Effects of adrenalectomy and subsequent corticosterone replacement on rat sleep state and EEG power spectra

MARGARET J. BRADBURY, WILLIAM C. DEMENT, AND DALE M. EDGAR
Sleep Research Center, Department of Psychiatry and Behavioral Sciences, Stanford University School of Medicine, Stanford, California 94305

Bradbury, Margaret J., William C. Dement, and Dale M. Edgar. Effects of adrenalectomy and subsequent corticosterone replacement on rat sleep state and EEG power spectra. Am. J. Physiol. 275 (Regulatory Integrative Comp. Physiol. 44): R555–R565, 1998.—Individual effects of corticotropin-releasing hormone (CRH) and glucocorticoids on sleep have been difficult to discern due to the feedback effects each hormone exerts on the other. In addition, it is not known whether hypothalamic-pituitary-adrenal axis hormones alter sleep homeostasis or circadian influences on sleep propensity. We therefore analyzed sleep architecture and electroencephalographic (EEG) power in freely moving rats before and after removal of corticosterone (thus elevating endogenous CRH) by surgical adrenalectomy. Adrenalectomy reduced the amplitude of the diurnal rhythms of maximal and average sleep bout lengths (P < 0.001). After adrenalectomy, power from 1 to 4 Hz decreased (P < 0.042), whereas power from 9 to 12 Hz increased in the power spectra of the EEG recording (P = 0.001). Administration of physiological corticosterone replacement reversed some of these effects. Supraphysiological corticosteroid replacement in adrenalectomized rats reduced the amount of non-rapid-eye-movement sleep in the 24-h cycle (P = 0.001). During each endocrine condition, rats were sleep deprived for 6 h. Endocrine status did not alter the subsequent homeostatic response to sleep deprivation. Thus ADX and supraphysiological corticosteroid replacement each altered sleep architecture without a demonstrable effect on sleep homeostasis.

ACTIVITY of the hypothalamic-pituitary-adrenal (HPA) axis has been hypothesized to influence sleep quality (16). Sleep patterns of patients with endocrine disorders support this concept. For example, non-rapid eye movement (NREM) sleep and slow-wave electroencephalographic (EEG) activity were increased in patients with untreated Addison's disease and restored to normal values after corticosteroid replacement therapy (18). This Addison-induced sleep pattern was mimicked by administration of the adrenal steroid synthesis blocker metyrapone in normal subjects (18). Conversely, patients with abnormally high levels of cortisol due to untreated Cushing's disease slept less and had more nocturnal awakenings before therapeutic reduction in adrenal steroid production (21). Experiments in healthy, young human volunteers have also implicated a modulatory role for the hormones of the HPA axis in sleep-wake cycle regulation and sleep architecture. Corticosteroid effects on slow-wave sleep depend on the affinity of experimental corticosteroids for the two cloned corticosteroid receptors. Hydrocortisone and other corticosteroids that bind the high-affinity, type I receptor increased time spent in slow-wave sleep (for review, see Ref. 16). By contrast, dexamethasone, which preferentially occupies the lower affinity, type II corticosteroid receptor (9), increased the time spent awake or in shallow sleep during the night (16). Administration of exogenous corticosteroids also reduced the time spent in rapid eye movement (REM) sleep (16). Corticotropin-releasing hormone (CRH) has also been implicated in sleep-wake cycle regulation. Sleep in humans infused intravenously with CRH contained less stage 4 sleep (16, 34), less REM sleep (16), and more frequent nocturnal awakenings (16, 34) compared with vehicle-infused controls. Together, these results suggest that elevated concentrations of both corticosteroids and CRH can impair sleep quality.

Frequent waking and early rising are common complaints of the elderly population (5) and may be exacerbated by age-related changes in HPA axis activity (10, 35). The mechanism and onset of age-induced HPA axis influences are not known. The homeostatic sleep drive results in increased NREM sleep time, slow-wave EEG activity, and increasingly consolidated sleep as a function of prior wakefulness (2, 12). Suprachiasmatic nucleus-generated maintenance of alertness is hypothesized to oppose the homeostatic sleep drive during the activity portion of the day (13). HPA axis hormones may interact with either of these processes. The age at which HPA axis-induced changes in sleep commence is also not known. The circadian rhythm of CRH mRNA expression in the paraventricular nucleus of the hypothalamus (PVN) (6) and concentrations of free and total plasma corticosteroids (30) in rats demonstrated age-related changes as early as 13 mo. Thus HPA axis influences on sleep regulation may vary as a function of aging and may be evident by midage (12–18 mo) in the rat.

Feedback pathways, both positive and negative, within the HPA axis complicate clarification of the individual roles that corticosteroids and CRH play in sleep-wake regulation. For example, CRH injections increase corticosteroid secretion through the production of pituitary ACTH, whereas exogenous corticosteroids reduce CRH production through occupation of B receptors both within the HPA axis and in other areas of the central nervous system (9). Conversely, removal of the negative-feedback influence of corticosteroids by adrenalectomy disinhibits CRH production for up to 60 days in rats (33). Animal models allow examination of corticosteroid and CRH effects on sleep while manipulating or controlling feedback regulation. Results from animal studies generally corroborate the notion that both CRH and corticosteroids can each reduce sleep quality. For example, intracerebroventricular injection...
of CRH into rats reduced slow-wave sleep and the power in EEG frequencies up to 4 Hz (6) (15). In addition, intracerebroventricular injection of CRH-receptor antagonists reduced wake time at the onset of the activity phase in rats (28). Intracerebroventricular injections of CRH-receptor ligands were not thought to stimulate the release of ACTH or corticosterone, although these hormones were not directly measured. Finally, corticosterone (B) clamped in adrenalectomized (ADX) rats at levels seen during stress increased the amount of active waking and decreased REM sleep during the activity portion of the light cycle compared with ADX controls (26).

Although CRH and high levels of B may each alter different aspects of sleep, few comprehensive studies have examined the effects of these hormones on sleep state, sleep continuity, and EEG spectral power obtained simultaneously. Furthermore, it is not known whether CRH and corticosteroids influence the homeostatic sleep drive or the circadian regulation of waking. Finally, although aging is associated with reduced sleep quality, the effects of aging on the interaction between sleep and HPA axis hormones have not been studied. We therefore tested three hypotheses concerning HPA axis activity and sleep quality. First, we employed a repeated-measures design to examine the effects of high levels of CRH and of exogenous B on sleep patterns in ADX rats. Sleep architecture and EEG power in rats were measured before and after elevation of CRH levels and elimination of B by adrenalectomy. ADX rats were subsequently given B replacement at a dose designed to restore CRH levels (1 X B) or to suppress CRH and damp plasma B concentrations at supraphysiological levels (2 X B). Second, we tested the hypothesis that high levels of these hormones would interfere with homeostatic regulation of sleep. To examine this, we analyzed sleep state, the EEG spectral power, and continuity of sleep after sleep deprivation during each endocrine condition. Third, to test the hypothesis that the sleep patterns of midaged rats would be more susceptible to the effects of HPA axis manipulation, we studied both young adult and midaged rats.

METHODS

Animal Surgery

Male Wistar rats (250–400 g at time of surgery; Charles River Laboratories, Wilmington, MA) were anesthetized with pentobarbital sodium (Nembutal, 60 mg/kg) and surgically prepared for continuous EEG, electromyogram (EMG), body temperature (Tb), and nonspecific locomotor activity (LMA) recordings (14). The sterilized surgical implant consisted of two frontal EEG (stainless steel screws; 3.9 mm anterior to bregma and 2.0 mm bilateral to sagittal midline), two occipital EEG (stainless steel screws; 6.4 mm posterior to bregma and 5.5 mm bilateral to sagittal midline), and two EMG (Teflon-coated stainless steel) recording leads. The EMG wires were placed under the nuchal trapezoid muscles. The implant was affixed to the skull with cyanoacrylate and dental acrylic. A sterilized biotelemetry transmitter (Mini-Mitter, Sunriver, OR, or Barrows, Palo Alto, CA) was surgically implanted into the abdomen for Tb and LMA measurements. Adrenalectomy was performed using a dorsal approach in Metofane-anesthetized rats. Sterilized B pellets were inserted subcutaneously in Metofane-anesthetized ADX rats (15–21 days after adrenalectomy). B pellets (90–115 mg) were made from mixed molten B (Aldrich) and cholesterol (Steraloids) as previously described (1). Physiological B pellets (1 X B) consisted of 40% B–60% cholesterol. Supraphysiological pellets (2 X B) consisted of 80% B–20% cholesterol. Rats recovered for a minimum of 3 wk before sleep-wake monitoring.

Recording Environment and Automated Monitoring

Rats were housed individually within modified Nalgene microisolator cages equipped with filter top risers and commutators (Biela Engineering, Irvine, CA). These cages were located in a sound-attenuated, stainless steel chamber that consisted of individual, ventilated compartments. A 24-h light-dark cycle (12:12 LD, lights at 30–35 lx in cage) was maintained throughout the study. Lights on was defined as zeitgeber time (ZT 0). EEG/EMG recording cables connected the EEG/EMG implant to the commutator. Food and water were available ad libitum.

After a 2-wk adaptation to the EEG/EMG recording environment, SCORE, a microcomputer-based sleep-wake and physiological monitoring system (14), was used to tabulate arousal state (NREM, REM, wake, and E-dominated wake) and LMA in 10-s epochs and Tb every minute. This system monitored amplified EEG potentials across frontal and parietooccipital cortices (band pass 1–30 Hz, digitized at 100 Hz) and integrated EMG (band pass 10–100 Hz). Templates for each arousal state were created from individually taught EEG epochs. The system software used feature extraction from these EEG templates, integrated EMG values, and a contextual algorithm to score the arousal state each 10-s epoch. Tb and LMA were detected by a receiver (Data Sciences, St. Paul, MN) located under the cage.

EEG Analysis

All data were digitized for offline analysis. If, after offline examination of the data, the automated scoring performance was unsatisfactory, EEG arousal state templates were refined with a SCORE algorithm. Arousal states for all 10-s epochs from a given recording were reassigned based on the new templates. EEG traces with 5% recording artifact in NREM and REM sleep were discarded. The same scoring criterion was used for each rat across all of the endocrine conditions to be described below. Sleep-wake state was analyzed for percentage NREM and REM sleep per hour. Sleep-wake state was also analyzed for length of sleep (NREM and REM) and wake (wake and E-bouts per hour (SBL and WBL, respectively). A complete sleep bout was defined as a minimum of three sleep epochs. Bouts were terminated by a minimum of three consecutive wake or E-epochs. In instances in which one or two wake or E-epochs separated two sets of three or more sleep epochs, a single bout was counted. Wake bouts were defined analogously. EEG recordings from NREM epochs were screened twice for artifacts (first automatically and then visually) and analyzed with Hartley's modification of the fast Fourier transform (3). The resultant EEG power spectra were binned in 1-Hz increments.

Study Design

Treatment groups. The 1 X B rats entered the study design when they were young (3–6 mo old, n = 7) or midaged (12–18 mo old, n = 7). Three 24-h EEG recordings were made when rats were undisturbed (baseline): 1) before adrenalectomy
Three 6-h EEG recordings from a subset of the rats were made during recovery sleep after 6 h of sleep deprivation: 1) before adrenalectomy (con), 2) day 8 after adrenalectomy, and 3) day 5 after B pellet implantation. The 1 × B group was studied in two parallel groups, with three or four young and three or four midaged rats in each group. ADX + 2 × B rats entered the study design while they were young (n = 9) or midaged (n = 2). Recordings were made as above. The 2 × B group was studied in two parallel groups, with four or five young rats in each group and both midaged rats in one group.

Sleep-wake recordings. During baseline sleep, rats were completely undisturbed for 24 h before and throughout the studied period. Sleep deprivation began at ZT 6 (6 h after lights on) and ended at ZT 12 (lights off). EEG traces and real-time sleep-wake scoring from all rats were visible to experimenter as a guide for application of wake stimulus. Initially, the chamber doors were opened, and individual cages were partially withdrawn to awaken the rats. When more stimuli were required, novel objects (e.g., pieces of paper towel) were added to the cages. Near the end of the sleep deprivation, noise (e.g., tapping with pencil) was applied to individual cages as required. Rats were not physically handled during the sleep deprivation unless cable maintenance was required. Data were continuously collected for 24 h after the end of sleep deprivation while the rats were completely undisturbed.

Blood collection and plasma B determination. ADX, ADX + 1 × B, and ADX + 2 × B rats were anesthetized with metofane. Inhalation anesthetic was used as a stress stimulus (4) for the detection of functional regenerated adrenal tissue in rats with incomplete adrenalectomy. Small blood samples were taken from a lateral tail vein to verify adrenalectomy 1–7 days after the end of the baseline ADX recording. Blood samples were also taken to evaluate the dose of B provided by the pellet 5 days after the end of the sleep deprivation recording. Blood (100–300 µl) was collected with heparinized capillary tubes, mixed with 15 µl EDTA in chilled Eppendorf tubes, and then transferred to serum separation gel packs (Becton-Dickinson, Franklin Lakes, NJ). Plasma was collected after 2–3 days of separation and then frozen at −80°C. Before assay for B concentration, plasma was centrifuged at 10,000 rpm. Plasma B concentrations were determined by RIA (ICN, Costa Mesa, CA). Intra-assay coefficient of variation was 5.6% at 10 µg B/dl plasma.

Statistics

Baseline sleep. Within the 1 × B group, endocrine and age effects on all sleep variables were tested with two-way ANOVA corrected for repeated measures (RMANOVA). Within the 2 × B group, endocrine effects on all sleep variables were tested with one-way RMANOVA. Significant sources of variance were identified with the Newman-Keuls post hoc test.

Recovery sleep after sleep deprivation. Only a subset of rats from each endocrine group was sleep deprived. Furthermore, no midaged, ADX + 2 × B rats were sleep deprived. Therefore repeated measures were not used. Within each young and midaged endocrine group, t-tests were used to compare percent NREM in time-matched baseline and recovery sleep recordings. Two-way ANOVA were used to detect age and endocrine effects on sleep deprivation responses (recovery sleep – baseline sleep) in the Con, ADX, and ADX + 1 × B groups. The Newman-Keuls post hoc test was used to identify sources of significant variance.

In instances in which the data were not normally distributed, ANOVA was applied to rank transforms of the data.

RESULTS

Adrenalectomy reduced plasma B concentrations to 0.2 ± 0.2 µg/dl plasma, a level near the limit of detection of the RIA (0.1 µg/dl plasma; n = 23). In two rats, adrenalectomy was incomplete as defined by RIA. Sleep recording data from these rats were not included in the analysis below. Ten days after B pellet implantation, 1 × B replacement in ADX rats resulted in B concentrations of 4.7 ± 1.4 µg/dl plasma (n = 11).

### Table 1. Baseline endocrine effects on NREM and REM sleep

<table>
<thead>
<tr>
<th></th>
<th>Con</th>
<th>ADX</th>
<th>ADX + 1 × B</th>
<th>ADX + 2 × B</th>
<th>RMANOVA</th>
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<tr>
<td><strong>% NREM/h</strong></td>
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<td>Lights on</td>
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<tr>
<td>1 × B group</td>
<td>55.1 ± 1.2</td>
<td>57.2 ± 1.4</td>
<td>55.9 ± 2.0</td>
<td>48.7 ± 1.9</td>
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<td>2 × B group</td>
<td>53.4 ± 1.1</td>
<td>54.3 ± 1.0</td>
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<td>Lights off</td>
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<td>1 × B group</td>
<td>34.9 ± 1.1</td>
<td>36.8 ± 1.3</td>
<td>34.3 ± 1.3</td>
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<td>33.6 ± 1.6</td>
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<td><strong>% REM/h</strong></td>
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<td>Lights on</td>
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<td>1 × B group</td>
<td>7.7 ± 0.5</td>
<td>6.4 ± 0.7</td>
<td>7.6 ± 1.0</td>
<td>8.9 ± 0.7</td>
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<td>8.8 ± 0.8</td>
<td>7.1 ± 0.9</td>
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<td>Lights off</td>
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<tr>
<td>1 × B group</td>
<td>3.3 ± 0.3</td>
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<td>4.1 ± 0.9</td>
<td>2.3 ± 0.3</td>
<td>NS</td>
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<tr>
<td>2 × B group</td>
<td>4.2 ± 0.4</td>
<td>5.0 ± 1.0</td>
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Values are means ± SE and are 12-h averages. Groups with corticosterone replaced at physiological and supraphysiological levels (1 × B and 2 × B, respectively) were analyzed separately. Con, control; ADX, adrenalectomized; 1 × B group, 2-way repeated-measures ANOVA (RMANOVA) was applied, with age as 1 factor and endocrine status the 2nd, repeating factor. Age was not a significant factor for these variables. Thus data for young and midaged rats in 1 × B group were grouped. 2 × B group, 1-way RMANOVA, with endocrine status as the repeating factor, was applied. *Results of post hoc contrasts (Newman-Keuls).
Endocrine and Age Effects on NREM and REM During Baseline Sleep

The percent NREM per hour and the percent REM per hour were each averaged in 12-h bins corresponding to lights on (rest phase) and lights off (activity phase; see Table 1 for statistical detail). Adrenalectomy did not change the percent NREM during lights on or off in either group of rats. The $2 \times B$, but not $1 \times B$ replacement after adrenalectomy, reduced the percent NREM at both times of day (Table 1). Figure 1 shows that recordings made from $ADX + 2 \times B$ rats had fewer hours of NREM in 12 h compared with control (Con) or ADX recordings during both the lights on ($F(2,29) = 18.5, P = 0.001$) and lights off ($F(2,29) = 6.0, P = 0.011$) portions of the light cycle. Taken as a percentage of the Con recording for each rat, the reduction in NREM time during $ADX + 2 \times B$ was greater during the lights off phase (paired $t$-test, $P = 0.027$). The percent REM during lights on decreased significantly after adrenalectomy in the $2 \times B$ group (Table 1); this trend was not significant in the $1 \times B$ group ($P = 0.06$). The $2 \times B$ replacement after adrenalectomy restored the percent REM during lights on. During lights off, $2 \times B$ replacement into ADX rats reduced the percent REM below that measured when rats were Con or ADX. Aging to 12–18 mo did not effect percent NREM, hours of NREM in 24 h, or percent REM.

Endocrine and Age Effects on Sleep and Wake Continuity During Baseline Sleep

The mean SBL per hour and the maximal (max) SBL per hour were each averaged in 12-h bins corresponding to lights on (resting phase) and lights off (activity phase; see Table 2 for statistical detail). In general, adrenalectomy fragmented sleep through shorter SBL during the resting phase, whereas SBL were longer during the activity phase. During lights on in the $2 \times B$ group, adrenalectomy reduced the length of mean SBL and max SBL compared with Con. This fragmentation of sleep by adrenalectomy was not ameliorated with $2 \times B$ replacement. In both the $1 \times B$ and $2 \times B$ groups, adrenalectomy increased the mean SBL compared with the Con condition during lights off. Neither B-replacement dose reversed this effect. ADX rats had significantly longer max SBL during lights off than Con rats in the $1 \times B$ group. The $1 \times B$ replacement did not reverse this effect. In the $2 \times B$ group, $2 \times B$ replacement into ADX rats reduced the max SBL during lights on compared with ADX rats. Max SBL and mean SBL were unaffected by aging to 12–18 mo at either time of day.

The mean WBL per hour and the max WBL per hour were each averaged in 12-h bins corresponding to lights on (resting phase) and lights off (activity phase; see Table 2). During lights on, adrenalectomy increased the length of the mean WBL and max WBL compared with Con...
The effects of age and endocrine status on the power spectra are shown in Fig. 4. The spectra were divided into the δ (1–4 Hz), θ (5–8 Hz), α (9–12 Hz), and β (13–20 Hz)-bands. For each rat, the power in each band measured during the ADX and ADX + B endocrine conditions was normalized to the Con condition (Con = 100%). The effects of age and endocrine status on each frequency band were analyzed with RMANOVA, with lights on (resting phase) and lights off (activity phase) considered separately. Endocrine status affected the δ-frequency band in the 1 × B group during lights on [endocrine effect: F(2,31) = 7.78, P = 0.007] and lights off [endocrine effect: F(2,31) = 4.6, P = 0.035]. Subsequent contrasts revealed that ADX rats had reduced δ-power at both times of day. Reduced δ-power was restored by 1 × B replacement during lights on but not lights off. δ-Power was not significantly affected by endocrine status in the 2 × B group. Endocrine status also affected the α-frequency band in both the 1 × B and 2 × B groups of rats during lights on [endocrine status...
Endocrine and Age Effects on Tb and LMA During Baseline Sleep

Endocrine status, age, and transmitter brand (see Methods) did not affect the amplitude of the Tb rhythm or the mean Tb within both the 1 × B and 2 × B groups. Endocrine status had no effect on LMA in either group. Aging to 12–18 mo tended to decrease LMA in the 1 × B group (P = 0.06). An age effect was anticipated, since 7-mo-old rats display more voluntary wheel-running activity than rats aged 12 mo and older (Bradbury and Edgar, unpublished results). The two different brands of transmitters used have different sensitivities to LMA. This may have increased variance in LMA measurements, thus reducing the statistical resolution of age effects.

Endocrine and Age Effects on Compensatory Responses to Sleep Deprivation

After sleep deprivation (ZT 12), rats were allowed to sleep ad libitum as data were collected. In all age and endocrine groups, t-tests demonstrated that rats slept 13.4–20.4% more during the 1st 6 h of recovery sleep than during the corresponding hours of baseline sleep (P = 0.001, data not shown). The percent NREM per hour, percent REM per hour, max SBL per hour, mean SBL per hour, and relative δ-power per hour for each rat during baseline sleep were each subtracted from the corresponding time-matched measurement made during recovery sleep after sleep deprivation for each endocrine condition. The differences were defined as responses to sleep deprivation. Figure 5 shows the percent REM (top), mean SBL (middle), and max SBL (bottom) responses averaged over the 1st 6 h of recovery sleep after sleep deprivation. Two-way ANOVA revealed that age but not endocrine status had significant effects on the REM response [F(1,39) = 6.9, P = 0.013], mean SBL response [F(1,39) = 6.7, P = 0.014], and max SBL response [F(1,39) = 6.6, P = 0.015], with more robust responses in young rats. In addition to these age effects, there was a significant age by endocrine status interaction on the max SBL response [F(2,39) = 4.3, P = 0.023]; sleep continuity in midaged ADX rats was significantly reduced compared with young ADX rats.
There were no age or endocrine effects on the percent NREM responses and the relative $\delta$-power responses (data not shown). Due to differences between young and midaged ADX rats, $T_b$ in these rats was examined. Figure 6 (top) demonstrates that young and midaged ADX rats had similar $T_b$ after sleep deprivation. Variance was in part due to differences in the mean $T_b$ for each rat. Therefore individual hourly mean $T_b$ was expressed as the difference from the 12-h mean (sleep deprivation and 6 h of recovery sleep) for each rat. These difference scores were not affected by aging to 12–18 mo in ADX rats (Fig. 6).

**DISCUSSION**

In the present study, HPA axis manipulations that elevated CRH and B concentrations each induced distinct sleep disturbances. Adrenalectomy, which elevates CRH in the PVN (20) for at least 60 days (e.g., Ref. 33), reduced relative and absolute $\delta$-power in the EEG while increasing $\alpha$-power during baseline recordings. Adrenalectomy also attenuated the normally robust baseline circadian rhythm in sleep continuity, with shorter SBL during the resting phase. When B concentrations were clamped near stress levels, however, ADX rats exhibited less NREM sleep in 24 h. These results suggest that the separate effects of CRH and B on sleep architecture combine to reduce sleep quality in instances of HPA axis hyperactivity, such as depression, chronic stress, and aging.

Elevated CRH after adrenalectomy is a likely cause of sleep disturbances in the present study, but other explanations are possible. CRH release from the portal blood vessel in the present study was not measured directly, because this cannot be performed in conscious, undisturbed animals. ACTH was also not measured, because sufficiently large tail vein blood samples from conscious rats could not be collected within 2.5 min, the time at which concentrations of this plasma hormone rise after the onset of stress (4). Thus, although not measured directly, the elevation of CRH is inferred from well-documented effects of adrenalectomy in rats. It is important to note that Wynn et al. (36) have demonstrated that adrenalectomy caused little change in binding of CRH to its neural receptors. In addition to increases in CRH, adrenalectomy also results in unoccupied B receptors, removes adrenal sources of catecholamines, and changes plasma osmolarity. These additional effects of adrenalectomy may also contribute to the reported changes in sleep parameters. B replacement into ADX rats restored $\delta$- and $\alpha$-powers and increased the amplitude of SBL rhythms. Because catecholamines were not replaced and B has little mineralocorticoid activity (17), B-replacement effects suggest that sleep disturbances after adrenalectomy were attributable in large part to direct HPA axis effects (i.e., increased CRH within PVN and/or termination of B production).

The relative contributions of elevated CRH and removal of B from its receptors on sleep parameters after adrenalectomy remain to be distinguished. Intracerebroventricular injections of CRH compounds, however, provide some comparison for the present effects of adrenalectomy on sleep parameters. CRH injected intracerebrally reduced $\delta$-power in rats (15), as was measured in ADX rats. Adrenalectomy effects on sleep continuity in the present study are novel and not directly comparable to previously published reports. Sleep continuity, however, is normally proportional to the amount of $\delta$-power in rats (12). Thus the effects of intracerebroventricular injections of CRH support the notion that the adrenalectomy-induced decreases in $\delta$-power were due to increased CRH release. Conversely, Opp (28) has shown that intracerebroventricular injection of a CRH-receptor antagonist increased NREM time at the onset of the activity period. Adrenalectomy had no effect on NREM time in the present study. Although adrenalectomy only increases CRH in the PVN (20), intracerebroventricular injections of CRH-receptor antagonists affect CRH receptors.
throughout the brain. Regional changes in CRH-receptor activation may explain differences in the present results and those of Opp. Restoration of δ-power in ADX rats after local injection of CRH antagonists into target areas of the PVN would verify that increased CRH production in ADX rats was responsible for changes in δ-power and sleep continuity.

The effects of B-pellet replacement on sleep in ADX rats were somewhat dose dependent, with 1 × B replacement reversing many of the effects of adrenalectomy and 2 × B replacement reducing NREM sleep time. The 1 × B dose (4.5 µg/dl plasma) was approximately nine times greater than reported circadian trough concentrations and, 80% of the estimated daily mean in adrenal-intact rats (9). Tonic B presented at levels near the daily mean maximally reversed the effects of adrenalectomy on thymus gland mass, ACTH concentrations in plasma, and pituitary, food intake, and weight gain in adolescent rats (1). Binding data suggest that this dose fully occupies type I receptors but only partially occupies type II receptors (9). By contrast, the 2 × B-replacement dose (~9.0 µg/dl plasma) approached typical circadian peak values (9). A similar dose of B replacement shrank the thymus gland, reduced ACTH production, and decreased weight gain and food intake (1). This dose probably occupied type II receptors at levels normally achieved near the circadian maximum of HPA axis activity or during stress (9). Accordingly, ligands for the different receptors have somewhat different effects on human sleep. Type I receptor-prefering agonists, such as cortisol and hydrocortisone, slightly increased slow-wave sleep.

Table 3. Baseline sleep: endocrine effects on relative δ-power

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<tr>
<th></th>
<th>Con</th>
<th>ADX</th>
<th>ADX + 1 × B</th>
<th>ADX + 2 × B</th>
<th>RMANOVA</th>
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<td>1 × B group</td>
<td>36.4 ± 1.1</td>
<td>31.5 ± 0.8</td>
<td>34.5 ± 0.9</td>
<td>F (2,32) = 11.4, P = 0.001</td>
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<tr>
<td>2 × B group</td>
<td>37.9 ± 1.2</td>
<td>34.7 ± 1.2</td>
<td>35.4 ± 1.3</td>
<td>F (2,29) = 6.1, P = 0.010</td>
<td>Con &gt; ADX, ADX + 1 × B*</td>
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<tr>
<td>1 × B group</td>
<td>36.2 ± 1.1</td>
<td>32.3 ± 1.2</td>
<td>33.8 ± 1.7</td>
<td>F (2,32) = 3.9, P = 0.042</td>
<td>Con &gt; ADX*</td>
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<tr>
<td>2 × B group</td>
<td>34.5 ± 0.9</td>
<td>31.2 ± 0.7</td>
<td>32.8 ± 0.9</td>
<td>F (2,29) = 4.1, P = 0.040</td>
<td>Con &gt; ADX*</td>
</tr>
</tbody>
</table>

Values are means ± SE and are 12-h averages. Relative δ-power was expressed as percentage of total power (1–20 Hz) in each 10-s non-rapid eye movement epoch corresponding to δ-frequency band (1–4 Hz). For statistical method, see Table 1. *Results of post hoc contrasts (Newman-Keuls).
(16). Removal of ligand from these receptors after adrenalectomy may compound the effects of elevated CRH on δ-power. Type II receptor-preferring agonists, such as dexamethasone, increased wake time (16), as seen in ADX + 2 × B rats. The 2 × B replacement did not increase the amplitude of the rhythm in SBL compared with ADX rats, whereas the 1 × B replacement did. This may reflect the reduced NREM sleep time in ADX + 2 × B rats. There may also be an inverted U-shaped curve relating the circulating B concentrations and sleep continuity, with maximal sleep bout lengths promoted by physiologically appropriate B replacement. Such a relationship exists between concentration of B and maximal weight gain in ADX rats (1).

It is not known how tonic B replacement affects sleep and circadian rhythm variables. Plasma B concentrations in pellet-treated ADX rats remain stable for up to 14 days, with negligible circadian variation (1). The lack of circadian rhythm of B in pellet-treated rats may explain the incomplete attenuation of the adrenalectomy effects. Several points, however, suggest that tonic B replacement provides a viable reversal of the consequences of adrenalectomy. First, in both ADX and ADX + B pellet rats, the circadian rhythm in CRH mRNA levels in the PVN persisted (23). Second, tonic and cycling B replacement were similarly effective in reversing the effects of adrenalectomy on endocrine end points (1). Finally, sleep in 2 × B-replaced rats contained less NREM time and less percent REM during lights off than that of ADX + 1 × B rats, demonstrating that tonic B effects were dose dependent. Nonetheless, the incomplete reversal of the effects of adrenalectomy...
on sleep variables may be due to the lack of circadian rhythm in B.

The potential mechanisms for CRH- and B-receptor effects on sleep parameters are many, since the receptors for these hormones are found throughout the brain. These hormonal effects may converge on monoaminergic systems that influence cortical activation. Curtis et al. (8) have demonstrated that CRH infused into the locus ceruleus (LC) increased the LC spontaneous discharge rate and the release of norepinephrine into the prefrontal cortex and reduced the synchrony of the EEG. The source of endogenous CRH that activates this circuit has not yet been identified conclusively. Adrenalectomy, which increased CRH mRNA specifically in the PVN (20), increased the spontaneous and stress-induced LC discharge rate and appeared to increase the sensitivity of LC neurons to CRH (29).

Corticosteroid effects on sleep parameters may also impinge on ascending monoaminergic projections. For example, increased binding capacity and postsynaptic effects in neocortical serotonin (5-HT\textsubscript{2C}) receptors by B (22) may account for the increased wake time in 2 × B-treated rats; 5-HT\textsubscript{2C}-receptor activation reduced synchronous, high-amplitude EEG activation and increased wake time in rats (11). Together, these results suggest that the consequences of HPA axis hyperactivity during depression, aging, and chronic stress may reduce sleep quality through effects on monoaminergic systems. Accordingly, disturbances in norepinephrine and 5-HT neuronal functioning have long been implicated in the pathophysiology of depression (19), and HPA axis changes have been hypothesized to interact with monoamine effects on mood disorders (27).

Sleep deprivation appears to be a stress that activates the HPA axis; 6 h of sleep deprivation caused an approximate doubling of plasma B concentrations (Bradbury and Edgar, unpublished results). It is not known whether these stress responses and compensatory sleep mechanisms induced by sleep deprivation interact. Adrenalectomy and clamping of plasma B at supraphysiological concentrations, manipulations designed to mimic the high CRH and B levels measured during stress, had little effect on recovery sleep in young rats, suggesting that endocrine and/or age-related changes in the diurnal rhythm of CRH gene expression in the paraventricular nuclei.

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Address for reprint requests: D. M. Edgar, Sleep Research Center, 701 Welch Rd., Suite 2226, Palo Alto, CA 94304. Received 30 October 1997; accepted in final form 3 April 1998.

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