Neuronal Activity in the Preoptic Hypothalamus During Sleep Deprivation and Recovery Sleep

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ABSTRACT

The preoptic hypothalamus is implicated in sleep regulation. Neurons in the median preoptic nucleus (MnPO) and the ventrolateral preoptic area (VLPO) have been identified as potential sleep regulatory elements. However, the extent to which MnPO and VLPO neurons are activated in response to changing homeostatic sleep regulatory demands is unresolved. To address this question, we continuously recorded the extracellular activity of neurons in the rat MnPO, VLPO and dorsal lateral preoptic area (LPO) during baseline sleep and waking, during 2 h of sleep deprivation (SD) and during 2 h of recovery sleep (RS). Sleep-active neurons in the MnPO (n=11) and VLPO (n=13) were activated in response to SD, such that waking discharge rates increased by 95.8%±29.5% and 59.4±17.3%, respectively, above waking baseline values. During RS, nonREM sleep discharge rates of MnPO neurons initially increased to 65.6±15.2% above baseline values, then declined to baseline levels in association with decreases in EEG delta power. Increase in nonREM sleep discharge rates in VLPO neurons during RS averaged 40.5±7.6% above baseline. REM-active neurons (n=16) in the LPO also exhibited increased waking discharge during SD and an increase in nonREM discharge during RS. Infusion of A2A adenosine receptor antagonist into the VLPO, attenuated SD-induced increases in neuronal discharge. Populations of LPO wake/REM-active and state-indifferent neurons and dorsal LPO sleep-active neurons were unresponsive to SD. These findings support the hypothesis that sleep-active neurons in the MnPO and VLPO, and REM-active neurons in the LPO are components of neuronal circuits that mediate homeostatic responses to sustained wakefulness.
INTRODUCTION

Sleep homeostasis refers to compensatory increases in sleep amount, sleep consolidation and/or sleep depth that occur in response to sleep deprivation (SD) (7). Brain mechanisms that regulate homeostatic responses to SD are not fully understood, but a role for endogenous sleep factors in sleep homeostasis is widely accepted (32). Increased production of adenosine, interleukin 1β, tumor necrosis factor-α, nitric oxide and prostaglandin D2 have all been implicated in increased sleep and increased EEG slow-wave activity (0.3-4 Hz) during sleep following SD (6; 27; 31; 32; 37). However, there is no consensus where sleep factors act in the brain, or how sleep-promoting and wake-promoting neuronal circuits respond to SD.

Neurons in the preoptic hypothalamus are important regulators of sleep onset and sleep maintenance (see (48) for review). Lesions of the preoptic hypothalamus cause chronic sleep loss and local administration of endogenous somnogens into this brain region can promote sleep (17; 24; 27; 29; 50; 51). Within the preoptic area, the ventrolateral preoptic area (VLPO) and the median preoptic nucleus (MnPO) contain high densities of neurons that are activated during sleep, as identified by single unit recordings and by sleep-related expression of c-fos (10; 43; 45; 47). Sleep-related c-Fos protein immunoreactivity (IR) in these nuclei occurs in GABAergic neurons and descending projections from the VLPO and MnPO to several nuclei implicated in generalized brain arousal have been demonstrated (9; 42; 44; 53). Activation of VLPO and MnPO neurons is hypothesized to promote sleep through GABA-mediated inhibition, of monoaminergic, hypocretinergic and cholinergic neurons located in the
posterior hypothalamus and brainstem (38; 48). VLPO GABAergic neurons also contain the inhibitory neuropeptide galanin (42).

The role of preoptic neurons in homeostatic responses to sleep loss is unresolved. Studies of c-Fos-IR in rats following SD indicate that VLPO neurons are not activated in response to SD unless animals are permitted recovery sleep (RS) (12; 43). Elevated Fos+ cell counts have been reported in MnPO neurons in rats that were sacrificed prior to opportunity for RS, suggesting that neurons in this nucleus are responsive to increasing homeostatic pressure for sleep during waking (12). Due to the time required for gene expression and synthesis of c-Fos protein and the time required to degrade synthesized protein, studies of c-Fos immunoreactivity provide poor temporal resolution of neuronal activity with respect to dynamic changes in homeostatic sleep pressure. Previous electrophysiological studies of preoptic neurons have focused on activity during spontaneous sleep and wakefulness (2; 45; 47; 49). To more precisely characterize the relationship of preoptic neuronal activity to dynamic changes in homeostatic sleep pressure, we have recorded the discharge of individual MnPO and LPO neurons across conditions of baseline sleep and waking, SD and RS.

MATERIALS AND METHODS

1. Subjects

Experiments were conducted on Sprague-Dawley male rats (300-350g) that were maintained at 12:12h light cycle (light-on at 8:30AM) and with food and water available ad libitum. The experiments were conducted in accordance with the National Research Council Guide for the Care and Use of Laboratory Animals and all experimental
protocols were reviewed and approved by the Institutional Animal Care and Use Committee of the V.A. Greater Los Angeles Healthcare system.

2. Surgical Procedures

Under surgical anesthesia (Ketamine, 80 mg/kg plus Xylazine, 10 mg/kg, i.p.) and aseptic conditions, rats were surgically prepared for chronic recordings of extracellular neuronal activity from MnPO or LPO and for assessment of sleep-wake state. Four screw electrodes were implanted over the frontal and parietal cortex to record the electroencephalogram (EEG). Two teflon coated stainless wires were implanted into the dorsal neck muscles to record the electromyogram (EMG). For the recording of extracellular unit activity, a single 23-gauge stainless-steel guide cannula attached to a mechanical microdrive was implanted stereotaxically such that its tip was positioned dorsal to the MnPO (AP = -0.26, L = 0.00, H = -4.00) or LPO (AP = -0.40, L = 1.20, H = -4.50) region (33). In a subset of animals used for pharmacological study, a microdrive-microdialysis guide cannula assembly, consisting of a single barrel mechanical microdrive and an adjacent guide cannula for microdialysis probe insertion, was implanted such that their tips rested 3mm above the dorsal aspect of the VLPO. Four pairs of microwires, each consisting of two 20µm insulated stainless steel wires glued together except for 2.0 mm at the tip and sharply cut at an angle, were passed through the microdrive barrel such that their tips protruded 3 mm beyond the tip of the guide cannula and into the MnPO or LPO. EEG and EMG electrodes and microwires were soldered to miniature plugs and the entire assembly was anchored to the skull with dental acrylic.
3. Data Acquisition

Experiments were carried out after allowing at least 10 days for recovery from surgery. During recovery, rats were placed in a cylindrical Plexiglas cage (Raturn System, USA) that was housed in a sound attenuated and electrically shielded chamber. During the final 2-3 days of the recovery period, rats were also acclimatized to the recording procedure by connecting them with recording cables for 2-3h daily. In recording chambers rats were maintained at the same 12:12h light:dark cycle (illumination intensity about 100 lux) and had ad libitum access to food and water.

All electrophysiological recordings were performed on un-anesthetized, unrestrained rats during early light phase of a 12:12 light dark cycle. EEG and EMG activity were recorded using a Grass Model 78 polygraph (USA). Neuronal activity was recorded using bipolar derivations from microwires that were amplified by a differential AC amplifier (model 1700, A-M System, USA) with low and high cutoff filters of 10Hz and 10kHz, respectively. Signals were continuously monitored on a digital storage oscilloscope (Hitachi, VC-6024, Japan). These signals were also digitized and stored on disk for subsequent offline analysis using a Power1401 data acquisition interface and Spike-2 Software (Cambridge Electronic Design, Cambridge, UK). During recording sessions the microwires were advanced in 20-30μm steps until isolated single units with signal/noise ratio ≥2 were located.

4. Experimental Procedures

4a. Total sleep-deprivation studies: First, after isolating individual cells, their baseline discharge activity was recorded across at least 3 sleep-wake cycles, i.e.,
during waking, non-REM and REM sleep. Selected neurons with stable discharge patterns were then recorded continuously during 2h of sleep deprivation (SD) followed by 2 h of recovery period. During SD, rats were closely monitored both behaviorally as well as with reference to EEG changes. Each time, the animal exhibited EEG synchronization for 5-10s it was gently awakened by taping on the cage or slightly turning the cage. Each attempt to prevent the animal from entering into sleep was recorded as an event. After SD, recording continued during a 2h opportunity for uninterrupted RS.

4b. Selective REM sleep deprivation: In another set of experiments, following the recording of the baseline discharge activity across 2-3 sleep-wake cycles, rats were subjected to two hours of selective REM sleep deprivation (REMD) (11). During REMD, rats were closely monitored for electrophysiological and behavioral signs of REM sleep. Each time, the animal exhibited electrophysiological signs of REM sleep, i.e., EEG desynchronization and lower EMG tone after NREM sleep, for 10s it was gently awakened by slightly touching the recording cable or turning the cage. Each attempt to prevent the animal from entering into REM sleep was recorded as an event. After REMD, rats were left undisturbed for 2 hrs of RS.

4c. Microdialysis perfusion: In the third set of experiments, we determined the effects of an adenosine A2A receptor antagonist, ZM 241385, on the discharge activity of the VLPO neurons across spontaneous sleep wake cycle as well as during total SD. In this case, at least 24h before the experiment, the stylet of the microdialysis guide cannula was replaced by a microdialysis probe (semi-permeable membrane tip length, 1 mm; outer diameter, 0.22mm; molecular cut off size, 50 kDa; Eicom, Japan), fixed with
dental acrylic, and flushed with artificial cerebrospinal fluid (aCSF; composition in mM, 145 NaCl, 2.7 KCl, 1.3 MgSO₄, 1.2 CaCl₂, and 2 Na₂HPO₄; pH, 7.2) at a flow rate of 2 µl/min. The time taken by the vehicle solution to travel from the reservoir to the tips of the probes was precisely calculated. The ZM 241385 was dissolved in 4% DMSO and therefore, aCSF containing 4% DMSO was used as control. Approximately 16 h prior to unit recording experiments, the dialysis probe was inserted into the guide cannula, fixed in place and perfused with aCSF (3).

In one set of experiments, the discharge rate of isolated VLPO neurons was recorded through 2-3 stable sleep-wake cycle with aCSF+DMSO perfusion as a baseline. After baseline recording, 50µM of ZM 241385 was microdialyzed adjacent to the recorded neurons for 10min so that the transient effects of this drug on the discharge activity of neuron(s) could be studied without triggering a strong behavioral response. After delivery of the drug, the perfusion medium was switched back to aCSF+DMSO and the recording continued for another 45-90 min as wash out or recovery. During the entire recording session, the animal was undisturbed except, if necessary, a stable episode of waking was achieved by lightly tapping or slightly moving the cage. In another set of experiments, the discharge of VLPO sleep-active neurons was recorded during baseline, and two hours of SD followed by RS. ZM 241385 was microdialyzed adjacent to the recorded cells for 10 min at the end of SD to determine the effects of A2A receptor blockade on the SD-induced changes on VLPO neuronal activity.

5. Histology
At the end of all recording sessions, rats were deeply anesthetized with pentobarbital (100mg/kg, i.p.) and micro-lesions were made at the tip of two or three microwires by passing DC current (20 μA, 15-20s). The microwires that yielded the highest number of cells were selected for micro-lesion and for tracking of anatomical localization of the recorded neurons. The animals were then injected with heparin (500U, I.P.), and perfused through the heart with phosphate buffered saline (PBS; PH 7.4) followed by fixative containing 4% paraformaldehyde in PBS. The brains were removed, postfixed for 24h at 4°C in the same fixative, and then placed in PBS containing 30% sucrose for 48h at 4°C. Forty micrometer thick coronal sections were cut serially on a freezing microtome and stained for Nissl (Cresyl violet). Reconstructions of microwire tracts were made with the aid of a Neurolucida imaging system (Microbrightfield, Colchester, VT, USA) guided by a rat brain atlas (33).

6. Data Analyses

States of waking, non-REM sleep and REM sleep were identified on the basis of EEG and EMG patterns using standard criteria. The mean discharge rate of neurons in waking, NonREM, and REM sleep was calculated from at least 3 episodes of each state ranging in duration of 30-300s. In order to determine the sleep-wake discharge profiles of the recorded neurons, a minimum of 25% change in discharge rate criterion was used. Neurons were classified as “wake-active” if their non-REM/wake discharge rate ratios were <0.75; “sleep-active” if non-REM/wake discharge ratio was >1.25, Neurons were classified as REM-active if REM/wake and REM/non-REM ratios were >2.0.
Neurons exhibiting <25% change in discharge rate across sleep-waking states were classified as state-indifferent.

The 2 hour SD and RS periods were divided into 30min quartiles. Sleep-wake discharge rates were calculated during each quartile to determine the effects of the duration of SD and RS on the discharge rate of various neuronal groups within MnPO and VLPO. Waking discharge rates during SD were calculated during 30-60s periods of wakefulness accompanied by a fully desynchronized EEG and elevated EMG activity, comparable to baseline periods of waking. Episodes of transient EEG synchrony associated with sleep onset attempts were excluded from determinations of waking discharge rate. During SD, discharge rates during waking were expressed a % change from baseline waking rates (Figures 3, 4 and 9). During RS, discharge rates during nonREM and REM sleep were expressed as % change from baseline rates during those states. The number of attempts to initiate sleep, as evidenced by EEG and behavioral criteria described above, was counted for each 30 min period during SD as a measure of increasing homeostatic sleep drive (Figures 3 and 4).

During RS, declining EEG slow-wave activity (SWA) in nonREM sleep was used as an indicator of diminishing homeostatic sleep pressure (Figures 3, and 4). Digitized EEG signals were subjected to a fast Fourier transform algorithm, after which a power spectrum was computed for the delta frequency range of 0.75 - 4.0 Hz (CED1401, Spike 2 software) This was done for consecutive 10-s epoch of scored nonREM sleep during the first two consolidated episodes of nonREM sleep (≥2min in duration) occurring in each 30 min quartile of the RS period. NonREM sleep EEG SWA was expressed as a percentage of values calculated during 2-4 consolidated nonREM sleep
episodes occurring during the baseline recording period prior to the initiation of sleep deprivation.

Changes in waking-related neuronal activity across the SD period and in nonREM sleep- and REM sleep-related neuronal activity during RS period were assessed with one-way repeated measures ANOVA, followed by Holm-Sidak test for multiple comparisons (SigmaPlot 11.0). For microdialysis drug delivery experiments, comparisons of waking and nonREM sleep discharge rates during baseline perfusion of aCSF and rates following perfusion of ZM241385 were by paired t-test (Figure 6).
RESULTS

1. Summary of Recorded Neurons

A total of 25 neurons in the MnPO and 128 neurons in the dorsal to ventral extent of the LPO were recorded across 8 animals. Table 1 summarizes the number and types of cells recorded in each brain region and the mean discharge rates of each cell type during waking, nonREM and REM sleep.

A total of 91 cells were successfully recorded during baseline sleep and waking, during 2 h of SD and during 2 h of RS. Results of these recordings for each of the major functional cell types (sleep-active, REM-active, wake/REM-active and state indifferent) are summarized in the following sections.

2. Sleep-Active Neurons

2.1 Sleep Active Neurons in the Median Preoptic Nucleus.

The activity of sleep-active neurons localized to the MnPO (n=11) were quantified during baseline, SD and RS. The spontaneous sleep-wake discharge and locations of these neurons are shown in Figure 1. An example of continuous recording of an MnPO sleep active neuron across the three conditions is shown in Figure 2. During the baseline period, the cell exhibited elevated discharge rates during nonREM and REM sleep compared to waking (Figure 2B). At the start of SD, only infrequent interventions were required to keep the animal awake (Figure 2A top trace), and discharge rate of the cell was uniformly low at <1 spikes/sec (Figure 2C). As homeostatic sleep pressure increased with continuing SD, as evidenced by the increasing number of interventions required to maintain wakefulness (Figure 2A), the discharge rate of the cell increased. By the end of 2 h of SD, the waking discharge rates was approximately double that
during baseline sleep (Figures 2B and 2D). Discharge of the cell remained elevated compared to baseline during early RS (Figure 2B and 2E) but returned to baseline levels after 2 h of unrestricted sleep (Figure 2A).

Figure 3 summarizes findings from all MnPO sleep-active neurons studied in the same manner as in Figure 2. Mean discharge rates, calculated during 30 min quartiles of the SD period, expressed as % change from baseline waking discharge rates, are plotted in Figure 3A. Discharge rates increased with increasing duration of SD, and were significantly elevated during the last 2 quartiles of the SD period, compared to earlier time points (Figure 3A). There was also a progressive increase in the number of attempts to initiate sleep with increasing duration of SD (Figure 3B).

Mean nonREM sleep discharge rates were elevated during the first 30-60 min of RS and returned to baseline levels after 2 hours (Figure 3C). EEG slow-wave activity in nonREM sleep showed a similar pattern (Figure 3D).

Mean discharge rates of MnPO sleep-active neurons during REM sleep did not change significantly across the conditions of baseline (3.3±0.5 s/s), early RS (3.6±0.6 s/s) and late RS (3.17±0.5 s/s; F(10,2)=2.69, ns).

2.2 Sleep Active Neurons in the Lateral Preoptic Area

The discharge rates of 13 sleep-active neurons localized to the VLPO and of 9 sleep-active neurons localized to the dorsal LPO (Figure 1) were quantified during baseline, SD and RS. The effects of SD on VLPO sleep-active neurons are summarized in Figure 4. Similar to MnPO neurons, waking discharge rates increased
with increasing duration of SD (Figure 4A) in association with increases in the number of attempts to initiate sleep (Figure 4B).

During RS, VLPO sleep active neuronal discharge during nonREM sleep was initially elevated compared to baseline nonREM sleep discharge rates, and gradually returned to baseline levels after 2 hours (Figure 4C), in association with the decline in nonREM sleep SWA (Figure 4D). This decline in nonREM sleep discharge rates during RS was not as rapid as that observed for MnPO sleep-active neurons as discharge rates during the first quartile of the RS period did not differ significantly from values recorded during the second and third quartiles (Figure 4C). Similar to MnPO sleep-active neurons, mean discharge rates of VLPO sleep-active neurons during REM sleep did not change significantly across the conditions of baseline early RS and late RS (Table 2).

Baseline sleep-wake discharge profiles of the 9 sleep-active neurons localized to the dorsal LPO were similar those of VLPO sleep active neurons (Figure 1). However, sleep active neurons in the LPO neurons did not exhibit significant changes in waking discharge rates during 2 hours of SD, (Figure 4A), in spite of significant increases in the number of attempts to initiate sleep across the SD period (Figure 4B). Changes in EEG SWA across the RS period for the experiments involving the 9 dorsal LPO neuronal recordings were similar to that observed during recordings of VLPO neuronal activity (Figure 4D). However, in contrast to VLPO neurons, there was no significant effect of RS on nonREM discharge rates of dorsal LPO sleep-active neurons (Figure 5C and Table 2).
2.3 Effects of Adenosine $A_{2A}$-Receptor Antagonist on VLPO Sleep-Active Neuronal Discharge.

Adenosine (AD) is implicated as an endogenous regulator of homeostatic sleep drive. The hypothalamus and adjacent basal forebrain are sites of action of the sleep regulatory effects of AD (6; 30; 35). Extracellular levels of AD in the magnocellular basal forebrain, immediately adjacent to the LPO, increase during SD and decline during subsequent RS (36). Sleep induced by central administration of $A_{2A}$ receptor agonists is associated with increased c-Fos expression in VLPO and MnPO GABAergic neurons (22; 40). Central administration of ZM-241385, an $A_{2A}$ adenosine receptor antagonist, suppresses SD-induced expression of c-Fos in MnPO and VLPO neurons (22). Therefore, we examined whether $A_{2A}$ receptor-mediated adenosinergic signaling contributes to the activation of sleep-active neurons in the VLPO during SD.

Figure 5 shows an example of the effects of ZM-241385 on neuronal discharge during SD and RS in a VLPO sleep-active neuron. As was typical for VLPO sleep active neurons (Figure 4A), this neuron exhibited increased waking discharge during the second hour of SD, compared to baseline waking activity. ZM-241385 was delivered for a 10 min period just prior to the end of SD (indicated by closed and open arrows at top of the figure) via a microdialysis probe located adjacent to unit recording electrodes. ZM-241385 perfusion evoked a short latency suppression of waking discharge during SD, and also suppressed the expected increase in nonREM sleep-related discharge of the cell during early RS (Figure 5C). There was eventual recovery of nonREM sleep discharge rates after approximately 75 min following the end of drug perfusion (Figure 5D). Effects of ZM241385 perfusion during SD and RS was examined in 4 sleep-active
neurons, and the effects of drug perfusion on spontaneous sleep-waking discharge rates was examined in 2 additional sleep-active neurons. There was a significant decrease in both waking and nonREM sleep discharge rates of these 6 cells following ZM241385 perfusion compared to vehicle perfusion (Figure 6A). In contrast, ZM241385 perfusion had no effect on state-dependent discharge in a group of neurons with wake-related discharge (Figure 6B).

3. REM Sleep-Active Neurons

3.1 Effects of Total Sleep Deprivation on Discharge of REM Sleep-Active Neurons.

A total of 16 neurons with REM sleep-active discharge were recorded during baseline, SD and RS. Mean spontaneous sleep-wake discharge rates and locations of these neurons are shown in Figures 7A and 7B, respectively. A representative example of continuous recording of a REM-active neuron during baseline, SD and RS is shown in Figure 8. Similar to what was observed in MnPO and VLPO sleep-active neurons, waking discharge of this REM-active neuron increased across the 2 hour SD period (Figure 8A and 8B). There was a transient increase in nonREM discharge rates in this cell early in the RS period, compared to baseline (Figure 8C). Discharge rates during REM sleep were similar in the baseline and RS conditions (Figure 8A and 8D). Figures 9A and 9B summarize the effects of SD on waking and nonREM sleep discharge in 16 REM-active neurons. Waking discharge rates, expressed as %change from baseline, were significantly elevated in the late versus early stages of SD (Figure 9A). NonREM sleep discharge rates were maximal during the first 30 min of recovery sleep, but returned to baseline levels as RS progressed (Figure 9B). There were no
changes in mean discharge rates during REM sleep across the conditions of baseline
sleep-wake the first 30 min of RS and the final 30 min of RS for these 16 REM-active
cells (Table 2).

3.2 Effects of REM Sleep Deprivation on Discharge of REM Sleep-Active Neurons.

In an attempt to determine if changes in discharge rate of REM-active neurons
during total SD were a consequence of increasing homeostatic pressure for REM sleep,
we studied an additional 10 REM-active neurons in the LPO during baseline, 2 hours of
selective REM sleep deprivation (REM-SD) and subsequent RS. REM-SD was
achieved by gently arousing animals at the initial electrographic signs of REM sleep
onset, as described previously (11). As shown in Figures 9C and 9D, although the
general trend of changes in waking discharge rate during 2 hours of REM-SD and of
changes in nonREM sleep discharge rate during 2 hours of RS was similar to that
observed in REM-active cells as a consequence of total SD, these changes did not
reach statistical significance. This argues that REM sleep loss alone cannot account for
the changes in neuronal discharge of LPO REM-active neurons that were observed in
response to total SD.


Discharge of 28 wake/REM-active neurons and 14 state-indifferent neurons were
recorded during baseline sleep-wake, SD and RS. The mean discharge rate of these
neurons during baseline waking, nonREM and REM sleep are shown in Figure 7A. The
reconstructed locations of these neurons in the LPO are shown in Figure 7B. There
were no significant changes in mean waking, nonREM and REM sleep discharge rates for wake/REM-active and state indifferent neurons across the conditions of baseline, SD and RS (Table 2).

DISCUSSION

The preoptic hypothalamus is recognized as a critical sleep regulatory region of the brain. Within the rat preoptic area, the MnPO and VLPO contain sleep-active neuronal populations that are hypothesized to have important sleep regulatory functions. The extent to which MnPO and VLPO neurons are activated in response to expression of sleep as opposed to homeostatically-driven sleep regulatory demands has been controversial. Here we report for the first time that waking discharge of MnPO and VLPO neurons increases during SD in parallel with behavioral indices of increasing homeostatic sleep pressure. NonREM sleep related discharge of these neurons is elevated during initial RS, in association with elevated EEG SWA, an electrophysiological biomarker of homeostatic sleep pressure. NonREM sleep discharge of MnPO neurons rapidly decreases across the recovery sleep period, in parallel with the decline in EEG SWA. Elevated nonREM discharge rates during recovery sleep were more persistent in VLPO neurons. A population of sleep-active neurons located in the dorsal LPO did not demonstrate changes in wake- or sleep-related discharge during SD and RS. Wake/REM active and state-indifferent neurons in the LPO did not exhibit significant changes in discharge rate in response to SD. These findings demonstrate that the activity of MnPO and VLPO sleep regulatory neurons is
reflective of changes in homeostatic sleep pressure in animals subjected to short-term sleep deprivation early in the light phase.

We also recorded the activity of a population of REM sleep-active neurons located in the LPO. The anatomical distribution of these neurons partially overlap with neurons in the so-called extended VLPO, previously identified by REM sleep-related c-fos expression and excitotoxic lesion-induced REM sleep suppression (23; 24). Here we report the novel finding that discharge of REM-active cells in the LPO is strongly responsive to changes in homeostatic sleep pressure, and that these cells exhibited both increases in waking discharge during SD and an initial increase followed by a decrease in nonREM sleep-related discharge during RS (Figures 8 and 9). To investigate if loss of REM sleep contributed to responses of REM-active cells to total SD, we recorded from a second group of REM-active cells before, during and after 2 h of selective REM-SD. We found no significant effects of REM deprivation on wake- or sleep-related discharge, indicating that loss of REM sleep during total SD did not underlie changes in discharge of REM-active neurons. However, the possibility remains that LPO neurons with REM-related discharge can be activated in response to increasing REM sleep pressure, but that selective REM sleep loss must be extended beyond 2 hours.

The neurotransmitter phenotype and anatomical projections of neurons in the dorsal LPO, where REM-active neurons were localized, are not completely known. Neurons that express Fos-IR during REM-enriched sleep and that are located dorsal to the VLPO core, express galanin and project to the dorsal raphe nucleus (DRN) and the locus coeruleus (LC) (23). Neurons throughout the LPO project to the ventrolateral
periaqueductal gray (PAG) (15), an area implicated in REM sleep regulation (25;26). If
REM-active neurons in the LPO are GABAergic and/or galaninergic, the increase in
nonREM sleep-related discharge rates in these cells could disinhibit REM generating
neurons in the pontine reticular formation via inhibition of monoaminergic neurons in the
DRN and LC and of GABAergic neurons in the PAG. (25; 26; 28).

Our findings on the temporal relationships between discharge of MnPO sleep-
active neurons and homeostatic sleep pressure (Figures 2 and 3) are largely consistent
with previously published reports. In studies comparing c-Fos expression in glutamic
acid decarboxylase (GAD)-positive MnPO neurons following 2 of hours spontaneous
sleep, 2 hours of SD and 2 hours of RS, Fos-GAD+ cell counts were highest following
SD without opportunity for sleep (12). Fos-GAD+ counts were higher in rats sacrificed
after 1 hour versus 2 hours of RS (12), a finding consistent with single unit activity
during RS (Figure 3). During spontaneous sleep, discharge of MnPO sleep-active
neurons is highest around wake-sleep transitions and early in the sleep bout, with
decreases in discharge rate occurring across sustained episodes of nonREM sleep
(45). This is consistent with the data reported here. The collective evidence indicates
that during the rest phase, discharge of MnPO sleep regulatory neurons accurately
reflects dynamic changes in homeostatic sleep pressure during both waking and sleep.

The relationship between the activity of VLPO sleep-regulatory neurons and
homeostatic sleep pressure has been less well-understood. Early studies of c-Fos
expression indicated that following 24 hours of SD, c-Fos+ cell counts in the VLPO were
not elevated unless animals were permitted RS prior to euthanasia (43). In rats
subjected to 12-14 hours of SD, waking discharge of VLPO neurons remained low, but
discharge rates during RS were elevated compared to sleep-related discharge of VLPO neurons recorded in nonsleep deprived animals (47). Comparisons of c-Fos-GAD+ cell counts in the VLPO following 2 hours of spontaneous sleep, 2 hours of SD and 2 hours of RS demonstrated that Fos-GAD+ cell counts were higher in both sleep conditions compared to the condition of SD without opportunity for RS (12). Collectively, these findings suggest that activation of VLPO neurons is strongly dependent on the expression of sleep and less reflective of homeostatic sleep pressure during waking.

The findings of the present study suggest that, while activation of VLPO neurons is strongly reflective of sleep expression, a subpopulation of these neurons is also responsive to changing homeostatic sleep pressure during waking, as evidenced by increased waking discharge after 60-120 minutes of SD (Figure 4A). The discrepancy with our previous studies of c-fos expression in VLPO neurons following short-term sleep deprivation (12) may reflect that Fos studies include the entire VLPO neuronal population which may have heterogeneous responses to SD, while a more homogenous population of VLPO neurons may have been studied here due to microelectrode sampling bias. The negative findings of increased waking-related activity of VLPO neurons after more prolonged periods of SD noted above (43; 47), may indicate that activation of VLPO neurons during waking may not persist when sleep restriction extends beyond a few hours.

In each of the three groups of neurons that exhibited increases in discharge rate during nonREM recovery sleep, discharge rates during REM sleep were unchanged compared to baseline values. This suggests that discharge patterns of SD-responsive
neurons during REM sleep are more stereotyped and not modulated by factors that
cause neuronal activation during sleep deprived waking and nonREM recovery sleep. It
remains possible that REM sleep discharge rates in SD-responsive preoptic neurons
might be altered under conditions of more sustained REM sleep loss.

The MnPO and VLPO are sources of descending GABAergic projections to
several wake-promoting regions in the posterior hypothalamus and brainstem. These
include the TMN, DRN and the LC (42; 44; 53). A subset of MnPN and VLPO neurons
that project to the DRN and the adjacent ventrolateral PAG, express Fos-IR during
sleep (15; 54). Projections from the VLPO and MnPO to the hypocretin neuronal field in
the perifornical lateral hypothalamus have also been described (55) and a subset of
these projection neurons exhibit sleep-related Fos-IR (52). The activation and
inactivation of MnPO neurons have been shown to suppress and activate, respectively,
wake-active neurons in the lateral hypothalamus (46). The combined activation of
sleep-active, presumed GABAergic neurons in the MnPO, and VLPO in response to
short-term sleep loss, as reported here, can be hypothesized to promote homeostatic
responses of decreased sleep latency and increased sleep continuity and sleep depth
through enhanced GABA-mediated inhibition of neuronal systems that promote
behavioral and electrographic arousal.

A population of sleep-active neurons located in the dorsal LPO (Figure 1E), did
not exhibit significant changes in wake- or sleep-related discharge during SD and RS
(Figure 4 and Table 2). The baseline sleep-wake discharge profile of these DLPO
neurons was identical to that of sleep-active neurons localized to VLPO (Figure 1A).
These findings indicate that not all neurons with sleep-related discharge have
comparable sleep regulatory functions. Simple characterization of activity patterns across spontaneously occurring sleep-waking states may not be sufficient to identify neurons as components of sleep-wake regulatory circuits.

Adenosine is an endogenous neuromodulator that has been critically implicated in sleep regulation (6; 35). The sleep-promoting effects of adenosine have been shown to involve $A_1$ adenosine receptor ($A_1$R) mediated inhibition of cholinergic and noncholinergic neurons in the basal forebrain (BF), which are located immediately lateral to the LPO (6). Extracellular levels of adenosine in the BF are elevated in response to acute sleep deprivation (18; 36). $A_{2A}$R-mediated activation of preoptic sleep regulatory neurons is an additional mechanism through which adenosine can promote sleep. $A_{2A}$R agonists delivered to the lateral ventricles or the subarachnoid space, ventral to the BF (SS-BF) promote sleep (39), increase Fos expression in VLPO neurons and decrease Fos-IR in the TMN (40). Micro infusion of $A_{2A}$R agonist into the lateral preoptic area, including the VLPO, promotes sleep (30). Bath application of adenosine in vitro reduces firing of some VLPO neurons via direct $A_1$ effects, but excites other VLPO neurons via effects on $A_{2A}$R (8). We have recently reported that microinjection of $A_{2A}$R agonist into the SS-BF increases c-Fos expression in GABAergic neurons in the MnPO and VLPO and that drug-induced activation of preoptic GABAergic neurons persists even if animals are prevented from sleeping during the post-injection period (22). Infusion of $A_{2A}$R antagonist into the lateral ventricle during the final 2 hours of a 3 hour period of sleep deprivation suppresses recovery sleep, suppresses EEG SWA during recovery sleep, and suppresses c-Fos expression in MnPO and VLPO GABAergic neurons during recovery sleep (22). Here we report that
local microdialysis perfusion of A\textsubscript{2A}R antagonist suppresses spontaneous and sleep deprivation-evoked discharge in VLPO sleep-active neurons (Figures 5 and 6).

In addition to adenosine, several other endogenous sleep regulatory substances, have been implicated in the regulation of sleep homeostasis, including PGD\textsubscript{2}, growth hormone releasing hormone (GHRH) and the pro inflammatory cytokines interleukin-1β and tumor necrosis factor-α (see (21; 28; 32) for review). Where and how sleep regulatory substances target sleep-wake regulatory circuits in the brain is poorly understood. Sleep-active neuronal populations with possible sleep regulatory functions have been identified in the perifornical lateral hypothalamus (13; 14; 20; 46) the neocortex (19) and the rostral medulla (4). Optogenetic activation of melanin concentrating hormone (MCH) neurons in the lateral hypothalamus promotes sleep (20). This is the first demonstration that selective stimulation of a targeted cell type is sufficient to increase sleep, and provides compelling evidence for a sleep-regulatory role of MCH neurons. However, of the known sleep-active neuronal populations, only MnPO and VLPO neurons have been shown to be responsive to multiple endogenous sleep regulatory substances, including adenosine acting via A\textsubscript{2A} receptors (22; 40), PGD\textsubscript{2} acting on DP\textsubscript{1} receptors (16; 41), GHRH (34) and interleukin-1β (1; 5). Here we describe for the first time that dynamic increases in MnPO and VLPO neuronal activity are positively correlated with behavioral and EEG biomarkers of increasing homeostatic sleep pressure. Collectively, this evidence supports the hypothesis that increased production/release of endogenous sleep regulatory substances in the ventral basal forebrain and adjacent hypothalamus that occur in response to either spontaneous or forced wakefulness, promotes homeostatic responses of increased sleep amount, sleep
continuity and sleep depth through excitation of GABAergic sleep-active neurons in the MnPO and VLPO.

Grant Support

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REFERENCES


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54. **Uschakov A, McGinty D and Szymusiak R.** Sleep active neurons within the ventral lamina terminalis project to the bed nucleus of the stria terminalis and the lateral hypothalamus. *Sleep* 28: A17, 2005.
Table 1; State-Dependent Discharge Rates of Sleep-Active, Wake/REM-Active and State Indifferent Neurons During Spontaneous Waking and Sleep

<table>
<thead>
<tr>
<th>Region</th>
<th>n</th>
<th>Median Discharge Rate (spikes/sec) ± S.E.M. (range)</th>
<th>Median Discharge Rate (spikes/sec) ± S.E.M. (range)</th>
<th>Median Discharge Rate (spikes/sec) ± S.E.M. (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Wake</td>
<td>NonREM Sleep</td>
<td>REM Sleep</td>
</tr>
<tr>
<td>Median Preoptic Nucleus</td>
<td>5</td>
<td>5.7 ± 2.5 (3.3-12.5)</td>
<td>3.9 ± 2.2 (2.3-8.9)</td>
<td>4.8 ± 3.9 (3.3-12.4)</td>
</tr>
<tr>
<td>Sleep-Active</td>
<td>13</td>
<td>1.5 ± 0.4 (0.6-3.2)</td>
<td>3.8 ± 0.6 (1.2-7.4)</td>
<td>3.7 ± 0.4 (0.8-6.1)</td>
</tr>
<tr>
<td>State-Indifferent</td>
<td>7</td>
<td>4.1 ± 1.4 (1.5-16.1)</td>
<td>3.8 ± 1.2 (1.2-14.4)</td>
<td>4.2 ± 1.4 (1.2-24.4)</td>
</tr>
<tr>
<td>Lateral Preoptic Area</td>
<td>48</td>
<td>7.9 ± 0.8 (1.3-26.2)</td>
<td>3.2 ± 0.4 (0.2-17.9)</td>
<td>8.2 ± 1.0 (1.0-42.8)</td>
</tr>
<tr>
<td>Wake/REM-Active</td>
<td>26</td>
<td>5.1 ± 2.1 (0.3-12.3)</td>
<td>8.2 ± 1.3 (1.5-27.9)</td>
<td>8.8 ± 1.8 (1.4-32.0)</td>
</tr>
<tr>
<td>Sleep-Active</td>
<td>29</td>
<td>4.9 ± 1.1 (0.3-22.8)</td>
<td>3.7 ± 1.0 (0.2-12.3)</td>
<td>13.1 ± 2.2 (1.0-48.3)</td>
</tr>
<tr>
<td>REM-Active</td>
<td>25</td>
<td>20.8 ± 5.9 (1.2-47.9)</td>
<td>19.3 ± 5.4 (1.2-43.8)</td>
<td>19.9 ± 6.2 (1.3-44.1)</td>
</tr>
</tbody>
</table>

All values expressed as mean discharge rate (spikes/sec) ± S.E.M. (range).
Table 2: Discharge Rates of Lateral Preoptic Area Neurons During Baseline, Sleep Deprivation and Recovery Sleep

<table>
<thead>
<tr>
<th>Wake/REM-Active</th>
<th>Baseline</th>
<th>SD 0-30 min</th>
<th>SD 91-120 min</th>
<th>RS 0-30 min</th>
<th>RS 91-120 min</th>
<th>F-ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Waking</strong></td>
<td>6.4±0.7</td>
<td>5.4±0.8</td>
<td>6.1±0.9</td>
<td>--</td>
<td>--</td>
<td>$F_{(27,2)} = 1.70$ ns</td>
</tr>
<tr>
<td><strong>NonREM</strong></td>
<td>2.7±0.4</td>
<td>--</td>
<td>2.8±0.6</td>
<td>2.2±0.5</td>
<td>$F_{(27,2)} = 2.02$ ns</td>
<td></td>
</tr>
<tr>
<td><strong>REM</strong></td>
<td>6.5±0.9</td>
<td>--</td>
<td>7.0±1.1</td>
<td>6.3±1.0</td>
<td>$F_{(27,2)} = 0.64$ ns</td>
<td></td>
</tr>
<tr>
<td><strong>State-Indifferent</strong></td>
<td><strong>Waking</strong></td>
<td>12.3±4.3</td>
<td>12.1±4.2</td>
<td>10.3±3.7</td>
<td>--</td>
<td>$F_{(13,2)} = 0.54$ ns</td>
</tr>
<tr>
<td><strong>NonREM</strong></td>
<td>11.7±4.2</td>
<td>--</td>
<td>11.0±3.7</td>
<td>9.7±3.4</td>
<td>$F_{(13,2)} = 1.49$ ns</td>
<td></td>
</tr>
<tr>
<td><strong>REM</strong></td>
<td>13.8±5.3</td>
<td>--</td>
<td>13.3±4.6</td>
<td>11.7±4.8</td>
<td>$F_{(13,2)} = 2.27$ ns</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>VLPO Sleep-Active (n=13)</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Waking</strong></td>
<td>4.5±1.1</td>
<td>5.4±1.3</td>
<td>7.2±2.1</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td><strong>NonREM</strong></td>
<td>8.8±2.0</td>
<td>--</td>
<td>12.2±2.2</td>
<td>10.1±1.6</td>
<td>$F_{(12,2)} = 5.06$ p&lt;.05</td>
</tr>
<tr>
<td><strong>REM</strong></td>
<td>9.7±2.5</td>
<td>--</td>
<td>10.1±2.0</td>
<td>9.2±1.8</td>
<td>$F_{(12,2)} = 0.26$ ns</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>DLPO Sleep-Active (n=9)</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Waking</strong></td>
<td>5.1±0.9</td>
<td>5.1±1.0</td>
<td>5.6±1.4</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td><strong>NonREM</strong></td>
<td>8.6±1.7</td>
<td>--</td>
<td>6.9±2.0</td>
<td>6.6±1.9</td>
<td>$F_{(8,2)} = 1.60$ ns</td>
</tr>
<tr>
<td><strong>REM</strong></td>
<td>9.3±2.8</td>
<td>--</td>
<td>8.3±2.0</td>
<td>6.7±2.1</td>
<td>$F_{(8,2)} = 2.00$ ns</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>REM-Active (n=16)</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Waking</strong></td>
<td>5.6±1.5</td>
<td>6.3±1.6</td>
<td>8.5±2.3</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td><strong>NonREM</strong></td>
<td>4.8±1.4</td>
<td>--</td>
<td>7.8±2.0</td>
<td>5.6±1.6</td>
<td>$F_{(15,2)} = 8.89$ p&lt;.01</td>
</tr>
<tr>
<td><strong>REM</strong></td>
<td>14.9±2.8</td>
<td>--</td>
<td>15.4±2.8</td>
<td>15.1±2.8</td>
<td>$F_{(15,2)} = 0.92$ ns</td>
</tr>
</tbody>
</table>

All values mean discharge rate (spikes/sec) ± S.E.M. Anatomical locations of Wake/REM Active, State Indifferent and REM Sleep-Active neurons shown in Figure 7B. Locations of VLPO Sleep-Active and dorsal LP0 Sleep-Active neurons shown in Figure 1E.
FIGURE LEGENDS

**Figure 1.** Sleep-active neurons in the MNPO and LPO.  **A.** Mean baseline sleep-wake discharge rates (DR) for groups of sleep-active neurons in the MnPO, VLPO and dorsal lateral preoptic area (DLPO) that were studied under conditions of SD and RS.  **B.** Coronal sections at the level of the rostral preoptic hypothalamus containing marking lesion (arrow) indicating the end of a microwire track targeting the MnPO.  **C.** Reconstruction of locations of sleep-active neurons in the MnPO. **D.** Coronal section at the level of the preoptic hypothalamus, showing the course of a dorsal to ventral microwire bundle pass through the LPO.  **E.** Reconstruction of the locations of sleep active neurons recorded in the VLPO (black circles) and DLPO (open circles).

**Abbreviations:** ac anterior commissure; BSTMV bed nucleus of the stria terminalis, medial ventral; HDB horizontal limb of the diagonal band of Broca; LPO lateral preoptic area; MnPO median preoptic nucleus; MPA medial preoptic area; ox optic chiasm; PS parastrial nucleus; VLPO ventrolateral preoptic area.

**Figure 2.** Responses of an MnPO sleep-active neuron to sleep deprivation. **A** From top to bottom, discharge rate histogram (spikes/sec), cortical EEG and neck muscle EMG recordings during baseline, SD and RS. Black and gray arrowheads at the top indicate start and end of SD, respectively. The dots between the arrows indicate times when the animal attempted to initiate sleep and the experimenter had to intervene to maintain wakefulness. Note how the frequency of sleep attempts increases with increasing duration of SD. See text for further explanation. **B-E** Expansion of areas labeled in **A**, showing 10 min of recording during baseline (**B**), the first 30 min of SD (**C**), the last 30 min of SD (**D**) and early RS (**E**). The waveforms at the right of **B-E** are...
superimposed and averaged action potentials recorded the 10 minutes shown in each
figure, demonstrating stability of unit recording across all three experimental conditions.

**Figure 3.** Summary of the effects of sleep deprivation and recovery sleep on the
discharge of MnPO sleep-active neurons (n=11).  **A.** Mean waking discharge rate,
expressed as % change from baseline waking values, across 30 min quartiles of the 2
hour SD period.  There was a significant overall effect of duration of SD on waking
discharge rates (F(10,3)=12.3, p<.001).  **B.** There was also a significant effect of duration
of SD on the number of sleep attempts (F(10,3)=114.0, p<.001).  For **A** and **B,** **significant**
different from all other values, Holm-Sidak test.  **C.** Mean discharge rates
during recovery nonREM sleep, expressed as % change from baseline nonREM sleep
values.  There was a significant overall effect of duration of RS on nonREM sleep
discharge rates (F(10,3)=10.9, p<.001).  **D.** There was a significant effect of duration of
RS on EEG SWA in nonREM sleep, expressed as a percentage of baseline nonREM
sleep values (F(10,3)=35.6, p<.001).  For **C** and **D,** **significant** different from all other
values; * significantly different from values at 61-90 and 91-120 min;  # significantly
different from value at 91-120 min, Holm-Sidak test.

**Figure 4.** Summary of the effects of SD and RS on the discharge of VLPO sleep-active
neurons (n=13) and dorsal LPO (DLPO) sleep-active neurons (n=9).  **A.** Mean waking
discharge rate, expressed as % change from baseline waking values, across 30 min
quartiles of the 2 hour SD period.  There was a significant overall effect of duration of
SD on waking discharge rates for VLPO sleep-active neurons (F(12,3)=13.3, p<.001).
There was no significant effect of duration of SD on waking discharge rates for DLPO
neurons (F(8,3)=0.6, ns)  **B.** There was a significant and comparable effect of duration of
SD on the number of sleep attempts for experiments involving both VLPO neurons 
(F_{12,3}=150.3, p<.001) and DLPO neurons (F_{8,3}=130.9, p<.001). For A and B, **
significantly different from all other values, p<.01; *significantly different from values at
1-30 min, Holm-Sidak test.  C. Mean discharge rates during recovery nonREM sleep,
expressed as % change from baseline nonREM sleep values, for VLPO and DLPO
sleep-active neurons. There was a significant overall effect of duration of RS on
nonREM sleep discharge rates for VLPO sleep-active neurons (F_{12,3}=4.6, p<.05), but
not for DLPO sleep-active neurons (F_{8,3}=0.6, ns). D. There was a significant effect of
duration of RS on EEG SWA in nonREM sleep, expressed as a percentage of baseline
nonREM sleep values, for experiments involving both VLPO neurons (F_{10,3}=12.7,
p<.001) and DLPO neurons (F_{8,3}=7.4, p<.01). For C and D, # significantly different
from values at 61-90 min and 91-120 min; * significantly different from values at 91-120
min, Holm-Sidak test.

Figure 5. Effects of local perfusion of A_{2A} receptor antagonist, ZM-241385, on
discharge of a VLPO sleep-active neuron during SD and RS.  A From top to bottom,
discharge rate histogram (spikes/sec), cortical EEG and neck muscle EMG recordings
during 40 min baseline waking and sleep, during 120 min of SD and 120 min of RS.
Just prior to the end of the SD period perfusion was switched from vehicle (aCSF+4%
DMSO) to 50 µM ZM241385 for 10 min (arrows), then back to vehicle for the remainder
of the recording.  In the baseline condition, discharge of the cell was strongly sleep-
related (B).  The cell increased waking discharge, compared to baseline waking,
during the second hour of SD (A). Perfusion of ZM241385 evoked suppression of
waking discharge during the final 10 min of SD.  The cell exhibited marked
suppression of nonREM sleep discharge during initial RS following ZM24185 administration, compared to baseline nonREM discharge (C and B). Discharge suppression persisted throughout RS, eventually returning to baseline levels during the final 30 min of the RS period (D). To demonstrate stability of unit recording before and after drug perfusion, figures at right of B-D are averaged action potentials and the superimposed action potentials on which the average waveforms were calculated.

Figure 6. Effects of perfusion of A2A receptor antagonist, ZM-241385, on the state-dependent discharge of VLPO sleep-active neurons. A Discharge rates of 6 VLPO sleep-active neurons were quantified during baseline perfusion of aCSF and following a 10-15 min perfusion of ZM-241385 (50µM in aCSF). For 4 cells, drug perfusion was initiated after 90-100 minutes of sleep deprivation and for 2 other cells drug was perfused during spontaneous sleep-wake occurring between ZT 2 and ZT4. There was a significant decrease in waking discharge rate (paired t-test, t=2.58, df=5, p<.05) and nonREM sleep discharge rates (t=3.46, df=5, p<.05) following ZM-241385 perfusion compared to baseline. B Perfusion of ZM-241385 had no effect on waking and nonREM sleep discharge rates in a group of 5 wake-active neurons recorded in the VLPO region.

Figure 7. REM sleep-active, wake/REM-active and state indifferent neurons in the LPO. A. Mean baseline sleep-wake discharge rates (±SEM) for the different LPO cell types that were studied under conditions of baseline, SD and RS. There was a significant overall effect of sleep-waking state on the discharge of REM-active neurons ($F_{(15,2)}=39.7$, $p<.001$), on the discharge of wake/REM-active neurons ($F_{(27,2)}=14.9$, $p<.001$), but not on the discharge of state indifferent neurons ($F_{(13,2)}=1.0$, ns).
* Significantly different from all other values, Holm-Sidak test.  

B. Drawing of a coronal section at the level of the preoptic hypothalamus showing the locations of REM-related neurons (black circles), wake/REM-active neurons (gray circles) and state-indifferent neurons (open circles).  

**Abbreviations:**  
- ac: anterior commissure  
- BSTMV: bed nucleus of the stria terminalis, medial ventral  
- HDB: horizontal limb of the diagonal band of Broca  
- LPO: lateral preoptic area  
- MnPO: median preoptic nucleus  
- MPA: medial preoptic area  
- ox: optic chiasm  
- PS: parastrial nucleus  
- VLPO: ventrolateral preoptic area.

**Figure 8.** Response of an LPO REM sleep-active neuron to SD.  

A. Continuous recording of EEG and EMG and rate histogram of unit activity during baseline sleep and waking, 2 hours of SD and RS.  

B. Expansion of areas labeled in A, demonstrating increases in waking discharge rate during the final 30 min of SD (B2) compared to baseline waking (B1). Waveforms at the right of each panel are superimposed (top) and mean (bottom) action potentials recorded during the 2 min shown.  

C. Expansion of areas labeled in A demonstrating increases in nonREM sleep discharge rate during the first 30 min of RS (C2) compared to baseline nonREM sleep (C1).  

D. Discharge rates during recovery REM sleep (D2) were similar to discharge rates during baseline REM sleep (D1).

**Figure 9.** Summary of the effects of SD and selective REM-SD on the discharge of REM-active neurons in the LPO.  

A. There was a significant overall effect of the duration of SD on the waking discharge of REM-active neurons ($F_{(15,3)}=9.4, p<.001$).  

B. There was also a significant overall effect of the duration of recovery sleep after SD on nonREM sleep discharge of REM-active cells ($F_{(15,3)}=6.6, p<.001$).  

C. In contrast to SD, there was no significant effect of duration of selective REM-SD on the waking discharge.
of a separate group of 10 REM-active neurons (F(9,3)=1.9, ns). D. There was also no
significant effect of the duration of RS that followed 2 hours of REM-SD on the nonREM
sleep discharge of REM-active neurons (F(9,3)=2.7, ns). * Significantly different from
values at 1-30 min; # significantly different from values at 31-60 min, Holm-Sidak test.
Wake NonREM Sleep REM Sleep

Discharge Rate (s/s)

MnPO Sleep-Active (n=11)  VLPO Sleep-Active (n=13)  DLPO Sleep-Active (n=9)

B

D

300μ

C

E
Discharge

EEG

EMG

Baseline

SD starts

SD ends

Recovery

0 4 8 12

Discharge (s/s)

Sleep deprivation (First 30 min)

Sleep deprivation (Last 30 min)

Recovery

Waking

NREM

REM

0 4 8 12

Discharge (s/s)

EEG

EMG

B

C

D

E

20 min

2 min
Sleep Deprivation

A. % Δ baseline waking DR
- VLPO sleep-active (n=13)
- DLPO sleep-active (n=9)

B. # of sleep attempts

Recovery Sleep

C. % Δ baseline nonREM DR

D. % Δ baseline nonREM SWA
Discharge (s/s)

A: Baseline  →  Sleep Deprivation  →  Recovery Sleep

B: Baseline (aCSF + 4% DMSO)  →  Waking  →  NREM

C: Recovery (0-30 min) 50 μM-ZM 241385  →  Waking  →  NREM

D: Recovery (90-120 min)  →  NREM  →  Waking
**SLEEP- ACTIVE NEURONS (n=6)**

- Vehicle
- ZM-241,385

**NonREM Sleep**

- **Wake**

**WAKE- ACTIVE NEURONS (n=5)**

- **NonREM Sleep**

Discharge Rate (s/s)

- **Wake**

- **NonREM Sleep**

**Rate (s/s)**

**Discharge Rate (s/s)**
REM-Active (n=16)
State-Indifferent (n=14)
Wake/REM-Active (n=28)

Discharge Rate (s/s)

A

B

REM-active neurons
State-indifferent neurons
Wake-, Wake/REM-active neurons

ac MnPO BSTMV PS MPA BO 3V HDB VLPO ox
Discharge (s/s)

EEG

EMG

A

Baseline

SD starts

SD ends

Recovery

B1: Baseline

(2.6 s/s)

Waking

(7.7 s/s)

B2: SD

C1: Baseline

(0.3 s/s)

NREM sleep

(1.2 s/s)

C2: Recovery

D1: Baseline

(13.1 s/s)

REM sleep

(11.6 s/s)

D2: Recovery

B: Baseline

20 S

C

D

20 S

Waking

REM sleep

Discharge (s/s)

EEG

EMG

Discharge (s/s)

EEG

EMG

Discharge (s/s)

EEG

EMG

Discharge (s/s)

EEG

EMG

Discharge (s/s)

EEG

EMG