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FOR VESTIBULAR FUNCTION STUDIES

Makoto Igarashi, Ashton Graybiel, and F. Robert Deane

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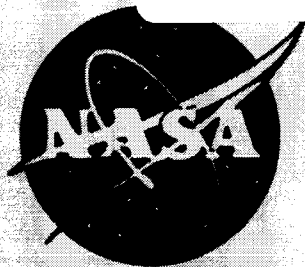
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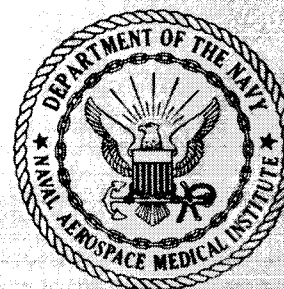
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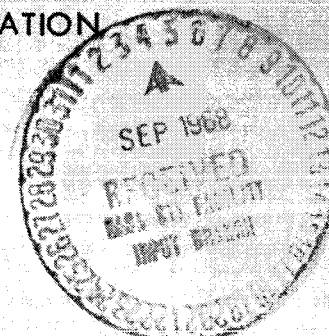
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FOR VESTIBULAR FUNCTION STUDIES^{*}

Makoto Igarashi, Ashton Graybiel, and F. Robert Deane

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NAVAL AEROSPACE MEDICAL INSTITUTE
NAVAL AEROSPACE MEDICAL CENTER
PENSACOLA, FLORIDA 32512

SUMMARY PAGE

THE PROBLEM

- Unrecognized spontaneous infection or other ear defects may present difficulties in the use of animal subjects for otological studies. In the case of the squirrel monkey, the problem became apparent during a previous experiment which demonstrated a return to normal threshold caloric test values and susceptibility to motion sickness following the administration of streptomycin sulfate in some animals in which injury to the cristae ampullares was seen in post-mortem histological examinations.

The present study was undertaken primarily to learn the incidence of otological disease in young healthy squirrel monkeys maintained under optimum laboratory conditions. A secondary objective was to extend our knowledge with respect to inferences which could be drawn from careful clinical and pathological correlations.

FINDINGS

No definite pathologic alterations were found in the middle ear except for residual inflammatory changes in the anterior pneumatization of two out of twenty-two ears examined. Morphologically, all inner ear end organs appeared normal. Therefore, it appears that young healthy squirrel monkeys with normal vestibular functions are unlikely to have disease or defect of these organs.

All threshold caloric test values were within the normal range. No significant variance was noted among the trials. Six out of eleven squirrel monkeys were not susceptible to motion sickness on repeated testings in the Slow Rotation Room. Thus, the lack of susceptibility to motion sickness does not necessarily reflect pathologic suppression of semicircular canal function.

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Experiments reported herein were conducted according to the principles enunciated in "Guide for Laboratory Animal Facilities and Care" prepared by the Committee on the Guide for Laboratory Animal Resources, National Academy of Sciences-National Research Council.

Dr. Igarashi's present address is Department of Otolaryngology, Baylor University College of Medicine, Houston, Texas.

INTRODUCTION

The squirrel monkey (Saimiri sciureus) is a small, easily handled and relatively inexpensive primate (1). Its usefulness as an animal subject has been demonstrated in space flight (3) and in the study of motion sickness and other behavioral responses which directly involve the function of the vestibular system. The question was raised whether it was possible to select animal subjects which would be free of disease or defect of the labyrinthine organs. Several studies of vestibular function previously have been performed to test the motion sickness susceptibility of this animal (6,9,11), but there was considerable individual variance in susceptibility among presumably normal animals. In addition, a previous general histopathologic evaluation of the squirrel monkey suggested that unrecognized pathology in the middle and inner ears existed to a significant degree in healthy-appearing members of the species (2).

The present study is an attempt to determine whether such pathology does exist in healthy-looking squirrel monkeys, and to correlate the results of vestibular function tests with possible vestibular end-organ pathology.

PROCEDURE

Eleven healthy-appearing squirrel monkeys, not previously exposed to any experimental procedure, were used for the present investigation. These squirrel monkeys were obtained from within a 25-mile radius of Letecia, Colombia, South America. After arriving in the United States, they were maintained for thirty days by the supplier at his primate holding compound. Upon arrival at the Institute, they were quarantined for thirty days, during which time they were tested for tuberculosis with intrapalpebral PPD 1st strength Tuberculin and treated for the removal of any intestinal parasites. The animals were housed together in stainless steel cages (22" x 22" x 16") that are equipped with a fount through which water runs continuously. No sex preference was made in selecting or housing the monkeys. They were fed Purina Monkey Chow and also received a small portion of fresh fruit daily.

Animals were placed in a cage 4 feet from the center of rotation in the Pensacola Slow Rotation Room (SRR). A previously used test method was standardized to consist of counterclockwise rotation at 10 rpm until the monkey actually vomited, or until fifteen minutes had elapsed. Stimulation by noise as needed kept the animal alert and active so that its head movements would produce Coriolis forces acting on the semicircular canal end organs and thus provide the stimulus for motion sickness. The three test periods for each animal were separated by a minimum of two days. The end point for determining susceptibility was the appearance of emesis, and the time of its occurrence was noted.

Threshold caloric tests (10) were performed bilaterally at least twice on each animal. When these two threshold caloric test values for a given ear were different by more than 0.6°C , a third measurement was carried out. Irrigation was first done with water at 36.8°C , and then the temperature was lowered in 0.2°C steps, with an

interval of at least ten minutes between tests, until nystagmic eye movements of more than four unequivocal beats appeared. During the irrigation, care was taken to keep the animal alert by tapping the restraint device or whistling. Irrigation was carried out in the dark with Frenzel goggles over the animal's face, and the eyeball movement was recorded on a Sanborn polygraph recorder by the corneoretinal potential technique.

The squirrel monkeys were subsequently sacrificed by intravital cardiac perfusion with Heidenhain's Susa fixative. The temporal bone block, including both middle and inner ears, was dissected from the skull and immersed in the same fixative for further fixation. The bone was thereafter decalcified by 5% trichloroacetic acid solution, and the end point of decalcification was chemically detected. Dehydration was done by processing the specimen in 30%, 50%, 70%, 80% (with iodine solution in it) 95%, 100% of ethanol and ether-ethanol (1:1). The specimen was processed to 3%, 6%, and 12% celloidin, and hardened very slowly. The temporal bone was sectioned serially in a horizontal plane at $20\ \mu$. One of each ten sections was stained in Hematoxylin-Eosin and examined by light microscopy (5,7).

RESULTS

Five squirrel monkeys were found to be "motion sickness sensitive." (See Table I.) Two of them vomited in all three trials and the other three in two out of three trials. Vomiting occurred within nine minutes of rotation. The remaining six monkeys were non-"motion sickness sensitive." Of these six, five never vomited, and one did so after eleven minutes of rotation only once out of the three trials.

The variance in threshold caloric test values in the initial two ^{trials} ~~tests~~ of each ear was less than 0.2°C on seventeen of the twenty-two ears, and less than 0.4°C on two other ears. A third measurement was made on each of the remaining three ears since threshold caloric test values in them varied from 0.6°C to a maximum of 2.2°C . The mean of twenty measurements on the ten ears of the five motion sickness sensitive squirrel monkeys was 34.6°C ; twenty-seven measurements on the twelve ears of the six non-motion sickness sensitive animals produced a mean of 35.1°C . No significant difference was noted in the threshold caloric test values of the left and right ears. The means of the threshold caloric test values for the less sensitive ears from five motion sickness sensitive and from six non-motion sickness sensitive monkeys were 34.2°C and 34.2°C , respectively.

Histopathological study (Table II) revealed no significant pathology in either middle or inner ear apparatus. This particular group of squirrel monkeys exhibited very clean ears (Figures 1-4). Six ears showed a little amount of acellular serous exudate, mostly between the anterior malleolar process and chorda tympani nerve. No cell infiltration to the surrounding tissues was observed. The two ears from one non-motion sickness sensitive monkey demonstrated probable residual inflammatory change in the limited area of the epitympanum; a slight thickening of middle ear mucosa and mucosal folds, and a small round cell infiltration were noted (Figure 5). However, these findings were not directly connected to the inner ear apparatus which was found

to be morphologically normal. No acute infection was found in any ear. All inner ear end organs were morphologically intact and thus it could be expected that they had normal function. Considerable densely stained (eosinophilic) perilymph immediately below the footplate (in the vestibular perilymphatic space) in six other ears, and in scala vestibuli and tympani of the cochlea in two others, was observed; however, this finding is presumed to be nonpathologic.

DISCUSSION

The results of the motion sickness tests in the SRR are the most interesting feature of the findings. On the basis of the threshold caloric test results and morphological findings, it can be assumed that the semicircular canals of all the monkeys functioned normally. In another study in this laboratory (6), injection of streptomycin sulfate in squirrel monkeys caused temporary suppression of canal end-organ function, as indicated by 1) ataxia, 2) threshold caloric values, and 3) cessation of vomiting during exposure to rotation at 10 rpm in the SRR. Prior to the suppression the animals had vomited on each such exposure. Thus, normal semicircular canal function in at least one ear, or even a part of one ear (4,6,9), is essential to cause emesis in the SRR. The present investigation, however, does not show this particular aspect sufficiently.

In screening squirrel monkeys for vestibular studies, several facts must be considered. A normal threshold caloric test value appears to indicate that histopathologic findings and semicircular canal function will also be normal. As shown in this study and supported by past experience, as exhibited in Appendix A, a normal threshold caloric response is not necessarily indicative of motion sickness susceptibility on the SRR, nor is the reverse true. However, the response of motion sickness sensitive animals (those that have emesis at least twice and usually three times in three consecutive SRR trials) is predictive of normal end-organ morphology, threshold caloric test value, and semicircular canal function in at least one ear. A lack of emesis on the SRR has no predictive value and provides little information for screening purposes. Threshold caloric test values, therefore, give the best help in screening the animals, while SRR emesis in two or more of three trials appears to be of considerable value in showing that at least one ear is normal, and as an indicator of semicircular canal function.

Histologically, the ear apparatus of this group of squirrel monkeys was relatively clean as compared to that of other species (8). Densely stained eosinophilic perilymph was found in six ears of the present series, but this cannot be termed a pathological finding as it is frequently seen in normal ears and is usually indicative of high protein or fibrin contents in the perilymph. Such a finding is more reasonably explained as a physiological one or as a result of the technical procedure.

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Table 1

Results of Slow Rotation Room and Threshold Caloric Tests in
a Group of Eleven Squirrel Monkeys

Motion Sickness Sensitive Monkeys	Incidence of Emesis in SRR* No. of Trials	Time of Occurrence (min.)	Threshold Caloric Test Values ($\pm 0.2^{\circ}$ C)		
			Right Ear	Left Ear	Lower**
N Y	2	5, 8	36.0	36.2	36.4
N Z	2	3, 5	34.4	34.6	31.8
O B	3	3, 9, 4	36.2	36.0	36.4
O C	3	3, 9, 5	34.2	34.2	34.0
O G	2	5, 7	33.6	33.6	33.0
			Mean: 34.6		34.2
Non-Motion Sickness Sensitive Monkeys					
N W	1	11	35.4	35.8	36.0
N X	0		33.8	33.8	35.6
O A	0		33.8	34.4	34.8
O D	0		34.6	34.8	35.2
O E	0		36.2	36.2	35.6
O I	0		34.2	35.4	34.0
			Mean: 35.1		34.7

* Number of times emesis was produced in a total of 3 trials and the time in minutes after rotation at 10 rpm at which it appeared.

** Mean of the two values for ear with lower threshold temperature.

Table II
Histopathological Findings in 22 Squirrel Monkey Ears

Monkey	Ear	Tympanic, Anterior, and Mastoidal Pneumatization	Cochlea	Semi circular Canals	Otolith Organs
N W	R	-	-	-	-
	L	*	-	-	-
N X	R	**	-	-	#
	L	**	-	-	#
N Y	R	-	-	-	-
	L	-	-	-	-
N Z	R	-	-	-	-
	L	-	-	-	-
O A	R	-	-	-	-
	L	-	-	-	-
O B	R	*	-	-	-
	L	*	-	-	-
O C	R	*	-	-	-
	L	*	-	-	-
OD	R	-	#	-	#
	L	-	#	-	#
O E	R	-	-	-	#
	L	-	-	-	#
O G	R	-	-	-	-
	L	-	-	-	-
O I	R	-	-	-	-
	L	*	-	-	-

* Acellular serous exudate-like fluid in epitympanum.

** Probable old inflammation in the limited area of epitympanum.

Eosinophilic perilymph.



Figure 1

Low magnification photomicrograph demonstrating intact middle and inner ear of a squirrel monkey. X8

A: Anterior pneumatization

Y: Incus

D: Middle ear cavity

R: Stapes

M: Mastoid pneumatization

F: Facial nerve

W: Carotid artery

L: Lateral semicircular canal

T: Tensor tympani muscle

P: Posterior semicircular canal

X: Malleus

S: Saccule



Figure 2

Photomicrograph showing normal organ of Corti and saccule of a squirrel monkey. X45

M: Organ of Corti, middle turn of the cochlea
 B: Organ of Corti, basal turn of the cochlea
 S: Saccule

G: Vestibular ganglion (Scarpa)
 F: Footplate of stapes
 T: Tensor tympani muscle



Figure 3

Photomicrograph demonstrating an extensive anterior pneumatization from a normal squirrel monkey. X22

A: Carotid artery

E: Eustachian tube orifice (opened)



Figure 4

Photomicrograph showing a good view of utricle-endolymphatic valve (V) from a squirrel monkey. No post-mortem change is seen both in organ of Corti of the basal coil (B) and in saccular macula (S). F is footplate of stapes. X55



Figure 5

Photomicrograph showing a site of mild inflammation at the anterior edge of the epitympanic pneumatization. Arrow indicates dense precipitate. X31

T: Tensor tympani muscle
 C: Chorda tympani nerve
 A: Anterior malleolar process
 I: Incus

F: Facial nerve
 R: Stapes
 S: Saccule

Appendix A

Results of Slow Rotation and Threshold Caloric Tests of Squirrel Monkeys Carried out During July 1964-April 1966

Monkey	Threshold Caloric Test Values ($\pm 0.2^{\circ}\text{C}$) [#]							
	1	2	3	4	5	6	Right Ear	Left Ear
OQ	0	5	9	6	8	11		
OR	0	0	0	0	0	0		
OS	0	0	0	0	9	11		
OT	0	0	5	0	4	11		
PA	0	0	0	0	0	0		
PB	0	0	0	14	0	3		
PC	0	5	4	11	5	5		
PD	0	15	4	6	0	11		
PE	0	0	0	0	0	11		
PF	0	0	0	11	0	0		
PG	0	0	6	0	0	13		
PI	0	9	9	8	0	0		
PJ	0	0	0	0	14	0		
PK	0	0	0	0	0	0		
PL	10	0	13	0	0	0		
PM	0	0	7	10	0	0		
PN	5	0	0	0	0	0		
PO	0	0	8	0	0	0		
PP	0	0	0	0	0	0		
PQ	0	11	0	0	0	0		
PR	0	7	8	0	0	0		
PS	1	3	3	13	8	5		
PT	0	0	0	0	0	11		
PU	0	0	0	0	0	0		
PV	2	2	1	3	7	13		
PW	0	0	0	0	0	0		
PY	0	9	8	6	9	0		
PX	6	13	0	0	0	0		
PZ	0	0	13	15	0	0		
A49	0	0	0	0	0		38.8	36.0
A50	0	0	0	0	0			
A51	14	6	11	10	0		31.4 31.4	31.6 31.2
A52	12	7	5	6	9		34.8 34.4	35.2 35.0
A53	0	0	0	0	0		32.0	(operated)
A54	0	0	0	0	0		35.0	34.6
A55	13	0	7	10	11		36.4 36.0	36.4 36.0
A57	10	6	5	5	4		35.6 35.4	35.4 35.4
A58	9	0	0	13	0		33.8	34.0
A59	0	0	0	0	12		33.8	32.0
A60	0	0	0	0	14		35.2	35.0
A63	0	0	0	0	0			
A65	3	6	5	11	10		35.8 35.6	35.8 36.0
A66	0	0	0	0	10		33.2	(operated)
A67	0	0	0	0	0		35.8	36.2
A68	0	0	0	12	0		36.0	36.0
A69	11	6	6	9	6		35.8 35.4	35.4 35.2

* Zero (0) indicates that emesis did not occur. Other numerals denote time of rotation, in minutes, until it occurred.

Threshold caloric test values not determined on first 29 animals listed.

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