

SLEEPJ, 2020, 1-9

doi: 10.1093/sleep/zsz296 Advance Access Publication Date: 10 January 2020

#### REVIEW

## A circuit perspective on narcolepsy

A. R. Adamantidis<sup>1,2,\*,•</sup>, M. H. Schmidt<sup>1,3</sup>, M. E. Carter<sup>4</sup>, D. Burdakov<sup>5</sup>, C. Peyron<sup>6</sup> and Thomas E. Scammell<sup>7</sup>

<sup>1</sup>Department of Neurology, Centre for Experimental Neurology, Inselspital University Hospital Bern, University of Bern, Bern, Switzerland, <sup>2</sup>Department of Biomedical Research, Inselspital University Hospital Bern, University of Bern, Bern, Switzerland, <sup>3</sup>Ohio Sleep Medicine Institute, Dublin, OH, <sup>4</sup>Department of Biology, Program in Neuroscience, Williams College, Williamstown, MA, <sup>5</sup>Department of Health Sciences and Technology, ETH Zürich, Zürich, Switzerland, <sup>6</sup>Center for Research in Neuroscience of Lyon, SLEEP team, CNRS UMR5292, INSERM U1028, University Lyon 1, Bron, France and <sup>7</sup>Department of Neurology, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA

\* Corresponding authors. Antoine Adamantidis, Department of Neurology, Inselspital University Hospital Bern, University of Bern, Freiburgstrasse, 18, 3010 Bern, Switzerland. Email: antoine.adamantidis@dbmr.unibe.ch; Thomas E. Scammell, Beth Israel Deaconess Medical Center, Center for Life Science, Room 705, 330 Brookline Ave., Boston, MA 02215. Email: tscammel@bidmc.harvard.edu.

## Abstract

The sleep disorder narcolepsy is associated with symptoms related to either boundary state control that include excessive daytime sleepiness and sleep fragmentation, or rapid eye movement (REM) sleep features including cataplexy, sleep paralysis, hallucinations, and sleep-onset REM sleep events (SOREMs). Although the loss of Hypocretin/Orexin (Hcrt/Ox) peptides or their receptors have been associated with the disease, here we propose a circuit perspective of the pathophysiological mechanisms of these narcolepsy symptoms that encompasses brain regions, neuronal circuits, cell types, and transmitters beyond the Hcrt/Ox system. We further discuss future experimental strategies to investigate brainwide mechanisms of narcolepsy that will be essential for a better understanding and treatment of the disease.

#### Statement of Significance

New techniques and recent findings suggest an imbalance in the activity of brain-wide circuitries controlling boundary state control and cataplexy in narcolepsy. This review summarizes current views on possible mechanisms and discusses future experimental strategies to further understand the brain-wide pathophysiological mechanisms of narcolepsy.

Key words: narcolepsy; cataplexy; hypocretins/orexins; neural circuits

#### Introduction

The year 2018 marked the 20th anniversary of the simultaneous, independent discovery of the hypocretin/orexin peptides by de Lecea et al. [1] and Sakurai et al. [2]. The former study described the cloning of the "hypothalamus-specific mRNA that encodes preprohypocretin, the putative precursor of a pair of peptides

that share substantial amino acid identities with the gut hormone secretin," expressed by a restricted number of cells in the lateral hypothalamus with widespread projections similar to modulatory systems of the brain, and the excitatory nature of the hypocretin peptides onto neurons in culture. The later reported "two novel neuropeptides, both derived from the same

Submitted: 20 August, 2019; Revised: 13 November, 2019

© Sleep Research Society 2020. Published by Oxford University Press [on behalf of the Sleep Research Society].

This is an Open Access article distributed under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs licence (http://creativecommons.org/licenses/by-nc-nd/4.0/), which permits non-commercial reproduction and distribution of the work, in any medium, provided the original work is not altered or transformed in any way, and that the work is properly cited. For commercial re-use, please contact journals.permissions@oup.com

precursor by proteolytic processing (...) with no significant structural similarities to known families of regulatory peptides (...) that bind and activate two closely related (previously) orphan G protein-coupled receptors" that stimulate food consumption when injected into the brain of rats.

Soon after the discovery of these novel neuropeptides, it was found that loss of Hcrt/Ox signaling results in narcolepsy (Figure 1). The cloning of Hcrt-2 receptor mutation in dogs with narcolepsy [3], the narcolepsy phenotype of Hcrt/Ox KO mice [2, 4], the detection of low Hcrt/Ox levels in human narcolepsy [5], and the severe loss of Hcrt/Ox neurons in the brains of people with narcolepsy [6–8] were critical discoveries. This work laid the foundation to establish the Hcrt/Ox system as essential for the regulation of arousal and REM sleep and opened up many new opportunities for the treatment of people with narcolepsy.

The seventh International Symposium on Narcolepsy (September 9-13, 2018) near Boston, MA (USA) gathered scientists and clinicians from all over the world with diverse backgrounds in genetics, cellular biology, systems neuroscience, immunology, psychology, psychiatry, and neurology to review progress and define unmet needs. The last two decades have substantially improved our understanding of Hcrt/Ox neurotransmission, Hcrt/Ox receptor pharmacology, and its clinical application, the synaptic connectivity of the Hcrt/Ox neurons, their causal control of sleep-to-wake transitions and (hyper-) arousal during wakefulness, the genetic causes and immunemediated pathophysiology of narcolepsy, and the refinement of the clinical criteria defining narcolepsy with or without cataplexy. Yet, as expected, the meeting emphasized knowledge gaps in both the basic mechanisms of Hcrt/Ox neurons in normal brain function and in the pathophysiological mechanisms underlying narcolepsy.

Reviewing the current state of knowledge led us to questions such as, What are the mechanisms underlying cataplexy, sleepiness and other symptoms of narcolepsy? How do these symptoms result from alterations of circuit-specific neuromodulation (norepinephrine, histamine, dopamine, acetylcholine, neuropeptides)?

How does circadian cycling influence brain activity and behavioral manifestation of cataplexy?

Below, we summarize our opinions and those of other researchers. While loss of Hcrt/Ox signaling is the primary cause of narcolepsy, we believe that the symptoms arise from a broader network centered on hypothalamic input–output connections. We also summarize the connectivity and dynamics of Hcrt/Ox cellular networks across sleep—wake states, and also locomotion, food intake and reward, and eventually integrate the novel concepts driving experimental research on the mechanisms of cataplexy.

# Lost in Transitions: Current Models of Cataplexy

Cataplexy is the most extraordinary symptom of narcolepsy type 1 (narcolepsy with cataplexy). Cataplexy is characterized by episodes of muscle weakness triggered by strong and generally positive emotions. In people with narcolepsy, cataplexy is often partial, resulting in face and neck weakness. Full episodes of cataplexy, in contrast, can produce severe global weakness in which an individual slumps to the ground, unable to move or speak for seconds to a minute or two, but consciousness is fully preserved. Additional abnormalities include frequent waking from sleep, inability to maintain wakefulness during the active (wake) phase accompanied with sleep attacks, and intrusions of REM sleep-like events, that is, cataplexy intruding into wakefulness, or the premature appearance of REM sleep soon after sleep onset, that is, sleep onset REM (SOREM) events (Figure 2).

The symptoms of narcolepsy, including cataplexy, have been interpreted as resulting from an instability of sleep—wake states often loosely referred to as "poor state boundary control" [9]. An area of current controversy regarding state instability pertains to its understanding, or definition, particularly as it relates to cataplexy. For example, cataplexy may be interpreted as resulting from the intrusion of REM sleep muscle atonia into wakefulness.

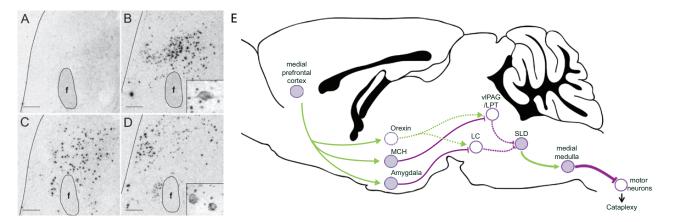


Figure 1: Pathophysiology of narcolepsy. (A–D) Hypocretin and MCH mRNA expression in the hypothalamus of a control subject and a person with narcolepsy. Preprohypocretin transcripts are detected in the hypothalamus of a control (B) but not in a narcolepsy (A) subject, while MCH transcripts are detected, intermingled with hypocretin in both control (D) and narcolepsy (C) samples. f, fornix scale bars = 10 mm. Adapted from Peyron et al. (2000). (E) Strong, positive emotions many activate neurons in the medial prefrontal cortex (mPFC) that excite neurons making orexin and MCH as well as neurons in the amygdala. Normally, the orexin neurons would then excite atonia-suppressing neurons of the ventrolateral periaqueductal gray/lateral pontine tegmentum (vlPAG/LPT), but in the absence of orexins, the inhibitory effects of MCH and the amygdala are unopposed. Lacking inhibition from the vlPAG/LPT, the sublaterodorsal nucleus (SLD) can then inhibit motor neurons via GABA and glycine-containing neurons of the medial medulla. Norepinephrine from the locus coeruleus (LC) normally inhibits REM sleep and cataplexy, and the LC is probably inhibited during cataplexy, perhaps via the amygdala and MCH neurons. This model is built upon research derived from many labs, but its basic components are still debated and remain to be definitively demonstrated. Pathways active during cataplexy are shown with solid lines; pathways inactive during cataplexy are shown with dashed lines. Inhibitory pathways are purple; excitatory pathways are green.

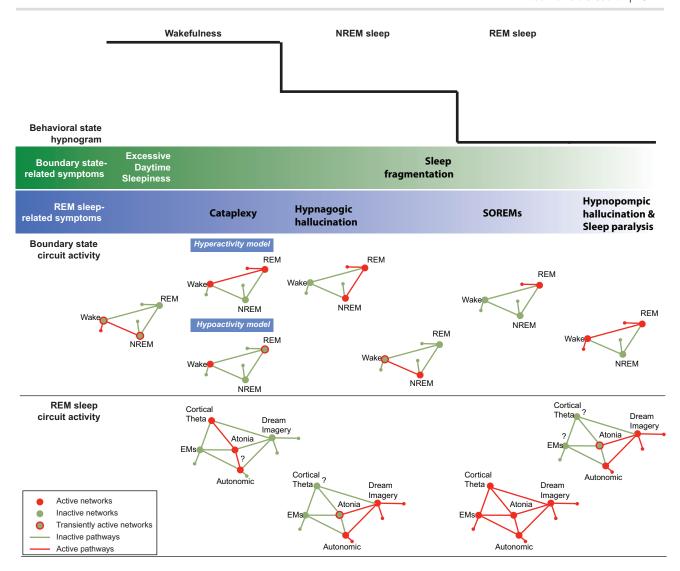


Figure 2: Circuit perspective of narcolepsy. Schematic representation of the physiological elements underlying the symptoms of narcolepsy, including excessive daytime sleepiness, sleep fragmentation, cataplexy, hypnagogic hallucinations, SOREMS and sleep paralysis. These are divided into symptoms related to boundary state control (highlighted in the green box) or REM sleep (highlighted in the blue box) and are distributed across the sleep-wake cycles (top part of the figure). The bottom part of the figure highlights possible mechanisms involving neural circuits of boundary state control (top) and REM sleep (bottom). In this schematic representation, primary circuit hubs controlling sleep wake state (top) or REM-related brain activity or phenomena (bottom) are represented by large dots connected to each other; smaller dots represent secondary output circuits. Color coding indicates active (red) or inactive (green) networks and pathways. Some of the symptoms of narcolepsy are thought to result from a weak boundary state control. For example, in the absence of excitatory Hcrt/Ox drive, excessive daytime sleepiness could arise from weak or inconsistent activity of wake-promoting systems, or inappropriate intrusion of NREM sleep-promoting regions. Similarly, sleep fragmentation could result from uncoordinated activity of wakepromoting neurons during sleep. Abnormal activity in REM sleep circuits may produce other symptoms of narcolepsy including cataplexy during wakefulness, hypnaagaic hallucingtion during NREM sleep, and SOREMS or sleep paralysis during REM sleep. In the context of this perspective, cataplexy may result from overactivity of REM sleep circuits during wakefulness (hyperactivity model) or hypoactivity of REM-suppressing circuits (hypoactivity model) and the activation of brain circuits governing theta rhythm or muscle atonia. Hypnagogic hallucinations occur at the transition from wakefulness to sleep and may result from the activation of REM sleep circuits that promote dreaming/imagery and sometimes muscle atonia (paralysis). SOREMs implicate a comprehensive activation of REM sleep circuits, including those responsible for theta rhythms, muscle atonia, eye movements, dreaming/imagery and the autonomic system. Finally, hypnopompic hallucinations and sleep paralysis may result from the co-activation of both wake and REM circuits, in particular those involved in muscle atonia, dreaming/imagery and the autonomic system.

This perspective would suggest that cataplexy is triggered when executive neural circuits driving REM sleep inappropriately activate during wakefulness, thus producing a "dissociated state" containing elements of both REM sleep and wakefulness. Alternatively, the underlying mechanisms of muscle atonia during cataplexy may be different from those of REM sleep, suggesting instead, that cataplexy is an entirely distinct state potentially unrelated to either REM sleep or wakefulness. Currently, these two models, or interpretations, of cataplexy are based on complementary sets of experimental evidence and interpretations (Figure 2).

On the one hand, researchers have speculated since the 1980s that cataplexy, hypnagogic hallucinations, and sleep paralysis are simply elements of REM sleep that mix into wakefulness, creating dissociated states [9, 10]. This longstanding hypothesis suggests that during an episode of cataplexy, some of the neural pathways involved in muscle atonia during REM sleep are transiently activated by, for example, strong positive emotions during wakefulness. Further support for this hypothesis is the observation that transitions between states of vigilance are more frequent in human narcolepsy and animal models of narcolepsy

[11] and that REM sleep, as well as cataplexy, seems to be under the inhibitory control of the Hcrt/Ox neuropeptides [12], despite a normal pattern of cortical EEG signals across wake, NREM and REM sleep transitions. In one popular model, REM sleep-active, glutamatergic neurons of the sublaterodorsal nucleus activate GABA/glycinergic pre-motor neurons which then strongly inhibit alpha-motor neurons to produce muscle atonia [13, 14]. Lacking contrary evidence, most researchers have assumed that cataplexy is mediated by the abnormal activation of these REM sleep atonia pathways during wakefulness. However, this common assumption remains to be confirmed experimentally.

In contrast, more recent data have suggested that cataplexy and REM sleep involve distinct states of brain activity and behavior [15]. This perspective is based on the finding that while EEG activity during cataplexy is similar to that of REM sleep, it also has some distinct features such as bursts of hypersynchronous theta activity [15]. Furthermore, this hypothesis is supported by the finding that, in contrast to REM sleep, the onset, maintenance, and termination of cataplexy is a multi-phased process involving a progression of behavioral states beginning with wakefulness. This hypothesis would be further supported if the muscle atonia mechanisms of cataplexy were found to be different than the control of muscle atonia observed in REM sleep. Thus, understanding boundary state control in narcolepsy will likely be the subject of future investigation.

Finally, there is a common manifestation of cataplexy across species, from rodents to humans, that is, cataplexy often transitions into a state indistinguishable from REM sleep. The difference between sleep-onset REM sleep (SOREM) and cataplexy is clearly defined in human narcolepsy, however, it remains difficult to differentiate in mice, particularly since intervening NREM sleep may be very brief. Some studies have tracked the absence of a masseter muscular tone to confirm cataplexy in mice [16], while others have distinguished "abrupt" versus "gradual" arrests when comparing cataplexy in Hcrt/Ox KO and OX2 receptor in mice [17], as it might differentiate SOREM versus cataplexy. These findings are indicative of a possible link between the behavioral states preceding cataplexy and the forthcoming cataplexy [17] that should be considered in future investigation in human.

#### Hcrt/MCH Duality in Cataplexy

Current models of narcolepsy mainly focus on the loss of Hcrt/Ox neuron signaling. However, other neurotransmitters and circuits likely contribute to the pathophysiology of the disease. Indeed, there is no clear understanding of how the loss of Hcrt/Ox signaling leads to the production of cataplexy. Elucidating these circuits is an area of strong interest that may open avenues of future clinical intervention for people with cataplexy.

One of the emerging themes in the field is the potential interplay between the MCH and Hcrt/Ox neuronal systems in the control of both REM sleep and cataplexy. These two neuronal populations are intermingled within the LH, a critical brain region that integrates diverse inputs related to energy balance, sleep pressure and circadian time for the output modulation of behavioral state and global shifts in resource allocations required for sleep or wakefulness [18–20]. MCH and Hcrt/Ox neurons show reciprocal, state-dependent, firing patterns with the former being maximally active during REM sleep and the latter predominantly active during wakefulness. In addition, GABA receptors on MCH and Hcrt/Ox neurons undergo dynamic

and differential changes during the sleep-wake cycle [21]. GABA receptors on Hcrt/Ox neurons appear to increase during waking and sleep deprivation, during which Hcrt/Ox activation is assumed to be prolonged, but decrease during sleep when Hcrt/Ox neurons are typically silent. Neighboring MCH neurons, in contrast, show the opposite pattern of GABA receptor expression, increasing during sleep when MCH neurons are most active and decreasing during wake when these neurons are typically silent. It was proposed that this reciprocal expression of GABA receptors on MCH and Hcrt/Ox neurons may play a role in the homeostatic adjustment of their respective activity patterns as a function of preceding sleep and wake duration [19]. The role of this differential expression in homeostatic sleep-wake regulation, and also cataplexy or REM sleep-like brain activity, are potential implications of this work that requires further investigation.

Another hypothesis is that a loss of Hcrt/Ox signaling may favor MCH neuron activation, potentially driving the increased REM sleep propensity found in narcolepsy. This hypothesis is largely based on the reciprocal firing patterns of these two neuronal populations suggesting a reciprocal inhibition [22, 23], given that MCH activity is maximal in REM sleep whereas Hcrt/ Ox activity is maximal in wake. Although a growing body of literature demonstrates that the MCH system plays an important role in REM sleep expression, a role for MCH in narcolepsy or cataplexy has remained unknown. Using a global measure of MCH level of release in the CSF, no difference was seen between people with narcolepsy, central hypersomnias or controls [24]. However, recently published data show that MCH neuronal activation in Hcrt-KO mice leads to an increase in both REM sleep and cataplexy, whereas administration of an MCH receptor antagonist decreases cataplexy [25]. These data would suggest that the MCH system can drive both REM sleep propensity and intrusions of its associated muscle atonia (cataplexy) into wakefulness. However, these data contrast with previous studies showing an increase of cataplexy in [Hcrt/Ox -MCH] double, as compared to Hcrt/Ox single, knockout mice [26].

Reconciling these opposing views regarding the role of MCH in cataplexy may potentially provide insight into mechanisms causing boundary state instability in narcolepsy. To illustrate, one can ask is cataplexy caused by overactivation of neural circuits responsible for muscle atonia, or brain activity typical of REM sleep? The answer to this fundamental question has significant implications. For example, if both REM sleep and cataplexy are triggered by similar mechanisms, then experimental conditions increasing REM sleep propensity would be expected to increase cataplexy by causing REM sleep-related phenomena such as muscle atonia to intrude into wakefulness as recently reported [27]. Another perspective is that acute changes in MCH signaling [27] may differ from chronic loss of MCH [28] on sleep boundaries. Alternatively, if cataplexy occurs when both Hcrt/Ox (waking) and MCH (REM sleep) systems are simultaneously hypoactive, then boundary state instability may ensue when neither the Hcrt/Ox nor MCH system predominates. If correct, then experimental conditions should thus be capable of dissociating these phenomena, that is, increasing cataplexy while decreasing REM sleep, or at least brain activity similar to REM sleep. In this latter perspective, activation of systems that drive REM sleep would shift network activity toward this sleep state and decrease the probability for cataplexy by stabilizing REM sleep and the boundary between REM sleep and wake.

More work is clearly needed to clarify the role of the MCH and Hcrt/Ox systems in both cataplexy and the increased REM sleep propensity of narcolepsy. With respect to the "mutual inhibition" models of sleep-wake states [29], it is conceivable that a number of circuit inputs to Hcrt/Ox cells, and possibly their post-synaptic targets, may ultimately contribute to sleep-wake instability or even cataplexy. Lack of Hcrt/Ox may induce pre- and post-synaptic changes that make muscle atonia circuits more responsive to atonia-promoting inputs other than those of Hcrt/Ox origin. Although this latter hypothesis is often discussed, it implies that the underlying circuits of REM sleep atonia are essentially "highjacked" in waking during cataplexy, an assumption that has yet to be experimentally demonstrated.

## Hcrt/Ox Neuron Connectivity and **Hypothalamic Control of Behaviors**

An open question remains as to whether Hcrt/Ox neurons form a homogenous cell population within the lateral hypothalamus, or whether there are distinct Hcrt/Ox cell clusters that exhibit different afferent/efferent projection maps, and therefore different activity patterns, physiological or behavioral phenotypes, which may ultimately deepen our understanding of the etiology and phenotype of narcolepsy.

Previous studies have examined Hcrt/Ox neuronal afferent and efferent projections using classically and genetically targeted neuroanatomical tracing methods in rodents. These studies reveal that Hcrt/Ox neurons integrate a wide variety of neural signals from multiple sources distributed all over the brain [30-32]. Quantitative estimates of the relative projection densities show dense monosynaptic inputs from the lateral and paraventricular hypothalamus, cerebral nuclei such as the nucleus accumbens and bed nuclei of the stria terminalis (BNST), as well as from the lateral habenula, midbrain reticular nucleus, and periaqueductal gray; some inputs from the cortex and brainstem have also been reported [30]. Accordingly, Hcrt/ Ox neurons are excited by a wide variety of neurotransmitters including glutamate [33], ATP [34], corticotropin releasing factor [35], thyrotropin releasing hormone [36], noradrenaline [37], and acetylcholine [33, 37], and inhibited by GABA [33] and adenosine [38, 39].

Recent studies have defined neurochemical inputs from specific sources, such as GABAergic [40], corticotropin releasing factor, and cholecystokin-projecting neurons from the BNST [41], to be essential for arousal modulation. Interestingly, local GABAergic control of Hcrt/Ox is depressed by cholinergic or noradrenergic input to the LH39. Moreover, although the inhibitory GABAergic interneurons are not affected by Hcrt, they appear inhibited by dynorphin which is co-released from Hcrt/Ox collaterals [40]. These and other data demonstrate how Hcrt/ Ox neurons may be disinhibited by other waking systems (i.e. acetylcholine) or by their own release of dynorphin.

In turn, Hcrt/Ox neurons project diffusely throughout the brain, with especially dense excitatory projections to brain areas regulating arousal (e.g. locus coeruleus and tuberomamillary nucleus), reward (e.g. ventral tegmental area and nucleus accumbens) and autonomic function (e.g. brainstem sympathetic and respiratory control centers). Postsynaptic excitatory responses to optogenetic activation of Hcrt/Ox terminals demonstrate the involvement of both Hcrt/Ox peptides and glutamate neuro-transmission/modulation of the activity of post-synaptic targets, for example histamine or noradrenergic

Electrical activity in Hcrt/Ox neurons correlates with states of heightened arousal, and, electrophysiological and calcium transient recordings from identified Hcrt/Ox neurons in vivo broadly suggest that these neurons are more active during states of wakefulness and silent during NREM sleep (but with some residual phasic activity during REM sleep). Importantly, Hcrt/Ox neurons also increase activity during the presence of salient environmental stimuli (e.g. predator odor, somatosensory stimulation, immobilization stress) and active behaviors (e.g. running) while decreasing activity during quiet, sedentary behaviors (e.g. eating, grooming) [44, 45]. Although the mechanisms by which these context-specific activity dynamics arise from specific neural inputs is an open question, recent and future studies examining the effects of stimulating specific afferent projections to Hcrt/Ox neurons will help dissect the contributions of input from distinct brain regions [41].

Some recent studies have proposed that Hcrt/Ox neurons can be anatomically and functionally clustered into at least two separate subpopulations: one that resides in the medial Hcrt/Ox field sends projections to the locus coeruleus and tuberomammillary nucleus to regulate wakefulness; and another that resides in the lateral Hcrt/Ox field and sends projections to the ventral tegmental area and nucleus accumbens to regulate reward [46]. Although further studies have found some evidence to support this hypothesis [47, 48], others have observed projections to locus coeruleus and the ventral tegmental area to be in equal frequencies across the Hcrt/Ox field [49, 50]. Most recently, Iyer et al. identified distinct Hcrt/Ox neuron subtypes that projected to both the locus coeruleus and tuberomammillary nucleus, or to the ventral tegmental area and nucleus accumbens, but these two categories of Hcrt/Ox neurons were intermingled among the Hcrt/Ox field [51]. At the cellular level, Hcrt/Ox neurons can be functionally subdivided into two populations based on their electrophysiological properties, but whether these categories of neurons participate in distinct behaviors will require future investigation [52]. Interestingly, a recent molecular study suggests a weak molecular heterogeneity amongst Hcrt/Ox neurons, indicating that circuit-specific Hcrt/Ox cell functions is rather due to connectivity (inputs-outputs) than molecular content [53]. This is in agreement with the fact that Hcrt/Ox neurons are all born at E12 [54] in contrast to MCH neurons that have clear 2 subpopulations (CART+ and CART-) but both seem to be involved in REM sleep [55].

Efforts to identify transcriptome profiles in more detail (e.g. RNA-seq or Drop-seq) of a genetically defined Hcrt/Ox neuronal population in animal models [56], together with a refined identification of functional clusters of Hcrt/Ox neurons based on their discharge patterns across sleep-wake states and goal-directed behavior will be essential in refining our understanding of the multiple aspects of Hcrt/Ox control of brain states, in particular during cataplexy and goal-directed behaviors.

## Activity of Brain Circuits in the Absence of a Hcrt/Ox System

Although the link between Hcrt/Ox cells and spinal motor neurons remains poorly defined [57], an important challenge remains to determine how the absence of Hcrt/Ox neurons leads to cataplexy and REM sleep-like brain activity at the circuit level. An equally important question is whether cataplexy results from a sudden or, instead, a progressive (long-term) cellular or circuit change requiring secondary or compensatory mechanisms. These changes of cellular and circuit activity in animal models of narcolepsy are summarized below (Figure 2).

A growing body of clinical and experimental evidence suggests a circuit mechanism for cataplexy that encompasses a larger network than just Hcrt/Ox neurons and their direct targets. Indeed, spiking activity of multiple neuronal populations exhibit altered activity during cataplexy in animal models of narcolepsy. On one hand, cataplexy is associated with an increased neuronal spiking in a subpopulation of non-cholinergic REM-ON neurons from the medial medulla (their activity during cataplexy is higher than during wake and similar to REM sleep) [58], dorsal raphe REM-OFF (presumably serotoninergic) and unidentified REM-ON neurons (their activity during cataplexy is similar to their activity during NREM sleep) [59], histaminergic wake-ON cells (their activity during cataplexy is similar to their activity during wake) [60]. In contrast, medial mesopontine neurons (their activity during cataplexy is similar to their activity during quiet wake) [61] and unidentified neurons from the locus-coeruleus (their activity during cataplexy is similar to their activity during REM sleep) [62] showed reduced activity at the onset of, and during, cataplexy relative to their activity during either REM sleep or active waking, respectively. Interestingly, transient silencing of LC noradrenergic cells is insufficient to induce cataplexy, although their sustained activation which may then be followed by depolarization block which induces behavioral arrests that resemble cataplexy [63]. Collectively, these findings suggest that cataplexy results from the activation of REM sleep atonia circuits during wakefulness, some of which may include the SLD neurons responsible for REM sleep atonia [64], and the cessation of activity of wake-promoting neurons such as the norepinephrine cells. A complete representation of brain activity during cataplexy will be essential to the understanding of the pathophysiology of narcolepsy.

At the cellular level, the lack of a functional Hcrt/Ox system in the brains of people with narcolepsy profoundly alters several neuromodulatory systems. In particular, hyper-sensitivity to cholinergic stimulation [65], a dependence on opiod [66] and histaminergic [60, 67] systems have been reported. Whether these translate into altered neuromodulatory tone, through alteration of their release, receptor expression or membrane trafficking remains unknown. Furthermore, drugs that enhance cataplexy reduced spontaneous LC activity during the time that the propensity for cataplexy was enhanced [62], suggesting that LC norepinephrine neurons, and disfacilitation of motoneurons, may be permissive to the onset of cataplexy. Accordingly, both prazosin and physostigmine greatly increased the propensity for cataplexy. Thus, these findings further support the implication of multiple neural circuits, and a possible role for compensatory mechanisms underlying the development of some symptoms of narcolepsy.

A remarkable observation is the association between positive emotions and the triggering of cataplexy in people with narcolepsy (laughing, joking, social interaction with friends and family), which has focused experimental investigation on the role of amygdala circuits in cataplexy. The amygdala is essential to the processing of emotional stimuli and has dense projections to brainstem regions regulating muscle tone and sleep. Two populations of cells within the amygdala show a significant change in spiking activity with respect to cataplexy [68]; a first sub-population of sleep-active

neurons located in the central and basal nuclei increase discharges prior to, and during, cataplexy, while a second sub-population of wake-active cells from the cortical nucleus exhibits the inverse response. Lesions of the amygdala in mice with narcolepsy decrease cataplexy triggered spontaneously, or with high arousal or positive emotions, without altering their sleep-wake cycle [10]. In 2017, GABAergic neurons of the amygdala were found to be necessary for cataplexy [69] (but see Ref. [16]), suggesting that these inhibitory cells may be implicated in cataplexy induced by positive emotions. Whether other cell types in the amygdala, or extra-amygdala circuits, are involved in the initiation, maintenance and termination of cataplexy attacks remains to be investigated. Of interest, reversible suppression of medial prefrontal cortex activity substantially reduced cataplexy induced by chocolate, but did not affect spontaneous cataplexy [70]. In addition, neurons in the medial prefrontal cortex innervate parts of the amygdala and hypothalamus that contain neurons active during cataplexy and that innervate brainstem and spinal motor systems. In this context, the implication of dopamine [71], together with the oxytocinergic system in emotion and social interactions, in triggering cataplexy remains to be investigated.

At the level of circuit oscillation, electroencephalographic (EEG) features of cataplexy were recorded from people and mice with narcolepsy [15, 72, 73] where cataplexy was divided into three distinct stages. The initial "wake-like" stage involved tonic suppression of EMG activity, full postural collapse and a waking-like EEG spectrum. A second "REM-like" stage differed from the previous one by the onset of hypersynchronous hippocampal theta activity and a REM-like EEG spectrum (large amplitude EEG signals and high peak theta frequency). The final transitional stage to wakefulness or NREM sleep is accompanied with EEG activities of mixed amplitude and frequencies (but see Ref. [15]). Finally, long periods of cataplexy may evolve into REM sleep [15]. Altogether, these data somewhat contradict the hypothesis that cataplexy shares identical EEG and behavioral characteristics with REM sleep. Despite their differences, murine cataplexy shares many EEG features with REM sleep, or the pre-REM sleep spindling [4, 26]. Once cataplexy is fully established, the mouse cortical EEG is dominated by REM-like high amplitude theta activity with transient prefrontal theta bursts not of hippocampal origin [15] and also seen in histamine deficient mice [74]. Consistent with this observation, wakefulness preceding cataplexy displays rich theta and gamma rhythms possibly due to higher locomotor activity and low delta wave activity. Of importance, theta activity is higher during cataplexy than wakefulness in mice [75].

Currently, this research provides an incomplete map of single cell and circuit activity in narcolepsy. One way to provide missing information would be to simultaneously track the activity of multiple brain circuits, including those implicated in the control of sleep–wake states, muscle tone, and emotion (e.g. dopamine, serotonin) at single-cell resolution in healthy and people with conditions.

#### **Future directions**

Cataplexy results from complex, yet unclear, pathophysiological mechanisms that involve neural circuits distributed throughout the brain, in particular in the amygdala, prefrontal cortex and brainstem. Whether cataplexy is a simple intrusion of REM sleep, or some aspect of it, into wakefulness, or rather represents a unique state that is distinct from REM sleep remains to be determined.

Furthermore, questions remain such as, From where does this pathological circuit activity arise? and How can one accurately restore a naturalistic activity in these circuits? The activation of cataplexy-silent neurons (e.g. locus coeruleus norepinephrine neurons), or silencing of cataplexy-active neurons may be sufficient to block some of the narcolepsy symptoms including cataplexy, yet this remains to be investigated. Addressing these questions requires a much more precise understanding of the cellular and circuit dynamics of a "narcoleptic brain" than what is available thus far. Equally important is the need to harmonize methodological approaches across research labs. For example, scoring of sleep-wake states in rodents in a recent study [25] was based on 12-s epochs, whereas many labs score sleep-wake data based on 4-s epochs. Thus, longer epochs are more likely to contain transitional states such as NREM sleep. This is important since REM sleep and cataplexy demonstrate very similar polysomnographic features, and their differentiation relies heavily on the presence or absence of intervening NREM sleep, as well as distinct cellular activity amongst circuits described in this perspective.

In this context, brain-wide simultaneous recording/imaging of multiple circuits across states of higher arousal (e.g. during goal-directed behaviors), sleep or cataplexy in animal models of narcolepsy is expected to shed light on the pathophysiological mechanisms. Long-term perspectives aim at better understanding the molecular, cellular and circuit substrates of narcolepsy symptoms through a particular attention to the longitudinal aspect of the disease and the brain compensatory response to the absence of a functional Hcrt/Ox system.

#### **Funding**

A.R.A. was supported by the University of Bern, Bern University Hospital, Inselspital, the Human Frontier Science Program (RGY0076/2012), Inselspital University Hospital Bern, Swiss National Science Foundation (31003A\_156156), European Research Council (725850), Sinergia (CRSII3\_160803), and the University of Bern. M.E.C. is supported by the National Institutes of Health (DK105510) and National Science Foundation (1652060). C.P. was supported by the CNRS. T.E.S. is supported by grants from the NIH (R01 NS106032), WakeUpNarcolepsy, Takeda Pharmaceuticals, and Merck. M.H.S. is supported by the University of Bern, Bern University Hospital (Inselspital), Insel grant (84801184), the Sleep Medicine Research Foundation, and the Ohio Sleep Medicine Institute.

#### Acknowledgments

We thank the organizers and sponsors of the 7th International Symposium on Narcolepsy: Harmony Biosciences, Jazz Pharmaceuticals, Takeda Pharmaceuticals, and Merck. All the authors discussed and wrote the manuscript. Conflict of interest statement: None declared.

## References

de Lecea L, et al. The hypocretins: hypothalamus-specific peptides with neuroexcitatory activity. Proc Natl Acad Sci USA. 1998;95(1):322-327.

- Sakurai T, et al. Orexins and orexin receptors: a family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behavior. Cell. 1998;92(4):573-585.
- Lin L, et al. The sleep disorder canine narcolepsy is caused by a mutation in the hypocretin (orexin) receptor 2 gene. Cell. 1999;98(3):365-376.
- Chemelli RM, et al. Narcolepsy in orexin knockout mice: molecular genetics of sleep regulation. Cell. 1999;98(4):437-451.
- Nishino S, et al. Hypocretin (orexin) deficiency in human narcolepsy. Lancet. 2000;355(9197):39-40.
- Peyron C, et al. A mutation in a case of early onset narcolepsy and a generalized absence of hypocretin peptides in human narcoleptic brains. Nat Med. 2000;6(9):991-997.
- Thannickal TC, et al. Reduced number of hypocretin neurons in human narcolepsy. Neuron. 2000;27(3):469-474.
- Crocker A, et al. Concomitant loss of dynorphin, NARP, and orexin in narcolepsy. Neurology. 2005;65(8):1184-1188.
- Broughton R, et al. Excessive daytime sleepiness and the pathophysiology of narcolepsy-cataplexy: a laboratory perspective. Sleep. 1986;9(1 Pt 2):205-215.
- 10. Burgess CR, et al. Narcolepsy: neural mechanisms of sleepiness and cataplexy. J Neurosci. 2012;32(36):12305-12311.
- 11. Diniz Behn CG, et al. Abnormal sleep/wake dynamics in orexin knockout mice. Sleep. 2010;33(3):297-306.
- 12. Roman A, et al. The inappropriate occurrence of rapid eye movement sleep in narcolepsy is not due to a defect in homeostatic regulation of rapid eye movement sleep. Sleep. 2018;41(6). doi:10.1093/sleep/zsy046
- 13. Valencia Garcia S, et al. Genetic inactivation of glutamate neurons in the rat sublaterodorsal tegmental nucleus recapitulates REM sleep behaviour disorder. Brain. 2017;140(2):414-428.
- 14. Valencia Garcia S, et al. Ventromedial medulla inhibitory neuron inactivation induces REM sleep without atonia and REM sleep behavior disorder. Nat Commun. 2018;9(1):504.
- 15. Vassalli A, et al. Electroencephalogram paroxysmal  $\theta$  characterizes cataplexy in mice and children. Brain. 2013;136(Pt 5):1592-1608.
- 16. Snow MB, et al. GABA cells in the central nucleus of the amygdala promote cataplexy. J Neurosci. 2017;37(15):4007-4022.
- 17. Willie JT, et al. Distinct narcolepsy syndromes in Orexin receptor-2 and Orexin null mice: molecular genetic dissection of Non-REM and REM sleep regulatory processes. Neuron. 2003;38(5):715-730.
- Latifi B, et al. Sleep-wake cycling and energy conservation: role of hypocretin and the lateral hypothalamus in dynamic state-dependent resource optimization. Front Neurol. 2018;**9**:790.
- 19. Briggs C, et al. Sleep deprivation distinctly alters glutamate transporter 1 apposition and excitatory transmission to orexin and MCH neurons. J Neurosci. 2018;38(10): 2505-2518.
- 20. Grivel J, et al. The wake-promoting hypocretin/orexin neurons change their response to noradrenaline after sleep deprivation. J Neurosci. 2005;25(16):4127-4130.
- 21. Toossi H, et al. GABA receptors on orexin and melaninconcentrating hormone neurons are differentially homeostatically regulated following sleep deprivation. eNeuro. 2016;3:ENEURO.0077-16.2016.
- 22. Apergis-Schoute J, et al. Optogenetic evidence for inhibitory signaling from orexin to MCH neurons via local microcircuits. J Neurosci. 2015;35(14):5435-5441.

- 23. Gao XB, et al. Melanin concentrating hormone depresses synaptic activity of glutamate and GABA neurons from rat lateral hypothalamus. *J Physiol.* 2001;533(Pt 1):237–252.
- 24. Peyron C, et al. Melanin concentrating hormone in central hypersomnia. Sleep Med. 2011;12(8):768–772.
- Naganuma F, et al. Melanin-concentrating hormone neurons contribute to dysregulation of rapid eye movement sleep in narcolepsy. Neurobiol Dis. 2018;120:12–20.
- Willie JT, et al. Orexin and melanin-concentrating hormone (MCH) double knockout mice: compensatory role for MCH in narcolepsy-cataplexy. Sleep. 2003;26:A50.
- Vetrivelan R, et al. Melanin-concentrating hormone neurons specifically promote rapid eye movement sleep in mice. Neuroscience. 2016;336:102–113.
- 28. Tsunematsu T, et al. Optogenetic manipulation of activity and temporally controlled cell-specific ablation reveal a role for MCH neurons in sleep/wake regulation. *J Neurosci.* 2014;34(20):6896–6909.
- Saper CB, et al. Sleep state switching. Neuron. 2010;68(6):1023–1042.
- González JA, et al. Awake dynamics and brain-wide direct inputs of hypothalamic MCH and orexin networks. Nat Commun. 2016;7:11395.
- Sakurai T, et al. Input of orexin/hypocretin neurons revealed by a genetically encoded tracer in mice. Neuron. 2005;46(2):297–308.
- 32. Yoshida K, et al. Afferents to the orexin neurons of the rat brain. *J Comp Neurol.* 2006;**494**(5):845–861.
- Henny P, et al. Innervation of orexin/hypocretin neurons by GABAergic, glutamatergic or cholinergic basal forebrain terminals evidenced by immunostaining for presynaptic vesicular transporter and postsynaptic scaffolding proteins. J Comp Neurol. 2006;499(4):645–661.
- Wollmann G, et al. Direct excitation of hypocretin/orexin cells by extracellular ATP at P2X receptors. J Neurophysiol. 2005;94(3):2195–2206.
- 35. Winsky-Sommerer R, et al. Interaction between the corticotropin-releasing factor system and hypocretins (orexins): a novel circuit mediating stress response. *J Neurosci.* 2004;24(50):11439–11448.
- 36. Hara J, et al. Thyrotropin-releasing hormone increases behavioral arousal through modulation of hypocretin/orexin neurons. J Neurosci. 2009;29(12):3705–3714.
- Bayer L, et al. Opposite effects of noradrenaline and acetylcholine upon hypocretin/orexin versus melanin concentrating hormone neurons in rat hypothalamic slices. Neuroscience. 2005;130(4):807–811.
- Thakkar MM, et al. Orexin neurons of the hypothalamus express adenosine A1 receptors. Brain Res. 2002;944(1-2):190-194.
- Liu ZW, et al. Adenosine inhibits activity of hypocretin/ orexin neurons by the A1 receptor in the lateral hypothalamus: a possible sleep-promoting effect. J Neurophysiol. 2007;97(1):837–848.
- Ferrari LL, et al. Regulation of lateral hypothalamic orexin activity by local GABAergic neurons. J Neurosci. 2018;38(6):1588–1599.
- Giardino WJ, et al. Parallel circuits from the bed nuclei of stria terminalis to the lateral hypothalamus drive opposing emotional states. Nat Neurosci. 2018;21(8):1084–1095.
- 42. Schöne C, et al. Coreleased orexin and glutamate evoke nonredundant spike outputs and computations in histamine neurons. Cell Rep. 2014;7(3):697–704.

- Carter ME, et al. Mechanism for hypocretin-mediated sleep-to-wake transitions. Proc Natl Acad Sci USA. 2012;109(39):E2635–E2644.
- 44. Mileykovskiy BY, et al. Behavioral correlates of activity in identified hypocretin/orexin neurons. *Neuron*. 2005;46(5):787–798.
- Hassani OK, et al. Orexin neurons respond differentially to auditory cues associated with appetitive versus aversive outcomes. J Neurosci. 2016;36(5):1747–1757.
- 46. Harris GC, et al. Arousal and reward: a dichotomy in orexin function. *Trends Neurosci.* 2006;**29**(10):571–577.
- Harris GC, et al. A role for lateral hypothalamic orexin neurons in reward seeking. Nature. 2005;437(7058):556–559.
- Richardson KA, et al. Lateral hypothalamic orexin/ hypocretin neurons that project to ventral tegmental area are differentially activated with morphine preference. J Neurosci. 2012;32(11):3809–3817.
- España RA, et al. Organization of hypocretin/orexin efferents to locus coeruleus and basal forebrain arousal-related structures. J Comp Neurol. 2005;481(2):160–178.
- González JA, et al. Convergent inputs from electrically and topographically distinct orexin cells to locus coeruleus and ventral tegmental area. Eur J Neurosci. 2012;35(9):1426–1432.
- 51. Iyer M, et al. Identification of discrete, intermingled hypocretin neuronal populations. *J Comp Neurol.* 2018;**52**6(18):2937–2954.
- Schöne C, et al. Dichotomous cellular properties of mouse orexin/hypocretin neurons. J Physiol. 2011;589(Pt 11):2767–2779.
- Mickelsen LE, et al. Neurochemical heterogeneity among lateral hypothalamic hypocretin/orexin and melaninconcentrating hormone neurons identified through single-cell gene expression analysis. eNeuro. 2017;4:ENE URO.0013-17.2017.
- 54. Brischoux F, et al. Ontogenetic development of the diencephalic MCH neurons: a hypothalamic 'MCH area' hypothesis. Eur J Neurosci. 2001;13(9):1733–1744.
- 55. Hanriot L, et al. A combination of LongSAGE with Solexa sequencing is well suited to explore the depth and the complexity of transcriptome. BMC Genomics. 2008:9:418.
- Yelin-Bekerman L, et al. Hypocretin neuron-specific transcriptome profiling identifies the sleep modulator Kcnh4a. Elife. 2015;4:e08638.
- 57. Yamuy J, et al. Hypocretinergic control of spinal cord motoneurons. J Neurosci. 2004;24(23):5336–5345.
- 58. Siegel JM, et al. Neuronal activity in narcolepsy: identification of cataplexy-related cells in the medial medulla. *Science*. 1991;**252**(5010):1315–1318.
- Wu MF, et al. Activity of dorsal raphe cells across the sleepwaking cycle and during cataplexy in narcoleptic dogs. J Physiol. 2004;554(Pt 1):202–215.
- John J, et al. Cataplexy-active neurons in the hypothalamus: implications for the role of histamine in sleep and waking behavior. Neuron. 2004;42(4):619–634.
- 61. Siegel JM, et al. Activity of medial mesopontine units during cataplexy and sleep-waking states in the narcoleptic dog. *J Neurosci.* 1992;12(5):1640–1646.
- Wu MF, et al. Locus coeruleus neurons: cessation of activity during cataplexy. Neuroscience. 1999;91(4):1389–1399.
- Carter ME, et al. Tuning arousal with optogenetic modulation of locus coeruleus neurons. Nat Neurosci. 2010;13(12): 1526–1533.

- Luppi PH, et al. Not a single but multiple populations of GABAergic neurons control sleep. Sleep Med Rev. 2017;32:85–94.
- Nishino S, et al. Muscle atonia is triggered by cholinergic stimulation of the basal forebrain: implication for the pathophysiology of canine narcolepsy. J Neurosci. 1995;15(7 Pt 1):4806–4814.
- Thannickal TC, et al. Opiates increase the number of hypocretin-producing cells in human and mouse brain and reverse cataplexy in a mouse model of narcolepsy. Sci Transl Med. 2018;10:eaao4953.
- 67. Valko PO, et al. Increase of histaminergic tuberomammillary neurons in narcolepsy. Ann Neurol. 2013;74(6):794–804.
- 68. Gulyani S, et al. Cataplexy-related neurons in the amygdala of the narcoleptic dog. Neuroscience. 2002;112(2):355–365.
- 69. Mahoney CE, et al. GABAergic neurons of the central amygdala promote cataplexy. J Neurosci. 2017;37(15):3995–4006.
- Oishi Y, et al. Role of the medial prefrontal cortex in cataplexy. J Neurosci. 2013;33(23):9743–9751.

- Burgess CR, et al. Dopaminergic regulation of sleep and cataplexy in a murine model of narcolepsy. Sleep. 2010;33(10):1295–1304.
- Kushida CA, et al. Electroencephalographic correlates of cataplectic attacks in narcoleptic canines. Electroencephalogr Clin Neurophysiol. 1985;61(1):61–70.
- 73. Bastianini S, et al. High-amplitude theta wave bursts during REM sleep and cataplexy in hypocretin-deficient narcoleptic mice. J Sleep Res. 2012;21(2):185–188.
- 74. Bastianini S, et al. High-amplitude theta wave bursts characterizing narcoleptic mice and patients are also produced by histamine deficiency in mice. *J Sleep Res.* 2016;25(5):591–595.
- 75. Vassalli A, et al. Hypocretin (orexin) is critical in sustaining theta/gamma-rich waking behaviors that drive sleep need. Proc Natl Acad Sci USA. 2017;114(27):E5464–E5473.