EFFECTS OF CENTRIFUGE DIAMETER & OPERATION ON RODENT ADAPTATION TO CHRONIC CENTRIFUGATION

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INTRODUCTION

Background & Significance

Gravitational Biology is concerned with the functional and structural alterations that occur in biological systems exposed to altered gravitational fields. These have been observed in studies using a variety of experimental approaches, some ground-based and some in Earth-orbit [8].

The principal physical change in orbiting vehicles is the removal of the effects of Earth gravity -- weightlessness, and understanding this phenomenon is of critical importance to the continuing development of gravitational biology. The environment of spacecraft also have other factors that may modify biological function, such as solar and cosmic radiation, forces and materials produced in the space station (noise, vibration, environmental contaminants, etc.), illumination schedule, etc. These secondary factors may produce separate biological effects, or modify the effects of weightlessness. An interaction of the effects of ionizing radiation and gravitational fields has been demonstrated in rats [2,3] and also in plants and micro-organisms in orbiting satellites [7]. In short term orbital experiments these extraneous factors may not significantly affect results, however, in the protracted exposure anticipated with the space station, their cumulative effects may seriously interfere with the research. It is of critical importance that any influence of these extraneous factors upon biological experiments in the space station be identified so they can be separated and
not be confused with the effect of weightlessness. Provision must therefore be made for a suitable control as a part of space station experiments. Such requirements can only be fulfilled by an on-board centrifuge operating at 1 G. If the only biologically significant factor in the space station environment is weightlessness, the responses of on-board 1 G controls should be the same as those exhibited by equivalent ground-based controls. The use of on-board 1 G-controls on the space station should be continued until all variables in the space station environments are identified and determined not to have interfering biological effects.

The Space Station centrifuge facility will also be utilized for specimen holding so that the transition to weightlessness and return to 1 G can be made in a controlled manner permitting careful and repeated observations of the immediate responses to a 1 G change in the ambient acceleration field. For this, biological material will be maintained at 1 G from launch and provision made for its ready transfer to the Space Station environment. Similarly, it must be arranged for materials maintained under conditions of weightlessness to be readily transferred to the 1 G centrifuge. Animals and plants could also be preadapted to other fields: e.g., Lunar, 0.16 G; or Martian, 0.35 G.

Other potential uses of Space Station centrifuge include: (1) investigation and provision of counter-measures for gravitational deadaptation of astronauts; and (2), studies of labyrinthine function in weightlessness. Potentially, periodic exposure to a 1 G field could prevent
or limit the severity of the decompensations seen in circulatory, skeletal and muscular systems that occur with continued residence in weightlessness.

In order to determine gravitational effects satisfactorily, it is essential that a Space Station centrifuge be capable of providing multiple fields between 0 and 1 G. Observations in weightlessness will establish the nature of mass-determined biological function, that which is retained in weightlessness. The difference between observations made at Earth gravity and in weightlessness will establish the weight-determined biological function, that which develops under a 1 G gravitational load. However, such information will be based on only two points of observation, and as such it cannot be generalized -- and if space biology is to have scientific merit it must be amenable to generalization. Observations in at least three, and preferably more, gravitational fields will be necessary for the development of any generalization, such as determining the kinetics of gravitational responses.

A multi-field capability of a space station centrifuge is also essential in determining threshold fields for gravitational responses. It can be demonstrated that for some biological processes an acceleration field greater than minimum strength is required to elicit a gravitational response. Understanding such thresholds is essential to the development of a coherent science of gravitational biology. Understanding of thresholds for adaptive responses will also provide information of biomedical concern. The "permanent presence of man" in space may be jeopardized by human
inability to adapt to chronic weightlessness. Artificial inertial fields (artificial gravity) in future spacecraft or stations may provide the required countermeasure for our successful habitation of space. Understanding the threshold-G for various physiological processes will be an essential contribution in establishing the appropriate fractional-G for such countermeasures.

**Centrifuge Characteristics**

In addition to providing an inertial field, centrifuges have other characteristics that may, if not controlled, confuse the gravitational effects [9]. The basic physical relationships of a centrifuge are:

\[ a = r\omega^2; \text{ or} \]

\[ G = \frac{a}{g} = \frac{r\omega^2}{g} \]

where:
- \( a \) is the acceleration (inertial) field;
- \( r \) is the radius of rotation;
- \( \omega \) is the rotation rate (radians/time);
- \( G \) is the field characteristic (the weight-to-mass ratio); and,
- \( g \) is the Earth's gravitational constant.

The reciprocal relationship between radius and rotation rate allows the same field to be developed at infinite combinations of radius and rotation rate. Whether or not rotation rate has any effect, separate from field strength,
upon long-term gravitational experiments has never been adequately determined. In practice, the possibility of such interference has been recognized, but avoided by providing a large diameter (usually 4-6 m) which minimizes any separate effect of rotation. However, in the Space Station, centrifuge diameter may be limited and any separate rotatory influence must be identified by ground-based experiments. It is important that any interaction between rotation rate and G-field be thoroughly explored. Such information may provide suitable correction factors, or indicate the maximum fields (for a given diameter) that do not develop interference from rotatory effects.

Recent work has shown an effect of centrifuge diameter on the rate of adaptation of feeding and drinking of squirrel monkeys [4]. During chronic centrifugation, after an initial decline, mean daily levels of feeding and drinking returned towards precentrifugation levels. These variables adapted towards a steady state level faster on a larger diameter centrifuge. The amplitude of the body temperature rhythm was similarly affected.

The ratio of specimen stature ("height") to the diameter of rotation also is important because of potential interference from "head-to-foot" G-gradients in experimental subjects. This complication also has been recognized by investigators using centrifuges, and similarly avoided by providing a large diameter of rotation. In a Space Station there will be dimensional limits upon centrifuge size potentially introducing artifacts in biological experiments. Consequently, it is imperative that ground-based
research be initiated to identify the biological effects of head-to-foot G-gradients. Such information may provide suitable correction factors, or indicate the maximum G-gradient that will interfere with experimentation. The latter may limit the numbers of species (on the basis of stature) amenable to centrifugation on the Space Station.

The movement of organisms within a rotational environment also produce additional forces which are a function of the direction of movement [10]. For example, any motion in the plane of rotation of the centrifuge causes a 90° cross (Coriolis) acceleration equal to twice the relative velocity times the angular velocity. Thus, this force, which is proportional to angular velocity, is proportional to centrifuge diameter. Whether Coriolis forces will be of sufficient magnitude to have an influence on the adaptation and movement patterns of specimens such as rodents in a small diameter centrifuge, such as that being planned for space station is unknown.

Ground based studies [1] indicate that there is a time-intensity summation for gravitational effects, so that brief interruptions do not greatly affect the results of a biological experiment. Experiments which involve a daily 15 minute interruption in centrifugation (about 1% of a day) will yield results similar to those from experiments in which the centrifugation is continuous, but at 99% of the field strength. The variability in biological response to ground-based acceleration is such that this difference should not be detected. However, this relationship may not apply in a space station where the suspension of centrifugation will involve weightlessness,
potentially producing a disorientation that may induce separate and significant biological effects.

**Project Description**

Data from spaceflight and ground based experiments have clearly demonstrated the importance of Earth gravity to normal physiological function in man and animals. The physiological systems that are affected by exposure to altered gravitational environments include those responsible for regulation of temperature homeostasis, sleep and biological rhythms. Understanding the mechanisms of these effects and developing rational and effective counter-measures to astronaut deconditioning in space require the investigation of these gravity-dependent systems and functions under conditions of long-term weightlessness/microgravity. In order to fully understand the nature of the interaction of biological systems and gravity, it is necessary to make repeated observations at several field strengths between 0 and 1+ G. A centrifuge in an orbiting spacecraft provides the only method for studies of these gravity-dependent phenomena between microgravity and Earth gravity. This centrifuge must be capable of accommodating experimental subjects -- both acutely and chronically. However, such rotational facilities are not without technical and physical limitations, which may also prove to be severe biological limitations.

For example, G-gradients are inherent in all gravitational fields. In man on Earth there is a calculable head-to-foot G-gradient. So the problem of G-gradients in animals on centrifuges is a quantitative, rather than
qualitative, one. Centrifuge geometry and operation also produce several irregularities in acceleration fields (i.e., Coriolis forces, cross-coupled accelerations, starting and stopping accelerations, etc.). These potentially adverse aspects of centrifugation have not been critically examined; yet, where rotational radii must be limited, such as in the spacecraft, these factors may interfere with research objectives and they must be considered either during design of such centrifuges or as a factor in their experimental usage. This program examined the influence of variable diameter centrifuges (1.8 to 6 m) and their operation on the physiology and behavior of rats. These studies measured the responses of these rodents to centrifugation in a constant acceleration field, but at different radii.
METHODS

In this program we compared the influence of centrifuge diameter (1.8, 2.5 and 6.0 m) and stopping frequency (daily for one hour or weekly for seven hours) on the physiology and behavior of the rat. The studies were performed by measuring the responses of these rodents to centrifugation in a fixed acceleration field, but at different radii. These experiments provide data on the influence of: centrifuge diameter, Coriolis forces and stopping frequency on the adaptation of rats to chronic acceleration.

Subjects: Male Sprague Dawley rats (325g) served as subjects for these studies. All animals were maintained in an LD 12:12 cycle (12 hours of light followed by 12 hours of darkness) and a relatively constant ambient temperature (22-25°C) throughout all phases of the experiment. Each animal was surgically implanted with a biotelemetry transmitter to allow the collection of body temperature and activity data. Food and water were available ad libitum. Animal husbandry chores were performed weekly.

Protocol: Each study consisted of: a 27 day baseline (1 G), 57 days of the experimental condition (1.5 G, 1.05 G, or 1 G), and a 28 day postcentrifugation period (1 G). Animal-experimenter interactions occurred on the same weekly schedule during all portions of the protocol, animal. During the altered gravitational environment phase of the experiment, the centrifuges were either stopped daily for one hour (Daily) or once per week for a 7 hour period (Weekly). Thus, the total time of 1.5G exposure per week
was the same for both the Daily and Weekly studies. In all, 10 groups (n = 8) were studied; centrifuge diameters of 1.8, 2.5 m and 6.0 m were used to generate a 1.5 G ambient force. Two additional control groups were examined. The first was a 1 G static control group and the second was a rotational control (SRC) group exposed to 10 rpm on the 6.0 m centrifuge (1.05 G). Thus, the groups examined were:

1.8m - Daily & Weekly Stop
2.5m - Daily & Weekly* Stop
6.0m - Daily & Weekly Stop
SRC - Daily & Weekly Stop
Control - Daily & Weekly Stop

*Note: Equipment failure resulted in the experimental portion of the weekly stop at this diameter being shortened to 29 days. However, by this time, all parameters had returned to baseline levels.

Data collected: Animals were weighed weekly (at the time of cage changing) and measures of food and water consumption were made at this time. While its cage was being changed, several behavioral tests that have been used previously to test vestibular integrity [5] were performed on each animal. Four tests of neurovestibular function were performed weekly on each subject. These were:

Air-Righting Reflex. Rats were held 45 cm above a padded table in a supine position and dropped. Normal rats would turn and land on their feet, while rats with vestibular dysfunction would fail this reflex test and land on their backs.
Contact-Righting Reflex. Rats were positioned in a supine position and their back in contact with the table surface. A flat sheet of Plexiglas was placed in contact with the feet. Normal rats would turn to right themselves, while rats with vestibular dysfunction would attempt to step or walk along the Plexiglas sheet.

Head Movement Reflex. Animals were held in the air by the base of the tail for 5 seconds. Normal rats would move their head ventrally in order to climb upward. Rats with vestibular dysfunction would move their head dorsally or attempt to spin in a circular rotation.

Elevated Platform Test. Rats were placed on a 15 x 15 cm platform elevated 45 cm above the table. Rats were tested for the latency to step off the platform onto the table.

As an additional measure of behavior and to allow for examination of possible responses to Coriolis forces, 4 animals in each group were videotaped for 50 minutes at the start of the light portion of the LD cycle.

Activity and body temperature were continuously monitored by telemetry and these data were collected by microcomputer at 10 minute intervals. Collecting at this frequency allowed us to examine both the homeostatic levels of these variables and the daily, or circadian, rhythms. These data were subjected to cosinor analysis in order to determine the daily mean, amplitude and phase of the rhythms. ANOVA for repeated measures was used to compare results between groups.
Results

The process of adapting to a hypergravity environment is typically triphasic: there is an initial response, a period of adaptation and, finally, a new steady-state level is achieved [6]. These experiments were designed to allow us to examine both the acute response to 24 hours of centrifugation, and the adaptation to the new G environment. In all variables examined, with the exception of neurovestibular function which did not appear to be affected by the level of G-force that was used in these experiments, there was an immediate response followed by a rapid return back to baseline values. Each individual variable is discussed below.

Body mass: The average weekly change in body mass is plotted in Figures 1 (Daily Stop) and 2 (Weekly Stop). The period of 1.5 G is indicated by the dark line on the x-axis. Onset of centrifugation resulted in a loss in body mass that is reflected as a negative delta. There was no significant difference between the responses at the different diameters. The SRC did not differ from the stationary Control.
DAILY STOP DELTA WEIGHT

Fig. 1

WEEKLY STOP DELTA WEIGHT

Fig. 2
Delta body mass is plotted for the Daily and Weekly regime at each diameter in Figure 3. At 2.5 and 6.0m the change was significantly different.
between the Weekly and Daily stopping regimens ($p \leq 0.01$). Recovery of weight gain took longer when the centrifuge was only stopped once per week than when it was stopped daily. This was not the case at the smallest diameter (1.8m). These same data for the SRC and Control are shown in Figure 4.

![Graph showing weight gain comparison between Daily and Weekly regimens](image)

**Fig. 4**

**CONTROL**

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<th>DELTA (GMS)</th>
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Daily: 
Weekly:

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17
Food consumption: Food intake was also decreased at the onset of centrifugation. The average weekly food consumption is plotted in Figures 5 (Daily Stop) and 6 (Weekly Stop). The period of 1.5 G is indicated by the dark line on the x-axis. Again, there was no significant difference between the responses at the different diameters. The SRC did not differ from the stationary Control. Recovery of food intake rate was achieved by the 3rd week of centrifugation.
Weekly food consumption is plotted for the Daily and Weekly regime at each diameter in Figure 7 and for the Control and SRC in Figure 8.
Fig. 8

CONTROL

SRC

Daily
Weekly
**Water consumption:** Water intake presents a much more irregular pattern than body weight or food consumption. Average weekly water consumption is plotted in Figure 9 (Daily) and Figure 10 (Weekly). Water consumption did decrease with the onset of centrifugation, but recovery was accomplished more quickly. Again, neither centrifuge diameter nor stopping frequency appeared to affect this variable.
DAILY STOP WATER CONSUMPTION

WEEKLY STOP WATER CONSUMPTION
Weekly water consumption is plotted for the Daily and Weekly regime at each diameter in Figure 11 and for the Control and SRC in Figure 12.

**Fig. 11**

- **1.8m**
  - Daily
  - Weekly

- **2.5m**
  - Daily
  - Weekly

- **6.0m**
  - Daily
  - Weekly
Fig. 12

CONTROL

SRC

WEEK OF STUDY
Neurovestibular tests:

Air-Righting Reflex. There were no abnormal responses to this test.

Contact-Righting Reflex. There were no responses to this test that indicated neurovestibular dysfunction.

Head Movement Reflex. The only abnormal responses to this test were seen in the 1.8m diameter subjects. Over the seven week period of centrifugation, there were a total of four abnormal responses during the Daily Stop regime and two during the Weekly Stop regime.

Elevated Platform Test. This test produced the most “abnormal” responses and the results are summarized in Table 1. However, given the following: nearly one-third of the responses occurred in the three weeks of baseline data collection, approximately one-fifth of the responses occurred in the stationary Control group and the testers’ observed that the rats were often “sliding down the leg of the platform like a fireman,” we have concluded that these results do not indicate neurovestibular compromise.
Table 1

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<th>1.8m Wkly</th>
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<th>2.5m Wkly</th>
<th>6.0m Dly</th>
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We would attribute these results to the relatively low hypergravitational force that is being introduced.

**Movement analysis:** A preliminary analysis of the video records was made examining: linearity of movements, number and direction of rotation movements, total movement time, length of distance traveled, and pattern of movement. This analysis of the videotapes revealed an initial (one day) suppression of activity caused by exposure to centrifugation. However, after activity resumed, it did not appear that any of the analyzed variables were altered. Given the responses to the neurovestibular tests, these data were unsurprising and further analysis of the tapes was deemed unproductive.
Body temperature: These data were subjected to cosinor analysis; a cosine curve was fitted to each successive 24 hour interval of data. The mean, amplitude and acrophase (time of peak of the cosine curve) were determined. Each of these variables is discussed below.

Daily Mean Body Temperature: Data from days 21-49 are shown in Figures 13 (Daily) and 14 (Weekly). This time period encompassed the last 6 days of precentrifugation and the first 22 days of 1.5G exposure. There was an initial depression of the daily mean body temperature at the onset of centrifugation. This depression occurred at all diameters. Recovery back to baseline mean body temperature was generally accomplished within one week.
**DAILY STOP - TEMPERATURE MEAN**

Fig. 13

**WEEKLY STOP - TEMPERATURE MEAN**

Fig. 14
The mean daily body temperature is plotted vs. day of the study for the Daily and Weekly Stopping at each diameter in Figure 15.

**Fig. 15**

The charts show the temperature fluctuations over time, with daily and weekly stopping points highlighted. The temperature range is from 35.5°C to 38.0°C, with specific days marked for comparison between daily and weekly stopping.
The mean daily body temperature is plotted vs. day of the study for the Control and Slow Rotational Control in Figure 16.

Amplitude of the Body Temperature Rhythm: Onset of centrifugation resulted in a reduction in the amplitude of the body temperature rhythm. Rhythm amplitude gradually returned to pre-centrifugation levels. These data are shown in Figure 17 (Daily Stopping), Figure 18 (Weekly Stopping), Figure 19 (Daily vs. Weekly by diameter) and Figure 20 (Control and SRC).
DAILY STOP - TEMPERATURE AMPLITUDE

Fig. 17

WEEKLY STOP - TEMPERATURE AMPLITUDE

Fig. 18
Fig. 19

1.8M

AMPLITUDE

0.8

0.6

0.4

0.2

0.0

0 7 14 21 28 35 42 49 56 63 70 77 84 91 98 105

DAY

0.8

0.6

0.4

0.2

0.0

0 7 14 21 28 35 42 49 56 63 70 77 84 91 98 105

AMPLITUDE

0.8

0.6

0.4

0.2

0.0

0 7 14 21 28 35 42 49 56 63 70 77 84 91 98 105

AMPLITUDE

0.7

0.6

0.5

0.4

0.3

0.2

0.1

0.0

0 7 14 21 28 35 42 49 56 63 70 77 84 91 98 105

DAILY

WEEDY
Phase of the Body Temperature Rhythm: The timing of the body temperature rhythm was also altered at the onset of centrifugation, and as did the mean and amplitude, the timing of the rhythm also recovered back to the baseline values. These data are shown in Figures 21 (Daily Stopping), 22 (Weekly Stopping), 23 (Daily vs. Weekly for each diameter) and 24 (Control and SRC).
Fig. 23

1.8M

- Daily
- Weekly

2.5M

- Daily
- Weekly

6.0M

- Daily
- Weekly
Activity: The activity data were also subjected to cosinor analysis. In general, the response of the circadian rhythm of activity paralleled that of the body temperature rhythm, i.e. an initial decrease in mean level and amplitude coincided with altered phase control. These variables all returned to baseline levels.

Daily Mean Activity: Data from days 21-49 are shown in Figures 25 (Daily) and 26 (Weekly). As in the body temperature rhythm, at all
diameters, an initial depression of daily mean levels was followed by a recovery back to pre-centrifugation levels.

**DAILY STOP - ACTIVITY MEAN**

![Graph showing daily stop-activity mean with lines for 1.8M, 2.5M, 6.0M, and SRC.]

**WEEKLY STOP - ACTIVITY MEAN**

![Graph showing weekly stop-activity mean with lines for 1.8M, 2.5M, 6.0M, and SRC.]

38
The mean daily activity at each diameter is shown in Figure 27:

Fig. 27

1.8 M

COUNTS/10 MIN

0 7 14 21 28 35 42 49 56 63 70 77 84 91 98 105

Daily
Weekly

Daily
Weekly

2.5 M

COUNTS/10 MIN

0 7 14 21 28 35 42 49 56 63 70 77 84 91 98 105

3.0 M

COUNTS/10 MIN

0 7 14 21 28 35 42 49 56 63 70 77 84 91 98 105

Daily
Weekly

Daily
Weekly

6.0 M

COUNTS/10 MIN

0 7 14 21 28 35 42 49 56 63 70 77 84 91 98 105

Daily
Weekly
Mean daily activity for the Control and SRC groups are plotted in Fig. 28.

**Fig. 28**

**Amplitude of the Activity Rhythm:** Data from days 21-49 are shown in Figures 29 (Daily) and 30 (Weekly). As in the body temperature rhythm, at all diameters, an initial depression of rhythm amplitude was followed by a recovery back to pre-centrifugation levels.
Activity amplitude data for each diameter centrifuge are shown in Fig 31.
Activity amplitude data for the Control and SRC groups are shown in Fig 31.

**Fig. 32**

**CONTROL**

**SRC**

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**Phase of the Activity Rhythm:** These data are shown in Figs. 33-36, following the same pattern; i.e. Daily stop for all groups (33), Weekly stop for all groups (34), Daily vs. Weekly for all centrifuges (35) and Daily vs. Weekly for Control and SRC (36).
DAILY STOP - ACTIVITY PHASE

Fig. 33

WEEKLY STOP - ACTIVITY PHASE

Fig. 34
Fig. 35

1.8M

2.5M

PHASE (HRS)

PHASE (HRS)

PHASE (HRS)

DAW
Fig. 36

PHASE (HRS)

Daily
Weekly

0 7 14 21 28 35 42 49 56 63 70 77 84 91 98 105

DAY

PHASE (HRS)

SRC

Daily
Weekly

0 7 14 21 28 35 42 49 56 63 70 77 84 91 98 105
SUMMARY

This study examined the responses of rats to centrifugation in a constant acceleration field (1.5 G). Centrifuge diameter (1.8m, 2.5m or 6.0m) and schedule of operation (Daily or weekly stop) varied between groups. Body mass, food consumption, water consumption and neurovestibular function were measured weekly. Body temperature and activity were continuously monitored using telemetry. A subset of subjects were videotaped (50 minutes per day) to allow for movement analysis.

Exposure to a hyperdynamic field of this magnitude did cause the expected depression in the physiological variables monitored. Recovery was accomplished within a relatively rapid time frame; all variables returned to precentrifugation levels. In general, the magnitudes of the changes and the rate of recovery were similar at different centrifuge diameters and stopping frequency. There were cases, however, in which the magnitude of the response and/or the rate of recovery to a new steady-state were altered as a result of centrifuge diameter.

In summary, these results indicate that stopping frequency has little, if any, effect on adaptation to chronic centrifugation. However, the angular velocity (ω), and therefore centrifuge diameter is an important consideration in the adaptation of an organism to chronic centrifugation.
BIBLIOGRAPHY


