THE ROLE OF CORTICOTROPIN-RELEASING HORMONE (CRH) IN
STRESSOR-INDUCED ALTERATIONS OF SLEEP IN THE RAT

Fang-Chia Chang¹ and Mark R. Opp²

¹ Neuroscience Laboratory, Department of Neurology, China Medical College Hospital, 2 Yu-Der Road, Taichung, Taiwan and ² Department of Anesthesiology, The University of Michigan, Ann Arbor, MI 48109-0615, USA

Running head: CRH, stress, and waking in rat

Correspondence:
Mark R. Opp
Department of Anesthesiology
M-7433 Medical Sciences Building 1
1150 West Medical Center Drive
Ann Arbor, MI 48109-0615

Tel: (734) 763-5260
Fax: (734) 764-9332
Email: mopp@umich.edu

Copyright 2002 by the American Physiological Society.
Abstract

Corticotropin-releasing hormone (CRH) mediates responses to a variety of stressors. We subjected rats to a one hour period of an acute stressor, physical restraint, and determined the impact on subsequent sleep-wake behavior. Restraint at the beginning of the light period, but not the dark period, increased waking and reduced rapid eye movements sleep (REMS) without dramatically altering slow wave sleep (SWS). Electroencephalogram (EEG) slow wave activity during SWS and brain temperature were increased by this manipulation. Central administration of the CRH receptor antagonist astressin blocked the increase in waking after physical restraint, but not during the period of restraint itself. Blockade of CRH receptors with astressin attenuated the restraint-induced elevation of brain temperature, but not the increase of EEG slow wave activity during subsequent SWS. Although corticosterone increased after restraint in naïve animals, it was not altered by this manipulation in rats well-habituated to handling and injection procedures. These results suggest that under these conditions central CRH, but not the HPA axis is involved in the alterations in sleep-wake behavior and in the modulation of brain temperature of rats exposed to this stressor.

Keywords: HPA axis, EEG, neuropeptide, waking, stress
Introduction

Corticotropin-releasing hormone (CRH) is well documented as the major, although not only mediator of behavioral, physiological, and autonomic responses to a variety of stressors. CRH, expressed in widely distributed regions of the central nervous system (CNS), mediates the hypothalamic-pituitary-adrenal (HPA) axis and central autonomic components of responses to stressors (11,30). Sleep is a fundamental CNS process that is regulated by complex interactions between neural and humoral systems, and is altered in response to a variety of stressors [reviewed (26)]. Previous reports indicate that brief periods of exposure to physical restraint at the beginning of the dark period increase rapid eye-movements sleep (REMS) in rats (6,10,32). This REMS enhancement is modulated by the CRH receptor antagonist α-helical CRH₉₋₄₁ (α-hCRH) (10), suggesting a role for CRH in these responses. Recently a new CRH receptor antagonist, astressin, has become commercially available. Astressin, cyclo(30-33)[D-Phe¹²,Nle²¹,³⁸,Glu³⁰,Lys³³]-rat/human-CRH₁₂₋₄₁, is more potent than α-hCRH and exhibits little if any intrinsic agonist activity (15). Astressin effectively blocks responses to a variety of stressors, including CRH- and alcohol-induced increases in ACTH (33), and stressor-induced alterations in gastric and colonic motor function (20). The present study was designed to further elucidate the role of CRH in responses to physical restraint by determining the effectiveness of a different CRH receptor antagonist in modulating responses to this stressor.

In addition, we have previously shown that the impact of immune challenge on sleep-wake
behavior depends on the time of day at which the challenge occurs (28,29,36). In this study we extend previous observations of responses to physical restraint to include manipulations at a different circadian time, the beginning of the light period of the light:dark cycle. We now report that 1-h of physical restraint differentially impacts subsequent sleep-wake behavior, electroencephalogram (EEG) slow wave activity (SWA) during slow wave sleep (SWS) and brain temperature, depending on the timing at which the stressor is applied. Selective CRH receptor blockade with astressin abolishes or attenuates some, but not all responses to this stressor. Our results suggest that under the conditions of this study central CRH, but not the HPA axis is involved in restraint-induced waking and, in part, in the stressor-induced elevation of brain temperature.

Methods

Substances. Stock solutions of astressin, (Bachem, Torrance, CA) were prepared in pyrogen-free saline (PFS). Aliquots of these stock solutions were stored at -70 °C until use, when they were then brought to an appropriate injection volume. We used 2.5 µg (0.7 nmol) astressin in these experiments. This dose of astressin does not alter spontaneous sleep-wake behavior during the first 6-h after ICV administration (7).

Animals. Male Sprague-Dawley rats (250 - 300 g; Harlan, Indianapolis, IN) were
anesthetized (ketamine/xylazine; 87/13 mg/kg), and injected with an analgesic (butorphanol tartrate [Torbugesic]) and a broad spectrum antibiotic (penicillin G benzathine [Bicillin LA]).

Depending on the particular protocol (see below), surgical procedures included the implantation of electroencephalogram (EEG) screw electrodes, a guide cannula directed into the lateral ventricle, and a calibrated 30-kΩ thermistor (model # 44008, Omega Engineering, Stamford, CT) to monitor brain temperature (Tbr) at the surface of the cortex. All procedures have been previously described (27). The animals were allowed to recover for seven days prior to the initiation of experiments. The rats were housed in individual recording cages, two cages in each environmentally controlled chamber (model # 352601; Hotpack Corporation, Philadelphia, PA), and were maintained at 23 ± 1 °C with a 12:12 h light:dark cycle (25 W incandescent bulb; approximately 200 lx at cage height). Food and water were available ad libitum. All procedures performed in these studies were approved by the local Animal Care and Use Committee in accordance with the United States Department of Agriculture Animal Welfare Act and the United States Public Health Service Policy on Humane Care and Use of Laboratory.

On the second postsurgical day, the rats used in behavioral studies were connected to the recording apparatus (see later) via a flexible tether. Three days after surgery, the patency and free drainage of the ICV cannulae was assessed by administering 200 ng angiotensin II [human angiotensin II octapeptide; Peninsula Laboratories, Inc.]; angiotensin elicits a drinking response
mediated by structures in the preoptic area (12). For the next 4 - 5 days the animals were
habituated by daily handling and ICV injections of pyrogen-free saline (PFS) timed to coincide
with scheduled experimental administrations. At the end of each experimental protocol, all rats
were again injected with angiotensin; only data from those rats that exhibited a positive drinking
response were included in the subsequent analyses.

Apparatus and Recording. Signals from the EEG electrodes and thermistors were fed
into amplifiers (Colbourn Instruments, Lehigh Valley, PA; models S75-01 and S71-20,
respectively). The EEG was amplified (factor of 3,000) and analog bandpass filtered between
0.1 and 40 Hz (frequency response: ±3 dB; filter frequency roll off: 12 dB / octave). Gross
body movements were detected by custom-built ultrasonic detectors (Biomedical
Instrumentation, University of Tennessee, Memphis). These conditioned signals (EEG, Tbr), as
classified as well as those from the movement detectors, were subjected to analog-to-digital conversion with
12-bit precision at a sampling rate of 128 Hz (AT-MIO-64F5; National Instruments, Austin, TX).
The digitized EEG waveform, the Tbr samples, and integrated values for body movements were
stored as binary computer files until subsequent analyses.

Postacquisition determination of vigilance state was done by visual scoring of 12-s
epochs using custom software (ICELUS, M. R. Opp) written in LabView for Windows (National
Instruments) as previously described (27). The animal’s behavior was classified as either SWS, REMS, or waking based on previously defined criteria (27).

**Experimental Protocol.** Two experiments were conducted in this study. **Experiment 1** was conducted to determine the effects of 1-h restraint on subsequent sleep-wake behavior.

Rats used in this experiment (n = 16) were divided into two sub-groups, a **dark onset group** (n = 8) and a **light onset group** (n = 8). These rats were handled each day, and habituated to injection procedures by receiving ICV injections of vehicle at the same times when experiments were scheduled to begin. This habituation period lasted one week, after which 24-h baseline recordings were obtained from undisturbed animals. On the day after baseline recordings, four rats of each group were injected ICV with vehicle, and the other four rats received 2.5 µg (0.7 nmol) astressin. These rats were then immediately placed individually in plexiglas Broome-style rodent restrainers (Kent Scientific Corporation, Litchfield, CT) for 1-h. During this period of restraint, the restrainer was placed in the rat’s home cage, and the animals were connected via the tether to the recording system. After restraint, the rats were removed from the restrainer, and placed back in their home recording cage. Recordings were made during the one-hour restraint period and for 23-h following restraint. Two days later, this protocol was repeated, but the substance injected was switched. Thus, each rat was restrained only twice and served as its own control. The injection volume used for these animals was 3 µl, and the
injections were given over approximately a two-min period.

In Experiment 2, we determined the effects of 1-h restraint on circulating corticosterone.

All animals used in this experiment were maintained under the same conditions, in the same environmental chambers as those animals used in Experiment 1. Two groups of rats were used in this experiment. A group of naïve rats (n = 36) was composed of animals that were not subjected to any surgical procedure, and were not handled or otherwise habituated prior to experiments. These naïve rats served as controls for handling and injection procedures. Habituated rats (n = 72) were surgically implanted only with an ICV guide cannula. During the one week post-surgical period, these rats were habituated in the same manner as the rats used in Experiment 1; they were handled daily and given ICV injections of vehicle at the same time experiments were scheduled. They were not, however placed in, or habituated to the restrainers prior to the beginning of the experiment. The experimental protocol for this experiment consisted of sacrificing animals either before the planned period of restraint (T = 0), immediately at the end of the restraint period (T = 1), or 1-h after the end of restraint (T = 2). Naïve rats were simply placed into the restrainers and sacrificed at the indicated times with no additional manipulation. Habituated rats were injected with either vehicle (n = 36) or with astressin (n = 36) 15-min before restraint. This 15-min interval was designed in this protocol to match the time interval between injections and beginning of physical restrain used in Experiment 1. This
15-min interval is the time required in Experiment 1 to handle, inject and place groups of rats into the restrainer tubes, and connect them to the recording system. As such, the protocol used in Experiment 2 was identical to that of Experiment 1. Six rats were sacrificed at each time point (T = 0, 1, 2) for each condition (vehicle, astressin). All manipulations in this experiment were conducted twice; once at the beginning of the light period, and once at the beginning of the dark period using separate groups of rats. Trunk blood was collected into EDTA-containing tubes (Vacutainer, Franklin Lakes, NJ), and centrifuged for 15-min at 4°C. Plasma was then aliquoted and stored at –80 °C until radioimmunoassay.

**Corticosterone Radioimmunoassay.** Total plasma corticosterone was measured using a commercially available radioimmunoassay kit (ICN Biomedicals, Inc., Costa Mesa, CA).

**Statistical Analyses**

**Experiment 1.** All values are presented as the mean ± SEM. Repeated measures analyses of variance (ANOVA) were used to reveal differences in the duration of each vigilance state (SWS, REMS, Waking), for EEG slow wave activity during SWS, for Tbr values, and for sleep architecture parameters. The primary analyses were done across the 11-h recording period following physical restraint. Secondary analyses consisted of repeated measures ANOVA restricted to specific time blocks comprising the major periods of the study: the 5-h
immediately after restraint (hours 2 – 6 of protocol clock time), and the 6-h time blocks comprising the remainder of the recording period. Secondary analyses of data obtained during the 1-h period of physical restraint were done using one way ANOVA. In all ANOVA, the main effect (between subjects) consisted of manipulation (undisturbed, ICV vehicle + restraint, ICV astressin + restraint). If statistically significant differences were detected, Scheffe’s post hoc multiple comparisons test was used to determine which manipulation during experimental conditions deviated from values obtained from the same animals during control conditions. An \( \alpha \) level of \( p \leq 0.05 \) was taken as indicating a statistically significant departure from values obtained during control conditions.

**Experiment 2.** Circulating corticosterone concentrations are presented as the mean ± SDEV in units of ng/ml. One way ANOVA was used to reveal statistically significant differences between the values obtained in response to restraint. The main effect consisted of manipulation (naive, ICV vehicle + restraint, ICV astressin + restraint). If statistical significance was achieved, Scheffe’s post hoc multiple comparisons test was used to determine which manipulation contributed to the variance. An \( \alpha \) level of \( p \leq 0.05 \) was taken as indicating a statistical significance.
Results

**Experiment 1.**

**Effects of restraint during the light period.** Physical restraint for 1-h at the beginning of the light period altered sleep-wake behavior of rats (Figure 1). While in the restrainer tube, rats spent more time awake and less time asleep relative to values obtained from the same animals when undisturbed in their recording cages (Figure 1). Post-hoc analyses indicated that the amount of time spent in WAKE did not depend on whether the animals had been injected with vehicle or with 2.5 µg (0.7 nmol) astressin, ie., this CRH receptor antagonist did not alter WAKE during the period of restraint. Alterations in sleep-wake behavior were apparent for five hours after the animals were removed from the restraining device. During this 5-h time block after physical restraint (protocol hours 2 – 6), the amount of time spent awake increased and REMS was reduced; SWS was not statistically altered (Figure 1). This restraint-induced increase in the amount of time spent awake was blocked by astressin, while the restraint-induced REMS suppression was not (Figure 1). During post-manipulation hours 7 – 12, sleep-wake behavior was not statistically altered, although there was a tendency for increased REMS when the rats were injected with vehicle prior to physical restraint (Figure 1).

Analyses of sleep-wake architecture parameters were restricted to time blocks after the physical restraint ended. During post-manipulation hours 2 – 6 under control conditions (ICV...
vehicle + restraint) the duration and number of SWS bouts were not altered relative to the values obtained from baseline (Table 1). The increase in waking observed under control conditions after 1-h restraint was due to an increase in WAKE bout duration, not bout numbers, whereas the reduction in REMS during this post-manipulation period was due to a reduction in both the number and duration of REMS bouts (Table 1). Treatment with 2.5 μg (0.7 nmol) astressin prior to restraint resulted in a modest increase in SWS bout duration without altering restraint-induced reductions in REMS bout numbers or duration (Table 1).

In addition to alterations in sleep quantity and architecture, EEG slow wave activity during SWS was significantly increased during both time blocks after physical restraint (Figure 2). This restraint-induced increase in EEG slow wave activity during SWS was not altered by astressin (Figure 2).

Brain temperature increased during the 1-h period of restraint and remained elevated during post-manipulation hours 2 – 6 (Figure 2). Astressin attenuated, but did not completely abolish this restraint-induced increase in brain temperature (Figure 2).

**Effects of restraint during the dark period.** One hour of physical restraint at the beginning of the dark period did not alter subsequent sleep-wake behavior, although REMS was
reduced during the period of restraint (Figure 1).

Analyses of sleep-wake architecture parameters indicated that the number of WAKE bouts was modestly increased during the first 5-h post-manipulation time block, although WAKE bout duration and the time spent in WAKE were not altered. In addition, transitions from one vigilance state to another were increased after 1-h restraint, indicating that the sleep that did occur was fragmented (Table 1). Astressin did not alter the restraint-induced changes in sleep architecture parameters. EEG slow wave activity during SWS was not altered by 1-h restraint during the dark period (data not shown). Brain temperature was increased in response to physical restraint, and this increase in brain temperature was not altered by astressin (data not shown).

Experiment 2.

Effects of restraint on circulating corticosterone concentrations

In naïve unoperated rats that were not handled or disturbed prior to restraint, corticosterone concentrations increased regardless of the timing of the manipulation (Figure 3). Corticosterone concentrations for naïve rats returned to basal concentrations one hour after restraint ended (T = 2, Figure 3). Corticosterone concentrations of habituated rats injected with vehicle 15-min prior to light onset and then sacrificed at light onset (T = 0, Figure 3) were
greater than those of naïve rats sacrificed at the same time. The differences in corticosterone between these groups of rats at this time point are due to handling of rats that were injected. However, the tendency for increased corticosterone after 1-h restraint during the light period in habituated animals injected with vehicle did not achieve statistical significance. Corticosterone concentrations did not differ between naïve and habituated animals when the manipulations were done at dark onset. ICV administration of 2.5 µg astressin did not alter circulating corticosterone concentrations after restraint during either the light or dark period (Figure 3).

**Discussion**

There is now an abundance of evidence that CRH mediates endocrine, autonomic, immune, and behavioral responses to acute and chronic stressors (1,4,5,11,13,16,26). Sleep is altered in response to stressors of many modalities (31). Chronic stress significantly decreases the amount of SWS and REMS, the length of individual SWS and REMS sleep episodes, and alters the circadian patterning of sleep (18). Some acute stressors, such as physical restraint are reported to primarily induce a REMS rebound (21,31,32). Rampin et al., first reported that a 2-h period of physical restraint applied to rats at the beginning of the dark period induces a significant increase in REMS during the remaining 10 h of the dark period, without altering SWS (32). Subsequent reports by this same group indicate that ICV administration of the CRH receptor antagonist α-hCRH reduces the increase in REMS that follows restraint at the beginning
of the dark period, indicating that endogenous CRH may be involved in these responses (10).

Our present results contrast in some respects these previous reports of del C Gonzalez et al., and Rampin et al (10,32). In this present study, 1-h restraint at the beginning of dark period (active period) did not alter subsequent SWS, WAKE, or REMS, whereas the same manipulation during the light period increased waking and reduced REMS for 5 h post-manipulation. To allow recordings of the EEG and other parameters to be obtained during the period of physical restraint, the rats were restrained in their home recording cages. Under these conditions, the rats slept while in the restrainer tubes, albeit less than normal. One potential explanation for differences with respect to the impact of restraint on REMS observed in our present study and those of the aforementioned reports may be due to methods and/or location in which the animals were physically restrained. Novelty is a critical feature of constructs that describe the continuum of responses to stressors. The continuum of responses to stressors ranges from arousal to pathology, depending on the magnitude and duration of the stressor [see e.g. (19)].

There are numerous studies indicating that novel environments, such as, for example, exposure to an open field or elevated plus maze (17,22), constitute stressors for rodents, and that the CRH system is involved in mediating responses to these stressors. There is no information provided in the previous studies to which we refer concerning the environment in which the manipulations occurred. If, for example, rats are placed in restrainer tubes outside their home recording cages
in the general laboratory environment, the stressor would include a component in addition to physical restraint, i.e., being in a novel environment. We did not intend at the outset of this study to develop a stressor that was of more- or less magnitude than those previously used. We restrained the animals in their home recording cages to allow an assessment of responses during application of the stressor itself. As such, we believe it likely that our methodology resulted in a stressor of less magnitude than to which the animals would have been subjected had the manipulation been carried out in an environment outside the home recording cage. The impact on subsequent sleep-wake behavior was, as such, reduced.

The fact that many procedures used as stressors also result in sleep deprivation is often thought problematic for interpretation of results from such studies [but see, for example (2)]. Although beyond the scope of this discussion, one philosophic response to such suggestions is that no attempts should be made to separate the impact of acute stressors from that of loss of sleep per se, insofar as sleep-wake behavior may be viewed as a reflection of the status of the whole animal. Nevertheless, under the conditions of this present study EEG slow wave activity during SWS increased for the duration of the recording period after restraint when the manipulation occurred at the beginning of the light period. EEG slow wave activity during SWS is thought to reflect both sleep debt and sleep intensity (3), and is a characteristic homeostatic response to sleep deprivation. As such, the increase in slow wave activity during
SWS we observed could conceivably be a response to sleep loss per se, rather than to physical restraint. Our findings that EEG slow wave activity increases after restraint at the beginning of the light period are in agreement with previous observations by Meerlo and colleagues (21) of mice manipulated during the middle of the light period. We do not feel the increase in SWA during subsequent SWS observed in our present study is due to sleep loss however, because relative to the amount of SWS the rats obtained during the first hour of the light period when undisturbed in their home cages, SWS loss during the restraint period only amounted to 27-min, on average. We are not aware of sleep deprivation studies in which rats were deprived of so little sleep, but it is not likely that the increase in slow wave activity during SWS observed after restraint in this study is due to 27-min sleep loss. For example, Tobler and Borbély (35) demonstrate that three hours (180-min) total sleep deprivation of rats by gentle handling at the beginning of the light period does not alter slow wave activity during subsequent SWS, and has little impact on the amount of time spent in vigilance states. Furthermore, 1-h physical restraint of BALB/cJ mice during the middle of the light period increases SWA during subsequent slow wave sleep by an amount greater than that observed after 1-h sleep deprivation by gentle handling (21). For these reasons, we believe the increase in slow wave activity during SWS after physical restraint observed in this study is a response to the acute stressor rather than loss of sleep per se. Astressin does not affect the increases of EEG slow wave activity during SWS, although it reduces restraint-induced increases in waking. These observations suggest that
increased slow wave activity during SWS is not mediated by the central CRH system, although additional studies are necessary to elucidate the precise mechanisms responsible for these effects.

We have previously targeted the CRH system of well-habituated rats in their home recording cages (7,8). Two consistent finding emerge from these studies. First, under the conditions of our studies, antagonizing the CRH system in the absence of overt stressors selectively alters waking and SWS, not REMS. Similar results are obtained when using antisense knockdown strategies to modulate CRH peptide expression; waking is reduced and SWS is increased (Chang and Opp, unpublished observations). The fact that none of the strategies we have employed to target the CRH system in spontaneously behaving rats alters REMS suggests to us that the CRH system plays little, if any role in the regulation of this arousal state. As such, our interpretation of data from previous studies reporting an increase in REMS after acute periods of physical restraint is that these responses are likely mediated by other systems. There are several candidate neuropeptides that could mediate the effects of acute physical restraint on subsequent REMS. One of these candidate neuropeptides is prolactin (PRL). PRL is well-documented for its ability to enhance REMS (24,25,34). PRL is elevated in response to stressors, including restraint (21), and has been implicated in ether vapor stress-induced increases in REMS (2). Because acute periods of physical restraint increase PRL (21), and PRL enhances REMS (24,25,34), restraint-induced increases in REMS may in fact be
mediated by this peptide. In addition, CRH induces PRL secretion (23). Collectively, these observations that PRL secretion is induced by CRH, that CRH and PRL are elevated by physical restraint, and that PRL enhances REMS, suggest that findings of Valatx and colleagues (10), may be attributed to PRL rather than CRH directly. As such pretreatment with the CRH receptor antagonist $\alpha$-hCRH may block restraint-induced increases in REMS (10) because CRH receptor blockade reduces the impact of CRH on PRL release.

The second finding from our previous studies that is relevant to this discussion is that blockade of CRH receptors of well-habituated, non-stressed rats with appropriate doses of selective CRH receptor antagonists alters sleep-wake behavior only if injections are given prior to the dark (active) period of the light:dark cycle (7,8). Under the conditions of this present study, 1-h of restraint impacts subsequent waking and sleep only when the manipulation occurs at the beginning of the light period of the light:dark cycle. The differences in responses to restraint depending on timing of the manipulation may be due to the circadian rhythmicity of the CRH and/or other neurotransmitter/neuropeptide systems. During the dark period, the activity of the CRH system of entrained rats is at its highest [see e.g., (14)]; any additional modulation by an acute stressor may result in only a minimal increase in CRH activity with little subsequent influence on waking. Conversely, an acute period of restraint during the light period is expected to increase CRH activity to a greater extent, since the activity of the system is at its
lowest during this time. Increasing CRH, a potent inducer of waking, at a time when it would normally be low would be expected to increase waking and reduce sleep. This point is illustrated by responses of rats to ICV administration of CRH; the proportional increase in waking after low doses of CRH is greater when injections are given prior to light onset than when given prior to dark onset (7). Although circulating corticosterone concentrations, reflective of CRH activity, increase in naïve animals regardless of the timing of restraint, the proportional increase is greater when restraint occurs prior to the light period. These restraint-induced increases in corticosterone of naïve, unhabituated rats suggest that the alteration of sleep-wake behavior in response to this manipulation may be due to either increased central CRH activity and/or HPA axis activity. However, rats habituated to handling and injection procedures do not exhibit as large a change in corticosterone after restraint as naïve rats, and blockade of CRH receptors by ICV administration of astressin into habituated rats reduces restraint-induced increases in waking without altering circulating corticosterone concentrations. The finding that under the conditions of this study ICV administration of the CRH receptor antagonist astressin blocks restraint-induced increases in waking, but not circulating corticosterone, suggests that alterations in waking and sleep by this acute stressor are not greatly modulated by the HPA axis. As such, these responses are likely mediated by other neuropeptide systems, such as PRL as previously mentioned, or central mechanisms, such as CRH actions on the locus ceruleus, which has been implicated in restraint-induced increases in
REMS (9). Additional experiments are necessary to determine if there are differences in restraint-induced increases in PRL depending on the timing of the manipulation, and whether or not the differential responsiveness of CRH and the HPA axis to this manipulation impacts PRL secretion.

In conclusion, our results indicate that under the conditions of this present study exposure of rats to physical restraint for 1-h at the beginning of the light period, but not the dark period increases waking and EEG slow wave activity during SWS, and reduces REMS. Blockade of central CRH receptors abolishes restraint-induced alterations in waking, but not REMS, a finding consistent with our previous observations that antagonizing CRH in non-stressed rats does not alter REMS. Collectively, the data derived from this study implicate CRH in some, but not all responses to this acute manipulation.

**Perspectives**

The concept of stress and responses to stressors has proven difficult to precisely define; there is often disagreement as to what does, or does not constitute a stressor and how the magnitude of a stressor may best be determined. One aspect of research on responses to stressors that is generally agreed upon is that stressor-induced increases in HPA axis activity reflect the magnitude of the stressor, as perceived by the animal. Although autonomic markers
may be used to define responses to stressors, it is ACTH and glucocorticoids that are most frequently referred to as “stress hormones”. If the impact of stressors on behavior is the focus of study, there are multiple levels at which outcome measures may be obtained. The selection of readout parameters other than ACTH and/or glucocorticoids does not invalidate the use of the HPA axis as a measure of responses to stressors, nor does the use of other outcome measures redefine the concept of stress. Results presented in this study illustrate some aspects of this approach. Our finding that rats sleep when placed for the first time in a restrainer tube in their home recording cage suggests to us that under these conditions this manipulation is not perceived by the rat as overly stressful. It is difficult to conceptualize how a healthy animal that is not subjected to a drug would sleep during some event perceived as stressful. Therefore, we believe the extent to which sleep and other complex behavior is altered during, or after manipulations thought by the investigator to be stressful reliably serves as a readout of the perception of the animal to that manipulation.

Acknowledgments

The technical assistance of Ms. Kristi Overgaard, Mr. William Dalmeida, and Mr. Steven Fullwood is gratefully acknowledged. This work was supported, in part, by the National Institute of Mental Health (MH-52275).
References


Figure Legends

Figure 1. Restraint-induced alterations in sleep-wake activity after ICV administration of the CRH receptor antagonist astressin. Rats were injected ICV with either vehicle or 2.5 µg (0.1 nmol) astressin and then restrained for one hour beginning at light onset (n = 8) or at dark onset (n = 8). During the light period, waking was increased and REMS decreased during and after restraint period. Restraint-induced increases in waking were blocked by astressin, whereas REMS suppression was not. Restraint at the beginning of the dark period had little effect on subsequent behavior. The bars depict the mean ± SEM values. Open bars represents values obtained from the same animals during undisturbed baseline recording session and are matched to time of day for values obtained after restraint. Solid bars are values obtained after restraint and ICV administration of vehicle (pyrogen-free saline), whereas hatched bars denote values obtained when the rats received ICV astressin and restraint. * = p ≤ 0.05 vs. undisturbed baseline; # = p ≤ 0.05 vs. restraint plus vehicle.

Figure 2. Effects of ICV administration of astressin on restraint-induced increases in brain temperature (Tbr) and slow wave activity (SWA) during slow wave sleep (SWS) when rats were restrained for one hour at the beginning of the light period. Brain temperature and SWA during SWS were increased by this manipulation. The restraint-induced increase in brain temperature was attenuated by astressin, whereas the increase in SWA during SWS was not. Values are the
mean ± SEM from eight rats. Open bars depict values obtained during undisturbed baseline recordings. Solid bars indicate the responses of rats to restraint when they were injected ICV with vehicle (pyrogen-free saline) prior to the restraint period. Hatched bars denote values obtained from the same animals when ICV administration of 2.5 µg astressin (0.7nmol) preceded the one hour restraint period. * = \( p \leq 0.05 \) vs. undisturbed baseline; # = \( p \leq 0.05 \) vs. restraint plus vehicle.

Figure 3. Effects of restraint and ICV administration of astressin on circulating corticosterone concentrations. Bars are the mean ± SDEV values obtained from naïve rats (not handled or manipulated in any way prior to restraint; open bars), rats injected ICV with vehicle (pyrogen-free saline; filled bars), or rats injected ICV with 2.5 µg astressin (0.7 nmol; hatched bars). Six rats were used for each manipulation / time point. Corticosterone concentrations of naïve rats were significantly elevated (* = \( p \leq 0.05 \) vs. \( T = 0 \)) after restraint irrespective of the timing of the manipulation. Rats that were handled and injected ICV with vehicle 15-min prior to the beginning of the light period and then sacrificed at light onset, had significantly greater concentrations of corticosterone than did naïve rats sacrificed at \( T = 0 \) (# = \( p \leq 0.05 \) vs. naïve rats at \( T = 0 \)). This increase in corticosterone was due to the interval between handling and sacrifice (see text). Circulating corticosterone concentrations were not statistically altered by one hour of restraint in habituated rats injected ICV with vehicle or astressin.
Table 1.  Effects of 1-h restraint on sleep-wake architecture parameters of rats.

<table>
<thead>
<tr>
<th>Manipulation</th>
<th>Hour</th>
<th>L:D cycle</th>
<th>Number of bouts(^1)</th>
<th>Bout duration(^2)</th>
<th>Transitions(^3)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>WAKE(^7)</td>
<td>SWS(^7)</td>
<td>REMS(^7)</td>
</tr>
<tr>
<td>Undisturbed</td>
<td>2-6</td>
<td>L</td>
<td>6.5 ± 0.4</td>
<td>11.5 ± 0.5</td>
<td>3.3 ± 0.2</td>
</tr>
<tr>
<td>Restraint + Vehicle</td>
<td>2-6</td>
<td>L</td>
<td>7.7 ± 0.6</td>
<td>11.8 ± 0.7</td>
<td>1.9 ± 0.3*</td>
</tr>
<tr>
<td>Restraint + Astressin</td>
<td>2-6</td>
<td>L</td>
<td>6.6 ± 0.4</td>
<td>11.1 ± 0.7</td>
<td>2.3 ± 0.3*</td>
</tr>
<tr>
<td>Undisturbed</td>
<td>7-12</td>
<td>L</td>
<td>6.2 ± 0.3</td>
<td>10.9 ± 0.6</td>
<td>3.0 ± 0.2</td>
</tr>
<tr>
<td>Restraint + Vehicle</td>
<td>7-12</td>
<td>L</td>
<td>7.6 ± 0.4*</td>
<td>11.7 ± 0.5</td>
<td>3.8 ± 0.3*</td>
</tr>
<tr>
<td>Restraint + Astressin</td>
<td>7-12</td>
<td>L</td>
<td>6.9 ± 0.3</td>
<td>11.7 ± 0.5</td>
<td>3.2 ± 0.3</td>
</tr>
<tr>
<td>Undisturbed</td>
<td>2-6</td>
<td>D</td>
<td>4.6 ± 0.5</td>
<td>7.4 ± 0.7</td>
<td>2.0 ± 0.3</td>
</tr>
<tr>
<td>Restraint + Vehicle</td>
<td>2-6</td>
<td>D</td>
<td>6.2 ± 0.5*</td>
<td>8.9 ± 0.9</td>
<td>1.6 ± 0.3</td>
</tr>
<tr>
<td>Restraint + Astressin</td>
<td>2-6</td>
<td>D</td>
<td>6.0 ± 0.6</td>
<td>9.1 ± 0.8</td>
<td>1.4 ± 0.3</td>
</tr>
<tr>
<td>Condition</td>
<td>Period</td>
<td>Time</td>
<td>Number of Bouts per Hour (Mean ± SEM)</td>
<td>Mean ± SEM Bout Duration (min)</td>
<td>Number of Transitions from One Behavioral State to Another (Mean ± SEM) per Hour</td>
</tr>
<tr>
<td>--------------------------</td>
<td>--------</td>
<td>------</td>
<td>--------------------------------------</td>
<td>-------------------------------</td>
<td>--------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Undisturbed</td>
<td>7-12 D</td>
<td></td>
<td>4.1 ± 0.4 6.5 ± 0.6 1.7 ± 0.3</td>
<td>9.1 ± 2.1 2.0 ± 0.1 1.0 ± 0.2</td>
<td>29.2 ± 2.9</td>
</tr>
<tr>
<td>Restraint + Vehicle</td>
<td>7-12 D</td>
<td></td>
<td>6.3 ± 0.6* 8.2 ± 0.8 1.6 ± 0.3</td>
<td>4.1 ± 1.1* 1.8 ± 0.2 1.1 ± 0.2</td>
<td>38.8 ± 3.2*</td>
</tr>
<tr>
<td>Restraint + Astressin</td>
<td>7-12 D</td>
<td></td>
<td>5.0 ± 0.5  6.8 ± 0.7 1.1 ± 0.2</td>
<td>6.0 ± 1.3  1.9 ± 0.2  0.8 ± 0.1</td>
<td>31.7 ± 2.8</td>
</tr>
</tbody>
</table>

Values are Means ± S.E.M. Differences were detected by one-way analyses of variance within the indicated time blocks. * denotes a statistically significant difference between values obtained during the undisturbed condition and those obtained after 1-h of restraint when animals were pretreated with vehicle (pyrogen-free saline, PFS). # denotes a statistically significant difference between values obtained after the restraint+vehicle condition and those obtained after 1-h restraint+astressin condition.

1. Number of bouts per hour (mean ± SEM) for each vigilance state.
2. Mean (± SEM) bout duration (min) for each vigilance state.
3. Number of transitions from one behavioral state to another (mean ± SEM) per hour.
4. Experimental manipulation: PFS = pyrogen-free saline (vehicle).
5. Manipulation time blocks after the restraint period.
6. Period of the light:dark cycle immediately prior to which restraint and injections were timed: D = dark period, L = light period.
7. Vigilance states: WAKE, wakefulness; SWS, slow-wave sleep; REMS, rapid eye movements sleep.
Chang & Opp, Fig. 1
Corticosterone (ng/ml)

Light Period

Dark Period

T = 0  T = 1  T = 2
(1-h restraint)  (1-h recovery)

T = 0  T = 1  T = 2
(1-h restraint)  (1-h recovery)

Chang & Opp, Fig. 3