ has a certain difficulty differentiating between the up-and-down directions along the vertical. I would like to know if these people are swimming sideways. The fact is that even if you take skindivers and put them under water and rotate them, they tend either to swim up or down, but they don't tend to swim sideways. That means that you have the idea of the vertical even if you do not find the up-or-down direction easily.

MAYNE: When we test a subject under a new situation, his responses correspond to his adaptation to normal body activity. We cannot assume that the response is a direct measure of the quality of the sensory data. This can be established only with controlled adaptation. It could be that L–D and normal subjects have adapted in very different ways to normal body activity, and the differences or similarities of their response are a measure of this adaptation as well as of the relative quality of otolith and kinesthetic sensory data.

WEISSMAN: I was wondering why Dr. Clark chose to first test the means of the backward and forward facing rather than using a three-way analysis of variance.

CLARK: Would you have suggested a four-way analysis?

WEISSMAN: No; a three way.

SESSION IV

Chairman: **WALTER H. JOHNSON**
Banting Institute

Cochairman: **MILTON A. WHITCOMB**
National Research Council

Does Linear Acceleration Modify Cupular Deflection?[1]

GEORGE H. CRAMPTON
Edgewood Arsenal

SUMMARY

Data from two experiments are described. In part I, human nystagmus was recorded with the head at the center of rotation and at a radius under a 1.06-g resultant. The magnitude of nystagmus, especially during constant velocity following angular acceleration, can be manipulated according to the orientation of the head with respect to the centripetal acceleration vector.

In part II, single cells responsive to angular acceleration were recorded from anesthetized cat brain stem with the head at the center of rotation and at a radius under a 1.03-g resultant. Consistent differences in discharge rates were not found according to various orientations of the head with respect to the centripetal acceleration vector.

It is concluded that these nystagmic changes are not due to direct acceleration effects upon the cupula, but are better explained in terms of a centrally converging otolithic influence.

INTRODUCTION

The torque applied to the endolymph within the semicircular canal during angular acceleration is quite independent of the distance of the canal from the axis of rotation. If this torque is the only force of importance for responses to angular acceleration, then these responses should not vary in any of their characteristics when elicited at some radius, as opposed to stimulation at the axis of rotation. A substantial argument has been made that if the cupula is of greater density than the endolymph, the acceleration will act directly on the cupula itself (refs. 1 and 2). If so, various orientations of the head, and thus of the cupula, will lead to unique responses when exposed to concomitant centripetal and angular acceleration.

The evidence is not in complete accord on this point. Behavioral observation techniques have failed to show centripetal effects at low g with the head alined with the radius in the frog and toad (ref. 3) or the turtle tested at 1.02 g (ref. 4). For conditions in which a man faces either radially or tangentially when seated at a radius during rotation, no subjective response changes could be attributed either to resultants of up to 1.06 g (ref. 5) or 3.07 g when facing the center (ref. 6). But some not altogether consistent data show a small reduction in the rotation-sensation durations when facing the center if the total series of magnitudes is extended to 4.81 g (ref. 7). It has been pointed out that judgments of duration of the subjective reaction are difficult at high g because the stress of the situation and the compelling character of the tilting sensation disrupt an orderly attention to rotatory effects.

The effects of centripetal acceleration on human nystagmus are much clearer. Benson and Whiteside (ref. 6) found that nystagmus is reduced at values of 3.07 g when the subject

[1] This work was carried out at the U.S. Army Medical Research Laboratory, Fort Knox, Ky.

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faces the axis of rotation. In 1962, Benson reported (ref. 8) an important variation of this experiment wherein the subject faced not directly toward the center but to the right or the left of the radius by 40°. With a positive acceleration [2] and terminal velocities producing 3.07 g, nystagmus from the head-left position was increased and of infinite duration. Under the same condition, but with the head in the head-right position, the horizontal component of nystagmus was reduced in magnitude, and actually reversed direction of beating when the terminal g was reached and continued throughout the high-g period. Crampton also reported (ref. 9) a series of observations of a very similar nature, except that the effects were demonstrated at accelerations as low as 1.06 g. In this latter case, the subject faced not right or left 40°, but 90° in either direction so that he faced along the tangent. These data, to be reported in more detail below, showed the enhancement and diminution of the nystagmic response in the same fashion as did Benson's data, although the low g was apparently insufficient to cause a reversal in the phasing of the beats. Lansberg, Guedry, and Graybiel (ref. 10) later demonstrated that a nystagmus reversal for subjects riding in the tangential facings could be produced at a moderate value of 1.66 g.

If the cupula in the ampulla of the lateral canal is hinged anteriorly, extends dorsally and is approximately parallel to the mesial plane, the nystagmus data just cited are in accord with the hypothesis that centripetal acceleration is acting directly on the cupula. But more specific evidence is required to confirm the hypothesis, because the otoliths are stimulated at very low values of linear acceleration, and otolithic stimulation is known to have clear influences on nystagmic function (e.g., refs. 11 and 12). The frequently cited direct evidence is an experiment by Gernandt (ref. 13) in which the discharge of single units within the cat vestibular nerve was studied during angular acceleration when the preparation was placed at a radius from the rotatory axis.

Gernandt found that a Type I unit which direction (determined solely by caloric irrigation) now performed like a Type II unit with previously fired with an increase in only one an increase in response to angular acceleration of either direction even at low linear acceleration values of less than 1.02 g. The implication is that centripetal acceleration served to drive the cupula in but one direction, overriding the normally directional response of the endolymph. The threshold data were consistent with this hypothesis in that the threshold in one direction (the normal acceleration direction for an increased discharge) was lower than thresholds for accelerations of the opposite sense. These facts have been interpreted as clear evidence for direct acceleration effects on the cupula, but these data are not so decisive as one would wish for a keystone in this inductive structure. For example, single units were not studied both at the center and at the periphery. Vestibular units are quite idiosyncratic in their discharge pattern and a single cell must be compared at the two locations. In addition, identification of a cell type by caloric means may be quite tenuous. Finally, the head position of the animal, the angular accelerations, their durations and the terminal velocities were not clearly specified.[3]

The purpose of this paper is to present two experiments. In part I, the human nystagmic data reported in 1962 are offered for publication in these proceedings for the first time. These data are of interest because they show that linear acceleration produces systematic changes in nystagmus at even the very low values of 1.06 g. In part II, an experiment is described in which single-unit activity from cells within the cat vestibular nuclei were studied both at the center of rotation and at a radius.

[2] A convention of analytical mechanics is observed in this terminology. The turntable, as viewed from above, undergoes a positive angular acceleration during a period of decreasing counterclockwise (CCW) velocity or increasing clockwise (CW) velocity. Similarly, the turntable undergoes a negative angular acceleration during a period of decreasing CW velocity or increasing CCW velocity.

[3] In subsequent personal communications with Dr. Gernandt, it was determined that the same cells have been studied at the center and at the periphery in a later unpublished study and with the same result, and that the orientation of the head was along the radius.

PART I. HUMAN NYSTAGMUS

Apparatus and Recording Method

The electrically driven turntable was 2.4 meters in diameter and controlled with suitable feedback circuitry to maintain the required acceleration programs (ref. 14). The chair for the subject was firmly attached to rails that transversed one full diameter of the table. The rails permitted positioning the subject's head over the axis of rotation, or at any radius up to 1 meter. Additional adjustments provided for setting the heading of the subject to any direction with respect to the radius. A biteboard assured that the subject's head was fixed, and the lateral canal alined with the plane of rotation.

The electrode assemblies were constructed of small plastic cups and filled with electrode jelly which was in contact with silver-silver chloride junctions. Electrodes were taped to the skin at the outer canthi to record the horizontal component of ocular nystagmus and above and below the left eye to record the vertical component. The ground electrode was taped to the forehead. Potentials were led from the turntable through sliprings and amplified with a 1.4-second RC time constant, and displayed on an Offner type T transistorized electroencephalograph.

Subjects and Procedure

Four adult male subjects participated, none of whom had any history of labyrinthine disorder. Two were very experienced with rotatory devices.

Test trials consisted of accelerations of 4.5°/sec^2 magnitude with 22-second duration, and all began from a base speed of 1 rpm. A trial consisted of, first, an acceleration, either positive or negative, followed by a 2-minute period of constant velocity at 17.5 rpm and then an acceleration of equal magnitude and duration, but of opposite direction, back to the 1-rpm base speed.

In section I of the experiment, all four subjects received eight trials, including two test trials at each of two head positions and for both initially positive and initially negative acceleration directions. One head position was directly over the axis of rotation and called the center position. The other was at a radius of 1 meter, facing the tangent such that the subject would be riding "into the wind" during a counterclockwise rotation with his left shoulder directed to the rotatory axis. This latter position was termed "position CCW."

In section II of the experiment, the 2 most experienced subjects, those who gave the most systematic and scorable nystagmic responses, were given 4 trials at each of 3 head positions and for initially positive and initially negative acceleration directions, making 24 trials for each subject. Two head positions were center and CCW as for section I, and the third position was at a 1-meter radius, facing the tangent such that the subject would be riding "into the wind" during a clockwise rotation with his right shoulder directed to the rotatory axis: position CW.

The order of presentation of trials within section I and section II was counterbalanced so that no particular condition was favored in the order for all subjects, thus obviating the possibility of biasing the data with complications from habituation. An experimental session consisted of four trials a day in section I and six trials a day in section II. At least 1 day intervened between sessions. A preliminary trial was given prior to each session, during which the recording system was adjusted and the subject refamiliarized with the situation.

All acceleration trials were conducted in complete darkness, and the subject was instructed to keep his eyes open and directed straight ahead. The subjects performed intense silent mental arithmetic during the trials. At a signal from the experimenter, just prior to the acceleration, the subject would start the arithmetic and continue with the problem until signaled to stop. The stop signal was given during the period of constant velocity and after the nystagmus had entirely subsided. The subject then rested and no data were collected during the return to base speed. One of two arithmetic problems was employed on each trial. One problem was to divide 80 and each successive quotient by 5, more precisely $80(5)^{-n}$. The other was: $x+1+2+3+ \ldots +n$, assigning a

different number for x on each occasion. Mental arithmetic tasks have been shown to be a necessary adjunct to vestibular experiments if maximum nystagmus with minimum variability is to be elicited (refs. 15 and 16).

Calibration

Calibration of the eye movements was undertaken, with the room lights on just prior to each trial. The subject would shift his gaze smartly, on command, from a point directly ahead to measured marks at 20° right, left, above, and below the center position.

Results

An example of nystagmus as recorded in this experiment is shown in figure 1. This example, recorded during and following negative angular acceleration at the center and in the CCW position, shows the principal features. The activity on the vertical channel is of interest. At the end of acceleration, the recordings made at the center show a very faint upward beating of the eyes in association with the fast-phase left beating of the horizontal component. This beating is evident, in spite of the larger and less regular disturbances caused by eye blinks and lesser lid movements and is probably due to slight misalinement of the vertical electrodes. The vertical recording taken at the radius CCW position does not show an up fast-phase, but does show a vertical fast-phase down component which is best seen some 10 seconds after the end of the acceleration. This shift in the direction of a small vertical component indicates that the plane of nystagmus had shifted, but this shift, if seen, was always small, and never larger than depicted here. In fact, the plane proved quite erratic at this low-g level and the shift shown here is not the same as found by Lansberg et al. (ref. 10). In their position D with a negative acceleration, the plane shifted to one with an upward-beating fast-phase instead of a downward one.

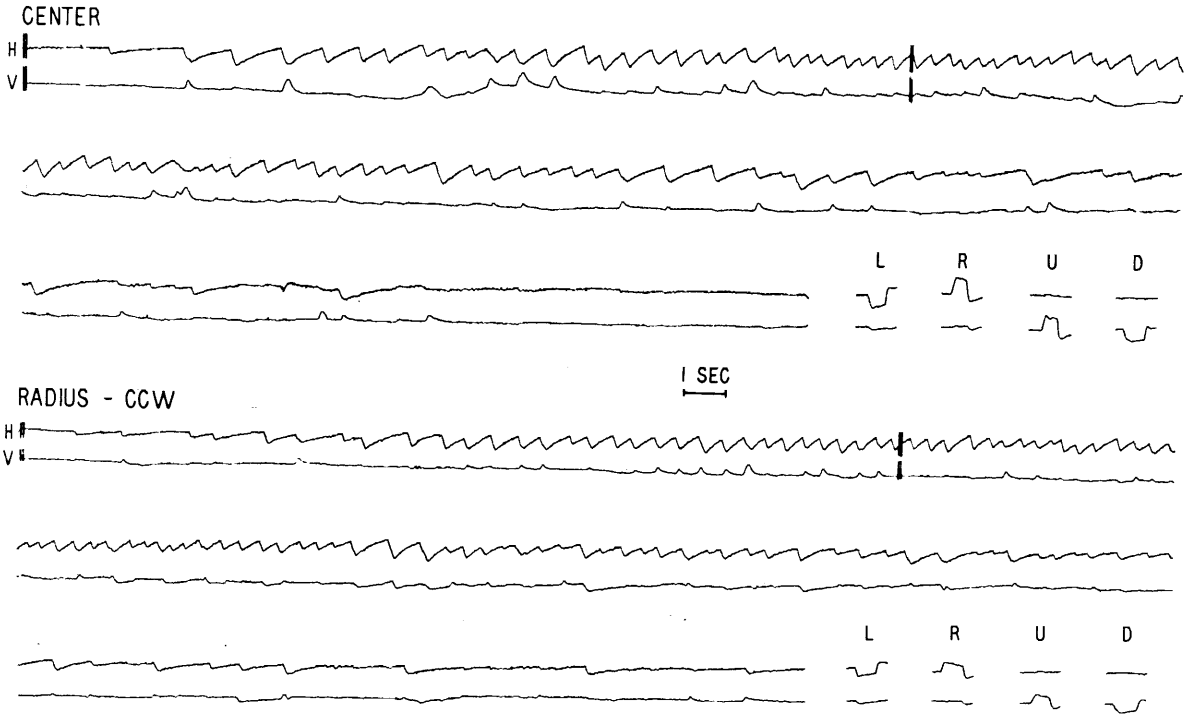

Figure 1.—*Nystagmus simultaneously recorded with horizontal (H) and vertical (V) electrode placements with a center and a radius CCW heading. The 22-second durations of the 4.5°/sec² negative accelerations are indicated by the heavy vertical lines in each recording. Calibrations at the end of the recordings were obtained by instructing the subject to shift his gaze to fixation points set at 20° up and down, and right and left from the straight ahead position.*

The horizontal component records for section I were scored by measurement over 2-second intervals of the magnitude of the slow-phase sweeps of the eyes. Measurement was continued over the entire duration of the primary nystagmus. Secondary nystagmus and recordings on the vertical channel were not scored. Measurements, first taken in millimeters, were converted to degrees per second by reference to the magnitude of the 20° calibration signals obtained after each recording trial. Finally, the data from all subjects were averaged and are plotted in figure 2.

As the left panel in figure 2 shows, no differences were found between the radius position and the center position when data from both positive and negative accelerations were averaged. It is of special interest to note, however, that whereas there was no difference in response magnitudes between positive and negative accelerations at the center (center panel), there was a distinct difference between the directions of acceleration at the radius (right panel). The response to the negative acceleration was decidedly greater during the following period of constant velocity, when the centripetal acceleration was at a maximum.

The difference between acceleration directions shown in the right panel of figure 2 could not be attributed to a directional imbalance, since an imbalance was not evident at the center position. But, because the difference was small, it was considered appropriate to repeat the experiment and to add the CW position to the observations. Section II was then carried out on two of the four subjects and the nystagmus measured as already described for section I. Figure 3, left panel, shows that the positive and negative accelerations produced responses which were essentially similar, with some small bias toward an imbalance in the direction of a greater response for the positive acceleration. In the center panel, positions CW and CCW have been compared for positive acceleration alone, and in the right panel, for negative acceleration alone. The positive acceleration enhanced the response at the CW position and depressed the response at the CCW position. The opposite occurred for negative acceleration. This difference was in the same direction that was found in section I. Again, the difference arose near the end of the acceleration and continued throughout the period of the cupular return, a period during which the centripetal acceleration was at a maximum.

PART II. SINGLE-CELL RECORDINGS FROM CAT BRAIN STEM

Head Positions

Figure 4 shows the body positions and figure 5 indicates the right lateral semicircular canal, and the cupula in each case, assuming that the

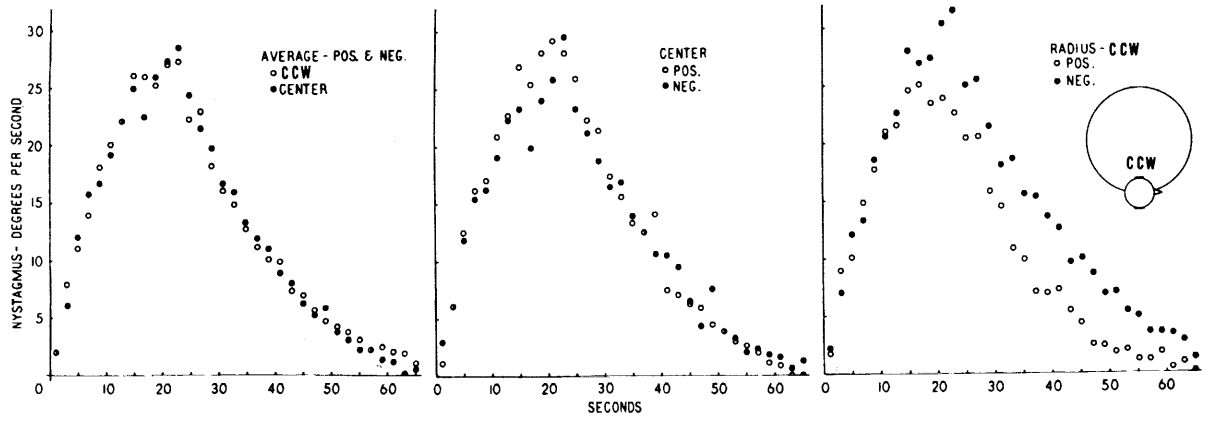

Figure 2.—*Average slow-phase nystagmic output during and following the 22-second 4.5°/sec² acceleration. The directions of the accelerations are indicated by the* POS and NEG *designations. The subject rode at the center or at 1-meter radius in position CCW as shown. Note that negative acceleration produced more nystagmus than positive acceleration in the CCW position.*

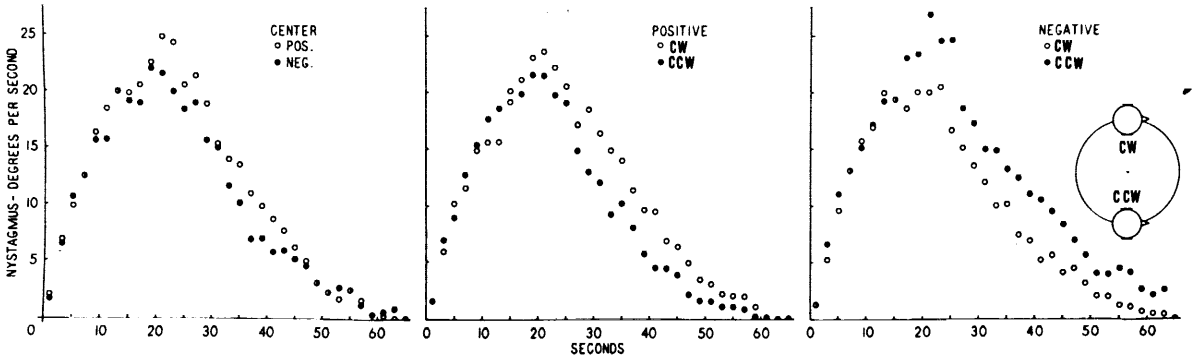

Figure 3.—*Average slow-phase nystagmic output during and following the 22-second 4.5°/sec² acceleration. The directions of the accelerations are indicated by* POSITIVE and NEGATIVE *designations. Note that negative acceleration produced more nystagmus than positive acceleration from the CCW position (right panel), but positive acceleration produced more nystagmus than negative acceleration for the CW position (center panel).*

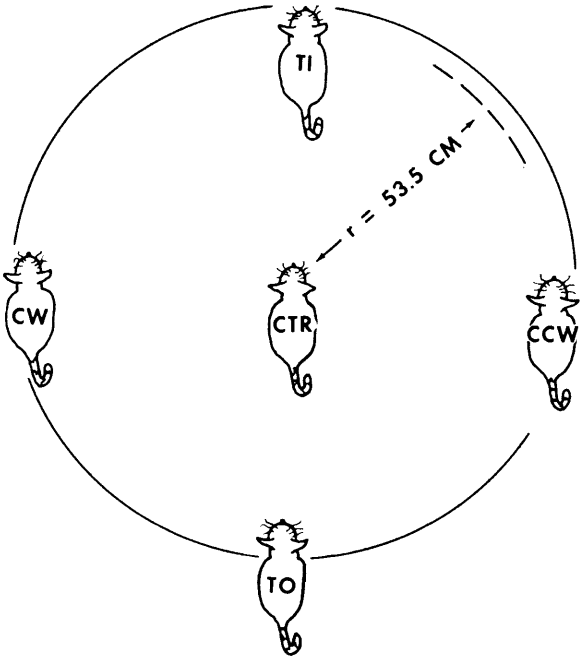

Figure 4.—*Body and head positions employed in radius experiments. The CW and CCW headings indicated here are the same employed for man in part I, except for the shorter radius.*

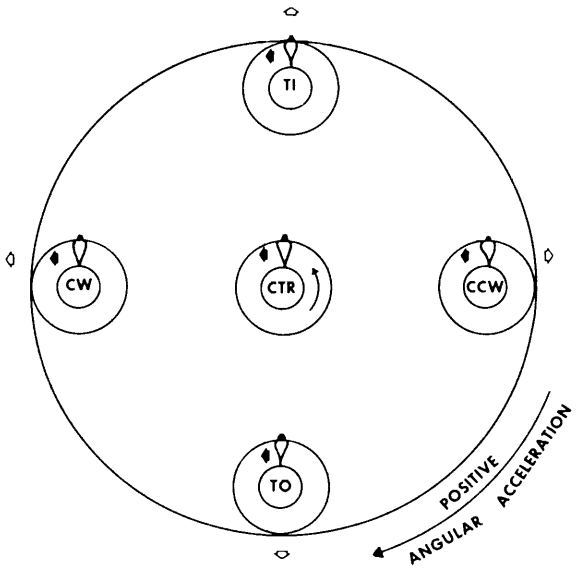

Figure 5.—*Schematic representation of the right-lateral semicircular canal at each of the positions. A positive angular acceleration is indicated which establishes a relative endolymph motion in a counterclockwise direction as shown by the curved arrow in the center figure. This endolymph displacement moves the cupula in the direction indicated in each figure by the heavy black arrow. Also shown in each figure with the open arrow is the direction which the cupula should be displaced if linear acceleration acts directly on this structure. Note that with the assumption the cupula is hinged anteriorly as depicted here, cupular displacement should be increased for the CW position, but diminished at the CCW position with positive acceleration. Cupular displacement at positions TI and TO might well be ambiguous, but a smaller displacement would be predicted for these orientations as compared to the center.*

cupula is hinged anteriorly, extends dorsally and is approximately parallel to the mesial plane. The white arrows show the direction of cupular displacement, if it is assumed that centripetal acceleration is effective on this structure. The black arrows indicate the direction of cupular displacement when the cupula is dis-

placed by the endolymph in response to angular acceleration. This diagram shows that with positive angular acceleration, the CW position should lead to an augmented response, whereas the CCW position under the same conditions should produce a diminished response, when either is compared to testing at the center. Such was the case for human nystagmus in part I, and the purpose of this second part is to examine single cells in the cat brain stem for the same effect.

Apparatus

A circular turntable 1.25 meters in diameter was mounted on a vertical shaft, and driven through a friction coupling by pneumatic wheel pressed against the rim (fig. 6). The precision-ground vertical shaft and the oil-bronze smooth radial and thrust bearings were enclosed in a column of oil. The drive system was a hydraulic pump and motor with servoamplifier control employing closed feedback loops from the slide block and a tachometer on the output. The turntable structure and the hydraulic motor were mounted within a radiofrequency-shielded, lightproof, and ventilated room. The hydraulic pump and control console were mounted outside the room.

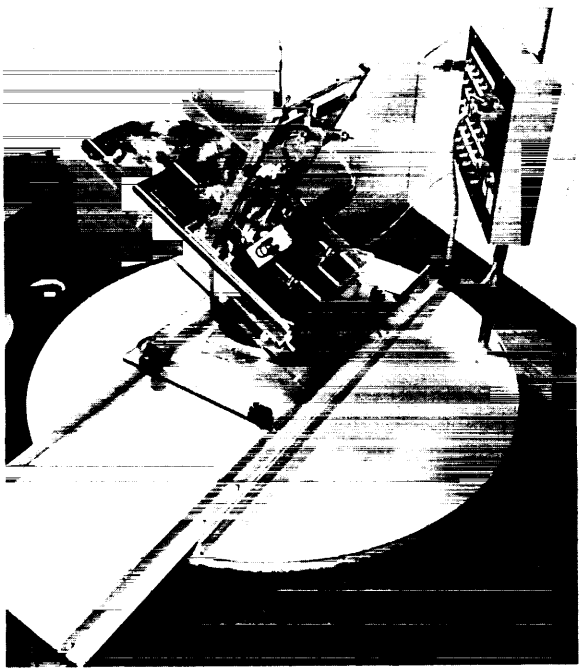

Figure 6.—*Rotatory device for single-cell recording. The tracks and wheeled frame permitted moving an animal in and out from the center position and also changing its heading without disturbing the electrode location.*

Procedure

Cats were deeply anesthetized with Nembutal and mounted within the stereotaxic head-holder. A small opening was made in the skull for electrode entry and then the animal was positioned on the turntable with the head centered over the axis, and the horizontal stereotaxic plane of the head tipped nose-down 30° from the horizontal plane of rotation. This orientation brings the lateral canals approximately into the stimulating plane. Further, the electrode was tilted back 30° from the stereotaxic transverse plane in order to avoid the tentorium when advanced in a parasagittal plane.

The friction drive wheel was disengaged from the turntable rim and the table gently oscillated between successive advances of the electrode manipulator until a single cell from the right side of the brain responded to angular acceleration and was sufficiently isolated for definitive recording. The friction wheel was then reengaged to the turntable rim, the shielded room closed, and the stimulus series begun.

The stimulus series consisted of a number of constant angular accelerations beginning at zero velocity and proceeding to 20 rpm, followed by 5 minutes of constant velocity and then returning to zero velocity with an equal but opposite acceleration. The standard series employed only clockwise rotations with accelerations of 4°/sec^2 over 30 seconds' duration. High-threshold cells were tested over the same velocity range, but at 8°/sec^2 accelerations of 15 seconds' duration. Some particularly sturdy cells were recorded during CCW rotations as well. Following evaluation at the center position, the animal was rolled along the tracks to a peripheral location, with the head 53.5 centimeters from the axis and the cell response evaluated in both the CW and CCW facings. The experiment was recommenced with the center position and continued as long as a

cell could be held. A series was also completed on one other animal but using the tail-in (TI) and tail-out (TO) positions.

Recording

Electrodes were electro-polished-steel insert pins prepared according to the method of Green (ref. 17), but insulated with successive coats of baked Formvar. Electrode tips were less than 6 microns in diameter, and tested with saline immersion under a microscope to observe if a small bubble was produced at the tip when a current was passed through the electrode. The single-ended signal from the electrode was led through a cathode follower mounted on the stereotaxic instrument, through instrument sliprings and thence to amplifying and recording equipment outside the turntable room. The signals were filtered through a frequency band between 300 and 3000 cps, and photographed from an oscilloscope at a 50-mm/sec film speed.

We found it difficult to obtain technically adequate records over long periods of time when the animal was placed under centripetal acceleration. If the animal is kept over the axis of rotation, successful recording sessions of over 1 hour are possible in more than half the attempts (ref. 18). Fewer than 1 success in 10 attempts was achieved with the procedure described here. In figure 7, recording events shown in lines A, B, and C led to a termination of the experiment at that point. In line A, the unit evidently shifted away from the electrode during the acceleration (indicated by the heavy band below the recording). When the cat returned to a stop, the brain reverted to its original position and the cell was recorded from again. The return of a unit in this fashion was a most rare occurrence. In line B, the more usual deterioration of cell isolation is shown. In line C, activity at the death of a cell was recorded showing the characteristic rapid firing. In line D, the normal discharge of the cell is shown during and following an acceleration which turns off the resting discharge. Here it is seen that only the rate, and not the amplitude as seen in A, is affected. The cell returned to its normal firing rate after the acceleration.

Histology

Following the recording, a small current was passed through the electrode by attaching the anode of a dc source to the electrode. The current in microamperes and its time of application in seconds were adjusted so that the product of the two amounted to approximately 150; more correctly, 0.00015 coulomb. The animal was then perfused with saline solution followed

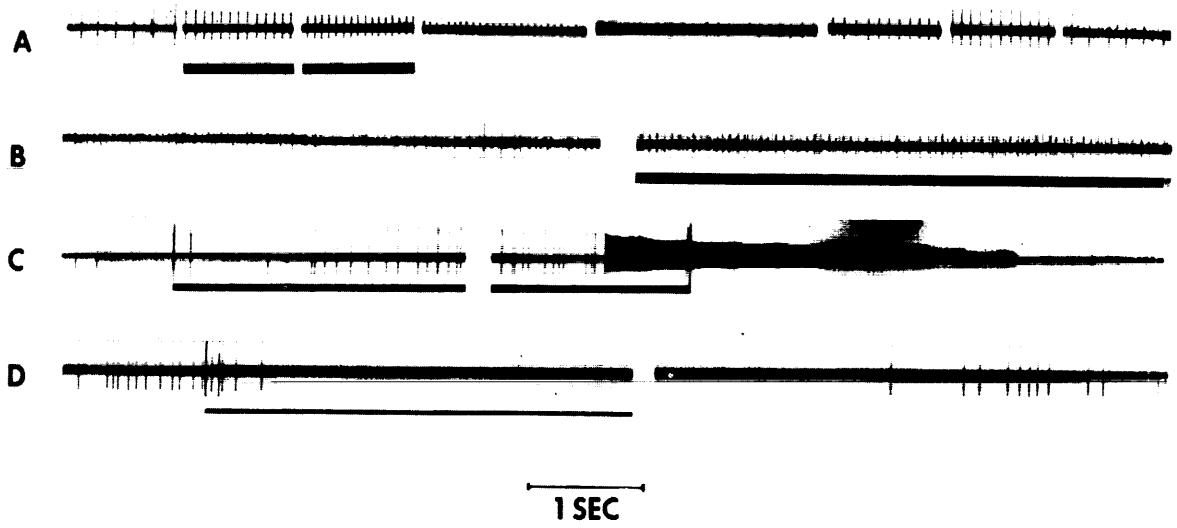

Figure 7.—*Examples of recording incidents.* (A) *The brain shifts and moves cell from electrode.* (B) *Cell isolation is lost by small brain or electrode movements.* (C) *The cell dies.* (D) *Usual inhibition of a resting discharge of the cell and its return to normal firing rate.*

by 10% formalin, to which 0.5% potassium ferrocyanide and 0.5% potassium ferricyanide had been added. The brain was removed and placed in this perfusing solution for not less than 3 days before subsequent processing. This procedure, adapted after the method of Green (ref. 17), produces, by virtue of the prussian blue reaction, a small blue spot at the electrode tip site which just can be detected by the unaided eye while preparing frozen sections. A series of 40-micron sections in the region of the dyed spot was then stained alternately with crystal violet and neutral red. Identification of electrode locations was made by reference to Brodal and Pompeiano (ref. 19), and Brodal, Pompeiano, and Walberg (ref. 20).

Cell Response Categories

Just as described previously (ref. 18), we find two major cell types when recording in this fashion. The most frequently found type follows the classical description for the function of the lateral canal as described by Ewald (ref. 21), in which it was deducted that utriculopetal movement of the cupula should produce a maximum response, and that utriculofugal movement of the cupula should produce but little response. This first category was termed "Type I" by Gernandt (ref. 22), but we prefer to term it an "Ewaldian" unit and denote that it increases its firing rate with positive acceleration and decreases its resting rate (if any) with a negative acceleration, when the electrode is on the right side and recording activity originates in the right ear. The opposite obtains for an electrode on the left side; that is, an increase in firing rate with a negative acceleration and a decrease in the resting rate (if any) with a positive acceleration.

We prefer to call cells from a second broad category "non-Ewaldian" units: units which increase their discharge rate with a negative acceleration and decrease their resting discharge (if any) with a positive acceleration when the electrode is on the right side and recording activity originating from the right ear. The opposite obtains for an electrode on the left side; that is, an increase in firing rate with a positive acceleration and a decrease in resting rate (if any) with a negative acceleration. Duensing and Schaefer (ref. 23) and Shimazu and Precht (ref. 24) found them in the vestibular nuclei and called them Type II, which confuses the issue because the new Type II does not agree with the former Type II designation of Gernandt's.

We believe that the non-Ewaldian reacting unit is reflecting activity originating in one of the vertical canals of the labyrinth on the same side as that in which the electrode is positioned. Lowenstein and Sand (ref. 25) have shown that a vertical canal will produce what appears to be non-Ewaldian activity with horizontal rotation of the head, and Ross (ref. 26) observed that even a small misalinement of the vertical canal from a true orthogonal orientation with the horizontal plane led to adequate stimulation.

Only Ewaldian units, those referred to as Type I by Gernandt, have been studied in this experiment.

Results

Twelve units have been studied under these adverse conditions, including one in a "tail-out and tail-in" series. All electrode sites were histologically verified.

Table 1 indicates the direction a response should have taken if centripetal acceleration acted directly on the cupula, as shown in figure 5. In not one single case was a response altered in its direction of change as predicted by figure 5 and table 1. If this were the case, the CCW response to all positive accelerations shown in the accompanying figures should have been in the "turnoff" direction or, at the very least, reduced in magnitude when compared to the center condition. Likewise, for responses to negative acceleration in figures 8 and 9, the CW response should have "turned on" if the hypothesis held or, at the very least, have been a lesser inhibition or turnoff than for the center condition. The one successful "tail-in and tail-out" animal also did not show differences between the center, CW, and CCW positions.

Careful examination of all records was made to determine if subtle response changes might have been introduced by linear acceleration.

For example, although the linear acceleration might not be sufficient to reverse entirely the direction of movement of the cupula, it might be adequate to modify the deflection to a lesser extent. But the differences between conditions were very much the same as the trial-to-trial reliability, and consistent differences were not seen. Thus, figure 8 shows a cell response in accord with Gernandt; the CW discharge is greater. Figure 9 shows just the opposite.

Table 1.—*Predicted Responses for Cupula and Type I Discharge From the Right Lateral Ampulla According to the Positions Shown in Figure 5*

Acceleration and position	Deflection	Discharge
Positive:		
Center	Utriculopetal	Increase from resting rate
CW	Utriculopetal>center	Increase from resting rate>center
CCW	Utriculofugal	Decrease from resting rate
TI	Utriculopetal<center	Increase from resting rate<center
TO	Utriculopetal<center	Increase from resting rate<center
Negative:		
Center	Utriculofugal	Decrease from resting rate
CW	Utriculopetal	Increase from resting rate
CCW	Utriculofugal>center	Decrease from resting rate>center
TI	Utriculofugal<center	Decrease from resting rate<center
TO	Utriculofugal<center	Decrease from resting rate<center

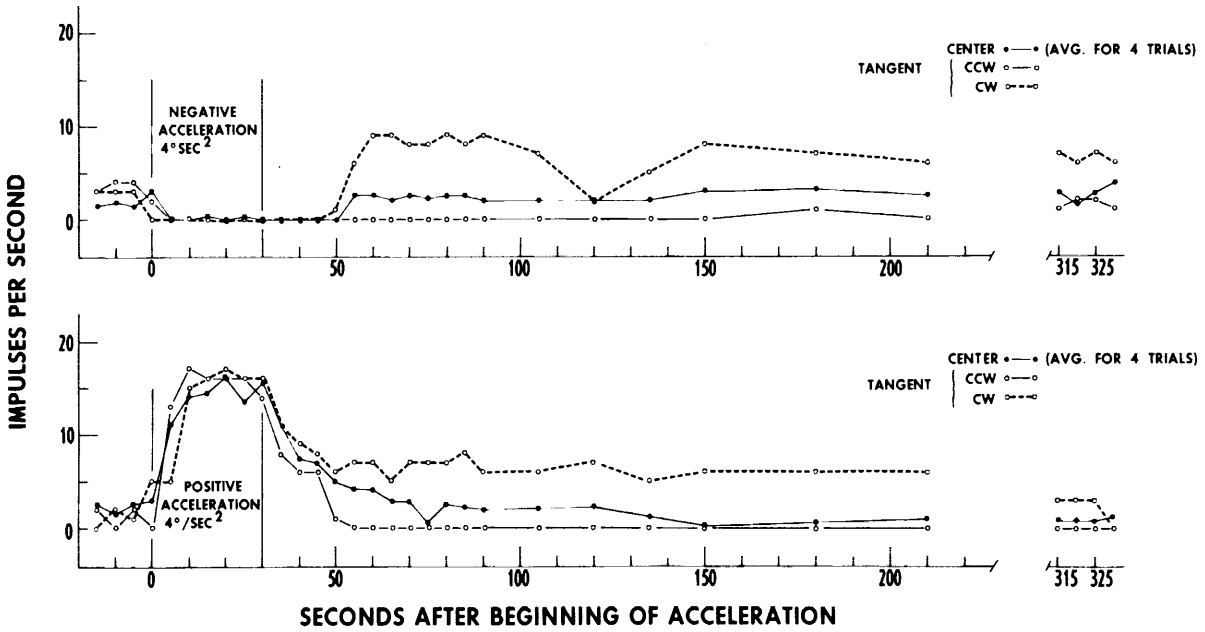

Figure 8.—*Angular accelerations commenced at zero rpm and terminated at 20-rpm CCW for negative accelerations and 20-rpm CW for positive accelerations. This recording from a cell within the right-medial vestibular nucleus shows that the type I character of this Ewaldian unit remained unchanged at all positions. The fact that the CW position produced greater responses than the center and that CCW produced even less when the cat was at 20 rpm is the direction of the hypothesis outlined in figure 5. All CW and CCW data from this and succeeding graphs are single trials, not averages.*

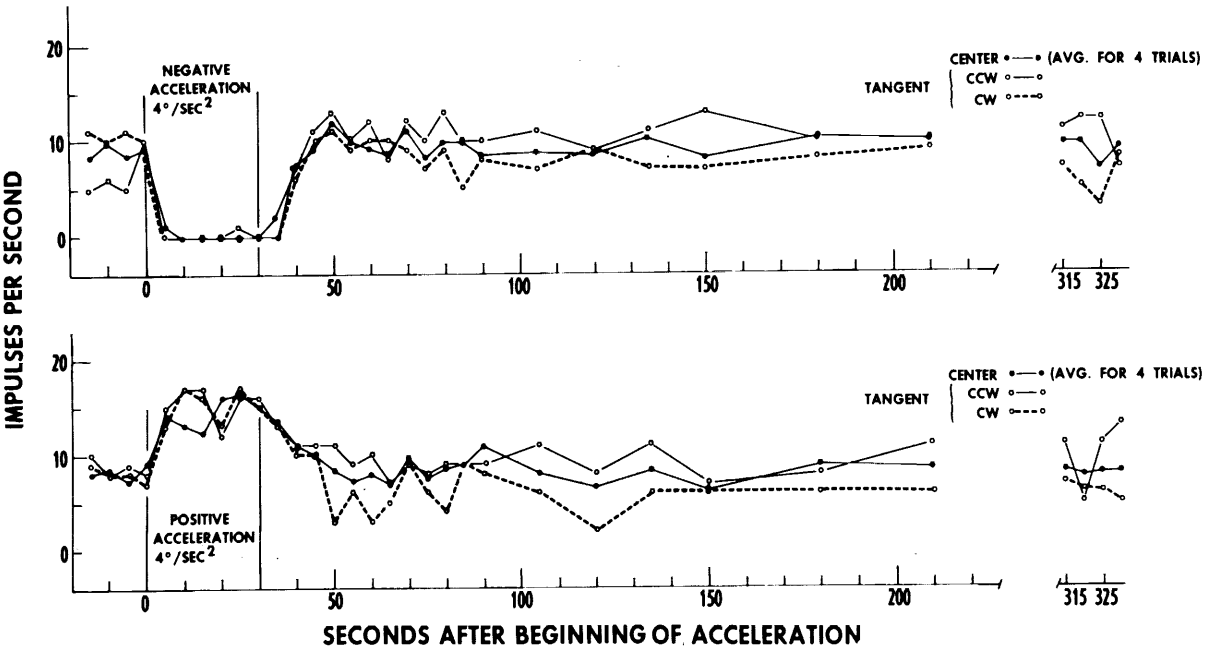

Figure 9.—*This recording from a unit within the right-descending vestibular nucleus shows that the type I character of this Ewaldian unit remained unchanged at all positions. Note that the relative differences between the positions when at constant velocity are the reverse from those found in figure 8, and not even in the direction specified by the hypothesis in figure 5.*

Similarly, in figure 10, the top graph does not agree with the hypothesis that centripetal acceleration is a factor, but the bottom one does. One must be careful not to base conclusions on too few cases. By and large, the most frequent finding is that shown in figure 11 for two additional animals. No clear-cut difference between conditions is evident.

DISCUSSION

Part I showed that human nystagmus recorded at resultant values as low as 1.06 g indicated clear effects attributable to centripetal acceleration. Such a low value is of the order of magnitude of 1.02 g in which Gernandt (ref. 13) reported centripetal effects in single-cell recordings, and the 1.03 g used here in part II describing our own single-cell recordings. Note the difference between these low values and the high ones at which dramatic nystagmic effects are found: 1.66 g (ref. 10) and 3.07 g (ref. 8).

Although these nystagmus experiments have results compatible with the hypothesis that linear acceleration acts directly on the cupula, other data are not in accord. In particular, if a man in a horizontal position is subjected to angular acceleration about his long axis, as on a roasting spit, a horizontal nystagmus is very much prolonged if a constant angular velocity is maintained following the angular acceleration (refs. 27 and 28). If a stop follows the angular acceleration, the nystagmic poststimulation duration is very short. Here, the otoliths are probably playing a regulatory role in nystagmic control; certainly, the prolonged duration cannot be due to direct gravity effects on the cupula.

Part II contained an experimental design specifically modified from Gernandt's to include those positions found to be critical for nystagmus experiments (CW and CCW). Other features of the procedure, including assessment of the same cell at the axial position as well as at a radius and employing a resultant even higher than that of the earlier experiment, were expected to confirm Gernandt's findings in a most explicit manner. Although the part II experiment is not an exact replication of

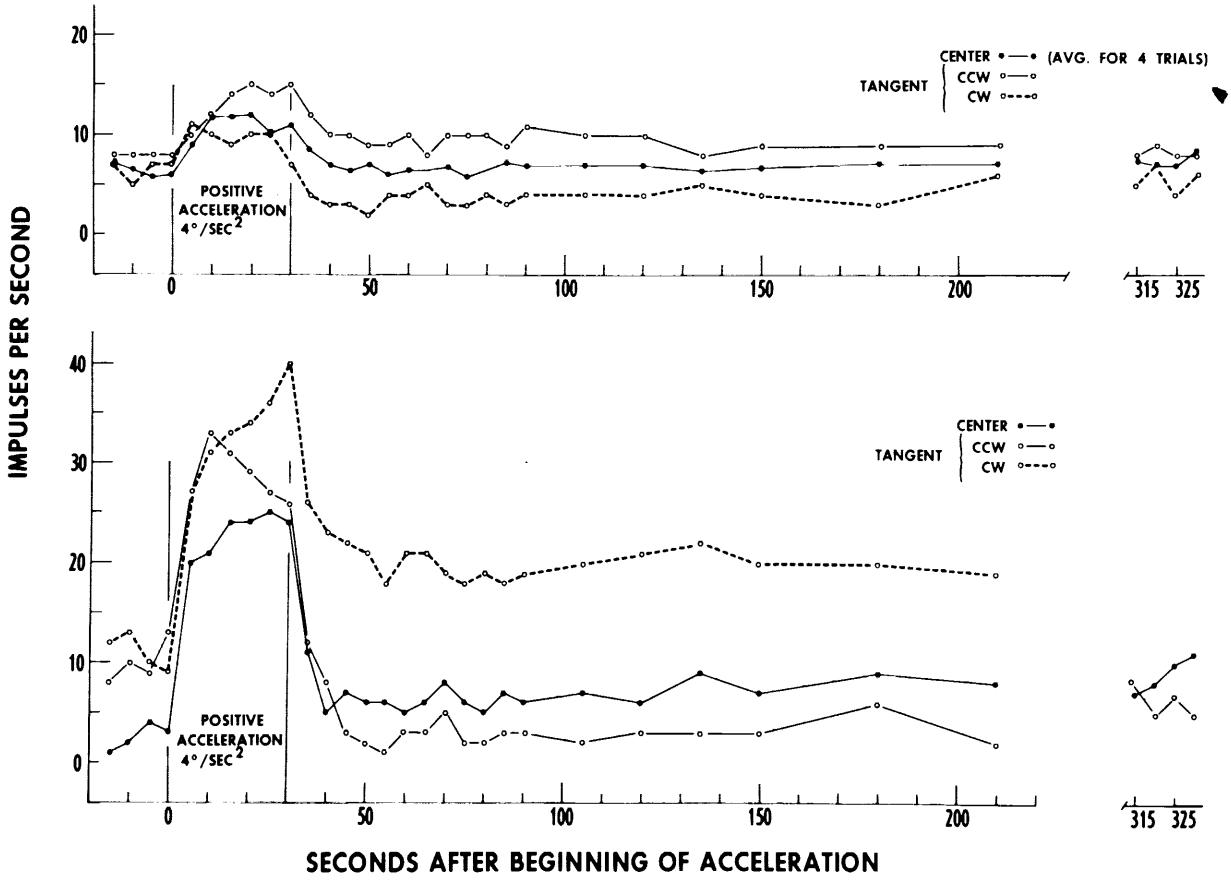

Figure 10.—*The cell for the upper record was within the right-medial vestibular nucleus. The response does not follow the hypothesis that centripetal acceleration is a factor. But the lower record, from a cell within the right superior vestibular nucleus, is in the direction predicted by the hypothesis.*

Gernandt's procedure, it is not evident why comparable results were not obtained. This is especially true for the one "tail-in and tail-out" animal which more nearly duplicates the earlier technique.

There are certain differences between the Gernandt experiment and part II of our work. He employed a shorter radius, 40 centimeters compared to our 53.5 centimeters. The terminal velocity in the earlier experiment is not known precisely, but he refers to 15–20 rpm as "extreme speeds." All terminal velocities in part II were at 20 rpm. Angular acceleration and acceleration durations cannot be determined from his text, and we may only assume that accelerations were comparable in the two experiments. It has since been confirmed that the cat was facing along the radius (our tail-out or perhaps tail-in position).

The recording sites are clearly different. In part II all cells were within the medulla itself. Gernandt directed his electrodes to the vestibular portion of the eighth nerve, just at its exit from the internal auditory meatus. He was able to isolate "units" sufficiently well to record from them under these rather adverse conditions. The nature of these units found in that segment of neural tissue remains in doubt, although it has been confirmed that unit activity can be recorded in this manner from cat, dog, and rabbit (ref. 29). Our own somewhat clumsy early attempts to record from the same location were singularly unsuccessful, and our subsequent recordings have all been from the

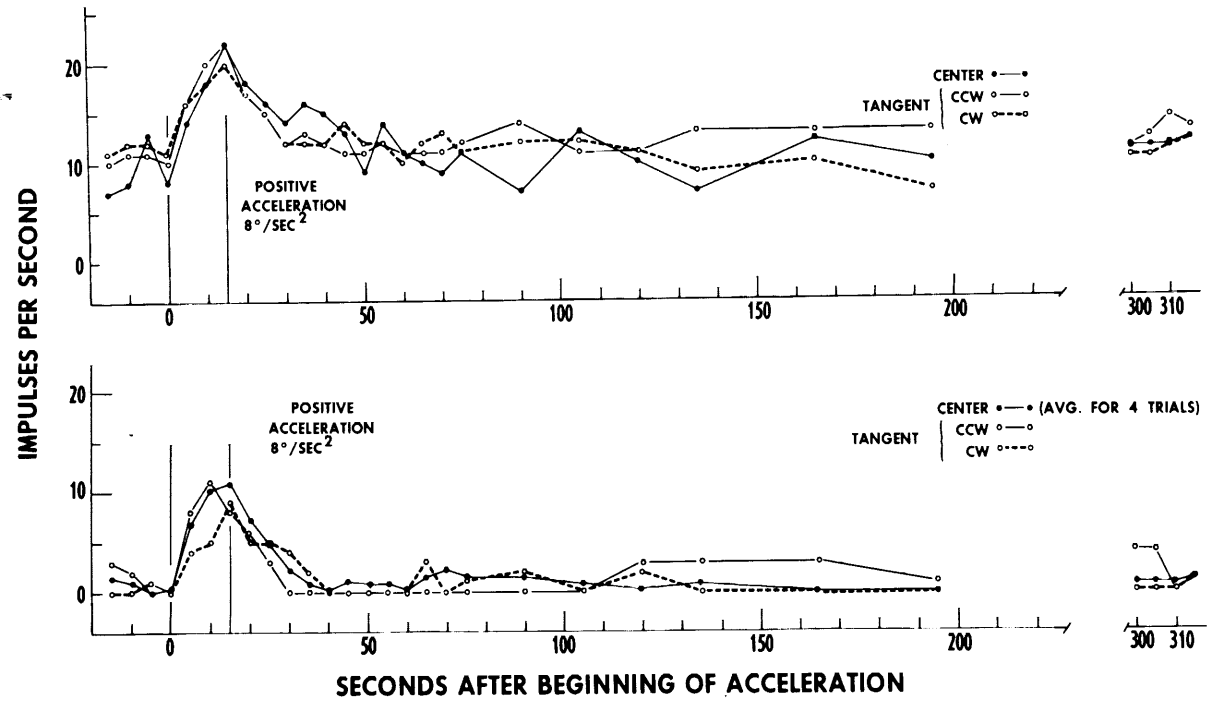

Figure 11.—*The cell for the upper record was at the upper margin of the right superior vestibular nucleus, and for the lower record, was in the right medial vestibular nucleus. These records represent the most typical finding in that there are no distinct differences among recordings when the head is at the various positions.*

vestibular nuclei and related central structures. This difference in recording sites may be a significant factor. Further, the Gernandt experiment was performed on decerebrate cats, with the lateral portion of the cerebellum aspirated to permit direct observation of the recording site. Part II experiments were performed under deep barbiturate anesthesia and with minimal brain exposure.

When one collates the data which bear on the question, "Does linear acceleration modify cupular deflection?" it appears that the answer is "No." Only Gernandt's experiment supports an affirmative response, but this frequently cited unhandy set of findings has here failed verification, or at the very least, depends upon a narrow and restrictive set of conditions. The theoretical analyses of Ter Braak (ref. 1) and Timm (ref. 2) are yet to be incontrovertibly confirmed. Nystagmic effects seen with changes in magnitude and direction, and even of direction alone, of the linear acceleration resultant are perhaps better explained in terms of a centrally converging otolithic influence.

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DISCUSSION

LOWENSTEIN: When you had your acceleration terminating in constant-speed rotation on the turntable, the long tail of your discharged frequencies was during this constant-speed rotation of the turntable, where you would have expected the return to the normal resting discharge level; isn't that so?

CRAMPTON: Yes.

LOWENSTEIN: And what you showed was that in some cases, it didn't quite return to the normal resting discharge, but it remained above, and in other cases became depressed below.

Concerning the question of the specific gravity of the cupula, Nature, let's put it metaphorically, tried to design a cupula which is roughly of the same specific gravity of the endolymph. Nature didn't manage, but Nature got away with it because man wasn't made for angular or linear accelerations larger than running to begin with. And it will take a few million years before man will become adapted to space flight.

HIEBERT: I'd better restate my earlier remarks and say that if you are recording from the vestibular nuclei, you have to be very careful in making any judgments that the nuclear discharges exhibit a point-to-point relationship with what is happening at the end organ.

I think that the "caprice" that you see in these cells in repetitive trials of identical stimuli of the end organ is due to modulation, so that you cannot draw a conclusion from them as to what might be happening at the cupula.

CRAMPTON: If linear effects on the cupula are prominent, we should have seen them.

MONEY: In my study of the bony canals in cats, according to the shape of the bony ampulla, the cupula is hinged at the front and points almost directly back but slightly laterally. I was able to get much the same impression from a study of histological sections. Dr. Igarashi made a similar study and found about the same thing, only he found the cupula to be pointed slightly medially instead of slightly laterally. In any event, it is fairly close to pointing straight back and it is hinged anteriorly in the cat.

CRAMPTON: How about in man?

MONEY: I have never studied it in man, but probably Dr. Igarashi could tell you this. You did find some units that did respond differently in the periphery compared to their response in the center. Why did you decide that for these units, there was no evidence of an effect of linear acceleration on cupular deflection?

CRAMPTON: Some of the units showed a small effect. It would take several years of work with this technique to obtain a meaningful statistical answer. I think that the significant point is that the profound change found by Dr. Gernandt was not seen. No effect was found consistently for all units. And indeed, that is why I presented the data in this individual fashion, rather than offering averages.

MONEY: Yes, because according to some hypothetical models, it would be very handy to have some units which did respond in this way and others that didn't.

SCHUKNECHT: If you are having some difficulty handling this idea of the cupula having a high specific gravity, permitting it to respond to gravitational force, I might suggest that the anatomy of the vestibular labyrinth is such that you might adopt a little different concept about this. The static labyrinth consists of the two structures, utricle and saccule, as you know. The saccule is located against the vestibular bony wall and can't move, but part of the utricular macula juts out into the center of the vestibule. If the labyrinth is subjected to gravitational or centrifugal force, it is reasonable to expect the utricular macula to move in the vestibule. The three canals enter the utricle and the ampullae are in very close anatomical position. The crista probably can move a bit in the line of gravitational force. And if the ampullary wall moves, I think it is reasonable to expect the cupula, which lies under it, to move a bit. In other words, the gravitational or centrifugal movement of the utricle would be transmitted to the crista.

CRAMPTON: At very low acceleration levels of 60 milli-g?

SCHUKNECHT: I don't know how to assess the force, but from the anatomical standpoint, I think this makes sense.

LOWENSTEIN: I wonder whether it is true in man as it is in other vertebrates that the semicircular canals are extremely well anchored within the bony canal by connective-tissue strands.

SCHUKNECHT: In all mammals there are very fine fibrillar strands which extend not only from the canals to the bony walls but from the ampullary wall itself. But these are very flimsy, and in many areas very scarce. I doubt that they would have very much of a retaining or fixing effect on the membranous labyrinth.

SMITH: Just a comment in reference to Dr. Schuknecht's statement. The anterior portion of the macula of the utricle, the anterior curved portion, is attached to the bony wall, whereas the posterior larger end is relatively free. Therefore, at least half of the macula of the utricle is well anchored and this is the part attached to the superior and horizontal ampullae. The ampullae of the semicircular canal are fitted rather closely into the bone so that it would be easier, I imagine, for the cupulae to be moved relative to the ampullae than for the entire structure to move. Although there might be some movement if the force were great enough to divert the straight posterior portion of the macula of the utricle.

CRAMER: It would be very easy to dismiss the inconsistencies you found as artifacts. You were centrifuging not only the ear but the brain and the electrode. Possibly, if you would examine your records with the nose-in, nose-out positions, you might find some differences in which you are applying tangential acceleration against the plane of the cupula. It might help resolve this.

CRAMPTON: Would you please clarify that?

CRAMER: You are using centrifugal force alone here, hypothetically, to modify your cupular response. Try the tangential force that is applied when you accelerate your animal and compare results. It might help to resolve these inconsistencies which everybody finds.

CRAMPTON: At these low angular accelerations, the tangential component is very small.

CRAMER: Use some bigger ones.

CRAMPTON: Linear components are linear components, and I am perfectly satisfied with the centripetal components.

CRAMER: You would be applying it in a different direction for a shorter time. The effect of tangential acceleration would appear as a difference in the growth of the response. It might help to tell you whether you have a genuine change here or just an artifact.

MAYNE: It seems that a number of hypotheses are possible regarding the phenomena reported by Colonel Crampton. In the first place, it could result, as indicated by Dr. Lowenstein, simply from a slight error of Nature in matching the density of the cupula to that

of the endolymph. Or again, it may be related to the fact that eye movements are sometimes controlled as a function of velocity and other times as a function of acceleration or rate of change of acceleration. It could be, also, that nystagmus, or whatever signal is related to it, is determined by perception rather than directly by the semicircular canals and that the test situation is subject to different perceptual interpretations. All these are, of course, guesses.

MONEY: In the pigeon, at least the specific gravity of the endolymph is greater than that of the perilymph. That makes it possible that gravity acting on the utricle, on the membranous ampulla, or on the membranous canal could cause some sort of movement and stimulate the semicircular canal. I wouldn't want to suggest that this is a normal physiological thing, but under unusual conditions, such as turntable experiments, it could be a significant thing.

CRAMPTON: You are referring then to a displacement of the membranes because of differing densities of the two fluids, but all within an unchanging volume. I think this "slosh" theory is a very appropriate thing to consider and will come up again with the discussion of Dr. Benson's paper.

Influence of Linear and Angular Accelerations on Nystagmus

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SUMMARY

Nystagmus and subjective responses were compared in experimental situations involving rotation at 10 and 30 rpm about an Earth-horizontal axis. At 10 rpm, responses were continuous throughout the period of rotation. At 30 rpm, responses showed a cyclic variation about a zero baseline after about 35 seconds. At this point, sensation of rotation was equivalent to that reported by labyrinthine-defective subjects in an earlier experiment. At both 10 and 30 rpm, cyclic variations in the slow component of nystagmus had a systematic phase relation to the cyclic variation in the direction of gravity. An experiment by Niven, Hixson, and Correia in which nystagmus was produced by horizontal linear acceleration revealed a consistency in results between the present experiment and several previous experiments. It is proposed that the prolonged nystagmus at 10 rpm and the cyclic variations in nystagmus (noted at all rotation rates) are an indication of separate mechanisms. Other experimental findings are discussed in relation to the proposed hypothesis.

INTRODUCTION

Linear acceleration can alter nystagmus initiated by angular acceleration (refs. 1–7). Because aerospace flights involve unusual combinations of angular and linear accelerations (including weightlessness), this line of scientific inquiry can have practical implications for space ventures.

By way of introduction, a review will be made of several previous experiments which are related to the new data presented below. Rotation about an Earth-horizontal axis as shown in figure 1 produces a continuous reorientation relative to gravity, whereas rotation about an Earth-vertical axis does not involve reorientation relative to gravity. Figure 2 shows that nystagmus produced under these two conditions is quite different, the horizontal axis yielding a

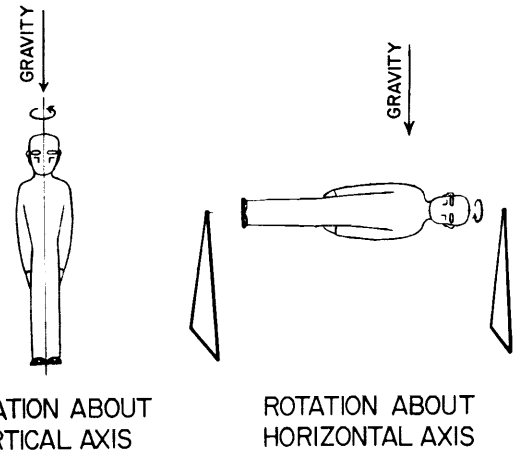

Figure 1.—*Horizontal semicircular canals were in plane of rotation in both situations, but only horizontal axis involves continuous reorientation of body relative to gravity.* (*From ref. 1.*)

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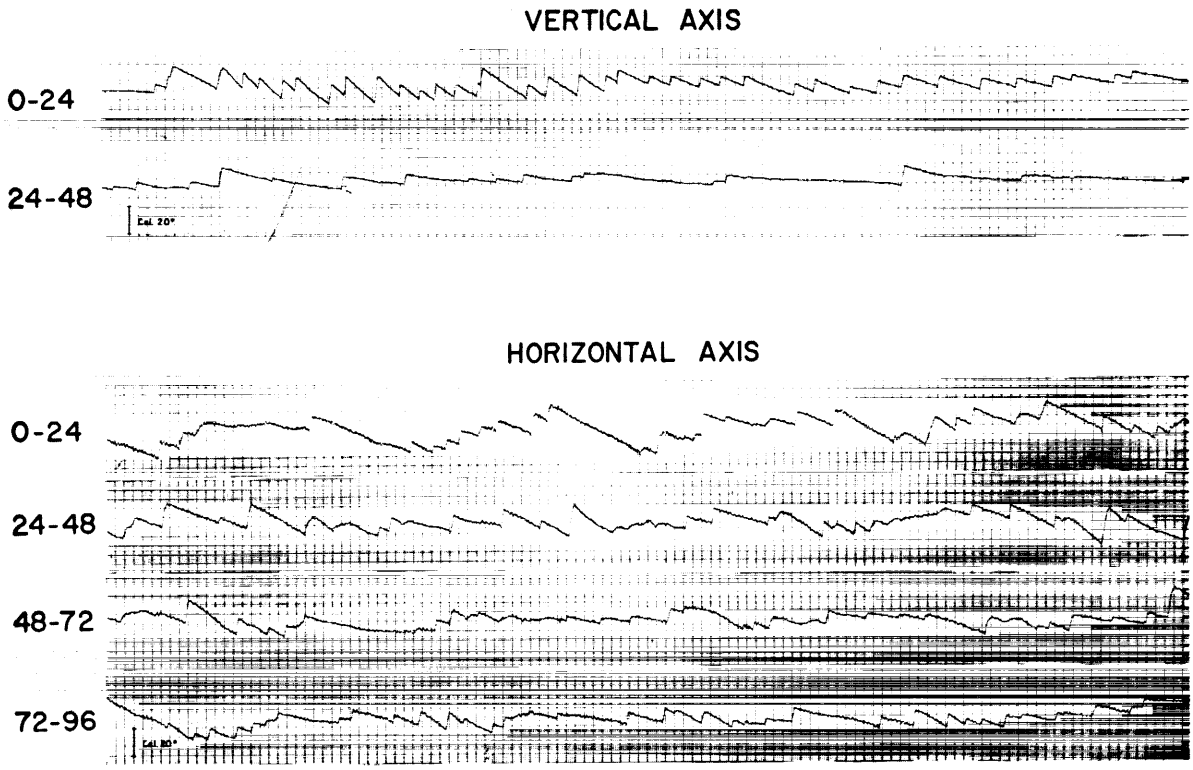

Figure 2.—*Nystagmus responses produced by rotation (10 rpm) about vertical and horizontal axes. (From ref. 1.)*

prolongation of the nystagmus response during rotation long after the response to angular acceleration would be expected to end. Figure 3 illustrates that whereas the response during rotation is maintained for a long time, the response after rotation is greatly attenuated when the subject is in horizontal position. The subjective effects are similar: horizontal axis rotation greatly prolongs sensation of turning during rotation, whereas after rotation about a horizontal axis, the sensation of turning is almost completely suppressed (ref. 1).

Subjects without vestibular function, or with at best greatly reduced vestibular function, (L–D subjects), do not exhibit continuous nystagmus or experience rotation about the horizontal axis (ref. 1). Four of eleven L–D subjects showed a weak, reversing nystagmus, but only 1 of 11 showed continuous nystagmus, and this was very weak. Apparently the prolongation of unidirectional nystagmus and of the sensation of turning is in some way dependent upon vestibular function.

Recently Benson and Bodin (refs. 5–7) have reported several experiments of similar nature. Several of their results are particularly pertinent to the present experiments. They found that the slow phase of the prolonged unidirectional nystagmus during clockwise rotation about a horizontal axis is proportional to the angular velocity attained and also that the unidirectional nystagmus showed a cyclic variation in slow component velocity, the maxima and minima being reached at approximately 270° (left ear down) and 90° (right ear down) positions, respectively (ref. 5). As will become apparent, our results confirm this finding.

The present experiment by Correia and Guedry compares responses of subjects during rotation at both 10 rpm and 30 rpm around an Earth-horizontal axis. The purpose of the experiment was to determine whether or not the system responsible for the prolongation of nystagmus and the sensation of rotation at 10 rpm (60°/sec) would continue to follow at cyclic rates up to 30 rpm (i.e., 180°/sec or 0.5 cps).

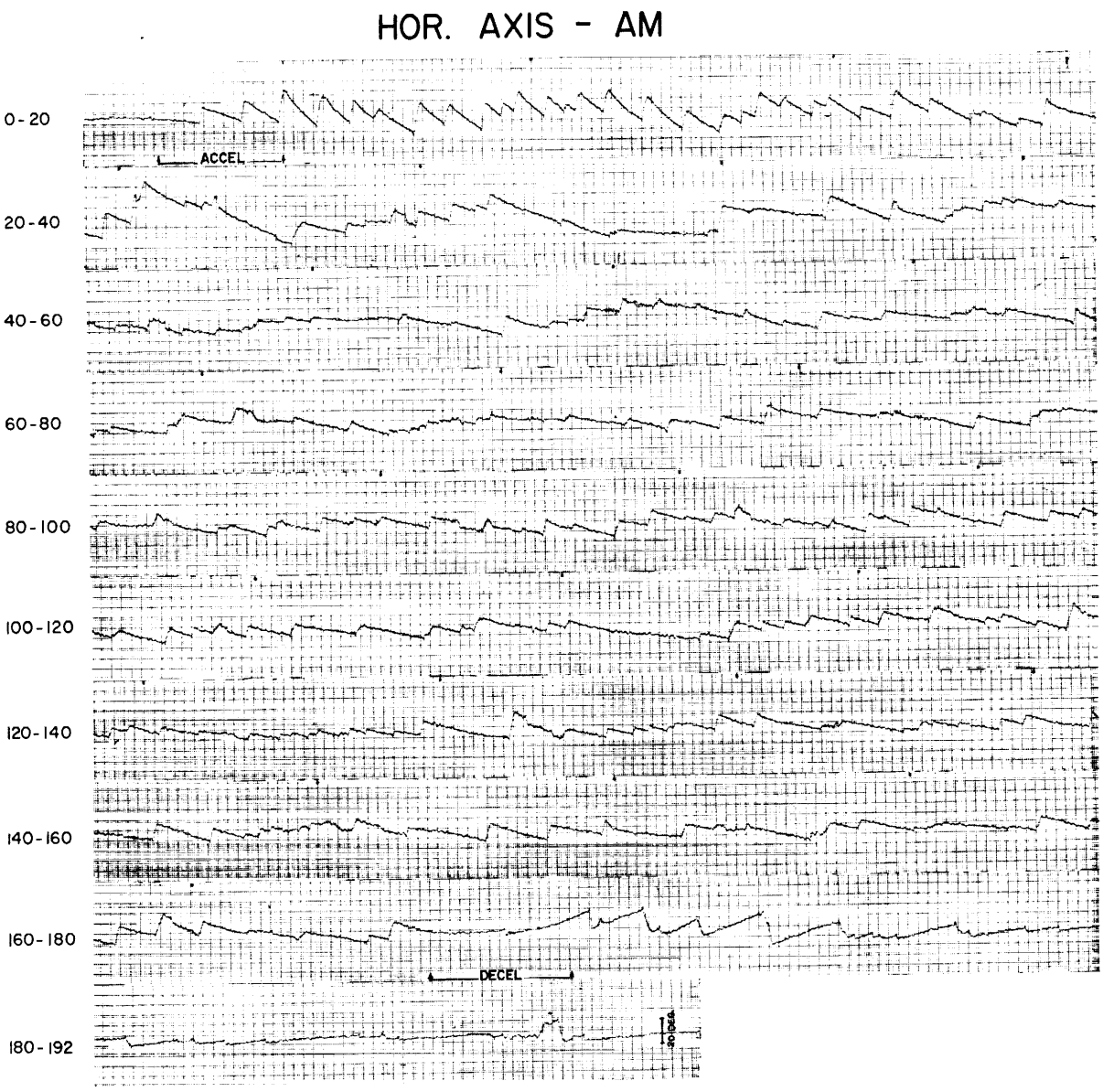

Figure 3.—*Prolonged unidirectional nystagmus during rotation (10 rpm) about horizontal axis and short response after deceleration. Dots mark nose-up positions. (From ref. 1.)*

BRIEF PROCEDURE

Eight subjects completed the experiment in which they were rotated with the cephalocaudal axis alined with the axis of rotation which was horizontal. This configuration placed the horizontal canals in the plane of rotation. Direction of rotation and angular velocity (10 and 30 rpm) were varied, each subject receiving all four combinations; order of presentation was counterbalanced among subjects. Altogether, 20 subjects were run to obtain 8 completions. Of the 12 who could not complete, all were attempting to form a mental image of the body motion relative to the Earth. Of the eight who completed, four were doing mental arithmetic

and the other four were attempting to signal nose-up, nose-down orientation.[1]

The capsule of the rotation device (see ref. 9 for description) was dark throughout each trial. Eye-movement calibration lights were used just before and after each trial. Corneo-retinal potential was used for recording vertical and horizontal components of eye movements.

RESULTS

Figure 4 shows horizontal nystagmus during rotation about a horizontal axis at 10 and 30 rpm from the same subject. Examination of the 10-rpm response throughout its course reveals that nystagmus remains unidirectional. Examination of the 30-rpm response throughout its course reveals that after 30 to 40 seconds, the nystagmus is no longer unidirectional but commences reversing with each rotation cycle. Figure 5 is a plot of the average slow component of nystagmic eye velocity for each second through the 10- and 30-rpm stimuli. From this plot it is apparent that at 10 rpm, nystagmus remains unidirectional in most subjects. The subjective experience of rotation was also continuous and was apparently about correct. The few points across the zero velocity line in figure 5 were contributed by single subjects who had opposing spontaneous nystagmus. Figure 5 also shows that, at 30 rpm, nystagmus commenced reversing, for most subjects between 30 and 50 seconds. There was close correspondence between commencement of the nystagmus reversals and the change in subjective experience. When the subjective reports change during the 30-rpm stimulus, they are very similar

[1] This has been a consistent finding in several experiments (ref. 2; Correia and Guedry) involving horizontal axis rotation and in another experiment involving the vestibular Coriolis canal reaction (ref. 8). Mental tasks requiring calculations or precise estimates seem to reduce sickness. It appears to be feasible to manipulate the "mental factor" in motion-sickness studies.

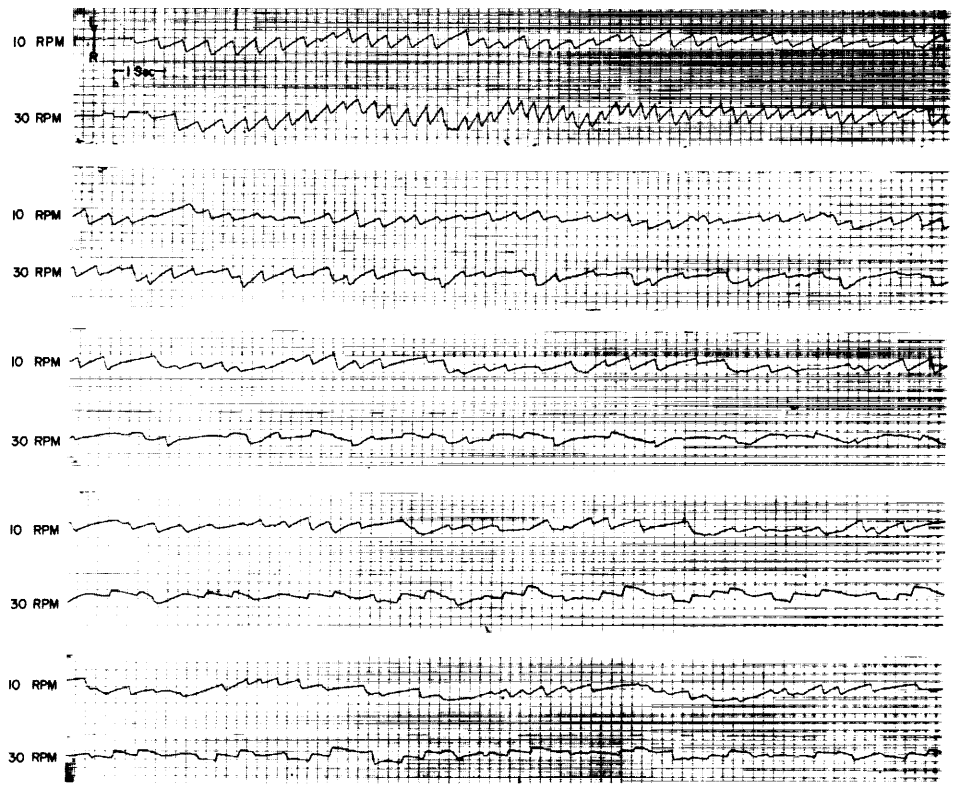

Figure 4.—*Continuous unidirectional nystagmus at 10 rpm and reversing nystagmus after about 35 seconds of rotation at 30 rpm (horizontal axis).*

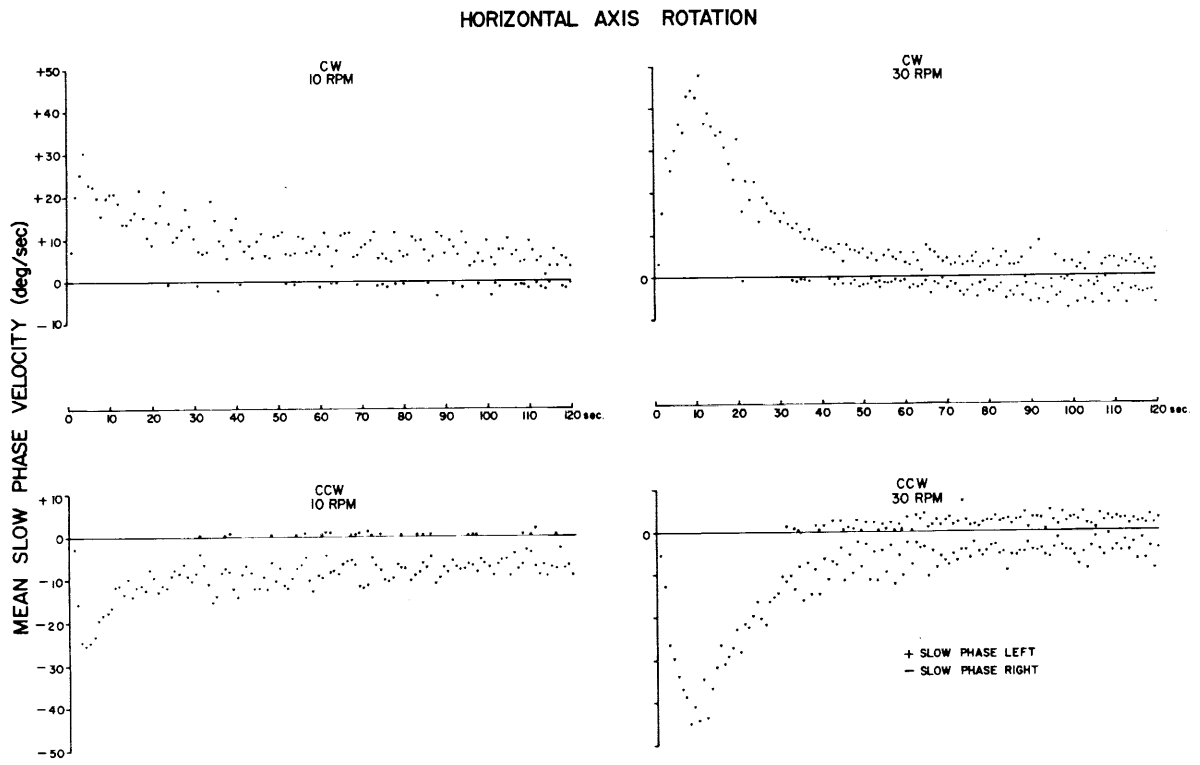

Figure 5.—*Average nystagmus slow component velocity for each second for 120 seconds of rotation at 10 rpm and at 30 rpm.*

to those of L–D subjects during a 10-rpm stimulus. Figure 6 shows the experiences of L–D subjects at 10 rpm from a previous experiment (ref. 1). These subjects do not experience true rotation in that the nose is always pointing in the same direction and the perceived cephalocaudal body axis may acquire almost any orientation relative to gravity. At 30 rpm normal subjects show this same range of responses.

Figure 7 shows responses from normal subjects at 10 rpm, also from a previous study (ref. 1). Notice that most of the normal subjects experience "nose-up/nose-down" rotation throughout the rotation period. BU is one of the exceptions; he experienced rotation and had continuous nystagmus during CCW rotation. During CW rotation, however, he experienced CW rotation for about 30 seconds during which he had continuous nystagmus; but after 30 seconds his experience of true rotation stopped and his nystagmus started reversing; after this, he experienced only a nose-up cylindrical motion without rotation. Figure 8 shows a 15-second sample of nystagmus obtained from this subject after 105 seconds of continuous rotation. In this figure the arrows indicate nose-up positions so that the reversing nystagmus during the CW rotation is beating with fast component right, when the left ear is down, and with fast component left, when the right ear is down. BU had a spontaneous nystagmus left. Thus far, all of our "normal" subjects who have shown a reversing nystagmus and loss of the sensation of continuous rotation at 10 rpm in less than 100 seconds of rotation have had either a spontaneous or a positional nystagmus.

A cyclic variation in the slow component of nystagmus has been apparent in most subjects tested with the axis of rotation horizontal at all rates of rotation used. Benson and Bodin (ref. 5) reported that nystagmus slow component velocity during CW rotation was maximum at about 270° position (left ear down) and minimum at the 90° position (right ear down). Our

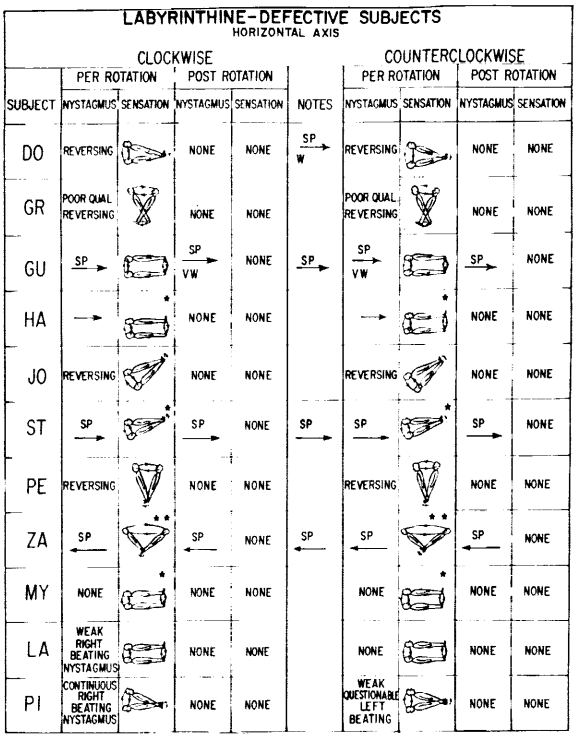

Figure 6.—*Summary of nystagmus and subjective data from L–D subjects when axis of rotation was horizontal. (From ref. 1.)*

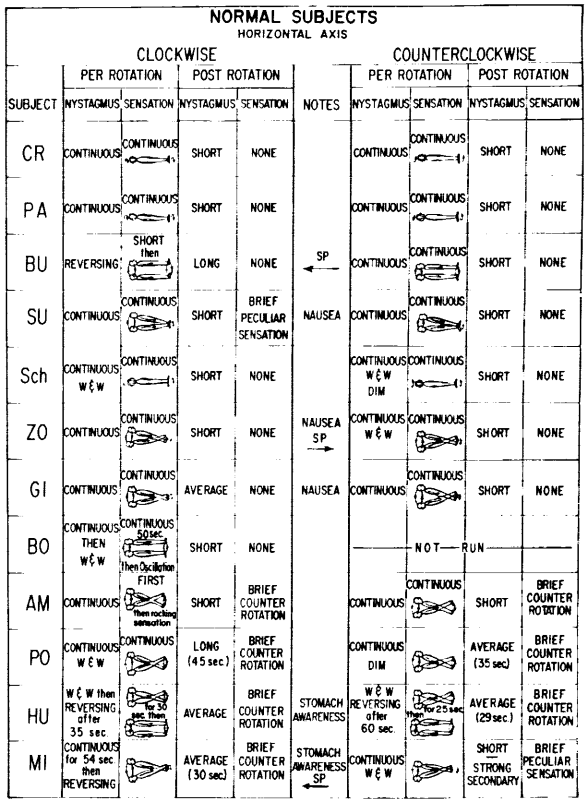

Figure 7.—*Summary of nystagmus and subjective data from normal subjects when axis of rotation was horizontal. (From ref. 1.)*

results confirm these points for the approximate maxima and minima in the cyclic variations and, as a matter of fact, indicate that whether rotation is clockwise or counterclockwise, the maximum is reached at about the 270° (between 200° and 270°) position; but note that when rotation is clockwise, 270° is the left-ear-down position and when rotation is counterclockwise, 270° is the right-ear-down position. At 30 rpm, when the cyclic variations reach the zero baseline, they become manifest by reversing nystagmus, with the original nystagmus direction occurring at about 270° position and nystagmus in reverse direction at about the 90° position. This is apparent in figure 9, which also shows the breakdown in subjective signals at the point the nystagmus commenced reversing. Actually some subjects stopped keypressing altogether when nystagmus started reversing. When questioned about the keypress patterns, all subjects reported that the task was easy at first, but that after a while they lost the sensation of rotation about a horizontal axis and attempted to keypress on the basis of pressure cues.

COMPARISON OF RESULTS WITH OTHER EXPERIMENTS

In regard to the cyclic variation in the slow component of nystagmus during rotation about a horizontal axis, there is an interesting correspondence between the points of response maxima and minima in this experiment and the orientation of subjects relative to linear acceleration vectors in several other experiments in which nystagmus was either elicited or modulated by linear acceleration.

Figure 10 illustrates the several experimental situations under consideration. The uppermost panel represents the present experiment and shows that when the gravity vector, represented as an upward-directed vector, is to the subject's

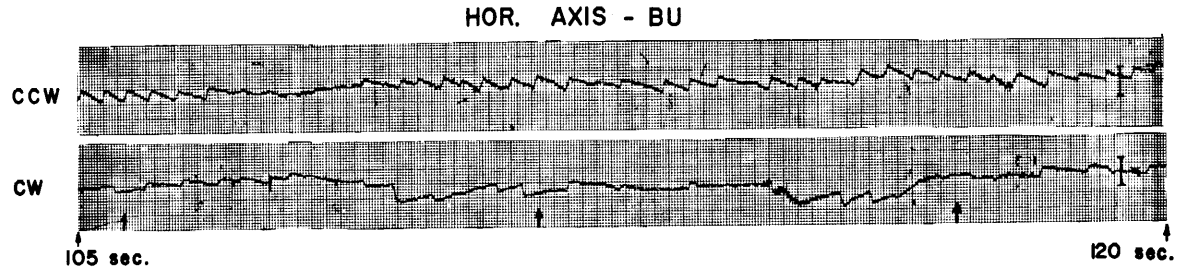

Figure 8.—*Responses of subject with spontaneous nystagmus. Direction of spontaneous nystagmus was left-beating; i.e., same direction as normal reaction to CCW rotation. Arrows on record mark nose-up position. (From ref. 1.)*

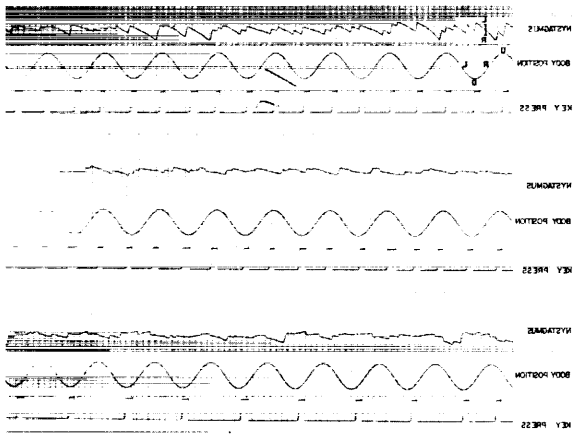

Figure 9.—*Showing reversing nystagmus in lower panel at same point as change in keypress signals of nose-up and nose-down positions.*

left, nystagmus left is augmented; and when it is to the subject's right, nystagmus left is diminished. After a period of rotation at 30 rpm, linear acceleration (g) to the left produces nystagmus left; "g right" produces nystagmus right.

The second panel represents an experiment by Niven, Hixson, and Correia (ref. 10). This experiment was performed on a track which permitted horizontal linear oscillation. A variety of subject orientations were used, and it was found that lateral oscillation produced nystagmus right when the subject accelerated right and nystagmus left when the subject accelerated left. Figure 11 shows horizontal nystagmus produced by lateral linear oscillation. Forward and backward oscillation (as well as a variety of head orientations in which the skull's sagittal plane was alined with the linear acceleration vector) failed to yield horizontal or vertical nystagmus. For the lateral oscillation, phase relations as well as maximum slow-component velocity of nystagmus were about constant for a frequency range between 0.2 cps and 0.8 cps. An interesting result of this experiment was that during lateral oscillation, subjects experienced little or no lateral tilt, although the resultant linear acceleration was angularly displaced from the body axis by as much as 30°. Rather, linear velocity was experienced. This confirms results of earlier experiments on parallel swings (ref. 11). In the fore-and-aft oscillation, more tilting was experienced. This experiment by Niven and colleagues provides a key for examining a number of other experimental findings. The

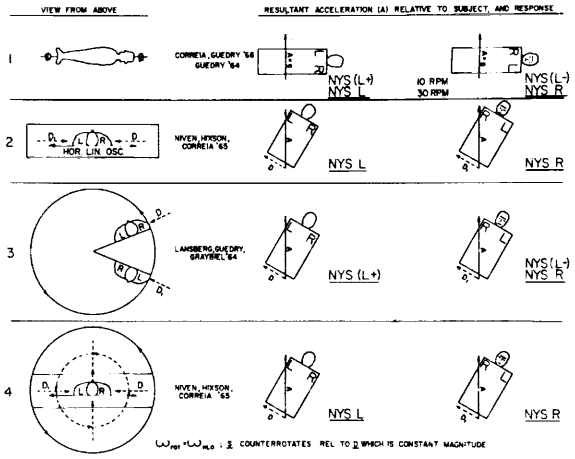

Figure 10.—*Comparison of several experiments which indicate that lateral linear accelerations produce nystagmus in the direction of the linear acceleration which can augment or diminish nystagmus produced by angular acceleration.*

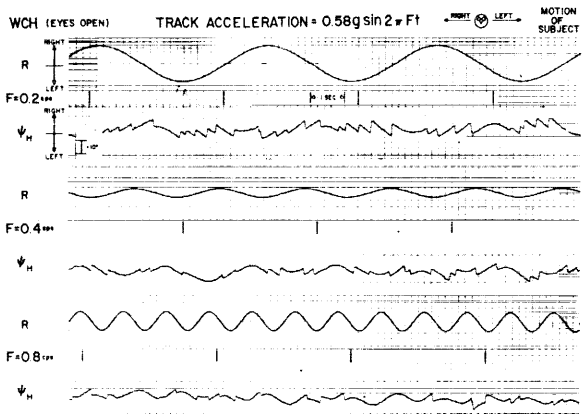

Figure 11.—*Nystagmus produced by lateral horizontal linear acceleration. (From ref. 10.)*

correspondence between cyclic oscillation during rotation about a horizontal axis and the horizontal linear oscillation experiment is apparent in figure 10.

Panel 3 of figure 10 represents a centrifuge experiment by Lansberg et al. (ref. 4). Again when the resultant linear acceleration vector was to the subject's left (D in fig. 10), nystagmus left was augmented, and when the resultant linear acceleration vector was to the subject's right (D_1 in fig. 10), nystagmus left was diminished and then reversed to right-beating nystagmus. Figure 12 shows the direction and magnitude of nystagmus in the D and D_1 positions.

Panel 4 in figure 10 illustrates an experiment by Niven et al. (ref. 10) in which subjects were simultaneously rotated and oscillated radially on a track. After constant rotation is attained, if the cyclic rate of rotation equals the cyclic rate of track oscillation, a resolution of the variation in (1) linear acceleration relative to the track, (2) centripetal acceleration, and (3) Coriolis acceleration produces a linear acceleration vector which changes its direction relative to the subject in a manner which is equivalent to counterrotation of a subject about a fixed vertical axis away from center on a vertical-axis centrifuge. In this case also when the linear acceleration was left, nystagmus was left, and when linear acceleration was right, nystagmus was right. Figure 13 shows nystagmus produced in this situation.

In figure 10, columns 2 and 3 show the consistencies between these various experiments; viz, laterally directed linear acceleration tends to produce nystagmus in the direction of the linear acceleration, and this can augment or diminish nystagmus produced by angular accelerations.

SOME PROBLEMS TO BE RESOLVED

Although there were consistencies between these experiments, there are also puzzling combinations of findings. For example:

1. Forward and backward linear oscillation does not produce nystagmus (ref. 10), either vertical or horizontal, yet head-over-heels rotation about a horizontal axis (Hixson, personal communication) produces prolonged vertical nystagmus (beyond the duration of the "expected" cupula return).

2. Unidirectional nystagmus produced by rotation about a horizontal axis when the rate of rotation is 30 rpm stops after 30 to 50 seconds and the sensation of rotation changes at this point. Both the sense of rotation and conception of axis of rotation are lost and any of a variety of orientations is perceived (almost as one might expect in a weightless state). Yet long after this point in time, the cyclic variations in nystagmus about a zero baseline (in other words, reversing nystagmus) continues. It is as though one mechanism can follow this stimulus frequency, whereas another mechanism cannot. These two points suggest that one mechanism may account for the cyclic variation in nystagmus and another for the long-continued nystagmus and sense of rotation at lower rotation rates. Further evidence which may be adduced for this possibility is that with horizontal linear oscillation, the slow component velocity of nystagmus is about the same for stimulus frequencies between 0.2 and 0.8 cps, whereas with angular accelerations within this frequency range there is at least a fourfold change in slow component velocity. Furthermore, for 0.2-cps oscillation of angular acceleration there is a phase lag of 80° to 90° between stimulus and response, whereas for linear acceleration of 0.2 cps, the phase lag is about 30° (ref. 10).

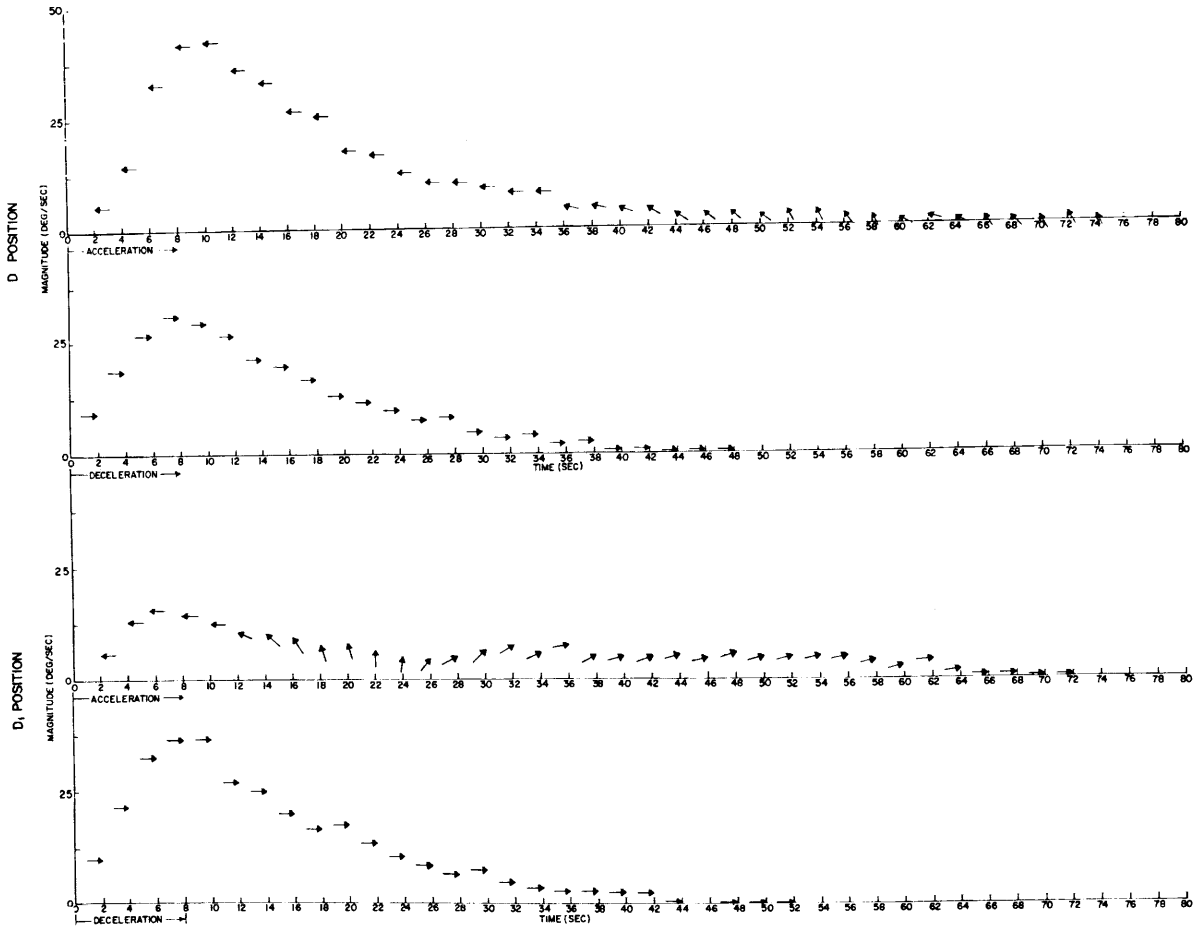

Figure 12.—*Left-beating nystagmus produced by comparable angular accelerations in subject positions D and D_1. (From ref. 4.)*

It is possible that several modes of canal response in addition to other systems are involved in the nystagmus recorded during various combinations of angular and linear acceleration. Prolonged unidirectional nystagmus during "horizontal axis rotation" at 10 rpm cannot be explained by a density difference between cupula and endolymph in the lateral canals. If this were true then, due to the alternating direction of the g-vector, nystagmus should commence reversing after 30–40 seconds with the 10-rpm stimulus. This does not occur in most subjects, but other operating modes of the canals as well as other systems may be involved. Another operating mode of the canals, in addition to known responses to angular acceleration, was suggested by Benson and Bodin (ref. 5). A density difference between endolymph and perilymph could cause a traveling wave around the membranous ring which would maintain a flow of endolymph during rotation about a horizontal axis. Recent experiments by Money (personal communication) can be interpreted as supporting this general idea of an alternative operating mode for the canals. However, some system other than the canals is probably also involved in the modulation of nystagmus and sensation. This is strongly supported by the fact that nystagmus and the sensation produced by cessation of rotation with the axis horizontal are greatly suppressed, as compared to the vertical-axis configuration, irrespective of stopping

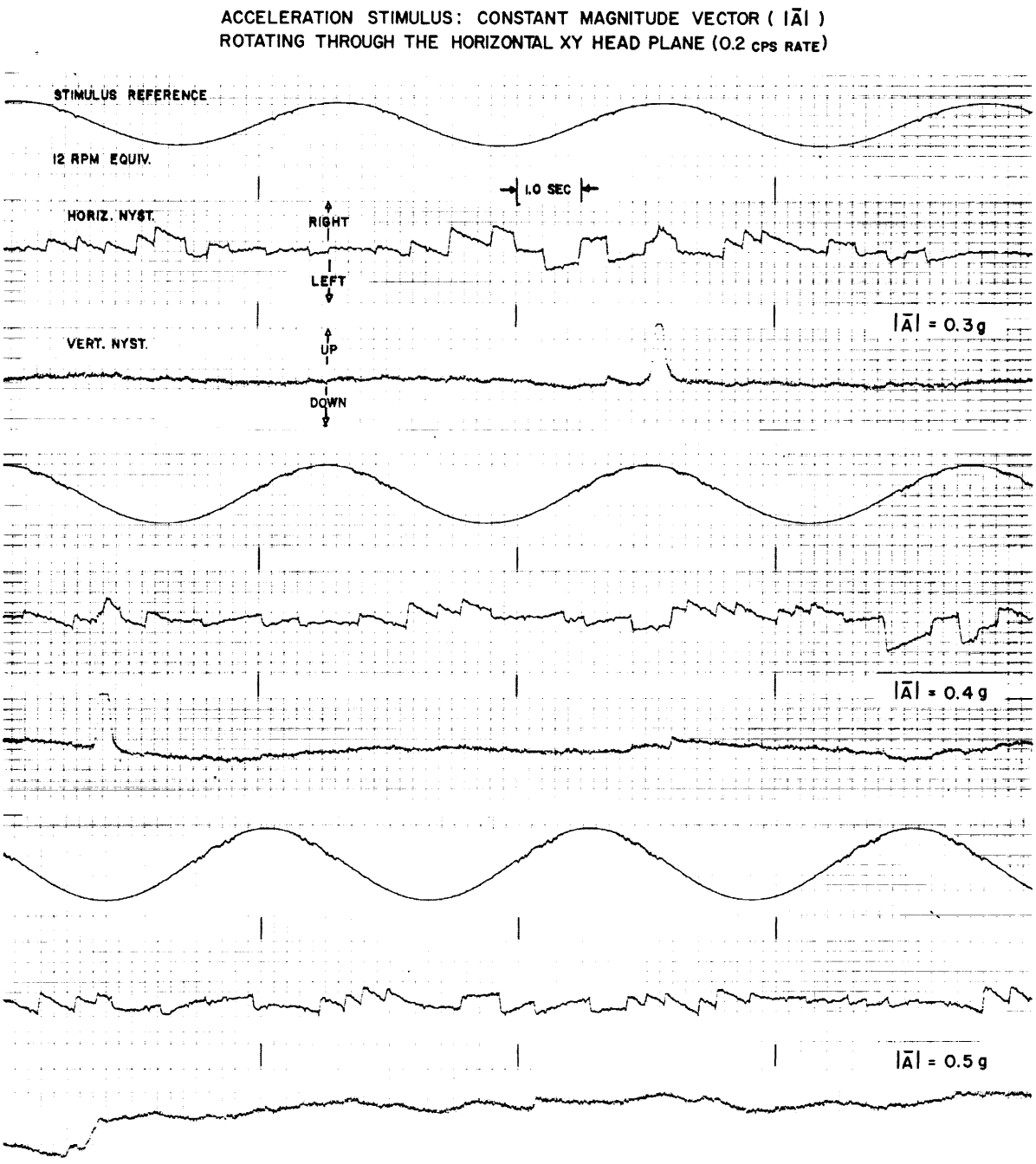

Figure 13.—*Nystagmus produced by horizontal linear oscillation relative to track at a frequency of* ω_{hlo} *during rotation of the track at a constant angular velocity,* ω_{rot}. *Circular frequency of* ω_{hlo} *equaled* ω_{rot}.

position (left-ear-up, right-ear-up, nose-up, or nose-down) (refs. 2 and 6). In other words, irrespective of how the linear acceleration vector is oriented relative to the canals, postrotational responses to horizontal-axis stimulation are suppressed. There may be slight differences in the amount of suppression in these various stopping positions (refs. 2, 6, and 7) and, if so, then a minor part of the modulation of nystagmus by linear acceleration may be attributable

to a cupula-endolymph density difference, but other interpretations would be equally plausible.

3. Another curious result is the absence of the sensation of tilt during lateral oscillation (refs. 10 and 11), even though the angle between the resultant force and the body axis changed by as much as 30°. This was for frequencies between 0.2 and 0.8 cps. However, for the first 30 to 50 seconds of rotation about a horizontal axis, change in orientation relative to gravity seemed fairly accurately perceived at a frequency of 0.5 cps (30 rpm). In other words, during the period of classical cupula response to angular acceleration, veridical perception of orientation relative to gravity was maintained.

One of many possible interpretations of these results is that the otolith system can provide information on angular position relative to gravity at fairly high rates of change of orientation, but this sensory input is correctly perceived only when otolith and canal information are coordinated. At 30 rpm, after the canal response to angular acceleration has subsided, the alternative mode of canal operation is not capable of following the rapid alternation of the stimulus; in this situation the otolith system yields a reversing nystagmus and is not capable, alone, of maintaining an accurate perception of the reorientation relative to gravity. At lower rotation rates (horizontal axis) the semicircular canals maintain unidirectional response by the alternative mode of operation, and together with the otoliths maintain an accurate perception of the continuous reorientation relative to gravity. The cyclic variation in nystagmus during horizontal-axis rotation is attributable to other systems, probably the otoliths and tonic neck reflexes. When the otoliths are stimulated by lateral horizontal linear oscillation (without angular acceleration), sensation of linear velocity and nystagmus are produced primarily by dynamic otolith stimulation; the change in angular displacement of the body relative to the resultant linear vector (i.e., "tilt") is underestimated due to the absence of coordinated canal information except at low frequencies (i.e., low rates of reorientation of the resultant linear vector). At very low frequencies the otoliths with the aid of pressure cues maintain a veridical perception of "tilt," without coordinated canal input. When the resultant linear vector is changed in one plane while the semicircular canals are stimulated in another plane, the change in angular displacement of the body is underestimated except at very low frequencies, which would account for the lag effect; i.e., long indication time (cf. ref. 12), reported by Clark and Graybiel (refs. 13–15) for the oculogravic illusion.

Finally, it is proposed that the otoliths yield sensory information which is necessary to the perception of the orientation of the axis of rotation relative to gravity while the canals determine the sense and magnitude of the perceived angular velocity relative to the otolith-determined axis. If the canals respond according to classical concepts, i.e., if they are stimulated solely by angular acceleration, then they alone cannot possibly determine the perceptual axis of rotation relative to gravity. If the canals are in some way relatively insensitive responders to linear acceleration, then it still seems unlikely that they could adequately signal simultaneously both the orientation of the rotation axis relative to gravity and the magnitude and sense of rotation about that axis.

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DISCUSSION

JOHNSON: In order to open this discussion, I would like to point out that we at Toronto have been trying to determine some basic experimental evidence to show just where the linear accelerations might cause this nystagmus, whether it is directly on the otoliths only or on the canals or indirectly from the otoliths affecting the canals. Dr. Money will just briefly show some of our results.

MONEY: We have found this question, of nystagmus continuing for a long time at constant angular velocity when the axis of rotation is horizontal, to be fascinating for a couple of years now. We wanted to find out whether we could get this without the initial angular acceleration. We have a device in our laboratory at the Defence Research Medical Laboratories which rotates the subject on the periphery of the turntable on a smaller turntable. The smaller turntable turns in the opposite direction from the big one at the same rate, so that the subject always points in the same direction. If he starts out pointing north, he keeps pointing north, but he goes around a circle. The effect is a rotating linear vector as applied to the subject. In figure D1 is shown the sort of response that we have seen in a human subject with this stimulus. It is a rotating linear acceleration which doesn't cause any movement in a ring of fluid within a sealed glass tube, and presumably no stimulus of the semicircular canals in the classical way. But nevertheless, Dr. Graybiel, Dr. Johnson, and I found a nystagmus in some human subjects on this device. In fact we got some nystagmus in 6 out of the 10 we have so far tried. You will also note in figure D1 that there is modulation of the speed of the slow component, which has been reported with the rotation about a horizontal axis. In some cases the nystagmus is not a constant one, in one direction, in man. Instead, the nystagmus reverses, as was demonstrated by this subject. The figure shows a left-beating nystagmus which changes to right beating, left, left, left, changes to right, right, left, left, left, right, right, left. So we do get this reversing as well in some humans on our revolution-without-rotation device, as we call it.

Another piece of information we have on this is with cats in which we have never found the reversing nystagmus. The stimulus in cats always gives a nystagmus in one direction for one direction of rotation

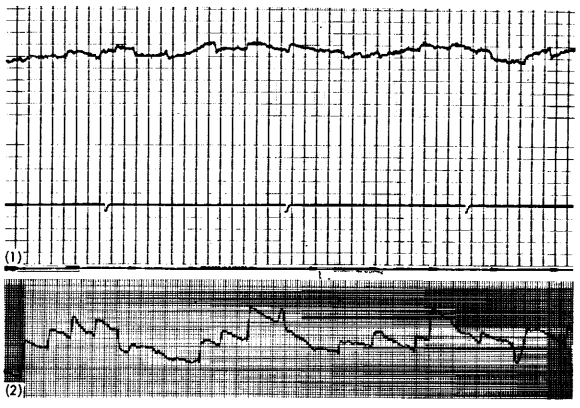

Figure D1.—*Two kinds of responses to a linear acceleration of changing direction.* (1) *Reversing nystagmus.* (2) *Nystagmus in one direction.*

of the main turntable, and the opposite nystagmus for the opposite direction of rotation of the main turntable. In cats it is a large obvious nystagmus of this variety rather than the smaller one, probably because we put needle electrodes into the cat. We have their heads fixed with steel plates embedded in the skulls with four screws, which are put in at least 3 months before testing, plus the wire-through-the-teeth technique of Fernandez and Henriksson. Their heads aren't flopping about; they are firmly fixed. But they have nystagmus without any angular acceleration, and it goes on for as long as the table rotates. We tried it for up to 15 minutes, and it just keeps right on going. We also have some white cats with otolithic defects; and although Hallpike's laboratory has shown that these white cats have utricular otoliths, some of ours have no response whatever to our standard otolith test. So these cats are at least grossly defective from the otolith point of view and, in fact, we suspect they might be entirely lacking otoliths. These cats have this nystagmus (fig. D2). It is huge in some of them, and can't be missed. We took some cats and plugged their horizontal canals. I shouldn't say some. We have only done this on two so far, but on both of these cats we find none of this nystagmus. The white cats with defective otoliths have the nystagmus and, so far, cats without canals haven't. This is an experiment which is just beginning. It doesn't have the virtue of being finished, but it has the virtue of being current.

YOUNG: The only MIT experiments relative to nystagmus resulting from pure linear stimulus were on a linear track only in the left-right, not in the fore-aft plane. We do find responses similar to the one Dr. Guedry showed over most of the frequency range.

Am I correct that you found the reversal effect somewhere between 10 and 30 rpm?

GUEDRY: We have not explored between 10 and 30 rpm. We jumped from 10 to 30 rpm. Now we have to go back and find out where it starts coming in. We did find one subject at 30 rpm who had continuous nystagmus, and a few subjects, since this reported experiment was completed, who have had reversing nystagmus at 10 rpm. Different subjects respond differently. Both positional and spontaneous nystagmus can change the response to rotation. If there is either spontaneous or positional nystagmus, there may be reversing nystagmus. If the rotation stimulus opposes spontaneous nystagmus, then we find a reversing nystagmus. Positional nystagmus can be seen to modify nystagmus produced by horizontal axis rotation as subjects rotate through the critical position.

YOUNG: The related data, which I will go into in a little detail tomorrow, I think bear on this. We investigated the phase of subjective perception of linear velocity versus sinusoidal frequency, trying to get linear sensor frequency responses, and Dr. Meiry found that the response at 3 radians per second, which is about 30 rpm, is approximately 150° behind that at the low frequency. Thus at 30 rpm you are spinning

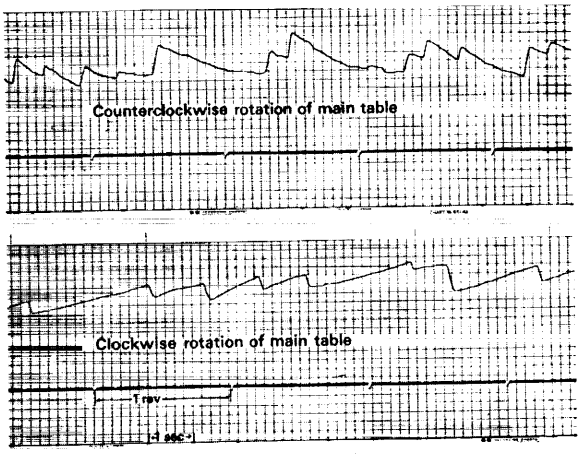

Figure D2.—*Horizontal nystagmus of cat 256 on the counterrotating table. Nystagmus to the right with counterclockwise rotation of the main turntable; to the left with clockwise rotation of the main turntable (20 rpm, constant angular velocity, complete darkness). The cat is white and has defective otolith function.*

a man at such a frequency that the linear acceleration sensors give a response which is phase reversed from that at low frequency. Consequently, that might explain the actual reversal of nystagmus that you find.

GUEDRY: I liked the otolith explanation at first, but then these data with the two cats described by Dr. Money bother me.

YOUNG: We are not saying that this is necessarily otolith. We are just saying it is linear response.

BERGSTEDT: It seems reasonable to me that the otoliths may be responsible for the curious new findings. Could the reverse nystagmus obtained on cessation of horizontal rotation be the usual so-called central compensation nystagmus, a pronounced nystagmus in one direction?

GUEDRY: Do you mean when we stop around the horizontal axis?

BERGSTEDT: Yes.

GUEDRY: We have a very brief response.

BERGSTEDT: Yes; but this brief response corresponds to the intensity of nystagmus during stimulation as shown. The brief response could be compared with what you gain after a constant angular acceleration, for example, or a long-lasting optokinetic nystagmus. Nystagmus disappears during stimulation, and then there is nystagmus in the other direction.

It lasts about the same time in your tests. I mean it could reasonably be the so-called central compensation. Have you tested persons with unilateral labyrinthectomies in this device? I don't think it will give you anything new, but it should be only just more interesting.

GUEDRY: That is something we would like to do. We have had just one person who had reduced function in one ear, but caloric tests did show that this person did have some function. I just don't have any information.

BERGSTEDT: When a man is placed in a pivoting cabin in a centrifuge and exposed to an angular acceleration which is above threshold for both sensation and nystagmus, for example 10°/sec/sec, and he closes his eyes when you start the centrifuge, he doesn't have even the slightest sensation, except perhaps for the first few seconds, about going around the hub of the centrifuge. But after the initial turns, he has only the feeling of linear acceleration and going forward and upward. If the speed is decreased, he has a feeling of going downward and not the slightest feeling of rotation around the centrifuge, except perhaps during the last turn. This is strongly in favor of an interaction between the otoliths and cupula. In that situation the otolith organs damp the cupula or damp the sensation. It is also possible that the otoliths are those organs which perceive rotation. From old results obtained by cupulometry, you know that the sensation cupulogram and the nystagmus cupulogram are not parallel. This has been a matter of discussion for many years, and it is usually said that the sensation and nystagmus signals go different ways. This could be the reason for the lack of parallelism. One explanation could be that, in regular cupulometry, sensation has its genesis in the otolith organs and nystagmus in the cupula organs.

GUEDRY: I originally was in favor of an otolith explanation for the continuous sensation of rotation and the continued nystagmus during rotation about a horizontal axis and would still like to get back to it; but I am concerned about the data that Dr. Money has obtained in cats with plugged canals. I think we need to have more evidence before we can choose a final interpretation of this.

BERGSTEDT: I made a study of linear acceleration on the centrifuge on patients with positional nystagmus and also normal subjects, who were later intoxicated with alcohol so they got positional alcohol nystagmus. In this study I was mainly interested in the state of increased g; so I observed the nystagmus movements during acceleration, usually 20 to 40 seconds, but I didn't include these in my report. But I often observed nystagmus while going up to the desired g-level where the nystagmus stopped. I did not go on to analyze if it was the result of angular acceleration or Coriolis force acting on the cupula organs.

The results I got in 1961 in studying these groups of different kinds seem not to contradict the results discussed here. I might say looking upon Dr. Guedry's results speaks in favor of them. I think, too, it is a question of otolith stimulation, presumably. Dr. Money's results that 6 out of 10 subjects got nystagmus in his counterrotation device and my results correspond. If you keep a normal young individual in a lateral position for a long period with his eyes closed, and if you then turn him to the other side, he will very often show nystagmus in this new position. This is so usual that it must be looked upon as close to a normal finding. It is especially pronounced in young people. I believe that if you increase to as much as 2 g in shorter time than 10 to 20 seconds, for example, you will increase the percentage of these normal persons who show nystagmus after so-called otolith stimulation or linear acceleration.

Modification of Per- and Post-Rotational Responses by the Concomitant Linear Acceleration

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SUMMARY

The nystagmus and sensation of turning engendered by an angular acceleration are modified by the direction and magnitude of the concomitant linear acceleration.

With a linear acceleration of 1 g, the rate of decay of postrotational responses was increased when otolithic and other gravireceptor signals were not in accord with signals from canal receptors. Responses to angular stimuli in yaw suffered a greater decrement than those in pitch or roll.

From experiments in which the subject's orientation to gravity was changed immediately following an impulsive deceleration, it was concluded that these effects were brought about by inhibition, within the central nervous system, of canal afferents by competing gravireceptor signals.

However, for higher linear accelerations and rotating linear acceleration vectors, it is not possible to exclude peripheral mechanisms.

INTRODUCTION

In aerospace flight, man is exposed to angular and linear accelerations of a temporal and spatial pattern which can fall outside the normal functional range of the vestibular receptors. As a result, the sensations and reflex figures engendered by the vestibular signals may be either inadequate or inappropriate. Many of the illusory sensations which constitute spatial disorientation in conventional flight have been described and are to be explained on the basis of our present knowledge of the behavior of vestibular receptors (refs. 1–3), in which the validity of the functional differentiation of semicircular canal and otolith organ has not been doubted. But with the advent of space flight, speculation about the effect of weightlessness on vestibular responses has raised questions about the behavior of these specialized receptors, questions which could not be answered with certainty on the basis of experimental observations made in the presence of the acceleration of gravity.

It was to be expected that during weightlessness, afferent signals generated by the sensory receptors of the otolithic maculae would, on movement of the head, differ appreciably from those which are elicited when the same head movements are made in the presence of gravity. However, an alteration of semicircular-canal responses cannot be predicted with the same certainty. While accepting the primary functional differentiation of semicircular-canal and otolith organs, there is a body of experimental evidence which suggests that the activity usually attributed to semicircular-canal receptors is modified by the direction and magnitude of the linear acceleration (refs. 4–13). Conversely, other workers, particularly those who used subjective responses as indicants of semicircular-

canal activity, have failed to demonstrate any effect when the linear acceleration vector was changed (refs. 14–17).

EFFECT OF LINEAR ACCELERATIONS GREATER THAN 1 G

Experimental investigations in this problem area began, at the RAF Institute of Aviation Medicine, with an examination of per- and post-rotational responses in the human centrifuge. It was found (ref. 8) that the nystagmus produced by an angular acceleration to constant velocity decayed more rapidly when the subject was at the end of the centrifuge arm and exposed to a resultant acceleration of 3.1 g, than when he was close to the axis of rotation, and cupula restoration occurred at 1 g (figs. 1 and 2). In this experiment the subject faced the center of rotation so that at constant speed, the resultant linear acceleration lay in a postero-anterior direction. However, when he faced

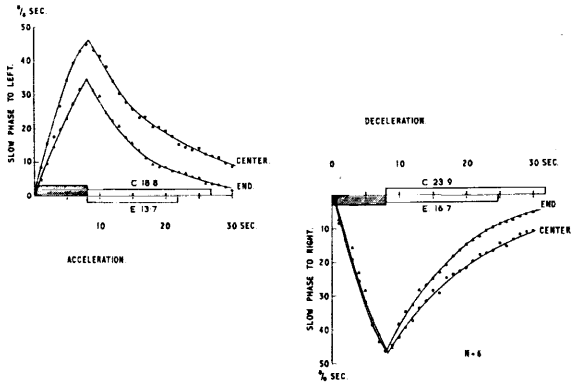

Figure 1.—*Comparison of horizontal nystagmus in subjects at end of centrifuge arm and when close to axis of rotation.*

Each point is the mean angular velocity of the slow phase of nystagmus, recorded in six subjects, each of whom had two runs at both center and end positions. The angular stimulus was a constant acceleration of 10°/sec² from 25°/sec to 105°/sec. Deceleration, of similar time course, followed after 60 sec at 105°/sec. Rotation was always clockwise. At the end of the centrifuge arm, subjects sat facing the center, with head vertical. Attitude did not change during rotation. The resultant linear acceleration was 1.02 g, 11° from vertical, at 25°/sec, and 3.1 g, 71° from vertical, at 105°/sec.

The mean durations of the sensation of turning are indicated by horizontal blocks on the abscissa. Sensation times did not differ significantly from one another between conditions.

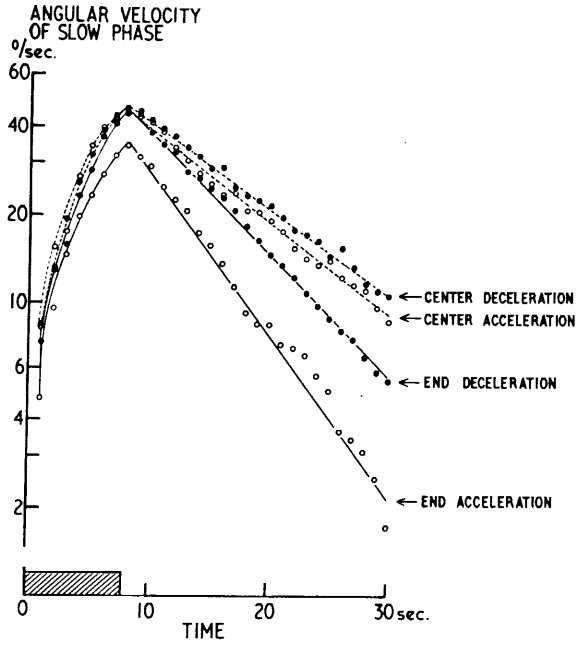

Figure 2.—*Logarithmic plot of decay of horizontal nystagmus from mean values displayed in figure 1.*

Analysis of variance of slopes of individual regression equations revealed that nystagmus decayed more rapidly (p=*0.001*) *at 3.1 g (end of acceleration) than in the other three conditions where the slopes did not differ significantly from each other.*

40° to the right or left of the radius, not only was the time constant of decay of nystagmus reduced but a sustained nystagmus was observed which beat to the right or left according to the subject's orientation to the resultant accelerations (ref. 4) (figs. 3 and 4). From this observation, confirmed and extended by Lansberg, Guedry, and Graybiel (ref. 13), it was tentatively concluded that a linear acceleration could modify the dynamics of the canal-cupula-endolymph system and also produce a sustained cupula deflection by the direct action of the linear acceleration on the end organ. Though it was not possible to exclude a neural mechanism in which the activity engendered by ampullary signals was modified as a result of the change in otolithic and other somesthetic signals, it was considered that the alteration of canal dynamics was a simpler hypothesis which received support from electrophysiological studies of the behavior of ampullary afferents in the cat (ref. 18) and the frog (ref. 19) on alteration of the linear acceleration.

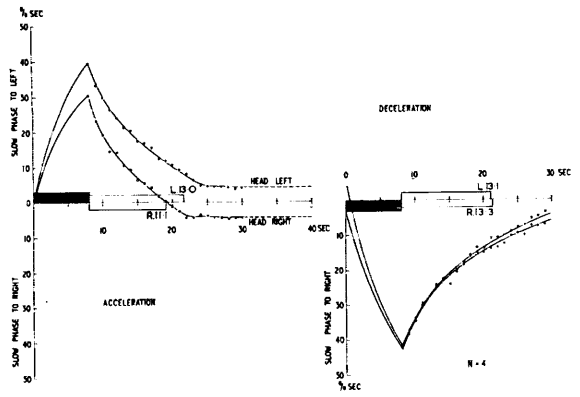

Figure 3.—*Modification of horizontal nystagmus by the direction of a linear acceleration of 3.1 g. Each point is the mean slow-phase velocity obtained in eight runs from four subjects.*

The subject sat at the end of the centrifuge arm, with head vertical, and faced 40° to the right or left of the radius.

Angular motion was the same as in figure 1. The differences between the duration of the sensation of turning, indicated on abscissa, in the four experimental conditions were not significant.

Note sustained nystagmus at 3.1 g.

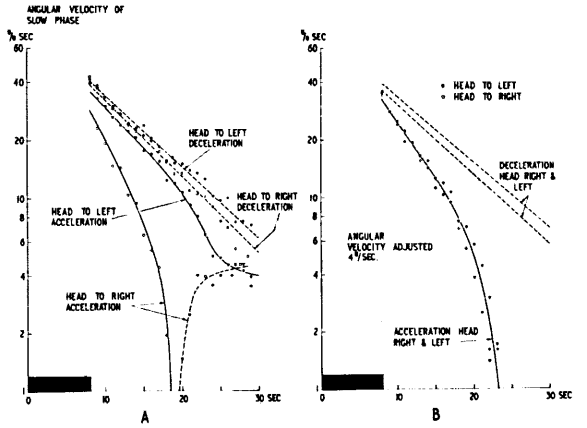

Figure 4.—*A. Logarithmic plot of decay of nystagmus obtained from mean values displayed in figure 3. Reversal of nystagmus in head right position at 3.1 g, indicated by interrupted line. B. When nystagmus recorded at 3.1 g was adjusted by +4°/sec and −4°/sec, in head right and left positions, respectively, the decay followed a similar time course which was not a simple exponential function.*

EFFECT OF 1 G ON PER- AND POST-ROTATIONAL RESPONSES

Rotation About a Horizontal Axis

Following the demonstration that a sustained, high, and it may be argued unphysiological, linear acceleration could alter nystagmus engendered by an angular acceleration, it was desirable to find out if a linear acceleration of 1 g, which should not evoke abnormal mechanisms, was capable of modifying canal responses. Both Guedry (ref. 11) and Benson and Bodin (ref. 5) approached this problem by comparing the response elicited by angular stimulus in yaw (z body axis) when the axis of rotation was vertical, and when horizontal. The angular stimulus to the canals was identical in each situation, but in the former the orientation of the vestibular apparatus to gravity was constant, while in the latter the direction of the linear acceleration vector in the transverse plane of the head changed continually.

When subjects were rotated about a horizontal cephalocaudal body axis, and so exposed to the rotating 1-g vector, horizontal nystagmus persisted for as long as rotation continued (fig. 5). This was in contrast to the exponential decay and disappearance of nystagmus, after some 40 seconds, produced by an equivalent angular impulse, when the axis of rotation was vertical. Although a rapid acceleration (300°/sec²) to constant velocity was employed routinely in these experiments, the sustained per-rotational nystagmus was similar when the stretcher was accelerated at a near-threshold rate (1°/sec²) to the same speed (ref. 5).

A continuing per-rotational nystagmus was not unexpected in this experimental situation, but if the cupulae of the horizontal (lateral canals) were deflected directly by the linear acceleration, or by shifts in the distribution of endolymph and perilymph as postulated by de Kleijn and Magnus (ref. 20), the direction of nystagmus should have alternated during each revolution of the stretcher. Whereas a regular modulation of nystagmus slow-phase velocity occurred during each revolution (figs. 6 and 7), it was only at the slowest speed of rotation (10°/sec) that a change in the direction of nystagmus occurred, and this only in a minority of individuals examined. From a polar plot of nystagmus slow-phase velocity (fig. 8), it was ap-

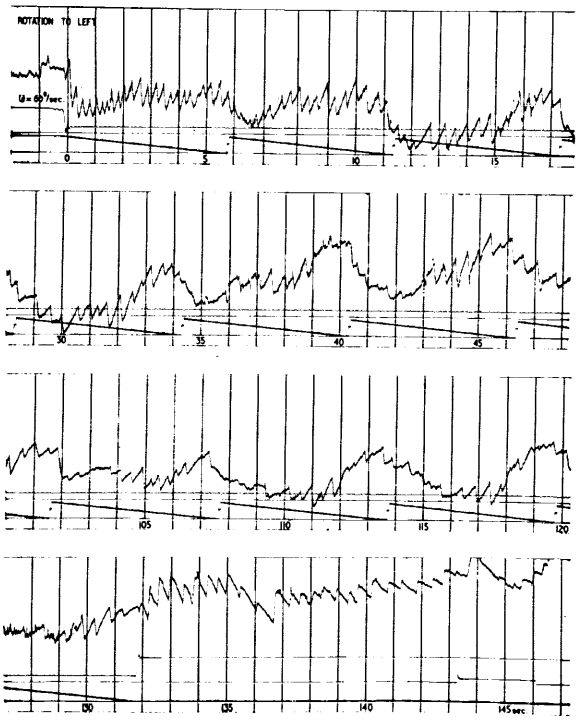

Figure 5.—*Horizontal nystagmus recorded during, and after, rotation of a subject about a horizontal, cephalocaudal (z) axis at 60°/sec. From above downward, the traces are: eye position, angular velocity of rotating stretcher, subject's event marker, and stretcher position. Vertical lines are at 1-sec intervals. Rotation began at t=0 sec and ended at t=132 sec.*

parent that it was only during the period in which the subject moved from the prone toward the supine position that the speed of rotation made an appreciable contribution to nystagmus velocity. As the typical per-rotational response was not observed in labyrinthine-defective subjects (ref. 11), it was concluded that normal canal and otolith function was necessary. But was the nystagmus engendered by stimulation of ampullary or otolithic receptors? It was apparent that it was not brought about by a mechanism which subsumed a difference in density between cupula and endolymph (refs. 21 and 22), or shifts in fluid distribution in the manner proposed by de Kleijn and Magnus (ref. 20). However, because nystagmus has not been observed in experimental animals as the result of direct stimulation of otolith organs (refs. 23 and 24), a mechanism in which semicircular-canal receptors were stimulated was favored. Benson and Bodin (ref. 5) suggested that redistribution of the endolymph within the membranous canal might occur (refs. 25 and 26) during rotation about a horizontal axis in such a manner as to produce a differential fluid pressure across the cupula for as long as rotation continued. Guedry (refs. 11 and 12), on the other hand, favored an otolithic mechanism and considered that the sustained nystagmus was a manifestation of the activity of otolithic and other somesthetic receptors, which conveyed information about continued rotation in yaw and in consequence evoked a compensatory nystagmus in that plane. Some support for this hypothesis was afforded by the pattern of modulation of nystagmus velocity which was similar, in its polar distribution, to the discharge of otolithic receptors described by Lowenstein and Roberts (refs. 27 and 28).

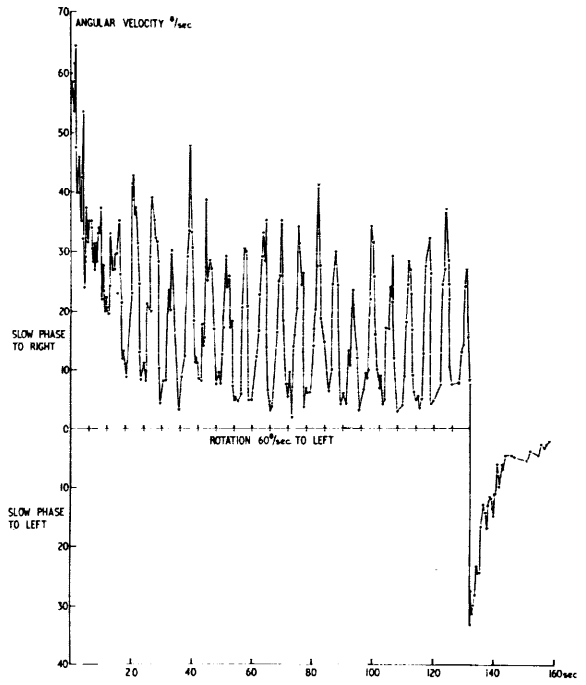

Figure 6.—*Linear plot of angular velocity of slow phase of nystagmus during, and after, rotation about a horizontal, cephalocaudal (z) axis at 60°/sec. Note modulation of nystagmus velocity during each revolution of the stretcher, indicated by vertical marks on zero abscissa.*

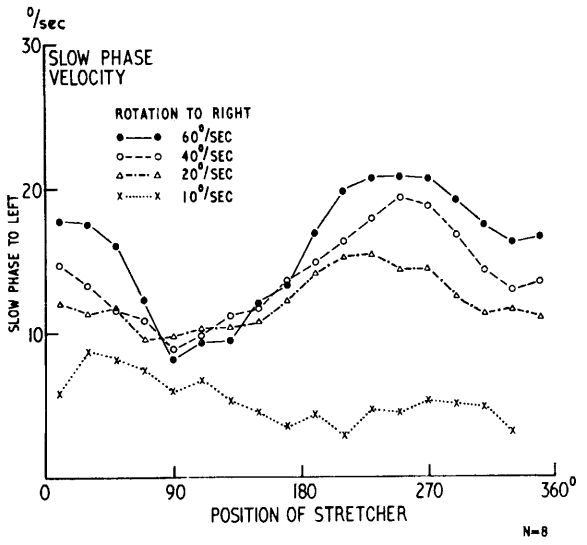

Figure 7.—*Effect of speed of rotation, and position, on slow-phase velocity of sustained nystagmus during rotation about a horizontal axis. Each point is the mean nystagmus velocity recorded during 20° rotation of the stretcher at 10°, 20°, 40°, or 60°/sec. Mean values from 10 revolutions in each of eight subjects. At 0° and 360°, the subjects were supine; at 180°, prone.*

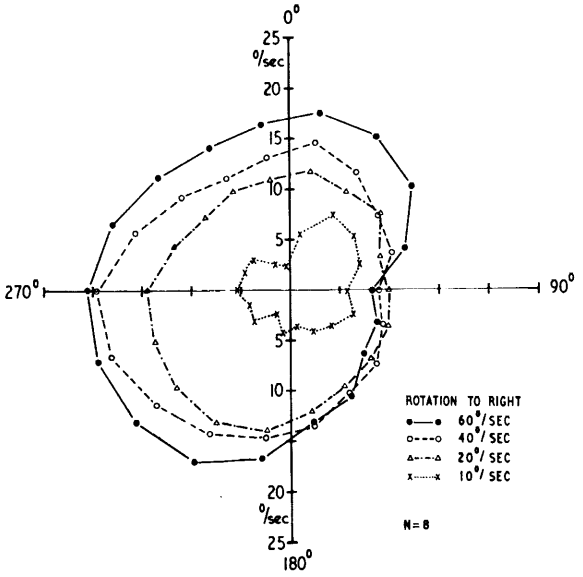

Figure 8.—*Plot in polar coordinates of slow-phase velocity of nystagmus against stretcher position, for the eight subjects depicted in figure 7.*

On examination of the decay of nystagmus following rotation about a horizontal axis, Guedry and Correia (ref. 10) demonstrated a significant reduction in nystagmus output in comparison with the postrotational response when the axis of rotation was vertical, which Benson and Bodin (ref. 6) showed was due to a reduction in the time constant of decay (fig. 9). Provided the axis of rotation was horizontal, the position in which the subject was stopped was without effect on the time constant of decay (π/Δ), although small differences in nystagmus output were observed which could be accounted for by an alteration in nystagmus slow-phase velocity with position during the per-rotational period. Furthermore, it was clear that the factor responsible for the modulation of per-rotational nystagmus was not manifest during the postrotational period and presumably did not influence cupular restoration. As with the mechanism underlying the per-rotational nystagmus, the large decrement in the time constant of decay of postrotational nystagmus, which occurred when the accelera-

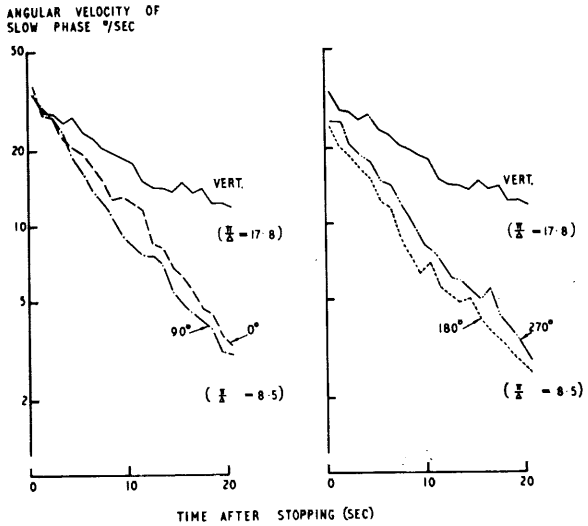

Figure 9.—*Comparison of decay of nystagmus following rotation in yaw (z axis), when the axis of rotation was vertical and when horizontal. Mean values from 14 subjects, each of whom experienced an impulsive deceleration from 60°/sec at t=0 sec. Angular velocity of slow phase is plotted on a logarithmic scale. Time constants of decay (π/Δ) were consistently shorter (p=0.001) when the axis of rotation was horizontal than when vertical. There was no significant effect attributable to the position in which the stretcher was stopped: 0°=supine, 180°=prone, 90° and 270°=right- and left-side-down, respectively.*

ion vector lay in the transverse plane of the head, could be regarded as a manifestation of either an alteration of the dynamics of the canal-cupula-endolymph system or the interaction of otolith and other somesthetic afferents with the signals from canal receptors.

Reorientation to Gravity Following Rotation

In the experiments in which subjects were rotated about a horizontal axis, the angular stimulus to the cupulae of the lateral canals was combined with a continual change in the direction of the linear acceleration vector. In a further series of experiments, these components were separated. Subjects were rotated about a vertical axis so that there was no change in the direction of gravity during rotation or on deceleration, but 1 or 2 seconds after the turntable was stopped, and the cupulae had commenced to return to their equilibrium position, the subject was reorientated to the gravitational vertical. Irrespective of whether the subject was moved from the vertical to the prone, supine, left-side-down, or right-side-down position, the time constant of decay of postrotational nystagmus was approximately half of that observed when the subject remained in the vertical position (ref. 7) (fig. 10). The

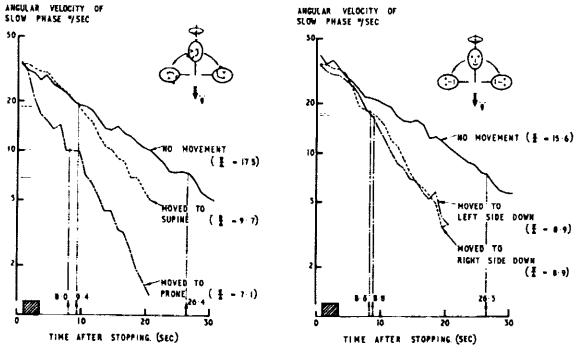

Figure 10.—*Effect of orientation to gravity on decay of nystagmus following rotation in yaw (z axis) about a vertical axis, at 60°/sec. The shaded block on the abscissa indicates the period in which the subject was moved from the vertical to a horizontal position. Figures on abscissa indicate the mean durations of the aftersensation in seconds. π/Δ=mean time constant of decay in seconds. Values obtained from eight subjects.*

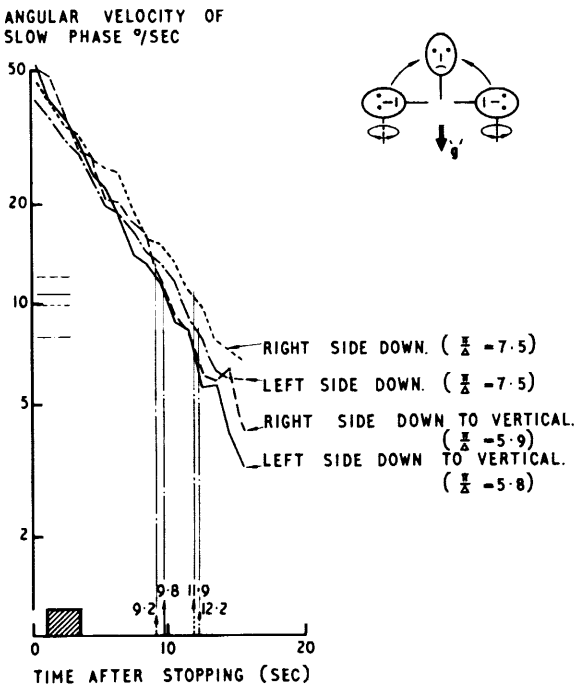

Figure 11.—*Effect of orientation to gravity on decay of nystagmus following rotation in pitch (y axis) about a vertical axis, at 60°/sec. Mean results from eight subjects. Other details as in figure 10.*

π/Δ values obtained in this experiment were comparable to those in which the axis of rotation was horizontal and cupula restoration occurred with the subject in identical orientations to the gravitational vertical. The only disparity between the two experiments was that in the second, where the subject was reorientated following deceleration, the time constant of decay in the prone position was slightly less (p=0.05) than in the supine position. The time constants in the right- and left-side-down positions were similar and were intermediate to those obtained in the prone and supine positions, from which they did not differ significantly.

In contrast, when the subject lay on his side during the initial rotation, so that on stopping he received an angular impulse in pitch, on being moved through 90° to the vertical the reduction in the time constant of decay of vertical postrotational nystagmus was proportionately much smaller than that produced by an equivalent change in the g vector on the postrotational response in yaw (fig. 11). The mean π/Δ

value was 7.5 seconds when the subject remained in the right- or left-side-down position and 5.9 seconds when moved to the vertical. These objective measures of modification of postrotational responses by the direction of gravity were paralleled by reports of the duration of the after-sensation of turning. Following initial rotation in yaw, the after-sensation was reduced from a mean value of 26.5 seconds when the head was vertical to 8.7 seconds when reorientated through 90°. In pitch, a mean change of 12.1 seconds to 9.5 seconds was observed; while in roll, where the subject lay in the prone or supine position during the initial rotation, reorientation to the vertical position reduced the after-sensation from 15.6 seconds to 12.3 seconds.

The greater effect of reorientation on the postrotational responses in yaw over those observed in pitch and roll was considered to support a peripheral mechanism, for it was in accord with the hypothesis, elaborated as the result of the horizontal axis rotation experiments, that the time constant of decay was greatest when the linear acceleration vector was normal to the plane of the stimulated canal, and least when it was coplanar with the canal. Thus it was proposed that the larger reduction in the time constant of decay following an impulse in yaw, compared with one in pitch, occurred because the increment in the coplanar g vector of the horizontal canals was greater than that in the vertical canals when the subject's orientation to gravity was changed in the postrotational period.

However, otolithic mechanisms cannot be excluded. Following rotation in yaw, the subject experiences an after-sensation of turning about the body axis, the spatial relationship of this sensation to the body being maintained irrespective of the subject's orientation to the gravitational vertical in the postrotational period. Thus when the head remains in the position occupied during the initial rotation, otolithic and somesthetic information does not conflict with the illusory sensation engendered by the ampullary signals; but when the body is placed, say, in the supine position after rotation with head vertical, the cues from gravireceptors are not in accord with the illusory sensation, and hence may, by a central neuronal mechanism, suppress those signals which are erroneous.

This, however, does not explain why the after-responses in yaw suffer a greater suppression than those in pitch or roll. One reason for this difference may be that in normal life, angular movements in pitch and roll stimulate both ampullary and otolithic receptors.. Thus an angular impulse in either of these axes without an appropriate alteration of the linear acceleration vector may evoke a smaller response than when there is concordance of the signals from canals and gravireceptors. Indeed, it may be that the shorter time constants of decay of postrotational responses in pitch and roll observed by Jones, Barry, and Kowalsky (ref. 29), as in the experiments here reported, are a manifestation of gravireceptor inhibition rather than differences in the dynamic behavior of the vertical and horizontal canals. If it is accepted that the postrotational responses in pitch and roll are suppressed when the axis of rotation is vertical, then the introduction of competing otolithic and somesthetic signals is likely to have a smaller effect than on the postrotational response in yaw to which otolithic signals do not normally contribute. In addition, it might be argued that otolithic and somesthetic cues are more intense on moving to the horizontal position than the converse case, in which the subject is returned to the normal, head vertical, sitting position, in the postrotational period. Thus the magnitude of the depression of the postrotational responses in yaw may reflect, in part, the unfamiliarity and relative discomfort of the four positions employed.

Effect on Postrotational Response of Orientation of Horizontal Canals to Gravity

The experiments already described were confined to the study of the effect of a change of 90° in the direction of gravity on postrotational responses about the three orthogonal body axes, and were not concerned essentially with the orientation of the stimulated canals to the linear acceleration vector. Accordingly, further experiments were performed in which the effect of the orientation of the lateral canals to gravity

in the postrotational period was examined in greater detail. In the first series of experiments, the subject was tilted 30° forward in order to bring the plane of the lateral canals normal to the gravitational acceleration. Immediately following rotation about a vertical axis, he was titled backward through 30°, 60°, 90°, or 120°. The average results from 10 subjects, who each experienced rotation at 60°/sec in the clockwise and anticlockwise directions and 5 orientations to the gravitational vertical, are shown in figure 12. This demonstrates the progressive increase in the rate of decay of postrotational nystagmus as the subject's position in the postrotational period deviated from that occupied during rotation. The relationship was sigmoidal, with the maximum change in π/Δ value occurring between the 30° and the 60° back positions (fig. 13).

Now if the time constant of decay were, in part, determined by the magnitude of the coplanar g vector, then it was to be expected that

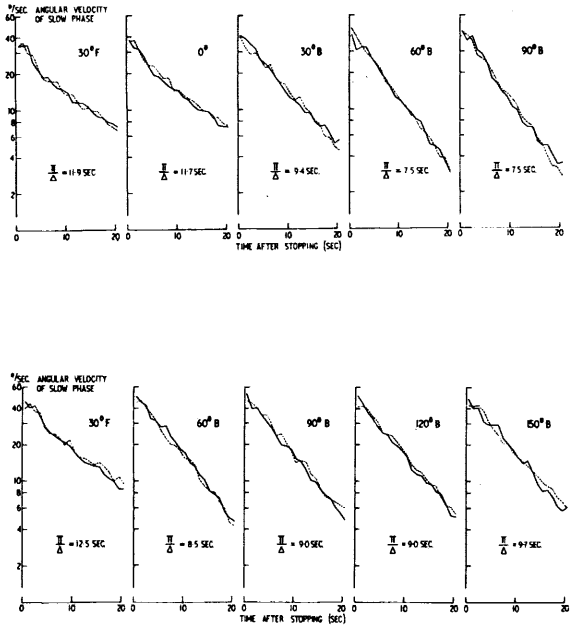

Figure 12.—*Effect of orientation to gravity on decay of nystagmus following rotation at 60°/sec about a vertical axis. During rotation, subject sat with head tilted 30° forward. One second after stopping he was tilted backward through* n×*30° to assume the position indicated. The upper and lower group of graphs are the mean values from two experiments each performed on 10 subjects.*

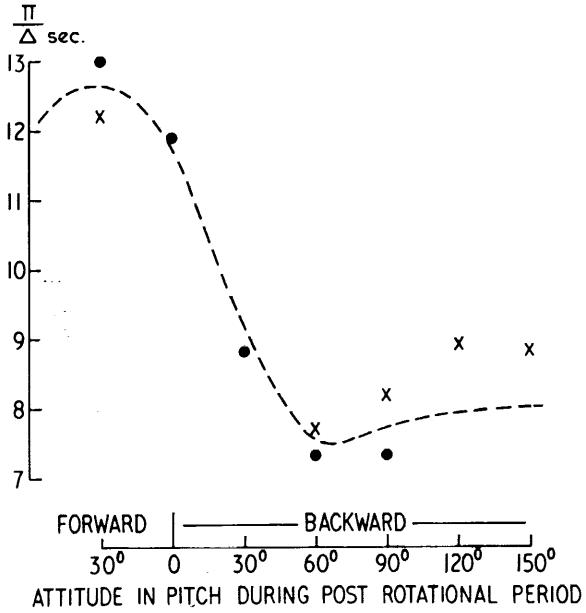

Figure 13.—*Change of time constant of decay* (π/Δ) *with position during postrotational period. Mean values from two groups of subjects* (● *and* ×), *10 subjects in each group.*

the minimum time constant of decay would have been found in the 60° back position, and that when the subjects were tilted 90° back, the time constant would have been similar to that observed in the 30° back position. As the π/Δ values in the 60° and 90° back positions did not differ significantly from one another, there was no evidence of such an inflection. Accordingly, the observations were extended to the 120° and 150° back positions in a further experiment, similar in design to the first except that postrotational responses were examined following reorientation in pitch to the 60°, 90°, 120°, and 150° back positions. Although the subjects in the first and second experiments differed, the π/Δ values obtained without repositioning in the postrotational period and when tilted to the 60° and 90° back positions did not differ significantly between the two experimental groups. This enabled the data from the two experiments to be combined in order to calculate the curve in figure 13 which indicates the general relationship between position and time constant of decay. The curve was not of the predicted sinusoidal form, for there was only a small increase in time constant between the 60° back and

150° back positions. The relationship thus demonstrated apparently supported neither hypothesis, for both would have predicted a sinusoidal relation between position and time constant of decay, in which the π/Δ value in the 150° back position would be similar to that obtained in the 30° forward position. When tilted 150° back, gravity was again normal to the plane of the stimulated canals, while the plane of the illusory sensation of turning was horizontal and not in conflict with otolithic and somesthetic cues.

Although of interest, these results do not materially assist in the understanding of the mechanisms by which linear acceleration modifies semicircular-canal responses. However, a small modification of technique yields an experimental situation in which the two hypothetical mechanisms would predict differing responses in the postrotational period. If a subject is rotated in a horizontal plane with the head vertical and on stopping moved in pitch to the 30° forward or 30° back positions, in the former the horizontal canals are brought into a plane normal to the gravitational acceleration, while in the latter position they lie at 30° to the vertical. If the magnitude of the linear acceleration in the plane of the canals is of importance, then the time constant of decay should be increased in the 30° forward position and decreased in the 30° back position. Conversely, in either of these two positions, otolithic and somesthetic receptors will carry information that the signals of the ampullary receptors of the horizontal canals are inappropriate and should, as Guedry proposed, be suppressed.

This experiment was carried out on 12 subjects. Immediately after rotation at 60°/sec in a clockwise or anticlockwise direction with the head vertical, the subject was moved to the 30° forward or 30° back position. The order of presentation of the stimuli was determined by a Latin-square design which was also balanced for order effects. Subjects were asked to perform mental arithmetic during the postrotational period in an attempt to maintain a constant level of arousal.

In all but one of the 12 subjects, nystagmus decayed more rapidly in both the 30° forward and 30° back positions than when they were not repositioned immediately after stopping (fig. 14). The mean rate of decay was apparently somewhat greater in the 30° forward position, but on analysis of variance of the individual π/Δ values, there was no significant difference between the reduction of time constant which occurred in the 30° forward and the 30° back positions. The decrement in π/Δ values on reorientation to gravity was however highly significant ($p=0.001$).

Thus it must be concluded that the alteration of the decay of postrotational nystagmus according to the direction of the linear acceleration vector is governed principally by activity of otolithic and other sensory receptors which signal the direction, and change in direction, of the linear acceleration, rather than by a direct mechanical disturbance of the dynamics of the canal-cupula-endolymph system.

The inhibition of the ampullary afferent signals cannot be described by a simple subtractive or proportional function, for with

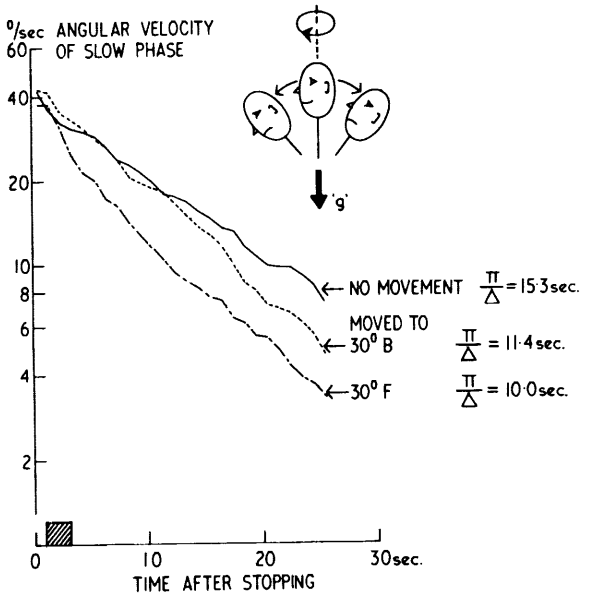

Figure 14.—*Effect of orientation to gravity on decay of nystagmus following rotation at 60°/sec about a vertical axis. During rotation, subject sat with head vertical. On stopping, he was moved in pitch, at time indicated by bar on abscissa, 30° forward or 30° backward. Mean values from 12 subjects who each experienced rotation in clockwise and anticlockwise directions.*

either of these inhibitory mechanisms a sudden fall in nystagmus velocity should have occurred coincident with the appearance of a competing otolithic signal on reorientation of the subject. Furthermore, proportional inhibition, should have yielded nystagmus which decayed with the same time constant, irrespective of the intensity of the inhibitory signal. Accordingly, it is necessary to propose that the inhibition increases in intensity as an exponential function of time, and that the exponent itself is a function of the intensity of the signal from otolithic and other gravireceptors. A symbolic diagram of the conceptual inhibitory mechanism is shown in figure 15.

Groen (refs. 30–32) has drawn attention to the differences which exist between the decay of the sensation of turning and of nystagmus following impulsive stimulation, and has proposed a variable adaptive coupling between the peripheral signal and the responses engendered by this signal. On the basis of the model here proposed, the strength of the coupling depends upon the exponent of the recurrent inhibitory signal which acts on the vestibular projections within the central nervous system.

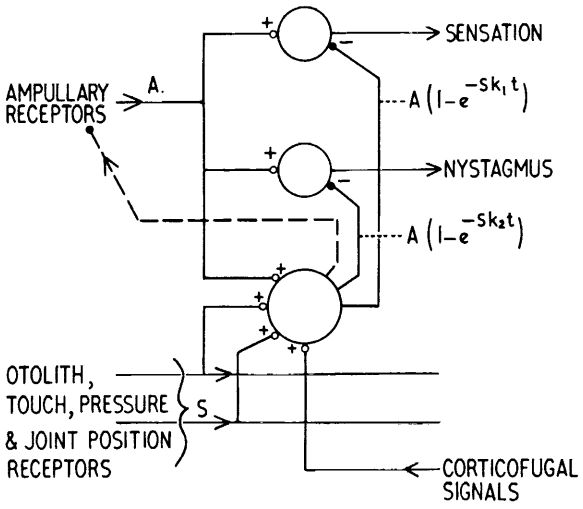

Figure 15.—*Conceptual scheme of possible inhibitory mechanisms in vestibular pathways. A and S are the intensity of signals from ampullary receptors and gravireceptors, respectively;* k_1 *and* k_2 *are arbitrary constants.*

While it is acknowledged that inhibition may be mediated through the efferent innervation of the vestibular receptors, which, in common with other sensory systems, can regulate the afferent signal (refs. 33 and 34), it is considered that such a peripheral mechanism must be of minor importance in the regulation of canal responses by linear accelerations. For otherwise it is difficult to explain why the various manifestations of the postrotational vestibular response, e.g., somatic muscle activity, sensation of turning, and nystagmus, do not have the same time constant of decay. Indeed, the dissociation of the sensation of turning and nystagmic response by an alteration of the direction of gravity was apparent in the experiments here reported as it was in other studies of vestibular function (refs. 9, 35, and 36).

CONCLUSIONS

Within the limitations of the procedures employed, the experimental evidence supports with certain qualifications the "Grundprinzip" of functional differentiation elaborated by de Kleijn and Magnus (ref. 20). It would appear that an acceleration of 1 g does not, in the normal individual, cause a significant deflection of the cupula or modify directly the dynamics of the canal-cupula-endolymph system. However, when one looks at the behavioral responses normally regarded as manifestations of afferent signals from semicircular canals, in particular sensations of turning and nystagmus, it is apparent that these are not independent of the concomitant signals from other receptors. Thus the response elicited by a particular motion stimulus depends upon the nature of the effective stimulus to both groups of vestibular receptors as well as signals from other somesthetic receptors. If information from gravireceptors is not in accord with that from canal receptors, then the response engendered by the inappropriate ampullary signals is inhibited. Conversely, if otolithic and somesthetic receptors signal rotation of the body, even when this is not supported by signals from the semicircular canals, as presumably occurs during rotation about a horizontal axis at constant velocity,

then an appropriate compensatory nystagmus is generated. Whether the interplay of canal and otolithic signals generated by motion in pitch and roll occurs in the same manner as motion in yaw awaits further investigation. There may well be differences, for angular motion in pitch and roll is normally associated with a change in the direction of gravity, while motion in yaw does not normally stimulate gravireceptors. At present, there is a lack of fundamental data, not only on the dynamics of the response to angular stimuli particularly in pitch and roll but also on the manner in which these responses are modified by the concomitant linear acceleration vector.

The demonstration of a significant modification of canal responses by linear accelerations should introduce caution to the prediction of responses in man when exposed to the complex motion of aerospace flight (ref. 29). While it is not denied that consideration of the separate responses to the resolved linear and angular components of a particular motion stimulus may at times be of value, such a method of analysis ignores the integration of cues from canal, otolithic and somesthetic receptors, which together determine the final response.

The use of experimental observations made in the presence of the gravitational acceleration to predict vestibular responses during weightlessness is not without considerable limitations, yet such extrapolations can be justified, especially if they expose problems which are amenable to investigation either on the ground or during space flight.

The corollary of the various manifestations of the modification of canal responses by gravireceptor signals is that, during weightlessness, the response to a particular angular stimulus could well be different from that evoked by the same angular stimulus in the presence of gravity. Unfortunately the lack of a detailed analysis of the contribution of linear and angular accelerations to the vestibular response, and in particular the relative importance of the synergism of gravireceptor and canal signals for motion in yaw, pitch, and roll, prevents an accurate assessment of the magnitude and time course of the vestibular reaction to a complex-motion stimulus.

In everyday life, the stimulation of gravireceptors by angular motion in yaw is small, therefore it is perhaps to be expected that the absence of gravity will have little effect on the response to an angular stimulus in this axis. This speculation is supported by the qualitative observation of nystagmus produced by rotation in yaw during zero-gravity maneuvers in a conventional aircraft (ref. 16). However, motion in pitch and roll may evoke a different response during weightlessness from that evoked in the presence of gravity, for the normal synergism of canal and otolithic signals will be disturbed.

Apart from the absence of normal gravireceptor information during angular motion in pitch and roll, in the zero-gravity environment an atypical sensory inflow from otolith organs may be engendered by angular and translational movements of the head. The linear accelerations produced by normal head movements are usually small so that, in the presence of gravity, there is little change in the magnitude or direction of the resultant. But in the absence of gravity these accelerations may prove to be an adequate and at the same time bizarre stimulus to the otolith organs. Thus otolithic afferents may engender inappropriate vestibular responses which could interfere with the operational efficiency of man in the space environment. From reports of abnormal vestibular sensations by Titov, Fekstitov, and Yegerov, and of nystagmus in Tereshkova during the 38th–45th orbits, it may be argued that the vestibular reactions peculiar to weightlessness can be of significance in individuals who are not well habituated to complex-motion stimuli.

Measurement of the response to controlled angular and linear accelerations during orbital flight would provide a better understanding of the underlying vestibular mechanisms and, in particular, the role of otolithic afferents in the regulation of nystagmus and sensations of turning which have been regarded as the prerogative of stimulation of semicircular-canal receptors.

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DISCUSSION

LOWENSTEIN: I myself would not be quite so diffident as Dr. Benson about his last flow diagram. It has to me the great appeal that it pays attention to the Cinderella, the central nervous system. Historically speaking, of course, we have seen a change in attitude. At first, one thought everything was sensation, and then peripheral mechanisms seemed to account for everything. We became accustomed to leaving the central nervous system out of our consideration. This flow diagram is recommended because it does in fact pay attention to the central nervous system under conflicting impact from peripheral signals.

When you have rotated your subject in the yaw plane, and then you stop the rotation and you change his position, you have to take into account that you now also have introduced a stimulation of the vertical canals.

BENSON: Yes; I appreciate that. This stimulus is very transient because there is acceleration followed shortly by deceleration, which should just flip the cupula one way and back again.

LOWENSTEIN: What sort of acceleration did you use in this flip?

BENSON: The movement took 2 seconds at the most. I agree that this could possibly influence the results but, on the other hand, I would have thought that the stimulus to vertical canals associated with the movement would be relatively small and of short duration.

GUALTIEROTTI: I wonder if there isn't an additional force here which is very minute and has not been discussed up to now. When you endure these kinds of movements, besides other acceleratory forces, a Coriolis accessory acceleration exists, resulting from the composition of Earth rotation and individual rotation. I don't know right now what kind of value this Coriolis force would have, but at 1 or 2 g rotatory acceleration, it might be just above threshold for the semicircular canals. There are two components of this kind of Coriolis acceleration, one is vertical and the other is horizontal. It is the principle on which the gyroscopic compass is based. It might be so minute not to have any importance, but it should be mentioned and some calculations be made.

BENSON: I agree. I would take any engineer's or mathematician's calculations and accept them. However, I wouldn't have thought it was very large and not really likely to influence the results that were here presented.

VALENTINUZZI: I would like to go back to the comment made by Dr. Lowenstein and to the question; aren't the vertical canals stimulated also when the head is turned? And this is very important, I believe, because if we go back to the changes of this quotient π/Δ, and we think of the physical meaning of pi and delta and also of the moment of inertia of the endolymph, we expect that, according to the physical or biophysical characteristics of the canal, those constants wouldn't change; therefore, that relationship wouldn't change. But why does it change in this case? I think that we are calculating this relationship for the complex information which is involved in the nystagmus, thinking of the equation as only applied to one canal. If we consider that the fundamental equation is applied to each of the semicircular canals on both sides, then we have to think that those constants remain constant. That relationship should remain the same according to the biophysical characteristics of the canal. That apparent change, I would say, is due to the composition of the inputs each canal is sending to the central nervous system. Beside that, another thing, which Dr. Gualtierotti has mentioned, is the participation of the Coriolis force. If we consider not only the tangential but also the Coriolis force, taking into account its magnitude and direction, it means that this change, I would say, is an apparent change because we are applying this equation to which these coefficients belong to something which now in this experiment is a complex result of not only one canal, but of the whole vestibular system.

BENSON: Yes. I must really apologize for using the expression π/Δ. If I just called it time constant, this wouldn't have worried you? However, if you want to take these values and substitute them in the equations of motion of the canals, this is clearly not justified. As an excuse, may I say that I have used it in the same way as Van Egmond, Groen, and Jongkees. They

have applied π/Δ to the slope of both the sensation cupulogram and the slope of nystagmus cupulogram, which differ by a factor of 2!

VALENTINUZZI: Yes; I agree with this. I should say that it is better to keep the expression "time constant." Maybe an analytical investigation of the integration of all the inputs, considering the six semicircular canals, would explain that variation, thinking again that the physical constants of the canals do not change.

With the permission of Dr. Fernandez, I would like to say that he and I have started to work in this direction, trying to develop an analytical program in order to get as much information as we can. We are taking into account the behavior of each canal with respect to different accelerations, different velocities, different inclinations, and, therefore, different Coriolis forces, et cetera.

BENSON: Yes; I think this is very valuable. We have spent too long looking at isolated responses of horizontal canals or vertical canals. We have forgotten to look at them all together. I'm even asking for an examination not only of the canals all togegther, but also the effect of stimulation of gravireceptors at the same time.

BERGSTEDT: Were these subjects rotating constantly?

BENSON: Barbecue-spit-wise.

BERGSTEDT: And then you stopped?

BENSON: Yes.

BERGSTEDT: To find out if there was any action from the cupula organ, did you try different values of retardation of this stopping movement? If you got different nystagmus duration after different degrees of retardation, this could speak in favor of a cupula mechanism. But it seems, from Dr. Guedry's talk and from your presentation, too, that you haven't paid so much attention to it. Presumably it doesn't matter.

BENSON: If one was expecting g to act on the cupula and to alter its process of decay, then we should have seen it in this experiment, I would have thought. We are flipping it over with a sudden deceleration and then we are following the time course of decay of the post-rotational nystagmus.

BERGSTEDT: Yes. But you never tried to give different values to this sudden deceleration.

BENSON: No. We have only one lever on our machine which either stops or starts it, and we can't control angular acceleration rates. Perhaps Dr. Guedry has information on this because his machine will accelerate and decelerate at controlled rates.

BERGSTEDT: I think, too, as Dr. Lowenstein does, that this speaks in favor of a central compensation in the nystagmus and sensation you get. It is very similar after different rotation directions, and so on. The change you get when you change position afterward is just the result of different impulses coming from positional proprioceptor organs.

BENSON: Yes; this I would certainly agree with entirely.

GUEDRY: We have used relatively slow stops, about $15°/sec^2$ as compared with a very high magnitude stop, about $100°/sec^2$. We haven't actually estimated time constants for these two stops, but in both cases the nystagmus is greatly suppressed. Sensation is stopped almost as soon as the person stops. In connection with another point which was brought up, I believe some of your durations of rotation were relatively low in your experiments; were they not?

BENSON: In the study of postrotational responses; yes. We only ran for about 40 seconds.

GUEDRY: Some people had the idea that perhaps our short afterresponses were due to the very prolonged rotation periods which preceded the stop. We find a short nystagmus upon stopping rotation about a horizontal axis, irrespective of the duration of rotation. Although we haven't actually carried out formal experiments, we have tested a number of subjects to check this point, and I thought your data showed the same thing.

BENSON: Yes. I think the parallelism between the decay of nystagmus following horizontal-axis rotation and the decay when you spin the man vertically and then reposition him into equivalent position, really says that all these other effects which have been talked about are not of any great importance, although we cannot completely disregard them.

LANSBERG: I am sure there have been brought up so many points that we could all go on discussing this for a very long time, but as the Earth is rotating, the day is changing to night eventually. That brings me to the point that Dr. Gualtierotti has brought up. He seems to object to this rotating speed of the Earth. I think I can soothe his mind a little bit, I hope so, as the exact value of it is 1/240th degree per second. Sensitive as our vestibular organ is, it's not that sensitive that it might influence it, I'm sure. Although, I think it's wonderful that you bring up this point, I don't really think that it can in any way influence the effect that Dr. Benson has brought up.

MAYNE: One cannot help wondering whether the interaction between the otoliths and the semicircular canals is an artifact or whether it serves any function and, if so, what function. As a possible lead to this problem, have you attempted to determine the subjective velocity sensation for the two cases?

BENSON: You mean did we follow the decay of post-rotational sensation by doing subjective cupulograms?

MAYNE: Yes; for the two conditions.

BENSON: No; we haven't looked at this. Certainly in these repositioning experiments the variability and the difficulty subjects have in reporting durations of sensations is quite high, but as soon as they are repositioned the sensations are annihilated or very seriously attenuated. Subjects may have a feeling of turning, but often this is of turning without getting anywhere. They feel they are rolling over on one side,

but they do not report any sensation of continued motion.

Your previous point about the functional significance of these findings is, I think, quite relevant. Clearly, if you have an inappropriate sensation and this is not in accord with gravireceptor information, then it is quite reasonable that the inappropriate canal signal should be suppressed and that the other reflex effects which are normally associated with this signal should likewise be inhibited.

SESSION V

Chairman: **HENNING E. VON GIERKE**

Aerospace Medical Research Laboratories
Wright-Patterson Air Force Base

Cochairman: **WILLIAM E. COLLINS**

Federal Aviation Agency

Control Engineering Approaches to Human Dynamic Space Orientation[1]

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JACOB L. MEIRY,
AND
YAO T. LI
Massachusetts Institute of Technology

INTRODUCTION

This paper reviews briefly some of the research that has been underway at MIT in the last few years, at the Man-Vehicle Control Laboratory, in the Aeronautics and Astronautics Department. It covers work on mathematical descriptions of the input-output relations characterizing the vestibular mechanisms and some work on eye stabilization, including the influence of vestibular inputs, neck proprioceptive inputs, and fixed-head visual tracking. Finally, results are presented relating the vestibular research to description of man as a member of a closed-loop control system controlling the orientation and position of a vehicle.

From the point of view of the control engineer, the nature of a pilot in a vehicle orientation task may be depicted as in figure 1. This block diagram functionally divides the investigation into study of the sensors, study of the central system for control and compensation, and study of the motor mechanisms by which the human transfers information back to the vehicle. The ellipses on this diagram represent those areas that we have chosen to concentrate on. These areas are visual input, tactile inputs, and vestibular inputs, when these are either in agreement or in conflict. At the output end, we consider not only the usual joystick hand control, but also postural control as a possible output mechanism. Another study deals with the adaptive mechanisms for manual control. Naturally, the purpose of this type of study is to achieve a sufficient mathematical description of the human as an input-output device or set of devices, including all the nonlinearities and the statistical nature of the random components, to permit the control-systems engineer to make rational, quantitative estimates of the reactions of a man in a piloting-type task.

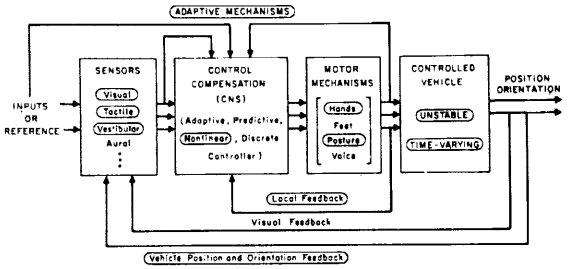

Figure 1.—*General block diagram of the man-vehicle control problem. Ellipses represent major areas under investigation at MIT.*

SEMICIRCULAR CANALS

The well-known torsion-pendulum model of the horizontal canal has been remarkably successful in describing most of the subjective and objective responses, and our experiments on

[1] This research was supported in part by NASA under Grant NsG–577.

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thresholds for low angular accelerations provide further quantitative checks on this model. In his doctoral thesis, Meiry pursued the hypothesis that the dynamics of the vertical canals were different from those of the horizontal canals and that these differences in dynamics in some way could explain the subjective feeling in an aircraft that the instantaneous axis of rotation is different from that of the objective instantaneous axis of rotation (ref. 1). The primary tool in this study was the measurement of latency of sensation to constant angular acceleration around a vertical axis as a function of acceleration level. Fitting the data with the exponential relation resulting from the torsion-pendulum equation yields the long-time constant. The long-time constant for the sensation of rotation about the sagittal (roll) axis was approximately 7 seconds, compared to the 10–12 seconds found for the horizontal canals. The vertical canal threshold was found to be approximately 0.5 deg/sec^2 compared to approximately 0.14 deg/sec^2 for the horizontal plane.

LINEAR MOTION SENSORS

Control-system descriptions of the gravireceptors have been almost nonexistent. Experiments have been difficult to perform when they require stimulating the linear acceleration sensors without simultaneous stimulation of the semicircular canals, and consequently quantitative linear sensor data that would be useful in a dynamic orientation model were quite limited. This is the area in which Meiry placed his primary research effort. To develop a pure linear acceleration as the stimulus, we constructed a 32-foot horizontal track on which a vehicle is driven under fine position control with uncertainties in acceleration of the order of a thousandth of a g. The maximum acceleration is limited to 0.3 g, and the frequency response is flat to above 1 cps, providing a useful tool for studying response to low-level acceleration. Notice that the studies correlating human input-output data using such a device define the dynamic response of the linear-translation sensors, and can only inferentially be applied to the otolith characteristics.

An important early experiment was to establish the phase relation between the subjective sensation of linear velocity and the objective linear velocity for sinusoidal linear oscillation. The subject was seated in the cart, which was covered to eliminate visual cues, and accelerated to the right and left sinusoidally with controlled frequency and amplitude. With a hand control stick he would indicate when he felt he was moving to the right, when he felt he was moving to the left, and when he was not moving. No meaningful results were expected in terms of the subjective amplitude of velocity, but we did find that the phase the subject felt that he was reversing direction could be determined accurately.

Figure 2 is a plot of subjective phase lag versus frequency of stimulation. The phase lag is the phase difference between the time that the cart actually reversed its velocity and the time the man indicated the change of velocity. At very low frequencies the subject leads the stimulus velocity, in agreement with the finding of Walsh (ref. 2). Over a fairly wide range of frequencies he has approximately the correct phase relationship (zero lag), and as frequency increases, he develops more and more phase lag, approaching 90° at high frequencies. These phase data can easily be fitted by a linear minimum phase model which does not include any pure delay. The resulting transfer function shown in figure 2 corresponds to a second-order differential equation identical to the form of the torsion-pendulum model of the semicircular canals. The major difference lies in the value of the short-time constant, which is 0.66 second compared to approximately 0.1 second

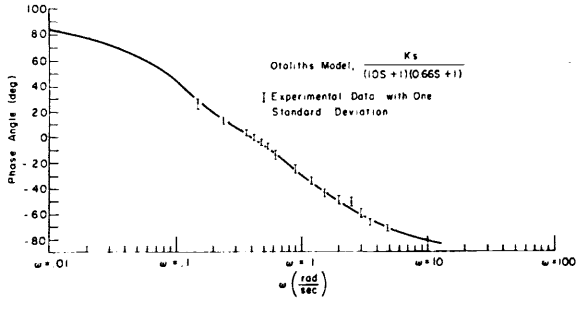

Figure 2.—*Subjective perception of motion reversal; phase versus frequency.*

for the canals. Curiously, the long-time constant of 10 seconds is approximately the same as that of the horizontal semicircular canal. Note that the model is in the form of an overdamped second-order system and, as such, is inherently a "linear velocity indicator" in the same sense that the semicircular canal is an "angular velocity indicator," over the physiological frequency range.

An independent check on the form of this linear motion model is provided by the threshold versus time experiment. Given a constant linear acceleration, how long will it take a man to indicate that he knows in which direction he is moving? That can be predicted from this model and checked experimentally. Using the theory that the shear components of specific force (gravity minus linear acceleration) stimulate the otolith, we can derive a theoretical curve of latency time versus acceleration level given in figure 3a. The experimental points fall exactly on this curve. (There was a degree of freedom in the amplitude of the model since the threshold was undetermined, allowing this curve to be moved up and down at will, but not moved left-right.) For the subject supine, an effective absolute threshold of about 0.01 g is found. The model now predicts exactly the latency time versus acceleration level for a subject in the upright position. Figure 3b shows the excellent agreement with experiments, and indicates an absolute threshold of 0.006 g for upright normals.

Most of the experiments in this paper have been repeated on labyrinthine-defective subjects in a joint project with Dr. Graybiel and the U.S.

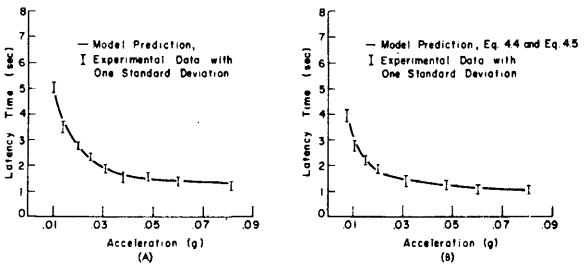

Figure 3.—(A) *Latency times for perception of horizontal linear acceleration, supine.* (B) *Latency times for perception of horizontal linear acceleration, upright.*

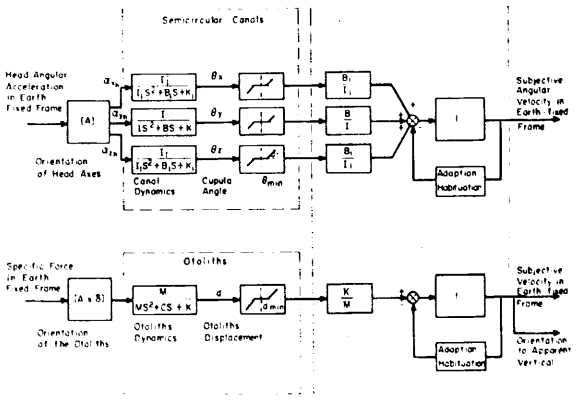

Figure 4.—*Control models for nonvisual motion sensation.*

Naval Aerospace Medical Institute. The results will be presented in the near future.

Figure 4 represents the current control models for nonvisual motion sensation, consisting of simple linear models, plus elementary nonlinearities. The semicircular canals are treated as three parallel blocks, corresponding to the three head-fixed axes, each of which is assigned its individual moment of inertia, damping-friction constant, and cupular-spring constant, and each of which is assigned an effective dead zone or threshold. Finally, the outputs are added to form a subjective angular velocity, which is damped out through an adaptation or habituation loop. The linear acceleration sensors, both otolithic and nonotolithic also have a second-order system description and a dead zone. Notice that the output of this system is twofold: subjective linear velocity in an Earth-fixed frame, and orientation to the apparent vertical. The choice of output obviously depends upon mental set and canal response, and can account for a number of upset illusions.

EYE STABILIZATION

A topic closely related to vestibular dynamics is the mechanism of eye stabilization. Figure 5 is a block diagram representation of the control systems which must be at work in controlling the angle of eye gaze. For the head fixed in space, the main driving block is the visual one, which responds to the difference between the target angle and the eye angle. This will be discussed in more detail below.

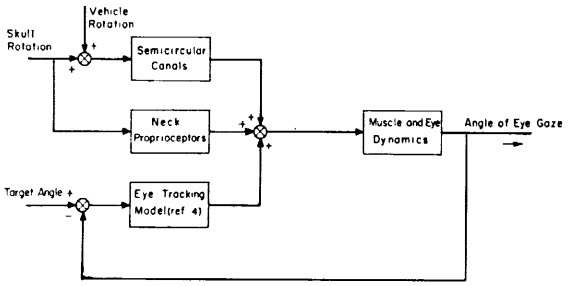

Figure 5.—*Block diagram of the eye movement control system.*

Inputs arise from rotation of the head in space and rotation of the head with respect to the trunk. (Our recent work also considers the eye response to linear acceleration of the body and head.) The semicircular canals respond to the net rotation of the head in inertial space, or the sum of the rotation of the head with respect to the body and the rotation of the body and vehicle with respect to space. The neck proprioceptors obviously respond to head rotation, movement of the head with respect to the neck. We performed input-output experiments to investigate each of these blocks in a quantitative manner. In an extension of some work that Hixson and Niven started some years ago, vestibular nystagmus was used as an indication of semicircular-canal phase relationships (ref. 3). Figure 6 shows some recordings taken by Dr. Meiry indicating the classical vestibular-nystagmus response to sinusoidal rotation of the head and body in a horizontal plane. By removing the saccades and fitting together all the slow-phase pieces of record, the curve which we call cumulative eye position results, making the determination of the phase relationships between eye movement and stimulus quite clear.

Eye movements were measured with a simple noncontacting commercial monitor based on the principle of differential reflection from the iris-sclera boundary.

The results of this vestibular investigation are shown in the frequency response of eye velocity compared with input velocity of figure 7. The data agree with the torsion-pendulum models in phase and extend the range of data found by Hixson and Niven to a higher frequency range. The next block to be considered is neck proprioception as a drive signal to the eye system. There have been almost no quantitative data in this area for humans. To separate the influence of any neck proprioception from vestibular input, we clamp the head to a frame which remains fixed and rotate the body underneath it, measuring the resulting eye movements. All experiments of rotating head and body, head only, and body only were performed in the dark and repeated with a fixation light fixed in the laboratory and one rotating with the subject.

Figure 8 indicates the small eye movements attributable to rotation of the neck. The cumulative eye position very definitely shows the relation to stimulation of the neck.

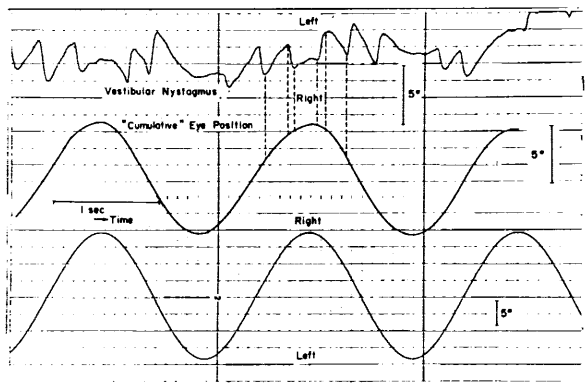

Figure 6.—*Vestibular nystagmus and "cumulative" eye position, f=0.5 cps. Note the correspondence of slow-phase vestibular nystagmus and "cumulative" eye position.*

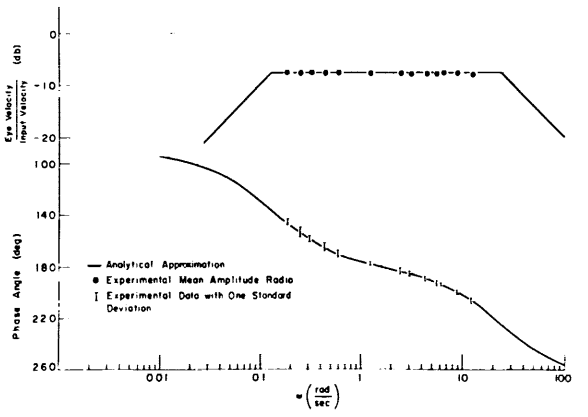

Figure 7.—*Bode plot of vestibular compensatory eye movements (slow phase).*

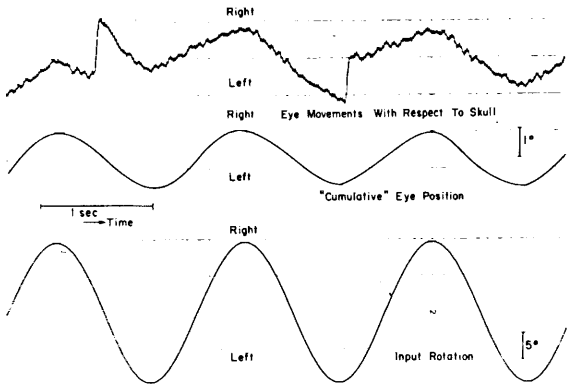

Figure 8.—*Compensatory eye movements by neck receptors, f = 0.6 cps.*

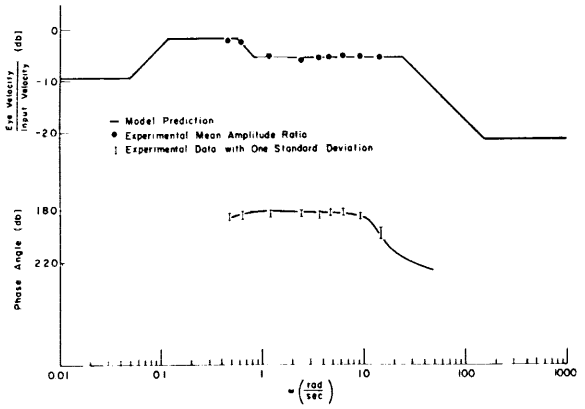

Figure 10.—*Bode plot of compensatory eye movements (vestibular and neck proprioception).*

Whether it is a feed forward in the sense of a Von Holst type or whether it is a proprioceptive feedback has not been determined. Notice that these eye movements are relatively small, which is one of the reasons that they are not readily observed. The frequency response of eye velocity with respect to input velocity for neck is shown in figure 9. The analytic approximation is

$$\frac{\text{Eye velocity}}{\text{Input velocity}} = \frac{0.325\ (1+0.43\ S)}{(1+1.74\ S)}$$

The form of this transfer function is a lag-lead network indicating the possibility of position-plus-rate proprioceptive feedback in the neck. A critical experiment is to combine this model for stimulation of the neck only with the previous model, stimulation of the vestibular system only, and to get the model which matches data for combination of head and neck movement. The result is perfect agreement as shown in figure 10, the frequency response for combined vestibular and neck stimulation.

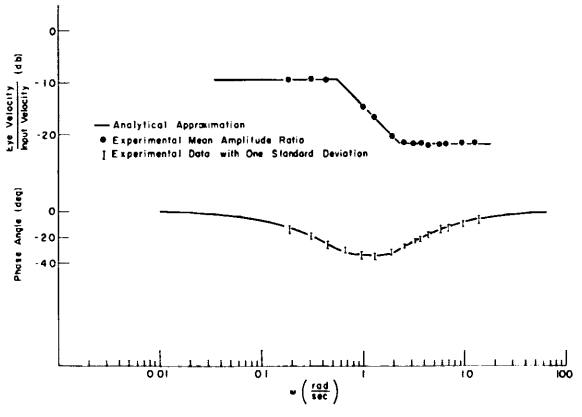

Figure 9.—*Bode plot of compensatory eye movements by neck proprioception.*

VISUAL EYE-TRACKING MOVEMENTS

Figure 11 is a model of eye-tracking movements developed in 1962 and since refined, which summarizes some of the information on the visual eye movement channel (ref. 4). The input is the target angle, the output is the eye angle, and the difference between these is the error which stimulates two paths. One is the saccadic movements path, used only for correction of the position of the eye. The other path is the pursuit movements path, used only for correction of the velocity of the eye. The "sampler" indicates that corrections in both eye position and eye velocity are not made on a continuous basis when we are watching a ran-

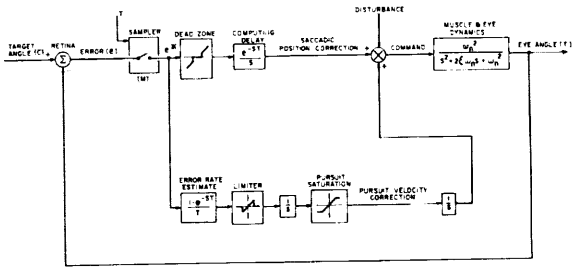

Figure 11.—*Block diagram of eye tracking movements.*

dom signal, but in fact are made on a discrete sampling basis in which samples are taken approximately every 0.2 second.

Figures 12 through 14 show the model predictions and experimentally observed eye movements in tracking horizontal target motions.

To check the model further, we wished to use the control engineer's technique of opening the feedback loop. Since the feedback of eye movement to error on the retina is fixed at unity, another parallel path was added (ref. 5). This was accomplished by measuring the eye movement through the monitor, feeding it back through an amplifier whose polarity and gain we could vary at will, and driving the target as a function not only of the input signal but as a function of the eye signal. (See fig. 15.) By making the parallel feedback unity, the eye position has no effect on the observed error,

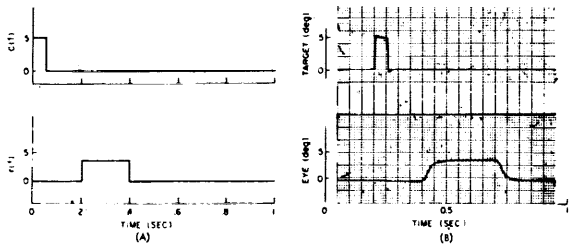

Figure 12.—*Pulse response:* (a) *model;* (b) *experimental.*

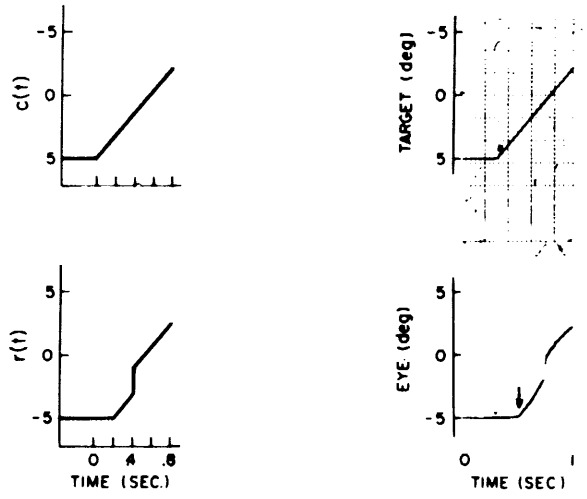

Figure 13.—*Theoretical and experimental ramp response.*

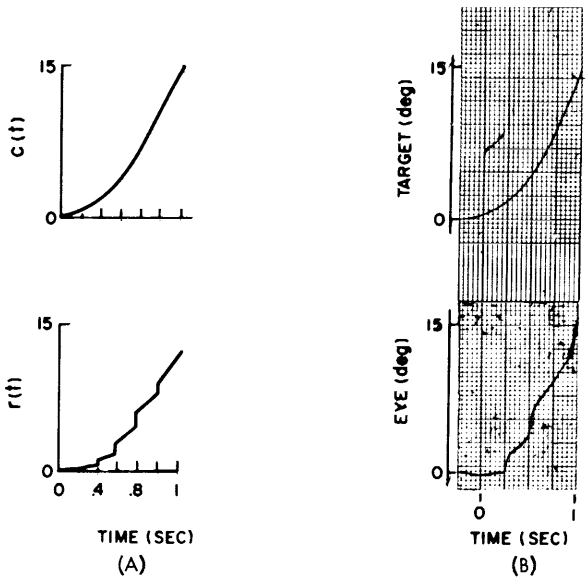

Figure 14.—*Parabola response:* (a) *model;* (b) *experimental.*

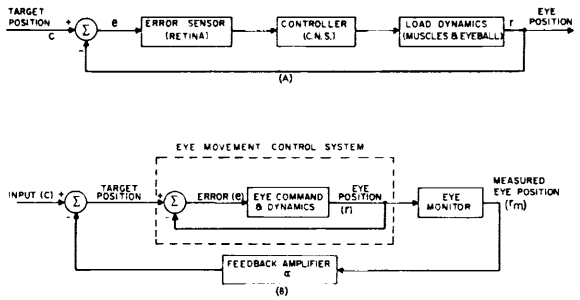

Figure 15.—*Eye movement control system block diagrams:* (a) *normal visual feedback;* (b) *method of varying visual feedback.*

permitting investigation of the open-loop characteristics. The model predicts a staircase, consisting only of saccadic movements of the eye in response to a step change in target input.

By experimenting with other values of effective visual feedback, further checks on the model predictions were obtained. Some of the step responses under altered visual feedback are shown in figure 16.

Figure 17 illustrates the predicted instabilities of the system when the effective visual feedback is positive (upper traces) or negative and greater than two (lower trace). Any external noise will start this system oscillating. This

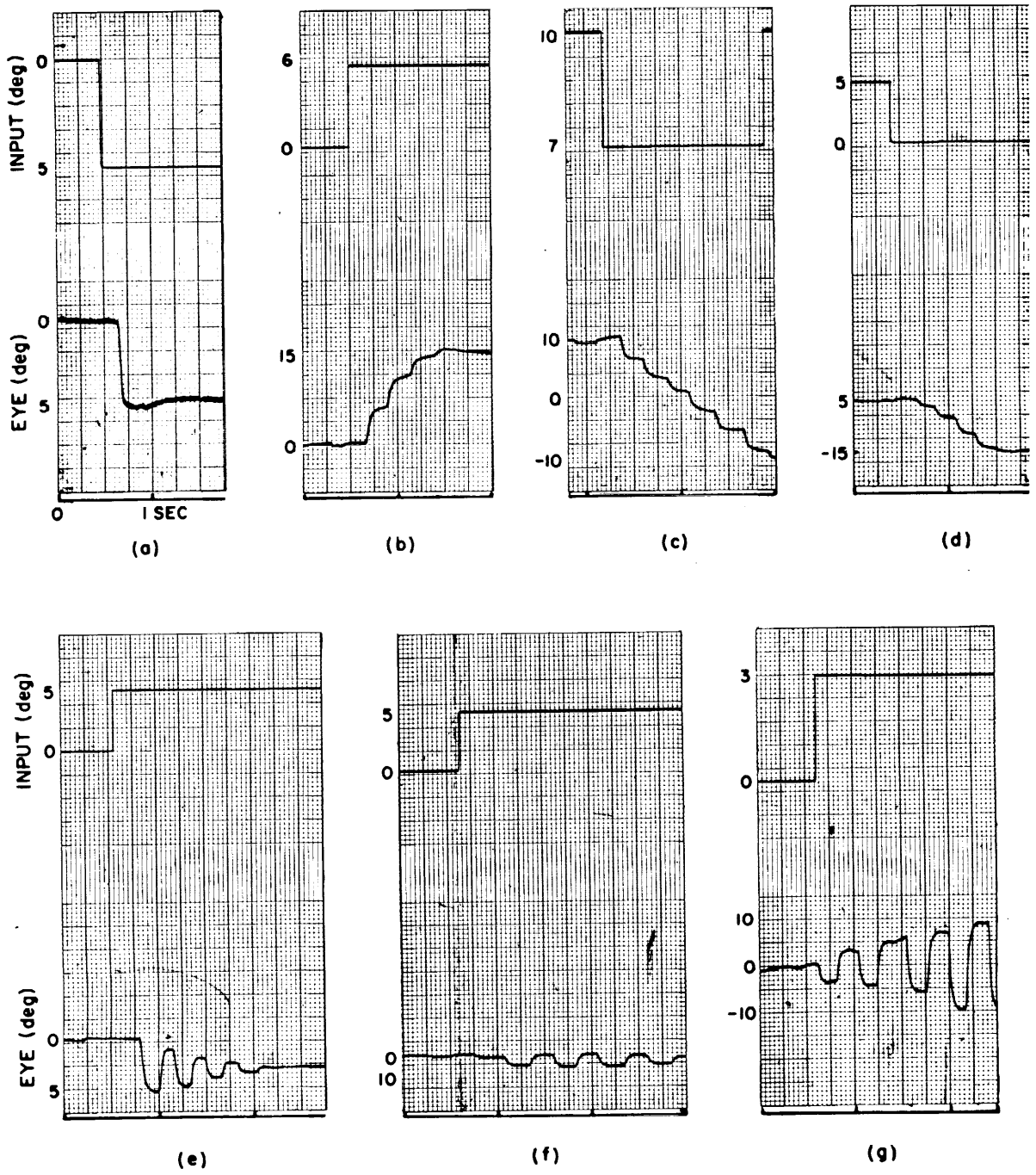

FIGURE 16.—*Experimental step responses under variable feedback.* (a) K=*1.0.* (b) K=*0.3.* (c) K=*0.0.* (d) K=*−1.0.* (e) K=*1.75.* (f) K=*2.0.* (g) K=*2.3.*

model for the visual tracking system response to random-appearing inputs complements the vestibular and proprioceptive input models of the oculomotor system described above.

MANUAL CONTROL OF VEHICLES

The practical application of our research is to determine the way men control vehicles, and, in particular, how they use motion cues, when

motion cues are helpful, and when they are harmful. What we have learned about both the otolith and the semicircular canals would indicate that they are very good velocity meters over a considerable frequency range. Consequently, whenever velocity information is important to a control task, the motion cues should be helpful.

Figure 18 shows schematically one of the experiments that we used to try to check this (ref. 6). The moving-base pitchroll simulator was programed as an inverted pendulum. A small control stick was used by the subject to return the simulator to its level orientation.

There is only one parameter which determines the difficulty of balancing such a system, and that is the length of the pendulum. It is very easy to balance a 12-foot broomstick, but very difficult to balance a pencil. The parameter ω represents the divergence frequency of the system and the difficulty of controlling it. Experiments were conducted on ability to maintain balance for various divergence frequencies under three conditions:

(1) When the subject is presented with visual cues only, sitting outside the simulator and watching it.

(2) Motion cues only, sitting inside the moving simulator with the hood over it.

(3) Combination of motion and visual cues, in which the subject sits inside the simulator but with the hood off so that he can see the laboratory background.

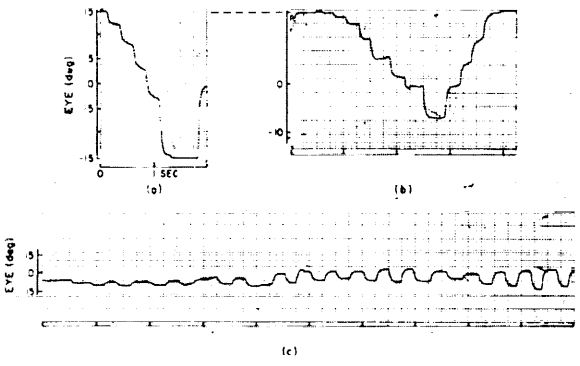

Figure 17.—*Positive feedback and high negative feedback instabilities, no input.* (a) K=—*0.3.* (b) K=—*1.0.* (c) K=*2.2.*

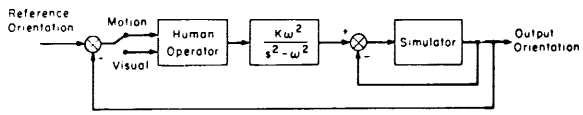

Figure 18.—*Control of inverted pendulum with visual or motion feedback.*

Figure 19 is a plot of rms error from orientation to the vertical as a function of divergence frequency (system difficulty) for the three test conditions. Notice that for a very easy system, where not much velocity compensation is necessary, the visual system and the combined system are better than the motion system alone. That is, we use the preciseness of the visual system to do very fine alinements. However, for very difficult systems in which the simulator moves violently and the subject must rapidly determine the direction of rolling motion in order to damp it, then the effect of motion is very clear in improving the response. Both the motion test alone and the combined motion and visual systems are clearly superior to the visual system alone for this difficult range.

When we analyze what the pilot is doing in a variety of flying tasks, we see that in fact he is able to use considerable velocity information in the higher frequency ranges of motion, and this information is evidently in large part attributable to the vestibular system (ref. 7).

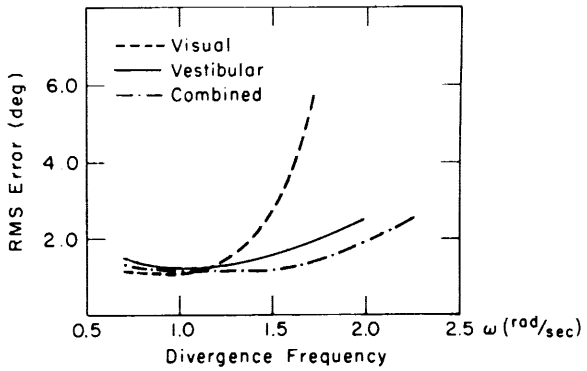

Figure 19.—*Rms error for control of inverted pendulum.*

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DISCUSSION

KELLOGG: I understand that you have been doing some work in caloric nystagmus effects; is that correct? If so, could you comment on it?

YOUNG: Well, yes and no. Yes; we have been considering the quantitative aspect of caloric stimulation and whether the convection-current theory is a sufficient description of the response. Mr. Steer is doing this as part of his doctoral dissertation. No; we don't have any results that I think are worth commenting on at this point.

STEER: I have one comment. In checking most of the convection current theories, we have found that the threshold levels of caloric stimulation correspond exactly to an equivalent angular acceleration of the threshold levels to angular accelerations that have been measured by Dr. Meiry and others. This is the primary result we have achieved. I am presently using the work of Cawthorne and Cobb who, in 1954, measured the time history of the temperature gradient across the semicircular canals with a step of caloric input. I am presently doing a transfer-function analysis from which we can predict other responses. Is anybody aware of any more recent temperature measurements of the temperature gradient across the semicircular canals than the work of Cawthorne and Cobb in 1954?

MAYNE: I would like to congratulate the speaker on a very clear presentation of the application of engineering to the operation of the vestibular system and the control of body movements. We believe, too, that the discipline of constructing models of biological systems as described by Dr. Young is a good one. There are, however, pitfalls of which the MIT group is surely aware in oversimplifying the system.

If we were to use a transducer defined by the transfer function of a semicircular canal as related to the classical differential equation, we would find that the system could not measure the amount of actual movement of the head by an integration of the signal. It would indicate a head movement of a magnitude somewhat less than the actual amplitude, then a slow return to the original position. This behavior of the canal must be compensated in the biological system by further processing or the development of compensatory reactions through adaptation.

[Curves showing response of semicircular canals were drawn on the board to indicate that an integration of the semicircular canal signal does not provide an accurate measurement of body displacement.]

YOUNG: We have tried to reach the same objective with what I think is a better experiment to do; that is, putting the subject in a closed-loop control on the linear track and having him try to maintain himself stationary in the presence of disturbance inputs; in other words, making it a psychophysical experiment rather than just a subjective estimation of displacement.

MAYNE: My reason for asking this question is that we find that if we move a subject by a small amount, he generally seems to sense first the movement in the proper direction, then a return to his original position. After a while he adapts to the situation and can estimate properly the amount of his displacement. This suggested to us the possibility that the compensatory reaction necessary to correct the error of an overdamped oscillatory system in measuring velocity is corrected by adaptation in the case of the semicircular canals but not in the case of otoliths. This is an interesting phenomenon well worth further investigations. We feel also that the biological system is at its very worst in the case of tracking a random function as in the objective test you describe. All the wonderful capacity of the system for nonlinear prediction is to no avail.

GUALTIEROTTI: I'd like to make a general comment on this kind of experimentation. I am sure that in these experiments we really do not study vestibular physiology. We study essentially the time reaction of

the central nervous system which is a value many times greater than whatever happens peripherally. So it is very difficult, I think in fact it is impossible, to build any kind of model of the vestibular system from experiments in which a long nervous system chain is involved. For example, if we take the simplest possible reflex that goes through the cortex, in man, like the blinking reflex, we know that we have a total reflex time of about from 100 to 150 milliseconds. The variability of this time is many times the absolute value of the receptor's time-response in the periphery. In your experiment, how can you establish what happens timewise at receptor level? I don't think you can, because measuring directly the output from the receptors, the delay is nearly insignificant in respect of your figures. The otoliths respond to acceleration with a delay which is no more than 3, 4, or 5 milliseconds, practically equivalent to the refractory time of the system. In your case you have to add at least 200 or 300 milliseconds for the central reaction time; and the variability of this would be of the order of 20 to 40 msec which is a multiple of the peripheral response time. So I will say that this very significant experiment involves the central nervous system reaction time and not much of the vestibular physiology.

YOUNG: I would fully agree with the basic point you are making, Dr. Gualtierotti. The title of our paper refers to human dynamic space orientation and not to vestibular physiology. We are dealing with input-output relations in which we are talking about human behavior responses to the kinds of stimuli which might occur in a space orientation problem. It is the task of you and the other gentlemen here to try to deduce from the basic vestibular physiology and whatever can be learned about the central processing those component descriptions, models, which fit our overall input-output experiments. I would not try to pass these off as experiments which necessarily show what is happening in the sensor. It is interesting certainly to correlate these experiments and models with the classic cupulometry and the other experiments which have been done and find relatively good agreement, but that is about as far as we want to push this.

MAYNE: The next point I would like to make about the problem of accurate simulation is that the semicircular canals and the otoliths do not appear to respond in the same manner to all situations for similar inputs. In the case of steady-state oscillations, as reported by Niven and Hixson, we have shown in our reports that the slow-phase nystagmus is of the right magnitude and direction to provide successive fixation points of the scene being surveyed. At the same time, there is a leading eye movement in the direction of the velocity by an amount proportional to velocity. There appears to be, also, a shift of the sensed body position ahead of velocity. All of this calls for a velocity signal.

In the case of a suddenly impressed movement, however, as found in data kindly supplied to us by Colonel Crampton, eye movements seem to be in response to an acceleration or a rate of change of acceleration. The displacement of an object during the oculogyral illusion calls for a lagging rather than a leading eye movement.

We have assumed that the differences between the two responses is one between anticipated versus nonanticipated movements and we find good functional reason why this should be so. The problem in model building is, then, to distinguish between these two cases and provide different responses for each. This only goes to emphasize the fascinating challenge of model building.

YOUNG: I don't think time would permit my responding to all the points, Mr. Mayne. Concerning the lead function with visual fixation of a sinusoidal input, which is predictable, Stark, Young, and Vossius in 1961, and Trincker a number of years before, showed that we are capable of putting in anticipation and lead in visual tracking. As far as the overall leading of the eye during rotation, Melvill Jones explained this very nicely in terms of what its utility would be for looking ahead of where the body position is.

MAYNE: The control function governing body movements, however, is the sensed body position by whatever means it is obtained, by vision or by internal spatial perception. A lead of sensed body position was suggested by us in the control of body movements. The problem is now to correlate this shift with eye movements, and the difficulty is that eyes sometimes lead and sometimes lag the head movements.

VON GIERKE: I just wonder how much confidence do you have in your otolith response function? Is there not the possibility that it is somehow altered by the proprioceptive cues? Did anyone try to simulate the shearing forces on the seat alone which are applied to the subject under this linear motion but keep the body stationary? For example, apply the same sinusoidal tangential forces to the buttocks which act on the body during the linear motion, but keep the man stationary. What kind of phase lag would you get then?

YOUNG: Our confidence that the input-output relationship indeed describes the otolith was greatly lowered in July 1965, when Dr. Graybiel's labyrinthine-defective subjects got into the linear acceleration cart. We found some of the subjects responded with what appeared to be almost normal responses to the sinusoidal drive functions. The preceding paper by Guedry presents a similar sort of paradox; something which we attributed to otolothic function seems to appear in labyrinthine defectives. We haven't tried the idea you mentioned last. I would have to think fairly carefully about how to do it.

GUEDRY: I have oscillated the same L–D subjects on a parallel swing and found that they were unable to estimate displacement of the parallel swing. They were not trying to estimate turning points. They apparently could do that very well, but they underestimated dis-

placement considerably in an oscillatory motion, whereas our normal subjects (who, incidentally, were blindfolded and carried into the building, so they didn't know the possible range of motion of the device) were about right. The period of oscillation was about 3.6 seconds which is about 1.7 radians/sec. At any rate, in connection with your last comment that they gave equivalent responses, is this based upon turning points?

YOUNG: The responses were not equivalent, but they were not so different as you might have expected. You certainly see the significant differences between labyrinthine defectives and normals. The results I referred to were based on both latencies to constant acceleration and detection of phase reversal for sinusoidal stimulation or turning point.

GUEDRY: We have the impression from our work that if we had been able to apply prolonged constant linear acceleration, a person would not experience velocity, linear velocity. I think Walsh reached the same conclusion. Do you agree with this? I wasn't clear on this.

YOUNG: The model we have would certainly indicate that for constant acceleration, constant input acceleration—

GUEDRY: Constant linear acceleration.

YOUNG: Constant l i n e a r acceleration, the model would predict that the man would feel constant linear acceleration and not velocity.

GUEDRY: He would only feel tilt in this case.

YOUNG: He would either feel a constant tilt or he would feel an increasing velocity in a steady state, depending upon his mental set, the conflict with canal sensation, and his instructions.

GUEDRY: On a centrifuge, if you apply, for example, a constant centripetal acceleration, you don't experience a constant linear velocity.

YOUNG: You experience tilt.

GUEDRY: Right. So I don't understand why you think that with a constant linear acceleration on a track, you should experience constant linear velocity.

YOUNG: I think we had best discuss this afterward privately.

Determination of Physical Constants of the Semicircular Canals From Measurement of Single Neural Unit Activity Under Constant Angular Acceleration

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SUMMARY

This presentation is a progress report on our attempts to correlate certain responses of the labyrinth to inertial stimulation with the mechanical properties of the semicircular canals.

INTRODUCTION

The commonly accepted model of the sense organ responsive to angular accelerations is a circular tube of small bore, filled with liquid, which is divided by a spring-loaded fluidtight flapper valve, the cupula. During angular acceleration, the inertia of the fluid causes it to lag behind the angular displacement of the canal, thus deflecting the cupula through the resulting hydrostatic pressure. This motion is impeded by both the viscous friction between the fluid and the wall of the tube, and by the elastic resistance of the cupula. Upon cessation of the stimulus, the cupula returns to its neutral position, with the viscous friction now opposing the movement.

Deflection of the cupula causes nerve stimulation which informs the organism of the change in its inertial environment.

For purposes of preliminary analysis, the cupula-canal system has been considered as a damped spring-mass system with a single degree of freedom. However, its representation by a nonhomogeneous second-order differential equation has not always been correct.

Figure 1 shows the behavior of a damped mass-spring system. In figure 1(a) the system is at rest, and in figure 1(b) its support is subjected to a constant acceleration which causes the mass to be displaced toward the point of attachment of the spring and damper. It ap-

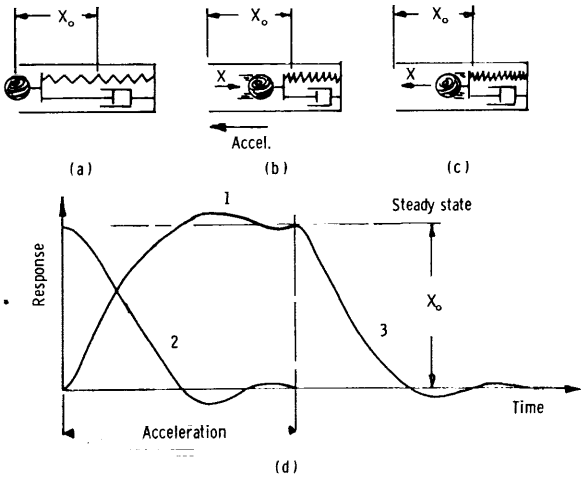

Figure 1.—*Simplified mechanical model of semicircular canal in resting state* (a), *during linear acceleration* (b), *and during return phase* (c).

proaches a final position located a distance x_0 from its original position. Therefore, dynamically, the motion of the mass may be considered as a return to an equilibrium position, following an initial displacement x_0. Thus, while in figure 1(d) the response of the cupula to a constant acceleration, as derived from nystagmus and nerve impulse firing experiments, is represented by curve 1, the motion of the cupula corresponds to the difference between the maximum or steady-state response and the actual response, as shown by curve 2.

The decreasing response to continued stimulation can thus be simply explained by the mechanical properties of the system, without invoking adaptation.

Upon cessation of the acceleration stimulus, the mass returns to the original equilibrium position shown in figure 1(c) and curve 3. Theoretically, for a linear system, curves 2 and 3 should be identical; the slight differences actually found might be used to determine the degree of nonlinearity.

The motion of the mass with respect to the support is represented by the homogeneous differential equation:

$$\ddot{x}+\frac{D}{\tau}\dot{x}+\frac{K}{\tau}x=0 \qquad (1)$$

where D/τ and K/τ are the damping-to-inertia and spring-constant-to-inertia ratios, respectively. Note that the acceleration does not occur explicitly; it is introduced in the solution by the initial conditions:

$$x_0=\frac{\alpha s}{K/\tau}$$

$$=\frac{\alpha s}{\omega_n{}^2} \qquad (2)$$

where s is a scale factor that relates the dynamic behavior of the system to the observed output variable, such as the velocity of slow-phase nystagmus or the number of nerve impulses per second. It may also be compared to the gain, as will be brought out later; ω_n is the undamped natural frequency of the system.

The damping-to-inertia ratio of the human semicircular canals may be estimated from the data given by Melvill Jones and Spells (ref. 1), which gives the internal radius as 0.14 millimeter. The ratio of damping constant to polar moment of inertia of the liquid in a circular tube is given by:

$$D/\tau=\frac{4\mu g}{\rho r^2} \qquad (3)$$

where

μ is the viscosity
ρ is the density
g is the gravitational constant
r is the internal radius.

If the endolymph is assumed to have the viscosity and density of water, the value of D/τ in equation (3) is found to be about 200. This value is several magnitudes higher than the values derived from the data on which the present analysis is based.

These responses were obtained by Crampton from single nerve units in the anesthetized cat brain stem (ref. 2). The long period of stimulation, 45 seconds, made it possible to analyze these data on the basis of the model described previously, since the responses clearly approached the steady-state values required for quantitative analysis. Of the 29 sets of data, only 8 have so far been analyzed.

Typical response curves, with nerve impulses counted in 5-second intervals, are shown in figures 2 and 3. The curves were generated by an analog computer to pass through the data points. It is evident that the response is under-

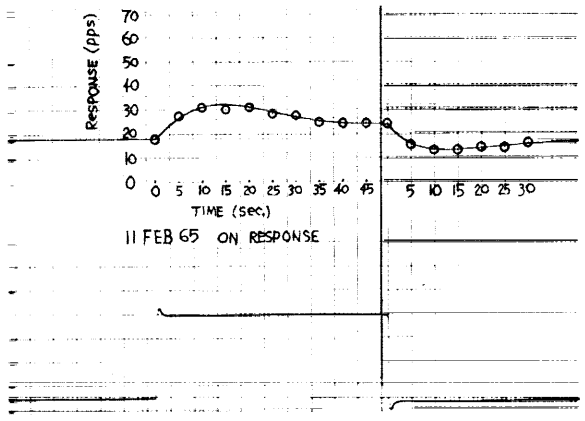

Figure 2.—*Analog computer simulation results: Nerve firing rate during and after angular acceleration of 4 deg/sec/sec. (Cat, Feb. 11, 1965.)*

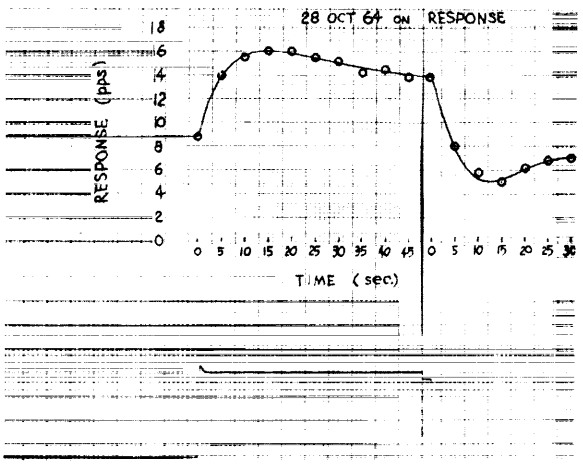

Figure 3.—*Analog computer simulation results: Nerve firing rate during and after angular acceleration of 4 deg/sec/sec. (Cat, Oct. 28, 1964.)*

damped during both the stimulation and the return phases.

Equations (4) to (6) show the relationship between the equations of the equivalent mechanical system (eq. (4)), the assumed proportionality between the mechanical model and the cupula system (eq. (5)), and the analog computer representation of the mechanical system (eq. (6)).

Mechanical System

$$\underset{\text{radians}}{x(s)} = \frac{x(0)[s+D/\tau]+\dot{x}(0)}{s^2+(D/\tau)s+K/\tau}$$

$$\approx x(0) f_1(\dot{x}(0) \text{ negligible}) \quad (4)$$

Physiological System

$$\underset{\text{impulses/sec}}{Y(s)} = s \times (s) \quad (5)$$

$$\therefore Y(s) = s \times (0) f_1$$

where s=scale factor or sensitivity impulses/sec per radian deflection.

Computer Model

$$E_{\text{out}}(s) = E_{\text{in}}(s) K\omega_n^2 \frac{s+\alpha}{s^2+2\zeta\omega_n s+\omega_n^2}$$

$$= E_{\text{in}}(s) K\omega_n^2 f_2, \quad (6)$$

where K=*gain.*

On the basis of the simplifying assumption that the transfer functions of the mechanical and computer systems are proportional, it is shown that the scale factor s relating the displacement of the cupula to the firing rate is proportional to the gain K of the analog computer model.

Correlation

If

$$f_1 \propto f_2 \quad \text{(assumed)}$$

and

$$Y \propto E \quad \text{(given)}$$

$$\therefore s \propto K\omega_n^2$$

The results are summarized on the following figures. Figure 4 shows the damping-to-inertia and spring-constant-to-inertia ratios. Most of the responses are underdamped during stimulation and overdamped during return, and the damping is almost invariably higher during the return phase. No clear difference between the types of cell termed "Ewaldian" and "non-Ewaldian" by Crampton is apparent.

Figure 5 shows a relatively narrow range of undamped natural frequencies with a mean of about one-eighth of a radian per second, or about 0.02 cps. A more exact analysis might reduce this range.

The maximum response of the cells varies over a wide range, from about 12 to almost 60 pulses per second above the resting discharge rate. This is equivalent to a large spread in the sensitivity (or scale factor, or gain.) It is most interesting to note, from figure 6, that a linear relationship exists between the sensitivity and the damping-to-inertia ratio, with only 3 of the 16 data points not falling near the straight lines. The figure also shows the increase in damping during the return phase, which may be related to nonlinearities. Thus, the properties of the system are consistent with servo design practice in which an increase in gain would be balanced by an increase in damping to prevent instability.

Confidence in the system constants derived from these data is increased by the fact that a considerable proportion of the nerve units showed remarkable symmetry of response under stimulation in opposite directions. An ex-

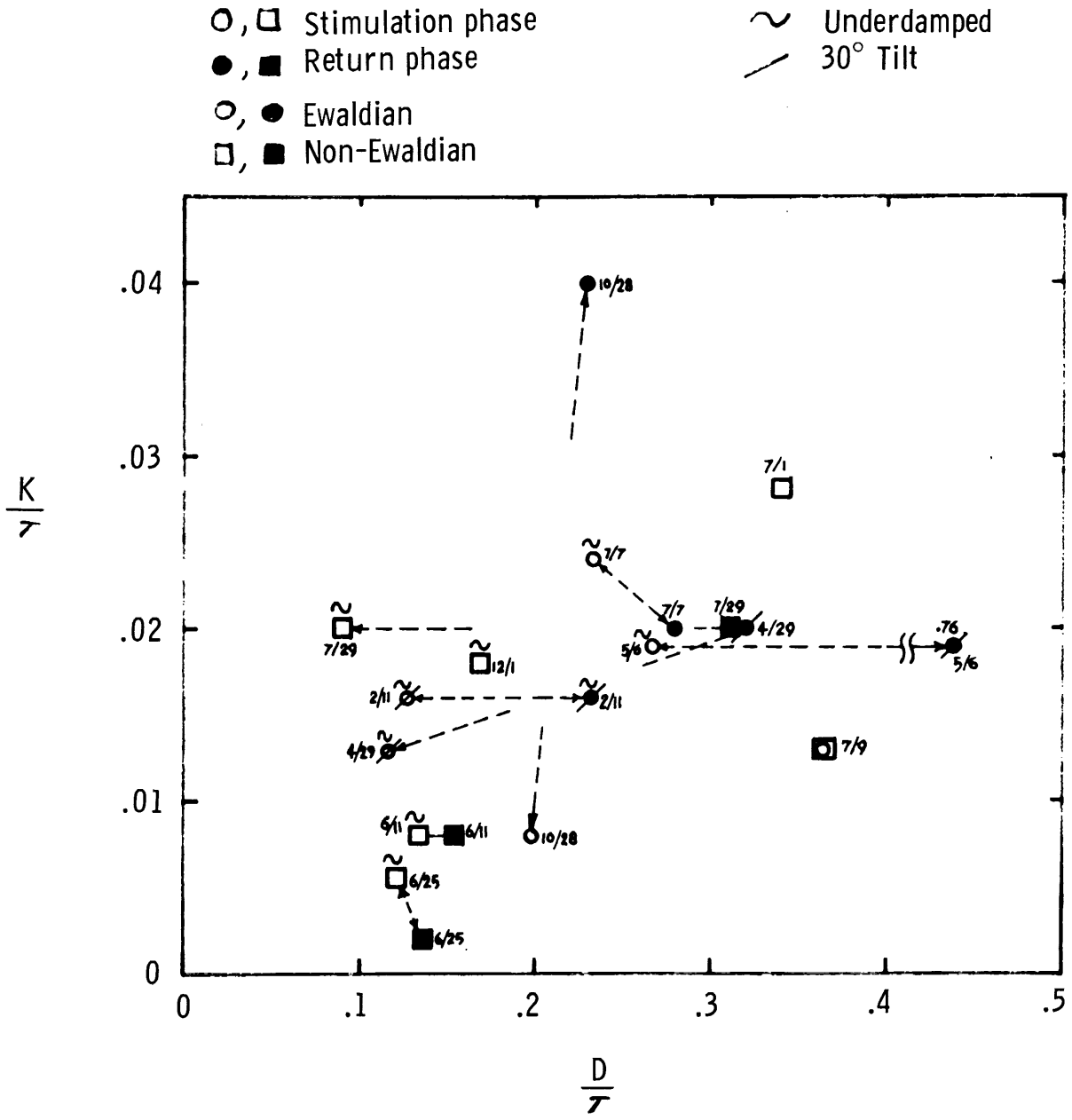

Figure 4.—*Summary of damping-to-inertia and spring-constant-to-inertia ratios derived from analog computer simulation of experimentally obtained nerve firing rates during and following stimulation by 4 deg/sec/sec angular acceleration.*

ample is shown in figure 7, in which the firing frequency is plotted with the resting discharge as a base line, which also represents the axis of symmetry. It appears from the data that the units exhibiting symmetry of response also have lower sensitivity and a higher resting discharge.

Most of the records obtained by Crampton show an additional high-frequency oscillation superimposed on the response discussed above. Second-by-second pulse counts result in response curves of which the one shown in figure 8 is representative. It has a frequency of about

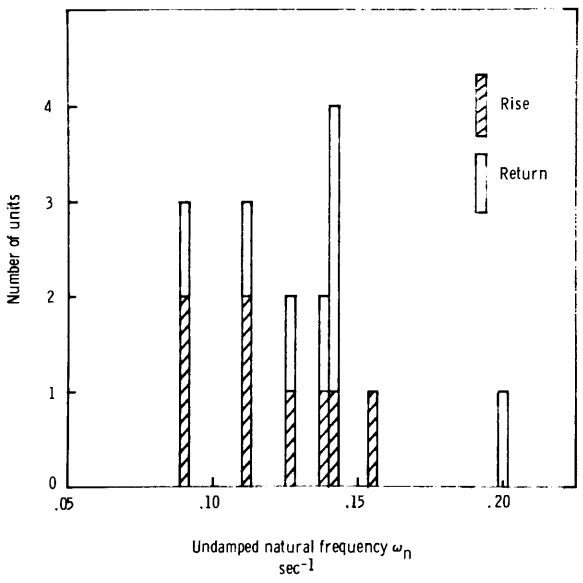

Figure 5.—*Distribution of undamped natural frequencies in eight experiments, as derived from analog computer simulation.*

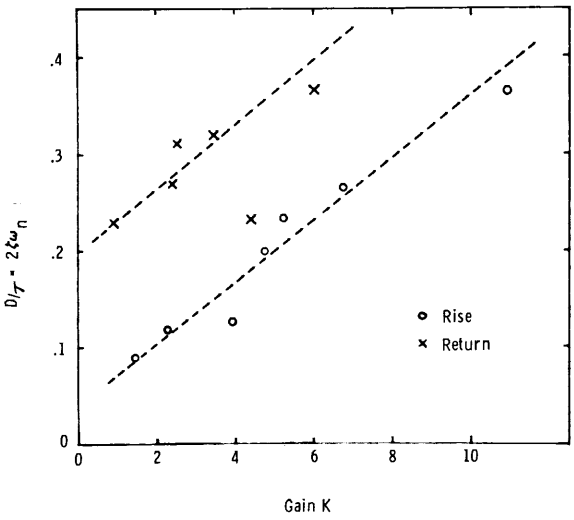

Figure 6.—*Relationship between sensitivity and damping in response, derived from analog computer simulation of nerve firing rate.*

0.20 cps, or an order of magnitude higher than the frequency of the responses previously discussed, and appears to be undamped. The order of magnitude is closer to that derived by Hixson and Niven (ref. 3) from nystagmic responses of human subject exposed to sinusoidal oscillations on the Human Disorientation Device.

As engineers, we must leave the interpretation of these results up to the biologists. We intend to analyze the remainder of the data obtained by Crampton along the lines discussed above. We will also attempt to derive similar relationships from records of nystagmic responses caused by angular accelerations of sufficient duration to make possible determination of the steady-state response value that has been shown to be necessary for derivation of the system constants.

We are also exploring the possibility that the

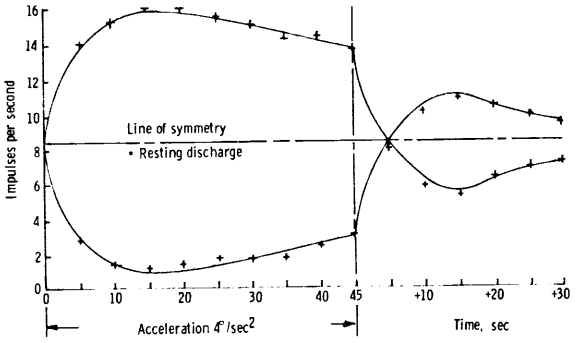

Figure 7.—*Showing symmetry in response during acceleration and return phases, typical of units having low sensitivity.*

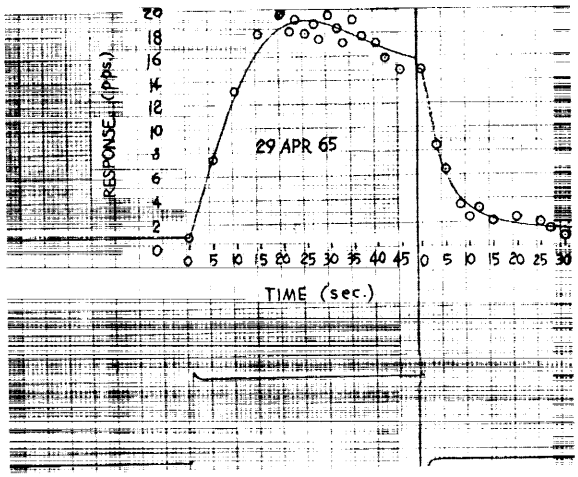

Figure 8.—*Analog computer simulation results showing undamped high-frequency response superimposed on more typical damped low-frequency response.*

cupula may be directly affected by inertial stimuli (both linear and angular accelerations), in addition to being deflected by hydrostatic pressure alone.

Finally, we should not take for granted that the second-order system characterized by our analysis of Crampton's data is necessarily mechanical.

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DISCUSSION

WERSÄLL: I just want to point out that you draw some conclusions about the behavior of the end organ itself which might not at all be valid as you are recording from second order neurons.

VON GIERKE: My question is related to your figure which gives the ratio of D/τ (damping constant to polar moment) as a function of the gain. In the graph you showed different lines for the rise-and-return phase, and your interpretation was that the system has different damping for the two phases. Could one not hypothesize just as well that the mechanical damping of the system is constant, but that the system has a different sensitivity in the two directions?

CAPPEL: That is right.

VON GIERKE: Wouldn't this give a different interpretation of your data if one assumes the mechano-electrical transducer sensitivity to be different for the two phases? I think this would also be more in line with the interpretation of other electrophysiological findings.

CAPPEL: Yes. I overlooked that. You may be right.

BELSLEY: What was the experiment these data came from?

CRAMPTON: These data were collected from heavily anesthetized cats in a stereotaxic frame mounted over the axis of rotation. The angular accelerations were all of 4°/sec² and of 45 seconds' duration. The velocity programs were symmetrical about zero velocity, starting at a velocity either positive (CW) or negative (CCW), passing through zero and terminating in the other direction so that the linear acceleration component would be the same at the beginning and at the end of each acceleration. The spikes were counted by hand, usually for every fifth second. The reliability of the units, with but one exception, turned out to be very high. Some examples of the reliability are shown in NASA SP–77.

The microelectrodes were stainless steel and less than 6 microns in diameter at the tip. We directed the electrodes into the region of the vestibular nuclei and studied but one unit per cat, before marking the spot with the prussian blue reaction for later histological verification. No data were used that were not accompanied by an adequate nuclear identification. Most units of this sample were in the superior vestibular nucleus and many were in the medial. It was very unusual to find any responding units in the lateral nucleus and only once in a while would one be located in the descending nucleus.

A number of surgical measures were undertaken at various times, including section of the contralateral VIIIth nerve and ablation of the cerebellum.

BENSON: I would like to underline Dr. Wersäll's remark that here you are recording from somewhere in the vestibular projection. I just wondered about the general validity of arguing about the pattern of response in terms of the equation of motion of the end organ itself. Can you not equally argue that we are seeing a manifestation or adaptation within the central nervous system and that if you had looked at, say, sensation, you would have seen a very much more rapid decay of neural events underlying the phenomenon? This is really adaptation that you are looking at. Is it not rather dangerous to say that this is change of damping? If you say there is more adaptation in the deceleratory phase than in the phase immediately following acceleration, this is in accord with some experiments that I think Dr. Guedry and you did, in which, with constant angular acceleration inputs, the sensation of turning decayed—adapted more rapidly than the nystagmus.

CRAMPTON: We always offer the disclaimer that we do know our data are removed somewhat from direct events at the ear, but then we often continue to guess at what is taking place at the end organ. I may comment that it has been acceptable for years to measure nystagmus or sensation duration, usually with a stopwatch and often with clinical cases, and then to make inferences about the sense organ. But now, when data are offered from the vestibular nuclei, all sorts of objections are raised because the data are not from the cupula. I accept the objections, and the reason these recordings are not from first-order neurons is because my technique has not been good enough to record from them. I have attempted to record from the nerve itself in the manner after Gernandt, but without success, and therefore moved into the vestibular nuclei where everybody can be successful. With special deference to our Swedish guests, I wish to present a smorgasbord of data in the following figure.

In the upper left-hand panel is a curve derived from the critically damped torsion pendulum model with an RC value of 10 for a response to constant angular acceleration of 45 seconds' duration. In the next panel down are the nystagmic responses of man and cat recorded in total darkness during angular acceleration of 4°/sec² magnitude and 45 seconds' duration. The men were doing mental arithmetic, and the cats were treated with *d*-amphetamine. You may note that if maximum arousal is maintained, no decline in the response is found even for these long durations. Those of you who want a nonovershooting cupula may use these data. But those who use subjective data from man will infer that the cupula overshoots, or that there is adaptation in the system. In the third panel on the left are velocity judgments of men who signaled each time they believed they completed an arc of 90°. It

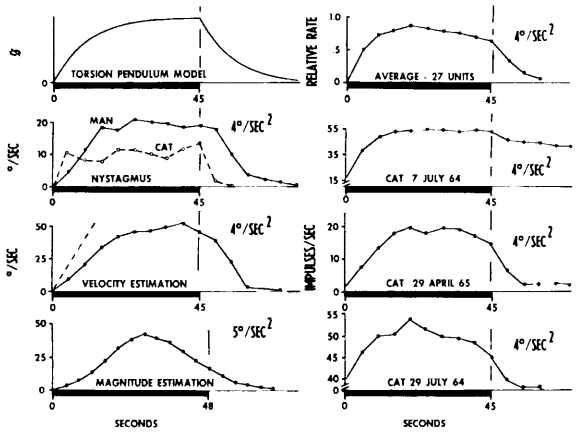

Figure D1.—*Response to constant angular acceleration, man and cat.*

is characteristic in experiments of this variety to find a decline in subjective velocity for these longer durations, a decline usually more prominent than found with this particular sample. Dr. Guedry and Dr. Collins have performed the definitive experiments on this phenomenon.

In the last panel on the left are results for estimations of the intensity of the sensation of rotation after the method of S. S. Stevens. We asked the men to select their own scale values and to verbally indicate their speed by their subjective scale. When this technique is used, one finds an even more prominent decline in the response. One must conclude that the inferred cupular mechanics depend upon which variety of remote response is chosen for study.

Units from the vestibular nuclei give responses which may support each of these several inferences about the cupula. In the lower three panels on the right are units which show little decline, a moderate decline, and a substantial decline. In the upper right panel is an average, a statistic I really cannot defend for these data, but which shows that a decline is probably representative of the aggregate. I would like to point out again that I do not really think I am studying the cupula directly, but I do feel that it is important to look at responses throughout the system and to try to build a total picture. Maybe next year I will have some data from the cupula to show.

GUEDRY: I must agree with Dr. Crampton on a number of points. In regard to the velocity estimation panel, I have found that if we use highly practiced subjects (and this velocity estimation is a difficult task), then we get a picture which is much closer to the magnitude estimation than is shown in the panel. I have tested subjects with several hours of practice and gotten essentially the picture of velocity estimation shown there for the same magnitude stimulus, incidentally, and for just about the same duration. In regard to the second panel which shows the man and cat, we have found essentially the same picture of nystagmus with the same magnitude stimuli and with lesser magnitude stimuli applied even for longer times, as long as we kept the person mentally active. If the person is not kept mentally active, almost anything can be found, but usually there is a rise and decline in nystagmus. Dr. Collins and I have just done an experiment with the cat, and found that cats without amphetamine show a surprisingly similar picture to the average human response for exactly the same magnitude stimulus. It even peaks at about the same point.

MAYNE: The evidence brought out by Mr. Cappel to the effect that there is more than one type of response of the semicircular canals to similar stimulation is very important. We have observed similar dif-

ferences in eye-movement responses. The nystagmus response to steady-state oscillation as reported by Niven and Hixson, for instance, is proportional to the velocity of the head, while the response to sudden movement as judged from data kindly forwarded by Dr. Crampton appears to be related to acceleration or rate of change of acceleration. We have attempted to differentiate between these two types of responses in terms of anticipated and nonanticipated movements. We have assumed that steady-state oscillations and willed movements belong in the class of anticipated movements.

There appear to be good functional reasons why the organism should not respond in the same manner to the two classes of movements. In the case of a willed movement calculated to bring the body from one position to another, conventional servo theory calls for a lead of sensed versus actual position by an amount proportional to velocity. At the same time, slow-phase nystagmus should provide accurate fixating periods. Both requirements call for velocity information. In the case of a suddenly impressed movement, such as our ancestors, the apes, may have experienced with the breaking of the limb of a tree, the dominant requirement is for an immediate warning of danger so that appropriate action may be taken. A velocity signal builds up too slowly, requiring as it does an integration of acceleration. Acceleration would be a better signal for the purpose; rate of change of acceleration would be even better.

I am looking foward to an opportunity to study Mr. Cappel's paper to see if these views can be reconciled with his findings.

CAPPEL: The damping-to-inertia ratio, as determined from the slow underdamped oscillation, is about three orders of magnitude lower than what one would compute from the characteristics of the canal, the physical dimensions of the canal and the fluid assumed to be approximately like water. This is a tremendous discrepancy.

MAYNE: A transducer of these characteristics, by whatever means they may be achieved, is an accelerometer, not a velocity transducer. The concept of two transducers incorporated in the semicircular canal sense organ would fit our concept of dual responses of these organs. We have suggested a similar idea for the otolith organs and proposed a mechanism to produce this dual response at the periphery. If the teleological argument mentioned previously is valid, the dual response must occur at the periphery. Only then can fast warning be given to the organism of an unexpected body movement. The processing of a velocity signal to give acceleration would result in further delay beyond that of producing the velocity signal. All of this lends weight to the often-suggested possibility that the semicircular canals are adapted to measure both acceleration and velocity.

CAPPEL: I wasn't making any predictions concerning the function of the organ. I was saying only that the response that is shown by Dr. Crampton's cats cannot be correlated with the physical characteristics of the end organ.

MAYNE: Yes; I understood this. I was only trying to interpret your very significant findings in terms of our own investigations and the likely functions of the vestibular systems.

Lack of Response to Thermal Stimulation of the Semicircular Canals in the Weightless Phase of Parabolic Flight

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AND

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SUMMARY

The objective of this study was to clarify the mechanism of caloric nystagmus in man by conducting the test during weightlessness. Eight subjects were selected on the basis of a strong nystagmus response to irrigation with ice water. Nystagmus was determined by oscillograph tracings and direct observation, and, in addition, subjective responses of the subject were obtained. The experimental evidence indicated that, under the conditions of this experiment, zero gravity completely suppressed caloric nystagmus. This supported Bárány's original hypothesis that caloric nystagmus was dependent on difference in specific weight of the endolymph in the horizontal canal.

INTRODUCTION

In 1906, Bárány (ref. 1) described caloric nystagmus and also advanced a hypothesis to explain the response. He wrote:

> Heat is conducted via the temporal bone to the semicircular canals, affecting first the horizontal canal. The result is a change in specific gravity of the endolymph in the canal's most lateral part relative to its innermost part. If the canal is not horizontal, this sets up a current in the endolymph which affects the cupula and leads to nystagmus.

Bárány's hypothesis has been widely but not universally accepted. The following is a summary of the leading theories which have been advanced to explain the reaction:

(1) Bartels (ref. 2) in 1911 suggested that the caloric reaction is due to a direct effect on the nerves, heat having a stimulating and cold a depressing effect.

(2) Kobrak (ref. 3) in 1918 theorized that the caloric response is caused by vascular reactions in which the vessels in the periphery of the labyrinth are constricted by a cold stimulus and the central vessels react with dilation. This, according to Kobrak, sets up a flow of endolymph and a consequent deviation of the cupula.

(3) Borries in 1920 and in 1925 (refs. 4 and 5) pointed out the importance of both the labyrinth as a whole and of the otolith specifically. He stressed experiments in which subjects whose semicircular canals were damaged or extirpated still showed clear caloric reactions.

(4) Brunner (ref. 6) in 1921 put forth the notion that the caloric reaction is not the result of deviation of the cupula but is of central origin.

(5) Van Caneghem (ref. 7) in 1946 suggested that a hot caloric stimulus might cause an increase of the intralabyrinthine pressure and a cold stimulus might cause a decrease. He felt that the increase of the intralabyrinthine pressure has its effect at the utricle.

In weightlessness, heating or cooling the endolymph cannot cause a change in specific weight; hence, endolymph flow for this reason would be an impossibility. On the other hand, conduction of heat would occur, and heat and cold would lead to expansion and contraction, respectively. Hence, conducting the caloric procedure in weightlessness would test many of the above-mentioned theories.

METHODOLOGY

Subjects

Eight subjects, ranging in age from 20 to 41 years, were used in the study. All eight were on flight status, implying they had met the USAF medical standards. One subject, G, manifested a 40-db hearing loss in the high-frequency range. None had experienced any spontaneous labyrinthine disturbances. All subjects had had extensive experience in military aircraft.

The Force Environment

A report by Weiss (ref. 8) describes in detail the force environment of the zero-gravity airplane in parabolic flight. The flight profile for each subject consisted of three consecutive zero-gravity maneuvers flown in the modified KC–135 (Boeing 707) (fig. 1). In each maneuver the aircraft was placed in a shallow dive followed first by a pullup generating 2.0 g-units and then a pushover into a ballistic trajectory with approximately 25 to 30 seconds of weightlessness or near-weightlessness. Recovery involved a second pullup generating 2.0 g-units followed by a brief period of level flight. The second and third maneuvers followed seriatim, the intervals ranging from one to several minutes.

Instrumentation

Standard corneoretinal extraocular electrodes were applied as shown in figure 2. Vertical

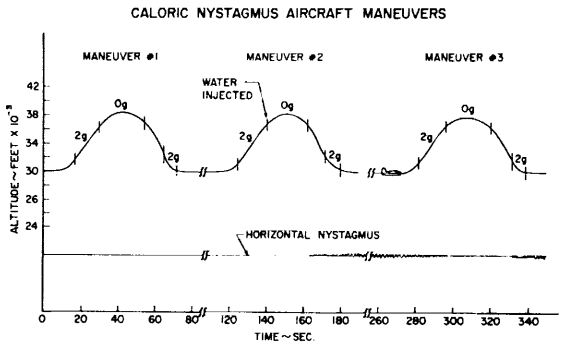

Figure 1.—*Caloric nystagmus aircraft maneuvers.*

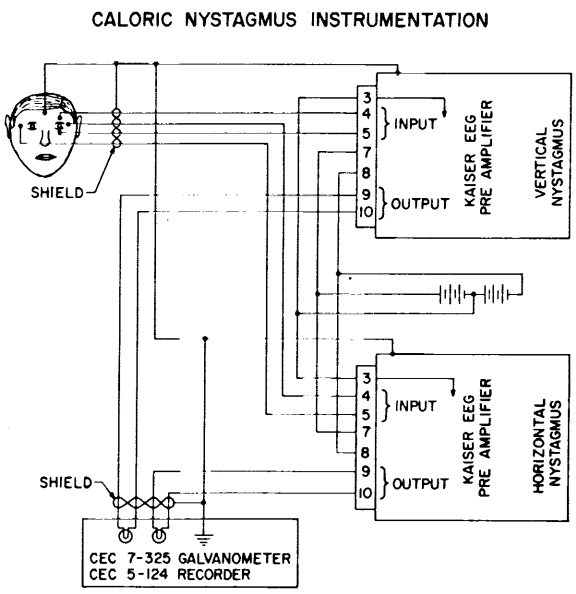

Figure 2.—*Caloric nystagmus instrumentation.*

and horizontal eye movements were recorded separately. Two Kaiser EEG miniature solid-state preamplifiers provided voltage amplification. The units were temperature compensated and had differential input circuitry such that, when the eye ceased moving, the tracing returned to the baseline; the response time was 1.2 seconds. The outputs of these amplifiers were passed directly to a CEC 5–124 oscillograph recorder equipped with CEC type 7–325 galvanometers. Sensitivity of the galvanometers was 2.92 mV/inch deflection. Movement of the eye in an upward direction produced an upward deflection on one graph, and movements of the eye to the right produced an upward deflection on the second graph. A third galvanometer was used to record aircraft normal

acceleration (g-level) as sensed by a Statham 2-g strain-gage accelerometer.

Procedure

The subjects were selected partly on the basis of a good nystagmic response to irrigation of the ear with ice water. Two or more baseline caloric tests were performed on each subject before flight tests were carried out. Since ice water was used for irrigation, the first ground-based test served to familiarize the subject with the experimental procedure. The subject, inclined backward 60°, was instructed to fixate on a convenient spot on the ceiling and note all of his subjective sensations. Thirty cubic centimeters of ice water were injected with a syringe directly into the external canal in approximately 3 seconds. One subject, H, had a remarkably short lag time, 9 seconds, before the appearance of nystagmus, while in the others lag time varied between 16 and 21 seconds. Inflight, the subject was inclined 60° backward from the visual vertical with respect to the aircraft which approximated the gravitoinertial vertical when this force was acting. The first maneuver served as a control. In the second maneuver, ice water was injected during the transition period from 2.1 to 4.5 seconds prior to the onset of weightlessness at which times the g loading was about 0.5 g-unit or greater; this minimized or prevented the tendency toward "airlock" due to the minimum energy configuration of fluid in zero g. Visual observation and sometimes recordings were continued throughout the third parabola. Immediately thereafter the subject was interrogated. Recordings obtained from subjects C and D were not wholly satisfactory, and chief reliance was placed on visual observation of eye movements.

RESULTS

The important findings are summarized in table 1. A nystagmic response was not manifested during the control parabolic maneuver. In the second maneuver, nystagmus was not observed during the weightless phase, although the total response period available, i.e., from the onset of irrigation to the end of the weightless phase, exceeded the ground-based nystagmus delay time by 0.1 to 16.5 seconds. On pullout, at approximately 1.5 g (4–8 seconds after the end of weightlessness), horizontal nystagmus appeared in every instance. During the zero-g phase of the third parabola, nystagmus disappeared in the few instances it was present in the pullup. The nystagmus always beat in the anticipated direction.

Subject H was an exceptionally good producer of nystagmus, and some details of the findings in his case are summarized in figure 3. During the first maneuver, there was little eye movement aside from blinking. During the second maneuver, nystagmus first appeared during pullout about 5 seconds after the end of the weightless phase and at which time the g loading was approximately 1.5 g-unit. Nystagmus continued during level flight after the second maneuver (fig. 3, *3*) and during pullup in the third maneuver (fig. 3, *4*), but disappeared in the weightless phase. On pullout a few beats appeared, but thereafter none appeared on the record.

There was a tendency for the subjects to be aware of the nystagmic beats during increased g loadings, and the impression was gained that spontaneous eye movements, aside from blinking, were reduced in weightlessness.

DISCUSSION

Although the observations just reported must be regarded as an experimental probe, yet they were clear cut. With eyes open and fixating a target, subjects did not manifest nystagmus during control parabolas; hence, any complicating positional nystagmus and nystagmus due to increased g loadings were avoided. Carrying out the irrigation prior to the onset of weightlessness, for the most part, not only had the effect of extending the zero-g phase but also of insuring good contact at the water and skin interface. The adequacy of the stimulus was demonstrated by the long-lasting nystagmus once the weightless phase had ended. The supranormal g loadings acted as an activator which not only served to extend the time during which nystagmus might be observed but also

Table 1.—*Caloric Nystagmus Responses in 8 Subjects Under Laboratory and Parabolic Flight Conditions*

Subject	Age	Ground-based observations, nystagmus delay time (NDT), [a] sec	Parabola I, control nystagmus response			Parabola II						Parabola III, nystagmus zero-g phase
			Pullup	Zero g	Pullout	Onset of irrigation, before zero g		Duration of zero-g phase, sec	Time from onset of irrigation to end of zero-g minus NDT, sec	Nystagmus response		
						Seconds	g level			Zero g	Pullout	
A	22	16	0	0	0	2.3	0.31	25	8.3	0	Positive	0
B	23	18	0	0	0	2.9	.58	26	7.9	0	Positive	0
C	20	19	0	0	0	2.2	.48	26	6.2	0	Positive	0
D	39	21	0	0	0	2.1	.45	22	.1	0	Positive	0
E	36	18	0	0	0	4.5	2.0	24	7.5	0	Positive	0
F	23	19	0	0	0	-------	-------	24	>2.0	0	Positive	0
G	41	20	0	0	0	2.6	.62	23	2.6	0	Positive	0
H	33	9	0	0	0	4.5	1.25	24	16.5	0	Positive	0

[a] NDT—nystagmus delay time; irrigation 30 cc ice water in approximately 3 seconds.

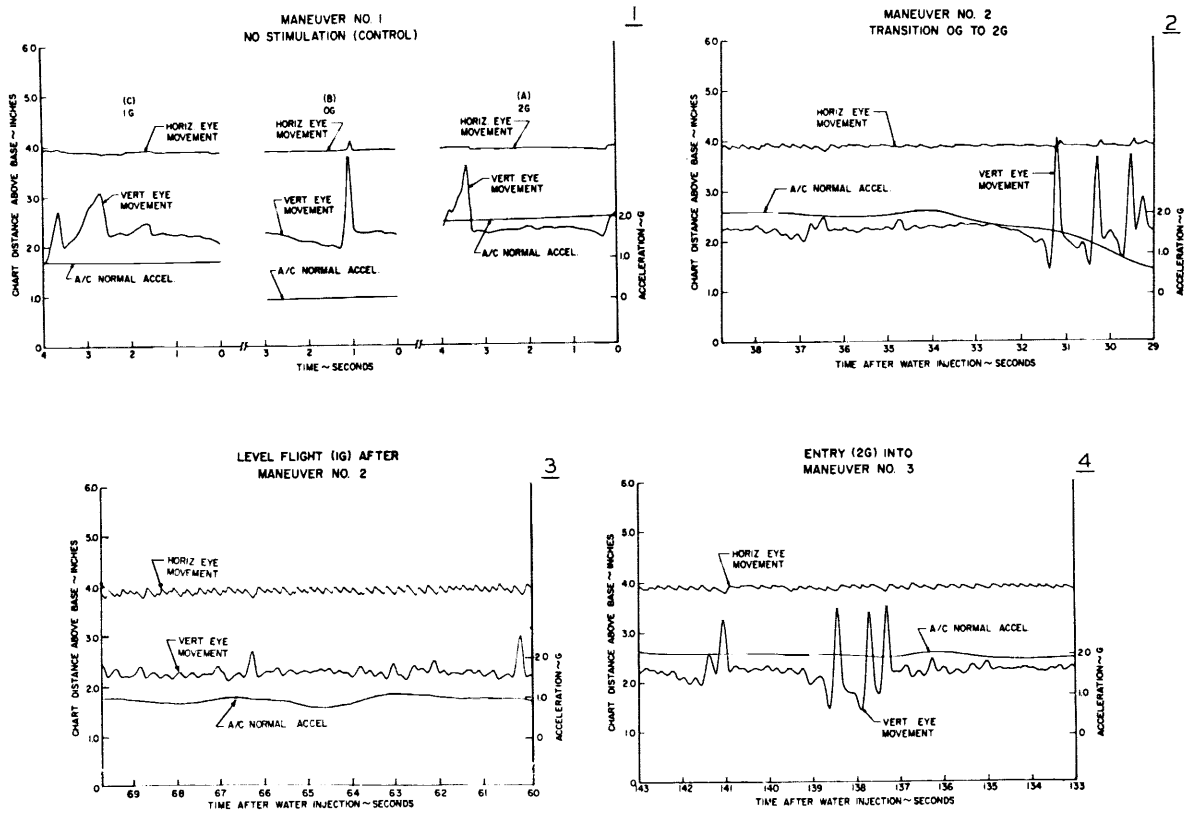

Figure 3.—*Oscillograph tracings from subject H. Read right to left. Nystagmograms in plate photographed; the others traced and photographed.*

emphasized the dramatic effect of weightlessness in abolishing caloric nystagmus.

Little attempt has been made to quantify the results inasmuch as improvements in procedure will make the task simpler. The short delay in appearance of nystagmus on transition out and into zero g suggests (1) that under ordinary conditions much of the delay following irrigation is due to conduction of the thermal stimulus from ear to canal, and (2) that little displacement of the cupula occurs, otherwise elastic restoration would continue well into the weightless phase with nystagmus a manifestation. By using a modified parabolic maneuver, it should be possible to determine the level of g required to evoke caloric nystagmus. Extrapolating the curve drawn from similar observations under supragravity conditions led Bergstedt (ref. 9) to predict that caloric stimulation in zero g would not evoke nystagmus. The present study indicates that his prediction was quite correct.

Direct stimulation of the nerve, as suggested by Bartels (ref. 2), does not seem tenable inasmuch as the reaction should have taken place regardless of the g level. The theory advanced by Kobrak (ref. 3) concerning vascular reactions seems equally untenable, since the vascular responses could not operate as fast as the eye-movement changes indicated in the changing acceleration fields.

Borries' position (refs. 4 and 5) is more difficult to counter, since the otolith is essentially deafferentated (ref. 10) during zero g. What effect this may have is still an open question. It is further difficult to explain the occurrence of caloric nystagmus in subjects with ablation of the canals. The theory put forth by Van Caneghem (ref. 7) is also difficult to dismiss, since it would seem that a change in the intra-

labyrinthine pressure would take place regardless of acceleration level.

The position taken by Brunner (ref. 6) (that of central origin) seems unlikely, since such a response would not seem to have a causal relationship with gravity changes. Gernandt, Igarashi, and Ades (ref. 11), moreover, have demonstrated in the squirrel monkey that very prolonged irrigation with ice water is required to evoke nystagmus which is of central origin.

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DISCUSSION

DOLOWITZ: When Bárány first reported the caloric test, he mentioned three things: nystagmus, pastpointing, and vertigo. Obviously, one couldn't do much with pastpointing in a situation such as Captain Kellogg described, but does he have anything to tell us about vertigo; what happens in that parameter?

KELLOGG: There was a certain amount of vertigo in the response. When the ice water was injected and when the subjects began to get the rapid caloric reaction, this was stronger in the airplane than it was on the ground. They got it very clearly, shifting the whole reference frame. They felt the room was moving back and forth. This was fairly constant in all the eight subjects; so there was definitely some vertigo present.

DOLOWITZ: Was it affected by the change in g?

KELLOGG: Yes. It was affected by the change in g in that when there was a higher level, the tendency for the vertigo was higher; they would feel the response more strongly. When we got to zero g, without fail things stabilized out. In other words, as you watched the eye, you would see the mode of movement going before entry into zero g. And as soon as zero g started, the eye would immediately stabilize; so would their reference frame. They would feel very, very secure and fixed when they got to zero g in comparison to the other phases of it.

YOUNG: Is the effect of a given caloric stimulus strictly proportional to gravity, or acceleration?

KELLOGG: You mean linearly related?

YOUNG: That is my question.

KELLOGG: I am not sure about that. I would have to look at it a little more carefully before I said that. But it certainly seems to be increasing with increasing g. How much, I am not prepared to say.

YOUNG: A related question is, do you know of any experiments showing a linear relation between the caloric stimulation temperature and the strength of the response; and finally, does anyone know of measurements on the thermal coefficient of expansion of endolymph?

MONEY: I don't really have any information and I don't think any work has been done on the thermal coefficient expansion of endolymph; however, when we were extrapolating our results of the specific gravity measurements with pigeon endolymph, from 23° C where we measured it to pigeon body temperature, we checked the behavior of pure water and sea water. As far as the 1-part-in-10 000 level is concerned, both of these substances changed the same way; so that it is

probably within the range of accuracy of most experiments to assume that this is so.

LOWENSTEIN: This remark isn't going to be very helpful, because it is just a rationalization of what we heard. I was very interested that the line in fact intersects zero point at zero gravity and I dare say the other nystagmic curves which didn't do so might easily be bent to produce this result. I just wonder in this connection whether the tonic influence of the otolithic organs is not a setting device in the absence of which nystagmus just cannot take place centrally, cannot be elicited centrally. That is to say, the question of missing nystagmus again merely refers the biologist to the fact that these organs were designed for a certain range of physiological conditions. One of them is that the nystagmus and the direction of the nystagmus are in fact perhaps governed by the static inflow from the statoliths. If this static inflow is absent, there is no, may I be teleological for a moment, there is no sense in a nystagmus because its direction can't be properly adjusted. This direction adjustment of nystagmus is extremely delicate with the tilt of the head, as everybody knows, with a vectorial switch between the eye muscles, et cetera. In the absence of static information, this is useless. There is no nystagmus. Wouldn't it also fit in extremely well with the wonderful performance of our space pilots at zero g, with the exception of some of their Russian colleagues?

BERGSTEDT: Jongkees has mentioned earlier that there are perhaps two types of sensations: rotation sensation and tilting sensation. You can thus divide the sensation from calorization into two components. I have tried to ask experimental subjects about these two things without biasing their answers, but couldn't get any really sure results. It was difficult for them to interpret. Jongkees has described positional nystagmus after calorization which can't be related to the temperature. I wonder if Captain Kellogg and Captain Graybiel could check this with their equipment. Could they do just caloric tests, wait a certain amount of time, and allow the peak of the weightlessness, the positive or negative g, to occur just when this nystagmus could be expected. Positional nystagmus after calorization could really be checked with this type of experimental apparatus.

Examination of a Possible Flight Experiment To Evaluate an Onboard Centrifuge as a Therapeutic Device

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INTRODUCTION

The effects of weightlessness on man's physiology during extended space missions, such as flights to Mars, are critical to man's effectiveness in performing such missions and returning safely. The recent flights of Gemini for 8 days and 14 days have extended our knowledge of weightlessness to these periods and indicate that lunar missions and possibly Manned Orbital Laboratory missions of 30 days can be performed by man (ref. 1). Mars missions, however, will extend the time in space by 50-fold to 100-fold over the Gemini flights.

In a recent joint meeting of representative members of the American Institute of Aeronautics & Astronautics and the Aerospace Medical Association in St. Louis, consideration was given to the problem of weightlessness for extended missions and means of protecting against any degradation that may occur. Although the results of this meeting are at this writing not available, the sense of the discussions was that debilitation and reduced performance possibly could occur and that measures should be taken to examine methods to prevent or rehabilitate from such effects in case they do occur. Many methods ranging from supplying artificial gravity by total vehicle rotation, to onboard centrifuges and to pressure cuffs and exercise are suggested to accomplish the desired effects. None, of course, is as yet totally proven, particularly for very long periods. Because of the potential debilitation and because, as yet, no clear and simple means for the prevention of debilitation exists, it behooves NASA to proceed with studies on this problem so as to be prepared for long missions. From the engineering standpoint, consideration must be given to all probable measures to cope with the problem so that spacecraft can be rotated or can adequately house a centrifuge if necessary.

The onboard centrifuge is simpler and cheaper than the provision of artificial gravity by total vehicle rotation. Although a centrifuge is considerably more complex than the limb cuffs that have been tried in space, the possibility that the proper stimulus or challenge can be given to human physiology by a centrifuge is great. The pioneering work of the Douglas Aircraft Co. (ref. 2) on the possibilities of the onboard centrifuge has indicated this potential through ground-based tests using bed rest to simulate weightlessness.

This paper examines the various factors, including some general engineering factors, involved in performing a flight experiment with an onboard centrifuge. Such an experiment would substantiate or refute the favorable potential of the centrifuge established by ground-based tests in preventing the possible debilitation of weightlessness in extended space missions.

SOME PHYSIOLOGICAL EFFECTS OF WEIGHTLESSNESS

Gemini V and VII flights, as previously noted, have extended man's experience in weightlessness to 8 and 14 days, respectively. As of this writing, no written reports of the Gemini VII flight are available. Those of Gemini V are available in reference 1. Some of the physiological effects found from Gemini V and presented in reference 1 are summarized herein.

The results indicate the adaptability of the crew, first to weightlessness and then to normal gravity on return. There were, however, some changes that appear to have direct application to the extended mission. These particularly involved the cardiovascular system. There was a 20-percent reduction of red-cell mass and a 4- to 8-percent reduction in plasma volume as measured after the flight relative to preflight values. One question of concern is, of course, whether these are stabilized values or whether continuing reductions are to be expected for longer exposures. The red blood cell changes may be attributed to the atmospheric environment and not to weightlessness. Mild fatigue following the flight was also reported in reference 1. Results of Gemini VII will give additional information on these factors.

Another factor from the postflight examinations of the Gemini V crew was their response to tilt-table tests. The results of these tests, from reference 1, are summarized in figures 1 to 6. The values of blood pressure and heart rate of reference 1 during the tilt test have been averaged over the tilt period for the results presented herein. The preflight values presented are the averages of three experiences of three different days prior to the flight. The results for the command pilot (Cooper) are on figures 1, 2, and 5, and for the pilot (Conrad) on 3, 4, and 6. Figures 1 and 3 are of the average heart rate and the pulse pressure and show a marked increase in heart rate, and a marked reduction in pulse pressure while tilted immediately after the flight as compared to the values prior to the flight. Figures 2 and 4 indicate the pulse-pressure reduction is primarily due to a larger reduction in the systolic pressure than the diastolic pressure. For both pilots, recovery to nearly preflight values occurred in about 1 day.

Figures 5 and 6 are ratios of the values during tilt to those just prior to tilt. Preflight ratios are also shown. The command pilot shows a more regular return to preflight values after the flight than does the pilot. These results show that the ratio of the pulse pressure when tilted to the pretilt value does not return

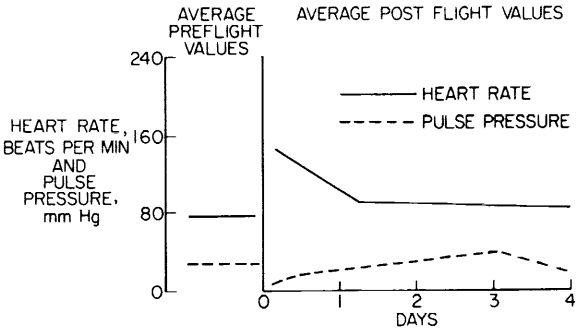

Figure 1.—*Gemini V average heart rate and pulse pressure during tilt-table tests.*

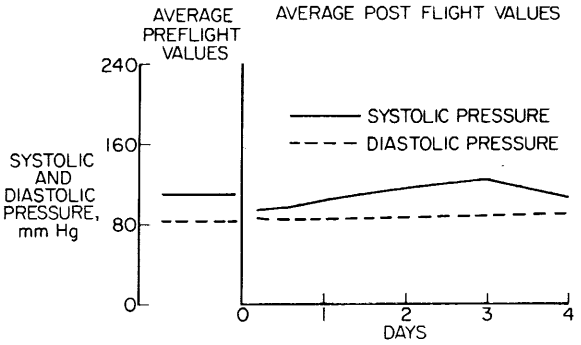

Figure 2.—*Gemini V average blood pressures during tilt-table tests.*

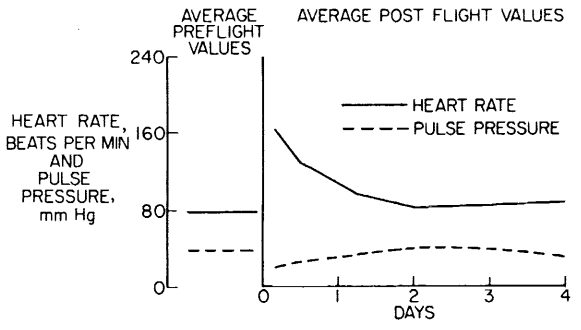

Figure 3.—*Gemini V average heart rate and pulse pressure during tilt-table tests.*

to the preflight value until more than 2 days following the flight. This result, with those of figures 2 and 4, implies an effect of the flight on the pretilt or normal pulse pressure and that recovery to normal may have taken more than 2 days.

These results indicate generally no serious changes from the flight and reasonably rapid recovery. The question remaining is: Are these changes in a transient phase or are they stable at the changes that were indicated? Further, if they were stable at the values shown for 8 days, will much longer periods cause additional changes?

It should be pointed out that the effects of bed rest, which has been extensively used to simulate weightlessness, are similar to the effects just discussed. These effects are decreases in plasma and blood volume, demineralization, and losses in tilt-table tolerance through increased heart rate, decreased pulse pressure, and syncope (refs. 1 to 6).

GROUND-BASED CENTRIFUGE TESTS

The Douglas Aircraft Co. under contract to the Air Force and through independent research has done considerable ground-based study of the potential of short-radius centrifuges in preventing debilitation due to weightlessness (refs. 2, 7, and 8). The sense of these studies was to examine the influence of riding a centrifuge on man's physiology as it relates to any degradation that may occur due to bed rest. As noted previously, during bed rest degradation of the same nature as is possible from weightlessness does occur.

Because of the confinement of space vehicles, spaceborne centrifuges will have, of necessity, fairly short radii. The consequences of short-radii centrifuges are the relatively high rotational rates necessary to obtain a specific g level and the relatively high gradient of force along the subject's body. Reference 8 contains an analysis of the acceleration gradient problem. Douglas (ref. 8) examined gradients of force along the body of 20 to 219 percent, using various radii. Discomfort in the legs was experienced at the shorter radii; but, in general, the tolerance to blackout was not appreciably differ-

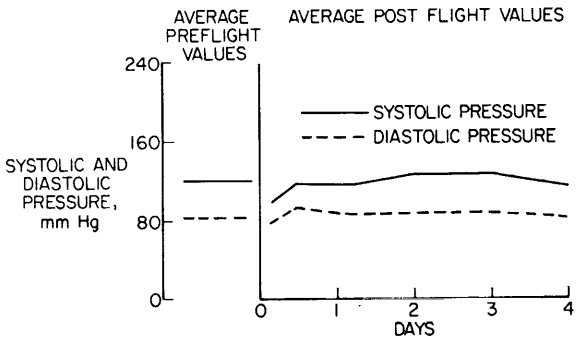

Figure 4.—*Gemini V average blood pressures during tilt-table tests.*

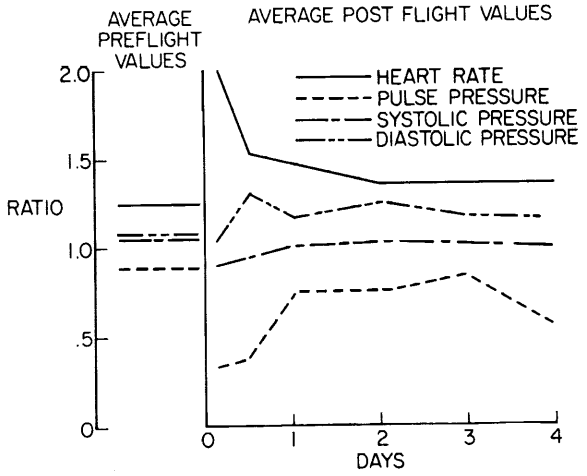

Figure 5.—*Gemini V ratios of cardiovascular factors during tilt-table tests to pretilt values.*

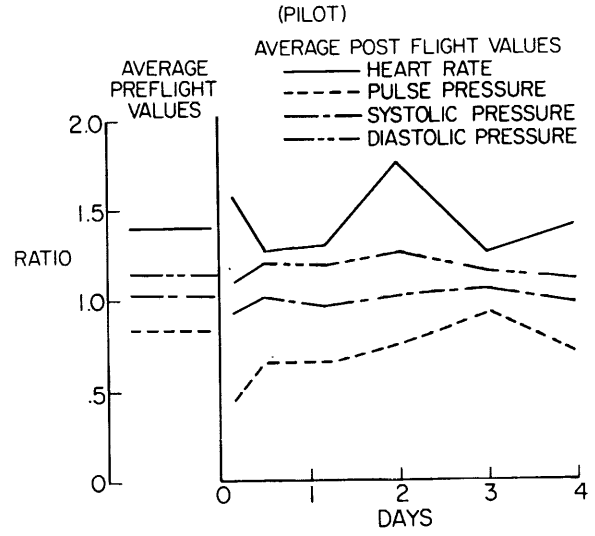

Figure 6.—*Gemini V ratios of cardiovascular factors during tilt-table tests to pretilt values.*

ent for the various radii, although somewhat less for shorter radii.

The studies of references 2 and 8 were made by Douglas to find directly the therapeutic and prophylactic value of short-radius centrifuges. The centrifuge used is shown in figure 7 (taken from ref. 2). This centrifuge had a 54-inch radius at the subject's feet. Two kinds of experiments were performed as reported in reference 2: one to determine the therapeutic value of the centrifuge by having subjects ride after having been degraded by bed rest, and another to determine the prophylactic or maintenance value of the centrifuge by having subjects ride during the entire period of bed rest.

The subjects of reference 2 were exposed to centrifugation for from ½ to 3-g-hours (the product of the g at the feet and the time in hours of exposure). This was accomplished by four exposures a day of 7.5 or 11.2 minutes per exposure. The results clearly indicated both a therapeutic and a prophylactic value of such exposures. During tilt-table experiments the narrowing of pulse pressures and elevation of heart rates were lessened in subjects using the centrifuge as a prophylactic device than those for controls. For these subjects, syncope did not occur as it did generally for controls. Subjects using the centrifuge as a therapeutic device had improved responses to tilt after several days of bed rest. Syncope was generally prevented after a few days' exposure on the centrifuge. Although there were generally losses in weight, blood, and plasma volume, these were less for the subjects using the centrifuge as a prophylactic device.

Samplings from some of the bed-rest studies, including those where a centrifuge was used, of the subjects' responses to tilt-table tests are compared with those of the Gemini V flight (ref. 1) in figures 8 and 9. Because of variability in the subjects, in their experience, and in the techniques, this comparison is only of tentative value. The values shown are averages for the period of tilt and are an average of the values for all subjects of each specific experiment. Syncope occurred with several of the subjects of the bed-rest studies during tilt, and although the average values of their response up to the time of syncope are included in the values shown, the imminence of syncope cannot be reflected in figures 8 and 9. Figure 8 shows the average heart rates during tilt and figure 9 shows the related pulse pressures. The data for the bed-rest experiments were taken from references 2 and 3; however, data from all experiments in these references were not used. The results in figure 8 show that in bed rest, heart rates during tilt increase but not to the magnitudes experienced after space flight. A fundamental result is that for all bed-rest experiments, except the 3-day exposure (ref. 3) and those for which the centrifuge was used as a prophylactic device (ref. 2), syncope was experienced. No syncope was experienced for the experiment where a centrifuge was used as a prophylactic

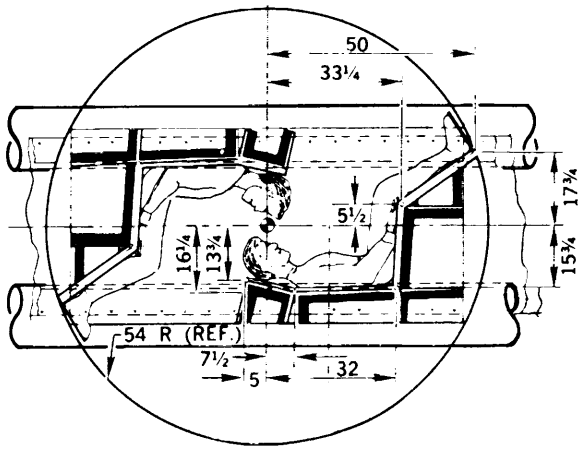

Figure 7.—*Douglas short-radius centrifuge.*

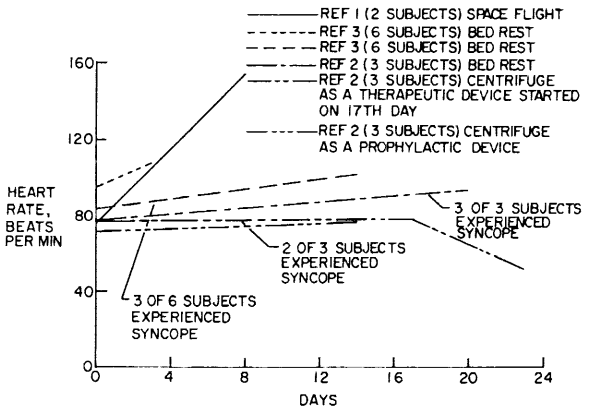

Figure 8.—*Heart rate during tilt-table tests for various experiments.*

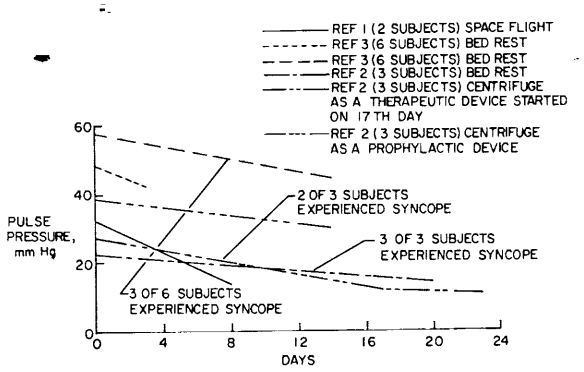

Figure 9.—*Pulse pressure during tilt-table tests for various experiments.*

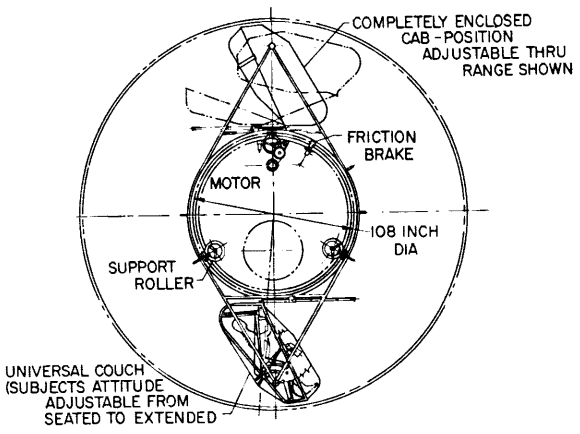

Figure 10.—*MORL internal centrifuge.*

device (ref. 2). Following 17 days of bed rest at which time syncope occurred in two of three subjects (ref. 2), the centrifuge was used as a therapeutic device. After 6 days of exposure to the centrifuge, while still in bed rest, no syncope occurred. The conclusion of reference 2 is that the centrifuge prevented and reversed the trend to syncope. The pulse pressures (fig. 9) show decreased values with exposure to bed rest and flight. The use of the centrifuge as a therapeutic device checked further decreases in this factor.

Many other factors, including bone density, blood and urine analysis, acceleration tolerance, exercise tolerance, etc., have been examined in bed-rest and flight experiments and should be correlated as a continuing examination of these problems.

The results of this Douglas and Air Force-sponsored research have indicated that the onboard centrifuge is probably useful as either a prophylactic or therapeutic device to prevent or retard the possible effects of weightlessness. These results encourage continued effort to study the onboard centrifuge, particularly in actual space flight.

ENGINEERING CONSIDERATION OF AN ONBOARD CENTRIFUGE

Studies sponsored by the Langley Research Center of the Manned Orbital Research Laboratory (MORL) led to the incorporation of an onboard centrifuge as a basic element of this proposed spacecraft (ref. 9). This centrifuge is designed to take maximum advantage of the 22-foot diameter of the MORL and is installed between the ceiling of the operations areas and the floor of the living area. The centrifuge assembly as shown in figure 10 is comprised of two completely enclosed cabs mounted 180° apart on a welded aluminum tubular truss. This truss design includes a 108-inch-diameter drive ring. The hub area is designed as a passageway between the living and operation areas and thereby prevents operation of the centrifuge from affecting passage from one compartment to the other.

A 1-horsepower electric motor with a solid-state motor control is proposed to drive the MORL centrifuge through the central ring. Centrifuge braking would use the dynamic braking effect of the motor. A mechanical brake would also be provided in case of a malfunction of the primary drive mechanism.

Each cab would have one universal couch which is adjustable to accommodate individual crewmembers and would be designed to take the loads of simulated reentry operation. The couch would be made adjustable to allow the subject to sit or lie down, and be adjusted to change the orientation of the acceleration vector applied to the subject.

The cab would be completely enclosed to eliminate the visual cues of motion. The centrifuge could be stopped at any time either by the subject or his monitor. Some of the characteristics of this centrifuge are listed in table 1. The diameter at the subject's center of gravity is 7 feet, whereas that at his feet can be 9

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Table 1.—*Performance and Design Parameters for 2 Short-Arm Centrifuges*

	MORL	Apollo application
Dry weight	172 lbs	317 lbs.
Spin rate at 1 g at c.g. of subject.	20.5 rpm	21.2 rpm.
Momentum transferred per event.	2940 lb-ft-sec	2580 lb-ft-sec.
Inertia about spin axis (I_z), centrifuge loaded.	1370 slug-ft^2	1160 slug-ft^2.
Spacecraft roll rate if torque is not counteracted.	0.1 rpm	0.8 rpm.
Propellant expended per event.	None	3 lbs (at 270 lb-sec per lb).
Average radius to c.g. of subject.	7 ft	6.5 ft.

feet. It is planned that the momentum of the rotating centrifuge be balanced by that of control-moment gyros on the MORL, thus involving little or no expenditure of energy for this purpose.

The purpose of this centrifuge is primarily therapeutic or prophylactic. A pattern of centrifuge exposure for the astronauts as previously discussed herein and reported in reference 2, that is, exposures of at least 7.5 to 11 minutes, four times a day, would be required for this purpose.

Because it is important to understand and verify the influence of an onboard centrifuge on human physiology and because a centrifuge in a space vehicle has a significant effect on vehicle design and payload, careful study must be made of the total design of a centrifuge installation if such installation is desirable. The MORL is, of course, only a study vehicle. Actual experience and studies with a centrifuge at an early date therefore involve the application or adaptation of a centrifuge to existing vehicle systems. The most practical of such an application would be on Apollo-LEM vehicles. Figure 11 is a sketch of such an application. The centrifuge fits below the LEM within the LEM adapter envelope. The figure depicts the system as it would be in a launch configuration. It is assumed that a normal Apollo-LEM transposition maneuver would be made after launch, placing the LEM with the centrifuge in front of the Apollo for orbital applications. Access to the centrifuge would be from the LEM, which would be used as a monitoring station for the centrifuge studies. Figure 12 is a sketch of the basic Apollo-LEM vehicle as it would appear in flight. The centrifuge as shown in figure 11 would be located below the LEM where the LEM lunar landing module and legs are shown. Figure 13 is a sketch of the Apollo-LEM on the Saturn 1B launch vehicle. This is the approximate configuration of the launch system as it may be used for the centrifuge experiment herein discussed.

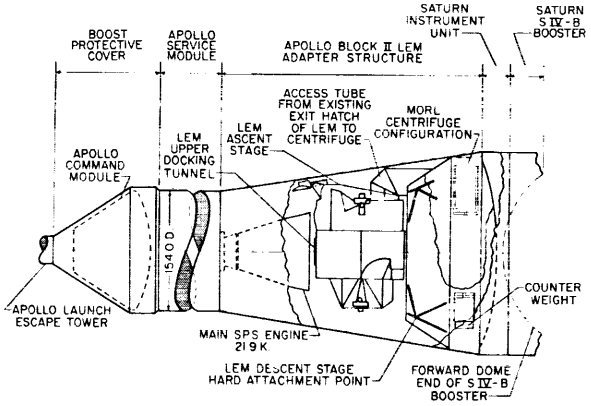

Figure 11.—*Possible centrifuge on the Apollo LEM vehicle.*

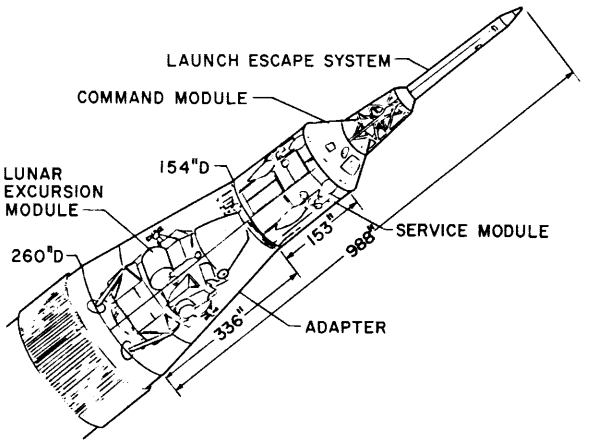

Figure 12.—*Apollo spacecraft.*

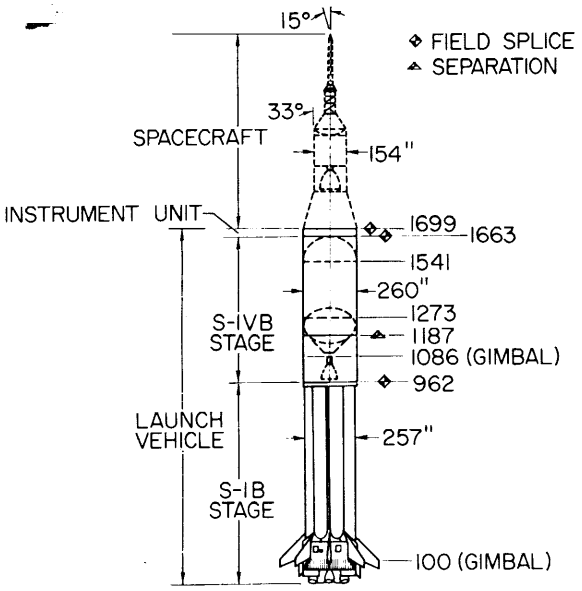

Figure 13.—*Saturn IB launch-vehicle configuration.*

This centrifuge design concept is similar to that of the MORL centrifuge but is designed for only one subject, requiring a counterbalance as shown in figure 11. Numerous other configurations are possible; the one shown is used, however, to evaluate some of the design parameters which would not vary appreciably for other designs. The performance and design parameters for this centrifuge are presented in table 1, along with those of the MORL centrifuge. This centrifuge is nearly as large as the MORL centrifuge and therefore has characteristics similar to it. The weight and inertias refer only to the centrifuge and not to the supporting or containing structures. The weight of the one-man centrifuge is greater than that of the two-man MORL centrifuge because the counterweight must be charged to the centrifuge; whereas, in the two-man case, the counterweight is largely made up by the second test subject.

The times expected for Apollo flights are appreciably less than those considered for MORL, and control-moment gyros are not now a part of the Apollo control system. The use of fuel expenditure to balance the angular momentum of the centrifuge as applied to Apollo therefore may be required. As noted in table 1, about 3 pounds of fuel per centrifuge event would be required. If one astronaut were exposed four times a day for 45 days, 540 pounds of fuel would be required. The Apollo-LEM vehicle, however, could be allowed to counterrotate to the centrifuge at 0.8 rpm without any fuel expenditure if such rotation did not interfere with other experiments or the other occupants of the vehicle.

The centrifuge, as depicted in figure 11, is contained in a separate pressurized chamber and is connected to the LEM by an appropriate pressurized passage. This would be the most complex arrangement for application to the Apollo-LEM. Pressure-suited astronauts riding in an unchambered, unpressurized centrifuge would be the least complex arrangement.

The safety of the astronaut riding the centrifuge is, of course, of prime concern. In an onboard centrifuge experiment, the possible dangers, both physiological and psychological, of the centrifuge are superimposed on the dangers normally imposed by space flight. One must consider fundamentally the recovery of an incapacitated astronaut from the centrifuge. A shirt-sleeved astronaut in a centrifuge within the confines of the basic vehicle as in the MORL would seem most ideal. The next apparently most desirable situation would be a shirt-sleeved astronaut in a centrifuge within a separate pressurized chamber with a clear passageway to the basic vehicle, such as is shown in figure 11. The least desirable situation would be a pressure-suited astronaut in an open centrifuge requiring tethered transfer through an airlock into the basic vehicle. Clearly, careful studies of the various problems involved, including the weight and power requirements and the logistics problem, must be made before a positive conclusion and decision is made on the actual configuration of a centrifuge experiment.

AN EXPERIMENTAL PROGRAM FOR AN ONBOARD CENTRIFUGE

Prevention of, or Recovery From, Degradation That May Occur From Weightlessness

The fundamental purpose of the onboard centrifuge experiments is, of course, to evaluate the prophylactic and the therapeutic value of

exposure to centrifugal force as a means of preventing debilitation from weightlessness for extended missions. The length of exposure is very difficult to determine. The level of gravitational force that would prevent degradation for normal full-time exposure as with continuous artificial gravity by total vehicle rotation is not known. It has been indicated that values of artificial gravity as little as ¼ g or ½ g would suffice. Others feel that at least ¾ g would be required. In reference 2, the exposure of subjects on the Douglas short-radius centrifuge was based on the product of the g's at the feet and the time on the centrifuge. Values to 3 g-hours were used. In normal Earth g, depending on man's activity, the g-hours at the feet may range from 10 to 18. Because some changes did occur during the centrifuge exposures reported in reference 2, it is possible that more than 3 g-hours at the feet are desirable. Further, because of the large gradient of acceleration along the body in the short-radius centrifuge, it is not clear that the value of force at the feet is necessarily the proper one for consideration. The larger radius of the centrifuge described in this paper than in that of reference 2 causes a gradient of only 40 percent from heart to foot rather than the 200 percent of reference 2.

This gradient of centrifugal force along the body as noted causes concern as to the desired value of acceleration and exposure time on the centrifuge to stress the physiological system as in 1 Earth g. The accelerations and pressures on a centrifuge, of a column of fluid of length equal to the height of a seated man and of a density equal to water, are shown in figure 14. Two sizes of centrifuges have been considered: a 5- and a 9-foot radius. For figure 14 the accelerations at the foot of the column were 1 g. The gradient of acceleration along the column is, of course, less and the pressures greater on the longer radius centrifuge than the shorter one. The mean pressure on the long centrifuge is 0.65 lb/in ^{2}, whereas that on the short centrifuge is 0.39 lb/in ^{2}. These mean pressures occur about 1 foot below heart level on the column. The mean pressure of the same column in Earth g is about 1.0 lb/in ^{2} and occurs

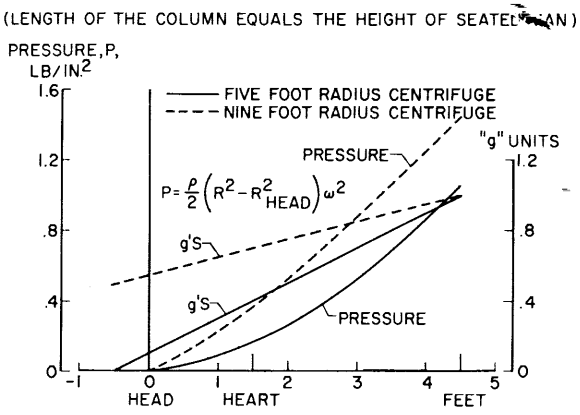

Figure 14.—*Pressure in a column of fluid on centrifuges.*

closer to the heart (about 9 inches below the heart). The pressure at the foot of this column on Earth would be about 2 lb/in ^{2}. If the cardiovascular system is affected by pressure variations similar to those discussed, a centrifuge with 1 g at the feet stresses the system much less than 1 Earth g. Physiologically it is not evident whether the mean or maximum pressure is significant, although the mean pressure is more indicative of the total workload imposed on the total system generally. To attain the same mean pressure on the centrifuges as in Earth g, accelerations at the feet of about 2.6 g and 1.5 g would be required on the 5- and 9-foot-radius centrifuges, respectively. Such factors must be considered in selecting centrifuge exposure times.

Ground-based studies with a centrifuge of the radius of the proposed centrifuge of this paper and with mean pressure hour exposures ranging up to 8 or 10 should be performed in a manner such as those performed in reference 2 in order to define the proposed flight experiment adequately. The flight experiment should then be based on such ground-based studies, probably with the exposure divided in four equal portions and confined to a 12-hour period of the day. In a 45-day orbital mission, one astronaut could use the centrifuge, beginning at the first day for the entire mission, as a prophylactic device. Another astronaut could start using the centrifuge after 30 days' exposure to weightlessness for the remaining 15 days as a thera-

peutic device. Clearly, more than one flight is desirable to attain sufficient data to verify the results and lend credence to ground-based studies.

Study of Threshold Levels of the Otolith Organs

The otolith organs are sensors which are definitely affected by weightlessness. On Earth these sensors are always stimulated by gravity regardless of the body orientation. The lack of stimulation of these sensors, as occurs in weightlessness, may cause a change in their sensitivity and thresholds of response. The threshold of response of the otoliths on Earth has been measured to be between 0.0003 and 0.01 g. In order to study the otolith response, centrifugal-force values of these magnitudes would be required at the vestibular system. In the type of centrifuge depicted in figure 11, the vestibular system would be about 5 feet from the center of rotation, and g's equal to about five times those listed on table 2 in the column of g-units per foot of radius would be experienced. At one-fourth rpm a value of 0.0001 g, well below the minimum threshold of 0.0003 g, would be experienced at the vestibular system. Very low rotational speeds with fine control would be required, however, and the threshold would be encountered as the speed of the centrifuge was increasing. Another way to experience threshold values of acceleration would require a somewhat different centrifuge wherein the subject could be placed at the center of rotation and moved from it with a constant rate of rotation of the centrifuge. The two columns in table 2 labeled "0.0003" and "0.01" centrifugal force levels indicate that rates of rotation of 1 to 30 rpm could be used for such studies with movement of the vestibular system from the center of rotation of about one-half inch to more than 7 feet. This method of operation may give better control and thus lead to more valid results than on a fixed-radius centrifuge. The complexity and weight of such a centrifuge would be greater than the fixed-radius type. Ground-based experiments evaluating both such methods should be performed before the final configuration of a centrifuge is selected, provided of course such experiments are desired.

Studies of the Responses From Semicircular-Canal Stimulation on an Onboard Centrifuge

Extensive studies have been made of the effects of semicircular-canal stimulation on ro-

Table 2.—*Centrifuge Characteristics for Research on the Vestibular System*

Centrifuge rotational rate		g-units per ft of radius	Centrifugal force levels, g-units						
rpm	rad/sec		0.0003	0.01	0.5	1	4	6	8
			Radius, feet						
0.25	0.0262	0.000021	14.0	---	---	---	---	---	---
.50	.0524	.000085	[b] 3.52	---	---	---	---	---	---
[a] 1.00	[a] .1047	[a] .000341	[b] .88	29.4	---	---	---	---	---
[a] 2.00	[a] .2093	[a] .00136	[b] .22	[b] 7.35	---	---	---	---	---
[a] 5.00	[a] .5235	[a] .0085	[b] .035	[b] 1.175	---	---	---	---	---
[a] 10.00	[a] 1.0466	[a] .0341	.0088	[b] .294	14.7	29.4	---	---	---
[a] 20.00	[a] 2.0933	[a] .136	---	[b] .074	3.675	7.35	29.5	---	---
30.00	3.1400	.306	---	[b] .033	1.635	3.27	13.1	19.62	---
40.00	4.1867	.545	---	.018	.915	1.83	[c] 7.32	[c] 10.98	14.64
50.00	5.2333	.850	---	---	.5875	1.18	[c] 4.70	[c] 7.05	[c] 9.40

[a] Ranges for semicircular canal studies.
[b] Ranges for otolith threshold studies.
[c] Ranges for reentry simulation studies.

tating vehicles. References 11 to 13 are but a few studies of this type. Studies to corroborate these results and verify man's response and sensitivity to rotation in flight are desirable. Rates of rotation from 1 to 20 rpm could be used for such studies as is indicated on table 2. The g forces at the subject's feet would range up to somewhat greater than 1 g. In such tests, nystagmus, eye counterrolling, and the astronaut's performance should be measured. In addition, as is done in the procedure of reference 13, the head positions and motion should also be measured. The additions to the centrifuge required for these experiments involve only the instrumentation suggested.

Reentry Simulation on an Onboard Centrifuge

The loss of proficiency in performing vehicle maneuvers such as reentry is recognized as a potential danger in extended space missions. In flights to date, the astronauts have practiced such maneuvers prior to flight, including reentries on a centrifuge, so as to be as proficient as possible at the time of the launch. For extended missions of several weeks or months, the desirability of maintaining proficiency during the flight is evident. The onboard centrifuge provides a simulator which permits an individual to actually experience some of the critical aspects of the reentry.

Figure 15 shows the accelerations encountered during Gemini V reentry. Included in figure 15 are the rates of rotation that would be required on the centrifuge shown in figure 11 in order to simulate this reentry. A maximum rate of rotation of 45.5 rpm is required. The maximum torque requirements of 50 foot-pounds for this simulation, also shown in figure 15, are within the capabilities of the 1-hp motor proposed for the subject centrifuge. Table 2 also shows ranges of radii and rates of rotation for the simulation of reentry. This information suggests that a variable-radius centrifuge, as previously discussed for the study of otolith response, may also be used for reentry simulation. An examination of these two kinds of centrifuges and possible ground-based studies with them seems desirable to determine which can lead to the best simulation. The variable-radius centrifuge is capable of reentry simulation with less variation in the centrifuge rate of rotation than is the fixed-radius centrifuge. The attendant disturbing effects of rotation may be minimized by this process, although Coriolis forces would be encountered and must be considered on the variable-radius centrifuge.

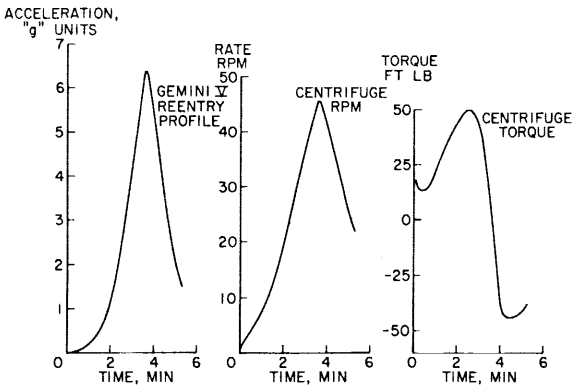

Figure 15.—*Centrifuge response for reentry simulation.*

Simulation with either centrifuge would require a computer with a control system, and display on the centrifuge. These requirements must be carefully examined and the weight and power requirements minimized during ground-based studies.

CONCLUDING REMARKS

The problem of the potential degradation of man in weightlessness for extended missions lasting for several months is not yet fully resolved. Recent Gemini flights have cast new light on the subject, but some projected missions, many times longer than have been flown, are of concern. Further, the astronauts of these Gemini flights have not been devoid of physiological changes when they returned to Earth. Some of these changes may not be attributable to weightlessness. Whether the changes encountered will stabilize at some new equilibrium such as that attained in Gemini and whether equilibrium has not as yet been reached by flights to date constitute unresolved factors of extended missions.

Technical preparations to provide prophylactic or therapeutic means of preventing any physiological degradation must be made if future flights prove it necessary.

Ground-based studies by Douglas with Air Force sponsorship have shown the onboard centrifuge to be capable of attenuating some of the expected effects of weightlessness as simulated by bed rest. Therefore, a flight experiment using an onboard centrifuge is proposed to substantiate the considered value of an onboard centrifuge both for therapeutic and prophylactic purposes.

The onboard centrifuge also provides a means of examining the vestibular system in flight and while being exposed to weightlessness. The sensitivity and thresholds of the otoliths may be examined and responses to stimulation of the semicircular canals can be evaluated and compared with those of ground-based studies.

As on the ground, the centrifuge in flight supplies a means of simulating space-flight maneuvers, particularly reentry. For the onboard centrifuge experiment, its use for reentry simulation with the intent of maintaining proficiency in the maneuver is also suggested.

The onboard centrifuge presents an experimental facility of multiple purpose. The experiments that can be performed are vital to future manned space missions and they are timely.

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DISCUSSION

NEWSOM: When we use a centrifuge of this type, we are really doing at least two things physiologically. One, we want to maintain what I will call the total orthostatic reflex; that is, when we push the blood down to the feet, we hope to get the vascular system to push it back up to the head. This might take only a short period of time on the centrifuge because reflexes are usually reinforced with rather small time

periods. The second, which is much more time consuming, is an attempt to maintain the body water. As Dr. Berry has pointed out, the diuresis that occurs, both at bed rest and at zero g, does change the fluid component. The internal centrifuge or a centrifuge concept might very well be used in combination with the lower body negative pressure or some of the drugs to maintain fluid, and then to be able to reduce the amount of time necessary on the centrifuge to a fraction of what is now being proposed.

BERGSTEDT: I presume there is room enough for head movements. In the picture you showed the head is quite close to the axis. The stimulus would be mainly the Coriolis acceleration. But will there be room enough for bending of the head and upper part of the body?

STONE: The picture you saw was a very first cut of the kind of centrifuge that would be used. Actually this would be designed to have sufficient room for any necessary positions to be studied and certainly for any instrumentation required to make the necessary measurements. This is a design problem we haven't really looked at.

JONES: Mr. Stone's proposal represents a new type of research tool—one in space, utilizing a new parameter, weightlessness. This device is receiving favorable consideration. I would urge all researchers who might have a use or who can foresee an experiment on a space centrifuge to come forward with their requirements for consideration in definitive stage of development.

Needs, Plans, and Identification of the Pathologic Effects of Prolonged Exposure to Weightlessness on the Vestibular Organs

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AND
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SUMMARY

There is immediate need for information on the effect of varied g-forces on the vestibular mechanism. While we do not know that damage to, or changes in the vestibular system will occur as a consequence of prolonged orbital flight, we do know that we must anticipate the possibility of such damage and do everything possible to prevent it. The present paper deals with methods and possibilities of evaluating disintegration of structural components inside the vestibular system. Many problems encountered are paralleled by those found in the evaluation of cochlear damage after exposure to noise or toxic agents. For that purpose we have developed special methods for analysis of the structural pattern and of the ultrastructure of the cochlear sensory epithelium. We recommend that a similar structural study be undertaken immediately on the vestibular epithelium. Substantial efforts must also be devoted to the problem of vascular supply in the vestibular labyrinth. No more than 3 years are available for this study, and it must, therefore, be pressed without delay.

INTRODUCTION

Our knowledge of the structure of vestibular sensory epithelia has been greatly advanced in recent years. At the present workshop, and at a similar meeting in Pensacola last year, detailed accounts of our present state of knowledge have been given and many new details added. Our knowledge of vestibular dimensions in man and squirrel monkey has been improved by the study of Beck and Bader (ref. 1) and by a preceding report by Makoto Igarashi. These studies have given us a wealth of information on ultrastructural details. The work of Lowenstein and Wersäll (ref. 2), Engström et al. (ref. 3), Flock (ref. 4), and Spoendlin (ref. 5) have shown that vestibular sensory cells display a distinct structural polarization. It has also been shown that this structural orientation is paralleled by a functional organization of primary importance, giving new impetus to the study of the total dynamic structural pattern inside the vestibular sensory epithelia.

We know that there are two separate forms of sensory cells (types I and II) in all mammalian vestibular epithelia, but we do not yet know their exact spatial interrelations. In the course of our analysis of the structural pattern of the organ of Corti, we have shown that the sensory cells of that structure are arranged in

a distinctive geometrical pattern. We have also shown that this pattern can be used for rapid and easily reproducible assessment of cochlear damage. Additional studies have elicited further evidence that an organized structural pattern may also be found in the vestibular sensory regions.

An important aspect of future studies in this field must be a systematic mapping of the two types of sensory cells relative to each other and to the sensory organ as a whole. Such mapping will include not only the presence of type I or type II cells but also systematic notation of the structural polarization of each (figs. 1–8). In addition, it is essential to carry out a concomitant analysis of neural components of the vestibular sensory regions. Experience with the cochlear analysis makes it clear that a combination of light- and electron-microscope methods will constitute the best approach to the vestibular analysis. Studies by Gacek (ref. 6) and others have shown that the total number of nerve fibers to the vestibular regions in the cat is about 12 000, of which only about 200 are efferent (centrifugal) fibers. Inasmuch as nerve endings of efferent type (type 2 endings) are widely distributed in the vestibular epithelium, contacting practically all the sensory cells, the distribution of both afferent and efferent neural elements in this region becomes a major interest in evaluation of every kind of functional damage. Such an analysis is a tedious and time-consuming affair but of utmost importance, from both a physiological and pathological point of view. That study is now being carried out in our laboratories.

There are many reasons to believe that moderate g forces will not severely damage the vestibular epithelia. For the planned orbital flight, available advance information indicates that forces above 10 g should not be expected; how-

Figure 1.—*General view of the macula utriculi seen from below with nerve fibers and one ampulla with its crista.*

Figure 2.—*Macula utriculi* (MU) *and macula sacculi* (MS). *This figure shows the relative positions of the two maculae in the same specimen.*

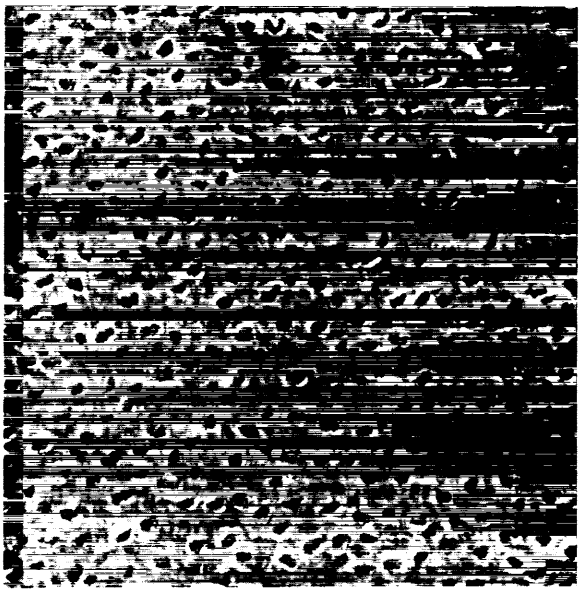

Figure 3.—*Surface view of the macula. It is feasible to count every sensory cell in such a specimen. The darker dots represent the hair tufts of the sensory cells.*

Figure 4.—*Higher magnification of corresponding area (compared with fig. 3) from another macula. It is possible to count the cell number per unit of surface area and to compare the populations of normal and damaged sensory regions. Structural polarization of the cells can also be discerned.*

ever, it has not yet been shown that minor damage or functional disturbance may not occur, and the damage-risk threshold for g forces is unknown. Likewise, it is not known where such damage may occur, nor what its physiological significance might be. The solutions to these problems must be found by parallel studies of morphological alterations and physiological changes, as is true in many aspects of vestibular physiology. It is reasonable to expect that the morphological study must utilize many different approaches such as normal histology, electron microscopy, histochemistry, including quantitative studies of single cells or specific regions of maculae and cristae. Single-cell techniques in this field have already been used by Hallén and collaborators (refs. 7 and 8) on the spiral ganglion and on cells of Deiters' nucleus. By such methods it is possible to evaluate small changes in cellular weight, RNA content, enzyme activity, and the like. Current histochemical methods may point the way for quantitative studies, but, because of their lack of precision, direct quantitation of the kind exemplified by Hallén's group must also be further developed and used.

A question of great interest in the study of vestibular disorders arising from radiation, Coriolis effects, and other factors, is motion sickness. Because of this, the pattern and origins of

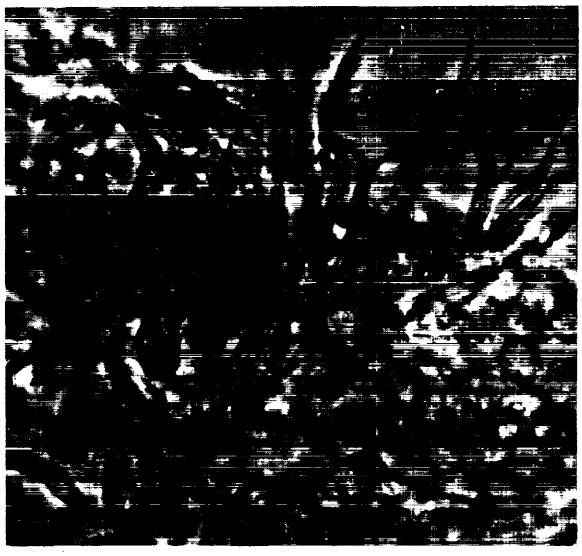

Figure 5.—*Macula utriculi, guinea pig. In this specimen the hair tufts are visualized.*

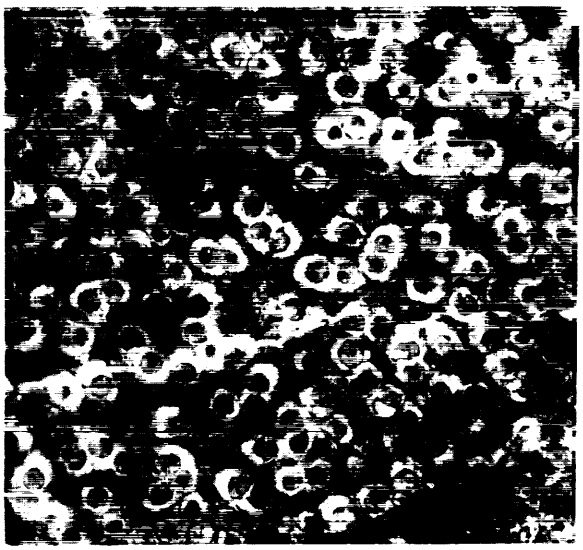

Figure 6.—*Surface view of macula utriculi. The focus of interest here is the population of different types of sensory cells. This and similar specimens show that the striola region is provided with greater numbers of large cells, mainly of type I, than the other parts of the macula.*

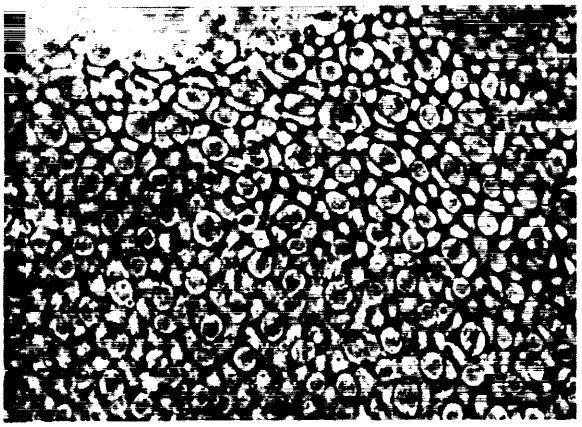

Figure 7.—*Pattern of structural organization on the macula surface. The surface of the epithelium shows rounded sensory cells, surrounded by supporting cells. In the upper left corner some sensory hairs are seen.*

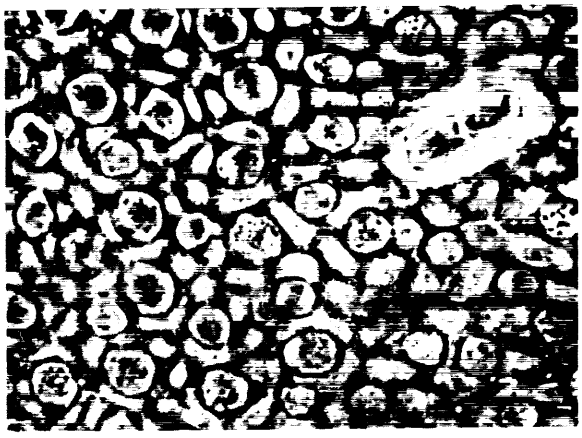

Figure 8.—*Pattern of sensory cells on macula utriculi with one statoconium, as seen in the upper right corner.*

autonomic innervation become an important area for investigation. The distribution of autonomic fibers in the vestibular sensory regions has been mentioned by several authors and especially by Spoendlin (ref. 5). Further studies using the Folk-Hillarp technique must be carried out.

A final problem which calls for careful exploration is that of the vascular supply of the vestibular end organs. Studies by Axelson, working with our group, have provided us with important new information on cochlear and vestibular blood vessels in animals and man. Circulatory disturbances under the influence of high g forces and under the influence of prolonged weightlessness can be expected, and may be of great importance in the study of postorbital changes. For this reason it is of great importance that the investigation of normal circulation be extended to and paralleled by similar studies under conditions of high g and weightlessness.

Finally, while the intensive study of vestibular morphology is essential to the prevention of unwanted effects in prolonged space flight, the functional implications remain as the paramount interest. It is essential that physiological and clinical experimentation and interpretation of the vestibular system be designed to parallel the morphological studies in such a way that neither lags behind the other, and each supports and extends the other.

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DISCUSSION

MEHLER: Dr. Engström, in respect to the streptomycin damage in the cochlea being essentially topistic in respect to the more apical high-tone regions of the cochlea, do you suspect that the damage and the pathology will again be topistic in a specific region of 17 millimeters of the vestibular epithelium, making the job a little easier for you to do serial sections?

ENGSTRÖM: I believe it has been shown already that there is a topistic form of innervation. As far as I understand, Dr. Spoendlin's studies have shown that there is a difference among different epithelia. I believe everyone who has worked in this field knows that there is both a topistic difference and a cellulotopic form, so that type 1 cells are damaged first. This was shown by Hawkins, who has taken part also in studies on the framework of the pattern of cells inside the cochlea.

SPOENDLIN: We do not have a precise idea what changes in the vestibular system we should expect to find during prolonged weightlessness. If we look around, we might find some hints from the eye. De Robertis described a reduced number of synaptic vesicles in the rods and cones of the retina when the animal was kept for a long time in the dark. The interesting question would be, however, whether we get a permanent damage when the proper stimulus for the sensory organ is lacking. I do not know at present whether the eye is permanently damaged if it is kept for years in darkness.

GUALTIEROTTI: There is one point in Dr. Spoendlin's proposition with which I don't agree. You can't compare the retina in actual darkness with the otolith, the gravitoceptors, in zero g, because even during weightlessness you still apply a number of stimulations to them. They are not only gravitoceptors, they are inertiometers. So every time you move the head, you get a stimulation. Every time the spacecraft spins, you have stimulation. Every time you have any maneuvering, you have stimulation. So I don't think that you can say that the otolith organ is deprived of stimulation in weightlessness. The problem here is not that we have a functional *deefferentation* of the system. The principal changes are the abnormal stimulation and the lack of a reference point which is the vertical. Thus, I do not think it can be compared with the absolute darkness on the retina.

COHEN: I think it is very important in regard to the many types of stimulations by rotation or otherwise to continue tests, especially where strenuous or powerful stimulations are made, for quite a long time after the cessation of the stimulation. I don't mean just for an hour or so, but for even a matter of days or weeks. There are some indications from certain centrifuge studies on humans of bizarre effects which might appear as long as 3 weeks after the severe exposure terminated. Would you have any speculation of the type of damage that one could expect to a sensory organ which might not appear at first but could account for effects of that order of time magnitude?

ENGSTRÖM: I fully agree with Dr. Spoendlin when he says he is not expecting too much by the increased g force, more from the absence of g force. It is possible, of course, that the modification of function modifies, for instance, the number of synaptic granules, but it will be very hard, if that modification is not pronounced, to find. I believe that by the study of structural details, you will find that the modification takes a certain time until it develops. This could be just like a man being poisoned. He is dying but you can't see it at first; only after a while does it become evident, and maybe certain functions are still in place in that dying man because he is not quite dead. You can even take the head of that man when he is dead, and you can get certain responses from him. I think it is the same in the vestibular apparatus, too.

VON GIERKE: Would it not be a very worthwhile supplement to studies in the weightless condition to do additional work at higher gravity and look for changes or damage there? Also, has anyone tried to raise animals in complete quiet and then look for any alterations in the cochlea?

ENGSTRÖM: I fully agree with you. I think that in many places centrifugation of animals in high g forces is used. The damage will not occur below a certain force, but you cannot always be quite certain where the border will be in your experiments. You might get more than 10 g one day. It would be of great interest to know what happens if you get 15 or 20 g in these regions.

VON GIERKE: I had in mind more the raising of animals at 2 g as has been done for years and then looking for changes in the cochlea and the vestibular organ.

ENGSTRÖM: I think Dr. Wersäll knows something about that.

WERSÄLL: There is one way of doing this; that is, to hatch chickens at various g forces. In the pathology department in Stockholm, Professor Torell has made a series of studies hatching chickens in a centrifuge producing 4 g, and we are just studying some of the specimens from these animals. One problem is that you have to help the chicken to knock through the shell because it cannot get out itself. We are just making a preliminary study of the anatomy; and unfortunately I cannot give you the anatomical results of that yet. When the chickens have come out of the shell, it takes 2 or 3 days before they really can walk. Still, after several days they apparently try to use their sensory

organ in relation to the 4 g instead of the 1 g of the Earth.

DOLOWITZ: One thing is bothering me. We are talking only of the specialized cells. We were told that there is a change of dehydration. This means there will be a change in the ground substance. We must remember all of these cells will depend on the ground substance for their nutrition and for their excretion of toxic wastes. It might be very important for us to study the connective tissue and the ground substance at the same time as we are studying the specialized cells. It may become possible later to alter pathology through alterations in ground substance. We may waste an awful lot of time if we don't study its actions and methods of controlling them.

ENGSTRÖM: One of the important developments in this field must be to make quantitative measurements at the organ itself and to study by histochemical and quantitative chemical measurements what kind of modification in ground substance, in enzyme contents, and so on, that you can find in the important regions, and also maybe in the fluid around them.

SESSION VI

Chairman: **CHARLES F. GELL**
Ling-Temco-Vought Aerospace Corp.

Cochairman: **WILLIAM M. HELVEY**
Lockheed Bioastronautics Organization

Experience With the Chimpanzee as an Experimental Space-Flight Animal

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6571st Aeromedical Research Laboratory
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SUMMARY

There are many risks associated with space flight that can be adequately investigated in ground-based laboratories or in animal space flights. As a result of the growing body of evidence which suggests that the chimpanzee is "man's closest living relative," the 6571st Aeromedical Research Laboratory has worked primarily with this animal.

The experience of the laboratory with the two Project Mercury animal flights indicated that the chimpanzee was an excellent surrogate for man in this environment. Further experiments have been carried out using a complex multiple-operant schedule to evaluate the performance of the subject before, during, and after a rapid decompression to 150 000 feet. No permanent brain damage in any subject has resulted from an exposure of 150 seconds at this altitude. Another experiment was carried out in cooperation with the Lockheed Missiles & Space Corp. to evaluate an Advanced Life Support Capsule. It was found here that major problems were the chronic restraint of the animal and the collection and disposal of urine and feces. Reliable solutions to these problems are needed before any prolonged animal flight can be considered. All experiments should require an evaluation of the ability of the subject to function in the new environment. The electroencephalogram, along with measures of psychomotor performance, has been found quite effective.

INTRODUCTION

It is in keeping with the Western philosophy that before man is subjected to a high-risk environment, it first be explored by a suitable animal surrogate. The use of animals in medical research is now so commonplace as not to require any comment. It is important, however, that a suitable analog or surrogate be selected. For many of the problems of space medicine, a member of the order Primates, suborder *Anthropoidea*, offers a suitable choice. Our laboratory has elected to work principally with the chimpanzee, *Pan* sp.

In the early days of space flight, we were primarily concerned with the eventual ability of man to withstand the rigors of the launch and reentry accelerations and the intervening period of weightlessness. Beyond the simple question of survival, we were then interested in knowing to what extent the pilot could act upon and interact with this new environment. Thus, while a lower animal would have afforded the opportunity to gather basic cardiorespiratory data, the all-important question of the integrity of the sensory and motor modalities would not have been answered. While it is true that operant conditioning techniques can be successfully employed with very simple animals, only the Primates have the manipulatory ability of the hand, with neural pathways presumably more closely related to those of man. It seemed rea-

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sonable, therefore, to employ a primate closely related to man. A young chimpanzee would appear to be the animal of choice.

There is a growing body of evidence that suggests in rather convincing fashion that the chimpanzee is indeed man's closest living relative (ref. 1). In fact, as one peruses the pertinent literature, the closeness of this relationship becomes fascinating. It has been suggested by Goodman (ref. 2), on the basis of serologic evidence, that the chimpanzee and gorilla be moved from the subfamily *Pongidae* to the subfamily *Hominidae*. The pioneering work of Köhler (ref. 3), and later the Yerkes group (ref. 4), presented evidence for higher cognitive functioning in these animals.

There are, though, several drawbacks to this species. Chimpanzees are in short supply, and the situation promises to get worse with the increasing demand. In fact, if we do not exercise some degree of caution and conservation, the species is threatened with extinction. Even a young chimpanzee, with only modest preparation, represents at least a $2000 investment. For space flight or simulated space-flight experiments, only a young animal is suitable. As an animal exceeds about 23 kilograms and about 6 years of age, he becomes highly unmanageable in a restraint situation. Until Miss Goodall's remarkable film was shown on national television in December 1965, most people had never seen an adult chimpanzee. Few investigators have had the dubious pleasure of working with a 70-kilogram male.

With these brief introductory comments about the animal, I would now like to review briefly our experience with the two Project Mercury flights made by Ham and Enos. Both of these flights have been described in detail by the investigators (ref. 5), and I will only touch upon the highlights. Ham (*Holloman AreoMed*) was launched atop a Redstone booster into suborbital flight on January 31, 1961. The flight lasted but a few minutes, with a total weightless period of 7.5 minutes. Due to a malfunction in the escape rocket system, the subject was exposed to 17 g on launch in contrast to the 7 g expected. During the flight, behavior was evaluated with a two-event task—a continuous avoidance schedule which called for a lever press in response to a visual cue at least once every 15 seconds, combined with discrete avoidance which called for a different lever press within 5 seconds of a second visual cue. Failure to respond was punished by a low amperage shock. Both the launch and reentry accelerations caused a slight decrease of performance, but there was no effect during weightlessness.

Enos was launched atop an Atlas booster into orbital flight on November 29, 1961. Two orbits of the Earth were completed, with a total of 183 minutes of weightlessness. Launch and reentry accelerations were less than 8 g's. In addition to the continuous-avoidance and the discrete-avoidance tasks, Enos also had to perform on the following three programs. To obtain drinking water, lever presses cued by a green light had to be spaced 20 seconds apart. If he responded prematurely, the clock would be reset and he would again have to wait 20 seconds before responding. To obtain food, the subject needed to press a lever 50 times in response to a yellow cue light. A banana pellet was delivered upon the completion of each 50 presses. Finally, to evaluate the subject's ability to solve a discrimination problem, he was required to select the odd symbol from a three-symbol presentation. There were 18 such problems, and failure to solve the problem correctly resulted in a mild shock applied to the soles of the feet, with the same problem presented until correctly solved.

The adaptability of the animal was rather dramatically demonstrated in this flight when a malfunction occurred in the oddity problem. Despite the animal's correct solution of the problem, a switch malfunction caused him to be punished. He tried the "correct" response several more times, but was again punished each time. He then began systematically pressing the other levers in an apparent attempt to find some other possible solution, all the time receiving a shock for each lever press. Finally, after having received 35 shocks, the switch functioned normally and he proceeded through the program. It is rather remarkable that this experience did not affect his subsequent ability to perform on any of the other programs. It is ex-

tremely doubtful if a lower animal could have functioned in so rational and composed a fashion.

Since the completion of the animal phase of Project Mercury, there have been no animal space flights launched by the United States. Despite this, there are still many space-related programs that can be investigated in ground-based laboratories. The complexity and very high cost of any space flight demands that we utilize ground-based experiments to the maximum extent consistent with good scientific judgment. We have therefore been pursuing, in cooperation with several agencies, a systematic study of some of the more obvious hazards. While the chimpanzee is our prime animal, and we maintain the world's largest colony, we still use various other monkeys where possible. We feel that the intelligence of the rhesus group has never been fully explored and that this animal has much to offer. Our experience indicates that the chimpanzee can learn a given task appreciably faster than the rhesus. However, to our knowledge, there has never been an adequate systematic study exploring the relative intelligence of these animals.

A chronic hazard of space flight and high-altitude flight is the possibility of a cabin or pressure-suit failure with exposure of the occupant(s) to a high vacuum. There have been dire predictions that an exposure to above 63 000 feet (the "Armstrong line") would result in death within about 15 seconds. It is of obvious importance to know whether or not these predictions are true. Further, if death did not ensue in a few seconds, how long could one survive and what would be the resultant damage, especially to the central nervous system? We set about studying this problem for NASA to aid in the design of mission rules for space flight as well as for the establishment of safety regulations in the operation of high-altitude manned space chambers. The details of this experiment have recently been reported by Koestler (ref. 6).

Briefly, a series of chimpanzees was trained on a fairly complex multiple-operant schedule. This allowed the evaluation of performance on continuous avoidance, discrete avoidance to several visual stimuli, discrete avoidance to an auditory stimulus, and problem solving on an oddity task coupled with choice behavior for food or water reward. Prior to the actual experiment, the animals had stainless-steel screws implanted in their skulls for cortical electroencephalography. After a satisfactory period of training, the animals were placed in a small altitude chamber where they were allowed to breathe pure oxygen for 3 hours for denitrogenation. The chamber was then depressurized to a simulated altitude of 35 000 feet where the animals were run through the complete performance schedule to insure that their behavioral and physiological indices were still within normal limits. They were then decompressed in about 0.8 second to 150 000 feet and remained at that altitude for periods varying up to 150 seconds. They were then recompressed to 35 000 feet in 30 seconds and held at that altitude until recovery ensued. In 16 such experiments we have had but one death and that appeared, at necropsy, to be attributable to preexisting cardiac disease. Not only were we surprised to find this lack of mortality, but we were also quite surprised to find no evidence of any permanent central-nervous-system damage. We found that we could predict reliably, from the time of exposure, the time of total behavioral impairment and the time of return of behavior to within normal limits. All animals are restudied at 3-month intervals up to 1 year to rule out chronic brain damage. To date, all animals are normal.

During the past year, our laboratory was also called upon to aid in the evaluation of the Advanced Life Support Capsule Project which had been developed by the Lockheed Missiles & Space Corp. (LMSC). This program evolved from a previous study of the LMSC, the Advanced Biomedical Program, later renamed the "Bioastronautical Orbital Space System" (BOSS). We were to test this system with a 50-pound chimpanzee for 30 days.

A much more complex work panel has been developed to better evaluate the performance of the subject (fig. 1) (ref. 7). As before, the animal had both a continuous avoidance task (one lever press per 5 seconds in response to a

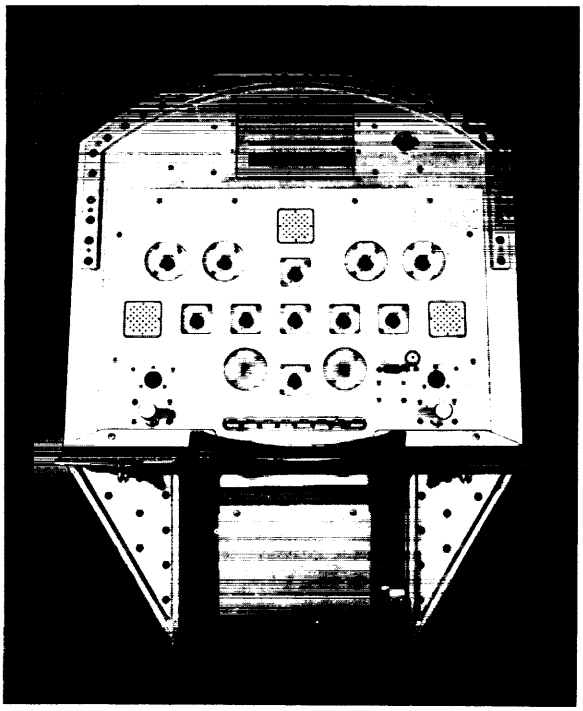

Figure 1.—*Work panel for evaluation of chimpanzee performance in a life-support capsule.*

red light) and a discrete avoidance task (lever press required within 1 second in response to a blue light). Instead of a single auditory stimulus, there were now three, at 512, 2048, and 8192 cps. There was thus a need to discriminate the tone, with a button press required within 3 seconds. The above problems were all motivated by shock avoidance. In the center of the panel there are five display lights and switches and a sample presentation window above. Initially, a colored light is presented in the sample window, and the animal must respond by selecting a pushbutton below, previously (in training) associated with this color. The schedule then moves to a temporal delay problem somewhat similar to that employed with Enos in Project Mercury. When a "square" symbol was presented in the lower, central window, the response had to be withheld until after 15 seconds and then made within the next 5 seconds. A "circle" symbol was next presented which doubled the delay time (to 30 seconds) and also doubled the time permitted for the response (e.g., from 5 to 10 seconds). Upon completion of this task, the program moves back to the central display windows which are used for sample program wherein the symbol (color or shape coded) presented is to be "matched" with a similar symbol in the five presentations below. An incorrect response on these two programs results in a shock, but a correct response permits the subject to choose a food (F) or water (W) reward by pressing the appropriate button. In effect, then, the chimpanzee was able to "tell" us whether he was hungry or thirsty. Following these programs, the subject then had to select one of two symbols, presented at the upper left, which he had been taught as correct. There were nine such problems. Finally, these same problems were then immediately presented on the displays at the upper right-hand portion of the panel, but now the answer that had been correct on the left was incorrect and the subject had to reverse his response.

We felt that a series of tasks such as these would permit a much better evaluation of the subject's condition. Any subtle effects of the experimental environment would then be more likely to be detected. Along with these behavioral measures, we also obtained cortical and subcortical EEG, ECG, respiration rate, body temperature, and blood pressure (arterial and venous) from indwelling catheters.

Briefly, we found that the environmental control system performed quite well. However, as we had suspected a chronically restrained chimpanzee presents many problems. Our largest single problem was cystitis and pyelitis resulting from catheterization of the urinary bladder. In all, we used four different animals to complete the 30-day test. Simply stated, the waste-management problem must be solved before the chimpanzee (or any animal) can be used for long-term flights (ref. 8).

In evaluating any hazardous, high-risk environment, we believe that it is most important to monitor performance on several sensitive variables, for it is upon performance that man's life will depend. Further, we have found the EEG to be the physiologic index most sensitive to impending change. At least in the chimpanzee, we have seen little change in conventional

cardiorespiratory data when both performance and the EEG were grossly abnormal (ref. 9). We would suggest that as the EEG is employed with greater frequency, its value will be more generally recognized and appreciated.

Finally, what are our own feelings about the requirements for animal space flights? There are three clearly defined situations: (A) Long-term flights of significantly greater duration than can be expected from the manned space-flight program in the near future. At present, 90 days would seem to be the minimum for such a flight. (B) Shorter flights with animals instrumented to obtain information that we cannot obtain from man. I am especially considering studies of the central nervous system with deep recording electrodes and possibly stimulating electrodes. Similarly, implanted flowmeters on the major vessels might yield valuable data. (C) Last, and perhaps most important, flights planned for basic or fundamental research without regard for the applied aspects of the program. By the very definition of basic research one cannot predict where it will lead, but that should be no deterrent. We, who are familiar with the problems of space flight, are charged with the responsibility of conceiving and executing those experiments which will allow us to move forward at an optimal and maximal rate.

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DISCUSSION

GELL: I certainly agree with the philosophy expounded in the latter portion of your talk.

MONEY: I suppose it is predictable at this meeting, but I was surprised that when you suggested space flights, you left out a space flight to investigate the problem of motion sickness. It seems to me that this is probably the most pressing, the most important biological problem in space flight, because 3 out of the 15 or 20 people who have been in space have had this sickness. I think that we don't really know why the Russians got it and the Americans didn't. Considering the fraction of the astronauts who have had it to date, this is a very serious problem and should be investigated to see if it is motion sickness and how it can be avoided.

KRATOCHVIL: I would not suggest that we should not look at it, certainly. In our studies, of course, we have an animal. I gather you are familiar with the work that has been done on motion-sickness drugs, and the whole problem of motion sickness in the human. The vestibular problems are extremely complex; emotion has a lot to do with the occurrence of motion sickness.

I do not know whether a chimpanzee would react in the same fashion, nor do I know how extrapolatable animal information would be to man. Certainly the use of tranquilizing drugs in animals has not worked out too well as compared to what we find in the human in motion sickness. This is just a difficult area to work in.

WEISSMAN: First, regarding the complexity of the tasks which the chimpanzee used: I would imagine that the purpose of these complex tasks was to differentiate effects of these environments. Were they ever

successful in differentiating any of the effects, or differentiating among any of the stresses? That is, did they perform differently on the timing tasks only, or did they perform differently on the avoidance tasks only? Second, would you elaborate on why you feel the EEG is so useful. You said, I believe, that the EEG and the performance were two useful measures. I was wondering, did you mean independently useful or were they useful dependently?

KRATOCHVIL: They tend to support each other. When the behavior starts to degrade as judged by the psychomotor performance, the EEG has already started to show changes a little bit ahead of this. And when the animal regained consciousness and started to work, the EEG recovery led the performance. But the point I'm trying to make here was that the electrocardiogram, through all of this, looked great. If you were looking only at the EKG, you wouldn't have known anything had happened. There was a little tachycardia and a little bradycardia, but nothing to get too excited about.

WEISSMAN: What I really meant was: Was there a one-to-one correlation between performance and the EEG?

KRATOCHVIL: Pretty well, yes, insofar as one can say one-to-one when you are looking at an EEG. Dr. Farrer may want to say something about whether reaction time or discrimination changed first.

FARRER: I might comment very briefly on the first part of Dr. Weissman's question which dealt with the variables involved in the various performance tasks which were related here. The effort involved in the variability of the performance test is to catch as many sense modalities as we possibly could; e.g., auditory, visual input, and so forth. A more regular work variable, the variation on the Sidman avoidance tasks as we call it, continuous avoidance, is an effort to get at perhaps a more continuous long-term work as opposed to reaction times, specifically, to auditory stimuli and reactions to visual stimuli. We do find, also, related to the first question, some difference between appetitively rewarded tasks and aversively rewarded tasks in that we find a greater variability in performance of those appetitively awarded tasks. The food-water rewards, we find, do have greater variability in performance as a function of environment insult such as the type Dr. Kratochvil describes. The aversively rewarded tasks do maintain a little more stable rate of responding. Reaction times hold up a little better; the Sidman avoidance behavior holds up a little better in the face of high-risk environmental insults.

DAVEY: I would just like to make a comment about the electrocardiogram and the electroencephalogram. There just isn't any question about the fact that the two of them scarcely measure the same things. Those of us who take care of patients who have suffered severe head injuries find ourselves often in the unfortunate situation of having patients who are still showing a normal electrocardiogram long after they have ceased to show any kind of cerebral function. I would also like to say that it is very difficult to establish any real point-to-point relationship between the electroencephalogram and the specific performance of the animal. I think all that the electroencephalogram demonstrates is a metabolic alteration. And if there is this alteration in the brain, there may very well be a change in performance.

KRATOCHVIL: I'd like to have Dr. Adey's comments here, but let me just say one thing. I'm sure that by watching alpha, beta, theta, or whatever you want to look at, you are not going to say that this animal or this man can write his name or cannot write his name. No; there is not that kind of one-to-one correspondence.

ADEY: I just cannot agree with Dr. Davey's last remark, that it is a metabolically related phenomenon, like putting cheese inside a green apple. The fact is, if one gets away from the anecdotal clinical remarks of Dr. Davey, does some sophisticated computer analysis of EEG data, and gets away from notions that by visual examination of the EEG, he can really tell what is going on, one comes to the conclusion that in man, with scalp leads alone, it is possible to distinguish many different states of consciousness in a quantified way. This is true in our experience for a baseline of 50 astronaut candidates. In fact, you can distinguish between EEG patterns during visual discriminations made in 3 seconds, as opposed to those made in 1 second. I know of no finer assay of the state of consciousness than the assessment of the EEG during a visual discrimination task of a type that accurately simulates the sort of tasks confronting a pilot or astronaut. Moreover, we have related the EEG recorded grossly on the scalp or brain surface to an intercellular wave process, so the EEG is certainly not metabolic in origin.

KRATOCHVIL: I was glad to hear Dr. Adey say this. We feel rather strongly about this, too.

GUALTIEROTTI: I'd like to discuss further the significance of the EEG. Suppose an animal increases his mistakes by 10 percent, or significantly, can you detect his significant change in the EEG?

ADEY: Yes, sir.

KRATOCHVIL: I think you can, Dr. Gualtierotti. I'm certainly not a professional EEG'er. But just by what I have seen in my rather gross experience, I think this is undoubtedly true.

GUALTIEROTTI: Second point is that the EKG by itself, as is known, doesn't mean much. Now, if instead of measuring the EKG, you'd measure the blood flow in the cortex, or the blood flow in the brain, that would be a completely different story.

KRATOCHVIL: But as you and I both know, nobody has successfully ever measured regional blood flow in the brain under physiologic conditions.

GUALTIEROTTI: You can in animals, of course. Anyway, there is no question that the EKG is of relative significance. Another point I was interested in is the experiment regarding rapid decompression. I didn't

quite follow the problem. Do you mean to say that the animal was decompressed to 2 mm Hg in 0.8 second?

KRATOCHVIL: Point eight; yes, sir.

GUALTIEROTTI: And what happens then?

KRATOCHVIL: He looks just about like a human in terms of time of useful consciousness.

GUALTIEROTTI: I know, but how long did he stay in this state?

KRATOCHVIL: He passes out, if you will, becomes unconscious, at about 10 to 15 seconds. We had one that lasted for 28 seconds; I don't know why. Let me run through how we approached this. We started out initially with just a 5-second exposure, because we didn't know what was going to happen. This was innocuous. The animal was back to normal almost immediately. We went to 30 seconds, 60 seconds; and the exposure was still so innocuous that we then settled down on 90, 120, and 150 seconds' exposure. Now we have replicated these experiments at least three times at each point. We now have four animals that have been at 150 000 feet for 2½ minutes, and all have come back to within baseline behavior within 4 hours. Further, all animals are held so that they are not used on any other experiments which could possibly perturb their behavior for at least 1 year. They are re-examined, both for their psychomotor performance and EEG's, every 3 months to make sure we don't have an epileptic focus developing, or something similar.

GUALTIEROTTI: Did you observe if the animal during these 2½ minutes made any kind of respiratory movements?

KRATOCHVIL: Yes, sir. He has gasping respiration; he initially has a bradycardia and then he goes to a tachycardia.

GUALTIEROTTI: And so we have to suppose a state of a near vacuum in the alveoli.

KRATOCHVIL: Yes, sir.

GUALTIEROTTI: Did you find any alteration in the alveoli, like blood?

KRATOCHVIL: You occasionally see a little. These animals are not necropsied; they are alive yet; so part of this we cannot answer. Occasionally you see bloody sputum, frothing at the mouth. But is this coming out of the alveoli or not? This is just a very gross sign, of course.

It is not a very pretty experiment. They swell up. Animals, you know, at this altitude, or man, do not look too good, but they do come back to normal. My explanation for this return to normal is: I think the embolization must be there and (we have some data on dogs that suggest that the heart is full of gas, not full of blood) that these are oxygen emboli. We are now going to look at the two-gas system, and my guess is that when we have nitrogen emboli, we are going to have real trouble. But this is only a guess. We have no data on this as yet.

Long-Term Performance of Squirrel Monkeys Under Space Simulation Conditions[1]

Part I: Characteristics of Approach and Capsule

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SUMMARY

The necessity and importance of orbital animal flights for a duration of 6 months is stressed. Such flights should closely simulate conditions in later manned space flight. Unrestrained animals, conditioned to perform certain tasks and observed by a television camera, are expected to furnish valuable physiological and behavioral information. A capsule housing life support and working stations for two squirrel monkeys was built by Ling-Temco-Vought Astronautics Division and tested in laboratory experiments. A low-residue liquid diet promises great savings in food supply and waste disposal. Some results of long-term laboratory experiments are described.

I should like to preface this report with some general comments intended to place our work in the proper perspective. The prudence of animal experiments preceding possibly dangerous human exposure is seldom in doubt. In the history of manned space flight, trail-blazing mice, dogs, and monkeys have served that purpose. The lagtime between animal and manned flights is often considerable and it took, for instance, 6 and 8 years, respectively, until the duration of Russian and United States manned flights equaled the Laika (Sputnik II) time in orbit (fig. 1). If the full benefit of animal experiments for long-term manned flights in the 1970's is to be realized, animal experiments of many months' duration should be started immediately. Just as the recent series of manned orbital flights benefited from the Laika and other animal experiments, so will future plans for manned flight profit from sucessful long-term animal exposure.

I should like to characterize briefly our approach to a long-term animal satellite experiment. Supported by the National Aeronautics and Space Administration, Office of Advanced Research and Technology, the U.S. Naval Aerospace Medical Institute, Pensacola, Fla., and Ling-Temco-Vought, Astronautics Division, Dallas, Tex., have made feasibility studies and ground-based experiments with squirrel monkeys in preparation for a 6-month orbital flight. During this extended period we hope to gain information on the effects of prolonged weightlessness on bodily mechanisms, systems, and organs, with particular emphasis on the vestibular organ. In addition, it is anticipated that the experiments will reveal possible psychological and behavioral changes. In future

[1] In conducting the experiments reported herein, the investigators adhered to the "Principles of Laboratory Animal Care" as established by the National Society for Medical Research.

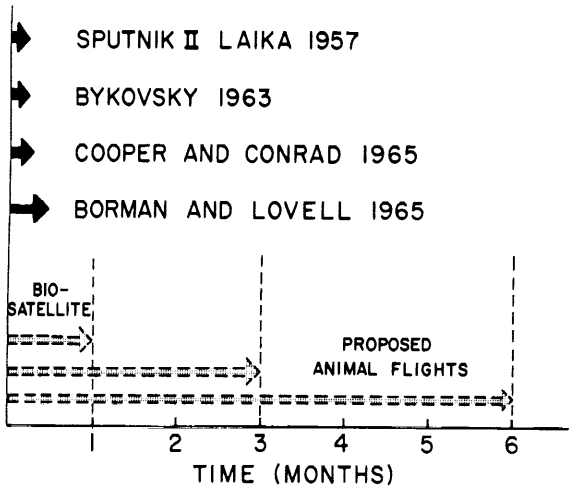

Figure 1.—*Duration of proposed animal flights in relation to previous animal and manned flights. Note the time delay between animal flight (Laika) and manned flights.*

space flight, man will move freely and without restraint in the space vehicle, and little is known about the effect of prolonged free movement in the absence of gravity. Our experiment is characterized by the use of unrestrained, free-moving, trained animals in preparation for manned flight.

In our present approach an egg-shaped capsule houses the animals and their life-support system (figs. 2 and 3). A total weight of the capsule at launch of about 400 pounds has been planned for a nonrecoverable 6-month orbital flight of two squirrel monkeys. The squirrel monkeys are located in separate but window-connected cages in the center part of the capsule. A fan forces the air through a waste filter, a charcoal filter, a carbon dioxide and water absorber (LiOH and LiCl). The air reenters the cage area close to the location of the television camera. The temperature regulating system, which operates flaps at the blunt end of the capsule, is not shown in figure 2. A mixture of oxygen and nitrogen is bled into the capsule from an outside storage tank, and a leak valve allows renewal of the atmosphere at set intervals. Liquid food and water are stored in pressurized bags in the capsule. The capsule is at present in the process of being tested in our laboratory.

Our choice of a liquid, low-residue diet was originally dictated by extreme weight restrictions, but it may merit more general attention. The liquid diet used in our experiment was supplied by Schwarz BioResearch, Inc., of Orangeburg, N.Y., and is composed of amino acids (9.4 percent); carbohydrates, mostly glucose (87 percent); fats (0.5 percent); vitamins (0.1 percent); and minerals (3 percent). The daily excretion of a squirrel monkey on this diet is only about 3 grams compared with 15 grams under normal solid food conditions. We carried out a separate study on the diet and found that

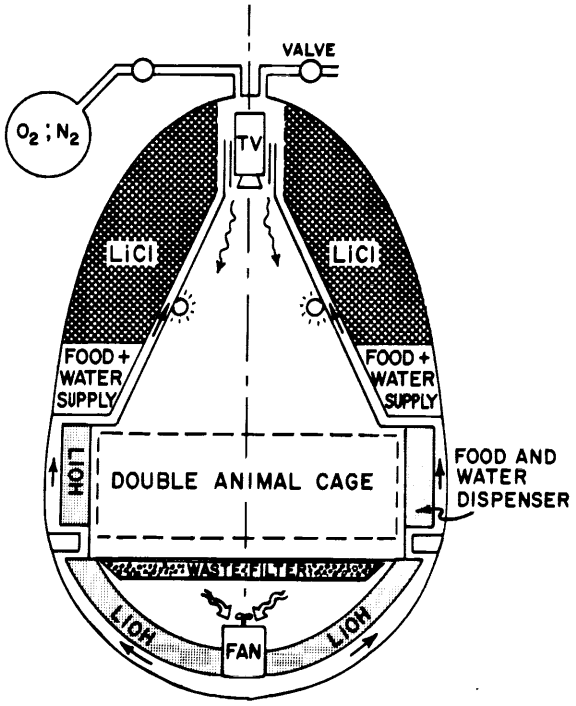

Figure 2.—*Arrangement of animal quarters and life-support system in Ling-Temco-Vought capsule.*

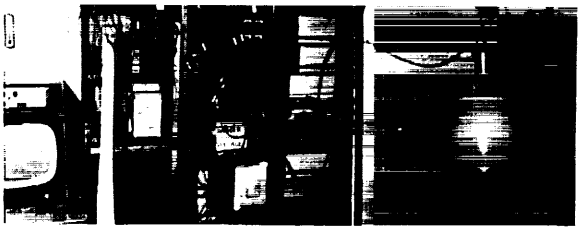

Figure 3.—*Capsule and monitoring station located in adjoining rooms.*

in its present composition, it is adequate to support the requirements of squirrel monkeys for about 3 months. In preparation for longer flights (6 months), additions of certain vitamins, minerals, or fats will be necessary to increase the present low survival rate in long-term feeding. Attempts are in progress to devise such a long-term artificial diet.

In the capsule, the animals receive their rations in measured proportions through lever-operated solenoids as a reward for the performance of certain tasks. We have selected the tasks so that a certain amount of physiological as well as behavioral information can be gained. The electrocardiogram or at least the heart rate can be monitored by training the animals to hold levers for a prolonged period of time, while thermistors embedded in the levers also measure the palm temperature. Attempts have been made to determine the muscular force necessary to pull the lever against a resistance and measure possible muscular deterioration in the long-term gravity-free state. There are practically no limits to the ingenuity of the behavioral scientist in probing functions of the conditioned animal. Interest is also concentrated on behavioral indices, and it is expected that valuable information on motivation, discrimination, adjustment, and learning can be gained during long-term exposure to the gravity-free state. The next paper will report on some results of ground-based simulation experiments.

I should like to emphasize that the capsule described in this report should be considered a temporary solution. With more space and weight available in a future vehicle, an improved approach should be planned. However, we would suggest retention of the following characteristics of our present concept:

(1) The animals should not be restrained. The possibility of free motion in work and play during the entire orbital time is essential.

(2) Arrangements for physiological and psychological measurements and observations should disturb the animal as little as possible, and methods of animal behavioral research are most appropriate for this purpose. These self-administered measurements allow an ingenious combination of work and exercise with a method of measuring food and water intake. The methods may be supplemented by implants of physiological sensors, and televised observation should be provided. All instrumentation should serve one supreme task: unobtrusive long-term observation of unrestrained animals in the gravity-free state.

(3) A minimum of two animals should be exposed, since companionship for animals on long-term travel is extremely valuable if not mandatory. In addition, observation of several animals results in data of greater significance.

(4) We also highly recommend recovery of the bioflights for physiological and pathological examination of the animals. Our consideration of a nonrecoverable, long-term flight was dictated by technical limitations at the time of experimental design.

Finally, the necessity of long-term animal flights in preparation for manned space flights is emphasized. Such animals flights will allow a considerably faster pace than is possible in gradually escalated manned flights. Preparations for long-term animal flights are extremely time consuming and should be forcefully supported at an early time.

Part II: Behavioral Technique

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SUMMARY

Behavioral measures were obtained from two squirrel monkeys under two-degree-of-space simulation conditions in order to evaluate the adequacy of the design. Relationships were established between work requirements and diet and water intake, weight, and efficiency of work. Extended and severe isolation appeared to reduce locomotor activity. When the monkeys lived under continuous light, their activity cycles increased to longer than 24-hour periods. The behavioral technique was sensitive yet stable, but limited by persisting problems with liquid diets.

The first task facing all zero-g researchers is to evolve a set of procedural baselines against which to measure the effects of weightlessness. For certain specific problem areas, such as the reaction of the human cardiovascular system, an extensive background is already available. Our concern has been somewhat different, the primary purpose being to demonstrate survival for a long period of time, and only secondarily to glean as much information as possible. Within the constraint of a 400-pound total payload, an environmental life-space and behavioral regime for two unrestrained, free-moving, conditioned squirrel monkeys is being developed in keeping with these goals.

Following a description of the physical arrangement and procedures, a selection of the results from the past year's simulation experiments will be presented. The first will show the effects of several working/eating conditions on weight; the second, factors affecting the principal behavorial measure; and the third, two interesting findings from a recently completed 45-day encapsulation run.

In figure 4 can be seen the general spatial relationships of the monkeys' living area, the work panel, the barred window, and one of the monkeys pulling on the two handles with which it earns food and water.

The choice of a behavior to occupy and to exercise the monkeys was dictated as much by a desire to acquire physiological information as

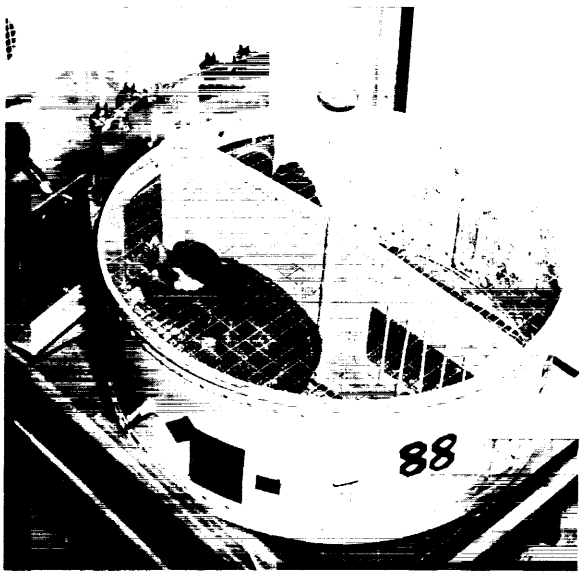

Figure 4.—*The MOBATS partially removed from the isolation chamber showing one subject pulling on the handles.*

by other considerations. The two handles the monkeys grasp and pull are knurled and require a 100-gram force to activate the switches. This assures satisfactory electrical contact for an EKG potential to be recorded. To obtain a half-milliliter reward of liquid diet or water, they must maintain this force for an accumulated time of 30 seconds, which allows ample time for an EKG tracing. In addition to serving as demand feeding devices and EKG electrodes, the handles have thermistors buried in them for recording palm temperatures, which peak 2° to 3° C below rectal temperature.

The effort required to pull hard on the handles for a certain number of seconds also insures that the monkey will consume the reward rather than waste it. This is, of course, necessary to determine the monkeys' intake accurately. Which reward they received, food or water, was programed on a time basis. During 15 minutes of each hour, an auditory clicker signaled that they would receive a water reinforcement for performing properly; during the other 45 minutes of each hour, lack of an auditory cue signaled that they would receive a diet reinforcement. Since they were exposed to this regime 24 hours per day, the monkeys could obtain diet or water at any time of the day or night.

For all but one of the experimental conditions, the monkeys lived in the monkey basic training simulator (MOBATS) of figure 4 or in an identical cage in the capsule itself. The MOBATS was kept inside a 2-foot-square, brightly lit, well-ventilated, sound-isolation chamber. To minimize disturbance from daily weighings, the monkeys were trained to walk from the simulator into a weighing case. When in the capsule, the monkeys were never removed or otherwise disturbed.

Table 1 shows the sort of relation obtained between various working/eating conditions, diet and water consumption, and the resulting weights of monkeys MH and A07. These are steady-state averages obtained during the 5 months that the monkeys lived in the simulator.

In the two central columns of the table are recorded the work requirements in the sequence in which they were imposed. Adjacent columns, MH on the left, A07 on the right, show the monkeys' weights expressed as a percentage of their maximum, and intake in milliliters of diet and water. By examining the first row it can be seen that under a relatively relaxed work requirement of 15 seconds for either diet or water, both monkeys held the same percentage of body weight, drank nearly as much water as diet, and consumed different total amounts, as would be expected because of their different absolute sizes, MH weighing 100 grams more than A07. To separate the effects of the diet and water work requirements, water was next provided ad libitum. This produced polydipsia in both monkeys, appreciably increased diet consumption, and resulted in similar weight

Table 1.—*Effect of Eating Conditions on Intake and Weight*

MH			Work requirements, sec.		A07		
Intake, ml/day		Weight, percent	Diet	Water	Weight, percent	Intake, ml/day	
Diet	Water					Diet	Water
44.9	51.5	89	15	15	89	32.7	31.1
52.5	115.3	94	15	[a]	93	38.2	100.0
77.5	42.0	96	[a]	[a]	95	40.8	41.0
----------	57.7	104	[b]	[a]	99	----------	79.8
38.2	75.3	90	30	[a]	90	41.7	105.0
35.7	16.7	86	30	30	91	42.6	34.8

[a] Ad lib.
[b] Chow.

gains. The eating/drinking interaction, demonstrated by an increase in diet consumption although the diet work requirement remained unchanged, is also worth noting.

Provision of ad libitum diet as well as water produced further increases in diet consumption and weight, but stopped the excess water drinking. Since neither monkey reached his previous 100 percent weight, both were given Purina monkey chow, the food on which the 100-percent ad libitum weights had been calculated.

After return to a moderate 30-second work requirement, both monkeys were reduced again in weight below that of the earlier 15-second diet, ad libitum water conditions, and again became polydipsic. Although MH earned less diet, as would be expected when more work was required, A07 earned more diet while weighing 3 percent less than before. When work was required for water, too, A07 revealed little effect, maintaining 91 percent weight and the same diet intake. This could have been due also to an increase in the amount of diet delivered per reinforcement. MH, however, earned barely enough water to survive.

Thus both monkeys' percentage weights responded nearly identically to changes in eating and drinking conditions. Both monkeys were initially affected more by removal of the water work requirement than removal of the diet work requirement, but only one, MH, was more greatly affected by resumption of the water work requirement. The relation between percentage weight and intake was less clear.

When these studies were designed, it seemed quite probable that diuresis and/or water intake might be a problem in space flight. Fortunately, results from the earlier astronauts were apparently disturbed by problems having nothing to do with weightlessness, such as low humidity and temperature control. However, the present design does allow the precise measurement of consumption and does suggest how we may encourage the subjects to increase their intake.

The primary index of the animal's condition was an efficiency index, or number of responses per reward. If the monkey pulled the handles continuously for the required time, his index, or number of responses per reward, would be one, but the monkeys were seldom that efficient. More often they released the handles one or two times before accumulating the time required for a reward. As shown in figure 5, the major determinant of the efficiency index is the size of the work requirement. Such factors as individual differences, amount of experience on the task, kind of liquid diet, water or diet reward, and MOBATS or capsule environment appeared to have little effect on the efficiency index at work requirements of 4, 8, and 16 seconds. At these lower three values the range of variation was no more than two-tenths of a response, but at the higher value, 30 seconds, variability increased.

Two factors contributing to this variation are identified in figure 6, a difference between subjects and a difference attributable to practice effects. The "individual" difference, appearing only at the 30-second value, will receive further comment later. The monkeys were well trained at the time of the first determinations, and showed no tendency to improve over several weeks. The increased efficiency resulting from training came about only after months of further experience under varying conditions.

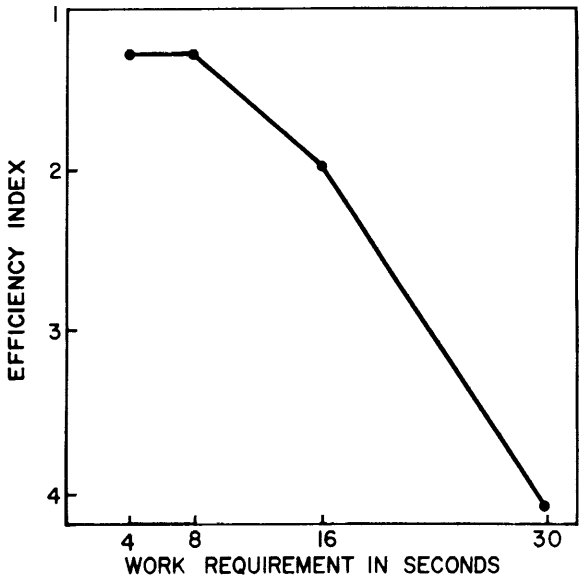

Figure 5.—*Relationship between work requirement and the efficiency index as obtained from subjects MH and A07.*

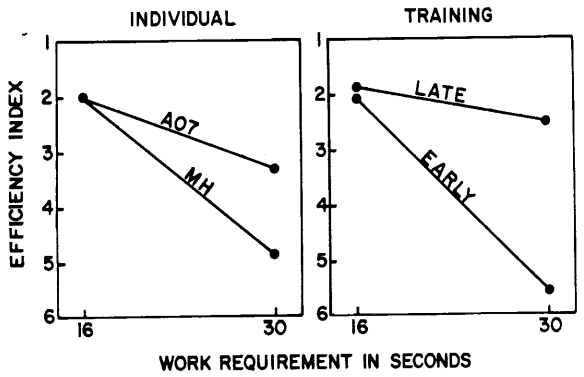

Figure 6.—*Two sources of variation in the effect of work requirement on the efficiency index.*

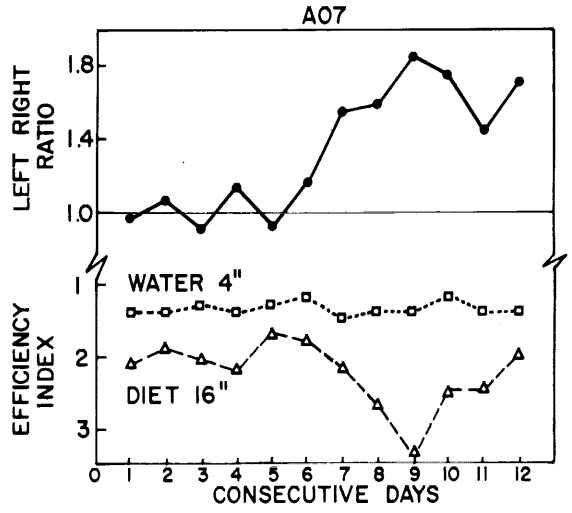

Figure 7.—*Effect of an increase in the force required to activate the right handle on the ratio of left responses to right responses and the efficiency index at a low (4 seconds) and a moderate (16 seconds) work requirement.*

Serendipity entered into the next finding, which shows the effect of another parameter on the efficiency index. Figure 7 displays diet and water indices separately, the water efficiency index, at a low (4-second) work requirement, being performed with a nearly 1:1 ratio of responses to rewards, and the diet efficiency index (16-second work requirement) at its normal value of 2 through the first 6 to 7 days. By approximately the eighth day, the monkey began making extra, unnecessary responses when working for diet, as shown by the decreasing efficiency index. The upper part of this figure shows that this change was preceded by an increasing proportion of left-handle to right-handle responses. For some unknown reason, A07 was not activating the right handle appropriately and this was reflected in the diet efficiency index. Note that at the much easier 4-second work requirement, the water efficiency index did not reflect the change, thus illustrating the common inverse relation between stability and sensitivity of a measure. Even though the left/right ratio remained unbalanced, the diet efficiency index returned to normal after 4 more days, evidencing successively that index's sensitivity and stability. When the capsule was dismantled, the cause of the shift was found to be a slipped setscrew, producing an increased force requirement on the right handle.

Here, a changed condition caused a transient efficiency index increase. The monkey was doing the same thing as usual but not getting the same results. When this happens to us, we might say we are "confused."

The left/right ratio also supported a suspicion we had concerning proper cage construction. The work panels, as noted in figure 4, were alined with respect to the circumferential arc of the cage perimeter rather than perpendicular to the cage-divider partition. If the monkey orients itself with the divider, as a larger monkey such as MH can scarcely avoid, one handle will be further away than the other. When the monkey pulls symmetrically on asymmetrically placed handles, one handle will hit the stop before the other handle. At least, such a possibility can explain the larger monkey's strong "handedness" (fig. 8), and perhaps as well could account for the "individual" differences appearing in the earlier studies on the relation between efficiency index and work requirement. This measure might also detect a change in a monkey's spatial orientation during space flight.

Previous corollary experiments have shown the efficiency index or nearly any sort of efficiency score to be little affected by motivational factors. The efficiency index detects effort, confusion, difficulty, and similar perturbations. If the animal eats or drinks less and the efficiency index remains normal, a motivational

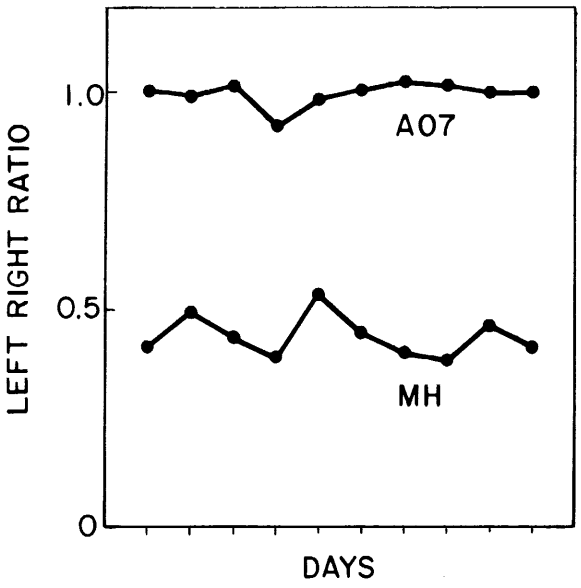

Figure 8.—*Right handedness of the larger subject, MH, compared to the neutral handedness of the smaller subject, A07.*

factor is involved. However, if the animal is confused, weak, drugged, or otherwise disturbed, the efficiency scores will be affected. If its motivation remains constant, the animal will compensate for its decreased efficiency with an increase in attempts, if the effort of the behavior required is within the appropriate range. Herein lies the importance of careful, detailed, and unfortunately usually tedious parametric studies. Only by observing the animal through several replications of widely varied parameters can the direction, magnitude, and probable variation of the response be determined with confidence.

This first part of the report was intended to give some impression of how precise behavioral measures may be used to investigate problems resulting from extended space flights.

The second part of the report presents some findings concerning activity and circadian rhythms during extreme isolation in the space capsule. Figure 9 presents for each monkey the total daily gross (photocell) activity counts for the 45-day period of encapsulation. Both show the same overall features: a high level after a few days, continuing for a few weeks, and then a gradual decrease to little movement. Removing the monkeys for 6 days and then returning them, as indicated by the first break in the baselines, produced the recovery seen, and a similar removal for 2 days at day 41 produced the last transient increase.

As was mentioned in part I, some monkeys are debilitated after several months on the chemically defined diet. This is probably not the complete explanation here, since one monkey, A07, weighed more when she came out than when she went in, and was in good health clinically.

What may have been revealed here is an effect primarily from extended, severe isolation, an effect not manifesting itself unless isolation was nearly absolute and not until 3 to 6 weeks had elapsed.

The last experiment might be cast in a time orientation frame of reference, a different kind of "orientation in space." The monkeys were kept on a schedule of 12 hours of light to 12 hours of darkness and then shifted to a continuous light schedule. The resultant activity changes are displayed in figure 10. Time of day is represented along the abscissa, and the beginning and end of their activity on successive days along the ordinate. The clear area therefore indicates when they were active, the stippled area when they slept. Four days of light/dark 12:12 were followed by continuous light. Both monkeys appeared to settle rather quickly into a 26-hour day as shown by the τ

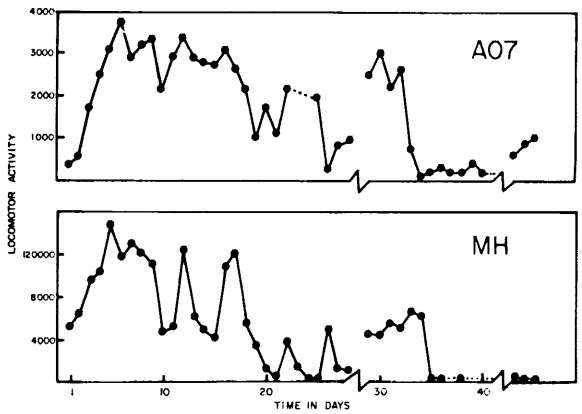

Figure 9.—*Locomotor (photocell) activity as a function of time in isolation. Breaks in the lines indicate removal of the subjects; dotted lines indicate missing data points.*

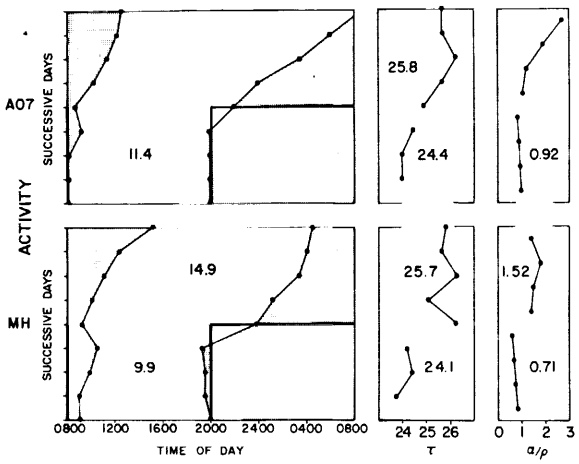

Figure 10.—*Effect of a change in lighting conditions on the diurnal activity cycle. Subject A07, upper half; subject MH, lower half. Day-by-day shift in "activity space," left panel; period length, center panel; activity/rest proportion, right panel.*

plots of the center-right panel. The data are presented in this format to emphasize the degree to which their activity space expanded when given free rein. MH was active for half again as much time per day (15 versus 10 hours) with the corresponding α/ρ ratios (activity/rest) doubling from 0.71 to 1.52 as shown in the far-right panel. A07 was more active than MH initially and her trend toward longer activity periods continued in the 4 days before the experiment was interrupted by apparatus failure. The data are presented in this format to show also that the monkeys' activity space grew by activity lasting later each day, rather than beginning sooner. This, of course, supports the hypothesis that for diurnal animals, it is light termination rather than light presentation which is the common zeitgeber.

The particular behavioral procedure used has been satisfactory, proving to be a sensitive yet stable and meaningful measurement tool, in addition to providing physiological data on food and water intake, temperature, and EKG. Strong interactions between work requirement, liquid-diet consumption, and water intake make unreliable any inferences concerning the monkeys' weight and state of well-being from the amount consumed. Further work is needed on the effects of severe isolation and on the effect of work requirement on free-running circadian rhythms. Although handicapped by limitations of the liquid diet, an exciting step forward could be made by flying this system for its half-year design period.

DISCUSSION

FARRAR: A two-part question: First, what influence would you hypothesize the social facilitation factor to be in your experiment? The traditional circadian rhythm experiment is done with the animal in isolation without the temporal cuing of, in essence, another zeitgeber, if you will, of the animal working next door. Secondly, have you found any relationship between performance in circadian rhythms; that is to say, do you find the circadian rhythm in behavior as well as in temperature? We seem to have some indication at our laboratory that there is perhaps a circadian variable affecting the variability of performance.

THACH: First, your question about social interaction of one providing cuing to the other or tending to hold both in synchrony with each other. In only four animals have I had opportunity to observe such phenomena, where conditions were optimal for it to appear and yet uncontaminated by other factors. It has not happened. If either the period length or activity/rest proportion differed, they continued to differ even though previously synchronized by the external factor, light. I have heard that Dr. Menaker's work in Texas with sparrows agrees with my experience.

Second, the variation of behavior with time of day. Of course, it has been well established, by Thor and others since, that human estimation of short time intervals varies with time of day. In our experiments we have not yet proceeded to such a fine-grain analysis as would allow detection of the effect. Up to now, we have been concerned with more basic problems, but circadian effects on timing are planned for our next series.

WEISSMAN: I have a couple of comments regarding some of the data that you have measured which seem very much related to the data we are obtaining at Ames. First of all, I notice that you had both food and water reinforcers and that you selected a 4-second response requirement on the water and a 16-second requirement on the food. Our experience has been that working for food is much better in terms of performance than working for water. It is surprising that even with this low-residue diet, you may be showing the same thing.

Secondly, much of our work here with higher organisms, baboons, shows that as we increase the work requirement, we get an increase in efficiency. This was first shown by Ferster with chimpanzees, and then Findley, and I later reported a similar finding with baboons. It is interesting to note that this differs from

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the effect that you obtained by increasing the work requirements. Thirdly, I would like to mention some detailed computer analyses of errors. Animals working on a task seem to cycle, first working for food and water and then taking a break, and then working for food and water and then taking a break. When they first begin to work, their performance is very accurate, and their efficiency in terms of the way you were using the word "efficiency" is about one-to-one. As they start to satiate on food and water, we find that they start to make errors, and the errors continually increase until they stop responding. It appears as though errorless responding, error responding, and no responding are on the same continuum. Have you tried any such analyses with your data?

THACH: You mentioned that performance was "better" with food than with water as the reinforcer. My experience with the chemical diet versus water has been similar: To attain equal diet and water intake, when physiological need is equal, the water work requirement must be much lower. This is indeed more surprising, since both diet and water are in liquid form. Just how these observations fit into other work on food and water regulation, I don't know. The difference has not to my knowledge been documented. The established interaction between the two makes such an evaluation difficult.

Your second statement, that efficiency increases as work requirement increases, appears to need qualification in the light of my work and that of many others. Your procedures are vastly different and the contingencies are very, very complex. For instance, you punish "errors." In my simpler procedure, there is not even an opportunity for "errors" to occur. A trouble seems to lie in your desire to equate errors in your discrete stimulus discrimination situation to extra responses in my free operant situation. Neither the present data nor history supports this interpretation. While oranges and apples may both be classed as fruits, so errors and extra responses may both be classed as indices of efficiency, while, nonetheless, remaining distinctly unique.

The same line of reasoning could explain why your errors are sensitive to motivational variables, while my extra responses are relatively free of them.

A Program for the Study of Long-Term Adaptation to a Weightless Environment Providing Three-Dimensional Freedom of Movement

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SUMMARY

The mechanical details of a working model of a proposed small-animal space station which is currently undergoing bench tests are briefly described, together with supporting evidence pointing to the feasibility of the program. The 12-cubic-foot canister weighs less than 300 pounds when loaded for a 9- to 15-month period. It would transmit data by slow-scan television, permitting determination of the growth and activity of a group of weightless mice. If feasible, animal recovery and/or substitution by rendezvous in orbit would supplement the data collected by television.

Inclusion of a small preliminary test capsule in a manned Gemini or Apollo flight would permit determination of the effectiveness of the life support, waste disposal, and nesting arrangements before commitment to a full-scale long-term flight. Animals born and raised in such a station would have lacked throughout half a lifetime the normal otolithic and proprioceptive information differentiating subjective space into an "up-and-down" and "sideways." Given recovery, measurement of the effect of such deprivation on their adaptation to horizontal and vertical mazes in a 1-g environment would be possible.

Some effects of such changed structuring of space on group interaction and on the definition of territory are also to be expected. Such effects could be studied by observing the animals' behavior while in orbit, with different configurations of the living area. If recovery were achieved, then the degree of preexisting stress could be compared with that of Earth controls by using established techniques.

INTRODUCTION

The next generation of experiments studying the effects of weightlessness on animals must seriously consider work with unrestrained animals. Indeed, during the first ballistic flights mice were photographed in a slowly rotating drum (ref. 1). They were included to find out how an organism will behave in the weightless state when free to move in a container which is large relative to its own body dimensions. Events have moved fast, yet the recent epoch-making observations of the weightless state for periods ranging up to 2 weeks have so far been confined to men and animals which were restricted physically (ref. 2). Furthermore, the responses to weightlessness had of necessity to be those of an organism which had spent its entire life, including the vital early formative period, under the influence of the Earth's gravity. In the approaching period of detailed

research into the effects of the weightless state which rapidly is becoming feasible, consideration should be given to reversing the procedure.

We propose to study the reactions to the Earth's gravity of mature vertebrates bred and reared in the weightless environment. This approach would use subjects that have been reared in a space large relative to the animal's size; i.e., in a space station as opposed to a capsule. Groups of such animals would thus grow up in a world in which a slight thrust induces a floating movement from place to place. The consequence, as Held and Freedman point out (refs. 3 and 4), may well be an observable change due to inadequacy of the sensory-motor feedback which is important for the development of the capacity to move surely in a three-dimensional environment. In addition to this loss, the absence of the orienting acceleration of gravity on the otoliths may be expected to alter the development of the vestibular pathways in the brain. Taken together, the two influences might affect the animal's later response to test situations in a gravity field.

The following proposal deals with the biological aspects of the problem. It briefly describes the engineering of a small space station in which mammals could be maintained in orbit in a relatively large free space throughout an entire life cycle. The information that could be transmitted from such a miniature space station is then discussed, together with the information that could hopefully be derived from the animals if their recovery were to be effected.

METHODOLOGY

Capsule Design

It is proposed to place in orbit a modest-sized (200 to 300 pounds and 12 cubic feet), self-contained capsule. An operational prototype of such a device has already been fabricated and is currently undergoing prolonged tests. It was designed to meet space and weight restrictions that would have permitted its use as a piggyback experiment on one of the Saturn engineering launches. The basic design efforts were directed toward maintaining both mechanical simplicity and minimum power requirement. The device being tested uses a 5-psia oxygen atmosphere which permits the use of a relatively simple gas-management system. The feeding system dispenses standard laboratory mouse chow, and the watering system takes advantage of a commercially available drinking valve. It was planned that the principal data would be collected by a television camera.

Figure 1 is a picture of the test environmental capsule. It is 15 inches in diameter, 60 inches high, and consists of three sections. The lower section contains the oxygen supply and the pressure-regulating equipment. The middle portion houses the gas-management components and the living area. The upper section holds the food and water supplies and also provides space for the television camera, telemetry transmitter, and associated electronic gear.

Figure 2 is a cross section of the entire capsule. As can be seen from the figure, the living

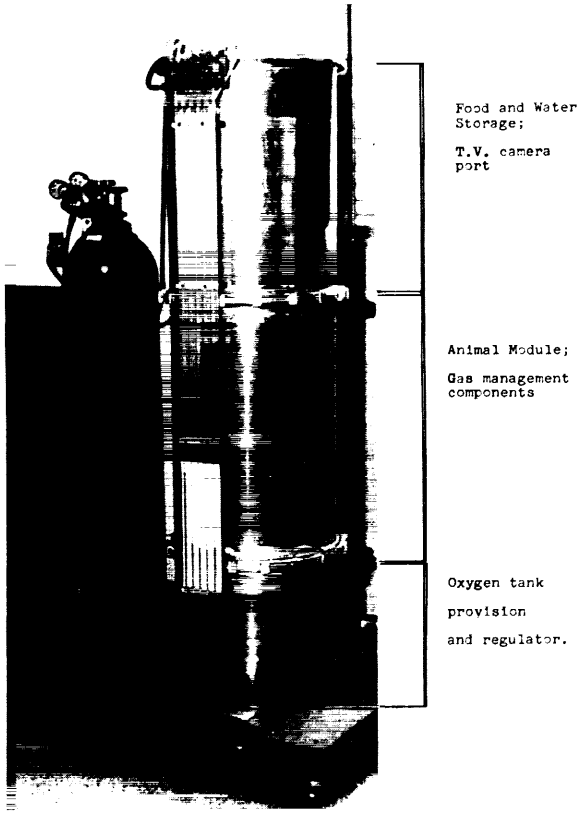

Figure 1.—*The mouse drum during a test run. Note the three-section construction and clamping rings.*

area is located above a funnel-shaped structure. Air is circulated through the living area at a velocity of one-half linear foot per minute. The restriction encountered at the funnel neck increases the airflow to about 25 ft/min. This arrangement of air circulation tends to move waste materials and water droplets to the bottom of the funnel structure. (The total calculated air pressure on the mouse at this velocity would be approximately 1/100 mg.) Appropriately placed screening removes the solid material from the airstream. Fans propel the air through the filter section.

The filter section consists of three compartments. The first in the line of airflow contains a Linde molecular sieve for water removal. The second contains lithium hydroxide for carbon dioxide adsorption, and the third contains activated charcoal for removal of certain trace gases.

Food and Water Supply

The food supply is in the form of cubes shaped to fit a series of thirty-six 18-inch tubes of approximately 1-inch internal diameter. Each tube holds 15 food cubes. The cubes are forced against a hook structure that gives the mice adequate access to the food. Figure 3 shows the food block as it is presented to the mice. The water is held in plastic bags which are in turn placed in a pressurized container. The modified Harada drinking valve permits the mice free access to water on demand. The present design allows a maximum of 10 pounds of food and 6 liters of water.

Data Collection

Observation of the mouse living area is to be provided by the television camera, together with a backup. A videcon tube will be briefly exposed by the actuation of a shutter, and the persisting image which is projected by the tube will be slowly scanned to provide a still picture which can be retrieved at any rate compatible with the capacity of the available data-retrieval system. Appropriately placed indicators of food and water supplies could be monitored by the camera. Such an arrangement would provide a relatively simple overall approach to the study of the growth and development of the mice. Other data could also be obtained by more conventional methods. This would include an appropriate measure of radiation as well as engineering data concerning the operation of the life-support system.

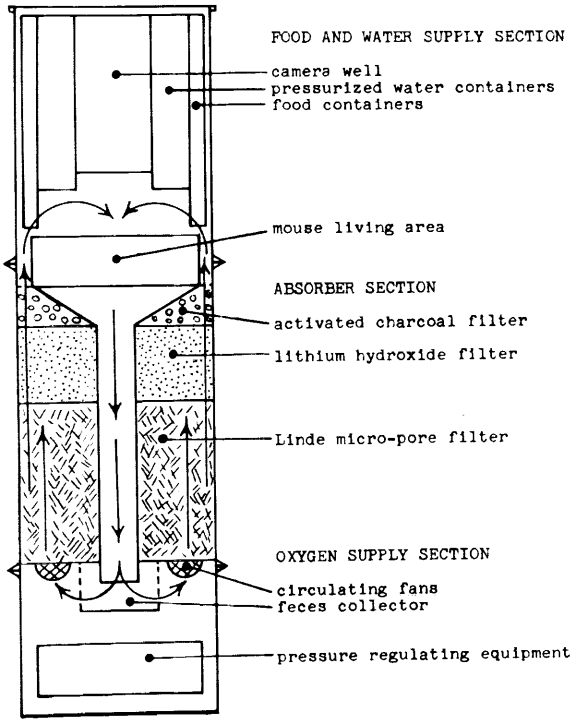

Figure 2.—*Cross section of environmental capsule. Arrows indicate direction of airflow.*

Total power requirements of the capsule are small. Continuous duty power is expected to be of the order of 4–5 watts and is determined primarily by the current required for the fan motor, lights, and continuous-duty electronic equipment. During data transmissions, intermittent requirement for 8–10 watts would be imposed. This level of power requirement makes the use of solar paneling very attractive, since such paneling could be effective without any necessity for continuous orientation of the capsule were it to be a free satellite.

Living Space Design

Initial design of the living area contemplates giving the animals a maximum of space in which to float freely. A series of nesting areas, each

Figure 3.—*Mice examining feeding and drinking station.*

with a mattress made of lengths of polyethylene film, will be provided. Tests have shown that mice will employ such material for nest building. The nesting regions would be dimly illuminated and accessible via holes in the wall of the main living area. The main chamber would be periodically illuminated more brightly and periodically completely darkened to promote development of a diurnal rhythm and to induce the animals to occupy the nesting area at times of "lights out." At the center of the main or floating chamber will be the food supply, while the water valves will be located peripherally in the general nesting area. In this way some movement can be forced on the animals as they alternately seek food and water.

These provisions could be evaluated by use of a model of the nesting area together with the feeding and drinking devices all in compact form suitable for inclusion in a manned flight.

Astronaut observation over a period of two or more days would establish whether any unexpected mechanical problems develop in the weightless state. By providing a fan to circulate the gas at the same rate as in the flight model, the effectiveness of the feces-removal system and the absence of significant wind pressure to "cue" the animals and give an undesirable direction to their living space could be determined.

By flying mice recently delivered of pups, performance of the mothers during the critical neonatal period, their retrieval, nesting, and nursing behavior could all be checked before commitment to a prolonged flight. Such a model of the living area could be provided with a separate charcoal-gas-filtration system to suppress odor. Its dimensions need not exceed the one-half cubic foot of a standard cage, and its weight could be held to less than a kilogram.

Finally, by photographing the animals during a free fall of the appropriate duration, i.e., from a high building or by an aircraft in parabolic flight, the effectiveness of the silhouette estimations of weight despite variation in posture could be determined before commitment to orbital flight.

Current Tests of Life-Support System

In tests currently in progress, the total pressure, oxygen percentage, nitrogen content, carbon dioxide content, temperature, and humidity are all being monitored. The results to date are satisfactory. The constant low nitrogen partial pressure of less than 2 percent of the total, despite the high content in the surrounding atmosphere, shows how free the capsule is from leaks. The constant carbon dioxide level of less than 1 percent and relative humidity of approximately 15 percent indicate the efficiency of the lithium hydroxide and the molecular sieve.

Growth Monitoring by Television

The prime method for determining the health and continued progress of these animals during the weightless state will be the "indirect" television coverage. A very-wide-angle lens is available which will be placed at the bottom of the camera well. This provides a view of the entire living area. The slow-scan television observes the silhouette of the animals. As figure 4 indicates, this area is directly proportional to body weight and gives a remarkably accurate measure. Much effort has been put into the design and validation of this technique by C. Lauprecht and J. Tsutsumi ("Studies of the Feasibility of Using Body Silhouette To Monitor Growth While in the Weightless State," in preparation), and its adequacy under Earth gravity conditions is now established. However, as has been indicated, further tests are planned to determine what difficulties, if any, arise during weightlessness and to solve these if they occur.

It is fortunate that the sensitive growth-weight curve can be picked up by measurement of body contours without prohibitive loss of accuracy. The method is simple and avoids mechanical problems connected with the use of some inertial measure such as the rate of oscillation of a spring-suspended container. The current plan is to rely on this silhouette measurement as the prime indication of growth and well-being. Backup indications of survival and evidence of continued food and water consumption will be available from strain-gage measures of the rate at which the spring tension in the food delivery tubes decreases as the food is eaten and from the corresponding fall in pressure in the closed water-supply system. These measures can be transmitted by commutation of a single channel of telemetry and so remain independent of the television channel.

One further measure of activity is planned. It is hoped to observe the movement of the animals from one region in the animal compartment to another by using heat sensitive elements; i.e., thermistors placed at various strategic locations. Their output can be readily commutated and so they represent a modest further load on the proposed one-channel telemetry system. By using thermistors at the feeding station, in the nesting area, and elsewhere in the living area, we anticipate a good backup of the slow-scan television. The cameras would, incidentally, be provided in duplicate to satisfy reliability requirements.

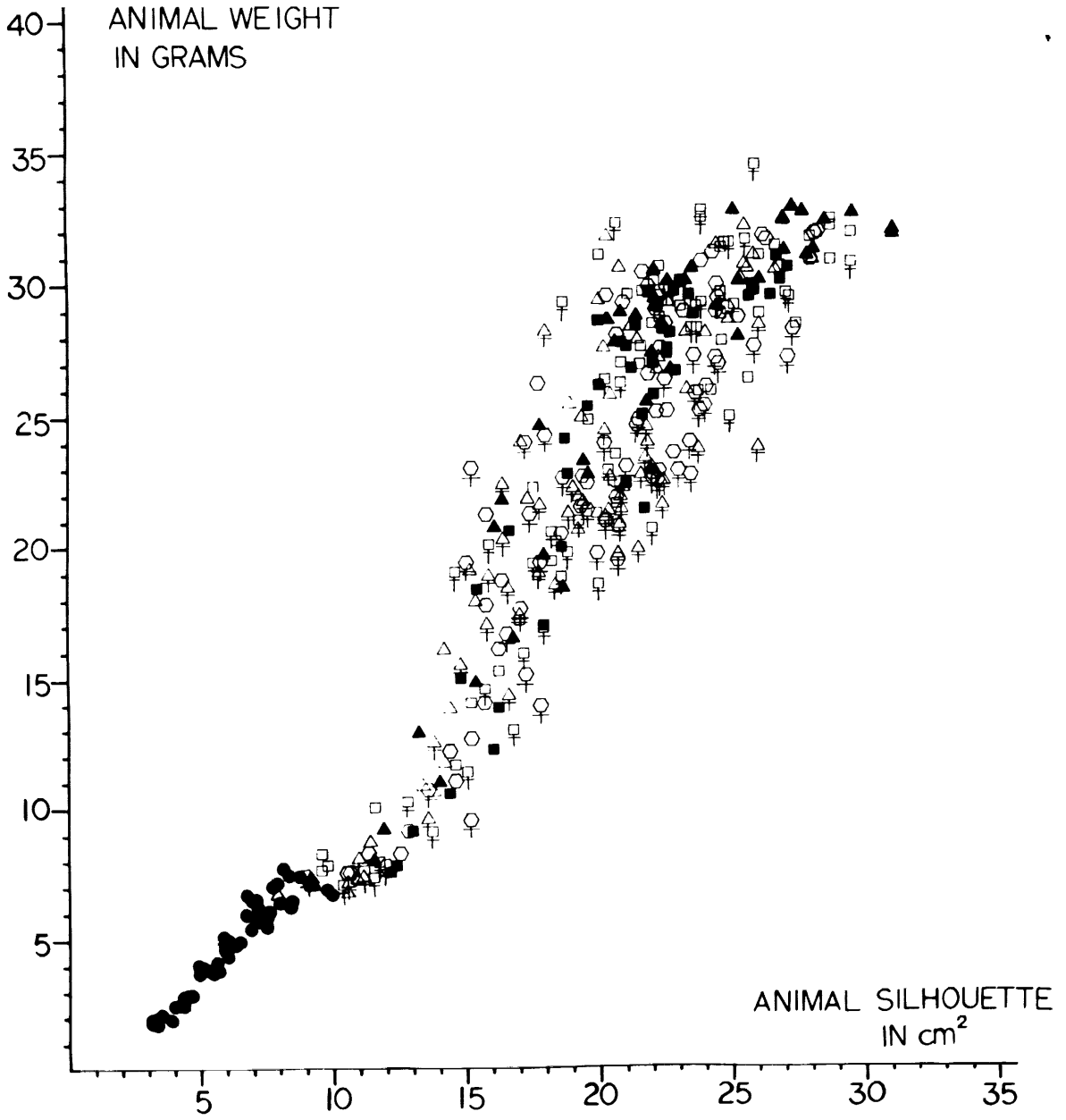

Figure 4.—*Correlation of silhouette area with weight for a litter of eight CBA mice. Solid circles represent newborn. Inflection of curve at 7 grams reflects onset of quadrupedal control. Thereafter, individual mice are followed by separate symbols.*

Possibilities of Recovery in Space by Rendezvous

A major objective of this experiment would be the recovery of mammals that had been raised in the weightless state. Such animals would have developed without gravity in utero as well as through the first months of their lives; that is, until they reached full maturity which occurs at 5 months. Rather than rely solely on television and telemetry from a nonrecoverable vehicle, it is hoped that the mice could be removed by an astronaut, following rendezvous. If the

design is successful, the mice could be induced to congregate in one restricted area, e.g., the nesting area, for a part of their day, and the television could verify their location. As has been mentioned, one way of establishing a gradient of preference for this location at certain times might be by imposing a diurnal rhythm and completely blacking out the main living chamber at the center of which the food is to be found. Rodents prefer a dim light to total darkness and would, hopefully, retire to the nesting areas which could be permanently dimly illuminated by use of phosphorescent lights. Odor may also be considered as a means of assuring the location of the animals within a small area which could then be removed after having been made gastight by the closing of a port. This removal of the mouse container could be executed by a simple manual action on the part of the astronaut. It would then be returned to Earth via the manned vehicle.

PROPOSED STUDIES

Primary Gains and Objectives: Establishment of Null Hypothesis

This design lends itself to a variety of experiments with small animals; for example, studies of the regeneration of tissues. Indeed, the successful survival and growth of normal mammals in the weightless state would in itself be an interesting observation from the viewpoint of the effects of this condition on cell growth and metabolism, as well as of its influence upon all bodily systems. A detailed study of the animals after recovery would be planned, contrasting the various systems of the body with those of normal animals. A normal growth curve during the weightless state would be presumptive evidence of satisfactory psychophysiological adaptation to the environment provided. If the growth curves were normal and unimpaired relative to ground controls, then neuroendocrine function and indeed the function of all major body systems could be presumed to be within normal limits. For example, the digestive tract must have compensated effectively for the lack of gravity, and the overall processes of cell metabolism and cell division must also have maintained adequate function. These conclusions supporting the null hypothesis that prolonged weightlessness in space flight would have no seriously deleterious effect could be drawn without need for recovery. However, successful recovery would greatly increase the information that could be obtained by permitting the use of sophisticated laboratory techniques.

Design of Living Area To Minimize Sensory Motor Feedback

As was stated in the first part of this paper, Held and Freedman (refs. 3 and 4) have described the importance of adequate feedback from the muscles and joints in the development of spatial discrimination. They speculated that organisms in the weightless state would not receive the proper quota of information as they move about because they are not kept in constant contact with the surface of their living area by gravity. This sensory-motor deficiency has added to it the absence of labyrinthine information from the otoliths. We believe that the severity of any resulting condition would be aggravated by exposure to weightlessness throughout the entire lifetime, including the prenatal period. Held and Freedman's observations have been made only with primates and cats whose plasticity and learning capacity are high. On the other hand, their experiments were necessarily brief in comparison with the lifetime of the individual; so, there is a possibility that if mice were exposed from conception to as unstructured a weightless environment as possible so that they had minimal opportunities for normal locomotion, their response might be grossly impaired. The demands of tasks such as maze running require accurate spatial localization. As Hydén (ref. 5) has shown, a device containing an inclined wire which must be negotiated to obtain food requires considerable labyrinthine cerebellar coordination. As the animal acquires facility in balancing, there are changes in the neuronal content of RNA, suggesting that adaptation to the need for learning is taking place. The design of the living area in a space station for small animals from which recovery is to be

arranged could either maximize or minimize these factors. A desirable initial objective would be to induce a maximum of sensory-motor deprivation by providing a small central feeding area with enclosed nests arrayed peripherally about a large empty space in which the animals could navigate by pushing off from the walls but could not progress normally by movements of the legs. At the other extreme the same space could be made "planar" by providing several layers of shelves between which the mice could progress and which, if made of resilient material or spring suspended, might press gently on the animals, giving them proprioceptive information, simulating that found on Earth.

An important study objective both during flight and following recovery would be observation of the group and the territorial behavior of these animals when they are confined in a relatively large three-dimensional space. Mice require a feeding area, and if the space is given them, they develop latrine and nesting areas as well (personal communication, C. Lauprecht). It would be interesting to determine how they respond to territorial requirements when in the weightless state. By studying their body contours, the tail biting typical of stress could probably be detected. After recovery, adrenal and thymic weights, renal glomerular interstitial histology, and especially resting blood pressure could be readily determined. These measures, as Christian (ref. 6) and Calhoun (ref. 7) have shown, will give a good idea of the extent to which the animals concerned have been exposed to stress.

Further Studies

It would be proposed that for follow-on flights, if the methods were successfully established, a division be made of the space available so that the equivalent of our new standard six-box intercommunicating system could be fitted into the vehicle. This box system consists of six intercommunicating cages, each of which is of the standard 11 inches by 5 inches by 5 inches, and will house a group of eight animals without evidence of social stress or elevation of blood pressure. By interconnecting the boxes and providing only one central food source, severe social stress can be induced as conflict for territory ensues (refs. 6 and 7). On the Earth at 1 g, this system is arranged on one flat plane; i.e., the interconnecting boxes are placed in a circle on a board, but modifications using three dimensions could be studied both in the 1-g environment and when weightless (refs. 8–9 and J. P. Meehan, J. P. Henry, and P. Stephens, "Blood Pressure as a Measure of Social Stress," in preparation). Mice, when crowded in the wild, will set up more runways in the same area rather than share them. The effects of having to share in a floating state a common large living space on a three-dimensional basis could be contrasted with the territory arrangements made by the mice in the relatively two-dimensional Earth-surface environment.

Performance Tests Following Recovery

If recovery were feasible, it would be planned to contrast the animal's on-the-ground-response to two- and three-dimensional mazes. Harcum (ref. 9) made such observations on rats over 10 years ago and found that animals deprived of experience of the vertical in early life showed impaired performance in a maze involving vertical movements. Hydén (ref. 5) has employed an inclined wire to "exercise" the vestibular nuclei and has demonstrated the consequent changes in neuronal RNA content. We would anticipate marked differences in animals brought up from conception without the inputs from the otoliths that orient them with respect to gravity. Their situation would be made worse by the absence from birth to maturity of the additional cues deriving from a use of the limbs under gravity loading (refs. 3 and 4). Such space-trained animals could be contrasted with those brought up under 1 g. It is not possible to predict to what extent the muscular coordination of small rodents is preprogramed. Conceivably, they need no experience during a critical learning period in order to develop effective responses in a gravity-dominated world. Certainly Held would expect primates with their very complex arm-and-hand movements to be more sensitive than rodents (ref. 4 and personal communication, R. Held). On the

other hand, work with such relatively rigid animals as birds shows that despite the clear evidence of their preprograming, they also have a critical learning period for the development of, for instance, their song. A failure of input during this period results in grossly inadequate performance (ref. 10). It remains to be seen whether half a lifetime spent by a mouse in the weightless state would not lead to serious deficiencies in motor performance because vital information had been lacking during a critical period.

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DISCUSSION

GRAYBIEL: How would you make them exercise?

HENRY: By making them go for their food to one place and for their water to another. A question has been raised, too, by the way, Dr. Graybiel, about the course of events if we have the temerity to put a pregnant female up and let her have her litter up there. How would you know whether the litter would be cared for, and so forth? This is why Dr. Meehan made the suggestion that perhaps we could do a preliminary study in a forthcoming manned flight using a very small container to establish that such care would be exerted by the mother. If we establish a little hole for them, a little nesting area with an exit into a small ⅓-cubic-foot cylinder which could be observed by the astronaut, I think we could demonstrate that the pups would be kept within that area. But we should establish this experimentally, with such a preliminary flight test. If the mother would not care for her pups when weightless, then you could put up a very young animal that is just of an age that could take care of itself, which is approximately 12 days.

WEISSMAN: I was curious as to the selection of species of mice for this test, particularly regarding susceptibility to audiogenic seizure at the time of blastoff.

HENRY: Various types of mice are available. For example, we have been in communication with Dr. John S. Barlow, of the Department of Neurology at Harvard, who is interested in animal navigation, by the way, in the weightless state, and he has commented that he thinks that it might be possible to produce animals with congenital vestibular defects, and these would be interesting to study in such an environment. But I can't think right now of what the relationship of audiogenic seizure to this situation would be.

WEISSMAN: I was thinking of blastoff.

HENRY: This can all be checked, of course, before flight.

WEISSMAN: What species are you going to use?

HENRY: A CBA, actually, Swiss whites, because they give good contrast for viewing on the television. But most of our work has been with the little, robust agouti CBA strain. As to this question of the effects of launch; fortunately, all this can be simulated. In practice neither the CBA nor the Swiss whites are unduly subject to audiogenic seizures.

MEEHAN: Also, any sound effects could be shielded out, so that wouldn't be any problem.

HENRY: Sound is not so serious a problem in launch as you might expect because the nose cone is so remote from the engines that the noise levels are attenuated to acceptable levels.

VON GIERKE: What is the probability that a capsule like this would go into spin and tumbling, if it is in orbit for a long time period?

MEEHAN: Its long shape would perhaps make it somewhat sensitive to that sort of thing. However, we are planning to redesign and get it shorter and squatter. By using the fan motor (you have to have motors to move air) and by selecting three blowers and placing them in such a manner as to operate at right angles to each other, you can achieve a gyroscope. While the force from this would not be great, again over a long period of time, it would probably give you enough stabilizing effect to prevent gross aberrations of motion.

HELVEY: Dr. Henry, did you indicate how long you kept them in the 1-g environment in such a container to evaluate the life-support system?

HENRY: As emphasized by Dr. Meehan, the period has been 1 month with a ninefold load and 3 months with a standard load, already accomplished and still running.

HELVEY: Does anyone foresee any technical reason why this couldn't very well be accomplished as indicated by these two gentlemen? We are always concerned, I think, by floating debris, such as feces, but with the airflow system which is addressed specifically to this problem, it looks like it might fly.

HENRY: We have adequate space, of course, for feces. We were worried about this and tried the liquid diet, but had problems with reproduction. We are relieved to find that others have also had problems with survival and breeding on liquid diets. But we have enough space for the residue from a standard diet, and as compared to primates, they do package their feces very neatly.

Central Nervous, Cardiovascular, and Visuomotor Studies Relating to Spatial Orientation in a 30-Day Primate Flight[1]

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SUMMARY

Central nervous mechanisms underlying orienting and visual discriminative functions are discussed. Interrelations of vestibular and optic sensory influxes with corticodiencephalic and limbic mechanisms as essential substrates for spatial orientation are reviewed. Techniques for central nervous, cardiovascular, peripheral nervous, and autonomic monitoring during the 30-day primate flight in Biosatellite D are discussed. A 6.8-kilogram *Macaca nemestrina* monkey will be tested in two behavioral tasks involving delayed matching-to-sample, and an eye-hand coordination test. Environmental support involves an oxygen-nitrogen gas system. Pellet feeding combines reward and *ad libitum* methods, with water provided from the fuel cell power system. Data acquisition and analysis techniques are reviewed.

INTRODUCTION

Spatial orientation on the basis of environmental cues has evolved in the mammalian organism to a sensitive and powerful integrative mechanism, dependent on simultaneous or sequential inputs in many sensory modalities. Visual, auditory, vestibular, somatic, and even olfactory cues all play important roles in information transacted in the subtle and continuous processes that relate the subject to a multidimensional environment. An essential parameter in this sensory integration, and one frequently overlooked, is the factor of timing, whether this be in such phenomena as the saccadic scanning of a visual field, or in multisensory barrages entering the nervous system in different modalities, and their subsequent temporal interplay in higher nervous structures. At an even more fundamental level, the coding of information in both slow waves and pulse-coded activity in cerebral tissue demands consideration of the vital role of spatiotemporal patterning in the transmission and processing of information within the brain. Temporal coding then, is truly the essential link between windows on the world without provided through sensory transducers, and windows on the world within through which we may peer, often "as through a glass, darkly," in sensing electrophysiological signals.

The cerebral cortex, in its interrelations with subcortical structures, may be likened to a screen on which the images of sensory experiences are cast in a transactional sense. It is also probably the site of their storage as permanent physiochemical changes in a "memory trace," with retrieval or recall dependent on reestablishment of electrical patterns in corticosubcor-

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tical circuits resembling those associated with the initial deposition of information. Here, the interplay of limbic structures of the temporal lobe with midbrain and subthalamic structures, and with the establishment thereby of requisite patterns of activity in corticosubcortical systems, is of vital importance. Spatial orientation draws heavily on such mechanisms, which are amenable in great degree to direct electrophysiological monitoring (refs. 1 and 2).

Specifically, integrity of the parieto-occipital cortex of the primate brain is essential to normal spatial orientation. The parietal lobe syndrome in man following damage to this region in the dominant hemisphere attests to its importance (ref. 3). It is characterized by severe impairment of navigation in previously familiar environments, and equally, by neglect of the contralateral half of the body in its normal participation in somatic and motor functions.

Yet, there is clear evidence that, in the hierarchical organization of cerebral structures, the focusing of attention to achieve spatial orientation, and the psychological processes of discrimination and judgment, require integrity of mechanisms relating hippocampal regions of the temporal lobe with subcortical structures. Participation by these systems in orientation and discrimination has been extensively investigated (refs. 4–8). In particular, it would appear that the isolated pursuit of vestibular functions, without regard to higher levels of neural integration in orienting mechanisms, may disregard quite fundamental aspects of these integrative processes in relation to space flight.

Evaluation of novel environmental stimuli and their precise spatial localization involves an essentially specific physiological response, the orienting reflex, first described by Pavlov (refs. 9 and 10). Its essential components involve turning of head and eyes toward the novel stimuli (ref. 11) and the selective extinction of separate components of the stimulus complex with repeated presentations. Sokolov has proposed an inverse relationship between the strength of orienting responses and the level of conditioning, from his studies of visual task performance in man. Evaluation of processes of spatial orientation must thus necessarily consider questions of perception, recent memory, learning, and recall (refs. 2 and 12).

In planning our flight experiment P–1001 in Biosatellite D of the Biosatellite Program of OSSA, we have attempted to run the gamut from direct assessment of vestibular functions in perception, to higher nervous functions in sleep and wakefulness, and in perception, recent memory, and visual discriminative performance. These central nervous studies have been combined with peripheral observations, including electro-oculograms, electromyograms, and galvanic skin responses. We have closely coordinated these baseline investigations with proposed cardiovascular monitoring by our coinvestigator, Dr. J. P. Meehan of the Department of Physiology, University of Southern California, and with catheterization procedures and urine analyses by Dr. A. T. K. Cockett, of the Harbor General Hospital. Inflight urine analysis will be undertaken by Dr. N. Pace, of the Department of Physiology, University of California, Berkeley, and by Dr. J. Rho, Jet Propulsion Laboratories. Calcium-balance studies will be performed by Dr. P. Mack, Texas Christian Medical Women's College. The test animal will be a *Macaca nemestrina* (pigtail macaque) monkey, weighing 15 pounds (6.8 kg) at launch.

EXPERIMENTAL DESIGN

Central Nervous Monitoring and Implantation Procedures

Implantation procedures have been described elsewhere (ref. 13), including details of histological controls on damage arising from brain movement relative to the electrodes (ref. 14). Bipolar electrodes formed of pairs of 29-gage stainless-steel tubing, insulated except at the tips, and separated by 2.0 millimeters, have been stereotaxically inserted into selected deep brain structures (fig. 1). Surface records are obtained from stainless-steel screws in the calvarium, and additional screws are used to secure the mass of acrylic covering the skull and enclosing connecting plugs.

It is planned to record 10 channels of EEG data. These leads have been selected, on the

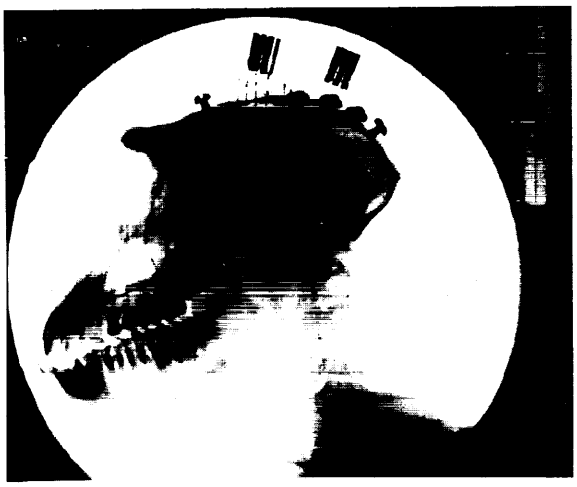

Figure 1.—*X-ray of head of* Macaca nemestrina *monkey, showing implanted electrodes of stainless-steel tubing (29 gage) stereotaxically implanted in deep brain structures. Screws in calvarium provide attachment for surface cortical EEG leads, as well as mechanical fixation.*

basis of our extensive studies in monkeys, chimpanzees, and man (refs. 15–18), as reflecting most sensitively the changing states of consciousness, including broad shifts in the range from emotional arousal and altered behavior, through drowsiness and fatigue, to actual sleep. They also specify appropriately the various stages of sleep, including dream states. In finer computational analysis, as described below, distinctive patterns can be reliably detected across decisionmaking states, ranging from simple vigilance tasks to difficult visual discriminations based on a 1-second visual task exposure ("Discriminating Among States of Consciousness by EEG Measurements," D. O. Walter, J. M. Rhodes, and W. R. Adey, in preparation). The leads are taken from the amygdaloid and hippocampal regions of the temporal lobe; from the midbrain reticular formation; and from surface leads overlying frontal, central, parietal, and occipital cortex.

Electro-Oculographic and Electromyographic Recording

Assessment of orienting responses places particular significance on monitoring head, eye, and trunk movements. Satisfactory long-term recording from electrodes implanted in soft tissues requires that they be resistant to shearing stresses imposed by movement in tissue planes. When implanted in muscles, they should not devitalize these structures to the point of inducing scar formation and loss of electromyographic activity.

A satisfactory solution to the shearing problem appears to have been found in the use of stranded stainless-steel wire, composed of 7 strands of 44-gage wire, insulated with silicone rubber. The bared terminal 1–2 centimeters of the wire have been loosely sutured through the muscles, and then threaded subcutaneously to the scalp where they are attached to the cranial plugs.

For electromyographic (EMG) recording in the neck, pairs of leads have been placed in adjacent portions of the splenius capitus and trapezius muscles. Similar leads in posterior and lateral trunk muscles have performed satisfactorily over periods of several months. Loss of tone in cervical musculature has been found a consistent accompaniment of dream-sleep states in animals and man (ref. 19), so that it will be important to assess any changes which may occur in tonic activity in cervical musculature in both waking and sleeping states during prolonged weightlessness.

Electro-oculographic leads are inserted through small holes drilled in the upper and outer margins of the bony orbit. EOG data will be valuable in monitoring eye movements during orienting responses and alerted behavior, as well as in the large and rapid movements of dream sleep.

Monitors of Autonomic Responses: Galvanic Skin Response, Impedance Pneumogram, and Electrocardiogram

Classic sensing techniques for galvanic skin responses (GSR) are not usually required to provide data for more than a few hours, so that special techniques were developed to record reliably for periods in excess of 30 days. A 2-cycle-per-second square wave, with an amplitude of a few millivolts, and applied to electrodes 1 centimeter square on the sole of the monkey's foot, has been found a reliable method for periods in excess of 30 days, with undiminished

responses to alerting stimuli, and in various sleep states, even after prolonged application.

The impedance pneumogram (ZPG) is attached to sensors in left and right midaxillary lines, and uses a carrier frequency of 50 kcs/sec with an applitude of 1 millivolt. This signal is compatible with the EEG signal conditioners, producing negligible interference in EEG records.

Electrocardiographic (EKG) records are secured from the same electrodes used for the impedance pneumogram. The location of these leads in the axillae differs from classical placements for precordial recording, etc., but qualitative information on the arrhythmias and alteration in conduction of the cardiac impulses is readily available.

Monitoring of Cardiovascular Functions

These investigations are under the direction of Dr. Meehan. His experience in the instrumentation of two chimpanzee space flights (by Ham and Enos) has provided an incomparable background in the design and performance of such experiments. Pressures will be recorded directly in femoral and carotid arteries, in the right atrium and left ventricle, by catheters connected to a total of six strain-gage transducers.

Much baseline data have been collected by Dr. Meehan in proving feasibility for a 30-day flight. Small impulse pumps, operating from a capacitor-discharge power source, inject approximately 0.003 milliliters of heparin solution into each catheter once each minute (fig. 2). Such small amounts are adequate to insure patency of the catheters, so that 1500 milliliters of solution provides an adequate reservoir for preflight preparations and flight requirements for all six catheters.

Catheterization for such extended periods requires meticulous asepsis in initial surgery and in all subsequent manipulations if infection is to be avoided. Additional prophylaxis has been provided by crystalline penicillin (1.0 μg/ml) in the heparin solution, and two subcutaneous depots of penicillin (4.0 ml, 2.4 million units each) in slowly absorbed form injected into

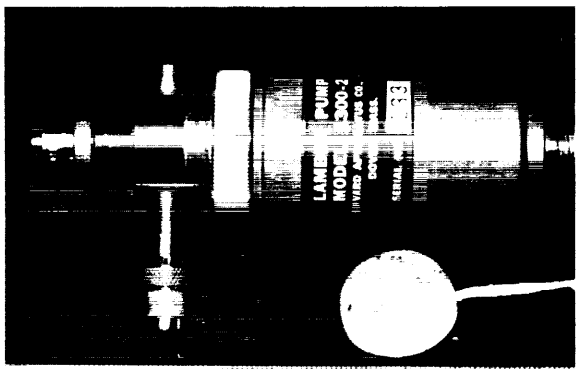

Figure 2.—*Impulse pump for injection of small amounts (0.003 milliliter) of anticoagulant solution at 1-minute intervals.*

subscapular regions. No clinical infection has occurred in catheterizations up to 50 days.

Urine and Feces Collection: Inflight Urine Analysis

As has been emphasized in relation to extended manned space flight, successful waste management ranks as a critical requirement. Moreover, urine and feces analyses provide vital information on whole body composition, against which changes in such functions as spatial orientation, discriminative performance, and biological rhythmicity must be equated if a realistic appraisal of performance capability is to be made.

Extensive investigations by Dr. Cockett have resulted in a technique of perineal urethrostomy which allows ready catheterization of the bladder, and an essentially watertight system of urine collection. He has performed extensive steroid analyses on urine so collected during couch restraint similar to that required for the flight animal.

Insufficient spacecraft power has prevented implementation of our plans to recover daily urine samples that would be fractionated and then frozen or lyophilized in flight. Other developments, however, may nevertheless make it possible to perform certain inflight urine analyses by fluorescence techniques. Dr. Pace and Dr. Rho have investigated the feasibility of measuring the concentration of calcium, urea, creatine, and creatinine in urine sampled en

route to storage in the adapter section of the vehicle. The readings will be telemetered every 6 hours.

Feces collection in the weightless state presents special problems. Our laboratory has evolved a technique which, in terrestrial testing, appears to insure reliable transfer to a collector can behind the couch. An accurately molded soft rubber pad is backed by a rigid plate, which is screwed to the ischial tuberosities. A flexible hose connects this plate with the collector, and is flushed with a disinfectant spray and air, injected perianally.

On recovery of the spacecraft, the calcium content of the feces will be analyzed by Dr. Mack, as part of her study of depletion of skeletal calcium in weightlessness, by wedge densitometry of the skeleton preflight and postflight. Dr. Mack has already made extensive baseline studies of the monkey skeleton by this method.

Behavioral Tasks, Including Visual Orientation: Food Reinforcement and Feeding Techniques

While it has been contended that investigation of weightlessness demands study of subjects in whom it is the only imposed variable, contamination of such an impressively simple situation in a primate experiment can be justified on the basis that the very perturbations introduced by tests, such as partial feeding by task performance on a scheduled basis, afford an opportunity to observe effects of a combination of variables on performance ability. Weightlessness then becomes the only variable not forming part of terrestrial baselines, and the paradigm then emphasizes the value of adequate preflight simulations.

We have included two tasks in this experiment. They will be scheduled successively both early and late in the 12-hour "day" imposed in the flight schedule. The first involves a delayed matching-to-sample test, and the second is an eye-hand coordination task.

In the first task, a symbol appears for 5 seconds in the center of a rectangular matrix, and is then extinguished (fig. 3). After a delay of 20 seconds, the whole matrix is illuminated for 10 seconds. The original symbol now appears embedded in the total matrix in a different location from that in which it was originally displayed. When it is touched by the animal, a food pellet reward is offered. Our experience indicates that this is an exacting task in recent memory and perception for the pigtail macaque, and attainment of a high-performance level takes approximately 2 months of daily training.

The second task tests coordination of eye and hand in a manner directly related to spatial orientation. Two corotating disks surround the periphery of the matrix board described above. A small window in the front disk allows access to the rear disk, on which is mounted a small red button switch (figs. 3 and 4). The disks rotate at different rates, so that the position of coincidence of window and button in successive encounters is constantly shifting in space. Our early experience indicated a surprising facility on the part of the monkey in performing this task, as well as a considerable motivation to succeed. Speeds of rotation were constantly increased to keep pace with increasing proficiency. It appears that the monkey can perform at over 80 percent correct with a window-disk rotation speed of 85 rpm, and a coincidence time for window and button of the order of a fifth of a second. To accomplish its goal, the animal has its head moving through a circular pattern at approximately the speed of rotation of the disk. This phenomenon alone

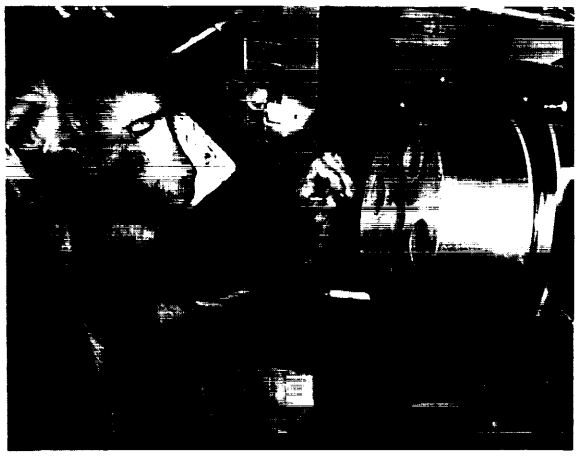

Figure 3.—*Arrangement of psychomotor test panel, showing windows for matrix of symbols used in delayed matching-to-sample task. Disks for eye-hand coordination test surround the matrix display.*

Figure 4.—*Performance in eye-hand coordination test. Subject must touch button on rear disk through window in front disk. Test monkeys' performance is more than 80 percent correct at disk rotation speeds around 90 rpm.*

suggests that vestibular disturbances associated with the rapid head movement in weightlessness may profoundly disrupt the performance, if frequent reports by astronauts and cosmonauts of disability in similar rapid movements provide a basis for comparison.

Feeding is by pellets dispensed from a feeder modified from a chimpanzee feeder, originally developed at Holloman Air Force Base for the Air Force Office of Scientific Research (fig. 5). This device carries 225 pellets on each of 8 tapes, to which the pellets adhere. Each tape is carried on a drum, and all drums are mounted on a single shaft. Pellets from each tape are dispensed separately and successively through a row of windows. Each pellet measures 2.0 centimeters by 2.0 centimeters by 5.0 millimeters, and has an energy value of 7.5 kcal. The animal may "win" 40 pellets per day by correct task performance. At the close of the "day," it may gain the remainder of a daily ration of 60 pellets on an *ad libitum* basis. To avoid hoarding, however, if more than two pellets remain unclaimed in the feeder windows, the feeder is disarmed until they are removed. Flavoring has been tested on the basis of continued attractiveness in the absence of other food.

Drinking water is provided from the General Electric Co. hydrogen-oxygen fuel cell,

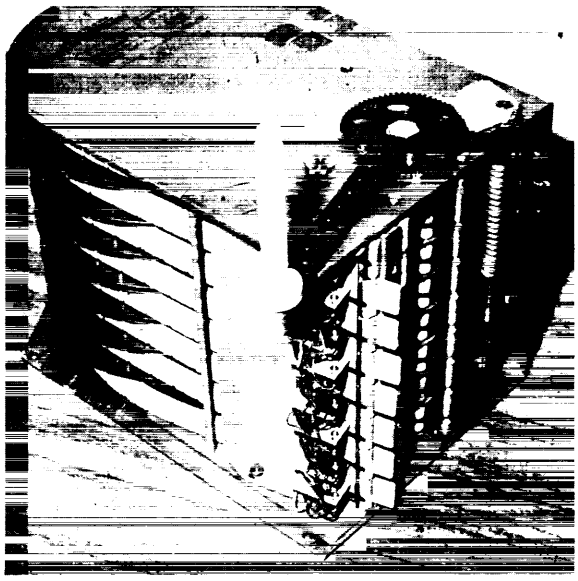

Figure 5.—*Pellet feeder, modified from Holloman AFB chimpanzee feeder. Eight tapes each carry 225 rectangular pellets on adhesive tapes. The pellets are dispensed by traction on the handle and appear in eight windows successively.*

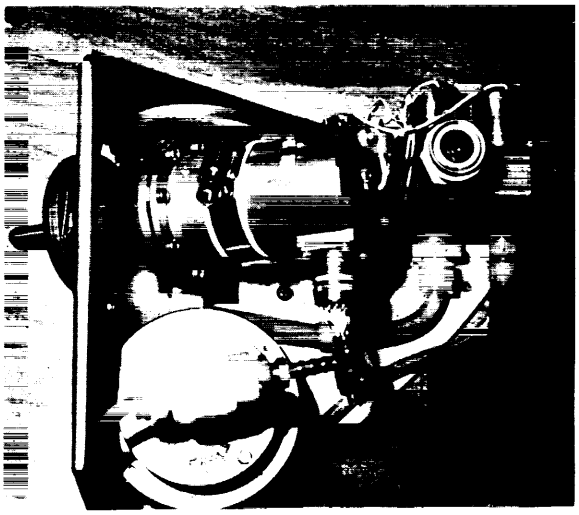

Figure 6.—*Water dispenser, having 30-milliliter capacity and solenoid controlled. This device requires suction by the animal, and is filled with filtered water from the fuel-cell power source.*

which powers the spacecraft. After filtration, the water is delivered to a nipple adjacent to the animal's mouth (fig. 6). Water rationing is at the rate of 30 milliliters per hour during the 12-

hour "day," and at one-third that rate during the "night," giving 540 milliliters per 24 hours. If telemetered data indicate dehydration, a ground command maintains "night" watering at the "day" rate, allowing 720 milliliters per 24 hours.

SIMULATION OF FLIGHT CONDITIONS RELATING TO SPATIAL ORIENTATION: EFFECTS OF ACCELERATION AND VIBRATION

In the context of this meeting, special significance attaches to those simulations testing vestibular functions and spatial orientation. Many of these studies have been reported elsewhere (refs. 1, 14, and 20).

Effects of Simple and Compound Linear Accelerations

Transverse accelerations to 5 g ($+g_y$ and $-g_y$) have little effect on this monkey's ability to perform an oddity discrimination task resembling the matching-to-sample task described here for the flight experiment.

The Thor-Delta acceleration profile for this flight imposes a peak transverse acceleration in the first-stage firing of 12 g, with the animal in the "eyeballs in" position ($+g_y$). In its initial formulation, the final injection into orbit required a vehicular spin to 100 rpm for 100 seconds, but this requirement has since been deleted. Simulations of this original flight profile revealed interesting EEG differences between simple centrifugal acceleration and compound acceleration in two planes. Evidence was also found of continuing changes in EEG and EKG patterns after the high-g pulse imposed by the simulated booster first-stage firing (fig. 7).

The effects in the visual cortex (fig. 7(C)) of the initial 12-g acceleration were minor, with a small peak in energy in middle-frequency bands from 6 to 25 cps as the acceleration reached its peak and suddenly declined. No significant peaks occurred in the low-g loading of the second stage. With stopping of the centrifuge (indicated by the 1-g vertical line), however, there was a rapid increase in energy levels between 6 and 13 cps, persisting for most of the coasting phase. Commencement of the final orbital injection phase, with 5 g of transverse acceleration and concomitant 100 rpm spin, evoked a quite different pattern of energy distribution from simple acceleration. Marked energy peaks occurred in the low-frequency bands from 3 to 8 cps. Energy distribution rapidly resumed the characteristics of control records at the end of the injection phase.

The amygdala (fig. 7(D)) showed similar changes. A moderate increase in energy in the low-frequency bands from 3 to 8 cps occurred in the "coasting" phase, with periodic peaks 30 to 50 seconds apart. Evidence of this periodic peaking was detectable at higher frequencies, but diminished progressively in the range from 13 to 45 cps.

In the hippocampus (fig. 7(B)), no significant changes in spectral distribution accompanied either initial or terminal phases of the boost simulation, although the energy levels in the coast phase rose moderately, and exhibited the cyclic changes described in the amygdala.

In summary, it would appear that changes lasting through the coasting phase may have been induced by the preceding high-g pulse in the first stage of booster acceleration, and may relate to cardiovascular readjustments and concomitant changes in cerebral oxygen tension occurring with such a pulse, as described by Kovalenko, Popkov, and Chernyakov (ref. 21).

Interrelations were noted between cardiac irregularity and paroxysmal slow-wave activity following high-g loading. In the simulated-booster profile, the pulse slowed as the acceleration approached the initial 12-g peak (fig. 7 (C)). During the following coasting phase, paroxysms of high-amplitude EEG slow waves appeared in many areas, including visual cortex, amygdala, hippocampus, and midbrain reticular formation (fig. 8(D)). Between these epochs the heart was regular, but missed beats appeared consistently during the paroxysms (ref. 20). No comparable abnormalities occurred after combined centrifuging and spinning at around 5 g (fig. 8(E)), and they may relate to readjustment in cerebral vascular mechanisms, since they always followed onset of the cerebral dysrhythmia.

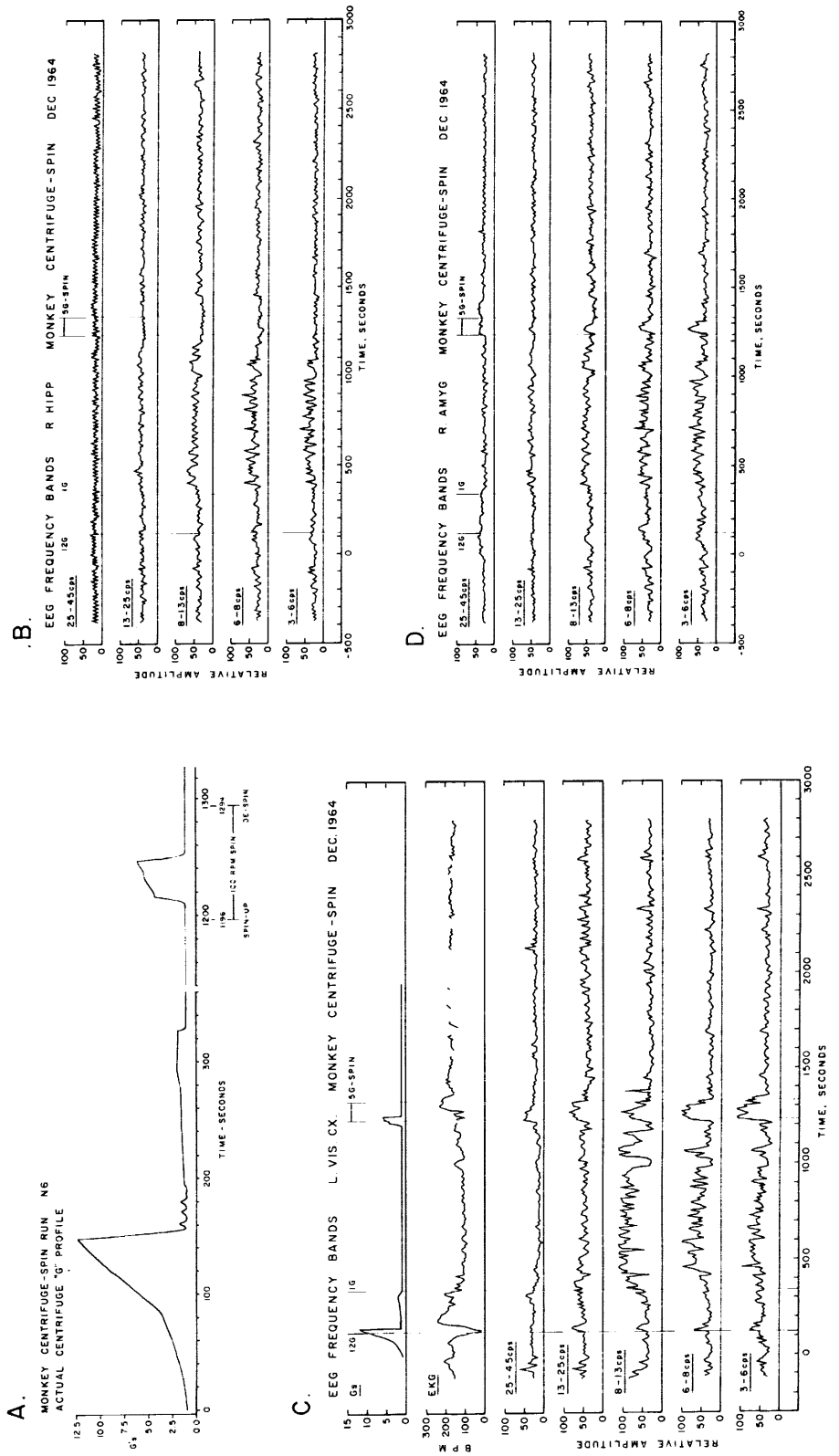

**Figure 7.—*Effects of compound transverse and spin accelerations on EEG during booster profile for attainment of orbital flight* (A). *Frequency analyses show major changes in energy distribution following high-g "pulse" in hippocampus* (B), *visual cortex* (C), *and amygdala* (D). (*From ref. 20.*)

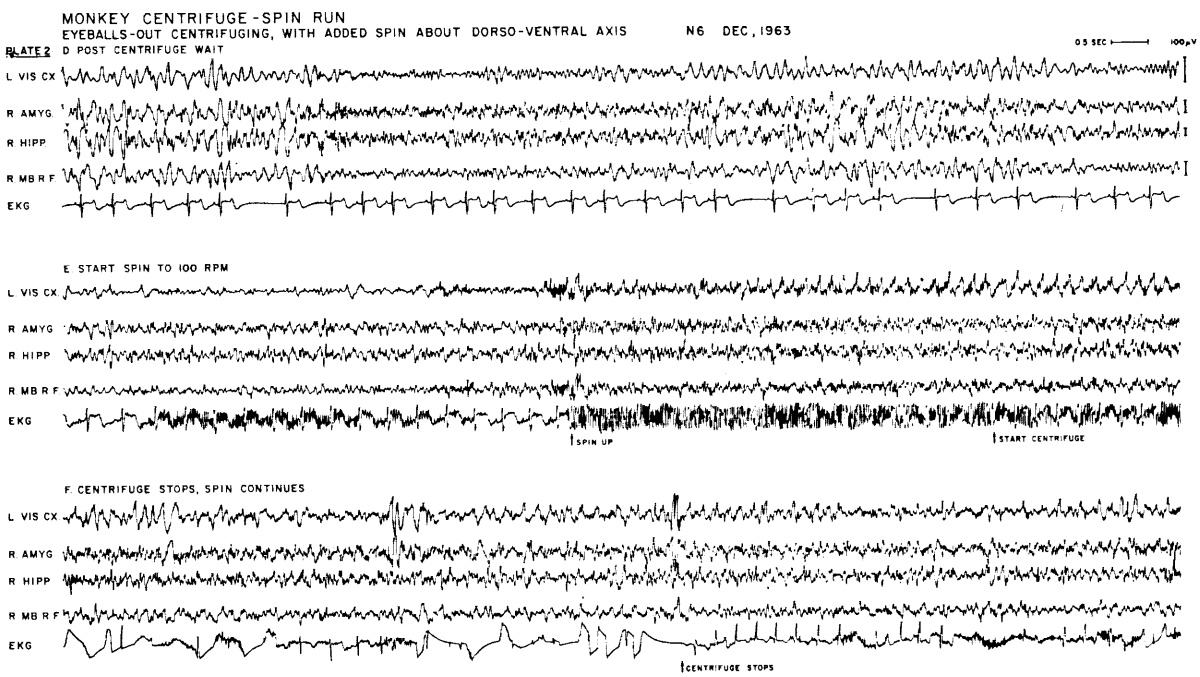

Figure 8.—*Paroxysms of high-amplitude slow waves in cortical and subcortical structures* (D), *during resting phase after 12-g acceleration, showing missed cardiac beats during EEG paroxysms, but not in intervening intervals. Effects of spin and centrifuge on EEG are clearly evident* (E). *Abbreviations; L. VIS. CX., left visual cortex; R. AMYG., right amygdala; R. HIPP., right hippocampus; R. MB. R.F., right midbrain reticular formation; EKG, electrocardiogram.* (*From ref. 20.*)

Characteristics of Cortical and Subcortical EEG Records During Whole Body Vibration: Effects of Bilateral Eighth-Nerve Section on Vibration-Induced EEG Driving

Results presented elsewhere have indicated the presence of a "driving" in EEG records from cortical and subcortical structures during whole-body vibration, at certain frequencies in the test spectrum of 5 to 40 cps, using 0.25-inch (6.2 mm) double amplitude between 5 and 13 cps, and 2-g peak-to-peak in the range from 13 to 40 cps (ref. 14).

More recent studies with extensive computer analyses (ref. 20) have confirmed earlier indications that this induced EEG rhythmicity has the characteristics of a physiological "driving," and that it appears distinguishable from superficially similar artifacts originating in connecting cables or loose plug connections. Autospectral density plots showed little or no evidence of EEG driving below 9 cps, despite violent head movements. Driving at the shaking rate was frequency selective and maximal in the range 10 to 15 cps. However, in many instances, maximum EEG energy peaks occurred at other than shaking frequencies, and without harmonic relationship to shaking frequencies (fig. 9).

Calculations of coherence (ref. 22), or linear predictability, were high between cortical and subcortical leads at EEG frequencies unrelated to concurrent shaking frequencies, and absent from baseline records before or after shaking (fig. 10). This may imply aspects of cerebral system organization with ephemeral sharing of activity elicited by the vibratory volleys. Coherence between head or table accelerometers and cortical and subcortical leads was below significant levels at fundamental driving frequencies below 11 cps, although significant coherence peaks appeared at other EEG frequencies. Shaking in the range 11 to 17 cps produced many coherent relationships at fundamental driving frequencies, and at harmonically related and unrelated EEG frequencies.

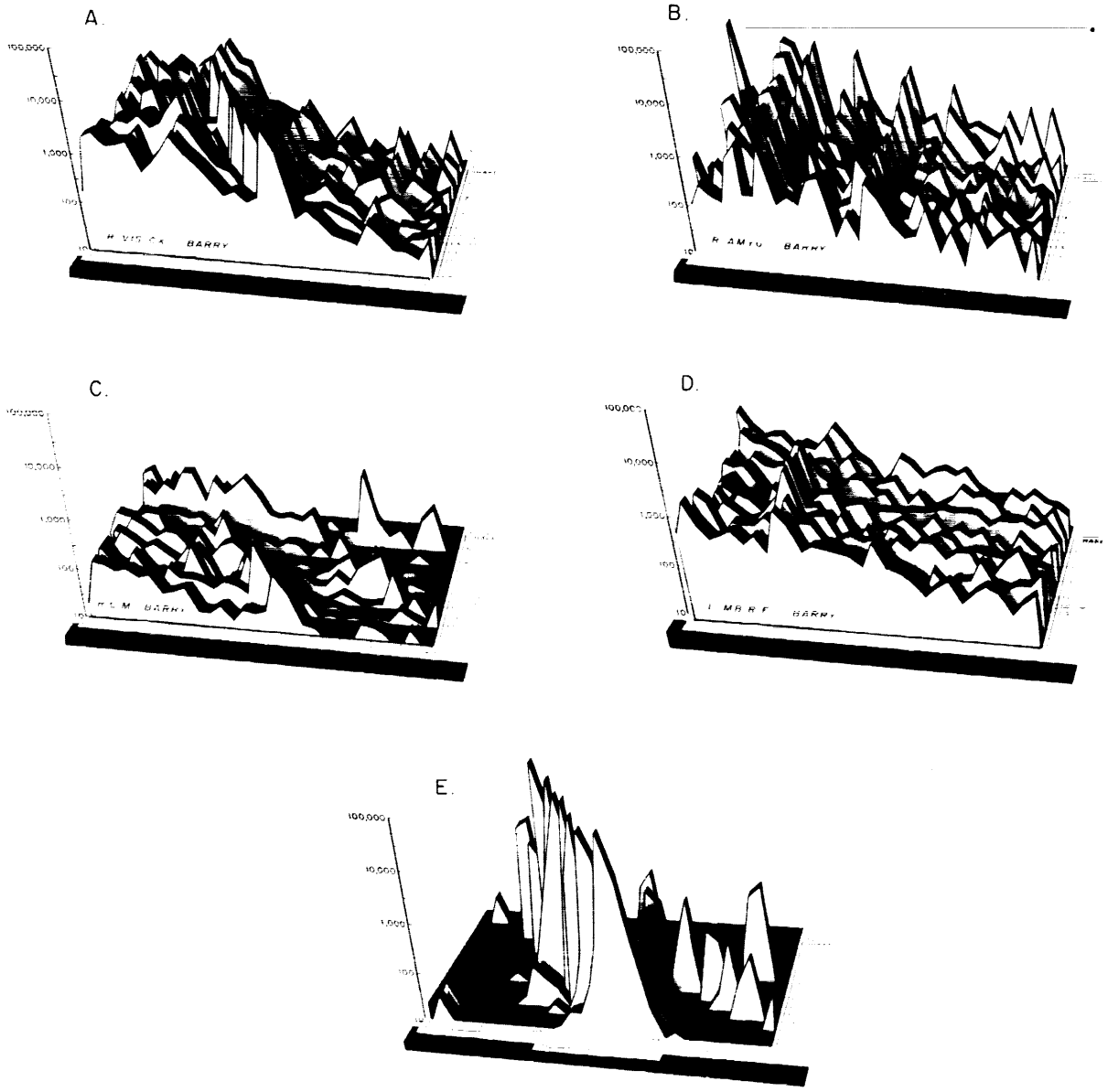

Figure 9.—*Models of autospectral contours in normal monkey before and during shaking at decreasing frequencies from 17 to 5 cps. EEG spectrum is depicted on ordinates, vibration spectrum on abscissae, and spectral power on z-axis (in microvolts²/cps/sec) for visual cortex* (A), *amygala* (B), *nucleus centrum medianum* (C), *midbrain reticular formation* (D), *and head accelerometer* (E). (*From ref 20.*)

After bilateral eighth-nerve section, there appeared to be an increased susceptibility to driving during vibration (at 11 to 18 cps) in midbrain reticular formation and nucleus centrum medianum, which showed a wide range of coherent frequencies with the table accelerometer (fig. 11). This possible increase in their driving may relate to their close relations to somatic sensory pathways. Certainly, the driving was not abolished by eighth-nerve section, although it was abolished or greatly reduced by anesthesia. Its frequency-selective characteristics suggest a physiological origin, and underlying mechanisms have been discussed elsewhere, in-

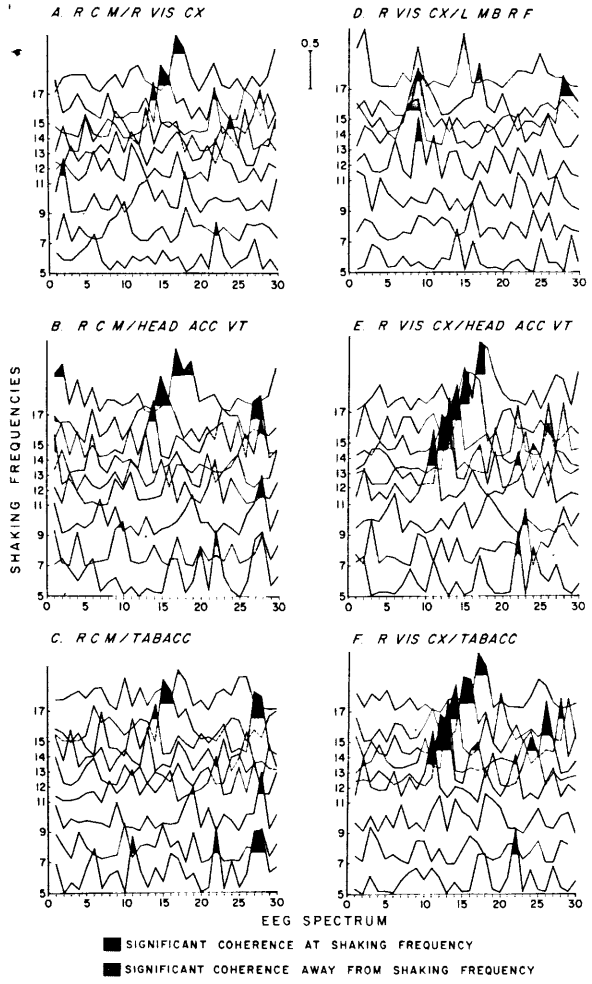

Figure 10.—*Plots of coherence (linear predictability) between centrum medianum and visual cortex* (A), *vertical head accelerometer* (B), *and table accelerometer* (C) *during vibration. Similar plots are shown between visual cortex and midbrain reticular formation* (D), *head accelerometer* (E), *and table accelerometer* (F). *Ordinates show EEG spectrum, abscissae the vibration spectrum, and z-axis the level of coherence. With 12 degrees of freedom, coherence levels were significant above 0.516. Significant coherence levels at the shaking frequency are shown in solid black, and at points away from the shaking frequency in stipple.* (*From ref. 20.*)

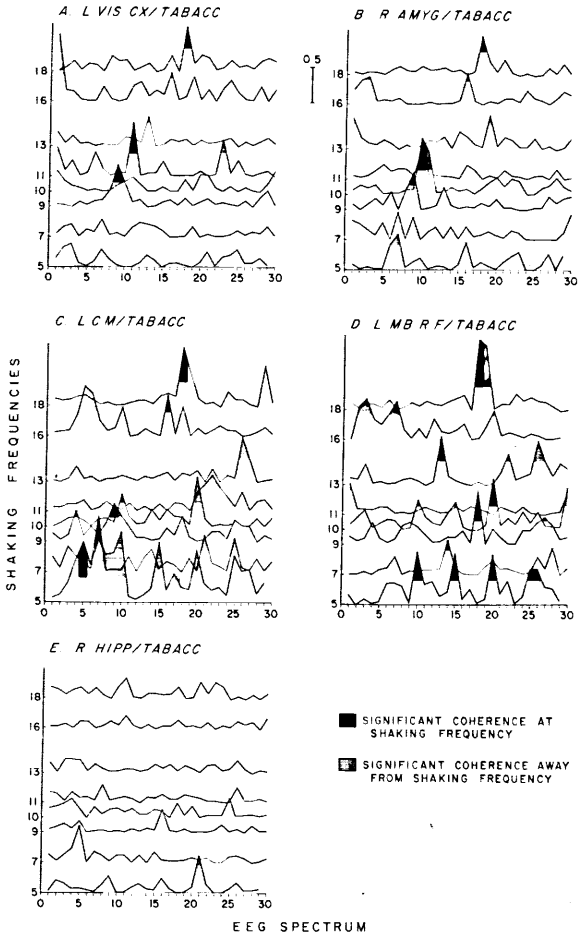

Figure 11.—*Plots of coherence as in figure 10 between the table accelerometer and visual cortex* (A), *amygdala* (B), *centrum medianum* (C), *midbrain reticular formation* (D), *and hippocampus* (E), *after bilateral eighth-nerve section. With 24 degrees of freedom, coherence levels were significant above 0.326.* (*From ref. 20.*)

cluding the role of abdominal, thoracic, and cervical tissues (ref. 20). Disruption of behavioral performance occurred at frequencies inducing EEG driving, with little or no behavioral effect at lower frequencies of vibration that also produced violent head movements (ref. 14).

DEVELOPMENT OF THE BIOSATELLITE CAPSULE FOR 30-DAY PRIMATE FLIGHT

The various systems necessary for this experiment have now reached a stage of development where system tests of substantial portions of the experiment are possible.

The couch support for the monkey is located centrally in the capsule, with the animal facing forward in the vehicle, with lower limbs flexed at hip and knee. An effective suit restraint has been developed by Wright-Patterson Air Force Base and provides for the animal to ride on a fabric sling to which the suit itself is attached

with Zipper fasteners. A torso harness assists in restraining the shoulder girdle against the couch. The animal's legs are contained in fabric sleeves and covered with a restraining apron (figs. 12 and 13).

On the animal's right are the pellet feeder and watering device. Positioned in front of him is the behavioral test panel, at about waist level. To his left, it is proposed to install a camera. Behind the couch are the feces-collecting can, and at higher level, the pumps for the cardiovascular sensors and associated signal conditioners. Also behind the couch will be the signal conditioner package for the EEG, EKG, EOG, EMG, and ZPG channels. The flight animal will be placed in the vehicle a few hours before launch, and the capsule sealed by attachment of the front hatch and heat shield. This cover will also contain batteries and tape-recording equipment.

Much effort has gone into the development of a mixed gas (oxygen-nitrogen) life-support system at 1 atmosphere. Such a system has not been previously used in U.S. animal or manned flight. Cabin temperature should be maintained within a narrow range around 70° F (21° C).

DATA ACQUISITION AND ANALYSIS

The overwhelming quantity of data gathered in such an experiment requires early and earnest planning of efficient data reduction and automated analysis. The techniques developed in our laboratory for handling massive amounts of neurophysiological data (ref. 23) and, more recently, in application of pattern recognition techniques to the initial computed outputs, lend themselves to treatment of both neurophysiological data gathered in this flight.

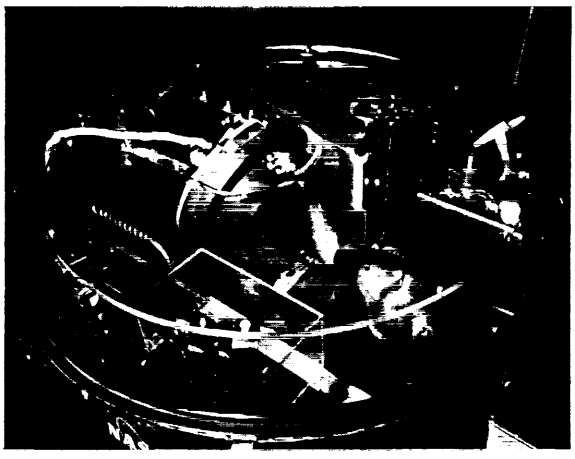

Figure 12.—*Oblique view of biosatellite mockup, showing disposition of animal on restraining couch, with behavorial programer before him.*

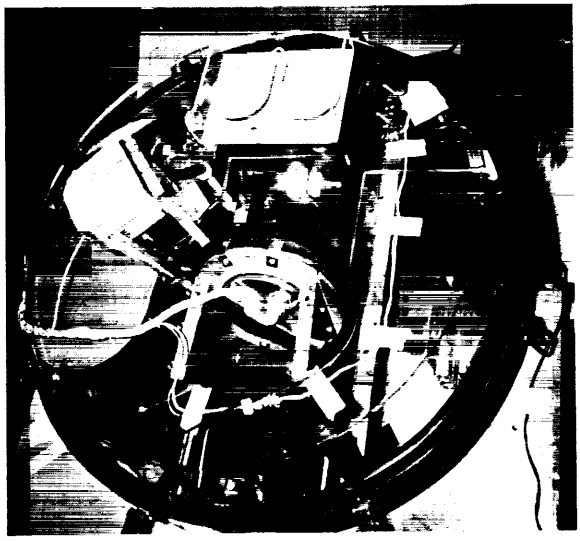

Figure 13.—*Frontal view of biosatellite mockup, showing relationship of animal to feeder and water dispenser.*

The basic procedures involve extensive spectral analyses, accomplished by digital computation, with calculation of auto- and cross-spectral densities, including phase angles, shared amplitudes, and coherence functions. These techniques, based on digital filtering methods, and endowed with great sensitivity and fine resolution, are among the most powerful mathematical tools available for evaluation of linear interrelations in wave-generating systems (ref. 22).

Analog signals from the physiological signal conditioners will be sampled at rates from 12.5 per second to 100 per second, depending on the bandwidth of signals involved, and converted to pulse code modulation (PCM) for telemetry. These signals will be acquired by tracking stations in North and South America for some part of each orbit. It is anticipated that, on this basis, approximately 10 minutes of data

will be acquired every 90 minutes. Additionally, an eight-channel tape recorder will acquire 100 hours of data in the course of the flight on a programed basis. Data telemetered in the continental United States will be retransmitted over microwave links to Goddard Space Flight Center for decommutation and transfer to a conventional digital format on magnetic tape. These tapes will be analyzed in our Data Processing Laboratory, as described above, within a short time of their receipt. It is expected that rapid analyses will keep pace with the course of the flight, meeting requirements for information on current and probably future status of the monkey.

CONCLUSION

This account of the 30-day primate experiment has reviewed the intricate and closely interrelated studies of central nervous, cardiovascular, and metabolic functions which have been painstakingly woven into a single entity.

Manifestly, orientation in space flight requires consideration, not merely of vestibular mechanisms and closely related ocular coordination, but of the whole hierarchy of functions in focusing of attention and visual discrimination. The former constitute the basic platform in a pyramid of increasingly complex central integration. The latter involve the interplay between cortical sensory systems and subcortical structures that are profoundly influenced by limbic activity. Limbic controls, particularly in the hippocampal system, appear essential to the fine focusing of attention necessary for the laying down of memory traces about spatially organized stimuli. Interference with such controls leads to degradation of spatial discriminative abilities in subtle but important ways, having particular relevance to problems of space flight.

It has been our joint purpose to monitor as comprehensively as possible within the frame of a single experiment the gamut of sensory, motor, and higher nervous functions that relate to visual coordination, spatial orientation, recent memory, and discriminative ability in prolonged space flight. We have been equally mindful of the need to consider the whole animal, insofar as maintenance of central nervous function depends so vitally on cardiovascular and metabolic integrity.

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DISCUSSION

GUALTIEROTTI: Just to clarify the issue, is it exact to say that the EEG changes are based mostly on consciousness changes?

ADEY: On consciousness changes?

GUALTIEROTTI: Yes. Do they not correspond to a status related to more or less alertness or more or less drowsiness? Or are they related to a specific function within the sensory area studied?

ADEY: The areas that we are examining in the brain are those that are concerned with the transaction of the information relating to this discriminative function.

GUALTIEROTTI: My point is a different one. Do your conclusions show a specific difference in the EEG for a specific function, or are they based on a general state of more or less alertness?

ADEY: No, sir; they relate to a specific function. There are differences that the computer discerns between a visual judgment and auditory judgment in just these circumstances.

GUALTIEROTTI: Let us consider, for instance, brightness discrimination against pattern discrimination. Can the computer determine if, given a certain alteration, this is a mistake based on pattern or brightness discrimination?

ADEY: There are differences of this type very clearly in both animals and man. For instance, in our chimpanzee studies, in fact, this sort of difference that you describe in a brightness judgment as opposed to that in which the animal makes a discrimination about patterns, and the EEG differences are very clear.

GUALTIEROTTI: If you have this marvelous tool based on the EEG, why did you go to all the trouble of actually having tests on behavior, and so forth? You might rely on your EEG alone, and just forget about pushing buttons, etc.

ADEY: For the very obvious reason that if you are going to design any form of behavorial experiment at all, it involves a performance by the individual. It isn't in the notion of merely looking at something in which the expression of a goal-directed performance can be tested.

GUALTIEROTTI: Yes; but if you have an animal just freely moving around and doing things, from your EEG analysis you should be able on this basis to determine how well he performed even if you don't have a direct measurement of the performance.

ADEY: I think we can, but the point is that most of us here are concerned with the pilot's functions in aerospace flight. We are concerned with the predictive quality of our physiological monitoring and, as I understand it, one of the prime functions of this series of experiments is to do those things which will have, at least in part, a meaningful application to problems of manned space flight. The area with which most of us are very truly concerned is, indeed, the very predictive quality of the present physiological data. I think most of us would agree that this is something in which the quality of the data and the predictability of physiological state could be vastly improved.

MONEY: Could you tell us what rates of rotation of the satellite you are anticipating? A second ques-

tion: In the selection of your animals, are you going to do any measurement of sensitivity to motion sickness?

ADEY: Yes, as a matter of fact. To answer the first part of your question, there will be some opportunity to control the slow development of spin in the spacecraft, such as will be induced by the psychomotor, for example. This device can induce precession as well as reaction, but the point is that this we think can be controlled.

Secondly, in terms of motion sickness, there is a most interesting difference between primate species in their susceptibility to motion sickness. The macaque genus is very insensitive, unlike the squirrel monkey, which is extraordinarily sensitive. I personally have never seen a *Macaca nemestrina* suffer motion sickness from horrendous, combined centrifugings and vibrations, or from multiplanar centrifuging and rotation. In other words, this animal is not a suitable animal in which to study motion sickness.

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