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READAPTATION OF RAT LOCOMOTOR ACTIVITY AND DEEP BODY TEMPERATURE CIRCADIAN RHYTHMS FOLLOWING EXPOSURE TO CHRONIC HYPERGRAVITY.

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Objectives: This study was conducted to evaluate the adaptation response of rat deep body temperature (DBT) and locomotor activity (LMA) circadian rhythms to acute hypergravity onset and chronic hypergravity exposure. Our study differs from previous reports (1,2) in that we provide a comprehensive mathematical analysis to quantitate the physiological adaptation of the circadian timing system to three chronic hypergravity intensities (1.25, 1.5 , and 2 G. and also provide a statistically quantitative evaluation of rhythmic readaptation and stabilization durations for several circadian rhythm metrics.

Methods: Two studies were performed on the 24 foot diameter centrifuge in the Center for Gravitational Biology Research at NASA-Ames Research Center with 24 Sprague-Dawley rats randomly assigned to three groups: Study 1: 1.0 G (stationary control), 1.5 G and 2.0 G ; Study 2:1.0 G , 1.25 G, and 1.50 G. The animals received food and water *ad libitum.*. Lights were on a 12:12 hour light:dark cycle with lights on at 06:00 A.M. DBT and LMA data were recorded digitally by telemetry at 5 minute intervals. Detailed methods are reported in another paper (3) at this symposium. For circadian rhythm analysis, non-linear trends, resulting mainly from the centrifugation induced hypothermic response, and telemetry artifacts were filtered from the DBT data by robust locally weighted regression. Circadian rhythm metrics evaluated were cyclic mean, phase, and amplitude (complex demodulates), group rhythm synchrony amplitude (based upon the circular statistics method of Batschelet (4)), cosinor 95% confidence limit ellipse and cross correlation coefficients. Post-hypergravity adaptation duration was defined as the interval between the time at which the data for the circadian metrics exceeded the baseline 95% nonparametric confidence limit (Tukey's lower fence) and the time at which the metric data reentered the confidence limit for at least one cycle. A post-hypergravity restabilization, or steady state, duration was similarly defined as the interval between centrifuge onset and the time at which the metric data reentered the post-hypergravity stabilization (days +12,13,14) confidence limit. Cosinor adaptation and restabilization was determined as the intervals during which the cosinor 95% error ellipses were distinctly separate. Circadian rhythm periodicity was evaluated using hanned and normalized periodogram spectra on four-cycle data windows at 6-hr moving increments to resolve transitional rhythm phenomena. Periodogram spectral statistical significance levels were determined from confidence limits established from 1000 spectra obtained from randomized input data sets.

Results: A centrifuge level dose response effect was evident in which the duration of readaptation to baseline levels increased progressively from 1.25 G to 2.0 G in most circadian rhythm metrics except the cycle mean level (Table 1). Certain metrics (DBT cross correlation, LMA mean and amplitude) did not readapt by day 14 but did stabilize by 8.6 days. This indicates that these rhythm metrics stabilized to new steady state levels. The duration of readaptation was markedly different for the different circadian metrics, and differed for a given metric between DBT and LMA data, but in general circadian rhythmic amplitude took longer to readapt and stabilize than circadian acrophase. Statistically significant circadian rhythm splitting in both DBT and LMA was observed following hypergravity onset (Figure 1). Significant rhythm splitting was identified as the occurrence of two statistically significant spectral peaks in the circadian periodicity range of 16-45 hours separated by non-significant spectral amplitudes and which were not circadian harmonics (e.g., 48 or 16 hours) or submultiples of each other. Centrifuge level dose response rhythm splitting occurrences were identified in 3/7, 3/8 and 6/8 rats at 1.25 G, 1.5 G and 2.0 G, respectively in DBT and in 1/7, 5/8 and 6/8 rats, respectively, in LMA.

Conclusions: This study is the first to show a dose dependent relationship between hypergravity level and duration of readaptation and stabilization. Durations of readaption in certain circadian rhythm metrics (e.g., DBT amplitude in 9.6 days, LMA stabilization in 7 days) were comparable to values previously reported studies (1,2). However, these studies used subjective estimates of adaptation duration while the present study utilized statistically reliable estimates for several circadian rhythm parameters. The phenomenon of rhythm splitting has only been reported for rat circadian rhythms following constant light to 12L:12D

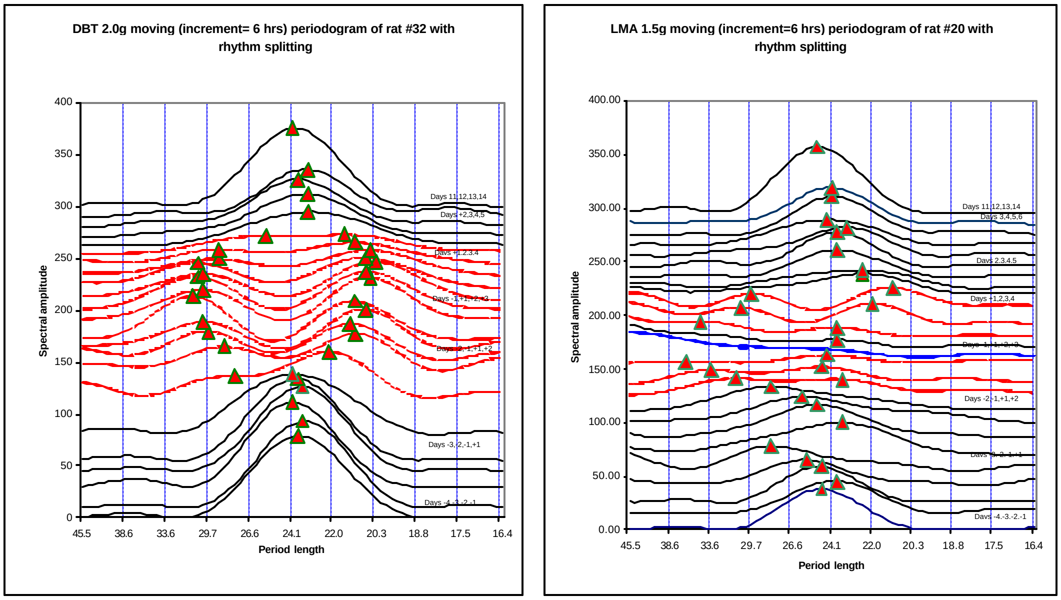

Figure 1. Moving periodograms for DBT and LMA during the hypergravity onset transitional period showing statistically significant incidences of circadian rhythm splitting (dotted lines) in rat #20 at 1.5 G. Periodogram spectral represent 4-day data windows incremented at 6-hr intervals.

Table 1.
Readaptation and steady state stabilization durations for several DBT and LMA circadian rhythm metrics at three different centrifugation levels (1.25 G, 1.50 G, and 2.00 G)

		Readaptation Duration (days)			Steady state (stabilization) duration		
Rhythm	Circadian metric	Centrifuge level			Centrifuge level		
		1.25 G	1.50 G	2.00 G	1.25 G	1.50 G	2.00 G
DBT	Amplitude	6.0	7.4	9.6	11.0	10.0	10.1
	Acrophase	0.9	7.9	6.8	3.6	5.8	6.6
	Cosinor	4.0	5.0	10.0	5.0	8.0	7.0
	Cycle mean	2.8	2.4	2.4	1.8	2.0	2.6
	Synchrony amplitude	4.6	4.6	6.3	4.6	6.1	8.1
	Cross correlation	**	8.5	**	1.1	8.5	8.6
LMA	Amplitude	13.6	**	**	4.5	11.1	7.1
	Acrophase	3.5	3.5	6.6	3.1	8.8	10.2
	Cosinor	3.0	3.0	5.0	3.0	4.0	1.0
	Cycle mean	**	**	**	11.0	8.0	6.8
	Synchrony amplitude	2.8	4.9	6.3	3.2	4.1	10.0
	Cross correlation	4.4	8.5	10.0	4.4	8.8	10.0

** metric did not readapt to baseline 95% confidence limits

transitions (5) and has never been reported in prior rat hypergravity studies. The reported loss in LMA rhythmicity at 2.0 G for up to 7 days (6) may indicate that eyeball or “macroscopic” examination of rhythm data cannot distinguish the presence of multiple periodicities in data, such as those detected in this study, which may appear to be arrhythmic. Circadian rhythmicity and internal rhythmic synchronization are profoundly disrupted by exposure to hypergravity in the rat. This may be the consequence of circadian phase shifts induced by the initial and daily hypergravity onsets (7), emotional fear in response to the unanticipated environmental changes (8), or attenuation of light/dark cycle entrainment (9).

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