DEVELOPMENTS OF ADVANCED RESEARCH ANIMAL HOLDING FACILITY (RAHF) FOR SPACE STATION:

II. Physiological and behavioral changes of golden hamsters in the fully enclosed environmental life support system.

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Introduction

An advanced Research Animal Holding Facility (RAHF) with a fully-enclosed life support system has been reported as a quite useful tool for life science experiments either on the ground or in the space (Sudoh et al. 1987). Since 1985, such a system has been developed by the Nagoya University and Kawasaki Heavy Industries. It consists of an animal holding part (glove box and air cleaning devices) and control part (further descriptions of the system see Shoji et al. 1989).

We have used this system for observations lasting either 4- or 8-weeks with golden hamsters and collected the data of their wheel running activities in two different environmental cases and compared these with the data obtained from normal controls put in the same room, but outside the fully-enclosed system.

Because the aim of our interest was to compare the influence of different settings on group activity, we could not pay attention to any biological rhythms. Thus, we analyzed first the influences of air compositions formed in the system to their activities. Moreover, for analyzation of physiological aspects, one male hamster was implanted with a transmitter in his abdominal cavity. The changes of some physiological factors, such as ECG, body temperature and general motor activity could be directly observed.

Further experimental planning using this life support system and the transmitter will be discussed.

Methods

In total 17 male golden hamsters (*Mesocricetus auratus*), 4 to 6 weeks old, were used in 8-weeks and in one case a three-months observations (Table 1). Animals were individually placed in cages equipped with an activity wheel and were exposed to a 12L:12D photoperiod (lights on 9:00 - 21:00). They could take food and water any time. Wheel running activity was recorded on an event recorder. The two main experiments were performed each eight weeks, respectively, from September 25 to November 20, 1987 and from December 8, 1987 to February 2, 1988. The preliminary one was done from November 5, 1988 to February 12, 1989. In the main experiments, activities were measured under two different environmental settings; with and without treatments of waste products. The number of wheel rotations was taken as a behavioral index, and their metabolic changes were also checked through a measuring of O₂ consumption and CO₂ accumulation.

In a preliminary case, from one subject, was implanted with a transmitter (Wt. 3.9 gms and Vol. 1.9 cc), body temperature, heart rate and general physical activity were collected at 5-minute intervals by a radiotelemetry system (Model TA10ETA-F20, DATA SCIENCES, INC. Minnesota), using microcomputer (NEC PC-9801VM) to control all aspects of data collection. Under sodium pentobarbital anesthesia (80 mg/kg), the transmitter, packed in biocompatible materials and sealed hermetically, was placed in the abdominal cavity and secured to the abdominal muscle. A fresh battery, placed

Table 1. Experimental design

		Number of Subjects	Duration of Observation (Weeks)	Experimental Settings
Case 1	Exp. Group	4	8	fully-enclosed life support system & with treatments of waste product
	Cont. Group	4	8	normal room cond.
Case 2	Exp. Group	4	8	fully-enclosed life support system & without treatments of waste product
	Cont. Group	4	8	normal room cond.
Preliminary Exp.		1	12	implanting of a transmitter

before implantation, provided a usable signal for up to six months. Telemetric signals were picked up either by external wire antennas placed around the cage or receivers with internal antennae (PhysioTel CTR 86) under the cage. After 3 months, the animal was again anesthetized with sodium pentobarbital and sacrificed. Damages in the abdominal cavity were carefully checked.

Figure 1. Wheel running activities of two different cases.

A: the case with waste treatment

B: the case without waste treatment

Results and Discussion

1. Comparison between two 8-weeks groups with and without waste treatments

1.1. Behavioral changes

Figure 1 shows the results of total wheel running activities obtained from the cases either with waste treatment (A) or without waste treatment (B). Comparing with total number of wheel running activities in both cases, the second case shows in general only a little different from the first case. This maybe due to seasonal effect, when the observations were started. Furthermore, the number of wheel running remarkably reduced at the first week to the tenth day, but after that it shows somewhat a transient increasing in both cases. As a course of event, their activities again reduced with the increasing number of experimental days. In the first case, the daily number of wheel rotation fluctuated before or after 30 days of observation, and in the second one, such changes occurred at about 36 days, but the later fluctuation was smaller than in the first one. There was, however, a clear differences between the experimental and control groups in both cases. A first indication of such difference was observed at the day seven to day ten. It might be correlated with some changes of gas compositions within the RAHF, especially on the increase of ammonium level (Fig. 2). There was, however, no precise relation between an increase of ammonium in the RAHF and animals' activities at all.

Figure 2: Ammonium accumulation in the system either with waste treatment or without waste treatment

It is difficult to bring to a conclusion from these two 8-weeks observation periods using a fully-enclosed life support system, what the real factor influenced to the animals' activities is. One possible way to find out such reason should be a direct measurement of the physiological indices.

1.2. Metabolic changes

The changes of their body weights during observation period were almost same tendencies in both cases, and there was no significant difference. In food intake and

Thus, the wheel running activity could not be affected, even though the ammonium level suddenly increased at the beginning of the seventh week. The other components like trimethylamine detected by a gas mass-spectrometer analysis were quite small amount throughout of the observations, so that those effect could be almost negligible. As one of the possible factors influenced to the animals' activities, a fully-enclosed environment could be assumed. That is known as a fact that animal's activity goes down such circumstance. Actually they were little influenced in comparison to the normal reared control group. Such a simple psychological element is maybe a reason of the activity differences between the experimental and control groups.

water consumption, both cases showed no clear changes throughout of the experiments. In addition, the metabolic rate of four hamsters placed in the RAHF was calculated through a measuring of oxygen consumption and accumulation of carbon dioxide gas. The obtained data were daily 15 grm/ animal for oxygen and 19 grm/animal for carbon dioxide gas.

2. Measurement of physiological and behavioral changes from the preliminary case.

Figure 3: The changes of body temperature and heart rate in three different environmental settings (see text)

HR: heart rate,
BT: body temperature

Before entering into the three-months period experiment, we implanted the transmitter in a hamster as a preliminary case and observed its physiological and behavioral changes in the different environmental settings in a 3 months-period. Figures 3 and 4 show, besides wheel running activities, some typical changes of heart rate and body temperature obtained from three different time periods. The animal was housed at first in a cage without wheel which was put in normal room condition, secondary was placed into a cage including a wheel and which was set in a fully-enclosed life support system and lastly in the same system, but with the front door taken off, the other conditions remaining the same. As a result, in a first stage we could not get any remarkable changes of biological rhythms even in heart rate and body temperature. If we introduced the wheel, the animal showed a quite clear day-night rhythm with corresponding heart rate and body temperature. This means that the introduction of wheel as a novel event stimulated the wheel running activities. However, a few days later it seems that such a stimulus has no more novel effect and his wheel running activities were splitted out as well as his physiological factors. The both mean body temperatures in the rest and active periods were $36.1^{\circ}\text{C} \pm 0.2$ and $38.3^{\circ}\text{C} \pm 0.4$, respectively, and the mean heart rates 218 ± 12.9 and 387 ± 31.1 , respectively. The both values were constant throughout of the observation period.

Figure 4: Free-running patterns of eight representative samples determined with two recording devices: running wheel and body temperature

The results of the preliminary study suggest that the hamster's body temperature and heart rate depend on its behavioral activity, and this, further, closely correlated with environmental settings whether the animal is housed with running wheel or not. Our data obtained at this time was, however, not enough to give a conclusion for any correlations between physiological indices and circadian rhythm, but the data necessary for this point should be gotten with a next long term observation starting from this April.

The semi-closed animal holding facility developed before by NASA had some problems of contaminations in the cabin of the Space-lab mission by cleaning cages and also by feeding occasions (for instance, SL-3). To avoid such contaminations, it was necessary to develop a fully-enclosed life support system, and we have firstly done bread board models. With using this ground model, several experiments with golden hamsters were performed until now and we found that the animals tested there showed no remarkable changes in both physiological and behavioral aspects. Thus, we have confidence that this system is quite effective for preventing any contaminations even in Space Station. We are planning a further experiment using this system and implanting other transmitters for blood pressure and EEG or directly recording unit activity from the brain. These points are, of course, indispensable to understand for physiological mechanisms in space.

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