The sleep switch: hypothalamic control of sleep and wakefulness

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More than 70 years ago, von Economo predicted a wake-promoting area in the posterior hypothalamus and a sleep-promoting region in the preoptic area. Recent studies have dramatically confirmed these predictions. The ventrolateral preoptic nucleus contains GABAergic and galaninergic neurons that are active during sleep and are necessary for normal sleep. The posterior lateral hypothalamus contains orexin/hypocretin neurons that are crucial for maintaining normal wakefulness. A model is proposed in which wake- and sleep-promoting neurons inhibit each other, which results in stable wakefulness and sleep. Disruption of wake- or sleep-promoting pathways results in behavioral state instability.

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During World War I, the world was swept by a pandemic of encephalitis lethargica, a presumed viral infection of the brain that caused a profound and prolonged state of sleepiness in most individuals. The victims could be awakened briefly with sufficient stimulation, but tended to sleep most of the time. A Viennese neurologist, Baron Constantin von Economo, reported that this state of prolonged sleepiness was due to injury to the posterior hypothalamus and rostral midbrain[1]. He also recognized that one group of individuals infected during the same epidemic instead had the opposite problem: a prolonged state of insomnia that occurred with lesions of the preoptic area and basal forebrain. von Economo further hypothesized that lesions of the posterior diencephalon could cause the disease we now call narcolepsy, in which individuals have a tendency to fall asleep at inappropriate times. Based on his observations, von Economo predicted that the region of the hypothalamus near the optic chiasm contains sleep-promoting neurons, whereas the posterior hypothalamus contains neurons that promote wakefulness.

In subsequent years, his observations on the sleep-producing effects of posterior lateral hypothalamic injuries were reproduced by lesions in the brains of monkeys[2], rats[3] and cats[4]; and the insomnia-producing effects of lateral preoptic–basal forebrain injuries were demonstrated in rats[3] and cats[5]. Injections of the GABA-receptor agonist muscimol into these areas in cats produced results similar to that of the lesions, suggesting that wakefulness is promoted by neurons in the posterior lateral hypothalamus and sleep by neurons in the preoptic area[6]. However, the basic neuronal circuitry that causes wakefulness was only clearly defined in the 1980s and early 1990s, and the pathways responsible for the hypothalamic regulation of sleep began to emerge only in the past five years. This article focuses on these hypothalamic switching mechanisms. Other recent publications are available that discuss the homeostatic and circadian control of sleep[7], the contributions of brainstem cholinergic–monoaminergic interactions to rapid eye movement (REM)–non-REM (NREM) sleep oscillations[8–10], and the role of the dopaminergic system in sleep regulation[11]. Our model of the hypothalamic switching circuitry provides an effector mechanism by which many of these other systems produce or prevent sleep.

The cholinergic and monoaminergic substrates of arousal

In the years after World War II, Moruzzi, Magoun and many others contributed to identifying an ascending pathway that regulates the level of forebrain wakefulness[12]. Transection of the brainstem at the midpons or below did not reduce arousal, whereas slightly more rostral transections at a midcollicular level caused an acute loss of wakefulness. The wake-promoting outflow from this crucial slab of tissue at the rostral pontine–caudal midbrain interface was traced by anatomical and physiological techniques through the paramedian midbrain reticular formation to the diencephalon, where it divided into two branches. One pathway innervated the thalamus, and the second extended into the hypothalamus. Although this arousal system was termed the ascending reticular activating system, in fact its origins were identified only recently by the availability of modern neuroanatomical tracer methods combined with immunohistochemistry (Fig. 1).

The main origin of the thalamic projection from the caudal midbrain and rostral pons was identified as the cholinergic pedunculopontine and laterodorsal tegmental nuclei (PPT–LDT)[13–15]. This population of cholinergic neurons projects in a topographic fashion to the thalamus, including the intralaminar nuclei[16–18], but also to the thalamic relay nuclei and the reticular nucleus of the thalamus. The reticular nucleus is thought to play a key role in regulating thalamic activity, and the cholinergic influence is thought to be crucial in activating thalamocortical transmission[19].

The activity of the PPT–LDT neurons varies with different behavioral states. During wakefulness, when the cortical electroencephalogram (EEG) shows low-voltage fast activity, many PPT–LDT neurons fire rapidly (Table 1). As the individual goes to sleep, the EEG waves become slower and larger; during this period, few PPT–LDT neurons are active. Periodically during the night, the individual enters a very

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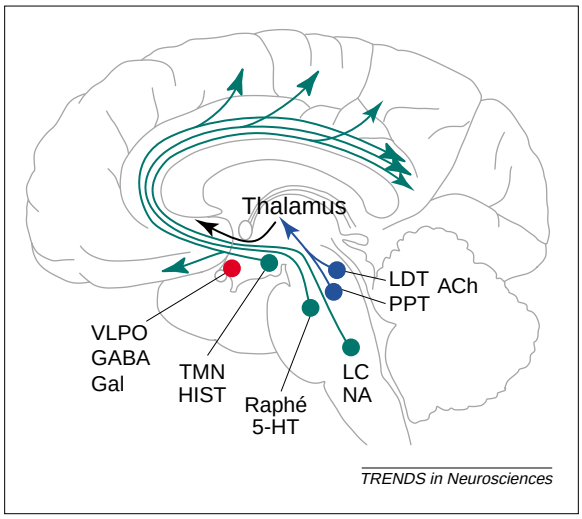

Fig. 1. The ascending arousal system sends projections from the brainstem and posterior hypothalamus throughout the forebrain. Neurons of the laterodorsal tegmental nuclei and pedunculopontine tegmental nuclei (LDT and PPT) (blue circles) send cholinergic fibers (Ach) to many forebrain targets, including the thalamus, which then regulate cortical activity. Aminergic nuclei (green circles) diffusely project throughout much of the forebrain, regulating the activity of cortical and hypothalamic targets directly. Neurons of the tuberomammillary nucleus (TMN) contain histamine (HIST), neurons of the raphé nuclei contain 5-HT and neurons of the locus coeruleus (LC) contain noradrenaline (NA). Sleep-promoting neurons of the ventrolateral preoptic nucleus (VLPO, red circle) contain GABA and galanin (Gal).

different state of active sleep, in which there are rapid eye movements (REM sleep), a loss of muscle tone, except for the muscles involved in respiration, and a low-voltage fast EEG, which resembles a waking state. The PPT–LDT are released from tonic monoamine-mediated inhibition and hence fire rapidly during REM sleep[8–10,20].

If the thalamocortical system is activated in both wakefulness and REM sleep, what is the difference between these two states? One key distinction is the activity in the hypothalamic branch of the ascending arousal system (Fig. 1). Cell groups in the caudal midbrain and rostral pons that contribute to this projection include the noradrenergic locus coeruleus and the serotoninergic dorsal and median raphé nuclei, as well as the parabrachial nucleus[21]. Their axons run through the lateral hypothalamus, where they are joined by histaminergic projections from the tuberomammillary nucleus (TMN). Other neurons in the lateral hypothalamic area, some of which contain the peptide neurotransmitters orexin (also known as hypocretin)[22] or melanin-concentrating hormone[23], join this projection, as do axons from the basal forebrain cholinergic nuclei (Fig. 1). Each of these pathways projects diffusely to the cortex of the entire cerebral hemisphere.

The neurons in the monoaminergic cell groups have been closely studied for their relationship to behavioral state. Neurons in the locus coeruleus, the dorsal raphé nucleus and the TMN all fire at relatively characteristic rates, which are state dependent[24–27]. All three groups fire fastest during wakefulness, slow down with the EEG during NREM sleep, and nearly stop firing during REM sleep. Hence, the differences in the firing of the cholinergic and monoaminergic ascending arousal systems characterize and probably regulate the production of the different behavioral states (Table 1).

Table 1. Sleep stages and physiological activity[a]

	Wakefulness	NREM sleep	REM sleep
EEG	Fast, low voltage	Slow, high voltage	Fast, low voltage
Eye movement	Vision related	Slow, infrequent	Rapid
Muscle tone	↑↑	↑	0
LDT/PPT	↑	0	↑↑
LC/DR/TMN	↑↑	↑	0
VLPO cluster	0	↑↑	↑?
VLPO extended	0	↑?	↑↑
Orexin/hyprocretin	↑↑	0?	0?

[a]Firing rates are as follows: two arrows = rapid firing, one arrow = slower firing, 0 = little or no firing. Question marks represent hypothesized firing patterns for which there is as yet no firm evidence. Abbreviations: DR, dorsal raphé nucleus; EEG, electroencephalogram; LC, locus coeruleus; LDT, laterodorsal tegmental nuclei; NREM, nonrapid eye movement; PPT, pedunculopontine tegmental nuclei; REM, rapid eye movement; TMN, tuberomammillary nucleus; VLPO, ventrolateral preoptic nucleus.

The 'off' switch

Because the firing of monoaminergic neurons is state dependent, understanding the sources of inputs to these cell groups provides a window into the mechanisms that regulate wakefulness. Sherin and colleagues have found two major inputs to the TMN core: (1) a population of diffusely distributed neurons in the lateral hypothalamic area; and (2) a dense cluster of neurons in the ventrolateral preoptic nucleus (VLPO cluster), surrounded medially and dorsally by a more diffuse extension from the nucleus (extended VLPO)[28,29]. Injections of an anterograde tracer have confirmed that the axons from the VLPO intensely innervate the cell bodies and proximal dendrites of the TMN, as well as less intensely innervating the dorsal and median raphé nuclei and the locus coeruleus[29,30] (Fig. 2). The axons from the VLPO also terminate within the cholinergic basal forebrain and PPT–LDT groups, but do not appear to contact the cholinergic cell bodies.

Nearly 80% of the retrogradely labeled VLPO neurons contain both the GABA-synthesizing enzyme glutamic acid decarboxylase and the peptide galanin[29]. Electron microscopy confirmed that the VLPO terminals onto TMN neurons were immunoreactive for GABA and make symmetric synapses[29]. Because galanin and GABA are known to inhibit both TMN and neurons of the locus coeruleus[24,31–33], the descending projection from the VLPO is likely to be inhibitory in nature[29,34,35].

To determine the relationship between VLPO activity and sleep–wake behavior, the expression of Fos protein immunoreactivity was examined, as a marker of neuronal activity in the VLPO across the wake–sleep cycle[28]. The number of Fos-immunoreactive neurons in

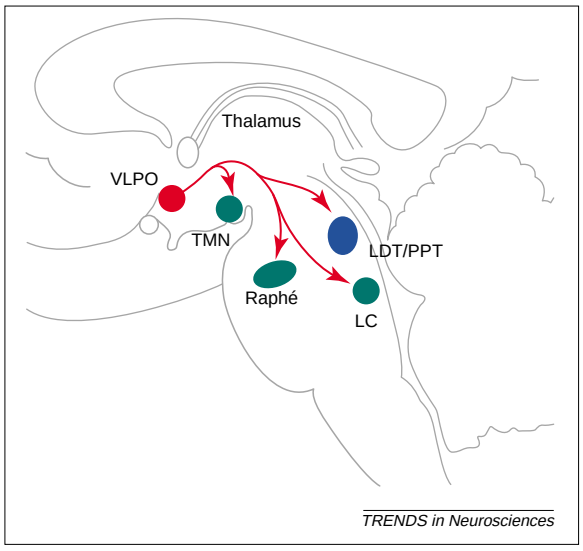

Fig. 2. The projections from the ventrolateral preoptic nucleus (VLPO) to the main components of the ascending arousal system. Axons from the VLPO directly innervate the cell bodies and proximal dendrites of neurons in the major monoamine arousal groups. Within the major cholinergic groups, axons from the VLPO mainly innervate interneurons, rather than the principal cholinergic cells. Abbreviations: LC, locus coeruleus; LDT, laterodorsal tegmental nuclei; PPT, pedunculopontine tegmental nuclei; TMN, tuberomammillary nucleus; VLPO, ventrolateral preoptic nucleus. The blue circle indicates neurons of the LDT and PPT; green circles indicate aminergic nuclei; and the red circle indicates the VLPO.

the VLPO correlated closely with the amount of sleep the animals experienced during the hour before death. Other animals were sleep deprived for 9 or 12 hours, to dissociate Fos expression from the circadian cycle. These animals showed the same correlation of Fos expression in the VLPO and sleep. However, the animals that failed to fall asleep following deprivation showed little or no Fos expression in the VLPO. Similar results have since been obtained in mice, cats, degus and Nile river rats (S. Gans *et al.*, unpublished)[36,37]. Electrophysiological recordings have similarly identified sleep-active neurons in the VLPO region[38,39]. The rate of firing of VLPO neurons was nearly doubled during sleep compared with waking, and it doubled again during the deep sleep that followed sleep deprivation. The firing rate of VLPO neurons was not increased after sleep deprivation until the animals actually slept, so VLPO firing rates probably are not related to the degree of sleepiness, but instead the production of sleep itself.

The chemical identity of the sleep-active VLPO neurons has recently been determined by combining *in situ* hybridization for galanin with immunocytochemistry for Fos (S. Gaus *et al.* and J. Lu *et al.*, unpublished). In sleeping rats, 80% of the Fos-immunoreactive neurons in the VLPO cluster (and 50% in the extended VLPO) also contained galanin mRNA, and about half of the galanin mRNA-containing neurons in both parts of the nucleus had a Fos-immunoreactive nucleus. Galanin-positive neurons of the VLPO were also sleep active in mice and cats (S. Gaus *et al.*, unpublished)[40]. A galanin-containing cell group in the VLPO has also been identified in monkeys and humans (S. Gaus *et al.*, unpublished), so this system appears to be a uniform feature of mammalian brains.

Is the VLPO necessary for sleep?

To determine whether the VLPO neurons are necessary for producing sleep, Lu and colleagues produced small excitotoxic lesions in the lateral preoptic area by microinjecting ibotenic acid[41]. Although previous studies have demonstrated insomnia after injury to this region, these lesions injured fiber pathways[3,5] or involved much of the preoptic area beyond the VLPO (Refs 42,43). In order to analyze the lesions, the numbers of remaining Fos-immunoreactive cell bodies in the VLPO cluster and the extended VLPO were compared with the changes in sleep behavior.

In animals with more than 70% bilateral cell loss in the VLPO cluster, the amounts of both NREM and REM sleep were reduced by about 55% (Ref. 41). The loss of neurons in the VLPO cluster correlated closely with the loss of NREM ($r=0.77$), but did not correlate significantly with loss of REM sleep. However, the loss of Fos-immunoreactive neurons in the extended VLPO correlated closely with the loss of REM sleep ($r=0.74$), but did not show a significant correlation with the loss of NREM sleep. Conversely, when rats were exposed to a period of darkness during the day, a condition that doubles REM sleep time, there was a concomitant increase in Fos expression in the extended VLPO, but not the VLPO cluster (J. Lu *et al.*, unpublished). Anatomical studies have shown that the projections to the locus coeruleus, dorsal–median raphé, and the PPT–LDT arise predominantly from the extended VLPO, rather than the VLPO cluster (J. Lu *et al.*, unpublished)[30,44]. These observations suggest that the VLPO contains specific subregions that are specialized for the control of REM versus NREM sleep.

The flip–flop and bistability

The relationship between the VLPO and the major monoamine groups appears to be reciprocal. The VLPO is innervated by histaminergic axons from the TMN, noradrenergic terminals from the locus coeruleus and serotoninergic inputs from the midbrain raphé nuclei[45]. Recordings from individual VLPO neurons in hypothalamic slices show that they are inhibited by noradrenaline and by 5-HT (Ref. 46). No responses to histamine were recorded, but TMN neurons also contain GABA and galanin, which might inhibit the VLPO (Ref. 47).

The model shown in Fig. 3 is based on the hypothesized mutual inhibition between the VLPO and the major arousal systems. Although the monoamine systems are emphasized, there might be other components of the arousal system that are not illustrated here, such as neurons in the lateral hypothalamic area, that would interact with the VLPO in a similar way. When VLPO neurons fire rapidly during sleep, they would inhibit the monoaminergic cell groups, thus disinhibiting and reinforcing their own firing. Similarly, when monoamine neurons fire at a high rate during wakefulness, they would inhibit the VLPO, thereby disinhibiting their own firing. This reciprocal relationship is similar to a type of circuit that electrical engineers call a 'flip–flop'[48]. The two halves of a flip–flop circuit, by each strongly inhibiting the other, create a feedback loop that is bistable, with

two possible stable patterns of firing and a tendency to avoid intermediate states. Such properties would be very useful in sleep–wake regulation, as an animal that walked about while half asleep would be in considerable danger. The net effect of this bistable switch is that during the course of the day, animals spend nearly all of their time in either a clearly waking or sleeping state, with relatively brief times spent in transitions.

The self-reinforcing firing patterns of the flip–flop switch produce a degree of resistance to switching when one side is firing briskly. This stability avoids inappropriate changes in wake–sleep state when input signals to the VLPO and the monoaminergic cell groups fluctuate transiently over the course of the day. However, large scale influences, such as circadian sleep drive or accumulated homeostatic need for sleep might gradually shift the relative balance of mutual inhibition. When this pressure to change becomes great enough, the same feedback properties that allow the flip–flop circuit to resist change will suddenly give way and rapidly produce a reversal of firing patterns. The flip–flop switch therefore changes behavioral state infrequently but rapidly, in contrast to the homeostatic and circadian inputs, which change continuously and slowly.

A crucial aspect of this bistable switch is that if the firing of neurons on either side is substantially weakened, the switch is less stable. For example, after lesions of the VLPO, the animals experience much more wakefulness, and the homeostatic drive for sleep might increase, forcing the balance in the circuit nearer to its transition point[41]. Thus, rats with VLPO lesions fall asleep more frequently, but because the self-reinforcing properties of the circuit are weaker, they switch back into wakefulness more frequently as well, with the result that both wake and sleep bouts are shorter after VLPO lesions.

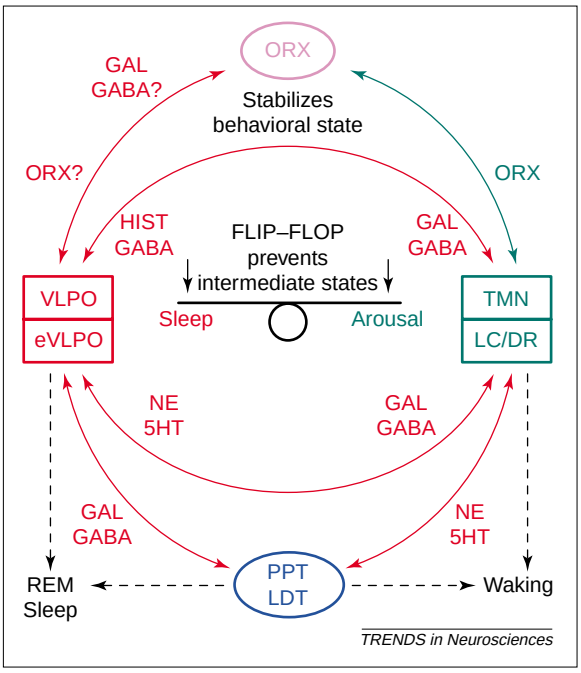

Fig. 3. A model for reciprocal interactions between sleep- and wake-promoting brain regions, which produces a flip–flop switch. Inhibitory pathways are shown in red, and the excitatory pathways in green. The blue circle indicates neurons of the LDT and PPT; green boxes indicate aminergic nuclei; and the red box indicates the VLPO. Aminergic regions such as the TMN, LC and DR promote wakefulness by direct excitatory effects on the cortex and by inhibition of sleep-promoting neurons of the VLPO. During sleep, the VLPO inhibits amine-mediated arousal regions through GABAergic and galaninergic (GAL) projections. Most innervation of the TMN originates in the VLPO core, and input to the LC and DR predominantly comes from the extended VLPO. This inhibition of the amine-mediated arousal system disinhibits VLPO neurons, further stabilizing the production of sleep. The PPT and LDT also contain REM-promoting cholinergic neurons. The extended VLPO (eVLPO) might promote REM sleep by disinhibiting the PPT–LDT; its axons innervate interneurons within the PPT–LDT, as well as aminergic neurons that normally inhibit REM-promoting cells in the PPT–LDT. Orexin/hypocretin neurons (ORX) in the lateral hypothalamic area (LHA) might further stabilize behavioral state by increasing the activity of aminergic neurons, thus maintaining consistent inhibition of sleep-promoting neurons in the VLPO and REM-promoting neurons in the PPT–LDT. Unbroken lines represent neuronal pathways described in the text. Broken black lines indicate influences of specific regions on behavioral states. Abbreviations: DR, dorsal raphé nucleus; HIST, histamine; LC, locus coeruleus; LDT, laterodorsal tegmental nuclei; PPT, pedunculopontine tegmental nuclei; REM, rapid eye movement; TMN, tuberomammillary nucleus; VLPO, ventrolateral preoptic nucleus.

Stabilizing the flip–flop

A similar deficit on the waking side of the mutually inhibitory flip–flop circuit might produce abrupt and unstable fluctuations in behavioral state in the disorder known as narcolepsy. Individuals with narcolepsy experience frequent and unwanted transitions into sleep during wakefulness, and they tend to awaken more frequently from sleep as well. When placed in a quiet environment, they fall asleep and transition into REM sleep far more rapidly than unaffected individuals. At times, they experience fragments of REM sleep intermixed with wakefulness, such as loss of muscle tone while awake, a condition known as cataplexy.

The origin of narcolepsy was not understood until a dramatic series of events that unfolded during the past three years. In 1998, two groups of investigators simultaneously discovered a family of peptide neurotransmitters that was made by neurons in the lateral hypothalamus. Sakurai and co-workers identified two peptides in a screen for ligands for orphan G-protein-coupled receptors, which they named 'orexin A and B', because the peptides appeared to promote feeding[49]. de Lecea *et al.*, meanwhile, described two hypothalamic-specific mRNAs coding for the same peptides, which they termed 'hypocretins' because they were hypothalamic peptides with sequence similarity to secretin[50].

However, when the full extent of the pathways containing the orexin/hypocretin peptides was revealed by immunocytochemistry[51–53], it became clear that the orexin/hypocretin neurons, like the VLPO, innervated all of the components of the ascending arousal system (Fig. 4). Orexin 1 receptors were found in the locus coeruleus, orexin 2 receptors in the TMN and basal forebrain, and both types of

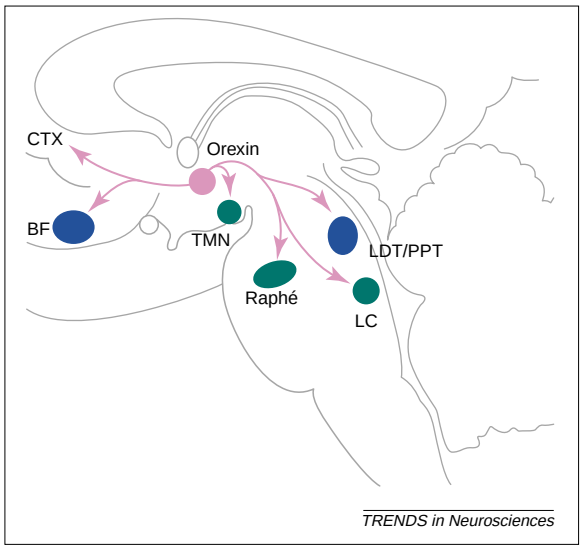

Fig. 4. Orexin neurons in the lateral hypothalamic area innervate all of the components of the ascending arousal system, as well as the cerebral cortex (CTX) itself. Abbreviations: BF, basal forebrain cholinergic nuclei; LC, locus coeruleus; LDT, laterodorsal tegmental nuclei; PPT, pedunculopontine tegmental nuclei; TMN, tuberomammillary nucleus. Blue circles indicate cholinergic neurons of the BF, LDT and PPT; green circles indicate monoaminergic nuclei.

receptors were found in the midbrain raphé nuclei and mesopontine reticular formation[54,55]. Because both receptors are mainly excitatory, these observations suggested that orexin/hypocretin might help maintain wakefulness by increasing the activity of the ascending arousal system.

In 1999, Chemelli *et al.* produced orexin/hypocretin knockout mice[53]. These animals suffered intermittent attacks during the active (dark) period, in which they would suddenly fall onto their sides for a few minutes, then get up and resume their activities. Polysomnographic analysis showed that these periods of behavioral arrest consisted of episodes of atonia associated with an EEG that was consistent either with wakefulness (i.e. cataplexy) or REM sleep, findings that are suggestive of narcolepsy. Simultaneously, Nishino *et al.* found that canine narcolepsy was due to mutations in the gene for the type 2 orexin/hypocretin receptor[56]. The combination of these two findings provide overwhelming evidence that the loss of orexin/hypocretin signaling via the type 2 receptor is sufficient to produce the symptoms of narcolepsy. The absence of orexin in the hypothalamus and in the spinal fluid of humans with narcolepsy has subsequently been confirmed[57–59].

The orexin/hypocretin neurons probably play an important role in producing normal wakefulness. Kilduff and Peyron have hypothesized that these neurons might be active during wakefulness and REM sleep[60], but we predict that these cells are predominantly wake active. Orexin neurons synthesize Fos protein during wakefulness, and the number of Fos-positive orexin-containing neurons correlates closely with the amount of wakefulness, whether it is naturally occurring, produced by sleep deprivation or caused by stimulant drugs, such as amphetamine or modafinil[53,61]. Extracellular recordings from neurons in the perifornical region, which contains the orexin/hypocretin cell bodies, confirm that cells in this area are predominantly wake active although some also fire during REM sleep (R. Szymusiak, unpublished). However, neither orexin-deficient animals nor narcoleptic humans have excessive amounts of sleep, but instead they have poor maintenance of both wakefulness and sleep, or dysfunctional switching.

What, then, can be the role of the orexin/hypocretin neurons in maintaining behavioral state? Recent studies have shown that the orexin/hypocretin neurons might influence both sides of the flip–flop circuit by direct projections to both the monoaminergic and cholinergic arousal cell groups, and to the VLPO region. Orexin/hypocretin increases the firing of neurons in the locus coeruleus[62], the dorsal raphé nucleus[63] and the TMN (H. Hass, unpublished). Although VLPO neurons do not appear to contain orexin/hypocretin receptors[53], injection of orexin/hypocretin into the preoptic area near the VLPO increases wakefulness and decreases both REM and NREM sleep[64], suggesting a presynaptic mechanism of action (perhaps on monoaminergic axons). Orexin/hypocretin neurons therefore might act as a 'finger', pressing the flip–flop switch into the 'wakeful' position, and preventing inappropriate switching into the 'sleep' position. In the absence of such an influence, as seen in narcolepsy, the switch would be less stable, and more susceptible to sudden and inappropriate transitions.

This model could also explain the rapid transitions into REM sleep, or fragments of REM sleep, that are seen in narcoleptics. The TMN, raphé nuclei and locus coeruleus contain orexin/hypocretin receptors[54], and all three groups inhibit REM sleep[10]. In the absence of an excitatory orexin input, the weakened arousal influence and increased activity of the extended VLPO would allow earlier and more frequent transitions to the REM state. Interestingly, like the animals with VLPO lesions, destabilizing the switch in narcolepsy also results in more frequent awakenings from sleep.

Concluding remarks

Advances over the past five years have largely borne out the remarkable predictions of von Economo, which were made over 70 years ago on the basis of clinical observations. The occurrence of insomnia in individuals with lesions of the preoptic area and basal forebrain was almost certainly due to the involvement of the VLPO in these cases. The hypersomnolent individuals clearly had lesions of the ascending arousal pathways at the midbrain–diencephalic junction. And von Economo's prediction that narcolepsy could be caused by lesions of the posterior diencephalon has been proven true by the recognition that this region contains the orexin/hypocretin neurons, the loss of which causes narcolepsy in humans. The recent progress in defining the components of the sleep switching system should allow us to understand better how slowly changing influences, such as homeostatic and circadian drives, can produce rapid and discrete changes in behavioral state.

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