Published in final edited form as:

J Sleep Res. 2012 April ; 21(2): 189–194. doi:10.1111/j.1365-2869.2011.00957.x.

Time-course of CSF histamine in the wake-consolidated squirrel monkey

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SUMMARY

Central nervous system (CNS) histamine is low in individuals with narcolepsy, a disease characterized by severe fragmentation of both sleep and wake. We have developed a primate model, the squirrel monkey, with which we can examine the role of the CNS in the wake-consolidation process as these primates are day-active, have consolidated wake and sleep, and have cerebrospinal fluid (CSF) that is readily accessible. Using this model and three distinct protocols, we herein report on the role of CNS histamine in the wake consolidation process. CSF histamine has a robust daily rhythm, with a mean of $24.9 \pm 3.29 \text{ pg}\cdot\text{mL}^{-1}$, amplitude of $31.7 \pm 6.46 \text{ pg}\cdot\text{mL}^{-1}$ and a peak at $17:49 \pm 70.3 \text{ min}$ (lights on 07:00–19:00). These levels are not significantly affected by increases (up to $161 \pm 40.4\%$ of baseline) or decreases (up to $17.2 \pm 2.50\%$ of baseline) in locomotion. In direct contrast to the effects of sleep deprivation in non-wake consolidating mammals, in whom CSF histamine increases, pharmacologically-induced sleep (γ -hydroxybutyrate) and wake (modafinil) have no direct effects on CSF histamine concentrations. These data indicate that the time-course of histamine in CSF in the wake-consolidated squirrel monkey is robust against variation in activity and sleep and wake promoting pharmacological compounds, and may indicate that histamine physiology plays a role in wake-consolidation such as present in the squirrel monkey and humans.

Keywords

Circadian; sleep; histamine; primate; pharmacology; locomotion

INTRODUCTION

Obligate consolidation of sleep and wake (i.e., a single prolonged period of wake and sleep each 24-hour cycle) is a phenomenon that occurs in humans and relatively few other

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Conflicts of Interest:

Dr. Zeitzer has no personal conflict of interest, financial or otherwise, to report.

Dr. Kodama has no personal conflict of interest, financial or otherwise, to report.

Ms. Buckmaster has no personal conflict of interest, financial or otherwise, to report.

Dr. Honda has no personal conflict of interest, financial or otherwise, to report.

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Dr. Mignot has no personal conflict of interest, financial or otherwise, to report.

mammalian species. It has been modeled as the integration of two processes – one circadian and the other homeostatic (Dijk and Czeisler 1994). The circadian drive for wakefulness, which is involved in the consolidation of wake, is disrupted in individuals with narcolepsy (Dantz *et al.* 1994), a disease characterized by dysfunction of the hypocretin (orexin) system (Taheri *et al.* 2002). Cross-species comparison of hypocretin physiology in wake consolidating and non-wake consolidating (polyphasic) species reveals relatively few differences, except in the enhanced responsivity of the hypocretin system to environmental stimuli in polyphasic species (Zeitzer and Mignot 2006).

One of the main wake-related output pathways of the hypocretin system is the histamine-expressing tuberomammillary nucleus (TMN) (Eriksson *et al.* 2002), which may be necessary for the alerting effects of hypocretin-1 (Huang *et al.* 2001). As CNS histamine must be studied in neural tissue or cerebrospinal fluid (CSF), studies of CNS histamine in humans are quite limited. The ubiquitous use of anti-histamines as sleep-promoting agents, however, offers *prima facie* evidence for the involvement of histamine in sleep/wake physiology. CNS histamine exhibits rhythmic release in rodents, with levels being elevated during the night (active period) and low during the day (Chu *et al.* 2004; Friedman and Walker 1968; Mochizuki *et al.* 1992; Prast *et al.* 1992). A similar pattern was observed in lateral ventricle CSF concentrations of the major histamine metabolite tele-methylhistamine in a single rhesus monkey, with levels being elevated throughout the day, and a rapid decline and nadir occurring at night (Prell *et al.* 1989). Tele-methylhistamine concentrations are also elevated during the day, relative to night, in the lumbar CSF of children (Kiviranta *et al.* 1994). Even less data have been published on the response of CNS histamine to a challenge of sleep/wake homeostasis (e.g., sleep deprivation). Six hours of sleep deprivation causes an elevation of CSF histamine in rats (Soya *et al.* 2008) and preoptic area microdialysate histamine in cats, though the increase is invariant across the six hours (Strecker *et al.* 2002). Stress and locomotion, concomitants of sleep deprivation in non-human mammals, also have been implicated in the regulation of CNS histamine in polyphasic animals (Mazurkiewicz-Kwilecki and Taub 1978; Mochizuki *et al.* 1992). Since *all* sleep deprivation studies in mammals involve stress, it is critical to examine the interrelationship of these variables as well.

Given the possible unique physiology of CNS histamine in wake consolidating species and the difficulty of studying CNS histamine in humans, we have developed a primate model, the squirrel monkey (*Saimiri sciureus sciureus*), in whom wake is consolidated (Edgar *et al.* 1993) and cerebrospinal fluid (CSF) is easily and repeatedly obtainable from the cerebellomedullary cistern (cisterna magna), obviating the issues of transport delay that encumber interpretation of protein concentrations in lumbar CSF (Di Chiro *et al.* 1973). Cisterna magna concentrations of CSF histamine are likely to be representative of general TMN tone rather than specific release of TMN at a single target. Given the extant literature on wake-promotion by histamine, we hypothesized that CSF histamine in squirrel monkeys would follow a diurnal pattern with a peak in the evening and be responsive to sleep and wake status. We herein report on CSF concentrations of histamine in the wake-consolidated squirrel monkey and its variation with time-of-day, locomotion, and pharmacologic manipulation of sleep (γ -hydroxybutyrate) and wake (modafinil). We have previously reported on the effects of these interventions on CSF concentrations of hypocretin-1 (Zeitzer *et al.* 2003; Zeitzer *et al.* 2004; Zeitzer *et al.* 2007; Zeitzer *et al.* 2009); the same CSF samples from the same monkeys are used in this study.

METHODS

Complete details of behavioral methodology can be found in previously published reports (Zeitzer *et al.* 2003, 2004, 2007, 2009). All animals were group housed (n=2–8) in single

sex cages. Food and water were provided *ad libitum* and replenished daily at 10:00, except on experimental days in Experiment 2 (below) when it was done at 07:00. Standard room lights were on 07:00–19:00; a dim yellow light was on 19:00–07:00. The presence of the yellow light at night did not affect the pattern of entrainment in these squirrel monkeys (Zeitzer and Buckmaster, unpublished observations). All husbandry and experimental procedures were reviewed and approved by the Stanford University Administrative Panel on Laboratory Animal Care (IACUC).

Experiment 1 – Diurnal Variation

To examine the daily pattern of CNS histamine, we collected cisternal CSF from adult, female squirrel monkeys (n=16) at 01:00, 03:00, 04:00, 10:00, 13:00, 16:00, 19:00, and 22:00. Each monkey was sampled at four different clock hours and collections were separated by at least two weeks. There was insufficient CSF volume to determine histamine concentrations in 11 of the 64 collections (CSF had previously been assayed for hypocretin-1 concentrations).

Experiment 2 – Effects of Locomotion

To examine the impact of locomotion, CSF samples were obtained from a group of ten adult female squirrel monkeys (subset of the 16 examined in Experiment 1) at 10:00, 13:00, 16:00, and 19:00, both under baseline conditions (same CSF samples as Experiment 1, no intervention) and after restricting activity for 3, 6, 9, or 12 hours (i.e., 07:00–10:00, 07:00–13:00, 07:00–16:00, and 07:00–19:00, respectively). Activity restriction consisted of placing five monkeys in a familiar transport box (46 cm³) in which little movement and no sleep occurred (verified through continuous monitoring via remote camera). Samples were also collected at 10:00 after environmental enrichment from 07:00 to 10:00 and at 10:00 after forced locomotion from 07:00 to 10:00. Environmental enrichment consisted of the investigator introducing novel toys (plastic couplers, foraging boards) and perching (plastic frames in various shapes) that were familiar to the monkeys but recently withheld. Changes were made every 20–40 minutes or when a decline in activity (observed through remote video camera) was observed. For forced locomotion, a single investigator (JMZ) remained in the cage from 07:00 to 10:00 and would rattle the perch upon which a monkey came to rest, thereby encouraging the monkey to move. This was done to increase locomotion for 15 out of every 30 minutes.

Experiment 3 – Pharmacologic Manipulation of Sleep and Wake

To examine the direct impact of sleep and wake, CSF samples were obtained from a group of six adult female squirrel monkeys. We tested two different drugs: γ -hydroxybutyrate (GHB) and modafinil. GHB is a GABA-B receptor agonist that can induce slow wave sleep (Crunelli *et al.* 2006). Modafinil promotes wakefulness likely through the inhibition of dopamine reuptake, though the exact mechanism of action is unknown (Wisor *et al.* 2001). CSF samples were obtained at 13:00 following oral administration of 16 mg·kg⁻¹ modafinil at 07:00 and 10:00. CSF samples were also obtained at 13:00 following subcutaneous administration of 200 mg·kg⁻¹ GHB at 06:00 and 175 mg·kg⁻¹ at 10:00. We have shown previously that this administration schedule of modafinil at this time has no behavioral effects while this administration schedule of GHB induces sleep (Zeitzer *et al.* 2009). CSF samples were obtained at 01:00 following oral administration of 16 mg·kg⁻¹ modafinil at 19:00 and 22:00. CSF samples were also obtained at 01:00 following subcutaneous administration of 200 mg·kg⁻¹ GHB at 18:00 and 175 mg·kg⁻¹ at 22:00. We have shown previously that this administration schedule of modafinil at this time induces wakefulness while this administration schedule of GHB has no behavioral effects (Zeitzer *et al.* 2009). All monkeys received both drugs at both time points with administrations being separated by at least two weeks.

Actigraphy

All animals wore a collar-mounted actigraph (Actiwatch-64, MiniMitter, Bend OR) to monitor locomotor activity. An actigraph is a small (17 gm) device capable of detecting three-dimensional movement through the use of an accelerometer. Data were saved on the actigraph memory chip as the integral of 60–120 seconds of activity and later offloaded to a personal computer where they were analyzed using Sleepwatch software (v. 2.82, Cambridge Neurotechnology, Cambridge UK).

CSF Collection

CSF was obtained by an experienced technician with a sterile 1 mL siliconized syringe and 25 G needle via a percutaneous tap of the cerebellomedullary cistern from anesthetized monkeys (intranasal 0.5 mg·kg⁻¹ diazepam + 10 mg·kg⁻¹ ketamine). It has been reported previously that diazepam can reduce histamine release (Chikai *et al.* 1993). As all of our animals were exposed to the same anesthetic regimen prior to collection of CSF, it is unlikely that the diazepam had a significant influence on our results except in the possible uniform reduction in absolute CSF histamine concentrations. The tap was not performed until the monkeys fully succumbed (areflexic) to the anesthetic. Up to 200 µL of CSF was collected, placed in a siliconized tube on ice, and transferred to a -80°C freezer within one hour of collection. There were at least two weeks between each CSF collection in a given monkey.

Histamine Assay

Histamine concentrations in the CSF were detected by a high performance liquid chromatography (HPLC ECD-300, Eicom, Kyoto Japan) fluorescence spectrometer system (L7485; excitation wavelength: 355 nm, emission wavelength: 450 nm; Hitachi, Tokyo Japan). CSF samples were separated in a reverse-phase separation column (SC-50DS, 3 mm internal diameter, 150 mm length, Eicom) using a mobile phase of 5% methanol in 0.16 M KH₂PO₄ containing 0.8 mM sodium octane sulfonate (pH 4.5) at a flow rate of 0.3 mL·min⁻¹. Next, elutants were subjected to post-column derivatization with a mixture of 2.5 M NaOH (0.06 mL·min⁻¹) and 0.05 % *o*-phthalaldehyde (0.06 mL·min⁻¹) in a reaction coil made of polytetrafluoroethylene tubing (5 m length, 0.25 mm internal diameter) at 42°C; then 2 M H₃PO₄ (0.13 mL·min⁻¹) was added to stop the reaction. The concentrations of histamine were quantified with PowerChrom (AD Instruments, Australia), using pure histamine standards (Sigma, St. Louis MO). All measurements were done blindly and each sample was measured twice. The detection limit was 10 pg·mL⁻¹ (50 fmol). Inter-assay variation was < 5%. All samples previously had a single freeze-thaw cycle for the removal of CSF to determine hypocretin-1 concentrations.

Cortisol Assay

All samples previously had a single freeze-thaw cycle for the removal of CSF to determine hypocretin-1 concentrations. To determine CSF cortisol concentrations, samples (10 µL) were diluted with 50 µL of RIA buffer and assayed using a commercially available radioimmunoassay (Diagnostic Products Inc., Los Angeles CA). All samples were assayed in duplicate. CSF cortisol data have been published previously (Zeitzer *et al.* 2004; Zeitzer *et al.* 2007), though the analyses presented herein are novel.

Statistics

All data are presented as mean ± SEM. Rhythmicity of histamine was determined using

ANOVA and a non-parametric sine wave fit: $y = y_0 + A * \sin\left(\pi * \frac{x - x_c}{12}\right)$, such that y_0 is the mean, x_c is the time of the nadir, and A is the amplitude from mean to nadir; the period was

fixed to 24 hours (OriginPro 8.0.63.988 SR6, OriginLab, Northampton MA). Curve fitting procedures used the Levenberg-Marquardt algorithm to minimize error. Effects of locomotor activity were determined using one-way and two-way ANOVA, as indicated in the text (OriginPro). Correlations were determined using linear models (OriginPro). Student's *t*-tests and summary statistics were calculated using Microsoft Excel (v.11.8169.8172, Microsoft, Redmond WA). All data were normally distributed.

RESULTS

Across the 24-hour day, cisternal CSF concentrations of histamine averaged 34.4 ± 2.75 pg·mL⁻¹. There was a significant daily variation ($p < 0.05$, ANOVA), with elevated levels being present during the day and levels becoming progressively lower after the onset of sleep (Figure 1). Daytime concentrations were statistically invariant from one another ($p = 0.25$, ANOVA). Fitting a sine wave to the data (adj. $r^2 = 0.53$, $p < 0.001$) indicated that the fitted mean was 24.9 ± 3.29 pg·mL⁻¹ with a peak-to-trough amplitude of 31.7 ± 6.46 pg·mL⁻¹ and an acrophase occurring at $17:49 \pm 70.3$ min.

As the diurnal rhythm may be secondary to locomotion, we examined the effects of removing locomotion. We reduced locomotor activity in the monkeys to $17.2 \pm 2.50\%$ of usual by placing them in transport boxes, instead of their home cages, from the time they arose (07:00) until the time of CSF collection. Despite this reduction in activity, CSF histamine concentrations averaged 38.4 ± 4.01 pg·mL⁻¹ (cf., 38.7 ± 3.12 pg·mL⁻¹ during the same hours with unconstrained movement). There was no effect of time of day ($p = 0.76$) or activity condition ($p = 0.99$, two-way ANOVA) (Figure 1). Thus, the elevated daytime histamine concentrations were not dependent on the occurrence of a significant amount of locomotor activity. Elevation of locomotor activity above normal also did not influence CSF histamine concentrations. While locomotion was increased $45.0 \pm 10.9\%$ in the environmental enrichment condition (CSF histamine = 49.0 ± 24.3 pg·mL⁻¹) and $161 \pm 40.4\%$ in the forced locomotion condition (CSF histamine = 57.5 ± 24.3 pg·mL⁻¹), there was no effect of activity condition on CSF histamine concentration ($p = 0.52$, one-way ANOVA; $p = 0.12$ one-way ANOVA including restricted activity condition). There was also no linear relationship between CSF histamine concentrations and the integrated amount of locomotor activity during the three hours before CSF was collected (07:00–10:00), using data from all four conditions ($r = 0.27$, $p = 0.15$, Pearson correlation).

Given the possible contribution of stress to the effects of sleep deprivation on histamine, we examined pharmacologic manipulation of sleep and wake that is *independent* of changes in stress. We, however, found no difference between control monkeys (*ad lib* sleep and wake) and monkeys with pharmacologically induced sleep (GHB during the daytime) or wake (modafinil at night) (Figure 2). There was no significant difference between CSF histamine concentrations at 01:00 after administration of modafinil (30.4 ± 4.75 pg·mL⁻¹, induction of wake) as compared with either controls (25.4 ± 2.47 pg·mL⁻¹; $p = 0.40$, *t*-test) or GHB administration (41.0 ± 9.84 pg·mL⁻¹; $p = 0.40$, paired *t*-test), both of whom were asleep during the five hours prior to CSF collection. There was also no significant difference between CSF histamine concentrations at 13:00 after administration of GHB (34.0 ± 11.1 pg·mL⁻¹, induction of sleep) as compared with either controls (30.7 ± 3.01 pg·mL⁻¹; $p = 0.79$, *t*-test) or modafinil administration (48.8 ± 9.61 pg·mL⁻¹; $p = 0.58$, paired *t*-test), both of whom were awake during the six hours prior to CSF collection. Since modafinil did induce a change in CSF histamine after daytime administration, when there is no stimulant-induced change in sleep/wake state, and GHB did not induce a change in CSF histamine after nighttime administration, when there is no hypnotic-induced change in sleep/wake state, there are no GHB- or modafinil-dependent actions on CSF histamine. Likewise, the lack of change in CSF histamine after nighttime administration of modafinil, when there is a

stimulant-induced change in sleep/wake state, and after daytime administration of GHB, when there is a hypnotic-induced change in sleep/wake state, indicates that there is also no pharmacologically-induced sleep/wake-dependent changes in CSF histamine concentrations.

DISCUSSION

Cisternal CSF concentrations of wake-promoting histamine have the predicted daily pattern in the diurnal, wake-consolidated squirrel monkey, being constantly elevated during the day and progressively falling throughout the night. This is the same pattern as is observed in a polyphasic mammal such as the rat (Friedman and Walker 1968; Mochizuki *et al.* 1992; Prast *et al.* 1992). In striking contrast, however, sleep and wake *per se* had no direct effect on CSF histamine concentrations in the wake-consolidated squirrel monkey. In polyphasic rats and cats, by contrast, six hours of sleep deprivation caused a sustained elevation of CSF histamine (Soya *et al.* 2008; Strecker *et al.* 2002). This is, to our knowledge, the first reported difference in sleep physiology between polyphasic and wake-consolidating mammals and may be a linchpin in this ethographical difference.

Our initial hypothesis was that, given previous anatomical evidence linking hypocretin-producing neurons in the lateral hypothalamus and histamine-producing neurons in the TMN, CSF histamine would follow a pattern similar to that of CSF hypocretin-1 – elevated during the daytime and responsive to sleep/wake state. To our surprise, however, CSF histamine was not responsive to change in sleep and wake. Thus, the increases in CSF hypocretin-1 at night secondary to induced wake and the decreases in CSF hypocretin-1 during the daytime secondary to induced sleep were unable to change the endogenous pattern of CSF histamine. These data imply that the TMN in the wake-consolidating squirrel monkey might receive a direct innervation from the SCN or one of its adjacent amplifying nuclei (*e.g.*, retrochiasmatic area) that is sufficient to drive the production of histamine in a sleep/wake state-independent manner. The data also imply that the pattern of CSF histamine is consistent of that of a circadian alertness signal.

While relatively simple in humans, challenging the sleep/wake homeostat with extended wake or sleep in mammalian species that cannot volitionally control sleep/wake state is fraught with behavioral confounds, notably changes in locomotion and stress. We found that systematic manipulation of locomotion, independent of changes of sleep and wake, has no impact on CSF histamine. When we used physical methods to induce even modest sleep deprivation (data not shown), however, the monkeys experienced increases in cortisol and a corresponding drop in CSF histamine concentrations. Using pharmacologic induction of both sleep and wake, which avoids the confound of stress, we observed no effect of extending wake into the biological night or extending sleep into the biological day. To control for state-independent effects of GHB and modafinil, we also administered GHB at night, when sleep was already occurring, and modafinil during the day, when wake was already occurring, and observed no effects of either drug on CSF histamine. Thus, any GHB- or modafinil-induced changes in CSF histamine would have been indirectly due to the effects of these drugs on sleep/wake behavior. This pharmacologic approach is also useful in that we were able to examine the effects of extended sleep, which is not readily achieved with behavioral methods in non-volitional wake-consolidated animals.

One important limitation of the current study is that the histamine concentrations are determined from cisternal CSF. While this likely more accurately reflects total brain histamine release and its temporal pattern than does lumbar CSF collection, it does not discriminate the source of histamine (*i.e.*, neuron versus mast cell or specific neuron). It will be important for future studies to examine localized release patterns of histamine using *in vivo* microdialysis or similar techniques to determine if the species differences we observed

with whole brain release of histamine are specific to particular brain nuclei or if there are changes within shorter time scales such as different stages of sleep. Microdialysis would also be useful in determining if spontaneous changes in sleep and wake, such as during a brief nocturnal arousal or the rare daytime nap, lead to changes in CSF histamine. A second significant limitation is that we did not determine sleep and wake using electroencephalographic techniques but, rather, through locomotor patterns that had been validated through visual observation. While the presence of motor activity can confirm modafinil-induced wakefulness, we are unable to conclusively demonstrate that all of the sedentary behavior present after GHB administration is truly sleep, or a mixture of sleep and quiet wake. Future studies using electroencephalography could be used to make this determination.

These data provide the first evidence that underlying variation in the responsiveness of the CNS histamine system to endogenous and exogenous stimuli may underlie the difference in wake-consolidating and polyphasic mammals. It has been recently suggested that variation in the homeostatic time constant (i.e., rate of dissipation of sleep pressure) and the drive on sleep centers (Phillips *et al.* 2010) is involved in sleep duration. Our data indicate, however, that it may not be the drive onto sleep or wake centers that varies between polyphasic and wake consolidating mammals, but the responsivity of these centers to various stimuli that differentiates the physiology in these two groups. As humans are inherently polyphasic at the two ends of the lifespan, alterations in the responsivity of the CNS histamine system may be a useful target to pharmacologically exploit, especially in older individuals in whom sleep disruption may be secondary to a failure to consolidate wake. In individuals with narcolepsy, pharmacologic interventions targeting the CNS histamine system are already underway (Lin *et al.* 2008).

Acknowledgments

We also wish to acknowledge our funding support: NARSAD (JMZ), Jazz Pharmaceuticals (JMZ), the Department of Veterans Affairs Sierra-Pacific Mental Illness Research, Education, and Clinical Center (JMZ), the National Institute of Mental Health (MH47573, DML), and the National Institute of Neurological Disorders and Stroke (NS23724, EM).

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Figure 1.

Average ($\pm$ SEM) cisternal CSF histamine concentrations over the 24-hr day under normal (■) and reduced-locomotion (○) conditions. Data points are connected by a hatched line as they were not collected continuously. Diurnal data (normal conditions) are double-plotted for visualization purposes. On the second day, data from monkeys who have experimentally-reduced locomotion are over-plotted. Dark bars represent time of darkness and typical sleep.

*: $p < 0.05$ vs. 19:00, ** $p < 0.01$ vs. 19:00, 22:00, and 01:00 (t -tests)

Figure 2. Average ($\pm$ SEM) cisternal CSF histamine concentrations after placebo (P, ■), modafinil (M, ●), or GHB (G, ▲) at both 13:00 (left panel) and 01:00 (right panel). Shading within the symbol indicates that the monkeys were awake prior to sampling. An open symbol indicates that the monkeys were asleep prior to sampling. There were no significant effects of either GHB or modafinil on CSF histamine, whether or not the pharmacologic administration was accompanied by a change in sleep/wake state.