Circadian and Homeostatic Regulation of Hypocretin in a Primate Model: Implications for the Consolidation of Wakefulness

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In humans, consolidation of wakefulness into a single episode can be modeled as the interaction of two processes, a homeostatic "hour-glass" wake signal that declines throughout the daytime and a circadian wake-promoting signal that peaks in the evening. Hypocretins, novel hypothalamic neuropeptides that are dysfunctional in the sleep disorder narcolepsy, may be involved in the expression of the circadian wake-promoting signal. Hypocretins (orexins) are wake-promoting peptides, but their role in normal human sleep physiology has yet to be determined. We examined the daily temporal pattern of hypocretin-1 in the cisternal CSF of the squirrel monkey, a New World primate with a pattern of wake similar to that of humans. Hypocretin-1 levels peaked in the latter third of the day, consistent with the premise that hypocretin-1 is involved in wake regulation. When we lengthened the wake period by 4 hr, hypocretin-1 concentrations remained elevated, indicating a circadian-independent component to hypocretin-1 regulation. Changes in the stress hormone cortisol were not correlated with hypocretin-1 changes. Although hypocretin-1 is at least partially activated by a reactive homeostatic mechanism, it is likely also regulated by the circadian pacemaker. In the squirrel monkey, hypocretin-1 works in opposition to the accumulating sleep drive during the day to maintain a constant level of wake.

Key words: **hypocretin; orexin; wake; sleep deprivation; squirrel monkey; homeostasis; circadian; cortisol; *Saimiri*; primate**

Introduction

Hypocretin is a newly discovered hypothalamic neuropeptide (de Lecea et al., 1998; Sakurai et al., 1998), neurons of which have widespread projections into brain areas implicated in the control of wake and sleep (e.g., locus ceruleus, tuberomammillary nucleus, basal forebrain) (Peyron et al., 1998; Date et al., 1999; Horvath et al., 1999; Moore et al., 2001). Given that the loss or dysfunction of hypocretin results in the sleep disorder narcolepsy in humans (Nishino et al., 2000; Peyron et al., 2000; Thannickal et al., 2000), dogs (Lin et al., 1999; Ripley et al., 2001), and mouse models (Chemelli et al., 1999; Hara et al., 2001), it has been hypothesized that hypocretin is essential for the normal expression of wakefulness. Intracerebroventricular injection of hypocretin increases wakefulness (Ida et al., 1999), and direct application of hypocretin onto wake-related nuclei (e.g., locus ceruleus, tuberomammillary nucleus, ventral tegmental area) is excitatory (Bourgin et al., 2000), indicating a possible wake-stimulating role for endogenous hypocretin.

Studies of narcolepsy suggest that hypocretin is integral to the circadian wake-promoting signal (Dantz et al., 1994; Broughton et al., 1998). The circadian wake-promoting signal is driven by the hypothalamic suprachiasmatic nucleus (SCN), peaks during the latter part of the active period, and is expressed independently of prior sleep or wake history (i.e., is not affected by sleep or sleep deprivation) (Borbély, 1982; Edgar et al., 1993; Dijk and Czeisler, 1994). Hypocretin-1, as measured in rat CSF (Fujiki et al., 2001) or brain dialysate (Yoshida et al., 2001) (*nota bene*, it is not consistently measurable in blood), peaks late in the active period, as expected for a circadian alertness signal. Rats, however, do not consolidate wake into a single bout as do humans and may, therefore, have a fundamentally different circadian wake-promoting mechanism. In the only study of the temporal profile of hypocretin-1 in human lumbar CSF, the neurotransmitter peaked during the inactive (sleep) period (Salomon et al., 2003), which would argue against its involvement in a circadian alertness mechanism. It is possible that the human data, however, represent a delay in brain hypocretin-1 reaching lumbar CSF (Di Chiro et al., 1976) or reflect spinal rather than brain hypocretin-1 release (van den Pol, 1999).

Given the limitations of studying hypocretin neurotransmission in the human brain, we investigated squirrel monkeys (*Saimiri sciureus*), New World primates that, as do humans, consolidate wake into a single, daily episode (Richter, 1968; Adams and Barratt, 1974; Erny et al., 1985; Wexler and Moore-Ede, 1985; Klerman et al., 1999). As with humans and other mammals, the squirrel monkey SCN is responsible for the daily rhythmicity in sleep and wake (Fuller et al., 1981; Lydic et al., 1982). Squirrel monkey CSF also can be collected from the cerebellomedullary cistern (cisterna magna), bypassing the difficulties of interpreting lumbar CSF. To determine whether hypocretin is a circadian alertness signal in a sleep-consolidating animal, we examined the temporal pattern of cisternal CSF hypocretin. We then examined the effects of sleep deprivation on hypocretin in the squirrel monkey, because a purely circadian signal would be unaffected by

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such a change in state. Because sleep deprivation is both stressful and causes an increase in motor activity, we also quantified cortisol levels and locomotion to determine whether the changes observed in hypocretin-1 concentrations during sleep deprivation were attributable to stress-induced activation of the hypothalamic–pituitary–adrenal (HPA) axis, locomotor activity, or wakefulness itself.

Materials and Methods

Animals and husbandry. Squirrel monkeys were housed in groups of five to seven (cage dimensions, $7 \times 8 \times 6.5$ feet) in an indoor facility with a 12 hr light/dark schedule (exposure to external light via skylights at longitude W122.1 and latitude N37.4, with artificial lighting between 7:00 A.M. and 7:00 P.M. to ensure 12 hr of light per day). During the hours of scheduled darkness, red-light/infrared heat lamps maintained ambient temperature. In the first experiment, we used a group of 30 adult female squirrel monkeys (2.8–15.5 years of age, 590–843 gm). In the second experiment, we used a different group of 10 younger, adult squirrel monkeys, five males (1.5–2.7 years of age, 658–823 gm) and five females (1.5–2.5 years of age, 546–618 gm). Animals had *ad libitum* access to food [New World Primate Diet (5040; PMI Nutrition International Inc., Brentwood, MO) supplemented with fresh fruits and vegetables] and water; food was replenished daily at 10:00 A.M. All husbandry and experimental procedures were reviewed and approved by the Stanford University Administrative Panel on Laboratory Animal Care.

Actigraphy. In both experiments, one monkey wore an actigraph (Actiwatch-64; MiniMitter, Bend, OR) to monitor locomotor activity. An actigraph is a small (17 gm) device capable of detecting movement through the use of an accelerometer. Preliminary actigraphy experiments with concomitant video monitoring (data not shown) indicated that (1) actigraph data accurately represent the relative activity of squirrel monkeys, (2) the activity of a single monkey closely corresponds with that of the group, and (3) our squirrel monkeys exhibit a consolidated active–inactive pattern similar to that observed in humans (Wehr et al., 1993) on a lengthened dark (>8 hr) schedule, without daytime napping. Actigraphy data were analyzed using Sleepwatch software (version 2.82; Cambridge Neurotechnology, Cambridge, UK). Because the collection of CSF and blood samples is known to cause significant disruption in monkey locomotor activity patterns, actigraphy data for the hour leading up to and the 12 hr after each sample collection were excluded from analyses of basal activity patterns.

CSF and blood collection. Using previously described procedures (Lyons et al., 1999), cisternal CSF was collected, using a siliconized syringe, from anesthetized monkeys. Anesthesia was induced by intrasaphenous injection of 10.0 mg/kg ketamine hydrochloride with 0.5 mg/kg diazepam and supplemented as needed with an intramuscular injection of 5.0 mg/kg ketamine hydrochloride. Up to 200 μl of CSF were collected and placed in a siliconized tube on ice. Within 1 hr of collection, CSF samples were placed in a −80°C freezer. In experiment 1, we were unable to obtain CSF during 2 of the 60 taps. In experiment 2, the control tap at 2400 in monkey 9905 produced only 0.3 cc, which was sufficient for assay of hypocretin-1 but was insufficient for assay of cortisol; monkey 9905 was not analyzed for cortisol changes at night.

In experiment 1, after the CSF sample was collected, a 1 ml femoral blood sample was taken using previously described procedures (Lyons et al., 1999). The blood was immediately stored on ice and within 1 hr of collection was spun in a centrifuge (4°C, 3000 rpm, 15 min), and the plasma was stored at −80°C. We were unable to obtain a blood sample from one monkey. Monkeys were monitored carefully for the next 7 d, and no untoward effects of the procedures were observed.

Assays. In preliminary experiments, we determined that 25 μl of squirrel monkey CSF is ideal for measuring hypocretin-1 using a commercially available RIA (detection limit, 100 pg/ml; 5% intra-assay variability; inter-assay variability adjusted with internal standard; Phoenix Pharmaceuticals, Belmont, CA). Preprohypocretin, the precursor to hypocretin-1, is also cleaved to form hypocretin-2 (de Lecea et al., 1998; Sakurai et al., 1998), which we assayed using a commercially available RIA (Phoenix Pharmaceuticals). As is the case in other species, we could not consistently detect hypocretin-2 in our CSF samples (up to 100 μl of unfiltered squirrel monkey CSF). Cortisol was assayed using a commercially available RIA (Diagnostic Products Inc., Los Angeles, CA). All samples were assayed in duplicate. Trace blood contamination in 13 of 58 CSF samples did not significantly impact our analyses.

Experiment 1: diurnal variation. To examine the daily pattern of hypocretin, we studied a group of 30 adult, female monkeys, subdivided into six groups of five. Each monkey in each group was sampled twice, with at least 2 weeks between sampling. Only one group was sampled on a given day, and there was at least 1 d between groups being sampled. Group 1 was sampled at 12:00 A.M. and 12:00 P.M., group 2 at 2:00 A.M. and 2:00 P.M., group 3 at 4:00 A.M. and 4:00 P.M., group 4 at 6:00 A.M. and 6:00 P.M., group 5 at 8:00 A.M. and 8:00 P.M., and group 6 at 10:00 A.M. and 10:00 P.M. Although there does not seem to be a substantial direct effect of light on whole-brain hypocretin-1 concentrations in the mouse (L. Ling, personal communication), when samples were obtained during the scheduled hours of darkness, monkeys were shielded from light with a custom-made blindfold.

Experiment 2: wake extension. A second group of five male and five female monkeys was used to examine the effects of an extension of the duration of wake on hypocretin. CSF samples were collected from each monkey at 12:00 A.M., at 12:00 P.M., at 12:00 A.M. after extending wake from 7:00 P.M. to 12:00 A.M., and at 12:00 P.M. after a wake extension control from 7:00 A.M. to 12:00 P.M. The wake extension control from 7:00 A.M. to 12:00 P.M. entailed continuous investigator presence in the colony room, with the investigator occasionally entering the group cage (males and females were kept in two separate cages) or rattling the exterior of the cage. Investigator interaction with the monkeys during the wake extension from 7:00 P.M. to 12:00 A.M. was similar to that during the morning interaction, except that two investigators were used (one for each group) and the investigators spent most of the time within the cage itself to ensure maintenance of wakefulness. At 12:00 P.M. or 12:00 A.M., all of the monkeys were placed in sex-specific transfer cages and brought to the procedure room for cisternal CSF taps.

Statistical analyses. Statistical analyses were done using Microsoft Excel (version 9.0; Microsoft, Redmond, WA), except for the Grubbs' Test (Extreme Studentized Deviate; java script on www.graphpad.com/calculators/grubbs1.cfm). Curve fitting and correlation analyses were done using a nonlinear least squares analysis on the basis of the Levenberg–Marquardt method (Origin version 6.0; Microcal Software, Northampton, MA). Diurnal variation in both cortisol and hypocretin-1 were estimated by curve fitting a cosine wave with a fixed 24 hr periodicity [$y = a + (b \times \cos[0.261799388 \times x + c])$]. All data, unless otherwise noted, are presented as mean ± SEM.

Results

Experiment 1

Hypocretin and cortisol in the squirrel monkey

The 24 hr average hypocretin-1 concentration in squirrel monkey cisternal CSF was 297.7 ± 7.642 pg/ml. As is consistent with other species thus far examined, there was no correlation between either weight ($r = -0.176$; $p = 0.186$) or age ($r = -0.002$; $p = 0.99$) and relative hypocretin-1 concentrations [concentrations converted to percentage of group ($n = 5$) average before correlation to account for time of day effects]. The 24 hr average CSF cortisol concentration was 13.6 ± 0.995 μg/dl, whereas plasma cortisol concentration averaged 121 ± 7.65 μg/dl. As with hypocretin-1 concentrations, there was no correlation between either weight ($r = -0.225$; $p = 0.090$) or age ($r = -0.190$; $p = 0.247$) and relative CSF cortisol concentrations. There was also no correlation between either weight ($r = -0.205$; $p = 0.119$) or age ($r = -0.155$; $p = 0.346$) and relative plasma cortisol concentrations. There was a significant correlation between plasma and CSF concentrations of cortisol ($r = 0.87$; $p < 0.0001$), with CSF concentrations being 11.1 ± 0.836%

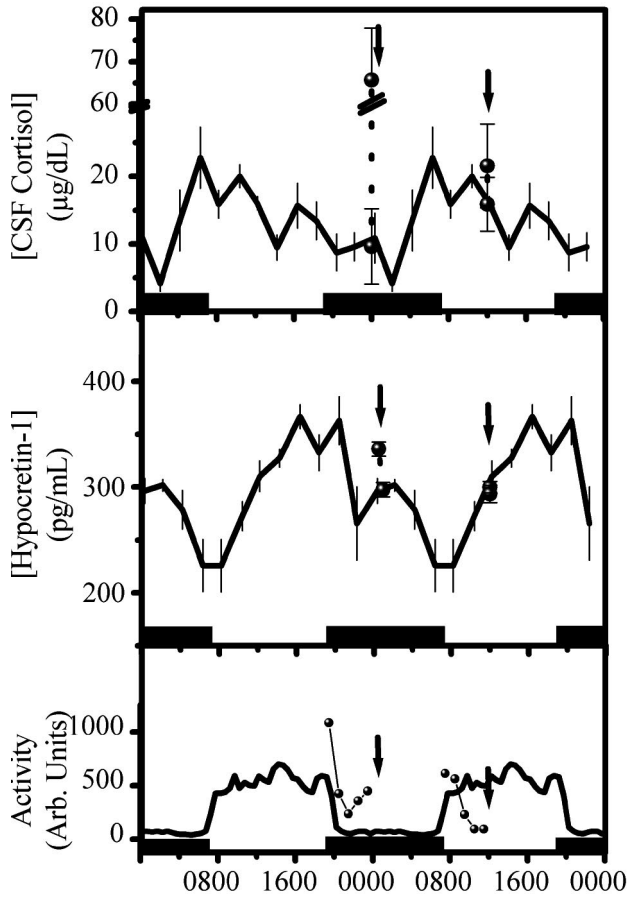

Figure 1. Daily variation in CSF concentrations of cortisol (top) and hypocretin-1 (middle) as well as locomotor activity (bottom). Data from experiments 1 (solid line) and 2 (circle) are shown as average ± SEM. A dashed line connects the control time point with a sample taken at the same time, but after 5 hr of wake extension or daytime control in experiment 2. Arrows indicate time of CSF sampling. An axis break is placed at the top to allow for viewing of all data without compression of the *y*-axis. Activity data are binned in 30 min intervals. Times of scheduled darkness are shown as dark bars along the *x*-axis. All data from experiment 1 are double-plotted along the *x*-axis.

of those found in the plasma (ANOVA of linear fit; values are ±SD; $y = mx + b$, where CSF = y and plasma = x, m = 0.11172 ± 0.00836; t value = 13.36391; $p < 0.0001$; b = −0.01948 ± 1.1286, t value = −0.01726; $p = 0.986$), similar to other primates (Murphy et al., 1967; Puri et al., 1980).

CSF and plasma cortisol exhibit expected daily oscillation

As expected, there was a robust daily oscillation in both CSF (Fig. 1) and plasma concentrations of cortisol (data not shown). Cortisol concentrations peaked just before wake time and remained elevated through the afternoon. In the evening, concentrations began to decline, reaching a nadir during the sleep period. Even with our relatively low sampling frequency, given the pulsatility of cortisol, we observed a significant time of day effect on both CSF (ANOVA; $p < 0.005$; df = 57) and plasma (ANOVA; $p < 0.0001$; df = 58) concentrations of cortisol. The cortisol data were also characterized by fitting with a cosine curve. The amplitude (half peak to trough) of the waveform ± SD fit to the CSF cortisol data was 4.71 ± 1.28 μg/dl, with a waveform mean ± SD of 13.5 ± 0.908 μg/dl. The peak ± SD of the fit CSF cortisol data was at 9:43 A.M. ± 37 min. The amplitude of the waveform ± SD fit to the plasma cortisol data was 41.1 ± 9.62 μg/dl, with a waveform mean ± SD of 121 ± 6.76 μg/dl. The peak ± SD of the fit plasma cortisol data was at 8:46 A.M. ± 31 min. There was, thus, an estimated 57 min delay between the blood and CSF in the diurnal accumulation of cortisol, as would be predicted from experiments with intravenous injections in canines (Fishman and Christy, 1965).

Hypocretin-1 exhibits robust daily oscillation

Hypocretin-1 concentrations exhibited a strong time of day effect, with lowest concentrations occurring around typical wake time (coincident with lights-on) (Fig. 1). After a few hours of wakefulness, concentrations began to rise linearly, reaching a plateau during the early evening. Elevated concentrations were maintained until just after the monkeys went to sleep, with concentrations falling throughout the night. ANOVA indicated that there was a significant effect of time of day ($p < 0.0001$; df = 57). We fit the individual hypocretin-1 data with a cosine wave, constrained to a 24 hr period. The mean ± SD of the waveform was 298 ± 7.64 pg/ml, with an amplitude ± SD of 66.7 ± 10.3 pg/ml, representing a 31% oscillation. The peak ± SD occurred at 6:11 P.M. ± 26 min, 2 hr before the typical sleep onset of 8:10 P.M., 8 hr and 28 min after the peak of CSF cortisol, and 9 hr and 25 min after the peak of plasma cortisol.

Cortisol levels are stimulated by the sampling procedures

Both plasma and CSF cortisol were affected by the sampling procedures. After fitting the cortisol data with a cosine wave, to account for time of day changes in concentration, the model residuals were plotted against the length of time between initiation of contact with a monkey and the time at which the blood or CSF sample, respectively, was obtained. A linear fit applied to the data indicated that there was a significant correlation between cortisol concentration and the latency between initiation of investigator contact and blood ($r = 0.597$; $p < 0.0001$) or CSF ($r = 0.620$; $p < 0.0001$) sampling (i.e., sample latency). For each minute of latency, plasma cortisol increased 1.84 ± 0.328 μg/dl and CSF cortisol increased 0.259 ± 0.0439 μg/dl.

Hypocretin-1 concentrations are not affected by the sampling procedures

We also ascertained the relationship between hypocretin-1 and the effects of sampling. We did this using two different statistical methods. In the first method, a residual analysis (as described above) of CSF hypocretin-1 was done, with time of day effects being accounted for by cosine curve fitting. Two time points (monkey D31 at 8:59 P.M. and monkey 1109 at 10:22 P.M.) were excluded as outliers from this analysis. A linear fit applied to these data indicated no effect of sample latency on hypocretin-1 concentration ($r = 0.00$; $p = 0.996$). To confirm this finding, we used a second method. The rank order of hypocretin-1 values at each time point ($n = 5$) was determined, as was the rank order of the times at which the sample was taken at that time point. ANOVA indicated that there was no effect of time order on concentration order ($p = 0.277$; df = 57).

Experiment 2

Daytime wake extension control does not affect hypocretin or cortisol

Because the first experiment established the daily pattern of CSF hypocretin-1, we subsequently determined whether CSF hypocretin-1 levels were influenced by wake extension. Hypocretin-1 concentrations at the 12:00 P.M. baseline were 299.9 ± 6.859 pg/ml, whereas cortisol concentrations at 12:00 P.M. were 31.91 ± 4.009 μg/dl (Fig. 2). After a 5 hr wake extension control (7:00 A.M. to 12:00 P.M.), compared with the baseline sample at 12:00 P.M., both

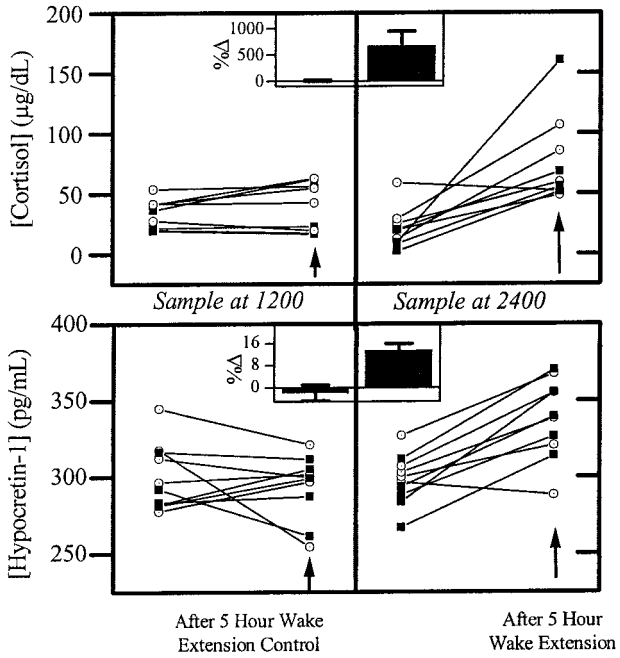

Figure 2. The effects of wake extension on CSF cortisol (top right) and CSF hypocretin-1 (bottom right) in individual squirrel monkeys are shown alongside the effects of the daytime wake extension control (cortisol, top left; hypocretin-1, bottom left) in the same monkeys. Males are represented by a shaded box and females by an open circle. The left side of each panel represents samples taken with no investigator presence preceding the CSF collection, whereas the right side of each panel is a sample taken at the same clock time, but after 5 hr of investigator presence. The average ± SEM percentage change for the group is shown for CSF cortisol (top inset) and CSF hypocretin-1 (bottom inset).

hypocretin-1 (293.4 ± 6.758 pg/ml) and cortisol concentrations (37.61 ± 6.213 μg/dl) were relatively unchanged (−1.81 ± 2.79 and +12.1 ± 10.9%, respectively). The daytime changes in both hypocretin-1 ($p = 0.46$) and cortisol ($p = 0.13$) were not significant (two-tailed, paired *t* test).

Wake extension increases hypocretin-1 and cortisol

At 12:00 A.M., baseline hypocretin-1 concentrations were 297.6 ± 5.200 pg/ml, and baseline CSF cortisol concentrations were 20.5 ± 5.59 μg/dl. After the extension of wakefulness from 7:00 P.M. to 12:00 A.M., there was an increase in hypocretin-1 concentrations in all but one (monkey 2015) and an increase in cortisol concentrations in all but another monkey (monkey 2011). Actigraphy data (Fig. 1) indicated that the monkey colony was typically awake from 7:00 A.M. to 8:10 P.M. The wake extension protocol from 7:00 P.M. to 12:00 A.M., therefore, lengthened wake by ~4 hr. After wake extension, hypocretin-1 concentrations increased to 336.0 ± 8.141 pg/ml, a change of 13.43 ± 2.372% compared with the 12:00 A.M. baseline sample (Fig. 2). Cortisol increased to 76.5 ± 12.3 μg/dl, a change of 671 ± 276% compared with the 12:00 A.M. baseline sample (Fig. 2). The wake extension-induced changes in hypocretin-1 ($p < 0.0005$) and cortisol ($p < 0.005$) concentrations were both statistically significant (two-tailed, paired *t* tests).

For comparative purposes, the values of hypocretin-1 and cortisol in the four conditions in experiment 2 are plotted with the data from experiment 1 (Fig. 1). Because absolute cortisol concentrations are seasonally variable in the squirrel monkey (Schiml et al., 1996) and the samples were collected at different times of year in the two experiments, data from the second experiment are normalized to the average of the corresponding time point in the first experiment. Although both hypocretin-1 and cortisol increase during the wake extension protocol, they seem to do so independently, because the percentage increase of hypocretin-1 and cortisol was not significantly correlated ($r = 0.29$; $p = 0.44$). Additional evidence for this independence is evident in Figure 2, because there was a single monkey (2011) that had a decreased CSF cortisol concentration (−11.4%) after wake extension. This monkey, however, had a normal increase in hypocretin-1 (+11.9%) after the wake extension. Likewise, another monkey (2015) exhibited a decrease (−3.46%) in hypocretin-1 after the wake extension, but this monkey had a normal (+584%) increase in CSF cortisol in response to the wake extension protocol.

Discussion

Our working hypothesis was that hypocretin is an integral part of the circadian wake-promoting signal. Although our data indicate that hypocretin-1 has a pattern of expression expected from a circadian alertness signal (i.e., low at wake onset, elevated late in the wake period, declines during the sleep period), the fact that hypocretin-1 concentrations are sensitive to wake extension demonstrates that noncircadian mechanisms are also involved. Hypocretin-1 remains low during the initial 1–3 hr of wakefulness in the squirrel monkey, despite elevated locomotor activity present from the onset of wake. This argues against hypocretin being necessary for the expression of wakefulness during these early hours of the day. This is also supported by data from narcoleptic humans, who lack hypocretin, that indicate that normal wakefulness is possible for up to 3 hr after sleep or a nap (Helmus et al., 1997). After this initial period, hypocretin-1 concentrations in monkeys increase linearly until they reach a plateau in the latter third of the wake period. This increase may represent a reactive homeostasis mechanism such that increased drive for sleep causes and is offset by a hypocretin-1-mediated drive for wake. Under entrained conditions, the onset of sleep and the concomitant relaxation of the sleep-drive causes a decrease in hypocretin-1 concentrations through the night.

A circadian wake-promoting signal may cause an augmentation of hypocretin-1 concentrations late in the wake period, to maintain the highest possible concentrations of hypocretin-1 and to offset the mounting sleep-drive. Variation of photoperiod length would change the phase of the SCN and, thus, the timing of the circadian alertness signal. In this manner, the SCN would be critical for maintaining wakefulness in varying day lengths, although this remains to be tested. There is an anatomical connection between the SCN and hypocretin-containing hypothalamic neurons (Abrahamson et al., 2001), supporting a role for the SCN in hypocretin regulation. If the SCN were to be involved, because both the diurnal (day-active) squirrel monkey and nocturnal (night-active) rat have elevated hypocretin-1 during the active period and both have SCN with peak metabolic activity during the day (Schwartz et al., 1983), there must be an intermediate nucleus (e.g., retrochiasmatic area, subparaventricular zone) that causes a phase inversion. Because our data were not collected under constant conditions, the involvement of a circadian signal still remains speculative.

Forced wakefulness caused hypocretin-1 concentrations to remain higher than if the monkeys had been allowed to sleep. This supports a direct effect of wakefulness per se on hypocretin tone. Alternatively, increased locomotion (compared with the inactivity of sleep) or stress-induced activation of the HPA axis could be responsible for the hypocretin-1 changes observed during wake extension. We addressed the contribution of the HPA axis on

hypocretin-1 physiology through an examination of the interaction between hypocretin-1 and cortisol. Unlike the increase in hypocretin-1, which represented a change of 31% of the fitted daily rhythm amplitude, the CSF cortisol increase was a change of 595% of the fitted daily rhythm amplitude. There was also no correlation between cortisol and hypocretin-1 changes with wake extension. Additionally, the CSF and blood sampling procedures increased both plasma and CSF cortisol levels but not CSF hypocretin-1 concentrations. These findings suggest that the hypocretin-1 increases observed during wake extension are unrelated to HPA axis activity.

Other investigators have proffered that activity directly increases hypocretin tone (Kiyashchenko et al., 2002; Wu et al., 2002). We attempted to have a daytime (7:00 A.M. to 12:00 P.M.) interaction with the monkeys that would stimulate activity in a similar manner to that which occurred during the wake extension (7:00 P.M. to 12:00 A.M.). As can be seen in Figure 1, however, locomotor activity was somewhat suppressed by investigator presence during the daytime. Even with this inhibition of locomotor activity, there was no significant difference in CSF hypocretin-1 concentrations obtained at 12:00 P.M. from monkeys with or without activity suppression. In addition, we observed that although locomotor activity is robust at wake time, hypocretin-1 concentrations remain low until ~3 hr after activity onset (Fig. 1). This may indicate that it requires ~3 hr of locomotor activity to begin to drive hypocretin-1 concentrations (Wu et al., 2002). There also remains the possibility that there is a delay of several hours between cortical release of hypocretin and its appearance in the cisterna magna. This, however, is unlikely given the phase concordance of hypocretin-1 concentrations in rat brain dialysate and cisternal CSF (Fujiki et al., 2001; Yoshida et al., 2001). Additionally, preliminary studies in humans indicate that diurnal variation of CSF hypocretin-1 is similar under conditions of bedrest and ambulation (Salomon et al., 2003). Given the unlikely direct influence of HPA axis activation or locomotor activity on the observed changes in hypocretin-1 during wake extension, the most parsimonious explanation would be that the mere act of being awake induces hypocretin-1 to remain at an elevated level, helping to ensure wakefulness in the face of a mounting sleep debt. The possible effects of locomotion will require additional study in the squirrel monkey, because unlike canine, feline, and rodent models, daytime stimulation of locomotion in the wake-consolidated squirrel monkey is possible without causing a concomitant sleep deprivation.

Hypocretin deficiency or dysfunction results in the sleep disorder narcolepsy. Sleep disorder may be a misnomer, because narcolepsy is as much a disruption of wake, if not more so, than it is of sleep. Unmedicated narcoleptic humans are unable to maintain wakefulness despite their ability to attempt volitional control of wake. Hypocretin is, therefore, a necessary component of the active maintenance of normal wakefulness. In a New World primate with a pattern of continuous wakefulness similar to that of humans, we have shown that this neuropeptide is regulated in such way that may best be explained as hypocretin-1 being a reactive homeostatic signal, with a circadian contribution that works in opposition to the sleep drive. In addition, the loss of hypocretin in humans is manifest in other clinical outcomes, such as cataplexy, hypnagogic hallucinations, and sleep paralysis. These symptoms are generally considered to reflect abnormal rapid eye movement sleep expression. An additional role of hypocretin in regulating various aspects of rapid eye movement sleep physiology was not addressed in our study and will require additional examination.

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