

Hypothalamic Regulation of Sleep

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The recent discovery linking narcolepsy, a sleep disorder characterized by very short REM sleep latency, with a neuropeptide that regulates feeding and energy metabolism, provides a way to understand how several behaviors may be disrupted as a result of a defect in this peptide. In this chapter we review the evidence linking hypocretin and sleep, including our own studies, and propose that a defect

in the lateral hypothalamus that also involves the hypocretin neurons is likely to produce a disturbance in sleep, mood, appetite, and rhythms.

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It is a privilege to write this chapter honoring Dr. Gillin who was a mentor to two of us (RSP and PJS). Dr. Gillin has been interested in the neurobiology of sleep and has published landmark studies on the relationship between sleep and mood disorders. His lesson to us was a simple one: Whatever you do make sure that it is hypothesis driven and clinically relevant. In his laboratory we examined the role of the cholinergic system in rapid eye movement (REM) sleep generation and how changes in the cholinergic system could influence mood. We focused on the pontine cholinergic REM sleep circuit because of the evidence that sleep-wakefulness and in particular REM sleep were generated from the brainstem, and we wanted to determine how such a circuit might produce the short-REM sleep latency that is evident in depression. However, in drawing up a hypothesis-driven neuronal circuit model we always found it difficult to link the brainstem sleep circuit with hypothalamic mechanisms that control other behaviors, such as, mood,

appetite, sexual drive, rhythms and endocrine function. We now believe that such a connection between sleep and hypothalamic behaviors can be made as a result of the recent evidence linking the hypothalamic peptide hypocretin with narcolepsy. In this chapter we review the recent evidence identifying hypothalamic sleep and wake neurotransmitter populations and propose that the hypothalamus plays a more important role in regulating states of consciousness than previously believed. By placing sleep regulation in the hypothalamus, it is easier to understand how a defect in this region could also produce a disturbance in other behaviors.

THE PREOPTIC AREA AND SLEEP

Nauta's experiments led him to conclude that there was a sleep center because discrete lesions of the preoptic area (POA) produced insomnia (Nauta 1946). In the succeeding years, a great body of evidence has accumulated to support Nauta's conclusions (for review see Shiromani 1998; Szymusiak 1995) and also has identified the neurotransmitter of the sleep-active neurons (Sherin et al. 1996).

Sleep active neurons are found in the POA and adjacent basal forebrain (BF) in rats, cats and rabbits (Findlay and Hayward 1969; Kaitin 1984; Szymusiak and

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McGinty 1986a, b; Koyama and Hayaishi 1994). These neurons begin to fire during drowsiness and peak activity is seen during non-REM sleep. The sleep active cells comprise about 25% of the recorded cells in the BF-POA and are intermixed with wake-active cells which predominate (Koyama and Hayaishi 1994). We have used c-Fos to identify sleep-active neurons located in the ventral lateral preoptic area (VLPO) (Sherin et al. 1996). The VLPO Fos-ir cells project to the histaminergic cells in the tuberomammillary nucleus (TMN) and to the brainstem monoaminergic groups (Sherin et al. 1998). The VLPO cells contain GABA and galanin and could inhibit the wake-active cells populations to which they project. Electrophysiology studies have shown that VLPO cells are sleep active (Szymusiak et al. 1998), and *in-vitro* slice studies have shown that these cells are inhibited by acetylcholine, serotonin and norepinephrine (wake-active neurotransmitters) (Gallop et al. 2000). Excitotoxic lesions of the VLPO produce insomnia (Lu et al. 2000).

Based on this evidence, sleep could occur as a result of increased activity of preoptic area and VLPO GABA neurons which project to and inhibit the wake-active neurons located in the posterior hypothalamus and brainstem.

POSTERIOR HYPOTHALAMUS, HYPOCRETIN AND WAKEFULNESS

von Economo (1930) believed that the posterior hypothalamus contained a "wake center" because he observed that patients suffering from the viral encephalitic epidemic of 1918 were excessively sleepy and post-mortem analysis revealed damage to the posterior hypothalamus. Since then the role of the posterior hypothalamus in wakefulness has remained largely unexplored except for a few lesion studies which have given inconsistent results. However, in the past year, the identification that narcolepsy is associated with a lesion of hypocretin neurons proves that von Economo was correct in concluding that the posterior hypothalamus contains neurons important for wakefulness.

HYPOCRETIN NEURONS AND RECEPTORS

The peptide hypocretin, also known as orexin, was discovered by two independent groups using different approaches (De Lecea et al. 1998; Peyron et al. 1998; Sakurai et al. 1998). Pre-prohypocretin is cleaved by proteolytic processing into two smaller peptides, hypocretin-1/orexin A (33 amino acids) and hypocretin-2/orexin B (28 amino acids). The distribution of hypocretin-containing neurons has been made in the mouse (Sakurai et al.

1998; Wagner et al. 2000), rat (De Lecea et al. 1998; Peyron et al. 1998; Nambu et al. 1999), cat (Wagner et al. 2000) and humans (Peyron et al. 2000; Thannickal et al. 2000). Neurons containing hypocretin are found only in the lateral hypothalamus (LH) from where they innervate virtually the entire brain and spinal cord (De Lecea et al. 1998; Peyron et al. 1998; Sakurai et al. 1998; Nambu et al. 1999). Because of the location of the hypocretin neurons in a region that has been implicated in feeding, this neuropeptide was initially thought to regulate appetite and energy metabolism (Sakurai et al. 1998). Application of hypocretin stimulates feeding (Dube et al. 1999; Sweet et al. 1999).

Two orexin receptors have been identified and the distribution of the receptor mRNA levels has been determined (Trivedi et al. 1998). Orexin 1 (hypocretin 1) receptor mRNA is more abundant in ventromedial hypothalamic nucleus, hippocampal formation, DR and LC. In the rat, orexin-2 receptor mRNA is mainly expressed in cerebral cortex, nucleus accumbens, subthalamic and paraventricular thalamic nuclei, and posterior pretectal nuclei (Trivedi et al. 1998; Date et al. 1999). The LC receives the heaviest projection of orexin-containing fibers and intraventricular administration of orexin A or hypocretin-2/orexin B excites LC neurons (Hagan et al. 1999; Horvath et al. 1999b). Orexin-containing terminals are also found in areas implicated in wakefulness such as the LC, TMN, the DR, and the basal forebrain (BF) (Peyron et al. 1998). Because of these projections to neuronal populations implicated in wakefulness, it is believed that orexin promotes wakefulness (Peyron et al. 1998).

Our group recently began to map the distribution of the hypocretin receptor protein in the dorsolateral pons, a site associated with REM sleep generation. Immunohistochemistry indicates that one or both of the receptor subtypes are expressed in the DR, the lateral dorsal tegmental nucleus (LDT), LC, the locus subcoeruleus, pontis oralis, Barrington's nucleus, the trigeminal complex (mesencephalic trigeminal and motor nucleus of the trigeminal nerve), the dorsal tegmental nucleus of Gudden (DTG), the ventral cochlear nucleus, trapezoid nucleus, pontine raphe nucleus and the pontine reticular nucleus. Complementary analysis of hypocretin-R mRNA with a cRNA probe shows that hypocretin receptor mRNA is also detected in areas that show protein immunoreactivity, suggesting the mRNA and protein are co-localized (Greco and Shiromani 2001).

Our results indicate that hypocretin-R1 is the predominant subtype expressed in the LC, DRN and the LDT, brain regions associated with REM sleep generation. On the other hand, the hypocretin-R2 is mutated in canine narcolepsy, which makes it unclear how the hypocretin-1 receptor on pontine neurons might regulate REM sleep. The presence of the receptor on a variety of neuronal phenotypes in the pons indicates that

the hypocretin system has a multi-functional role. These functions include mastication, bladder control, gastrointestinal function and arousal. Given these projection sites and the functions associated with these sites, we hypothesize that hypocretin acts to keep the animal alert and vigilant while engaged in feeding behavior.

HYPOCRETINS AND NARCOLEPSY

The hypocretins were linked to narcolepsy as a result of the finding that canines with narcolepsy possess a mutation in the hypocretin-2 receptor (Lin et al. 1999). This was then supported by the finding that mice with deletion of the hypocretin/orexin gene exhibit symptoms of narcolepsy (Chemelli et al. 1999). Recently, it was discovered that narcoleptic patients have a loss of the hypocretin-containing neurons (Peyron et al. 2000; Thannickal et al. 2000) and low CSF concentrations of HCRT-1 (Nishino et al. 2000).

Lesions of the posterior hypothalamus where the hypocretin neurons are located have produced inconsistent effects on sleep. Ranson (1939), Nauta (1946), and Shoham and Teitelbaum (1982) observed behavioral signs of sleepiness following electrolytic lesions of the posterior hypothalamus but these studies did not report the daily amounts of sleep which makes it difficult to conclude whether the behavioral symptoms were isolated or pervasive. Moreover, when Ranson and Nauta did their studies, REM sleep had not yet been discovered. Swett and Hobson (1968) reported finding increased slow wave sleep (SWS) accompanied by behavioral rigidity in some cats, but their electrolytic lesions encompassed the ventral tegmental area. They did not observe narcoleptic-like behavior in the cats. On the other hand, they observed that the cats were cataleptic, not cataplectic. McGinty (1969) recorded sleep EEG from rats with large electrolytic lesions of the posterior hypothalamus that included the lateral hypothalamus and observed hypersomnia in two of seven lesioned rats for 5–8 days after the lesion followed by hyposomnia in all rats; REM sleep was suppressed or decreased. Jurkowlanec et al. (1994) found that electrolytic lesion of the LH resulted in a decrease in SWS and REM sleep during the day cycle but the night cycle sleep was unchanged. They concluded that LH lesions produced hypersomnia. Ibotenic acid lesion, which would spare fibers of passage has also not produced consistent effects. In two studies from the same laboratory (Denoyer et al. 1991; Sallanon et al. 1988), ibotenic acid applied to the posterior hypothalamus produced a hypersomnia for 1–4 days followed by hyposomnia; REM sleep was increased only during the first 3–21 hours. In these lesion studies, no attempts were made to

specifically identify the phenotype of the neurons that were lesioned, and the inconsistent effects on sleep might have occurred because the lesion methods did not destroy the appropriate neurons.

To specifically test the hypothesis that lesions of the LH which result in loss of hypocretin-containing neurons produce symptoms of narcolepsy, we have created a neurotoxin by conjugating the ribosomal inactivating protein, saporin (Stirpe et al. 1992), to the hypocretin/orexin receptor binding ligand, hypocretin-2/orexin-B. The hypocretin-containing neurons have been shown to have an autoreceptor (Horvath et al. 1999a), and we reasoned that the neurotoxin would lesion these neurons. We found that when the neurotoxin was administered to the LH, the hypocretin-immunoreactive neurons were lesioned and the rats had sleep fragmentation, excessive sleepiness, increase in REM sleep, and sleep onset REM sleep periods (SOREMPs), symptoms which are characteristic of narcolepsy in humans, dogs and hypocretin null mice. The hypersomnia and SOREMPs were negatively correlated with the loss of hypocretin neurons. This finding demonstrates that a defect in the LH that also involves the hypocretin neurons produces narcoleptic-like sleep behavior in rats.

Is it the loss of the hypocretin neurons or the loss of the hypocretin innervation at target sites that causes the sleep disturbance in narcolepsy? The hypocretin neurons project to sites involved in wakefulness and REM sleep. The heaviest projections are to the LC (Figure 1) and the TMN. Hypocretin fibers also innervate the DR and BF. The BF, TMN, DR and LC have been implicated in wakefulness based on electrophysiological studies which have found that neurons in these regions are preferentially wake-active and quiescent during non-REM sleep. DR and LC neurons are silent during REM sleep (for review see Shiromani et al. 1987). Single-cell recording studies of the type conducted by sleep researchers are by definition correlational, yet investigators have been quick to infer a cause-and-effect. Moreover, historically, lesions of the DRN, LC or the BF have never been shown to produce hypersomnia, increased REM sleep, SOREMPs or other narcoleptic-like behaviors. Thus, it is not clear how activity of the LC, DRN, BF or the TMN produces wakefulness and inhibits REM sleep.

Alternatively, we suggest that the change in firing of the brainstem cholinergic and aminergic neurons including any imbalance in the aminergic versus cholinergic tone may actually result from a reduction in function of LH neurons, in particular neurons that contain hypocretin. If one supposes that the brainstem cholinergic and aminergic neurons are controlled by hypothalamic hypocretin neurons, then the emphasis shifts to the hypothalamus. This also enables a way to understand how an abnormality in neurons in the same brain region, i.e., hypothalamus, might produce disturbance in sleep, mood, feeding, sexual drive, and biological rhythms.

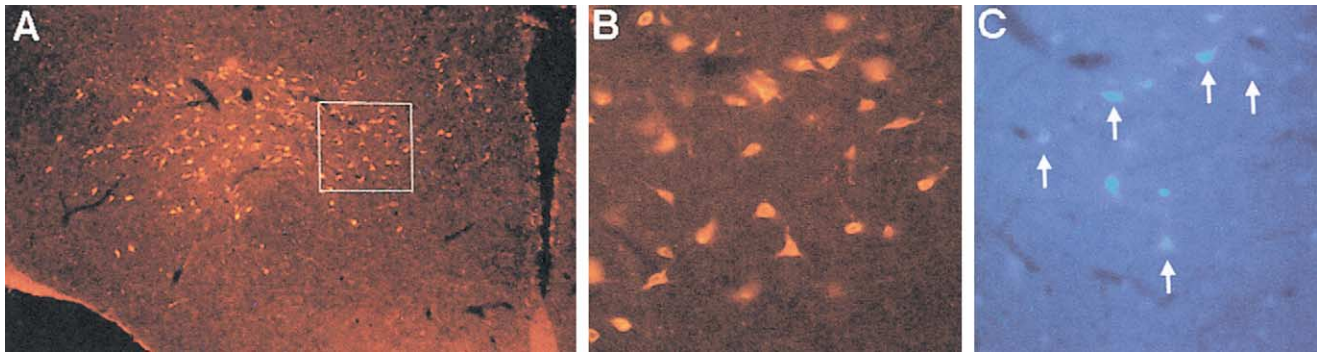


Figure 1. Hypocretin-containing neurons in the lateral hypothalamus project monosynaptically to the locus coeruleus. The retrograde tracer Fast Blue was injected using a glass micropipette into the LC (unilateral injection; 500 nl) and the lateral hypothalamus was examined for somata containing the tracer. **Panel A** depicts a coronal section (30 μ m thick) of the hypocretin-containing neurons in the lateral hypothalamus. The neurons in the region represented by the square are shown in **Panel B** and many of these neurons were found to contain the retrograde tracer Fast Blue (**Panel C**). White arrows in **Panel C** identify Fast Blue-containing cells that are also hypocretin-positive (**Panel B**).

HYPOCRETIN AND DEPRESSION

Patients with major depression have short REM sleep latency, increase in the length of the first REM sleep period, increased REM density, and a decrease in delta sleep (summarized in Shiromani et al. 1987). A disturbance in biological rhythms has been implicated in this disorder (Shiromani et al. 1987). Seasonal affective disorder, or "winter depression" is one example of how changes in external light cues affects mood. Light therapy has proven to be effective in treating this type of depression. REM sleep deprivation, as well as total sleep deprivation, also has an antidepressant effect, although the underlying mechanism is not known. As part of the clinical symptoms, a change in appetite and weight are reported in depressed patients. In winter depression, patients report a craving for carbohydrates and patients gain weight.

Because of the short REM sleep latency and the appetite disturbance we suggest that in depression, including winter depression, there might be a reduction in hypocretin secretion. Examining the levels of hypocretin across the 24h in patients with depression, including winter depression, could test this. The supra-chiasmatic nucleus projects to the posterior hypothalamus, including the LH (Watts et al. 1987; Watts and Swanson 1987). A defect in the lateral hypothalamus, including the hypocretin neurons, could result in an attenuated signal being received from the SCN, and this could produce abnormal rhythms.

Animal models of depression could be used to test the link between the LH, hypocretin and depression. For example, the Wistar-Kyoto (WKY) rats are hypoactive, have reduced exploratory behavior, and increased immobility in the Porsolt swim test (Pare 1994). WKY rats also have increased REM sleep (Dugovic et al.

2000). WKY rats have decreased hypocretin immunoreactivity (measured using RIA) and reduced levels of hypocretin mRNA, indicating a reduction in hypocretin (Taheri et al. 2001). It will be important to determine whether there is a phase-advance in circadian rhythms in these rats. Another model is the Flinders Sensitive Line (FSL) (for review see Overstreet 1986). The FSL rats have increased REM sleep and short REM sleep latency (Shiromani et al. 1988). The FSL rats also have phase-advance and shorter period of the temperature rhythm (Shiromani et al. 1991; Shiromani and Overstreet 1994). The hypocretin system has not been explored in these rats. Another rodent model of depression is the one developed by Vogel (Vogel et al. 1990) where administration of clomipramine in neonatal rats produces depressive-like symptoms in adulthood. In this model also, it will be important to determine whether there is a reduction in hypocretin function. Prenatal stress has been shown to produce symptoms of depression such as reduced latency to the onset of REM sleep, prolongation of the first REM episode, and diminished SWS (Rao et al. 1999). Because of the link between stress and depression, it will be useful to investigate whether there is a reduction in hypocretin levels during stress.

HYPOCRETIN AND NIGHT-EATING SYNDROME

Patients with night-eating syndrome display morning anorexia, evening hyperphagia, and insomnia (Schenk and Mahowald 1994). In a recent study, Birketvedt et al. (1999) surveyed 10 obese subjects with night-eating syndrome and 10 control subjects without NES, matched for age, sex and weight. The number of calories consumed by the two groups per 24-h interval was similar.

However, subjects with night-eating syndrome recorded an average of 9.3 eating episodes per 24-h interval, and this was significantly more than the average 4.2 episodes recorded by controls. Between 8 P.M. and 6 A.M. night-eating syndrome patients consumed 56% of their daily energy intake compared with 15% consumed by control subjects. Given the recent discovery of hypocretin and its relation to appetite and sleep, it will be very important to determine whether night-eating syndrome patients demonstrate a disturbance in leptin or orexin/hypocretin levels.

HYPOCRETIN AND OBSTRUCTIVE SLEEP APNEA (OSA)

Obstructive sleep apnea is a disorder in which, during sleep, the upper airway collapses and airflow is interrupted, which then forces the individual to awaken and resume breathing. As a result of the cessation of breathing during sleep, the individual is not able to maintain long bouts of sleep and fails to enter into the deeper stages of sleep, ie., stages 3 and 4. Obesity is a risk factor in that fatty deposits in the upper airway produce a narrowing of the upper airway, which then leads to cessation of airflow during sleep. The levels of orexin/hypocretin have not been measured in obese individuals. Obese patients have an increase in leptin levels (Halle et al. 1999; Scheen and Luyckx 1999). Although leptin is considered to be a satiety signal to stop eating, it is not clear why the increased leptin levels in obese individuals does not stop food intake. Hypocretin neurons possess leptin receptors (Hakansson et al. 1999; Horvath et al. 1999a), and this might be part of the feedback regulatory process that controls of food intake. One possibility is that the increased sleep fragmentation in these patients produces a sleep loss which increases energy metabolism. As a consequence the individual eats more, but this may only serve to occlude the upper-airway further, leading to more sleep loss. As such a vicious cycle of sleep loss and increased food intake may be occurring in these patients. In rats, sleep deprivation has been shown to increase energy expenditure and increased food intake (Rechtschaffen et al. 1989; Balzano et al. 1990). Measurement of orexin/hypocretin levels before and during CPAP treatment in these patients could provide clues about regulatory role of this neuropeptide in OSA.

CONCLUSION

The discovery of hypocretin, its link with sleep, and the fact that these neurons are located in the part of the brain that also regulates other behaviors, provides a way to understand psychiatric disorders where the ma-

nor symptoms are feeding disturbance, loss of sexual drive, abnormality in endocrine rhythms, hypersomnia and short REM sleep latency.

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