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CONDITIONED-REFLEX ACTIVITY OF RATS AT LATER PERIODS AFTER THE END OF FLIGHT ABOARD THE KOSMOS-605 BIOSATELLITE

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This is the second installment of the study of aftereffects of spaceflight aboard the Kosmos-605 biosatellite on the higher nervous activity (HNA) of rats. In the first installment, the period under investigation comprised the first two weeks after completion of flight (Item No. 4319). As in the preceding study, a five-lane maze with a feeding terminal was used, involving 10 experimental rats during the 15th to 50th day after the end of the flight, checking such factors as transfer of experience the habit and speed of reaching the goal in the maze, long-term memory, and the dynamics of errors. During the 3rd–7th post-flight week, functional disturbances in the rat HNA were manifested in the deterioration of the capacity for the transfer of experience and for locating the feeding compartment in the maze, thus indicating a general decrease of work capacity. The increased number of errors and failures pointed to exhaustion of higher nervous processes and to the weakened functional activity of the brain.
The study of higher nervous activity (HNA) in experiments on animals at later periods after prolonged space flight is of considerable interest. It makes inferences possible about the influence of prolonged flight on HNA and about re-adaptation to ground conditions. In the present paper the HNA of rats was investigated during the 15th to the 50th day after the conclusion of a flight aboard the biosatellite "Kosmos-605". We reported on the state of HNA of these animals during the first two weeks after the flight in an earlier publication [2].

**METHODOLOGY**

The experiments were performed on male rats of the Vistar strain, each weighing approximately 200 g at the beginning of the flight. In the experimental group there were ten, and in the control group, 13 rats. To study the HNA, a maze of the type described by Ya. Dombrovska [1] was used. The maze consists of five parallel lines. In each of the first four lanes there were four doors each. Three of these four doors were locked, and one was completely closed, but not locked, so that a rat could pass through it into the next lane. The fifth lane led into a food compartment, where food was available to the animals.

Before the flight the experimental and control rats developed the habit of reaching the goal in the maze. It was re-established in 8-9 days after the end of the flight.
From the 15th to the 50th day, inclusive, after the end of the flight the transfer of experience, the development of new habits, the dynamics of errors during the experiment and long-term memory were investigated.

To study the capacity to transfer experience, the door in each lane of the maze which was previously unlocked, was locked, and one of the previously locked doors was completely closed. The change in the speed with which new habits were established in comparison to the acquisition speed of the pre-flight habits was determined.

Long-term memory was tested by comparing the number of mistakes in the first run in the given experiment, and in the last run in the experiment performed the previous day [7].

The results of the investigations were compared with the results in two control experiments on the ground. In one of these, called the "synchronous" experiment, we studied the effect on the HNA of the rats due to their stay in a mock-up of the spacecraft which imitated given environmental conditions in flight [8]. For technical reasons, in the case of these animals there was no preliminary study of the HNA and they begin the habit development after the stay in the mockup. In another experiment, called the "mock-up", the rats were placed in a mockup of the spacecraft which did not imitate the given conditions occurring on board the biosatellite "Kosmos-605". Examination of these rats before and after being aboard the mockup was carried out according to the same plan as for the 'flight' animals. Simultaneously, with each of the three experiments mentioned we also investigated groups of rats under the usual vivarium conditions which we shall henceforth call control conditions.

The data obtained were processed in accordance with the criteria for comparing two regression series, "ϕ1" [4], the medians, and in individual instances, according to Student's criterion [6].

To analyze the number of errors and refusals to run the maze on the part of the experimental and control groups we selected rats for whom these factors were similar (henceforth they will be called partners). There was essentially no difference in the average time to run the maze in both groups prior to the flight; therefore in the statistical treatment data are included which was obtained from all animals.

During the course of the entire experiment it was observed that in the first days there were occasions when a rat would refuse to run
the maze; therefore during an analysis of errors, and the elapsed time for running the maze, it was assumed that training began on the day when the rats ran the maze for the first time.

Flight conditions on the biosatellite "Kosmos-605" were described previously [8].

Research Results

Development of the Second Habit. On the 15th postflight day, the development of the second habit of reaching the goal in the maze began. In the case of the vivarium control group, the capacity to transfer experience was clearly expressed. At all stages of the development of the second habit, the rats of this group made fewer errors than during the development of the first habit. In contrast to them, the flight rats did not use their previous experience at all during the first two days of training when developing the second habit, and in the following 3 days they used it significantly less effectively than did the control rats, (Figure 1B: R < 0.05).

The percent of refusals to run the maze during development of the second habit increased in both groups. But in the case of the control rats, the increase was not substantial and lasted for only 2 days. In succeeding days, the percent of refusals by the control rats became significantly less than during the development of the preceding habit. For the flight rats, the percent of refusals was extreme and definitely increased during the entire observation period (Figure 2B; R < 0.001).

The time for running the maze also turned out to be increased significantly during the development of the second habit in the case of the flight rats (Figure 3B, R < 0.001).
Development of the Third Habit. On the 21st day of the postflight investigation, the development of the third habit of reaching the goal in the maze was begun.

For 5 days the number of errors was practically identical in the flight and control groups. On the first day of training fewer errors
were made by both groups than during the development of the previous habits. But in the next two days the number of errors did not decrease as was observed in the preceding stages of the study - either it did not change, or it increased (Figure 1c). Only after the 4th and 5th day did the number of errors begin to decrease slowly, but it merely declined to the level of the first habit.

Thus, with respect to the given parameters, some deterioration of the capacity to transfer experience was observed not only in the flight group, but also in the control group. This agrees with the data in the literature [1]. This can be explained by the fact that each development of a new habit involves a conversion of the previous habit, and a second conversion is difficult for rats, as is well-known. Although the influence of the difficulty of a second conversion showed to an equal extent in the number of errors made by the rats in both groups, the superiority of the control rats over the flight rats was discovered by means of the other parameters.

The number of refusals to run the maze in the case of both comparable groups of rats during the first days of the development of the third habit was significantly less than during the development of the second habit, but then it increased (Figure 2b, 2c). The changes in the percent of refusals in the first 3 days of examination were identical, both as to dynamics and value. However, on the 4th and 5th day distinct differences appeared. At that time, as the number of refusals by the control animals approached zero, in the case of the flight animals it was still increasing. The differences from the control were authentic \( R < 0.001 \).

The time to run the maze was substantially the same in both comparable groups during the first three days of the development of the third habit. Beginning with the fourth day of training, the flight rats began to take significantly more time to attain the goal than did the control rats (Figure 3c).

The Change in the Number of Errors from Run to Run During the Experiment. Four basic types of error dynamics were observed in the course of the experiment: 1) a monotonic decrease in the number of errors from run to run; 2) a constant number of errors during the experiment, 3) a curvilinear dependence of the number of errors on the number of runs, 4) a monotonic increase in the number of errors during the experiment. Type 1 is optimal, and types 3 and 4 are the worst.
Their dynamics indicate exhaustion of the nervous processes in the higher branches of the central nervous system and a reduction in the capacity for training.

Tabulation of the instances of types 3 and 4 discloses the essential differences between comparable groups of animals. In the case of the control rats whenever a new habit was developed a successive reduction in the number of deviations from the optimum error dynamics (from 30 to 19%) was noted, although this trend could not be verified statistically. In the case of the flight animals during development of the second habit a change in the number of deviations from optimum error dynamics was not observed, but when the third habit was developed, a sharp authentic increase in the number of such deviations (up to 43%) was noted, both in comparison to the second habit, and in comparison to the control animals (R < 0.05).

Fixation of the Third Habit. Beginning with the 26th day after touchdown, experiments were performed on four flight rats only. In the control there remained the previous collection of animals. Continued fixation and improvement of the third habit were investigated. For convenience in analyzing the data were present on all subsequent figures the results of the experiments on these four animals, not only in the last period, but also during the preceding stages of the work. Here, as a control, we present the data obtained from all the rats, as well as from the control partners.

During the development of the first and second habits, and also during the 5 days of development of the third habit, the relationships in the changes of the parameters under study between the four flight animals and the control animals were analogous to those between the control group and the entire flight group. This provides the basis for assuming that the data obtained from the four animals until the end of the experiment and subsequently are in conformity with the average level of the parameters of the entire flight group.

During the first day of the development of the third habit the number of errors and refusals to run the maze were not essentially different for comparable groups. However, both parameters subsequently became markedly worse for flight rats than for the control rats (Figure 4c and Figure 5c; R < 0.001). Some normalization of the number of errors began with the 15th day in the case of the flight animals, and with the 20th day of the development of the third habit, with regard to the number
of refusals.

The difficulty in the development of the third habit which the control animals experienced in the first 5 days was also detected during its fixation.

In the case of this group the percent of refusals (Figure 5C) increased in undulating fashion and had not declined even by the 23rd day of the development of the habit. The flight animals preserved until the end of the observations the deviation from the control animals with regard to the magnitude of the given parameter and they demonstrated a very similar picture in its dynamics (especially in comparison to the partners: Figure 5c). It may be assumed that the change in the behavior of the two groups was caused by the action of common factors (internal and external). However, in the case of the flight animals the reactions to these factors were significantly acute.

Figure 4: Change in the average number of errors in the case of 4 flight rats (I), the control partners (II), and all control animals (III). Designations the same as in Figure 1.

Figure 5: Change in the average percent of refusals to run the maze in the case of 4 flight rats (I), the control partners (II), and all control animals (III). Plotted along the y-axis, % of refusals. The remaining designations as in Figure 1.

For the flight animals the time to run the maze in the period under consideration (Figure 3c) was higher than for the control animals (R < 0.001). Complete synchronization between the changes in the given
parameter and the number of errors was not observed.

Thus, in the 1st and 3rd day of the development of the habit the flight animals spent the same time in traversing the same, but made a markedly different number of errors. Yet an increase in the maze traversal time was recorded from the 9th to the 10th day and from the 16th to the 17th day, while the number of errors was unchanged (cf. Figure 3 and 4).

This shows that the increase in the time to traverse the maze in the case of the flight animals was caused not only by an increase in the erroneous movements, but clearly also by a decrease in their motor activity. This statement is confirmed by the behavior of the animal at the time of the experiment. During the first 3 days of the development of the third habit, the time to traverse the maze was minimal and differed little from that in the control. Here, the animals ran through the maze without delay, making no side movements unrelated to the search for the path. Subsequently, the behavior of the rats at the time of the experiment changed abruptly, the animals scratched a great deal, washed themselves, sat motionless, and occasionally fell asleep. At this time it was often noticed that the animals refused food which was in the compartment. The feeding behavior in the vivarium was normal. It is unlikely that such passive behavior of the animals was due to muscular fatigue.

An investigation of the spontaneous bioelectrical activity of the extensors of the rear extremities on the 22nd to the 23rd day after the end of the flight showed that the coefficient of correlation between these parameters and the number of errors in the maze was equal to -0.36, and between them and the number of refusals to run the maze, to -0.48. Both coefficients were doubtful. Consequently, if the inverse correlation holds, it is incomplete.

Long-Term Memory. Long-term memory was investigated according to the method of V. K. Fedorov [7] - by comparing the number of errors in the first run on a given day and in the last run in an experiment conducted the day before. No essential differences with respect to this parameter were detected between the flight and the control rats, at any stage of the investigation.
The Synchronous and Mock-Up Experiments

**Transfer of Experience.** The second habit (for technical reasons) was developed in the synchronous experiment only during 2 days. In addition, only half of the control animals were examined on the second day. In view of this fact it was not possible to judge the speed and features of the development. However, there is adequate justification for speaking about the use of previous experience by both groups of animals. On the 1st day of the development of the second habit, all animals made significantly fewer errors than on the corresponding day during the development of the first habit. Moreover, the experimental animals used their past experience somewhat better than the control animals. On the 2nd day of the development of a new habit the control animals made somewhat fewer errors than the animals in the synchronous experimental group. However, a comparison of the level of errors with the corresponding data for the first habit disclosed an approximately identical degree of use of past experience (on this day) by both groups of animals. The difference between comparable groups with respect to the given parameter is uncertain (R > 0.05).

The number of refusals in the first day of the development of the second habit in the experiment was reliably lower than in the control (R < 0.05); on the second day their difference was uncertain (R > 0.05).

The time to traverse the maze during the development of the second habit in the case of the animal group in the synchronous experiment was less on the 1st and more on the 2nd day of the development of a new habit in comparison to the control. The difference in both cases was statistically reliable (R₁ < 0.001, R₂ < 0.01).

In the mockup experiment during the development of the second habit no essential differences with respect to any one parameter were detectable between the rats in the mockup and the control animals.

**The Change in the Number of Errors from Run to Run During the Experiment.** In terms of the dynamics of the errors made each day of the investigation, the animals in the synchronous and mockup experiments differed in no essential way from the animals in the control groups (R > 0.05). Thus, no systematic deviations between the parameters of HNA in groups of rats in the mockup of the biosatellite and in the vivarium were observed.
DISCUSSION OF THE RESULTS

The feeding behavior of the rats in the vivarium during the period under consideration was normal. Structural changes in certain skeletal muscles still survived, while showing a tendency toward restoration [5]. The correlation between the spontaneous bioelectrical activity of the skeletal muscles and the number of errors and refusals to traverse the maze turned out to be incomplete and uncertain. Moreover, when the motor activity decreased, the number of errors in the maze usually did not increase. Therefore, we believe that deterioration in the capacity to transfer experience would not be determined completely either by the muscular weakness, or by the still lower degree of reduction in feeding excitability.

The increase in the number of errors in the maze and the exhaustion of the nervous processes (determined by the change in the dynamics of the errors during a standard experiment) point to a reduction in the functional activity of the higher centers of the craniocerebrum.

At the end of the period described there appeared a tendency toward restoration of the initial level which was most clearly expressed in the transfer of experience and in the number of errors in the maze during establishment of the third habit. This is the basis for regarding the change in HNA, already described, as functional and reversible.

The mock-up and synchronous experiments proved that the upkeep conditions of the rats did not cause changes in the HNA, even though these changes were remotely similar to those observed in the flight animals.

The fact that the composition of the microcontaminants in the atmosphere of the biosatellite and in the synchronous experiment were not strictly identical might have influenced the HNA of the flight rats.

After the flight, the rats were soiled; this also might have influenced the HNA. However, within a week after landing, the flight rats could not be distinguished in their outward appearance from the control rats. The disturbance in their behavior in the maze was observed for a significantly longer time. The duration of the aftereffects on the HNA is at present unknown.

An important factor affecting the animals is the suddenness of the gravitational changes in flight and after landing. While man was prepared for these changes, for the animals they were a powerful special stimulus, causing drastic internal inhibition. But, as is well-known,
the inhibiting effect of extraordinary stimuli is gradually suppressed when their action is prolonged. Both in flight and in the postflight period the gravitational factor continued to have an effect for a long time, which must have contributed to the suppression of the external inhibition. Dissection of the flight and control rats disclosed inflammation of the ear. The causes enumerated probably strengthened significantly the action of the factors common to all space flights. To clarify the extent to which the facts peculiar to the given flight only were participants in the disturbance in HNA requires a special analysis.

Data are just now available concerning the fact that an analogous experiment on the biosatellite "Kosmos-782" caused a less drastic change in HNA on the part of the parameters. In this experiment, a strain of rats was used which is not susceptible to inflammation of the ear, and the hygienic conditions involved in the maintenance of the animals were improved.

When considering the factors more or less typical of all space flights we may assume that the influence of accelerations and vibrations operating at the time of launch of the biosatellite were minimal. According to the data from the ground-based model experiments their after-effects were of brief duration \[2\]. Therefore, it may be assumed that the prolonged stay in a condition of weightlessness and the readaptation to normal earth gravity exerted the greatest influence on the postflight changes in HNA. The substantial effect of dynamic factors which were operative during re-entry are also not excluded, since the latter are more difficult to withstand after a period of weightlessness.

CONCLUSIONS

(1) From the 15th to the 50th day after the conclusion of the flight in the biosatellite "Kosmos-605" functional disturbances in HNA were observed in rats. These disturbances were attested by a decrease in the capacity to solve complex problems (transfer of experience) and a decline in the operating capability of higher centers of the cranio-cerebrum.

(2) In the period described, a tendency toward normalization of HNA with respect to a number of parameters was observed. This justifies the assumption that the disturbances observed were functional, i.e., reversible.
(3) In the changes in the behavior of rats in a maze after the flight a definitive role was played, apparently, by the reduction in motor activity and by muscular weakness during the flight period.

(4) The most essential causes for the changes in the behavior of rats in the maze were their stay in a weightless condition and the subsequent sudden transition to the strains and conditions of earth gravitation.

It is possible that the changes in HNA were intensified by the influence of certain supplementary factors which took place during the given flight.

REFERENCES


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