Chronic centrifugation (hypergravity) disrupts the circadian system of the rat

D. C. Holley,¹ C. W. DeRoshia,² M. M. Moran,² and C. E. Wade²

¹Department of Biological Sciences, San Jose State University, San Jose, 95192-0100; and ²Life Sciences Division, National Aeronautics and Space Administration Ames Research Center, Moffett Field, California 94035

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Holley, D. C., C. W. DeRoshia, M. M. Moran, and C. E. Wade. Chronic centrifugation (hypergravity) disrupts the circadian system of the rat. J Appl Physiol 95: 1266–1278, 2003.—The present study was conducted to evaluate the response of rat deep body temperature (DBT) and gross locomotor activity (LMA) circadian rhythms to acute hypergravity onset and adaptation to chronic (14 day) hypergravity exposure over three gravity intensities (1.25, 1.5, and 2 G). Centrifugation of unanesthetized naive animals resulted in a dramatic acute decrease in DBT (−1.45, −2.40, and −3.09°C for the 1.25, 1.5, and 2.0 G groups, respectively). LMA was suppressed for the duration of centrifugation (vs. control period); the percent decrease for each group on days 12–14, respectively, was 1.0 G, −15.2%, P = not significant; 1.25 G, −26.9%, P < 0.02; 1.5 G, −44.5%, P < 0.01; and 2.0 G, −63.1%, P < 0.002. The time required for DBT and LMA circadian rhythmic adaptation and stabilization to hypergravity onset increased from 1.25 to 2.0 G in all circadian metrics except daily means. Periodicity analysis detected the phenomenon of circadian rhythm splitting, which has not been reported previously in response to chronic hypergravity exposure. Our analysis documents the disruptive and dose-dependent effects of hypergravity on circadian rhythmicity and the time course of adaptation to 14-day chronic centrifugation exposure.

All animals have endogenous biological timing mechanisms that regulate physiology and behavior to optimize performance, metabolism, and survivability within ecosystems (12). In higher vertebrates, this system is subserved by a multioscillator-hierarchical complex that resides primarily in neural substrate and has been labeled the circadian timing system (CTS) (37). Abnormal function, or desynchronization of this system from external timing cues, can disrupt the normal timed sequence of coordinated physiological processes and lead to decreased ability to resist environmental challenges and disease, and it has been correlated with physiological and psychological disorders, including jet lag (55). Animal physiology and behavior have evolved under various selective pressures, including the static gravitational field and the dynamic periodic environ-mental fluctuations as a result of the Earth’s rotation (26). Although the gravitational environment is relatively stable, its influence on animal morphology and physiology as life transitioned from the buoyant aquatic world to a terrestrial existence should not be understated. The development of large-diameter centrifuges to chronically expose laboratory animals to hypergravity and the availability of microgravity conditions in near-Earth orbit have opened up the study of gravity as an independent variable in environmental physiology (9).

Öyama et al. (47), using biotelemetry in unrestrained rats, provided the first evidence that exposure to hypergravity (up to 2.5 G) results in a dramatic rapid fall (within minutes) in body temperature (2–6°C). Subsequent studies examined the combined effects of cold exposure and hypergravity-induced hypothermia (22, 36). The centrifuge-induced hypothermic response was also shown in dogs (46), monkeys (16, 18, 20), and mice (42, 43). Fuller’s research group has performed many hypergravity studies investigating the CTS (23, 24, 26, 32, 43). Rats exposed to hypergravity show initial decreases in heart rate, deep body temperature (DBT), and locomotor activity (LMA) rhythm amplitudes. Monkeys (17) and mice (43) exposed to chronic centrifugation show an initial decrease in amplitude and mean of DBT and activity circadian rhythms. Feeding rhythm phase and period length are also affected. However, in rats exposed to 1.75 G for 40 days under constant conditions, Lafferty (34) reported that there was no difference in the circadian free-running feeding rhythm. A. E. Ronca (personal communication) found that pregnant rat dams exposed to hypergravity had decreased activity only during the subjective active period (dark phase) but not during the subjective inactivity period (lights-on phase). Fuller et al. (20) reported a circadian difference in the hypothermic response to hypergravity with the greatest fall in temperature occurring during the animal’s active phase. Fuller et al. (25) also showed that 1-h G pulses (2 G) could entrain the free-running body temperature rhythm and that even acute 2-G pulses could phase shift body temperature rhythms in rats (28).

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Address for reprint requests and other correspondence: D. C. Holley, San Jose State Univ., 1 Washington Square, San Jose, CA 95192-0100 (E-mail: dholley@email.sjsu.edu).

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Previous studies of the effects of hypergravity on circadian rhythms have described circadian rhythm responses to multiple doses of hypergravity in beagle dogs (46) and squirrel monkeys (17) only. Investigations in rats have reported responses to one level of hypergravity except for one study (47) in which circadian body temperature data were collected at several G levels, but the data were not analyzed or discussed with respect to the effects of hypergravity on circadian rhythmicity. However, it has been suggested that the CTS that controls body temperature rhythms as well as the mechanisms involved in temperature homeostasis are continuous gravity-dependent functions from microgravity through hypergravity (8, 26). Our study tested the hypothesis that a proportional gravitational effect on the CTS will be observed over a range of three gravity intensities. Our project differs from previous reports on this topic in that we are evaluating the physiological adaptation of the CTS to three doses of chronic hypergravity intensities (1.25, 1.5, and 2 G) in the rat. Also, previous studies have not utilized time-series analysis to resolve sequential changes in rhythmic periodicity, amplitude, phase, and synchronization. In this study, we are providing a comprehensive mathematical analysis to quantify and differentiate the physiological adaptation of the CTS to these three doses of chronic hypergravity intensities. This will enable us to differentiate readaptation responses to baseline levels from stabilization responses to new homeostatic steady states. The application of sophisticated time-series analyses in this study (e.g., moving periodogram analysis (33)) enabled us to discover and quantify the phenomenon of circadian rhythm splitting, which had not been reported previously in hypergravity studies.

MATERIALS AND METHODS

The two experiments described herein were conducted on the 24-fl.-diameter centrifuge in the Center for Gravitational Biology Research at National Aeronautics and Space Administration (NASA)-Ames Research Center and approved by the NASA-Ames Institutional Animal Care and Use Committee (see also Ref. 39). Experiment 1 used 24 male Sprague-Dawley rats (initial weight 205 ± 1.4 g; Simonsen Laboratories, Gilroy, CA), and experiment 2 used 24 rats (initial weight 198 ± 1.1 g; Simonsen Laboratories). Animals were randomly assigned to three groups: 1.0 (stationary control), 1.5, and 2.0 G for experiment 1; 1.0 (stationary control), 1.25, and 1.5 G for experiment 2. The animals received food (Rat Chow no. 5012, Ralston Purina) and water ad libitum. Light and 1.5 G for experiment 2 randomly assigned to three groups: 1.0 (stationary control), 1.25, and 1.5 G (8 ft.) and the other to 1.5 G (12 ft.) in experiment 2. During hypergravity conditions, each cage was rotated outward with one degree of freedom by centrifugal force to an angle to the gravitationally referenced horizon proportional to the centrifugal force. For example, the cage floor parallel to the sides of the cage, such that ambulatory movements per minute. The LMA data thus obtained in digital pulses, proportional to the distance moved, which were not circadian harmonics (e.g., 48 or 16 h) or submultiples of each other.

Circadian rhythm metrics evaluated were cyclic mean, phase, and amplitude, and group rhythm synchrony ampli-
tude, cosinor (95% confidence limit ellipses), and cross-correlation coefficients. Circadian means, acrophase [phase of computed rhythm peak referenced to time of lights-on (0600)], and amplitude were estimated by complex demodulates (48, 50), which provide pergressive (point to point) circadian phase and amplitude estimates.

Rhythm synchrony analysis, based on circular statistical methods described by Batschelet (4) and Durand and Greenwood (14), establishes criteria for the statistical evaluation of circadian rhythm synchronization across a group of animals. The null hypothesis states that acrophases at a given sample time are randomly distributed across a group such that the group composite synchrony amplitude approximates zero. If the group is perfectly synchronized, then the acrophases are equal across the group and the group synchrony amplitude approximates 1.0. Thus group synchrony amplitude is a measure of group synchronization, and exposure to hypergravity would be expected to induce rhythmic disruption or desynchronization in individual animals such that the group synchrony amplitude would fall below statistical significance.

Group synchrony amplitudes were computed from individual rat circadian rhythm Fourier coefficients obtained from complex demodulate analysis.

A cross-correlational method modified from a procedure originally described by Horton and West (31) was used to quantitate rhythm adaptation. It is useful because it is independent of model assumptions (e.g., harmonic, exponential trajectory, etc.). In this method, the values from three cycles of baseline DBT or LMA data were cross-correlated with the values from the corresponding times on each cycle of post-gravitational-onset exposure data. The values in the cycle were then shifted forward in time by the sampling time interval, and a new cross-correlation coefficient was calculated. This process was continued until the last cycle in the postgravitational-onset data was cross-correlated. The correlation coefficients at each sample point for each baseline vs. postgravitational-onset cross-correlation were z-transformed, the z-transforms were averaged, and the mean z-transform was converted to a mean cross-correlation coefficient. To establish a readaptation criterion, all baseline cycles were similarly cross-correlated with each other.

Duration of adaptation for circadian rhythm parameters (daily mean, amplitude, acrophase, cross-correlation coefficient, and rhythm synchrony amplitude) after hypergravity exposure was computed by establishing nonparametric 95% confidence limits for each parameter during the baseline phase [Tukey's box and whisker lower fence, approximately equivalent to the lower 2.5% confidence limit of a set of normally distributed data (54)] and determining the time interval between the instant the parameter fell out of the lower 95% confidence limit after the gravitational onset and then reentered the 95% confidence limit for at least several hours. Because we expected that several circadian rhythm parameters would not recover to the baseline 95% confidence limits during hypergravity exposure, a hypergravity stabilization, or new steady-state duration, was also defined as the time interval between gravitational onset and the time at which the parameter level reentered the posthypergravity stabilization (days 12, 13, and 14) 95% confidence limits. Cosinor (44) error ellipses were evaluated for adaptation duration by determining the interval between the first postgravitational-onset day in which the error ellipse did not overlap the error ellipse from the last baseline day and the postgravitational-onset day in which the baseline and postgravitational-onset day error ellipses overlapped. The cosinor was also used to evaluate group LMA and DBT rhythm significance for each day in the study.

Statistical analysis of group differences between baseline days (days −3, −2, and −1) and centrifugation days 12, 13, and 14 was performed by repeated-measures ANOVA with post hoc comparisons by Tukey's honestly significant difference method. Paired comparisons were performed by using paired t-tests (Statistica, version 5, Statsoft, Tulsa, OK). Unless noted, values reported are means ± SD.

RESULTS

Effects of hypergravity exposure on mean DBT and LMA cycles. We used cosinor analysis to determine group DBT and LMA circadian rhythm significance. All animals demonstrated circadian rhythms of DBT (P < 0.0002) and gross LMA (P < 0.02) during the baseline control period (days −1, −2, and −3) (Fig. 1). The circadian rhythms in these parameters were significant for all days except day 1 (LMA, 1.5-G group of experiment 1), day 3 (DBT, 1.5-G group of experiment 2), day 4 (DBT, 1.25- and 1.5-G groups of experiment 1), day 5 (DBT and LMA, 1.5-G group of experiment 2), and day 6 (DBT, 2.0-G group of experiment 1). During the 3-day control period immediately before the beginning of centrifugation, there was no statistically significant difference (ANOVA) among the groups for LMA or DBT circadian rhythms (mean, amplitude, or acrophase).

Because the centrifuge speed differed between experiments 1 and 2 (21.1 and 16.06 rpm, respectively) and because the 1.5-G groups were at different centrifuge radii for the two experiments (8 ft. for experiment 1, 12 ft. for experiment 2), the 1.5-G group data were compared to see whether there was an apparent rotational effect. We could find no statistical difference between the groups in any of the DBT or LMA data, including means, amplitudes, or acrophases. Therefore, we used data for the 1.5-G group in experiment 1 when showing centrifuge dose responses in the following figures.

Figure 1 shows the immediate (gravity) dose-dependent drop in core body temperature of rats exposed to hyperdynamic fields of 1.25, 1.5, and 2.0 G in this study. Centrifugation onset resulted in an acute dose-dependent drop in group mean DBT of −1.45, −2.40, and −3.09°C for the 1.25-, 1.5-, and 2.0-G groups, respectively. After onset of centrifugation, the circadian rhythm amplitude and mean appear to be suppressed combined with the appearance of erratic waveform changes, all of which give the appearance of arrhythmia for the first 4–5 days (Fig. 1).

Figure 1 also shows group mean plots of the LMA data. This figure clearly shows the gravity dose-dependent decrease in activity after the onset of the hyperdynamic fields. Note that activity appears to be suppressed out to day 14 in all groups. This phenomenon is also observable in a representative raster plot (actogram) of data from rats in the 2.0-G group (Fig. 2). Animals exposed to centrifugation show a decrease in activity at centrifugation onset followed by a gradual increase in activity, which, by day 14, does not return to baseline. When the last 3 days of centrifugation (“post” days 12, 13, and 14) are compared with baseline days (“pre” days −3, −2, and −1), there is a statisti-
cally significant centrifuge dose-dependent decrease in total daily LMA in all centrifuge groups. Compared with their respective baseline days, the percent decrease for each group, respectively, was 1.0 G, −15.2%, *P* = not significant; 1.25 G, −26.9%, *P* < 0.02; 1.5 G, −44.5%, *P* < 0.01; and 2.0 G, −63.1%, *P* < 0.002 (paired *t*-tests between pre and post days means for all groups). When total time (minutes) active during the animals’ subjective awake periods is examined for experiment 1, there is a centrifuge dose-related decrease in total time active, 2 G > 1.5 G > 1.0 G (Fig. 3). Although the magnitude is less, there is also a decrease in time active during the animals’ subjective inactivity periods, 2.0 G ≥ 1.5 G ≥ 1.0 G. The LMA circadian rhythm shows similarity to the DBT circadian rhythm immediately after gravitational onset with apparent suppression of amplitude and mean, and erratic waveform giving the visual impression of arrhythmicity (Fig. 1).

**Effects on circadian rhythm acrophase, phase difference, and amplitude.** Group mean acrophases before centrifugation (pre days) were compared with the group mean acrophase postcentrifugation days in the four groups (0° reference time = 0600). DBT acrophases were pre 260 ± 15.8, post 251 ± 11.3 (1-G group); pre 266 ± 35.7, post 243 ± 14.3 (1.25-G group); pre 255 ± 12.4, post 248 ± 5.1 (1.5-G group); and pre 254 ± 12.9, post 255 ± 13.2 (2.0-G group). LMA acrophases were pre 261 ± 22.0, post 245 ± 17.8 (1-G group); pre 240 ± 41.0, post 245 ± 18.9 (1.25-G group); pre 277 ± 19.8, post 247 ± 24.2 (1.5-G group); and pre 273 ± 18.0, post 238 ± 16.1 (2.0-G group). Only the LMA acrophases for the 1.5- and 2.0-G exposures were significantly different (*P* < 0.002 and *P* < 0.0001, respectively, *t*-test for dependent samples), indicating that centrifugation resulted in a phase shift of 30–35°, which is equivalent to an ~2-h LMA peak phase delay.

Cosinor analysis indicated a severe DBT circadian rhythm disturbance after exposure to the hyperdynamic field with rhythm phase reversal occurring on days 2–5 in some animals. Immediately after exposure to centrifugation the internal phase relationship between DBT and LMA, circadian rhythm peaks changed significantly (Fig. 4). The DBT-LMA phase difference returned to baseline (within 95% confidence limits).
after hypergravity onset in 2.6, 3.0, and 4.5 days in the 1.25-, 1.5-, and 2.0-G groups, respectively.

Figure 5 shows the circadian rhythm group mean amplitudes for DBT and LMA. The DBT circadian rhythm amplitude in all three groups showed an immediate dampening after onset of centrifugation followed by a gradual increase through day 14, even in the control group. Note that in the 1.25- and the 1.5-G groups that the circadian amplitude returns to baseline or above by day 14, whereas in the 2.0-G group the amplitude is still slightly below baseline on day 14. Figure 5 indicates that the amplitude of the circadian rhythm of LMA does not recover by day 14 in all three groups, with the 2.0-G group showing the greatest suppression in circadian rhythm amplitude.

Quantification of readaptation time and time for establishment of new steady state. It is difficult, via macroscopic observation (visual inspection) of the data, to make any conclusions as to explicit changes in circadian rhythm metrics after centrifuge onset. Therefore, we used several mathematical methods to quantify LMA and DBT circadian rhythm disturbance and to estimate duration of readaptation. The readaptation duration method used the baseline period for reference data (see MATERIALS AND METHODS). Table 1 shows the recovery rate in days for readaptation of the DBT circadian rhythm mean, phase, and amplitude to the baseline range (95% confidence interval). There appears to be a centrifuge dose-dependent effect on rhythm amplitude and phase recovery but not on cycle mean. Note that the cycle mean values had returned to baseline by days 2 and 3 in all groups. Table 1 also shows the results of cosinor analysis and group synchrony analysis for group DBT circadian rhythm recovery. These methods gave comparable readaptation durations for the 1.25- and 1.5-G groups and also show a centrifuge dose-dependent recovery of the circadian rhythm of DBT. For the 2.0-G group, the group syn-
chrony method showed recovery by day 6, whereas the cosinor method showed recovery by day 10. Table 1 shows the similar analysis for the LMA circadian rhythm readaptation. Note that rhythm means and amplitudes do not return to baseline by day 14 in all groups. Also, there appears to be a centrifuge dose effect, but the magnitude is reduced compared with the DBT parameters in Table 1.

The steady-state duration method to quantitate circadian rhythm recovery used the last 3 days of the study (days 12, 13, and 14) as reference data. This was done because, as can be seen in Table 1, some of the LMA parameters (mean and amplitude) did not return to the baseline range (95% confidence limits) by day 14 (the end of the study). This method indicates that the LMA cycle means and amplitudes reach a centrifuge dose-dependent new steady state in 5–11 days.

The DBT data showed a transient recovery phenomenon that lasted from 1–3 days starting on the second day of hypergravity exposure. This is evident as the reappearance of rhythmic cycles in the DBT data plots (Fig. 1) and as a transient recovery of DBT amplitude to baseline levels (Fig. 5). This phenomenon was also observed in the DBT rhythm synchrony plots.

Periodogram analysis and rhythm splitting. Centrifugation-induced periodicity disturbances were seen in all animals immediately after centrifugation onset (days 1, 2, and 3). These disturbances were characterized by rhythm splitting, reductions in spectral amplitudes (>50%), and the appearance of noncircular periods (<23 h or >26 h). Figure 6 shows 4-day moving window periodograms of LMA data in a rat from the 2.0-G group. This rat exhibited the most pronounced arrhythmia after centrifugation onset. Maximum rhythm disruption occurred during days 1–5, in which the 24-h spectral power was attenuated.

Examination of moving periodogram spectra revealed the occurrence of statistically significant circadian rhythm splitting in both DBT and LMA after hypergravity onset in some animals. Centrifugation-induced DBT rhythm splitting was identified in three of seven, three of eight, and six of eight rats at 1.25, 1.5, and 2.0 G, respectively. LMA rhythm splitting was found in one of seven, five of eight, and six of eight rats at 1.25, 1.5, and 2.0 G, respectively. An example of DBT and LMA rhythm splitting is shown in Fig. 7. This figure is illustrative of the complex dynamics of rhythm splitting. The DBT rhythm in this animal showed pro-
gressive dissociation of the two circadian rhythm components from 24.0 h to ~18.8 and 33.6 h, respectively, followed by convergence back to 24.0 h and progressive lengthening of the circadian period followed by rhythm splitting, arrhythmia, or spectral amplitude modulation. However, the LMA rhythm in this animal showed a different pattern, with progressive circadian period lengthening, rhythm splitting, fusion of the split circadian components, and occasional arrhythmia occurring during the 4-day data windows (Fig. 7).

DISCUSSION

This study confirms previous findings that centrifugation of naive animals results in a dramatic acute decrease in DBT and gross LMA. We report a dose response in this effect (2.0 G > 1.5 G > 1.25 G), which has not been previously reported in rats. Furthermore, we have used relatively sophisticated time-series analysis to quantitate alterations in DBT and LMA circadian rhythm amplitude, phase angle, and DBT-LMA phase relationship, and to detect the phenomenon of circadian rhythm splitting, which has not been reported previously in response to chronic hypergravity exposure. Our analysis provides a comprehensive overview of the physiological response to centrifugation onset (gravity onset) and the time course of adaptation to 14-day chronic centrifugation exposure.
The experimental animals were in good general health throughout this study, as determined by the daily examinations by animal care technicians. Food consumption showed a transient decrease on centrifugation onset, which returned to normal in 4 days.

Our laboratory has previously reported for this study (39) that there were no statistical differences in food intake among groups on days 11–14 (food intake, g/body mass$^{2/3}$). This is consistent with a previous study (34) that indicated that average food consump-

Table 1. Recovery rate for readaptation and steady-state stabilization duration of DBT and LMA circadian rhythm mean, phase, and amplitude

<table>
<thead>
<tr>
<th>Rhythm</th>
<th>Circadian metric</th>
<th>Readaptation duration</th>
<th>Steady-state (stabilization) duration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1.25 G</td>
<td>1.50 G</td>
</tr>
<tr>
<td>DBT</td>
<td>Amplitude</td>
<td>6.0</td>
<td>7.4</td>
</tr>
<tr>
<td></td>
<td>Acrophase</td>
<td>0.9</td>
<td>7.9</td>
</tr>
<tr>
<td></td>
<td>Cosinor</td>
<td>4.0</td>
<td>5.0</td>
</tr>
<tr>
<td></td>
<td>Cycle mean</td>
<td>2.8</td>
<td>2.4</td>
</tr>
<tr>
<td></td>
<td>Synchrony amplitude</td>
<td>4.6</td>
<td>4.6</td>
</tr>
<tr>
<td></td>
<td>Cross-correlation</td>
<td>*</td>
<td>8.5</td>
</tr>
<tr>
<td>LMA</td>
<td>Amplitude</td>
<td>13.6</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td>Acrophase</td>
<td>3.5</td>
<td>3.5</td>
</tr>
<tr>
<td></td>
<td>Cosinor</td>
<td>3.0</td>
<td>3.0</td>
</tr>
<tr>
<td></td>
<td>Cycle mean</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td>Synchrony amplitude</td>
<td>2.8</td>
<td>4.9</td>
</tr>
<tr>
<td></td>
<td>Cross-correlation</td>
<td>4.4</td>
<td>8.5</td>
</tr>
<tr>
<td>DBT/LMA</td>
<td>Phase difference</td>
<td>2.6</td>
<td>3.0</td>
</tr>
</tbody>
</table>

Readaptation duration refers to the time interval between the exit of the circadian metric data from the baseline 95% confidence limit and reentrance back into the 95% confidence limit for at least 1 cycle. Stabilization duration refers to the time interval between centrifuge hypergravity onset and the reentrance of the circadian metric data into the posthypergravity stabilization (days 12, 13, and 14) 95% confidence limit. DBT, deep body temperature; LMA, locomotor activity. *Metric did not readapt to baseline 95% confidence limits.

The experimental animals were in good general health throughout this study, as determined by the daily examinations by animal care technicians. Food consumption showed a transient decrease on centrifugation onset, which returned to normal in ~4 days.
tion showed no significant variation when rats were exposed to 1.75 G for up to 40 days. Another indication that our animals were in good health was that urinary corticosterone excretion (days 11–14, ng/mg urinary creatinine) was similar among groups (39).

Gravity onset decrease in DBT and LMA. The initial DBT decrease observed at centrifugation onset (e.g., −1.45 and −2.40°C for the 1.25- and 1.5-G groups, respectively) was consistent with values reported originally by Oyama et al. (47) for rats exposed to 1.25 and 1.5 G (−1.68 and −2.63°C, respectively). The initial decrease in LMA at gravity onset is also consistent with previous rat studies (23, 24, 26). The mechanism responsible for the drop in DBT at gravity onset has been attributed to increased heat loss and/or decreased metabolism (47) and to reduced food consumption (47, 49). Recently, Bishop et al. (5, 6) found a similar acute drop in DBT when 21°C acclimated rats were exposed to 10% O₂ ambient environments. Also, Mortola and Seifert (40), in a similar study of rats exposed to 10.5% O₂ (hypoxia), presented circadian rhythm data showing hypoxia-onset effects remarkably similar to the gravity-onset effects presented herein, e.g., hypothermia, circadian amplitude suppression, and what looks like arrhythmia for 4–5 cycles. It is possible that gravity onset alters cerebral blood flow, leading to relative hypoxia of central areas involved in temperature regulation, thus resulting in the DBT hypothermic response noted at gravity onset. BRN 3.1 knockout mice with deficient vestibular apparatus do not experience the gravity-onset drop in DBT (41). This would implicate the vestibular apparatus as a possible site that

![Fig. 7. Six-hour moving-increment DBT (A) and LMA (B) periodogram analysis of rat 20 from the 1.5-G group showing circadian rhythm splitting and periodicity changes in response to centrifugation. The baselines for each periodogram spectrum are sequentially staggered in the plot to reveal changes in circadian period lengths. Rhythm splitting was characterized by progressive lengthening of the circadian period followed by dissociation of the 2 circadian rhythm components from 24.0 to −18.8 and 33.6 h, respectively, followed by convergence back to 24.0 h. Dashed line, rhythm splitting; ▲, circadian rhythm spectral amplitude peaks.](http://www.jap.org)
may be influenced by the gravitational-loading alteration in blood flow.

**Effect on DBT and LMA circadian rhythms.** Centrifugation onset has an immediate and marked effect on the DBT and LMA circadian rhythms (Fig. 1). Time-series analysis confirms what is seen in the raw data, specifically that rhythm mean and amplitude (Fig. 5) are greatly depressed with transient-phase desynchronization (Fig. 4) and period disturbances (Fig. 7). Although dampening of the DBT circadian rhythm amplitude has been reported in dogs (46), monkeys (18), mice (42), and rats (32), none of the previous investigators used time-series analysis to quantify the magnitude of the response and the duration of the disturbance. In the present study, several methods were used to estimate the time required for rhythms to readapt to baseline levels after centrifugation onset. For DBT, this ranged from 4 to 10 days (cosinor, cross-correlation, and group synchrony methods). For LMA, this ranged from 3 to 10 days. There was a centrifuge level dose response, with the 2.0-G group taking the longest time for the rhythms to be reestablished. The differences in readaptation and restabilization duration for the various circadian rhythm metrics reflect variance in the measures and the mathematical properties of the methods. The cosinor measures the bivariate distribution of rhythm phase and amplitude and is dependent on a harmonic model assumption. The cross-correlation method is independent of model assumptions but is dependent on the number of data points per cycle. Therefore, it is more sensitive to changes in circadian waveform than the cosinor. The group synchrony amplitude method is dependent on the harmonic model and is very sensitive to changes of phase synchronization across a group of animals.

After recovery from the initial hypothermia, DBT returned to baseline levels, indicating that thermoregulatory homeostasis is operational concomitant to the continuous environmental perturbation of increased gravity load. However, the chronic increased gravity load suppressed LMA directly and continued to affect LMA throughout the course of the study, resulting in the new steady-state condition for many of the circadian parameters. As previously mentioned, it is possible that the DBT hypothermia at the onset of hypergravity resulted from altered cerebral blood flow leading to relative hypoxia of central areas involved in temperature regulation and reduced metabolic rates combined with increased heat loss. However, the decline in LMA at the onset of hypergravity may have resulted from the direct suppressive effect of hypergravity on movement behavior. Also, hyperdynamic field exposure results in fluid shifts and vestibular responses, which initiate autonomic and limbic system changes that can affect the CTS (43). These physiological effects could have differentially altered the DBT and LMA circadian mechanisms.

We have no definitive explanation for the transient recovery phenomenon observed in the DBT rhythm starting on the second day of hypergravity exposure. The appearance of this phenomenon in the amplitude and rhythm synchrony data, but not the cross-correlation data, implies that the temporary reappearance of rhythmicity resulted from amplitude, not phase or waveform changes, because the cross-correlation method primarily detects changes in rhythmic wave form and phase. These transient amplitude changes may have been induced by hypergravity-onset masking effects on body temperature, which often lasted 8–10 h, and which were much longer than the 2-h masking effects induced by the animal maintenance procedures. These masking effects could have also produced the appearance of enhanced rhythm synchrony in the groups exhibiting the transient recovery phenomenon if the masking effects occurred simultaneously across the animals in the group, thus resulting in phase synchronization artifacts. However, we cannot explain why enhanced rhythm synchrony appeared in the experiment 1 but not in the experiment 2 groups. Future centrifuge studies, in which the daily centrifuge stoppage protocol for animal maintenance is eliminated or controlled, will be necessary to determine whether the transient recovery phenomenon is a masking artifact or the result of CTS responses to hypergravity.

Progressive increases in DBT circadian amplitude after initial exposure to hypergravity were observed in all groups, including the 1.0-G control group. This unexpected trend in the control group could represent progressive stabilization of the DBT rhythm as a consequence of adaptation to the habitat environment, since the baseline period of 3–4 days was relatively short. The trends observed in DBT amplitudes in the hypergravity groups could represent a combination of habitat adaptation and stabilization to new hypergravity steady states. The control group also showed a transient decline in DBT amplitude at hypergravity onset (Fig. 5) coincident with the same responses in the hypergravity groups. Because the control group animals were housed in the centrifuge facility during the centrifuge runs with the other rats, the transient decline in control group DBT amplitude may be a stress-induced artifact induced by exposure of the control group animals to noise and motion associated with the initial hypergravity onset within the centrifuge facility.

C. A. Fuller (personal communication) has suggested that gravity onset results in suppression of circadian rhythmicity and has implied that this is an overt expression of attenuation of the central circadian oscillator. Cosinor and moving periodogram analysis of our data indicate that gravity onset results in rhythm disturbance, including phase shifts and the appearance of multiple periodicities. This may result in raw data that look somewhat arrhythmic. However, nearly all rats that we examined via time-series analysis (cosinor, moving periodogram) showed statistically significant circadian periodicities that persisted during the transient period immediately after gravity onset. It may be that the exposure to this hyperdynamic centrifugation environment decreased the coupling between the central oscillator and the overt rhythms that we measured here. The decrease in rhythm amplitudes would support this view. It may be argued that our
experimental design, which included centrifuge maintenance stops of up to 1 h/day, resulted in data artifacts that may have given rise to the rhythmicity that we see in our data, i.e., the masking increase in DBT as a result of the daily animal maintenance. We believe that this is not the case since we carefully edited out the data sections at the maintenance times. Additionally, we believe that the finding of rhythm splitting is inconsistent with a centrifuge start-stop masking effect for the following reasons: 1) the rhythm splitting showed a centrifuge level dose response; 2) rhythm splitting was manifested by periodicity divergence and convergence, i.e., increases in period length followed by fusion; 3) no differences in circadian rhythm metrics and recovery rates were found in a study comparing daily and weekly centrifuge stops and restarts (19); 4) rhythm splitting can occur despite animal maintenance (10); and 5) the large decrease in group rhythm synchrony amplitude after hypergravity onset indicates the occurrence of nonuniform changes in rhythm phase and amplitude among individual animals. We believe that our findings are inconsistent with an effect that would be predicted by an animal maintenance centrifuge stop occurring at precisely the same time each day (i.e., 24.0-h interval).

There are a number of mechanisms by which exposure to a hyperdynamic field (via centrifugation) may acutely influence the circadian system and thus produce an effect on overt circadian rhythmicity. These include alterations in food intake and its potential effect on the circadian system (51). As noted above, our animals showed decreased food intake for ~4 days after gravity onset. The decrease in LMA may have a direct feedback effect on the circadian system and thus affect overt rhythmicity (15). Changes in circadian rhythms noted may be the consequence of phase shifts induced by the initial and daily hypergravity onsets (25), emotional fear in response to the unanticipated environmental changes (35, 53, 27), or attenuation of light-dark cycle entrainment (3). Our laboratory showed in a previous study that exposure to chronic 2 G elevates urinary corticosterone excretion for up to 4 days (45). This may have a direct effect on the CTS. The exposure to ambient hypoxia (10% O₂) resulted in dampening of the DBT circadian rhythm for up to 3 days (5, 40) in a dynamic pattern, which was very similar to the hypergravity effect that we show herein. It is possible that ambient hypoxia results in hypoxemia to brain areas involved in the CTS. This may also be the case after gravity onset.

The discovery of circadian rhythm splitting in this study, enabled by the persgressive spectral analysis, was serendipitous since this phenomenon was not reported in previous centrifuge studies. Rhythm splitting, in which the primary circadian rhythm dissociates into two circadian components with different period lengths, has previously been initiated in nocturnal rodents by bright constant light (10) and food restriction (1, 7) treatments. Splitting seems to be the consequence of paired and synchronized suprachiasmatic nuclei oscillators that become reorganized into two oppositely phased circadian oscillators (13) under bright constant light treatment or by decreased oscillator coupling force partially due to a decrease in melatonin secretion from the pineal gland in malnourished animals (1). In our study, rhythm splitting could have been induced by several factors, including 1) reduced food consumption at the onset of hypergravity (39); 2) hypergravity exposure, which resulted in dissociation of the circadian oscillators, similar to the effects of bright constant light exposure (25); and 3) the attenuation of light-dark cycle rhythm entrainment by stress induced by hypergravity onset, evidenced by elevated urinary corticosterone secretion (39). Stress-induced attenuation of rhythm entrainment has been previously reported (3). The observed individual differences in the occurrence and dynamics of rhythm splitting as a function of hypergravity level and rhythm variable probably reflect individual differences in rat circadian period lengths and thresholds for hypergravity-induced splitting effects and perhaps individual differences in hypergravity-induced declines in food consumption and stressor responses.

In conclusion, this study shows a dose-dependent relationship between chronic hypergravity level exposure and duration of readaptation and stabilization of DBT and LMA circadian rhythms in rats. Durations of readaptation in certain circadian rhythm metrics (e.g., DBT amplitude in 9.6 days, LMA stabilization in 7 days) were comparable to values previously reported (26, 21). The phenomenon of rhythm splitting has only been reported for rat overt circadian rhythms after chronic constant light exposure (2, 7, 10), after constant light to 12:12-h light-dark transitions (7), after low-protein malnutrition (1), after methamphetamine ingestion (29), and it has never been reported in prior rat hypergravity studies. The previously reported 2-G-induced loss in LMA rhythmicity for up to 7 days (21) may indicate that “macroscopic” examination of rhythm data cannot distinguish the presence of multiple periodicities that may exist in the data sets. Using time-series analysis, we have shown that 2-G exposure results in phase shifts and multiple periodicities in the data, which may give the impression of arrhythmicity in the raw data. DBT and LMA circadian rhythmicity and internal rhythmic synchronization are profoundly disrupted by exposure to hypergravity in the rat. Readaptation to a new rhythmic steady state occurs within 7–10 days.

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DISCLOSURES

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