Inhibiting cortisol response accelerates recovery from a photic phase shift

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Mohawk, Jennifer A., Katherine Cashen, and Theresa M. Lee. Inhibiting cortisol response accelerates recovery from a photic phase shift. Am J Physiol Regul Integr Comp Physiol 288: R221–R228, 2005. First published August 26, 2004; doi:10.1152/ajpregu.00272.2004.—Jetlag results when a temporary loss of circadian entrainment alters phase relationships among internal rhythms and between an organism and the outside world. After a large shift in the light-dark (LD) cycle, rapid recovery of entrainment minimizes the negative effects of internal circadian disorganization. There is evidence in the existing literature for an activation of the hypothalamic-pituitary-adrenal (HPA) axis after a photic phase shift, and it is possible that the degree of HPA-axis response is a determining factor of reentrainment time. This study utilized a diurnal rodent, Octodon degus, to test the prediction that the alteration of cortisol levels would affect the reentrainment rate of circadian locomotor rhythms. In experiment 1, we examined the effects of decreased cortisol (using metyrapone, an 11β-hydroxylase inhibitor) on the rate of running-wheel rhythm recovery after a 6-h photic phase advance. Metyrapone treatment significantly shortened the length of time it took animals to entrain to the new LD cycle (11.5% acceleration). In experiment 2, we examined the effects of increased cortisol on the rate of reentrainment after a 6-h photic phase advance. Increasing plasma cortisol levels increased the number of days (8%) animals took to reentrain running-wheel activity rhythms, but this effect did not reach significance. A third experiment replicated the results of experiment 1 and also demonstrated that suppression of HPA activity via dexamethasone injection is capable of accelerating reentrainment rates by ~33%. These studies provide support for an interaction between the stress axis and circadian rhythms in determining the rate of recovery from a phase shift of the LD cycle.

stress; circadian rhythm; metyrapone; dexamethasone; entrainment

The stress response, regulated by the hypothalamic-pituitary-adrenal (HPA) axis, can affect entrained circadian rhythms. Elevated glucocorticoid (i.e., cortisol or corticosterone) levels, often observed after the presentation of a stressor, have far-reaching effects on the circadian system. Studies have shown that stress exposure disturbs peripheral circadian rhythms of locomotor activity, body temperature, hormonal secretion, and food intake (2, 4, 14, 16, 22).

The dramatic effects of photic phase shifts and the effects of stress with the associated glucocorticoid release on entrained behavioral rhythms have been well studied. However, there has been little investigation of the relationship between the HPA axis (i.e., glucocorticoids) and reentrainment after a photic phase shift. In female rats, a positive correlation was found between stress-induced corticosterone levels and the number of days it took activity rhythms to reentrain after an 8-h phase shift (28). The animals were subjected to mild restraint stress and allowed to recover for 2 wk before the advance of the LD cycle and resulting reentrainment period. This relationship supports the conclusion that individual variabilities in time to recover from a photic phase shift may be due to differences in HPA-axis responsivity.

Studies of humans also provide some evidence for an interaction between photic phase shifts (and transmeridian air travel) and HPA-axis activation. Desir et al. (9) found that adrenocorticotropic hormone (ACTH) and cortisol reentrain at different rates subsequent to a photic phase shift. Typically, these two hormones are highly regulated by each other through negative feedback. In a study of airline workers, flight attendants on transmeridian flights (at least an 8-h time change) had significantly elevated cortisol levels compared with flight attendants on domestic flights and ground crew (7). In the laboratory, an 8-h phase shift was capable of raising the cortisol nadir above that observed under entrained conditions (6). These studies suggest that phase shifts are stressful events, capable of stimulating glucocorticoid release.

The present study sought to further elucidate the relationship between the HPA axis and the circadian system after a phase shift. Specifically, we wanted to determine whether activation of a glucocorticoid response affects the rate of locomotor activity reentrainment after a shift in the LD cycle. The Octodon degus, a hystricomorph rodent, is an ideal animal for this purpose. The degu provides a model for human circadian behaviors because it is diurnal, social, and has a rate of reentrainment after a shift in the LD cycle (jetlag recovery) of 1–3 days for each hour of phase shift (11, 12). Degus are responsive to both photic and nonphotic zeitgebers (“time-
injection.

degus’ cortisol levels to pressing the HPA-mediated stress response for up to 24 h after a receptor agonist, activates negative-feedback pathways, supersedes the normal circadian rhythmicity of the HPA axis, and increases the overall level of cortisol secretion (21).

animals were housed in 42.5 × 22 × 19-cm cages fitted with Nalgene running wheels (9 cm wide, 34.5 cm in diameter) and maintained in a 12:12-h LD cycle. Ambient room temperature was maintained at 18 ± 1°C. All procedures involving animals were approved by the University Committee for the Care and Use of Animals at the University of Michigan.

Activity data collection and analysis. We monitored running-wheel activity using Vitalview software and hardware (Minimitter, Sunriver, OR) and collected results in 10-min bins. Time of activity onset was recorded for each animal on each experimental day. Activity onset was defined as the first 10-min bin of activity at a level equal to or greater than 10% of the animal’s average daily activity rate (22). The phase angle of entrainment (the relationship between the time of lights off and the onset of morning activity) was defined as three continual 10-min bins of activity at a level equal to or greater than 25% of the animal’s average daily activity rate. The phase delay was defined as the amount of time between lights off and the time of activity onset.

Blood draw procedures in experiments 1 and 2, which were used to verify changes in cortisol, were a potential source of additional stress for the animals. This could have resulted in activation of the sympathetic nervous system or additional adrenocortical activity, thereby increasing the extent of the effect of metyrapone injection in experiment 1. Therefore, a third experiment (experiment 3) examined the effects of manipulation of the glucocorticoid response on reentrainment rate in otherwise undisturbed animals. Animals in experiment 3 underwent a photic phase shift with concurrent metyrapone injection, as in experiment 1, as well as an additional shift with concurrent dexamethasone injection. Dexamethasone, a glucocorticoid receptor agonist, activates negative-feedback pathways, suppressing the HPA-mediated stress response for up to 24 h after administration (3, 5). Data from our laboratory demonstrate that 5 mg/kg of dexamethasone is capable of suppressing degus’ cortisol levels to −2% of control values 6 h after the injection.

METHODS

Determination of cortisol rhythm. A necessary requirement of these experiments was that the circadian profile of cortisol secretion be established for the Octodon degus. In a preliminary study (Fig. 1), we determined the circadian rhythm of cortisol secretion in male degus by taking blood samples at six time points across the day and assaying for cortisol (n = 20). A peak exists around the time of lights on, with a nadir at zeitgeber time (ZT) 12 (Fig. 1).

Subjects. Adult male Octodon degus, born in a laboratory colony at the University of Michigan, were used in these studies. Animals (n = 49; 20 in experiment 1, 20 in experiment 2, and 9 in experiment 3) were between 2 and 3 yr of age (expected life span for degus is 6 yr) and weighed 185–250 g. They were maintained on a diet of Purina rodent chow (5001), with food and water available ad libitum. The animals were individually housed in 42.5 × 22 × 19-cm cages fitted with Nalgene running wheels (9 cm wide, 34.5 cm in diameter) and maintained in a 12:12-h LD cycle. Ambient room temperature was maintained at 18 ± 1°C. All procedures involving animals were approved by the University Committee for the Care and Use of Animals at the University of Michigan.

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Blood sampling procedure. The degus were brought into the experimental room one at a time and anesthetized with 2% isoflurane (Aerrane, Baxter Pharmaceutical, Deerfield, IL); 0.5 ml of blood was then collected via intracardiac puncture. With animals in running wheels, jugular catheters were not practical and rapid blood collection was not possible by tail nick or other commonly used methods. Samples were collected within 2 min of removal from the home cage. Blood was clotted on ice for 2 h, centrifuged, and then stored at −20°C before being assayed for cortisol.

Cortisol radioimmunoassay. We measured plasma cortisol concentration using a modified radioimmunoassay kit (GammaCoat cortisol 125I; DiaSorin, Stillwater, MN), which has been previously used for assay of degu cortisol as described by Kenagy et al. (18). The plasma was diluted 1:1 (5 μl of plasma, 5 μl of serum) in human serum blank (AB 1–40, DiaSorin). The minimum detection threshold was 0.21 μg/dl, and the inter- and intra-assay coefficients of variation were 9.2% and 7.0%, respectively. All samples from experiments 1 and 2 were measured using the same standard curve.

Pellet preparation. Cholesterol pellets were made by melting 100 mg/pellet of crystalline 5-cholesten-3β-ol powder (Steraloids, Newport, RI) into 4.5 × 4.5 × 5-mm molds (Ted Pella, Redding, CA).
Cortisol pellets were made in the same way with the use of 75 mg of hydrocortisone (cortisol; 17-hydroxycorticosterone) powder (Sigma, St. Louis, MO) and 25 mg of cholesterol. This dose was determined by pilot studies that used pellets of 50, 75, and 100% cortisol concentration. The 75% cortisol concentration reliably raised cortisol levels above nadir baseline to a concentration equivalent to that of peak daily cortisol release, which was greater than the cortisol concentration produced by a phase shift alone.

Surgical procedure. In experiment 2, animals were surgically implanted with cholesterol and cortisol pellets. Animals were anesthetized with 2% isoflurane, and pellets were implanted subcutaneously in the dorsal neck area. Incisions were closed with wound clips, and an antibiotic solution was applied to the wound.

Experiment 1: suppression of endogenous cortisol synthesis. Baseline cortisol levels were collected 1 h before the onset of subjective night (ZT11; clock time 1700–1800) in animals entrained to a 12:12-h LD cycle \( (n = 20) \). Two weeks later, the LD cycle was phase advanced 6 h (“control shift,” 12:12-h LD cycle, lights on at 2400). To control for the possibility that the injection procedure was a stressor, half of the animals \( (n = 10) \) were injected subcutaneously with 1 ml of saline for the first 5 days after the photic phase shift. The injection took place between 0600 and 0900 (corresponding to preshift ZT0;

Fig. 2. Actograms from representative animals in the control (A) and metyrapone injected conditions (B) from experiment 3. The top bar denotes the original light cycle; the bottom bar denotes the light cycle after the 6-h phase advance. ▲ Injection day (animal was injected with 300 mg/kg metyrapone between 2100 and 2200). The vertical gray bars denote phase angle of entrainment before and after recovery from the phase shift. The missing data on days 11 and 12 in A are due to equipment failure; the small bout of activity that appears to occur at midnight through day 8 in A is actually an artifact due to electrical surges affecting the data collection equipment.
see Fig. 3), a time when the cortisol rhythm would be expected to be at its peak, based on the previously entraining LD cycle. The other 10 animals remained unhandled. Blood samples were collected from all animals on day 3 of the new LD cycle between 1700 and 1800 (preshift ZT11). Blood was drawn at this time because this is when the daily cortisol nadir occurs (see Fig. 1); therefore, collections at this time would allow for the most noticeable observation of any glucocorticoid activation occurring in response to the phase shift. The degus then remained undisturbed until their running-wheel activity rhythms had reentrained to the new LD cycle.

After reentrainment of activity rhythms to the new LD cycle had been achieved, the LD cycle was again phase advanced 6 h (12:12-h LD cycle, lights on 1800). Animals were injected subcutaneously with 300 mg/kg metyrapone (Sigma) in 1 ml of saline for the first 5 days of the photic phase shift. Injections occurred between 2400 and 0200 (preshift ZT0), a time when the cortisol rhythm would be expected to be at its peak, based on the previously entraining LD cycle. Blood samples were collected from all animals on day 3 of the new LD cycle, between 1100 and 1200 (preshift ZT11). Animals (n = 15 animals successfully completing all phases of the experiment) remained undisturbed for ~3 wk to ensure that their running-wheel activity rhythms had reentrained to the new LD cycle.

After reentrainment of activity rhythms to the new LD cycle had been achieved, the LD cycle was again phase advanced 6 h (12:12-h LD cycle, lights on 1500). Animals were injected subcutaneously with 300 mg/kg metyrapone (Sigma) in 1 ml of saline for the first 5 days of the photic phase shift. Injections occurred between 2100 and 2200 (corresponding to preshift ZT0; see Fig. 3), a time when the cortisol rhythm would be expected to be at its peak, based on the previously entraining LD cycle. After the 5-day injection period, degus remained undisturbed until their running-wheel activity rhythms had reentrained to the new LD cycle.

After reentrainment of activity rhythms to the new LD cycle had been achieved, the LD cycle was phase advanced 6 h (12:12-h LD cycle, lights on 0900). Animals were injected subcutaneously with 5 mg/kg dexamethasone (Phoenix Scientific, St. Joseph, MO) for the first 5 days of the photic phase shift. Injections occurred between 1500 and 1600 (preshift ZT0), a time when the cortisol rhythm would be expected to be at its peak, based on the previously entraining LD cycle. After the 5-day injection period, animals remained undisturbed for ~3 wk to ensure that their running-wheel activity rhythms had reentrained to the new LD cycle.

A final phase advance was done to confirm that there was no effect of multiple phase shifts on the time to reentrain. The LD cycle was phase advanced 6 h (12:12-h LD cycle, lights on 0300). Animals were then left undisturbed until their running-wheel activity had reentrained.

Fig. 3. Experimental paradigm for experiments 1, 2, and 3. Top bar represents the preshift light-dark (LD) cycle, with the open portion being the light period and the filled portion the dark period. Bottom bar represents the postshift LD cycle (for all photic phase-shifts). Arrows indicate blood draw (at a time corresponding to preshift ZT11), which took place before the phase shift and on day 3 postshift in experiments 1 and 2. ▪. Drug or saline injection (at preshift ZT0), which took place for the first 5 days after the phase shift in experiments 1 and 3.
reentrain locomotor activity rhythms (14.7 ± 1.4 days with cortisol pellets compared with 13.6 ± 1.1 days in the cholesterol pellet condition, n = 9, P > 0.10). We found no significant change (P > 0.05) in body weight (average body weight of 225 ± 4.7 g) or total daily activity as a function of cortisol pellet implantation.

Experiment 3. No significant difference was detected in days to reentrain for animals in the control shift that were injected with saline compared with those that were not injected; therefore, the data for these two groups were pooled to form a single control group. Repeated-measures ANOVA (n = 9) revealed an effect of condition (control, metyrapone injected, or dexamethasone injected) on days to reentrain locomotor activity (F3,24 = 20.985, P < 0.001). Bonferroni adjusted pair-wise comparisons confirmed that metyrapone injection significantly accelerated reentrainment (by 39%) compared with either control condition (P < 0.001; Figs. 6 and 7). Similarly, dexamethasone injection resulted in significantly accelerated reentrainment (by 32.7%) compared with control conditions (P < 0.001; Fig. 6). There was no difference between days to reentrain in the metyrapone-injected condition and the dexamethasone-injected condition. There was no difference between days to reentrain in the first and second control shifts.

DISCUSSION

Inhibiting cortisol synthesis accelerated the rate of recovery of circadian locomotor rhythms after a 6-h advance of the photic phase. Metyrapone or dexamethasone injections for the first 5 days of the phase shift resulted in ~36% (experiment 3) faster reentrainment of locomotor activity rhythms than when animals were in the control condition (saline injection or undisturbed). Increasing cortisol levels at the time of a phase shift tended to lengthen the time to recover locomotor activity rhythmicity, although this effect did not reach significance. These experiments indicate that glucocorticoid concentrations may affect the rate of circadian reentrainment, with lower cortisol levels resulting in more rapid recovery of normal circadian entrainment. Alternatively, it may be that a lack of normal circadian fluctuation of glucocorticoid is accelerating reentrainment (24).
In experiment 1, metyrapone inhibition of cortisol synthesis during recovery from a photic phase shift resulted in levels of plasma cortisol similar to those seen under basal conditions. Although it is possible that there had been some shift in the cortisol rhythm by day 3 postshift (the time of blood collection), it is unlikely that the rhythm had shifted substantially. This is because activity and body temperature have typically only shifted 1–1.5 h by the third day after a phase shift (12). The data from this study corroborate this finding (see Figs. 2 and 7). What is more, the human literature does not support a rapid shift of the cortisol rhythm after a change in the LD cycle (6). The blood in experiment 1 was always collected at preshift ZT11; therefore, postshift, the blood sample was taken in the middle of the dark period. Had the cortisol rhythm fully shifted, we would expect to have seen much lower cortisol levels on day 3 than we did (see Fig. 1 for the normal circadian profile of the cortisol rhythm).

The results of experiment 1 suggest that decreasing cortisol responsivity to a phase shift accelerates the establishment of normal circadian entrainment. An alternative explanation for these results is that saline injections may have increased cortisol levels, thus resulting in the observed difference in reentrainment rates. However, there were no differences in cortisol levels between saline-injected and unhandled degus on day 3 postshift, making this explanation of the results unlikely.

In experiment 2, there was a delay in time to recover from a phase shift when cortisol levels were modestly elevated compared with a phase shift in which no exogenous cortisol was provided, although this effect did not reach significance. Animals’ cortisol levels on day 3 of the control shift were 2.33 times higher than basal levels, whereas cortisol pellet implants resulted in day-3 postshift cortisol levels that were 3.28 times those of baseline levels. Thus, although we successfully manipulated cortisol levels, it appears that the phase shift itself increased cortisol levels (because cortisol levels were elevated in the control, cholesterol-implant shift) to such an extent that an additional elevation would have required a much larger dose of exogenous cortisol. Further trials (in which increased concentrations and numbers of cortisol pellets as well as hydrocortisone injection were used) have failed to elevate degus’ cortisol levels above what was seen in experiment 2. However, it is known that degus, when trapped in the field, are capable of reaching cortisol concentrations higher than those reported in this study (18). It appears that future experiments will need to utilize physical and/or psychosocial stressors to exaggerate the stress response to a photic phase shift.

Experiment 3 replicated the finding of experiment 1 that metyrapone injection can accelerate reentrainment after a photic phase shift. However, the accelerating effects of metyrapone injection were amplified in experiment 3 compared with experiment 1 (39% vs. 11.5% acceleration of recovery time). This is likely due to an effect of the blood collection procedure in experiment 1 on stress levels. That is, the blood draw via intracardiac puncture (in experiment 1) provided a possible confound masking the profound effects of glucocorticoid synthesis inhibition on reentrainment of locomotor activity.

Experiment 3 further supported the hypothesis that ablation of the glucocorticoid response to stress accelerates reentrainment by demonstrating that 5 days of dexamethasone injection significantly reduces time to reentrain in degus. Although dexamethasone is a glucocorticoid agonist, the activation of glucocorticoid receptors is transient compared with the suppression of HPA responsivity that follows. It is hypothesized that the accelerating effects of dexamethasone on reentrainment rate are due to the suppression of the stress response. However, it is possible that dexamethasone injection at preshift ZT0 is phase advancing the animals, resulting in a synergistic effect with the photic cue [dexamethasone injection can result in phase shifting of temperature rhythms in the rat (15)]. However, there are several reasons why phase-shifting effects of dexamethasone cannot explain the results obtained in experiment 3. First, the dexamethasone injections that resulted in significant changes in phase in the previous study were of a dose twice that used in experiment 3. A lower dose (1 mg/kg of body wt) was unable to produce alterations in circadian phase in the rat. Additionally, the effects of dexamethasone on circadian phase were obtained from free-running animals and were greater in animals with restricted feeding schedules than...
those fed ad libitum. Perhaps most importantly, injections of dexamethasone given at the time of day corresponding with temperature acrophase resulted in no alterations of circadian phase. It is likely that the time of injection in experiment 3 (at least on the first day of injections) corresponded with temperature acrophase, since large alterations in body temperature rhythm do not occur immediately (12).

This research has demonstrated that suppressing the glucocorticoid response accelerates reentrainment after a shift in the LD cycle. The observation that glucocorticoid inhibition accelerates reentrainment rate provides further evidence that circadian desynchrony results in activation of a stress response. The experiments described herein suggest that the important neuroendocrine messenger in this effect is glucocorticoid.

Glucocorticoids may not be solely responsible for the negative effects on reentrainment rates resulting from desynchrony. A chemical messenger in the cortisol synthesis pathway may play for at least some of the symptoms associated with jetlag. For example, increases in corticotropin releasing hormone or arginine vasopressin (AVP) may act directly within the brain to alter the recovery rate of activity rhythms. Corticotropin releasing hormone and AVP increase the release of ACTH from the pituitary, which causes an increase in cortisol. Engelmann et al. (10) found that the amount of AVP released within the SCN can vary in response to a physiological stressor. A phase shift may affect the amount of AVP released in the SCN, which could have a direct effect on the desynchronization of circadian rhythms, while also altering glucocorticoid release. Eventually, increased exogenous cortisol would decrease activity of brain mechanisms via negative feedback, thereby altering the SCN function of phase-shifting animals. Such changes might increase or decrease the degree of internal desynchrony.

In humans, a plethora of stressors such as crowded airports, lost luggage, and desynchrony with environmental and social cues may add to the physical and emotional problems caused by photic phase shifts and transmeridian jet travel. Hence, the relationship between circadian desynchrony and the stress axis has serious medical implications. Decreasing cortisol levels may result in faster recovery from phase shifts in humans, and thus pharmacological manipulation of cortisol might be employed to treat shift workers and air travelers. Research in this area will also have implications for investigations of the interaction between circadian dysfunction and depression and aging. As our knowledge of circadian desynchrony improves, so will our ability to successfully combat its stressful effects. This research demonstrated that decreased cortisol levels aid in recovery from a phase shift in the LD cycle. Future experiments will be necessary to determine the mechanism of action of the HPA axis on recovery from jetlag.

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GRANTS

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