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Control of arousal by the orexin neurons

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Abstract

The orexin-producing neurons in the lateral hypothalamus play an essential role in promoting arousal and maintaining wakefulness. These neurons receive a broad variety of signals related to environmental, physiological and emotional stimuli; they project to almost every brain region involved in the regulation of wakefulness; and they fire most strongly during active wakefulness, high motor activation, and sustained attention. This review focuses on the specific neuronal pathways through which the orexin neurons promote wakefulness and maintain high level of arousal, and how recent studies using optogenetic and pharmacogenetic methods have demonstrated that the locus coeruleus, the tuberomammillary nucleus, and the basal forebrain are some of the key sites mediating the arousing actions of orexins.

Introduction

Survival depends on an animal's ability to stay awake and dynamically adjust its arousal level to meet environmental and physiological demands. Wakefulness is characterized behaviorally by consciousness, voluntary motor activation, and high responsiveness to environmental stimuli, and electrophysiologically by general activation of the cerebral cortex and increased neuronal excitability. Though often referred to as a single state, wakefulness encompasses many interdependent neuropsychological components, including arousal, awareness, attention, memory, motivation and emotions [1].

Arousal is the overall level of responsiveness of an animal, often measured by the degree of stimulation necessary to trigger a specific response [2]. High levels of arousal, for example caused by stimulating an animal with novel sensory stimuli, promote wakefulness [3]. In contrast, low arousal, such as the drowsiness caused by sleep deprivation or sedative medications, promotes transition to sleep and impairs performance and learning [4,5]. Maintenance of an adequate level of arousal is thus critical to trigger or sustain appropriate behavioral responses to the current environmental and internal conditions.

A variety of neurons promote arousal, and among these, the orexin-producing neurons are essential to promote stable periods of wakefulness and to sustain the alertness required for the expression of motivated behaviors. This review focuses on the neuronal pathways through which the orexin neurons promote wakefulness and help drive high levels of arousal.

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Brief overview of the orexin system

The orexin neuropeptides were discovered simultaneously by two independent research groups [6,7], one of which named them orexins and the other hypocretins; the terms are used interchangeably in the literature. Orexin-A and orexin-B (hypocretin-1 and hypocretin-2) are synthesized by a cluster of neurons in the lateral hypothalamus and produce excitatory effects on target neurons. The orexin-producing neurons also synthesize glutamate and the inhibitory neuropeptide dynorphin, though the physiologic importance of these cotransmitters remains to be defined [8,9,10••]. The orexin neurons receive a broad variety of signals related to environmental, physiological and emotional stimuli [11], and they innervate much of the brain and spinal cord [12]. Highlighting their key role in regulating arousal, the orexin neurons are reciprocally connected with all brain regions known to promote wakefulness and arousal [13], including the cerebral cortex, basal forebrain (BF), tuberomammillary nucleus (TMN), locus coeruleus (LC), and dorsal raphe (DR) [11,12] (Figure 1). The orexin neurons also innervate brain nuclei that regulate motivation and emotions [14,15], such as the ventral tegmental area (VTA), nucleus accumbens, prefrontal cortex, and amygdala [12]. In addition, the orexin neurons project to many brain regions that regulate motor and autonomic functions [16]. Thus, the orexin system is anatomically well positioned to coordinate many aspects of arousal.

The orexin neuropeptides selectively excite and depolarize target neurons via two distinct G protein-coupled receptors, OX1R and OX2R [7]. OX1R binds orexin-A with higher affinity (~100-fold) than orexin-B, whereas OX2R shows an almost equal affinity for both orexin-A and orexin-B [7,17]. OX1R couples to G_q and OX2R can signal through G_q or G_i/G_o, but coupling mechanisms seem to differ by cell type and have not been thoroughly examined in neurons [17]. The two receptors have partly overlapping but distinct expression patterns in the brain [18]. For example, the LC expresses only OX1R, the TMN produces only OX2R, and many other arousal-promoting brain regions express both receptors (e.g. BF, VTA, DR, and cortex).

The orexin neurons have physiologic properties that may promote sustained activity [for review, see [19]]. The orexin neurons are intrinsically in a depolarized state near their firing threshold, which likely promotes increased spontaneous activity [20]. They also excite other orexin neurons indirectly via local glutamate interneurons and perhaps directly via OX2R on orexin neurons [21,22]. In addition to helping sustain orexin neuron activity, this positive feedback mechanism may also promote recruitment of a larger number of orexin neurons. Furthermore, neighboring astrocytes may influence excitatory inputs to the orexin neurons [23•]. Altogether, these physiologic mechanisms may promote sustained activity in networks of orexin neurons which could then drive persistent activation of a variety of arousal-promoting brain regions.

Orexin neurons and the maintenance of arousal

Orexins are clearly important as they promote arousal and orexin deficiency causes narcolepsy. Central administration of orexin-A produces long wake bouts and increases locomotor activity, while reducing both rapideye movement (REM) and non-REM (NREM) sleep [24,25]). Conversely, a mutation in the OX2R gene in dogs or disruption of the prepro-orexin gene in mice causes sleepiness and cataplexy almost identical to that seen in people with narcolepsy [26–28]. In addition, nearly all people with narcolepsy have little or no detectable orexin-A in their cerebrospinal fluid due to extensive loss of the orexin neurons [29]. This topic is reviewed in detail by Dr. Sakurai in this issue.

Mouse models of narcolepsy have provided some of the most helpful information on the mechanisms by which orexin deficiency disrupts arousal. Mutant mice lacking the orexin

peptides, orexin neurons, or orexin receptors are unable to sustain long periods of wakefulness and have frequent transitions between sleep/wake states (Figure 2a) [27,28,30–32]. Survival analysis of the durations of wakefulness bouts (Figure 2b) reveals that mice lacking orexins wake normally from sleep but have difficulty producing long-lasting bouts of wakefulness [33] and transition frequently between wakefulness and sleep [34]. Perhaps, in the absence of sustained excitatory signals from the orexin neurons, other arousal-promoting neurons have uncoordinated or inappropriate patterns of activity, resulting in low thresholds for crossing between states and poorly sustained wakefulness. A lack of orexin signaling may also upset the mutually inhibitory balance between sleep-promoting and wake-promoting neurons [35,36•].

Unstable activity in arousal-promoting brain regions may also explain the attentional deficits observed in human narcolepsy [37]. People with narcolepsy have generally normal cognition and show normal phasic arousal during an alertness task that requires their attention for a short period of time [37]. However, they have worsening and increasingly variable performance during tasks lasting more than 10 min, indicating poor maintenance of vigilance. In addition to difficulty sustaining attention over time, patients with narcolepsy also have deficits in divided attention (attention capacity) and flexible attention (attention control). These deficits could be caused by dwindling vigilance due to sleepiness [38], but others have hypothesized they are caused by inappropriate processing of relevant stimuli [39]. Such deficits of selective processing could be a consequence of reduced basal forebrain cholinergic signaling to the cortex [40]. The role of orexins in the BF regulation of attention is discussed later in this review.

Similar to people with narcolepsy, the performance of orexin null mice deteriorates after 10 min of engagement in tasks motivated by food or water reinforcement. Such poor performance results from multiple pauses resembling drowsiness during operant tasks [41•]. Surprisingly, this performance deficit is apparent only during the light period, and more work is needed to explain why performance seems normal during the dark period when one would predict a greater impact of orexin deficiency.

The activity of orexin neurons follows a pattern consistent with promoting arousal, with maximal discharge during active wakefulness [42–44], especially during exploration and foraging. Conditions requiring increased alertness, such as during detection of auditory stimuli, are also associated with high levels of orexin neuron activity, despite little motor activation. Furthermore, the spiking activity of orexin neurons is positively correlated with EEG frequencies in the gamma range (>30 Hz), a frequency band linked to heightened arousal [42,43]. Orexin neurons fire less frequently during other waking behaviors requiring less arousal such as grooming or eating [43]. When arousal levels are even lower, such as during quiet inattentive wakefulness, orexin neurons fire much less, and during sleep, the orexin neurons are nearly silent except for occasional bursts of activity during REM sleep [42,43]. Altogether, these data indicate that orexin neuron activity varies with the degree of arousal and is linked to heightened attentional states.

In addition, the activity of orexin neurons appears to be linked to motivational and emotional states. Orexin neurons show little c-fos expression when an animal is confronted with situations likely to trigger negative emotions such as delivery of foot-shocks or even expectation of foot shocks [41•]. In contrast, situations likely to elicit positive emotions, such as expectation of palatable food or drug reward, are associated with high orexin activity [41•,45–47]. In humans, extracellular levels of orexin-A may be highest during positive emotions and social interactions and lower during quiet wakefulness, sleep, or postoperative pain [48••]. Thus, the emotional valence of a situation may influence orexin neuron activity, with higher activity during behaviors associated with positive affect and less activity during

conditions associated with negative affect such as pain. Furthermore, orexin neurons are activated in rats that develop conditioned place-preference for morphine or cocaine and orexin antagonists may reduce the rewarding aspects of amphetamines and other drugs of abuse [46,49]. Perhaps the orexin neurons are most active during motivated behaviors when positive emotions lead to sustained periods of identifying and seeking desired outcomes.

Some researchers have hypothesized that the orexin neurons may also mediate awakenings from sleep. Orexin neuron firing increases at the transition from NREM sleep to wakefulness and precedes other correlates of emergence from REM sleep [42–44]. In addition, optogenetic studies demonstrate that selective activation of the orexin neurons increases the probability of waking from either NREM or REM sleep [50], though this awakening is slow, often occurring about 25 s after stimulation. Further arguing against a principal role for orexins in driving awakenings, mice that lack orexins have a normal probability of spontaneously waking from NREM sleep [33], suggesting that most awakenings are mediated by other systems. Nevertheless, orexin signaling is clearly necessary for the maintenance of wakefulness because acutely reducing the firing of orexin neurons or blocking orexin receptors reduces wakefulness [51,52,53•].

Through which neuronal pathways do the orexin neurons promote arousal?

The studies reviewed above demonstrate that activity of the orexin neurons is closely related to various aspects of arousal and that orexin signaling is necessary for the expression of normal arousal. Which of the many target neurons innervated by the orexin system are necessary to produce stable wakefulness and high levels of arousal?

The locus coeruleus (LC)

The noradrenergic neurons of the LC receive some of the densest orexinergic innervation [12] and have long been hypothesized to promote arousal [for review, see [54]]. LC neurons discharge maximally during active wakefulness and are virtually silent during NREM and REM sleep [55]. In addition, LC neurons fire a few seconds before the onset of wake, suggesting that they may help trigger awakenings from sleep [55,56]. Acute blockade of cortical NE inputs prevents sustained depolarization of cortical neurons [57••], and LC neurons may also promote EEG desynchrony via their projections to the BF. In support of this hypothesis, optogenetic stimulation of LC neurons rapidly wakes a mouse from sleep and extends the duration of wakefulness [58••].

Because orexins strongly excite LC neurons and promote wakefulness when injected near the LC [25,59], Carter and colleagues hypothesized that the LC mediates the arousing effects of orexins [60••]. They demonstrated that optogenetic inhibition of LC neurons prevents the arousals from sleep caused by simultaneous orexin neuron photostimulation. Moreover, injection of an OX1R antagonist into the LC before orexin neuron photostimulation blocks the awakenings otherwise observed [60••]. Though these findings demonstrate that the orexin neurons can elicit arousals via the LC, this may not apply to spontaneous arousals from NREM sleep as LC neurons begin firing a few seconds (>2 s) before a spontaneous awakening, while orexin neurons become active at the time of the transition [55,56].

The optogenetic experiments described above were performed during the rest period. Future studies will need to examine how the orexin system interacts with LC neurons during the active period. For example, blocking orexin receptors can decrease LC firing during the active period, but has little effect during the rest period [61]. Future studies should also

examine whether LC activation by orexins is necessary to promote vigilance, as LC activity is closely linked to the level of performance on sustained attention tasks [62,63].

The basal forebrain (BF)

The BF is another possible site through which orexins may promote arousal by activating the cortex [40,64,65]. Neurons in the substantia innominata (SI) and other caudal regions of the BF are thought to modulate cortical activation through widespread cholinergic, GABAergic, and glutamatergic projections [66–68]. Cholinergic BF neurons discharge maximally during states of cortical activation, when cortical release of acetylcholine is high [69,70]. More specifically, microdialysis studies show that cortical acetylcholine levels are higher during active waking compared to quiet wakefulness and sleep, and increase even more under conditions of enhanced or sustained attention [71]. The BF also contains a large population of cortically projecting, wake-active GABAergic neurons that probably contributes to cortical activation by reducing the activity of inhibitory cortical interneurons [69,70]. Many BF wake-active neurons begin firing less than 0.5 s before the onset of wakefulness and then continue firing during wakefulness [56,70]. Considered together, these observations suggest that BF neurons contribute to spontaneous awakenings and sustained wakefulness.

Orexin signaling in the BF can promote many aspects of arousal. Orexin-A excites BF cholinergic neurons, and delivery of orexin-A into the BF increases prefrontal acetylcholine release and promotes wake states [64]. Orexin-A also excites most of the GABAergic and glutamatergic neurons of the BF [64,65]. In addition, orexins may enhance glutamate release in the BF by exciting glutamatergic nerve terminals because dialysis of orexin-A into the BF increases local release of glutamate [40,65]. During EEG desynchrony associated with cortical activation, orexin-A is released within the BF [72] where it can activate OX1R and OX2R [18]. Using mice globally lacking orexin receptors, we found that focal restoration of OX1R and OX2R in the SI partially rescues their ability to produce long bouts of wakefulness [73]. These mice continued to have low locomotor activity, suggesting that this aspect of arousal is mediated by other pathways.

Orexinergic inputs to the BF may promote increased attention. For example, cortical release of acetylcholine is greatest during demanding attention tasks [74] and cholinergic SI neurons have been shown to play a role in sustained [75] and divided attention [76] in rats. Orexin neurons selectively increase firing in response to motivationally salient external stimuli [43,77], further implicating orexins in the control of selective attention.

The cortex

Orexinergic projections to cortex may also help sustain vigilance or attention [78]. For example, orexins improve performance on an attention task by exciting the same thalamocortical synapses that are activated by acetyl-choline [79]. Orexins may also drive increased arousal via direct actions on cortical neurons. For example, orexin-A may increase cortical excitability by closing hyperpolarization-activated/cyclic nucleotide (HCN) — gated channels on the layer 1 distal dendrites of layer 5 cortical pyramidal cells [80]. In addition, orexin-A directly and selectively activates layer 6b pyramidal neurons via OX2R–mediated closure of potassium channels [80]. Activation of these layer 6b neurons may help coordinate global cortical activation via widespread lateral projections to layer 1 spanning several millimeters of cortex. Thus, through actions on inputs to layer 1 from nonspecific thalamic nuclei; distal dendrites of layer 5 cortical pyramidal neurons; and layer 1-projecting layer 6b neurons, orexins may exert coordinated effects on neuropil activity in layer 1 of cortex — a major putative site of ‘top-down’ cognitive and emotional influences on cortical processing [78].

The posterior hypothalamus

For almost 100 years, researchers have hypothesized that the posterior hypothalamus (PH) is essential for generating wakefulness [81]. The PH is a heterogeneous area that contains neurons producing glutamate, GABA, dopamine, histamine and a variety of neuropeptides [36•]. There is strong evidence that histaminergic neurons of the TMN promote wakefulness [for review, see [82,83]]. TMN neurons are richly innervated by the orexin neurons and express only the OX2R receptor. Orexin-A excites TMN neurons, and infusion of orexin-A near this region promotes wakefulness. TMN neurons discharge maximally during active or attentive wakefulness, and unlike most other wake-promoting neurons, they start firing only after the onset of wakefulness [84]. Along these lines, TMN neurons do not fire during the brief arousals from sleep that are usually associated with phasic body movements. They also do not respond, or respond slowly, to an arousing sound stimulus. These observations suggest that TMN neurons mainly play a role in the maintenance of wakefulness rather than in the initiation of wakefulness [84].

Orexin signaling in the TMN region seems important for producing long bouts of wakefulness. Central injection of orexin-A increases wakefulness, but this response is reduced in mice lacking the H1R histamine receptor [85]. Restoration of OX2R expression in the TMN and adjacent parts of the PH markedly improves the maintenance of wakefulness in mice otherwise lacking OX2R signaling [31••]. This observation suggests that signaling through OX2R in the TMN region is sufficient for the normal maintenance of wakefulness under baseline physiological conditions, although whether orexin effects on arousal depend solely on the histamine-producing neurons remains unclear. For example, optogenetic activation of the orexin neurons can still induce awakenings from sleep in mice lacking the ability to synthesize histamine [86], confirming that histamine does not rouse animals from sleep but probably contributes to the maintenance of established wakefulness.

Research on the TMN has also highlighted the role of glutamate in the orexin neurons. For example, orexin neuron axon terminals in the TMN appear to contain glutamate [87], and optogenetic stimulation of these terminals increases glutamatergic EPSPs in the TMN [10••]. Thus, orexin neurons may provide both fast (glutamate) and slower (orexins) drive to histamine neurons, and thus represent a potent source of driving input.

Conclusions

Collectively, these findings suggest that the orexin neurons potentiate the activities of various arousal-promoting nuclei to enhance arousal and sustain vigilance, allowing an organism to react adequately to challenges and opportunities in coordination with its physiological needs. Alterations in orexin signaling probably destabilize the activity of these arousal centers, leading to low levels of arousal and poor vigilance during motivated behaviors.

In this review, we have focused on three major wake-promoting neuronal systems, but additional regions, such the mesopontine cholinergic and the raphe serotoninergic systems, are likely to mediate some of the wake-related actions of orexins [88]. In addition, the promotion of drug-seeking or other reward-related behaviors by administration of orexins into the VTA or accumbens nucleus suggests that influences of orexin on mesolimbic targets might indirectly influence arousal [15,89].

Our understanding of how the brain promotes arousal has been substantially improved by research on the orexins. It is now clear that orexins promote wakefulness and other aspects of arousal through a variety of brain regions including the LC, BF, cortex and PH. Still, it remains unknown which of these regions are truly necessary and which contribute most to

the effects of orexins on arousal, but this can now be tested by focally deleting orexin receptors in each of these regions. In addition, while it is clear that orexins are necessary for the maintenance of wakefulness, it remains unclear just which aspects of arousal are influenced by orexins. Future studies using genetic and optical tools should shed light on the basic mechanisms by which orexins enhance alertness, vigilance, and other key aspects of arousal and cognition.

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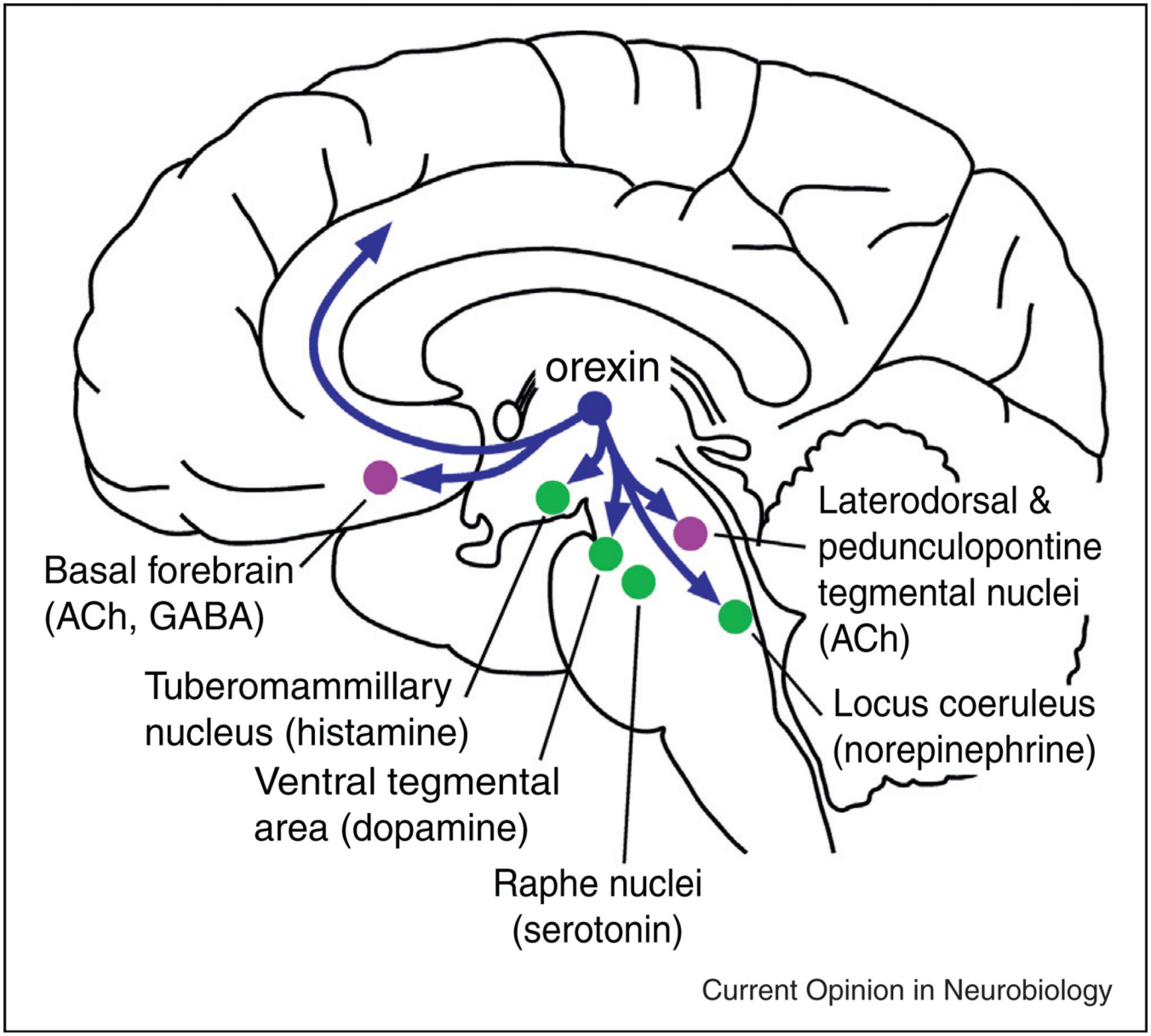

Figure 1.
Some of the key pathways through which the orexin neurons promote wakefulness. Orexin neurons innervate and excite monoaminergic brain regions such as the locus coeruleus, dorsal raphe, ventral tegmental area, and tubermomammillary nucleus. The orexin neurons also activate cortical neurons directly and indirectly via effects in the basal forebrain.

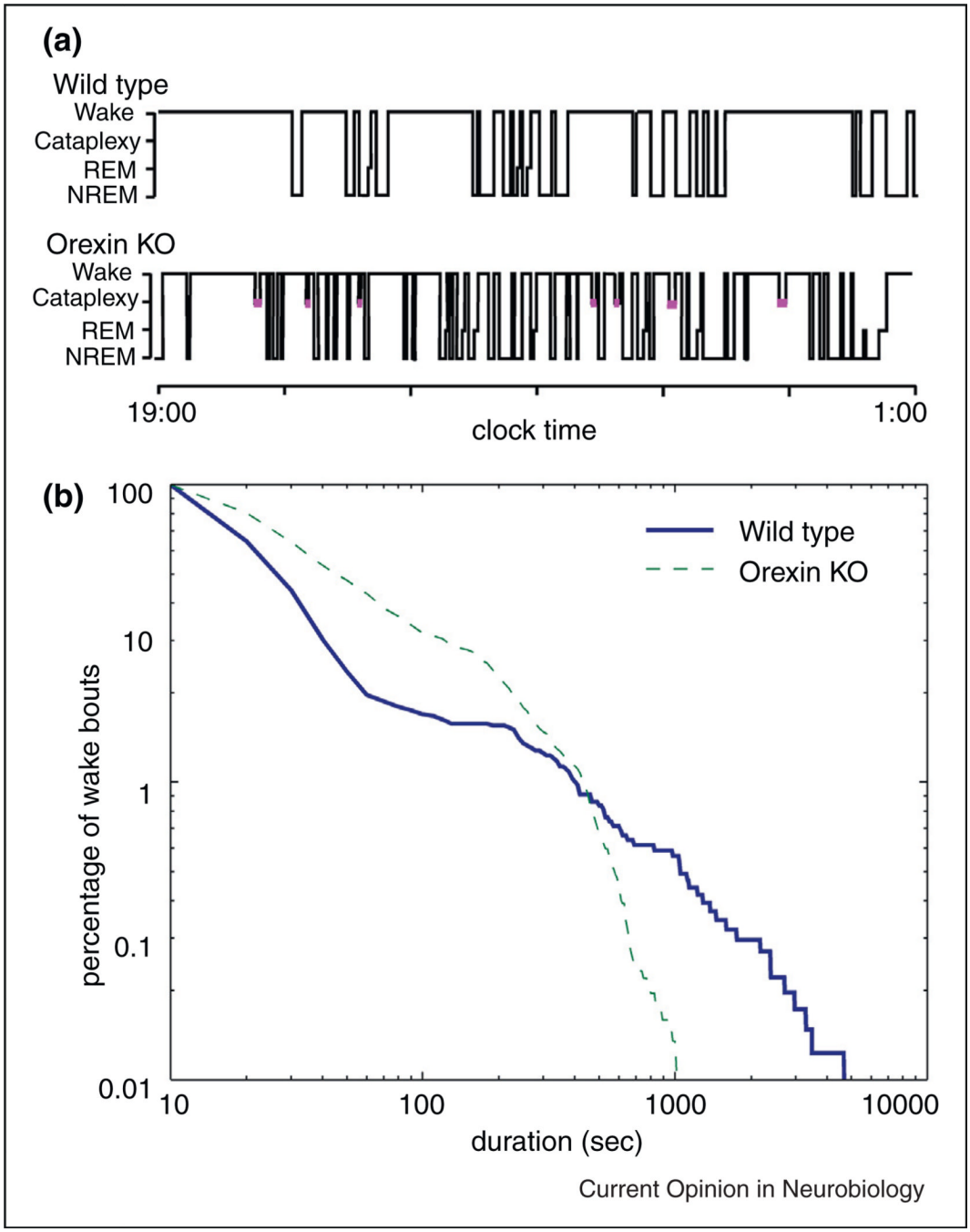

Figure 2.

Orexin peptide null mice (orexin KO) have fragmented wakefulness and sleep. (a) Representative hypnograms from the first 6 hours of the active period show that WT mice have long periods of wake and consolidated periods of sleep, whereas orexin KO mice have frequent transitions between wake and sleep. Cataplexy occurs frequently in orexin KO mice. (b) Survival curve analysis shows that during the active period, about 90% of all wake bouts end within 60 s in both mutant and wild-type mice, presumably representing just brief awakenings from sleep. After being awake for 60 s, wild-type mice generally remain awake, though after about 1000 s of wake, transitions into sleep became increasingly frequent. In contrast, wake bouts in orexin null mice more frequently fail after 200 s.

Hypnograms are courtesy of Dr. T. Mochizuki, and panel b is adapted with permission from Diniz Behn *et al.* [33].