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CHRONIC CENTRIFUGATION (HYPERGRAVITY) INFLUENCES THE CIRCADIAN SYSTEM OF THE RAT. **D. C. HOLLEY, C.W. DEROSHIA*, M.M. MORAN* AND C. E. WADE*, Department of Biological Sciences, San Jose State University, San Jose, CA, USA 95192-0100 (dholley@email.sjsu.edu), and *Gravitational Research Branch, Center for Gravitational Biology Research, NASA-Ames Research Center, Moffett Field, CA, USA 94035 (cwade@mail.arc.nasa.gov).**

Objectives: This study was conducted to quantitate the effect of chronic centrifugation on animal energy expenditure, and to evaluate the response of the DBT and LMA circadian oscillators (biological clocks) to acute hypergravity onset and adaptation to chronic hypergravity exposure. We report herein the analysis of the gross animal locomotor activity (LMA) data (which was measured as an indirect indicator of animal energy expenditure), and the deep body temperature (DBT) data. Our study differs from previous reports on this topic (2,3,6), in that we provide a comprehensive mathematical analysis to quantitate the physiological adaptation of the circadian timing system to three chronic hypergravity "doses" (1.25, 1.5 , and 2 G).

Methods: Two studies were performed on the 24 foot diameter centrifuge in the Center for Gravitational Biology Research at NASA-Ames Research Center. Both studies were approved by the NASA-Ames Institutional Animal Care and Use Committee. In Study 1 (ACS-1), twenty-four male Sprague-Dawley rats (initial weight 150 g) were obtained from Simonsen Laboratories (Gilroy, CA). In Study 2 (ACS-3), 24 rats (initial weight 156 g) were obtained from the same vendor. Animals were randomly assigned to three groups: Study 1, 1.0 G (stationary control), 1.5 G (centrifuged), and 2.0 G (centrifuged); Study 2 1.0 G (stationary control), 1.25 G (centrifuged), and 1.50 G (centrifuged). The animals received food (Rat Chow #5012, Ralston Purina) and water *ad libitum*. Lighting was provided by fluorescent lamps producing an average illuminance of about 45 lux within the animal cages. Lights were on a 12:12 hour light:dark cycle with lights on at 06:00 A.M. Temperature was constant 23 +/- 2 deg. C. All animals were implanted with a 7.5 gram telemetry transmitter (TA10TA-F40, Data Sciences International, St. Paul, MN) within the abdominal cavity via a procedure previously described (4). They were subsequently individually housed in standard vivarium cages for the seven-day recovery period. Data were recorded digitally at 5 minute intervals using the Data Sciences data acquisition system (Dataquest ART Gold). Following the 7-day recovery from the surgical procedure, each animal was placed individually into custom designed lexan metabolic cages (23"x14"x13") in the room housing the 24-foot diameter centrifuge. For seven days the animals were housed in their respective cages in the centrifuge room with the centrifuge turned off for base line data collection. Centrifuge rotation rate was 21.1 revolutions per minute (rpm) for Study 1 and 16.06 rpm for Study 2. The centrifuge groups were at a radius position on the centrifuge so that one group was exposed to 1.5 G (8 feet) and the other group was exposed to 2.0 G (12 feet) in Study 1; and 1.25 G (8 feet) and 1.5 G (12 feet) in Study 2. The control (1.0 G Group) cages were within the same room that housed the centrifuge, but were stationary. Once per day the centrifuge was stopped for 1 hour (08:00 to 09:00 hr) and body weights, water and food consumption, and urine volume and feces weights were recorded. The animals were exposed to the chronic centrifugation environment for 14 days. The daily (08:00 to 09:00 hr) animal maintenance procedure resulted in a spike artifact (masking effect) in the LMA and the DBT data between 08:00 hr and approx. 10:15 hr daily. Therefore, the LMA and DBT data were edited using a data folding technique. This was accomplished by folding data segments from 06:50 to 07:55 and from 10:20 to 11:25 into the 08:00 to 10:15 time period. For circadian rhythm analysis, non-linear trends resulting mainly from the centrifugation induced hypothermic response, were filtered from the DBT data using a 48 hour data window (robust locally weighted regression, RLWR, ref. 1). The detrended DBT data and the undetrended LMA data were then smoothed using a 3-hour data window to filter erratic fluctuations and telemetry artifacts. The data were further converted (decimated) from 5 minute samples to 30 minute means. Circadian rhythm significance was determined using cosinor analysis (5).

Results: Figures 1 and 2 show the group mean LMA and DBT hypergravity dose response data (1.25, 1.5, and 2.0 G) for the control period (d –3 to day –1) through day 14 of hypergravity exposure. All animals demonstrated circadian rhythms of deep body temperature (DBT, cosinor analysis $P<0.0002$) and gross locomotor activity (LMA, $P<0.02$) during the base line control period (Figures 1 and 2).

Since the centrifuge speed differed between Study 1 and 2 (21.1 and 16.06 rpm, respectively) and since the 1.5 G Groups were at different centrifuge radii for the 2 studies (8 feet Study 1, 12 feet Study 2), the 1.5 G Group data were compared to see if 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 show only data for the 1.5 G Group in Study 1 when showing centrifuge dose responses in the following figures.

Figure 1 shows group mean plots of the LMA data. This figure clearly shows the gravity dose dependent decrease in activity following the onset of the hyperdynamic fields. Note that activity appears to be reduced out to day 14 in all groups.

Figure 2 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. Following onset of centrifugation, the rhythm amplitude, phase, and mean were disrupted (Figure 2). We used several mathematical methods to quantify the rhythm disturbance and to estimate duration of readaptation (see companion paper by DeRoshia et al. in this symposium).

DBT and LMA circadian rhythm amplitude and acrophase estimates were determined at each sample point by the method of complex demodulates (7). Under baseline control conditions each group demonstrated DBT and LMA rhythm acrophases of between 254 and 280 degrees (0 degree reference time = 06:00 hr.). Following exposure to centrifugation the internal phase relationship between DBT and LMA changed significantly. The DBT vs. LMA phase difference returned to base line (within 95% confidence limits) in 2.6, 3.0, and 4.5 days, after hyper-G onset in the 1.25, 1.5 and 2.0 G Groups, respectively.

The DBT circadian rhythm amplitude in all three groups showed an immediate dampening following onset of centrifugation followed by a gradual increase through day 14. 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. 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.

Conclusions: Exposing rats to hypergravity via chronic centrifugation resulted in an acute gravity dose dependent decrease in both core body temperature, and gross locomotor activity. Circadian rhythm phase and amplitude were altered by the exposure to the hyperdynamic conditions on the centrifuge. The DBT/LMA phase difference relationship was altered returning to baseline in 2.6 to 3.0 and 4.5 days in the 1.25, 1.5, and 2.0 G groups respectively. The circadian rhythm amplitudes of DBT and LMA were decreased by exposure to hypergravity, with the greatest suppression found in the 2.0 G Group which lasted through day 14. Alteration of the ambient gravitational field effects the circadian timing system of the rat in a gravitational dose dependent manner.

Figure 1 LMA

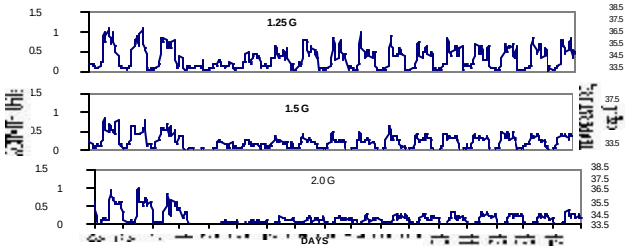
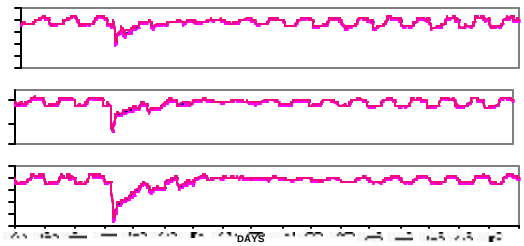


Figure 2 DBT



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