

Heterogeneity in the circadian and homeostatic modulation of multiunit activity in the lateral hypothalamus FREE

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Abstract

The lateral hypothalamus (LH) is a relatively large hypothalamic structure containing several neurochemically different, but spatially intermingled, neuronal populations. While the role of these neurons in the homeostatic regulation of diverse physiological and behavioral functions such as sleep/wake cycle has been studied extensively, the impact of sleep history on the electrophysiology of the LH and whether this effect is homogenous across LH is unknown. By combining multiunit activity (MUA) recordings in different regions of LH with electroencephalogram recordings in freely moving rats, we unravelled a heterogeneity of neural-activity patterns within different subregions of LH. This heterogeneity was evident in both the circadian and the vigilance state-dependent modulation of MUA. Interestingly, and consistent with this heterogeneity under baseline conditions, the magnitude of MUA suppression following 6 hr of sleep deprivation (SD) was also different within different locations of LH. Unlike the cortex and in contrast to the predictions of the synaptic homeostatic hypothesis, no correlation was found between the magnitude of activity increase during SD and the percentage of suppression of MUA during recovery sleep. These data provide in vivo evidence of a functional heterogeneity in the circadian and homeostatic modulation of neuronal activity in LH.

[sleep deprivation](#), [homeostasis](#), [synaptic plasticity](#), [hypothalamus](#), [health](#)

Statement of Significance

The lateral hypothalamus (LH) is a homeostasis center that concomitantly regulates several physiological functions such as food intake, metabolism, rewards-related behavior and sleep-wake states. We show that the modulation of neuronal activity by circadian and homeostatic sleep processes within LH is not homogenous. Our results suggest that the diverse physiological functions encoded within the different neuronal networks of LH might show different sensitivities toward alterations of both the circadian system and sleep history.

Introduction

The lateral hypothalamus (LH) is relatively a large portion of the hypothalamus residing anterior to the ventral tegmental area (VTA) and posterior to the preoptic area. Although anatomically not well differentiated, LH contains a number of different cell populations [1, 2]. Extensive studies have been conducted on the physiological role of this part of the brain [2]. Collectively, these studies demonstrated the importance of the LH in maintaining

homeostatic physiology and behavior [1, 2]. The regulation of vigilance states is among the functions tightly orchestrated by LH neuronal circuitries [2–4].

The LH contains several populations of neurons including Orexin-positive, melanin-concentrating hormone (MCH)-positive, GABAergic, glutamatergic, and neurotensin containing neurons [1]. In addition to this chemical diversity of neurons, a wide variety of electrophysiological patterns relative to sleep/wake states have been reported [3]. Although the basic physiological role of the different neural population in the LH in modulating sleep/wake states is well understood [2–4], the impact of sleep/wake alterations on LH physiology has received little attention. This question is more pertinent given the role of LH in the regulation of reward and feeding behavior and their integration within proper vigilance states [2] and the well-established association between metabolic disorders such as obesity and type 2 diabetes and short sleep duration [5]. Inspired by the neurochemical and electrophysiological heterogeneity of neurons in LH, we investigated whether LH display different patterns of electrophysiological activity during the different sleep/wake states.

Sleep deprivation (SD) increases the propensity of an animal to sleep [6]. Part of this homeostatic process of sleep regulation is mediated through the build-up of activity-dependent metabolites in the brain during prolonged waking and their subsequent clearance during sleep [7]. Because regional differences in the sensitivity of different brain areas to these metabolites was shown [8, 9], we subsequently tested whether different subregions in LH will respond differently to SD.

In addition to the neurochemical and electrophysiological heterogeneity of neurons in LH, the density of the different neuronal populations is not homogeneously distributed within LH [2, 10]. Furthermore, neuronal connections (both inputs and outputs) across different subcompartments of LH is not uniform [11]. This heterogeneity might potentially influence the electrophysiology of different subcompartments of LH. By recording electrical activity from different locations of LH, we aim to test this hypothesis by investigating the impact of vigilance states on LH electrophysiology.

To these ends, we recorded multiunit activity (MUA) in different subregions of LH extending from –1.3 to –4.3 mm posterior to bregma (Figure 1). Simultaneously to these recordings, electroencephalogram (EEG) and electromyogram (EMG) recordings were performed to investigate changes in sleep/wake characteristics before, during and after SD and their correlation with neuronal activity in different subregions of LH. Baseline recordings were performed under constant DD conditions to assess the potential circadian modulation of MUA in LH. Following these baseline recordings, rats were subjected to a 6-hr total SD in the beginning of their subjective day.

Figure 1.



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Depiction of electrode placement as confirmed by cresyl violet staining. The color code as well as the numerical references of the sites is preserved throughout all the figures. Atlas adapted from Paxinos et al. 1982 [15].

Materials and Methods

Animals

A total of 20 male Wistar rats (approximately 300 g at the time of surgery) were used for this study. The animals were purchased from Charles River. All the experiments were approved by of the Ethics Committee of the Leiden University Medical Center (DEC N° 13209, The Netherlands) and were carried out in accordance with the EU Directive 2010/63/EU on the protection of animals used for scientific purposes.

In vivo MUA, EEG, and EMG recordings

In vivo MUA and EEG and EMG were recorded as described previously [12–14]. In brief, for the MUA recordings, stainless steel tripolar electrodes (0.125 mm diameter; Plastics One, Inc., Roanoke, VA) were implanted in each animal under deep anesthesia. Two twisted electrodes were directed toward the targeted area of the LH with 0.4 mm space between the two electrodes. The third electrode was placed in the cortex as a reference electrode. Measurements were performed from one electrode at a time. The electrodes were placed to record from different subregions of LH (spanning from –1.3 to –4.3 relative to bregma; 2 mm lateral and with varied depth length (Figure 1). The coordinates were adapted from Paxinos et al. [15].

For EEG, electrodes were screwed into the skull above the dura over the right cortex (2.0 mm lateral to the midline and 3.5 mm posterior to bregma) and cerebellum (at the midline and 1.5 mm posterior to lambda). For EMG recordings, two wires with suture patches were inserted in the tissue between the skin and the neck muscle.

The animals were connected to the recording system via a flexible cable and counterbalanced swivel system, and the animals were acclimated to the setup in continuous darkness for at least 1 week before the start of the recording. The animals' behavioral activity (drinking and locomotion) was recorded continuously to obtain an estimate of the circadian rhythm.

Neuronal activity in the LH was amplified approximately 40000X, band-pass filtered (500–5000 Hz, –40 dB/decade), and processed further offline. Online, a window discriminator converted the action potentials into electronic pulses. A second window discriminator was set at a higher level to detect artifacts caused by the animal's movements. Action potentials and movement-related artifacts were counted in 10 s epochs. The analog EEG and EMG signals, which were recorded continuously, were amplified approximately 2000X, band-pass filtered (0.5–30 Hz, –40 dB/decade), and digitized at 100 Hz. All data were recorded simultaneously and stored on a computer hard disk. The stability of the multiunit signal and EEG recording was evaluated daily by visually inspecting the signal using an oscilloscope; the circadian rhythm in the signal and the amplitude of the EEG were monitored for 7 days before the baseline data were collected. After the experiments, the animals were sacrificed to verify the recording sites. To mark the location of the electrode tip, current was passed through the electrode, and the brain was perfused with a buffered solution containing 4 per cent paraformaldehyde and 8 per cent potassium ferrocyanide.

The brains were removed, post-fixed overnight in 4 per cent paraformaldehyde, and cryo-protected in 30 per cent sucrose solution. Free-floating coronal sections (40 µm thickness) were cut on a freezing microtome. The sections were stained with cresyl violet, mounted on gelatinized slides, dried, dehydrated in increasing gradients of ethanol, cleared in toluene, and cover-slipped with Depex.

Offline, the EEG power density spectra were calculated in 10 s epochs corresponding to the 10 s epochs of the action potentials of the targeted subregions of LH using a fast Fourier transform (FFT) routine within the frequency range of 0–25.0 Hz in 0.1 Hz bins. EMG signals were integrated over 10 s epochs. Three vigilance states—wakefulness, NREM sleep, and REM sleep—were determined visually based on standardized EEG/EMG criteria for rodents [12, 13, 16]. Wakefulness was scored when the EMG showed an irregular, high-amplitude pattern and the EEG signal was low in amplitude with relatively high activity in the theta band (6–9 Hz). NREM sleep was scored when EMG amplitude was low and the EEG amplitude was higher than during wakefulness, with high values in the slow wave range (1–4 Hz). REM sleep was scored when the amplitude of the EMG and EEG were low and the EEG showed relatively high values in the theta range. Epochs containing artifacts in the SCN electrical signal or in the EEG signal (observed during the scoring of the vigilance states) were excluded from our analysis of the neuronal activity and EEG spectral analysis.

Relative to NREM sleep, neural activity in LH is highly modulated by the behavioral repertoire of the animal during wakefulness [17–20]. For this reason, all MUA data and EEG power density data were calculated relative to the respective mean values recorded during NREM sleep over a 24-h period.

Sleep deprivation

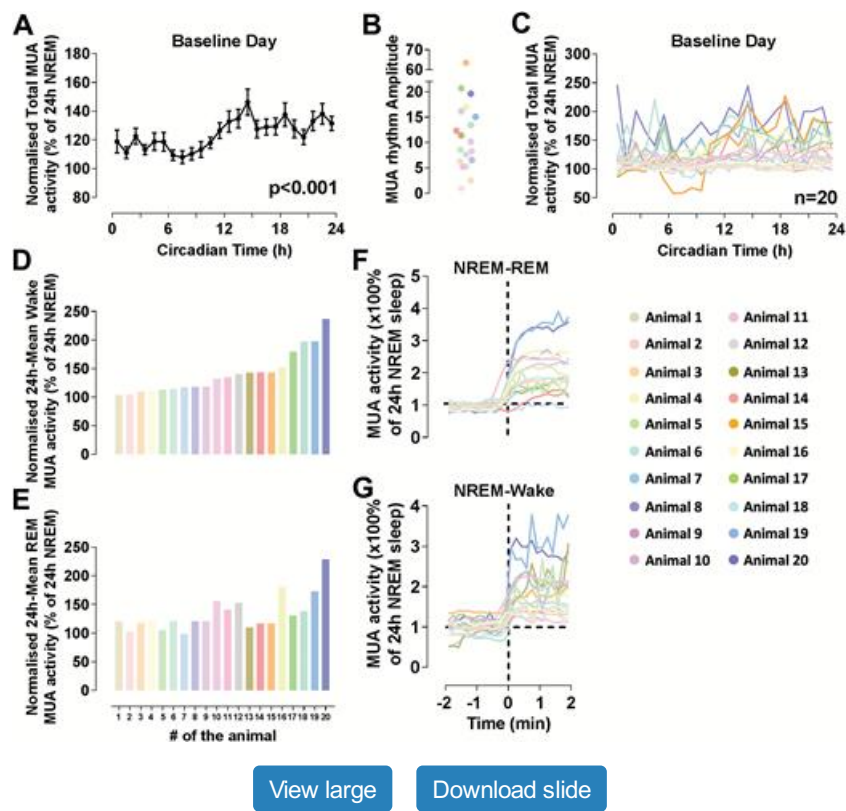
A previously validated method using an enriched, novel environment [21, 22] was used to stimulate spontaneous exploratory wakefulness without inducing stress. SD was performed during the first 6 hr in the subjective day. The experiments were performed in continuous darkness under constant temperature and humidity conditions. Clean bedding, food, water, climbing toys, and novel nesting materials were used as stimuli to stimulate wakefulness. During the 6 hr SD episode, the animals were monitored via their online EEG signal. Whenever the animals appeared to be entering

NREM sleep—or if an increase in slow wave amplitude was observed—new objects were introduced to the cage of the animal.

Statistical analysis

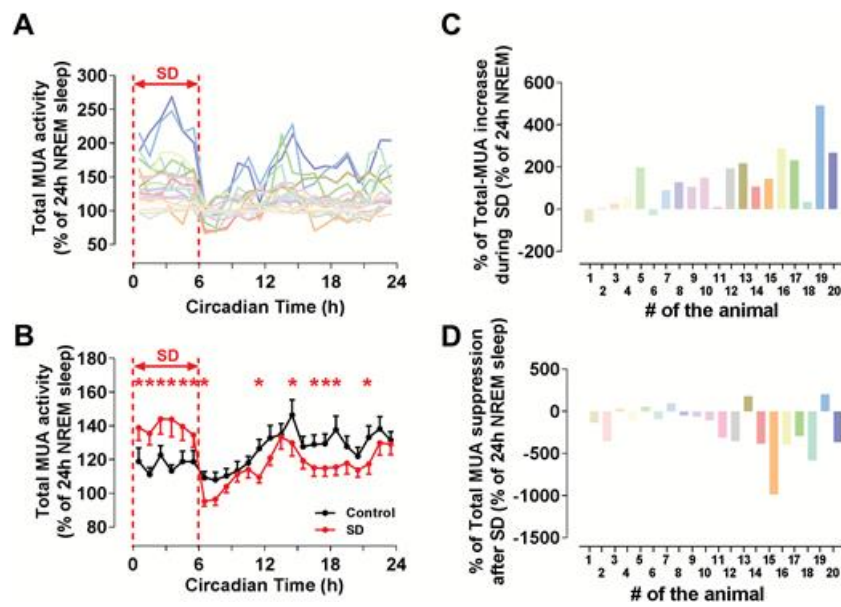
Data were analyzed using SigmaStat version 12.0. All summary data are reported as the mean \pm SEM. Statistical significance was determined using a repeated-measures ANOVA, with time, neuronal activity, sleep state, and power density considered as repeated measures coupled to Dunnett post hoc analysis in the case of significance (Figures 3B and 4A, C, and E; Supplementary Figure S2), paired Student's *t*-test (Figure 4B, D, and F), one-way ANOVA (Figure 2A; Supplementary Figure S1), or simple linear regression (Figure 5A, B, D, and F). *p*-Values are indicated in the text and the figure legends. Differences were considered significant when *p* < 0.05.

Figure 2.



Variability in the circadian amplitude and vigilance state-dependent modulation of neuronal activity in LH. Time course of mean (A) and individual (C) neuronal activity rhythms in the LH (*n* = 20). (B) Distribution of the amplitude of neuronal activity rhythms in all LH subregions. Amplitudes were extracted from the best Cosinor fit applied to original rhythms shown in (C). (D, E) Relative mean neuronal activity during wakefulness (D) and REM sleep (E) measured in all LH cases over 24 hr circadian day. (F, G) Time course of neuronal activity at the transition from NREM to REM sleep (F) and NREM sleep to wake (G) during 2 min before and after the vigilance state transition. All variables are expressed as a percentage of the mean activity within NREM sleep over 24 hr. Error bars in A represent SEM.

Figure 3.

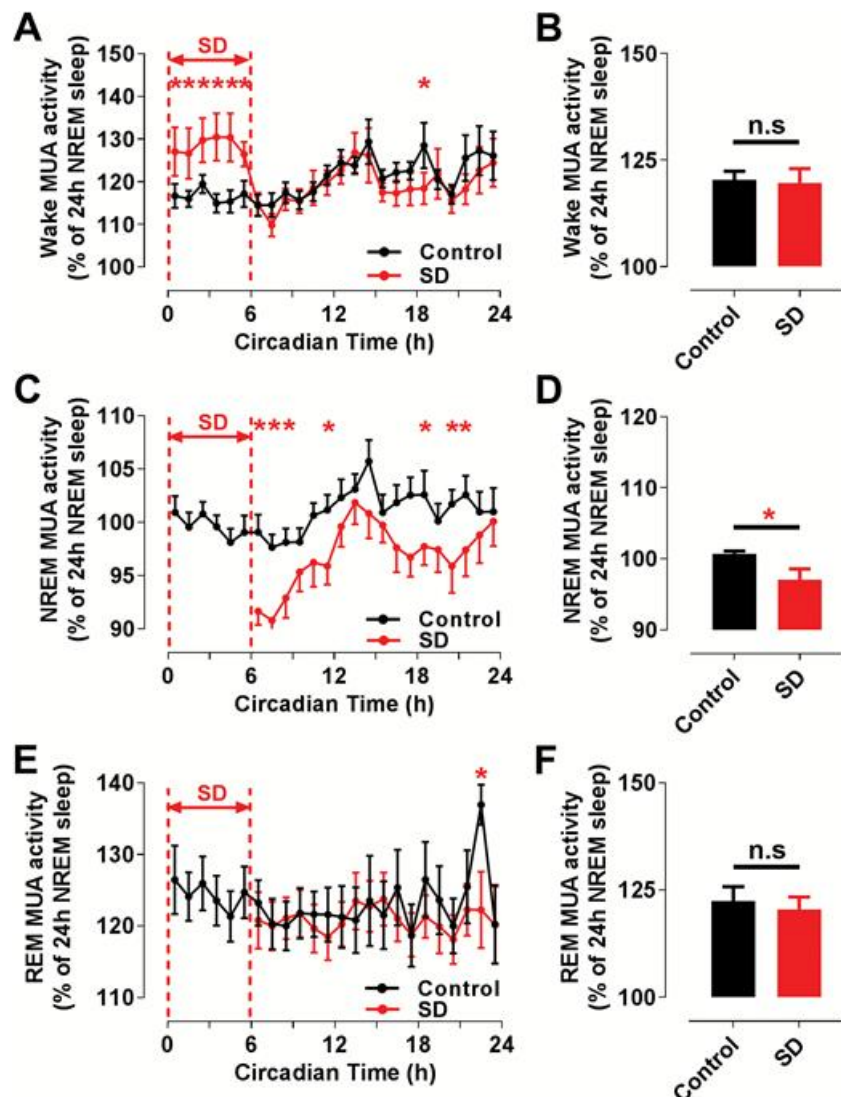


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Variability in the neuronal activity responses of LH subregions to SD. Time course of individual (A) and mean (B) neuronal activity in LH ($n = 20$) during and 18 hr following SD. In B, activity is displayed in 1 hr intervals as a percentage of the mean activity measured during NREM sleep during the baseline day. SD was induced during the first 6 hr of the subjective day. (C, D) Percentage of neuronal activity increase (C) and decrease (D) during respectively the 6 hr SD and the 18 hr following SD. Error bars in B represent SEM. $*p < 0.05$.

Figure 4.

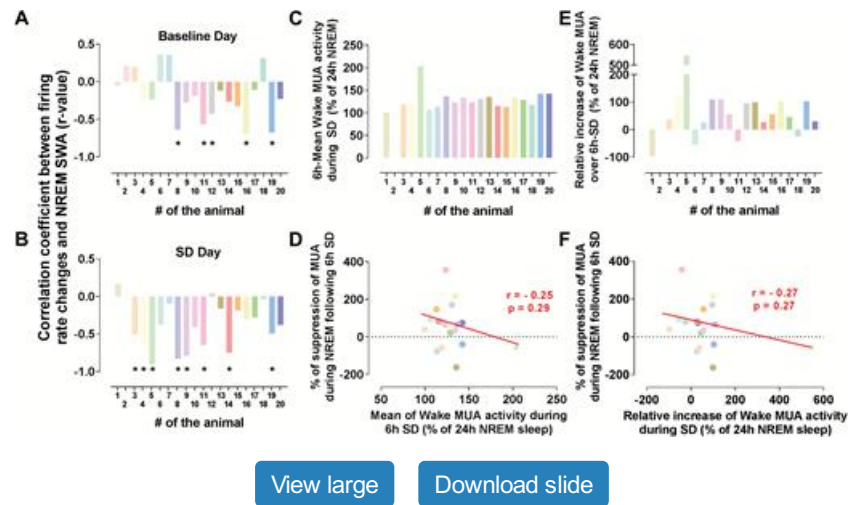


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The effects of SD on LH neuronal activity is vigilance state specific. (A, C, E) Time course of mean neuronal activity during wakefulness (A), NREM (C), and REM sleep (E) in the LH both during baseline and SD day. Activity is displayed in 1 hr intervals as a percentage of the mean activity measured during NREM sleep. (B, D, F) Relative 24 hr mean neuronal activity in LH during wakefulness (B), NREM (D), and REM sleep (E). Error bars represent SEM. $*p < 0.05$.

Figure 5.



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Effect of sleep/wake history on LH neuronal firing. Linear correlation analysis between firing rates and SWA (0.1–4 Hz) during NREM sleep in both baseline (A) and following SD (B). (C) depicts mean firing rates during wake states over the 6 hr SD in all animals. (E) Percentage of wake-related neuronal activity increase during SD relative to the mean firing rates during the corresponding 6 hr in the baseline day. (D, F) Relationship between the percentage of neuronal activity suppression during NREM sleep following SD and both the mean wake-related firing rates during SD (D) and the relative increase of neuronal activity during SD relative to the same circadian phase (CT1–CT6) during baseline (F). Lines in D and F depicts linear regression ($*p < 0.05$).

Results

Circadian and vigilance state-dependent modulation of MUA in LH

We recorded from a total number of 20 animals. The recording sites were all within LH and span from -1.3 to -4.3 mm posterior to bregma (Figure 1). As shown in Figure 2A, the average time course of MUA over all animals showed a significant circadian rhythmicity (Cosinor and ANOVA, $p < 0.001$) with high neuronal activity during the subjective night and low activity during the subjective day (Figure 2A). However, individual traces showed a large variability in the robustness of the circadian rhythm of MUA (Figure 2C). The amplitude of the rhythms ranged from 0.99 per cent to 63.4 per cent (Figure 2B).

Simultaneous recordings of MUA with EEG and EMG show a vigilance state-dependent modulation of neuronal activity in LH. The firing rates were higher during wakefulness and REM sleep compared to NREM sleep (Supplementary Figure S1). To appreciate the electrophysiological heterogeneity in the LH, we plotted the mean firing rates during wakefulness over the 24 hr baseline day for all 20 recordings we obtained from LH (Figure 2D). We found that the magnitude of increase in neuronal firing rates during wake compared to NREM sleep differed between the subregions in LH and ranged from no change (Animals #1 and #2) to increases of 137 per cent (Animal #20) (Figure 2D). The magnitude as well as the directionality of MUA change of firing rates during both wakefulness and REM sleep differed within LH (Figure 2E) but were significantly and positively correlated (linear regression analysis, $p < 0.0001$). This diversity of electrophysiological responses was also confirmed by examining the dynamics of neuronal activity at different vigilance state transitions (Figure 2F and G). The magnitude of increase seen at NREM sleep to REM sleep (Figure 2F) and NREM sleep to wake (Figure 2G) transitions was variable and ranged from no increase to an increase of approximately 250 per cent relative to NREM sleep firing rates (Figure 2F and G). Additionally, in the areas that showed significant increase of activity at these transitions, activity ramped up approximately 100 ms before sleep state transitions suggesting a causal contribution of LH neurons in the induction of transitions of sleep states from NREM sleep to either REM sleep or wakefulness. Collectively, these data suggest a functional, but spatially undefined, heterogeneity of neuronal activity in LH over different vigilance states.

SD induces variable electrophysiological responses within LH

LH is crucially involved in the homeostatic regulation of sleep/wake behavior [1–4]. Within LH, several neuronal subpopulations promote wakefulness [4]. To examine whether the electrophysiological heterogeneity impacts the response of LH to a sleep homeostatic challenge, we gently sleep deprived the animals for 6 hr starting at the beginning of the rest period. Electrophysiological recordings (EEG, EMG, and MUA) were obtained during and following SD. Relative to the baseline day, we found a significant decrease of the duration of time spent awake concomitant with a significant increase in the amount of NREM and REM sleep throughout the 16 hr following SD (Supplementary Figure S2). Sleep architecture recovered its baseline pattern during the second day following SD (Supplementary Figure S2). We also examined the power spectrum of the cortical EEG after 6 hr SD because the power density of delta EEG rhythm (0.1–4 Hz) is thought to reflect sleep pressure and its underlying homeostatically regulated recovery process [6], we found a marked increase ($p < 0.05$) in the delta power density over the first 7 hr following SD (Supplementary Figure S2D).

Next, we examined electrophysiological responses of the LH both during and following SD. Consistent with the wake-promoting role of many neurons in LH [3, 4], we found a significant increase of the mean MUA during SD (Figure 3A and B). When examining the individual responses, again a large variability was evident (Figure 3A). The percentage of change in MUA during SD was not homogenous throughout LH and varied between –61 per cent and 491 per cent relative to the mean firing during baseline NREM sleep (Figure 3C). The percentage of wakefulness (Supplementary Figure S3A) as well as the power density of cortical theta activity (6–9 Hz; Supplementary Figure S3B) during SD were comparable between all the animals suggesting that the difference in the SD-related electrophysiological responses was not related to differences in the overall arousal state of the animals during SD.

After SD, a sustained decrease of MUA relative to baseline was observed (Figure 3B). This decrease was significant at CT07 and CT12 and in intervals throughout the active phase (Figure 3B). By examining the individual responses, we again observed different responses to SD within LH (Figure 3A and D). With the exception of five recordings that showed an overall moderate increase in MUA after SD, all the other cases responded with a decrease in activity ranging from –48 per cent to –986 per cent (Figure 3D). These results demonstrate that the heterogeneity of LH electrophysiology is also evident in the responses to SD.

Additionally, all our MUA recordings during baseline and following SD fail to display a well-defined spatial organisation within LH. For example, areas that are juxtaposed to each other may display significantly different circadian amplitudes of MUA (site #13, amplitude = 11.39; site #17, amplitude = 20.68). Inversely, areas that are separated by 2.5 mm according to the rostro-caudal axis might display comparable amplitudes (site #5 at bregma –1.3 mm had an amplitude of 8.57 and site #10 at bregma –3.8 mm had an amplitude of 8.26). These results suggest that the patterns of circadian and sleep-related neuronal activity within LH do not display a clear and easily defined spatial organization.

The effect of SD on LH electrophysiology is vigilance state specific

Because neuronal firing rates are modulated across vigilance states (Supplementary Figure S1) and SD induced alterations in sleep/wake architecture (Supplementary Figure S2), the alterations observed in the MUA of LH (Figure 3) could be due to either changes in sleep/wake distribution or intrinsic changes in electrical activity caused by SD, or both. To assess the relative contribution of each factor, we analyzed the patterns of neuronal activity separately for wake, NREM, and REM sleep (Figure 4). We found that the decrease of neuronal activity in LH after SD was mainly evident during NREM sleep (Figure 4C and D). A sustained suppression of activity was observed immediately following and throughout the 16 hr following SD (Figure 4C) leading to significant reduction of the overall activity relative to baseline (Figure 4D). With the exception of CT19 for wake-related activity (Figure 4A) and CT23 for REM sleep-related activity (Figure 4E), the patterns of MUA following SD were indistinguishable from baseline. As a consequence, the overall 24 hr averages of MUA during the first day following SD for both wakefulness (Figure 4B) and REM sleep (Figure 4F) were not altered relative to baseline. Together, these results demonstrate that SD alters neural activity in LH selectively during NREM sleep.

The suppression of activity in LH during NREM sleep after SD is not correlated with the magnitude of activation during SD

One of the fundamental functions attributed to sleep is the downscaling of synaptic strength to offset the net synaptic potentiation induced during waking [23]. In the cortex, this homeostatic synaptic downscaling correlated with a homeostatic decline of cortical neuronal firing rates [24]. Importantly, changes in firing rates during NREM sleep correlated positively with changes in NREM sleep SWA suggesting that the dynamic of the downscaling in neuronal firing rates depend on the magnitude of activation during prior wakefulness [24]. The heterogeneity of activity we found in different subdivisions of LH during both physiological and sustained wakefulness allow us to test whether homeostatic regulation of neuronal activity applies also in a similar way to subcortical structure such as LH.

Under both baseline conditions and following SD, all significant correlations between changes in firing rates and NREM SWA were negative (Figure

5A and B). Notably, 15 and 12 out of 20 subregions correlations during respectively baseline (Figure 5A) and after SD (Figure 5B) were not significant suggesting different sensitivities of the different subdivisions of LH to sleep pressure. We then sought to evaluate whether this difference in the response to increased homeostatic sleep pressure is related to the magnitude of activation during SD. Although the mean wake-related firing rates during SD were comparable between LH subdivisions, the relative changes in wake-related firing rates were significantly different between LH subdivisions (Figure 5E). The correlations between the percentage of suppression in MUA during NREM sleep following SD and both the mean wake-related neuronal activity during SD (Figure 5D) and the relative increase in wake-related firing rate during SD (Figure 5F) were not significant. Together, these results demonstrate that different subdivisions of LH are differently sensitive to changes in homeostatic sleep pressure and that the magnitude of neuronal homeostatic changes during recovery NREM sleep was not correlated with the magnitude of activity-potential during prior wakefulness states.

Discussion

The present study demonstrates that the circadian pattern of MUA as well as vigilance-state related neuronal activity are heterogeneous within the LH. This functional heterogeneity is also evident in the electrophysiological response of the different subdivisions of LH to SD. Based on these results, we could not delineate anatomical subdivisions within LH where neurons display homogenous and comparable neuronal activity patterns. We additionally found that the effect of SD on LH neuronal activity was restricted to NREM sleep and was not correlated with neuronal activity during prior wakefulness.

Heterogeneity in the circadian pattern and vigilance-state related changes in neuronal activity

We found that baseline neuronal activity in LH shows a heterogeneity in the level of the circadian modulation and vigilance state-related changes. The LH occupies a relatively large portion of the hypothalamus and consists of an extended field of neurons and fibers with substantially low anatomical definition [25–27]. Both classical anatomical and electrical stimulation studies (reviewed in Stuber et al. [2]) as well as recent studies using optogenetic neurocircuit approaches [2, 4] have revealed the heterogeneous nature of neuron populations in LH. This heterogeneity is evident, not only on genetic and neurochemical levels [1], but also at the functional level in relation for example to sleep/wake states [3, 4]. Our results complement these studies and in addition demonstrate that the LH displays also a heterogeneity in the circadian modulation of MUA as well as in the relative neuronal activity of different subdivisions during the different vigilance states (Figure 2).

Previously, it was shown that the neurochemical identity of neurons expressing c-fos is heterogeneous, containing orexin positive, MCH-positive, and a majority of non-orexin and non-MCH neurons [10]. Our recording consists of MUA from the population of neurons surrounding the tip of the electrode regardless of the neurochemical identity of neurons. Compared to functional c-fos anatomy which is only able to take snap-shots of neuronal activity, our methodology has the advantage of monitoring neuronal activity of the same area in the same animal over time [12]. By using this technique, we show that the heterogeneity in LH neuronal activity is maintained over circadian time and across different vigilance states. Both light and electronic microscopies of the LH have shown a homogeneity of cell density across LH [27]. No definite aggregation of cells into nuclei areas of higher and lower packing density could be distinguished [27]. The functional heterogeneity we unravelled in LH is therefore unlikely to result from differences in neuronal densities between the recorded sites. The most parsimonious explanation of this heterogeneity is the un-even distribution of neurochemically and electrophysiologically diverse neurons across LH [1, 2]. This intermingling of diverse neuronal types would result into a specific electrophysiological signature within each subdivision of the LH. Taken together, our results demonstrate that LH acts as a multi-hub neuronal structure in which the different subregions probably contribute differently to the many physiological functions under the control of LH.

Variability within the LH in response to SD

Another finding of our study that emphasizes the functional heterogeneity in LH is the differential sensitivity to SD within the LH (Figure 3), suggesting that the effect of changes in homeostatic sleep pressure are heterogeneous in LH. As shown before, the 6 hr SD increased sleep pressure as evidenced by the significant increase in both the amount of NREM sleep throughout the 16 hr following SD (Supplementary Figure S2B) and the EEG delta power during NREM sleep (Supplementary Figure S2D) compared to baseline conditions [6, 13, 24]. The overall LH response was characterized by a significant increase in neuronal activity during SD and a significant decrease during the first hours of recovery from SD. Current models of sleep/wake regulation, such as the “flip-flop” model [28] and the activity-dependent metabolites homeostatic theory [7] suggest that the accumulation of activity-related metabolites over wakefulness increases sleep propensity and, eventually facilitates and promotes sleep by suppressing wakefulness. Adenosine stands as a potent sleep-promoting endogenous metabolite [8] and an in vitro study using patch-clamp recordings in

hypothalamic slices showed that application of adenosine inhibits activity of orexin-containing neurons in the LH [29]. Therefore, a potential mechanism for the suppression of neuronal activity after SD in LH would be the suppression of the activity of wake promoting neurons. Given the diversity of wake-promoting neurons in LH [4], future studies targeting single neurons should investigate the sensitivity of each subtype to sleep pressure.

The individual responses to SD were highly variable within the LH. The mechanism behind this heterogeneity in the electrophysiological responses to increased sleep pressure is unknown. Because regional differences in the pattern of extracellular accumulation of adenosine have been shown between multiple brain sites (including the preoptic nucleus of the hypothalamus) [8], similar difference either in the accumulation of extracellular adenosine or other cell metabolites or in the sensitivity toward these cell metabolites within LH may explain the heterogeneous response to SD in our study. In support of this hypothesis, the pattern of neuronal c-fos expression during recovery sleep following a 6 hr SD was found to be also heterogeneous within the LH [15].

SD affects neuronal activity in LH selectively during NREM sleep

After SD, only firing rate during NREM sleep was affected. Neuronal activity in the different LH regions was significantly reduced during NREM sleep, whereas during REM sleep and waking it was, on average, not different from baseline. Electrophysiological recordings as well as recent optogenetic manipulations of LH cells across the sleep/wake cycle identified a wide variety of neurons with different activity patterns in relation to the different vigilance states and with sleep and wake-promoting properties [1–4]. The activity of orexin neurons, for example, is typically low during quiet waking and highest during active waking, and ceases firing completely during NREM and REM sleep [17–19]. The activity pattern of MCH neurons is a mirror image of the activity of orexin cells with a maximal firing during REM sleep, low during NREM sleep and minimal during wakefulness [20]. Recently, a heterogeneous population of GABA-expressing neurons has been described [4, 30]. A subset of these GABA neurons was shown to act as potent wake promoting neurons [30] while others were most active during sleep predominantly during REM sleep [20]. Although a subset of these GABAergic neurons co-localizes with MCH, the precise overlapping map of these GABA neurons with the other markers in LH (Orexin, glutamate, and neurotensin) remains unknown. A surprising finding of our study is that neuronal activity after SD during wakefulness and REM sleep was not affected, presumably reflecting the evolution of the mean firing rates of wake- and REM sleep-active neurons (Figure 4). These results suggest that the firing rates of both wake-active and REM-sleep-active neurons are not affected by increased homeostatic sleep pressure which is at its maximum during the first hours following SD. In relation to wakefulness, this conclusion seems to contradict the *in vitro* study showing that adenosine reduces the firing rates of orexin neurons in LH [18]. Recently, however, by using functional c-fos expression and optogenetic targeting of orexin neurons, Carter et al. [31] showed that the neuronal c-fos response of orexin to optogenetic stimulation was not affected by SD and that the behavioral effects of SD were mediated mainly by inhibiting neuronal activity of downstream arousal-promoting locus ceruleus and tuberomammillary nuclei. These results suggest that *in vivo* compensatory signals may act on orexinergic neurons to sustain their activity under increased sleep pressure. Consistent with this conclusion is that multiple molecules that encode different ambient and behavioral stimuli including glucose, lactate, and dietary amino acids modulate activity levels of orexin neurons [18, 32]. During wakefulness, the synergistic action of these signals on orexin neurons could maintain their level of activity constant, whereas in sleep, orexinergic neurons are silent regardless of the level of sleep pressure [17–19]. Our results suggest that the overall mean of firing rates of all wake-active neurons in LH is not altered following SD. Alternatively, it is possible that SD affects the firing rates of the different wake-active neuronal populations differently without affecting the overall mean of the entire wake-active population. Our MUA recordings approach do not rule out this alternative, but the latter possibility is probably less likely.

The most dramatic effect of SD on neuronal activity in LH was observed during NREM sleep following SD (Figure 4C and D). A sustained decrease was observed throughout the 16 hr following SD. The pattern of neuronal activity during NREM sleep reflects the overall mean firing rates of all NREM-active neurons in LH. Among these, MCH neurons have been implicated in the induction of sleep [20, 33–36]. However, during sleep following SD, only a minority of c-fos+ cells in LH (~6%) are immune-reactive for MCH peptide [10] and no significant changes were found in c-fos expression of MCH neurons during sleep-enhanced periods induced by an adenosine A_{2A} receptor agonist (CGS21680) [37]. These results suggest that MCH neurons are not predominantly involved in the induction of NREM sleep. This conclusion was confirmed recently by physiological manipulation of MCH neurons using optogenetics which showed the crucial involvement of MCH neurons in the induction of REM but not NREM sleep [38]. However, the overall neuronal activity during REM sleep is not affected following SD (Figure 4E, and F). Together these findings suggest that the alterations in neuronal activity we found during NREM sleep following SD is more likely attributed to altered activity of non-MCH positive cells in LH. Future studies using targeted single cell recordings will be necessary to investigate the impact of SD on the neurochemically different neuronal populations within LH.

Homeostatic modulation of neuronal activity in LH

A prominent role attributed to sleep is the homeostatic modulation of synaptic plasticity [39]. An influential hypothesis proposed by Cirelli and Tononi postulates that sleep is associated with a global synaptic downscaling that offsets the net synaptic potentiation induced by prior waking experience [23]. Abundant direct and indirect evidence of vigilant state dependent synaptic scaling have been found [23, 40]. This synaptic re-scaling has been directly linked with the homeostatic modulation of neuronal firing rates [39, 41]. In the cortex, for example, a downscaling in firing rates was shown during NREM sleep after both normal and sustained episodes of waking [24]. Importantly, changes in firing rates during NREM sleep positively correlated with the changes in cortical SWA suggesting that the more potentiated the cortical neuronal network is in the course of waking, the strongest the downscaling is during subsequent NREM sleep [24]. So far however, experiments related to the synaptic homeostatic hypothesis of sleep have focused primarily on cortical regions and its generality to include subcortical areas has not been tested.

During SD, the relative increase in firing rates was different between the different LH subdivisions (Figure 5E). Yet, we found no significant correlation between the magnitude of activity increase during SD and the percentage of suppression of neuronal activity during subsequent sleep. In the cortex, and consistent with the synaptic homeostatic hypothesis [23], similar dynamics were found between neuronal activation and synaptic strength during both SD and subsequent sleep [24, 42]. In LH, the amplitude and the frequency of miniature excitatory postsynaptic currents (mEPSCs) in orexin neurons increased after SD suggesting a potentiation of glutamatergic synapses on orexin neurons [43]. Although consistent with the synaptic homeostasis hypothesis, it should be noted that changes in miniature EPSCs may not accurately reflect changes in neuronal activity. Rather, neuronal activity is modulated by several factors including the nature of neuromodulators and the balance between excitation and inhibition [24, 44]. Consistently, GABAergic neurotransmission is also found to be potentiated in orexin neurons after SD as shown by the increase in both the frequency and amplitude of miniature inhibitory postsynaptic currents (mIPSCs) on orexin neurons [45]. Therefore, and contrasting previous findings in the cortex [24], the changes in neuronal activity in LH following SD may not follow the pattern predicted by the synaptic homeostatic hypothesis which is primarily based on synaptic strength [23].

Conclusion

In summary, our findings extend the notion of heterogeneity of the LH in the neurochemical and molecular markers of its neuronal population [1, 2] showing that this heterogeneity is also found in the circadian and sleep homeostatic modulation of electrophysiological activity. We found a functional, but spatially undefined heterogeneity in LH neuronal activity in response to vigilance state changes. This heterogeneity was also evident in the homeostatic response of LH to SD. The LH is a homeostatic neuronal center that integrates a vast array of inputs to orchestrate food intake, energy balance, sleep/wake vigilance states, and food and drug reward mechanisms [1, 2]. Recent optogenetic studies revealed that different neuronal populations in LH encode different aspects of these behavioral functions [1, 2, 4, 46]. In this study, we did not monitor food intake during our experiments. However, it is well established that insufficient sleep adversely affects metabolism and lead to increased appetite, enhanced sensitivity to food stimuli and, ultimately, a surplus in energy intake [5]. It is likely therefore that the alterations in neuronal activity we measured in LH contribute to the metabolic dysfunctions associated with SD such as obesity and diabetes [5]. Unveiling the cellular and molecular mechanisms of the heterogeneity in the circadian and homeostatic neuronal responses in LH may contribute further in understanding the pathophysiological conditions linking sleep, reward, and metabolism [5, 47]. The present data show that endogenous circadian phase and sleep homeostatic changes are likely to influence the function of this important brain area.

Supplementary material

Supplementary material is available at *SLEEP* online.

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