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SLEEP-PROMOTING FUNCTIONS OF THE HYPOTHALAMIC MEDIAN PREOPTIC NUCLEUS: INHIBITION OF AROUSAL SYSTEMS

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INTRODUCTION

Current work on the hypothalamic preoptic area (POA) sleep-promoting mechanism has its roots in several landmark studies. First, more than 70 years ago, von Economo (45) reported that patients exhibiting insomnia in association with infectious encephalitis were found, *post mortem*, to have inflammatory lesions in the region of the POA. Later, Nauta (25) found that knife cuts bilaterally spanning the POA of rats were followed by sleeplessness, whereas posterior hypothalamic (PH) knife cuts were followed by somnolence. Rats with both POA and PH knife cuts were also somnolent. More recently, Sallanon *et al.* (32), from the laboratory of Prof. Jouvet, showed that acute inactivation of the PH could restore sleep in cats with insomnia produced by discrete POA lesions. Subsequent studies showed descending pathways from POA to posterior hypothalamus which could convey the sleep-promoting signals (15). These studies, and many other lesion studies (reviewed (22)), support a conclusion that the POA contains a sleep-promoting mechanism. Since the effects of PH lesions override effects of POA lesions they further suggested a hypothesis that the PH wake-promoting network was downstream from the POA sleep-promoting network and that the POA output inhibited the PH.

An important additional contribution was the demonstration by Sherin *et al.* (37) that the localization of POA sleep-active neuron could be identified using the c-Fos immunostaining method. This study suggested that a small site in the ventrolateral POA (VLPO) constituted a critical segregated sleep-promoting site. Using neuronal unit recording methods, we confirmed that the VLPO contained a high proportion of neurons with increased discharge during sleep (42). However, our lab and others have shown that sleep-active neurons, identified by electrophysiological methods, are found in throughout the lateral POA (reviewed (22)). Current studies are addressing several outstanding issues. Are POA sleep-promoting neurons localized or diffuse? What are the mechanisms and pathways by which POA sleep-promoting neurons control wake-promoting neurons? How is the sleep-promoting system controlled by homeostatic processes?

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METHODS

To further map the POA sleep-active neuronal network, we applied the c-Fos immunostaining approach in spontaneously sleeping rats that were sacrificed 3 hrs after "lights on". The numbers of c-Fos immunoreactive neurons were counted in standardized grids (13). Subsequently, the co-labeling of c-Fos and glutamic acid decarboxylase (GAD), the enzymatic marker of GABAergic neurons, was determined (14). Neuronal unit recording studies were used to confirm and extend findings derived from c-Fos studies and to assess interactions among critical sites. Unit recording was accomplished in freely-moving animals using the chronic microwire technique originally developed in our lab (6, 24, 40). In one study, stimulating electrodes (80 μm) were implanted in the median preoptic nucleus and responses of neurons in the perifornical lateral hypothalamus were studied. To assess homeostatic processes sleep deprivation was applied using an intermittent treadmill method (17). The treadmill moved at 10 cm/sec with a 3 sec "on", 12 sec "off" cycle. A control cycle of 15 min "on", 60 min "off" equates total treadmill movement in 24 hours, but permits sustained periods of sleep. The Internal Animal Care and Use Committee of the VA, Greater Los Angeles Health Care System, approved all experiments.

RESULTS

Gong *et al.* (13) confirmed the existence of a segregated group of sleep-active neurons in the VLPO and identified a second segregated group in the median preoptic nucleus (MnPN). The MnPN is a midline nucleus forming a cap over anterior pole of the third ventricle, extending dorsally around the rostral edge of the decussation of the anterior commissure (Fig. 1A). The numbers of c-Fos immunoreactive neurons in the rostral and caudal MnPN were highly correlated with the amount of sleep in the 2 hours prior to sacrifice. If animals slept in a warm ambient temperature, the number of sleep-related c-Fos labeled neurons was increased in MnPN, and was diminished in the VLPO.

We confirmed the existence of sleep-active neurons in the MnPN using electrophysiological methods (40). When electrode tracks were localized in the MnPN, 58% of recorded neurons exhibited increased discharge during both NREM and REM compare to waking (Fig. 1B). Additional neurons exhibited increased discharge selectively during NREM (10%) or REM sleep (8%). Neuronal discharge increased during sustained waking episodes prior to sleep, and sleep-related discharge was highest during the initial sleep episode and declined progressively during successive episodes. These last two findings suggested that MnPN sleep-related neuronal discharge was correlated with homeostatic drive.

Previous work suggested that VLPO sleep-active neurons contained the inhibitory neurotransmitter, galanin (36), and that galanin-containing neurons expressed glutamic acid decarboxylase (GAD) (21). We have recently examined the phenotype of MnPN sleep-active neurons by examining double-labeling for c-Fos and GAD (14). We found that following sustained sleep, 80% of c-Fos labeled neurons also exhibited immunostaining for GAD. After two hours of recovery sleep following 24 hours of sleep deprivation (SD), the percentage of GAD-expressing neurons in MnPN that also expressed c-Fos was increased compared to sleep-deprivation control animals. The recovery sleep following SD was characterized by increased EEG

delta power, a marker of homeostatic drive (44). These results suggest the hypothesis that elevation of sleep-related homeostatic drive involves the recruitment and activation of sleep-related GABAergic neurons in the POA. The hypothesis that the POA sleep-promoting system encodes homeostatic drive is consistent with reports that POA lesions that produce partial insomnia also reduce EEG delta power within residual sleep (20, 42).

Neurons expressing both c-Fos and GAD during sleep were not restricted to the MnPN and VLPO. Such neurons were also found diffusely in the POA, but wake-related c-Fos expression is also prominent in this region (31). However, preliminary results show that the numbers of c-Fos/GAD double-labeled neurons is higher in lateral and dorsal POA during recovery sleep following sleep deprivation than during waking.

Our lab (39) and others (15, 35, 46) found prominent projections from the POA to the sites of hypothesized wake-promoting neuronal groups including the histaminergic population, the serotonergic population of the dorsal raphe nucleus, the noradrenergic population of the locus coeruleus (LC) as well as diffusely in the posterior hypothalamus. Recent studies (30) have identified an additional putative wake-promoting neuronal population containing the peptide, orexin (hypocretin), localized in the perifornical lateral hypothalamus (pLH). Loss of the orexinergic neuronal population has been shown to underlie the human disease, narcolepsy (43). Using electrophysiological methods, we found that most neurons in the pLH field were wake-active, with low discharge in NREM sleep (6). Subsets of these wake-active neurons were either "REM-active" or "REM-off". As orexin-containing neurons are intermixed with non-orexinergic neurons, our method does not allow us to know which sleep-wake discharge pattern characterizes orexinergic neurons. However, we have recently shown, using anterograde tracing methods, that the MnPN contributes to the descending projections from the POA and distributes terminals in proximity to pLH orexin-containing-neurons (Fig. 1C) (12).

Since the MnPN contains a high density of GABAergic sleep-active neurons and projects to the pLH field, we can predict that stimulation of MnPN would induce inhibition of pLH wake-active neurons. Preliminary studies confirmed this prediction (Fig. 1D). Short MnPN stimulation trains also induced EEG synchrony and reduction of neck muscle tone (Fig. 1D).

We had previously shown that POA warm-sensitive neurons constituted part of the sleep-active neuronal population. Activation of warm-sensitive neurons by local POA warming is a potent sleep-promoting stimulus (reviewed (22)) and increases delta activity within sustained NREM sleep (23). We recently showed that local POA warming inhibits pLH neurons, even when EEG state is carefully controlled (24) (Fig. 1E). This is the latest in a series showing that POA warming inhibits putative wake-promoting neuronal populations, including PH arousal-related neurons (19), putative serotoninergic DRN neurons (16), and arousal-related neurons in the basal forebrain (3). Therefore, we can conclude that POA-induced inhibition of arousal systems originates, at least in part, in warm-sensitive neurons. Further evidence supporting a coupling of sleep to thermoregulatory processes is reviewed in detail elsewhere (22).

DISCUSSION

Previously, Nitz and Siegel showed that GABA release was increased during sleep in posterior hypothalamus (27), locus coeruleus (28), and dorsal raphe nucleus (26). Our findings suggest that this release may come, at least in part, from POA GABAergic neurons including MnPN neurons. Projections from the MnPN and VLPO to the sites of each of these arousal-related neuronal groups were identified. In this report we have emphasized the projection from the MnPN to the pLH field, showing anatomical and electrophysiological evidence for MnPN-induced inhibitory control over the putative pLH arousal system. We can hypothesize that the strong activation of the MnPN during spontaneous sleep contributes to the suppression of pLH neurons during sleep. There is evidence that inhibitory control of orexin neurons also originates in adjacent basal forebrain sites (33).

A comparison of the discharge across the spontaneous sleep-wake cycle of sleep-active neurons in the MnPN and VLPO with that of wake-promoting neurons, including pLH neurons, shows a reciprocal pattern. As sleep-active neurons become active (41, 42), wake-active neurons become inactive (6, 16, 38). Among wake-active neurons in putative arousal-promoting sites, some are also REM-active, but most neurons of the histaminergic, serotonergic, and noradrenergic populations, and some pLH field neurons are "REM-off". These findings suggest that there are two groups of sleep-promoting neurons, some inducing the "REM-off" pattern, but others permitting activity in REM. This suggestion is congruent with the electrophysiological data from both MnPN and VLPO, showing that some neurons are active in both NREM and REM, but others may be primarily NREM active.

The discharge profiles of MnPN and VLPO neurons within NREM are subtly different. MnPN neurons exhibit increased discharge following sustained waking and exhibit highest discharge during the initial sleep episodes, with declining discharge across sustained sleep. VLPO neurons show increasing discharge during sustained

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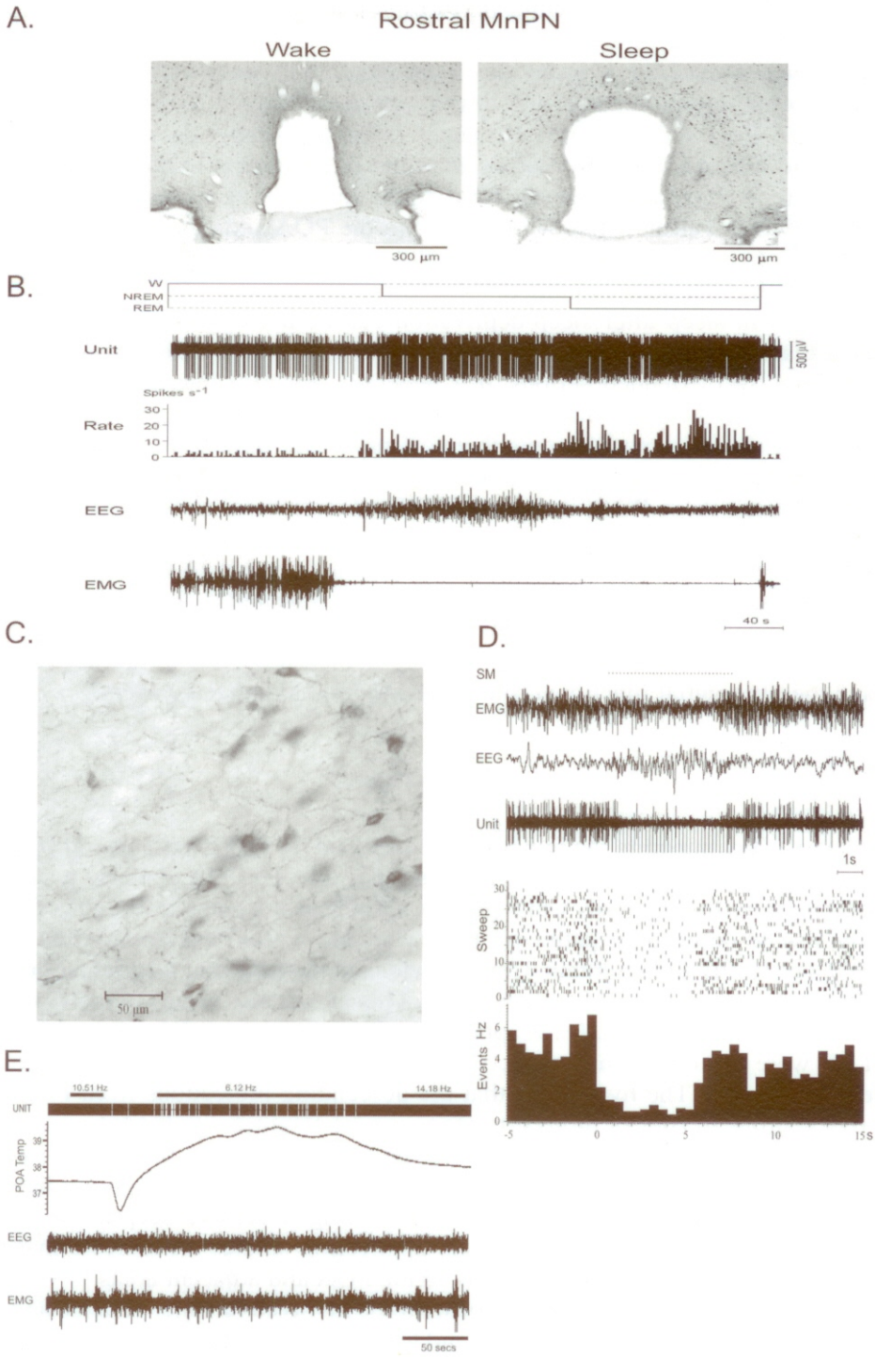
Fig. 1. A. *Example of c-Fos protein immunolabeling in the rostral median preoptic nucleus (MnPN) following 2 hours of waking or high spontaneous sleep.*
At this level c-Fos expression forms a cap over the rostral pole of the 3rd ventricle (from Gong *et al.*, 13). Recent work shows that GABAergic neurons in the MnPN express c-Fos following sleep.
B. *Neuronal unit recording from the MnPN, showing increased discharge during NREM and REM sleep (see top trace) compared to waking (from Suntsova et al., 41).*
C. *Anterograde tracer (biotinylated dextran) labeling among orexin-immunostained neurons in the perifornical lateral hypothalamus after iontophoretic application in MnPN. A strong projection from MnPN to the pLH was seen. (from Gong et al., 12).*
D. *Inhibition of perifornical neuronal activity during electrical stimulation of MnPN at 6 pulses/sec).*
The recorded neuron exhibited a wake-active discharge pattern. Stimulation pulses are indicated at the top (SM). The "sweep" plot shows neuronal discharge during series of trains. The "Events" plot shows average discharge rates. During train stimulation the EEG exhibited increased synchronization and neck muscle tone diminished. (unpublished data).
E. *Inhibition of pLH neuronal discharge in response to local POA warming.*
Discharge of this pLH wake-related neuron was reduced 42% during the warming pulse. In these studies, arousal was carefully maintained during the tests. During sleep, POA warm-sensitive neurons spontaneously increase discharge to the same extent as during 2° warming pulses during waking. (from Methippara *et al.*, 24).


A.
Rostral MnPN
Wake
Sleep
300 µm
300 µm
B.
W
NREM
REM
Unit
500 µV
Spikes s-1
Rate
30
20
10
0
EEG
EMG
40 s
C.
50 µm
D.
SM
EMG
EEG
Unit
1s
Sweep
30
20
10
0
Events Hz
6
4
2
0
-5
0
5
10
15s
E.
10.51 Hz
6.12 Hz
14.18 Hz
UNIT
POA Temp
39
38
37
EEG
EMG
50 secs

sleep episodes. These two systems may differentially facilitate sleep onset and sleep maintenance.

POA warm-sensitive neurons are intermixed with wake-active, cold-sensitive, putative wake-promoting neurons. Warm-sensitive and cold-sensitive neurons within the POA also exhibit reciprocal changes within the sleep-wake cycle (1, 2). Wake-active and putative wake-promoting neurons are prominent in the cholinergic field of the lateral POA and adjacent basal forebrain (5). The reciprocal discharge of wake-active and sleep-active neurons within the POA and adjacent basal forebrain suggests a hypothesis that these populations have inhibitory interactions, but this hypothesis has not been examined.

Changes in sleep after sleep-deprivation can be detected for at least 48 hours. Given that homeostatic processes involve the storage of the sleep-wake history of the animal over extended periods, it is generally assumed that homeostatic signals are encoded in neurochemical processes, possibly including gene expression. A number of sleep-promoting neurochemical processes have been explored in detail (reviewed (29)). Neurochemical modulation of sleep is found after local administration of the agents in POA, including 5HTP, adenosine agonists, prostaglandin D2, interleukin-1 (IL-1) and other cytokines, and growth hormone releasing hormone (GHRH) (reviewed (22, 29)). We have shown that lateral POA wake-active neurons are inhibited by the sleep-promoting agents, adenosine and IL-1 (4, 5). GHRH, 5HT, and NE directly affect POA GABAergic neurons *in vitro* (8, 11). For each of these signals, there is evidence for a role in sleep homeostasis, but we do not yet understand the differential functional roles of the several hypnogenic agents.

SUMMARY

Recent work supports the hypotheses developed by von Economo and Nauta and elaborated by Sallanon *et al.* (see introduction) that the POA contains a sleep-promoting output that opposes wake-promoting neuronal groups in the PH. The POA gives rise to descending pathways that terminate within wake-promoting populations in pLH, PH and midbrain. Current evidence suggests that this output originates in POA sleep-active GABAergic neurons. This output also seems to convey the signals of homeostatic drive. Disynaptic projections from the SCN to both MnPN and VLPO were recently identified (9, 10). These may regulate the circadian control of sleep propensity. The hypothesis that the descending projections from POA sleep-active neurons to sites of arousal-related neurons originates in GABAergic neurons must be confirmed. Also to be further clarified is the anatomical distribution of putative sleep-active GABAergic neurons within the POA. Segregated groups have been found in the MnPN and VLPO, but unit recording studies of sleep-active neurons, lesion studies and local neurochemical application studies all indicate that sleep-active neurons may be found diffusely in the POA and adjacent areas.

The MnPN has been shown previously to be involved in water balance and blood pressure regulation (for example (7)) and to be responsive to hyperthermia (34). Our

studies suggest that this nucleus also contains sleep-active, putative sleep-promoting neurons. However, interactions between sleep control and physiological variables must be considered. In particular, the details of neuronal basis of the coupling of warm-sensitive neurons in MnPN to the POA hypnogenic output has not been explored. It is also worth noting that both the VLPO and MnPN lie close to the ventricular and subarachnoid surface and are punctuated by radial arterioles. The possibility that the sleep-regulatory functions of these sites is coupled to physiological signals conveyed through epithelial cells has been suggested for the actions of PGD2 (18) but has yet to be explored in detail for other putative hypnogens.

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REFERENCES

1. ALAM, M.N., MCGINTY, D. AND SZYMUSIAK, R. Neuronal discharge of preoptic/anterior hypothalamic thermosensitive neurons: relation to NREM sleep. *Am. J. Physiol.,* **269:** R1240-R1249, 1995.
2. ALAM, M.N., MCGINTY, D. AND SZYMUSIAK, R. Thermosensitive Neurons of the diagonal band in rats: relation to wakefulness and non-rapid eye movement sleep. *Brain Res.,* **752:** 81-89, 1997.
3. ALAM, M.N., SZYMUSIAK, R. AND MCGINTY, D. Local preoptic/anterior hypothalamic warming alters spontaneous and evoked neuronal activity in the magno-cellular basal forebrain. *Brain Res.,* **696:** 221-230, 1995.
4. ALAM, N., MCGINTY, D., IMERI, L., OPP, M. AND SZYMUSIAK, R. Effects of interleukin-1 beta on sleep- and wake-active preoptic anterior hypothalamic neurons in unrestrained rats. *Sleep*, **24:** A59, 2001.
5. ALAM, N., SZYMUSIAK, R., GONG, H., KING, J. AND MCGINTY, D. Adenosinergic modulation of rat basal forebrain neurons during sleep and waking: Neuronal recording with microdialysis. *J. Physiol., London,* **521:** 679-690, 1999.
6. ALAM, M., GONG, H., ALAM, T., JAGANATH, R., MCGINTY, D. AND SZYMUSIAK, R. Sleep-waking discharge patterns of neurons recorded in the rat perifornical lateral hypothalamic area. *J. Physiol., London,* **538:** 619-631, 2002.
7. ARADACHI, H., HONDA, K., NEGORO, H. AND KUBOTA, T. Median preoptic neurons projecting to the supraoptic nucleus are sensitive to haemodynamic changes as well as to rise in plasma osmolity in rats. *J. Endocrinol.,* **8:** 35-43, 1996.
8. DE, A., CHURCHILL, L., OBAL, JR. F.S.S.M. AND KRUEGER, J.M. GHRH and IL1 beta increase cytoplasmic Ca2+ levels in cultured hypothalamic GABAergic neurons. *Brain Res.,* **949:** 209-212, 2002.
9. DEURVEILHER, S., BURNS, J. AND SEMBA, K. Indirect projections from the suprachiasmatic nucleus to the ventrolateral preoptic nucleus:a dual tract tracing study in the rat. *Eur. J. Neurosci.,* **16:** 1195-1213, 2002.
10. DEURVEILHER, S. AND SEMBA, K. Indirect projections from the suprachiasmatic nucleus to the median preoptic nucleus in rat. *Brain Res.,* **987:** 100-106, 2003.
11. GALLOPIN, T., FORT, P., EGGERMANN, E., CAULI, B., LUPPI, P., ROSSIER, J., AUDINAT, E., MUHLETHALER, M. AND SERAFIN, M. Identification of sleep-promoting neurons in vitro. *Nature,* **404:** 992-995, 2000.

12. GONG, H., MCGINTY, D. AND SZYMUSIAK, R. Projections from the median preoptic nucleus to hypocretin and forebrain cholinergic systems in rats. *Sleep*, **25:** A155, 2002.
13. GONG, H., SZYMUISAK, R., KING, J., STEININGER, T. AND MCGINTY, D. Sleep-related c-Fos expression in the preoptic hypothalamus:effects of ambient warming. *Am J. Physiol. Regulatory Integrative Comp. Physiol.,* **279:** R2079-R2088, 2000.
14. GONG, H., SZYMUSIAK, R., KING, J., SHIN, S. AND MCGINTY, D. Co-localization of c-Fos protein and GABA in preoptic area neurons following sleep. *Sleep,* **24:** A155-155, 2001.
15. GRITTI, I., MAINVILLE, L. AND JONES, B.E. Projections of GABAergic and cholinergic basal forebrain and GABAergic peroptic-anterior hypothalamic neurons to the posterior lateral hypothalamus of the rat. *J. Comp. Neurol.,* **339:** 251-268, 1994.
16. GUZMAN-MARIN, R., ALAM, N., SZYMUISAK, R., DRUCKER-COLIN, R., GONG, H. AND MCGINTY, D. Discharge modulation of rat dorsal raphe neurons during sleep and waking: Effects of preoptic/basal forebrain warming. *Brain Res.,* **875:** 23-34, 2000.
17. GUZMAN-MARIN, R., SUNTSOVA, N., STEWART, D.R., GONG, H., SZYMUSIAK, R. AND MCGINTY, D. Sleep deprivation reduces proliferation of cells in the dentate gyrus of the hippocampus in rats. *J. Physiol., London,* **549:** 563-571, 2003.
18. HAYAISHI, O. AND URADE, Y. Prostaglandin D2 in sleep-wake regulation: recent progress and perspectives. *Neuroscientist,* **8:** 12-15, 2002.
19. KRILOWICZ, B.L., SZYMUSIAK, R. AND MCGINTY, D. Regulation of posterior lateral hypothalamic arousal related neuronal discharge by preoptic anterior hypothalamic warming. *Brain Res.,* **668:** 30-38, 1994.
20. LU, J., GRECO, M., SHIROMANI, P. AND SAPER, C. Effect of lesions of the ventrolateral preoptic nucleus in NREM and REM sleep. *J. Neurosci.,* **20:** 3830-3842, 2000.
21. LU, J., BJORKUM, A.A., XU, M.G.S.E., SHIROMANI, P.J. AND SAPER, C.B. Selective activation of the extended ventrolateral preoptic nucleus during rapid eye movement sleep. *J. Neurosci.,* **22:** 4568-4576, 2002.
22. MCGINTY, D. AND SZYMUSIAK, R. Brain structures and mechanisms involved in the generation of NREM sleep: focus on the preoptic hypothalamus. *Sleep Med. Rev.,* **5:** 323-342, 2001.
23. MCGINTY, D., SZYMUSIAK, R. AND THOMSON, D. Preoptic/anterior hypothalamic warming increases EEG delta frequency activity within non-rapid eye movmement sleep. *Brain Res.,* **667:** 273-277, 1994.
24. METHIPARRA, M., ALAM, N., SZYMUSIAK, R. AND MCGINTY, D. Preoptic area warming inhibits wake-active neurons in the perifornical lateral hypothalamus. *Brain Res.,* **960:** 165-173, 2003.
25. NAUTA, W.J.H. Hypothalamic regulation of sleep in rats. An experimental study. *J. Neurophysiol.,* **9:** 285-316, 1946.
26. NITZ, D. AND SIEGEL, J. GABA release in the dorsal raphe nucleus: role in the control of REM sleep. *Am. J. Physiol.,* **273:** R451-R455, 1997.
27. NITZ, D. AND SIEGEL, J.M. GABA release in posterior hypothalamus across sleep-wake cycle. *Am. J. Physiol.,* **271:** R1707-R1712, 1996.
28. NITZ, D. AND SIEGEL, J.M. GABA release in the locus coeruleus as a function of sleep/wake state. *Neuroscience,* **78:** 795-801, 1997.
29. OBAL, JR. F. AND KRUEGER, J.M. Biochemical regulation of non-rapid-eye-movement sleep. *Frontiers Biosci.,* **8:** 520-550, 2003.
30. PEYRON, C., TIGHE, D.K., VAN DEN POL, A.N., DE LECEA, L., HELLER, H.C., SUTCLIFFE, J.G. AND KILDUFF, T.S. Neurons containing hypocretin (orexin) project to multiple neuronal systems. *J. Neurosci.,* **18:** 9996-10015, 1998.
31. POMPEIANO, M., CIRELLI, C., ARRIGHI, P. AND TONONI, G. c-Fos expression during wakefulness and sleep. *Neurophysiol. Clin.,* **25:** 329-341, 1995.

32. SALLANON, M., DENOYER, M., KITAHAMA, K., AUBERT, C., GAY, N. AND JOUVET, M. Long-lasting insomnia induced by preoptic neuron lesions and its transient reversal by muscimol injection into the posterior hypothalamus in the cat. *Neuroscience,* **32:** 669-683, 1989.
33. SATOH, S., MATSUMURA, H., NAKAJIMA, T., NAKAHAMA, K., KANBAYASHI, T., NISHINO, S., YONEDA, H. AND SHIGEYOSHI, Y. Inhibition of rostrol basal forebrain neurons promotes wakefulness and induces FOS in orexin neurons. *Eur. J. Neurosci.,* **17:** 1635-1645, 2003.
34. SCAMMEL, T., PRICE, K. AND SAGAR, S. Hyperthermia induces *c-fos* expression in the preoptic area. *Brain Res.,* **618:** 303-307, 1993.
35. SHERIN, J.E., ELMQUIST, J.K., TORREALBA, F. AND SAPER, C.B. Innervation of histaminergic tuberomammillary neurons by GABAergic and galaninergic neurons in the ventrolateral preoptic nucleus of the rat. *J. Neurosci.,* **18:** 4705-4721, 1998.
36. SHERIN, J.E., SHIROMANI, P.J., MCCARLEY, R.W. AND SAPER, C.B. Activation of ventrolateral preoptic neurons during sleep. *Science,* **271:** 216-219, 1996.
37. SHERIN, J.E., SHIROMANI, P.J., MCCARLEY, R.W. AND SAPER, C.B. Activation of ventrolateral preoptic neurons during sleep. *Science,* **271:** 216-219, 1996.
38. STEININGER, T.L., ALAM, MD N, GONG, H., SZYMUSIAK, R. AND MCGINTY, D. Sleep-waking discharge of neurons in the posterior lateral hypothalamus of the albino rat. *Brain Res.,* **840:** 138-147, 1999.
39. STEININGER, T., GONG, H., MCGINTY, D. AND SZYMUSIAK, R. Subregional organization of preoptic area/anterior hypothalamic projctions to arousal-related monoaminergic cell groups. *J. Comp. Neurol.,* **429:** 638-653, 2001.
40. SUNTSOVA, N., SZYMUSIAK, R., ALAM, M., GUZMAN-MARIN, R. AND MCGINTY, D. Sleep-waking discharge patterns of median preoptic nucleus neurons in rats. *J. Physiol., London,* **543:** 665-677, 2002.
41. SZYMUSIAK, R., ALAM, N., STEININGER, T. AND MCGINTY, D. Sleep-waking discharge patterns of ventrolateral preoptic/anterior hypothalamic neurons in rats. *Brain Res.,* **803:** 178-188, 1998.
42. SZYMUSIAK, R. AND MCGINTY, D. Sleep suppression following kainic acid-induced lesions of the basal forebrain. *Exp. Neurol.,* **94:** 598-614, 1986.
43. THANNICKAL, T.C., MOORE, R.Y., NIENHUIS, R., RAMANATHAN, L., GULYANI, S,. ALDRICH, M., CORNFIELD, M. AND SIEGEL, J.M. Reduced numbar of hypocretin neurons in human narcolepsy. *Neuron,* **27:** 469-474, 2000.
44. TOBLER, I. AND BORBELY, AA. Sleep EEG in the rat as a function of prior waking. *Electroencephalogr. Clin. Neurophysiol.,* **64:** 74-76, 1986.
45. VON ECONOMO, C. Sleep as a problem of localization. *J. Nerv. Ment. Dis.,* **71:** 249-259, 1930.
46. ZARDETTO-SMITH, A. AND JOHNSON, A. Chemical topography of efferent projections from the median preoptic nucleus to the pontine monoaminergic cell groups in the rat. *Neurosci. Lett.,* **199:** 215-219, 1995.