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Homeostatic, Circadian, and Emotional Regulation of Sleep

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ABSTRACT

A good night's sleep is one of life's most satisfying experiences, while sleeplessness is stressful and causes cognitive impairment. Yet the mechanisms that regulate the ability to sleep have only recently been subjected to detailed investigation. New studies show that the control of wake and sleep emerges from the interaction of cell groups that cause arousal with other nuclei that induce sleep such as the ventrolateral preoptic nucleus (VLPO). The VLPO inhibits the ascending arousal regions and is in turn inhibited by them, thus forming a mutually inhibitory system resembling what electrical engineers call a "flip-flop switch." This switch may help produce sharp transitions between discrete behavioral states, but it is not necessarily stable. The orexin neurons in the lateral hypothalamus may help stabilize this system by exciting arousal regions during wakefulness, preventing unwanted transitions between wakefulness and sleep. The importance of this stabilizing role is apparent in narcolepsy, in which an absence of the orexin neurons causes numerous, unintended transitions in and out of sleep and allows fragments of REM sleep to intrude into wakefulness. These influences on the sleep/wake system by homeostatic and circadian drives, as well as emotional inputs, are reviewed. Understanding the pathways that underlie the regulation of sleep and wakefulness may provide important insights into how the cognitive and emotional systems interact with basic homeostatic and circadian drives for sleep. J. Comp. Neurol. 493:92–98, 2005.

Indexing terms: ventrolateral preoptic nucleus; orexin; subparaventricular zone; dorosmedial nucleus; hypocretin

We spend about one-third of our lives asleep, and many animals, such as laboratory rodents, spend about half of their life sleeping (see Chou et al., 2003). A good night's sleep is one of the most satisfying human experiences, while sleeplessness causes stress and cognitive impairment. Yet the mechanisms by which cognitive and emotional stimuli interact with the basic homeostatic and circadian drives for sleep have only recently been the subject of detailed study. In this review, we discuss recent work that has identified a network of connections between the brainstem and hypothalamus that governs sleep and wakefulness. We also explore the role of the orexin neurons in the lateral hypothalamus, whose absence leads to narcolepsy. We then examine the ways in which homeostatic and circadian drives can interact with this sleep control system, and discuss how emotional and cognitive inputs can influence this system, producing extraordinary periods of wakefulness under challenging conditions, and unwanted wakefulness during times of stress.

AROUSAL SYSTEM

Fifty years ago, Moruzzi and Magoun and colleagues demonstrated the role of the upper brainstem in forebrain arousal (Moruzzi and Magoun, 1949; Starzl et al., 1951), but only recently have the specific structures that activate the forebrain been clarified. Although early investigators mainly emphasized the role of the reticular formation, many of the neurons that contribute to these pathways are found in clearly defined cell groups with identified neurotransmitters (Saper et al., 2001). A key component of this ascending arousal system is the cholinergic neurons in the pedunculopontine (PPT) and laterodorsal (LDT) tegmental nuclei in the mesopontine tegmentum. The PPT and LDT provide a major excitatory signal from the upper brainstem to the thalamic relay nuclei and the reticular nucleus (Levey et al., 1987; Rye et al., 1987), thus helping to gate thalamocortical transmission.

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A series of monoaminergic cell groups projects to the intralaminar and midline thalamic nuclei and also innervates the lateral hypothalamus, basal forebrain, and cerebral cortex (see Saper, 1987, for review). These groups include the noradrenergic locus coeruleus (Loughlin et al., 1982; Jones and Yang; 1985; Jones and Cuello, 1989), the serotoninergic dorsal and median raphe nuclei (Sobel and Corbett, 1984; Vertes, 1991; Tillet, 1992), the dopaminergic neurons in the ventral periaqueductal gray matter (Lu J. and Saper C.B., unpubl. obs.), and histaminergic neurons in the tuberomammillary nucleus (Takeda et al., 1984; Lin et al., 1988, 1994). Neurons in each of these cell groups fire faster during wakefulness than during non-REM (NREM) sleep, and most of them stop firing altogether during rapid eye movement (REM) sleep (Aston-Jones and Bloom, 1981; Fornal et al., 1985; Steininger et al., 1999).

The lateral hypothalamus contains at least three populations of neurons that contribute to the regulation of wakefulness. Neurons producing orexin (also called hypocretin) also contain glutamate and project to the cerebral cortex and basal forebrain as well as to the brainstem arousal system (Peyron et al., 1998; Chemelli et al., 1999; Abrahamson et al., 2001; Torrealba et al., 2003). These neurons are active during wakefulness (Estabrooke et al., 2001), and orexin increases the firing rates of neurons in the tuberomammillary nucleus, locus coeruleus, and dorsal raphe (Horvath et al., 1999; Eriksson et al., 2001; Takahashi et al., 2005). The neurons in the lateral hypothalamus that contain melanin-concentrating hormone (MCH) have similar projections, but are most active during REM sleep (Bittencourt et al., 1992; Verret et al., 2003). Many of these cells also contain GABA and probably reduce the activity of monoaminergic neurons (Gao and van den Pol, 2002; Boissard et al., 2003). In addition, cell-specific lesions of the lateral hypothalamus cause severe sleepiness that is not seen with knockouts of both orexin and MCH (Chemelli et al., 1999; Gerashchenko et al., 2003; Willie J.T. and Yanagisawa M., unpubl. obs.). Hence, additional neurons in the lateral hypothalamus probably help promote wakefulness. The neurotransmitters of this third population are unknown, and they are unlikely to project to the cortex, as the MCH and orexin populations account for virtually all of the cortically projecting cells in the lateral hypothalamus (Chou et al., 2004).

The basal forebrain also contains neurons that project directly to the cerebral cortex (Saper, 1984). Many of these are cholinergic neurons that tend to fire most rapidly during wakefulness (Lee et al., 2004). A separate population of basal forebrain neurons produces GABA and projects to GABAergic interneurons in the cerebral cortex; these cells fire most rapidly during wakefulness and REM sleep and are likely to disinhibit cortical activity.

THE VENTROLATERAL PREOPTIC NUCLEUS AND THE FLIP-FLOP SWITCH HYPOTHESIS

As compared to the large number of cell groups that are involved in arousal, relatively few populations of neurons are positioned to turn off the arousal system in a coordinated way, as happens during sleep. In a study of the inputs to the histaminergic tuberomammillary group, for example, large numbers of retrogradely labeled neurons were found only in the lateral hypothalamus (see section on orexin neurons below) and in the ventrolateral preoptic nucleus (VLPO; Sherin et al., 1996). Essentially all of these VLPO neurons contain GABA and most also contain the inhibitory neuropeptide galanin (Sherin et al., 1998; Gaus et al., 2002). The VLPO was found to contain two parts, a dense cell cluster, which projects especially densely to the tuberomammillary nucleus, and a more diffuse component, the extended VLPO, that projects preferentially to the locus coeruleus and the dorsal and median raphe nuclei (Lu et al., 2000, 2002).

Examination of the brains of animals that were asleep prior to death shows increased expression of the immediate early gene *c-fos* in VLPO neurons during sleep (Sherin et al., 1996; Gaus et al., 2002; Lu et al., 2002). Recordings of VLPO neurons across wake–sleep states confirm that they fire about twice as fast during sleep as during wakefulness (Szymusiak and McGinty, 1986; Szymusiak et al., 1998). Interestingly, expression of Fos in the extended VLPO is most closely associated with REM sleep (Lu et al., 2002). Lesions of the extended VLPO cause animals to lose more than half of their REM sleep, whereas lesions of the VLPO cluster mainly reduce NREM sleep (Lu et al., 2000). The loss of sleep correlates well with loss of neurons in the VLPO, and in some animals with especially effective VLPO lesions loss of sleep time approached 80%. Interestingly, the animals with VLPO lesions have much more frequent transitions between sleep and wakefulness, with very short sleep bouts. The neurons in the VLPO were also found to be inhibited by noradrenergic afferents, which mainly originate from the ventrolateral medulla, and serotoninergic inputs from the dorsal raphe nucleus (Gallopin et al., 2001; Chou et al., 2002). The tuberomammillary nucleus also innervates the VLPO, and although the VLPO neurons do not respond to histamine the tuberomammillary neurons also contain GABA, galanin, and endomorphin, all of which may be inhibitory in the VLPO (Vincent et al., 1983; Kohler et al., 1986; Ericson et al., 1991; Martin-Schild et al., 1999; Arrigoni E., Lu J., Saper C.B., unpubl. obs.).

This mutually inhibitory interaction between the VLPO and the components of the arousal system produces a circuit similar to what electrical engineers call a "flip-flop" switch (Saper et al., 2001; see Fig. 1). Such switches tend to produce discrete states with sharp state transitions, as any time a perturbation pushes the switch close to its midpoint (where both sides are about equally active), one side rapidly gains advantage over the other and turns the other off, thus causing a complete transition, instead of gradually moving through intermediate states. Such a switch has obvious advantages for animals, as moving about while not fully alert would pose numerous risks, including becoming an easy target for predators.

However, flip-flop switches also have the property of potentially undergoing unwanted state transitions when the switch is pushed close to its transition point by environmental perturbation (e.g., monotony while driving, or an innocuous sound while sleeping). Modeling studies show that behavioral states become less stable with a loss of neurons on either side of the switch (Chou, 2003). The weakened side is less able to inhibit the other side, and the switch seems to ride closer to its midpoint, where unwanted transitions occur even with small perturbations. Oddly, excess transitions occur in both directions, a prediction that is strikingly confirmed in animals with VLPO lesions, that awaken more often from sleep and also fall

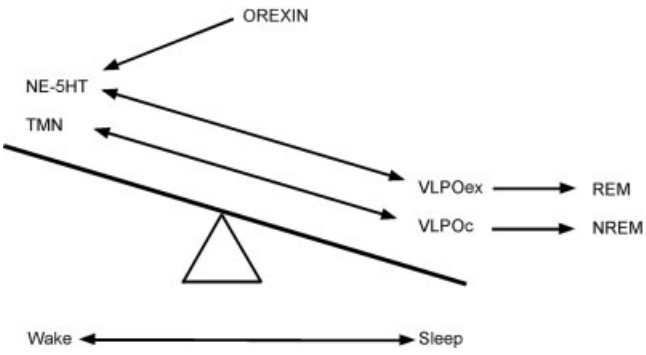

Fig. 1. A diagram to illustrate the relationships of the flip-flop switch between the ventrolateral preoptic nucleus (VLPO) and components of the ascending arousal system. The VLPO cluster (VLPOc) primarily inhibits the histaminergic tuberomammillary nucleus (TMN), whereas the extended VLPO (VLPOex) preferentially inhibits the noradrenergic (NE) locus coeruleus and the serotoninergic (5HT) dorsal and median raphe nuclei. In return, the VLPOex is inhibited by serotoninergic inputs from the dorsal raphe nucleus and noradrenergic inputs from the ventrolateral medulla and the locus coeruleus. The VLPOc is heavily innervated by the TMN, but has no histamine receptors; however, TMN cells also contain galanin, GABA, and endomorphin, which can inhibit the VLPOc neurons. The VLPOc and the TMN are thought to play important but opposing roles in regulating NREM sleep, whereas the VLPOex and the locus coeruleus and dorsal raphe nuclei are thought to be opposing forces in regulating REM sleep. The mutually inhibitory interactions of the VLPO and the monoaminergic neurons produces a state similar to a "flip-flop switch" in an electrical circuit. Such switches have sharp transitions, but may be unstable. The orexin neurons, which reinforce the monoaminergic systems by activating them, act to stabilize the flip-flop switch and prevent unwanted transitions.

asleep more often during wakefulness, perhaps because they are perpetually sleep-deprived (Lu et al., 2000).

ROLE OF THE OREXIN NEURONS

The other major input to the tuberomammillary nucleus comes from neurons in the lateral hypothalamus, particularly those that contain orexin (Sherin et al., 1996; Chemelli et al., 1999). Orexin neurons are mainly active during wakefulness, as indicated by their expression of Fos protein (Estabrooke et al., 2001). The orexin neurons have intense projections to the arousal system, and whereas they have a moderate projection to the VLPO, there do not appear to be orexin receptors in the region (Peyron et al., 1998; Marcus et al., 2001). On the other hand, monoaminergic afferents to the VLPO express orexin receptors, and thus orexin may inhibit the VLPO presynaptically by enhancing these inputs. The VLPO also provides a projection back to the lateral hypothalamus, which may inhibit the orexin neurons (Sherin et al., 1998; Yoshida et al., 2005; Sakurai et al., 2005). Hence, the orexin neurons do not appear to have a strong, mutually inhibitory relationship with the VLPO, but probably express their influence by means of their control of the monoaminergic systems. This relationship allows the orexin neurons to actively reinforce monoaminergic arousal tone, like a finger that holds the switch into the aroused position during wakefulness (see Fig. 1). Observations in animals that lack the gene for orexin, or its type 2 receptor, which have narcolepsy show the importance of this influence on the flip-flop switch (Lin et al., 1999; Chemelli et al., 1999: Willie et al., 2003; Mochizuki et al., 2004). Such animals have many more state transitions, including transitions into REM-like states of muscle atonia directly from wakefulness (cataplexy). Thus, the orexin neurons normally serve to reinforce the activity of the waking side of the flip-flop switch, which stabilizes it to prevent unwanted state transitions both during wakefulness and sleep.

HOMEOSTATIC DRIVE FOR SLEEP

Borbely and colleagues (Borbely and Tobler, 1985; Achermann and Borbely, 2003) have proposed a model for the regulation of sleep that includes a homeostatic process (sleep drive, process S) that accumulates during wakefulness and diminishes during sleep, as well as a circadian drive (process C, which is considered below). The nature of the homeostatic drive for sleep has been the subject of much consideration, and many researchers have hypothesized that adenosine promotes sleep (Radulovacki et al., 1984; Benington and Heller, 1995; Strecker et al., 2000). Sustained neuronal activity can increase adenosine levels in the brain, and adenosine levels rise in the basal forebrain during prolonged wakefulness and fall during sleep (Porkka-Heiskanen et al., 1997; Strecker et al., 2000). This relationship is not apparent in other brain regions, suggesting that adenosine acts as a local somnogen just in the region of the basal forebrain.

As the VLPO sits adjacent to the basal forebrain, the effects of adenosine on VLPO neurons have been examined. Infusion of the adenosine A2a agonist CGS21680 near the VLPO increases sleep and induces Fos in the VLPO neurons, suggesting that they are activated (Scammell et al., 2001). However, VLPO neurons have few A2a receptors, and this response is probably mediated via A2a receptors in the adjacent meninges (Scammell et al., 2001). Recordings from VLPO neurons indicate that adenosine may also act through adenosine A1 receptors to inhibit inhibitory synaptic inputs to the VLPO (Chamberlin et al., 2003). Thus, in an intact animal adenosine may activate VLPO neurons via both A1 and A2a receptor-mediated mechanisms.

In this model, then, the accumulation of adenosine during prolonged wakefulness would increase the activity of the VLPO neurons, pushing them closer to the transition point in their interaction with arousal systems. Similarly, if there were a buildup of adenosine around arousal neurons that had inhibitory A1 receptors (Rainie et al., 1994), the flip-flop switch would be pushed toward its transition point from both directions. At some point the arousal systems would be inhibited by the VLPO and a rapid transition into sleep would occur. Thus, the flip-flop model explains how the slow accumulation of adenosine or similar metabolic products, as in Borbely's model of process S, can ultimately produce a relatively rapid transition into sleep. Interestingly, the tuberomammillary neurons also contain adenosine deaminase (Sherin et al., 1996), which should metabolize adenosine. Thus, it is possible that the tuberomammillary innervation of the VLPO may also help modulate local adenosine levels to which the VLPO neurons are exposed. However, this hypothesis will require critical testing.

CIRCADIAN DRIVE FOR SLEEP

The circadian propensity for sleep, as hypothesized by Borbely, would increase during the sleep state, thus en-

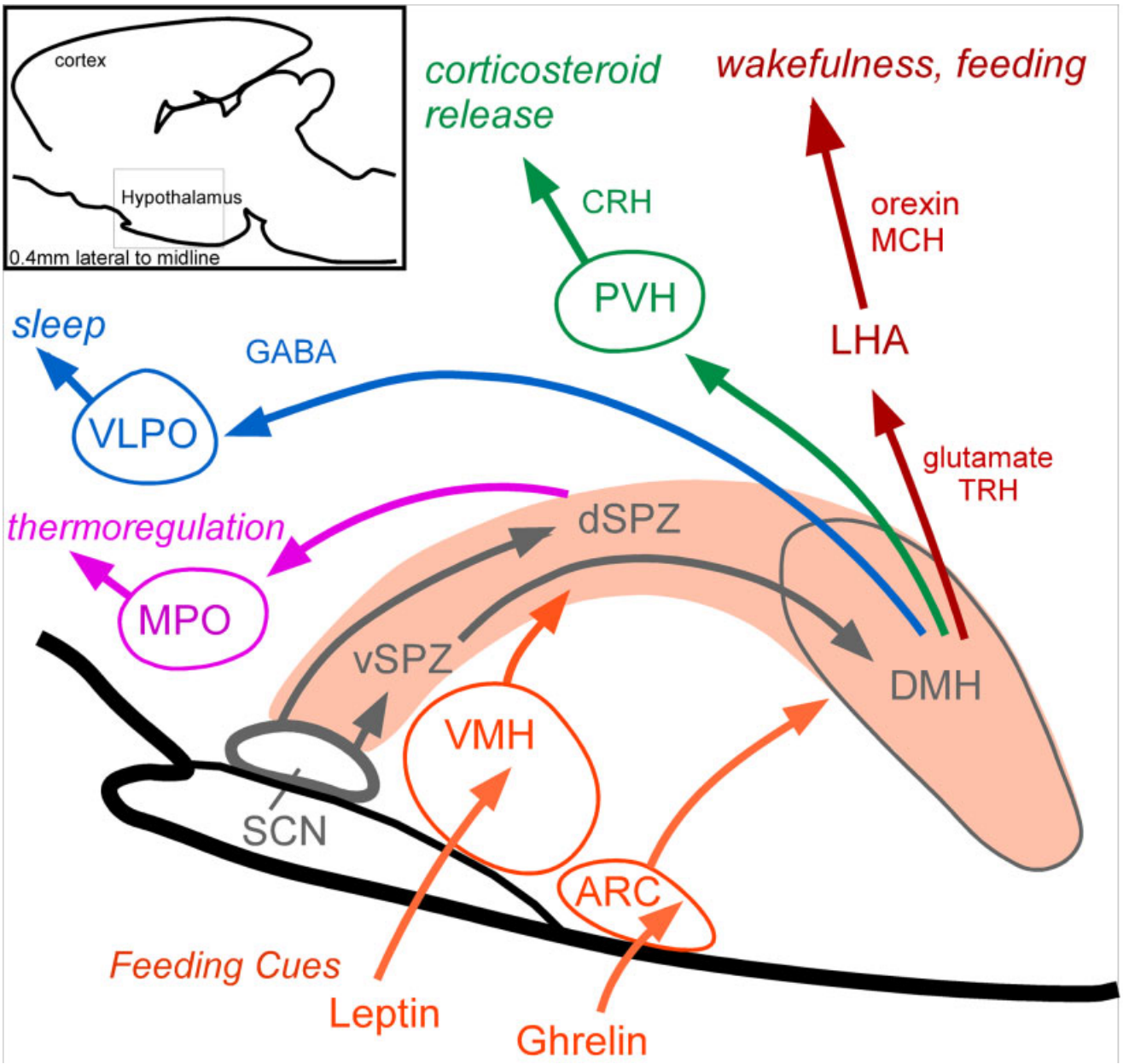

Fig. 2. A summary diagram to illustrate the major pathways that translate the output from the brain's biological clock (the suprachiasmatic nucleus) into circadian rhythms of sleep, feeding, corticosteroid secretion, and body temperature. The suprachiasmatic nucleus sends the bulk of its output into a column (the orange shaded area) that consists of the subparaventricular zone and the dorsomedial nucleus of the hypothalamus. Relays from the dorsal subparaventricular zone are necessary to organize circadian regulation of body temperature, which is controlled by the medial preoptic region. The ventral subparaventricular zone, which is important for regulating circadian rhythms of sleep and wakefulness, as well as locomotory activity, projects to the dorsomedial nucleus. The dorsomedial nucleus is critical for organizing circadian rhythms of sleep and wakefulness, feeding, locomotor activity, and corticosteroid secretion. The purpose of this three-stage integrator for regulating circadian rhythms may be that it allows flexibility in organizing daily schedules. For example, animals that are given food only during the normal sleep cycle soon invert their circadian cycle to be awake when the food is presented. Feeding-related signals such as leptin or ghrelin may enter the hypothalamus and be relayed by the ventromedial nucleus and arcuate nucleus to the subparaventricular zone and dorsomedial nucleus, to influence circadian rhythm organization. ARC, arcuate nucleus; CRH, corticotropin-releasing hormone; DMH, dorsomedial nucleus; dSPZ, dorsal subparaventricular zone; LHA, lateral hypothalamic area; MCH, melanin-concentrating hormone; MPO, medial preoptic nucleus; PVH, paraventricular nucleus; SCN, suprachiasmatic nucleus; TRH, thyrotropin-releasing hormone; VLPO, ventrolateral preoptic nucleus; VMH, ventromedial nucleus; vSPZ, ventral subparaventricular zone.

suring continued sleep despite the diminishing homeostatic need for it toward the end of the sleep cycle (Borbely and Tobler, 1985; Achermann and Borbely, 2003). Conversely, the circadian waking drive builds up during the day and is maximal during the hours just before sleep, when homeostatic sleep drive is nearing its peak. This

relationship has been dramatically demonstrated in humans by Dijk and Czeisler (1995) by placing subjects in a forced desynchrony protocol, where they were tested for wake and sleep propensity while living on a 28-hour "day" schedule.

The circadian system in mammals in the absence of external cues depends on the suprachiasmatic nucleus, whose neurons are driven by a transcriptional-translational loop that enforces a near-24-hour cycle of activity, even in the absence of external cues (see Jin et al., 1999; Reppert and Weaver, 2002, for review). The suprachiasmatic nucleus is entrained to the external light–dark cycle, firing most rapidly during the light period, under the regulation of a special class of light-sensitive retinal ganglion cells that contain the photopigment melanopsin (see Gooley et al., 2003, for review). However, the suprachiasmatic nucleus itself has only minimal direct outputs to the state regulatory system, mainly a sparse output to the VLPO and to the lateral hypothalamus (Watts et al., 1987; Chou et al., 2002; Deurveilher et al., 2002; Yoshida et al., in press).

The major output from the suprachiasmatic nucleus is to the adjacent subparaventricular zone, a region that extends through the medial part of the anterior hypothalamic area from the dorsal border of the suprachiasmatic nucleus, back under the wing of the paraventricular nucleus, and to the rostral end of the dorsomedial nucleus of the hypothalamus (Watts et al., 1987; see Fig. 2). Fibers from the suprachiasmatic nucleus give off terminals all along this course. Cell-specific lesions of the ventral subparaventricular nucleus substantially disrupt the circadian rhythms of sleep and wake, suggesting that neurons in the ventral subparaventricular zone must relay this influence (Lu et al., 2001). These subparaventricular cells also have very few direct projections to the wake–sleep system, but they intensely project to the dorsomedial nucleus (Chou et al., 2003).

Cell-specific lesions of the dorsomedial nucleus, but not adjacent structures, also caused nearly complete loss of the circadian rhythms of sleep and wakefulness, as well as feeding, locomotor activity, and corticosteroid secretion (Chou et al., 2003). Tracing studies show that the neurons in the dorsomedial nucleus are the largest source of (predominantly GABAergic) inputs to the VLPO (Chou et al., 2002, 2003). They also provide massive glutamate- and thyrotropin-releasing hormone-containing projections to the lateral hypothalamus, particularly to the orexin neurons (Chou et al., 2003; Yoshida et al., in press). The influence of the dorsomedial nucleus on other components of the arousal system such as the locus coeruleus (Aston-Jones et al., 2001) is likely to be mediated by these connections, as it has few if any direct inputs to those targets (Thompson et al., 1996). Thus, the dorsomedial nucleus is in a position to consolidate wakefulness at the appropriate circadian phase by exciting the orexin neurons and inhibiting the VLPO neurons.

Normally, these rhythms are mainly driven by the suprachiasmatic nucleus, but under some conditions the daily pattern of activity and rest can be altered, or even reversed. For example, when rats are given access to food only during the light cycle, when they normally are asleep, they invert their daily cycles of feeding, wake–sleep, locomotor activity, and corticosteroid secretion (see Stephan, 2002, for review). Under these conditions the pattern of Fos expression in the dorsomedial nucleus, which is normally greatest during the night when rats are awake, is shifted to match the new activity and sleep cycle (Saper et al., 2005). In fact, the greatest Fos expression is seen just before the time that the food is habitually presented. This pattern ensures that the animal is at its maximum state of arousal when food is expected, and in fact the animals begin to become more active a few hours before the food arrives (Saper et al., 2005). Cell-specific lesions of the dorsomedial nucleus also prevent this shifting of the circadian cycle of activity, body temperature, sleep, and wakefulness (Gooley J.J. and Saper C.B., unpubl. obs.).

EMOTIONAL AND COGNITIVE INPUTS AND INSOMNIA

As the pathways controlling the homeostatic and circadian drives for sleep have been identified, it has become clear that these drives are also shaped by emotional and cognitive inputs. Under some conditions, such as in shift work or during an emergency situation, this ability to overcome the more basic drives can be adaptive. However, when emotional states such as stress prevent sleep that is wanted, the process can be maladaptive. The mechanisms by which cognitive and emotional inputs may affect sleep are suggested by the afferents to the VLPO and orexin neurons, which include the infralimbic cortex, the lateral septum, the bed nucleus of the stria terminalis, the central nucleus of the amygdala, and the ventral subiculum (Chou et al., 2002: Yoshida et al., in press; Sakurai et al., 2005).

These data are beginning to identify the cognitive and emotional systems that may be able to override the normal homeostatic and circadian circuitry by means of inputs to the arousal system. These pathways may suppress the firing of VLPO neurons, thus disinhibiting the orexin and tuberomammillary neurons and thereby helping to overcome homeostatic sleep pressure. Such inputs may be critical to allow arousal during times of emergency or behavioral necessity (e.g., allowing an emergency room doctor to be wide awake when helping a sick patient in the middle of the night). On the other hand, activation of these circuits at inappropriate times may be a mechanism for insomnia (Nofzinger et al., 2004). Studying these pathways in conditions of behavioral arousal may allow us to understand in greater detail how cognitive and emotional states may affect sleep.

CONCLUSIONS

The last decade has seen a remarkable increase in our understanding of the basic circuitry underlying both wake-sleep regulation and the ways that circadian, homeostatic, and emotional/cognitive drives can shape it. More importantly, this work has identified specific neuronal pathways, transmitters, and receptors that are involved in this regulation, and which may serve as sites for pharmacological manipulation of the system. For those who have experienced sleepless nights, or sleepy days, the need to control our own wake–sleep cycles is clear. The ability to regulate wake–sleep cycles may also help ameliorate a variety of psychiatric disorders, from depression to mania to anxiety disorders. Although the reason we need to sleep is still poorly understood, the urgency of this necessity is felt by all, and is an integral part of the fabric

of human needs and desires, and like other aspects of the human "soul" is only partially under our voluntary control. Understanding that control, and how to exercise it more effectively, remains a lofty goal.

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