

## REVIEW

## Shining Light on Wakefulness and Arousal

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Alterations in arousal states are associated with multiple neuropsychiatric disorders, including generalized anxiety disorders, addiction, schizophrenia, and depression. Therefore, elucidating the neurobiological mechanisms controlling the boundaries between arousal, hyperarousal, and hypoarousal is a crucial endeavor in biological psychiatry. Substantial research over several decades has identified distinct arousal-promoting neural populations in the brain; however, how these nuclei act individually and collectively to promote and maintain wakefulness and various arousal states is unknown. We have recently applied optogenetic technology to the repertoire of techniques used to study arousal. Here, we discuss the recent results of these experiments and propose future use of this approach as a way to understand the complex dynamics of neural circuits controlling arousal and arousal-related behaviors.

**Key Words:** Arousal, hypothalamus, locus coeruleus, optogenetics, sleep, wakefulness

**A**lthough states of sleep and wakefulness are qualitatively and quantitatively easy to characterize, it is relatively difficult to define arousal. Sleep is characterized as a period of relative inactivity with stereotyped posture, higher sensory threshold, and unconsciousness. Wakefulness is a conscious state in which an animal can perceive and interact with its environment. States of both sleep and wakefulness can be quantitatively identified and characterized by patterns of cortical activity on an electroencephalogram (EEG) and intensity of motor activity on an electromyogram. The term arousal refers to the degree of vigilance and alertness during wakefulness. In mammals, it can be described as a state of wakefulness with increased motor activation, responsiveness to sensory inputs, emotional reactivity, and enhanced cognitive processing (1). Depending on the environmental circumstances, arousal systems increase the vigilance of an animal so that an animal can properly react to a stressor, initiate the flight-or-fight response, engage in courtship and reproductive behaviors, or engage in goal-directed behaviors that are mandatory for behavioral adaptation and survival.

Mild perturbations of arousal in humans are considered symptoms of many psychiatric disorders (2). For example, hyperarousal is a symptom of schizophrenia, addiction, and generalized anxiety disorder (3). In contrast, hypoarousal correlates with aggressiveness and attention-deficit/hyperactivity disorder (4,5). Although hypocretins, as well as other arousal circuits, participate to the setting of arousal threshold in hyperarousal or hypoarousal (6), it remains unknown whether those changes are causally linked to such symptoms. Therefore, elucidating the neurobiological substrates of wakefulness and arousal is important for understanding the intensity of vigilance, sensory responsiveness, and emotional reactivity that are often impaired in psychiatric conditions. Despite the enormous progress in identifying neural populations that regulate wakefulness and arousal, it is still unclear how these circuits are anatomically and functionally interposed to affect behavior in different environmental contexts.

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Arousal is regulated by distinct neural populations in the brain (Figure 1A,B). Activity in these nuclei is correlated with arousal: not only does their activity increase when an animal is awake compared with asleep, but this activity also increases during states of enhanced arousal, such as moments of high alertness or stress (7). These arousal systems include:

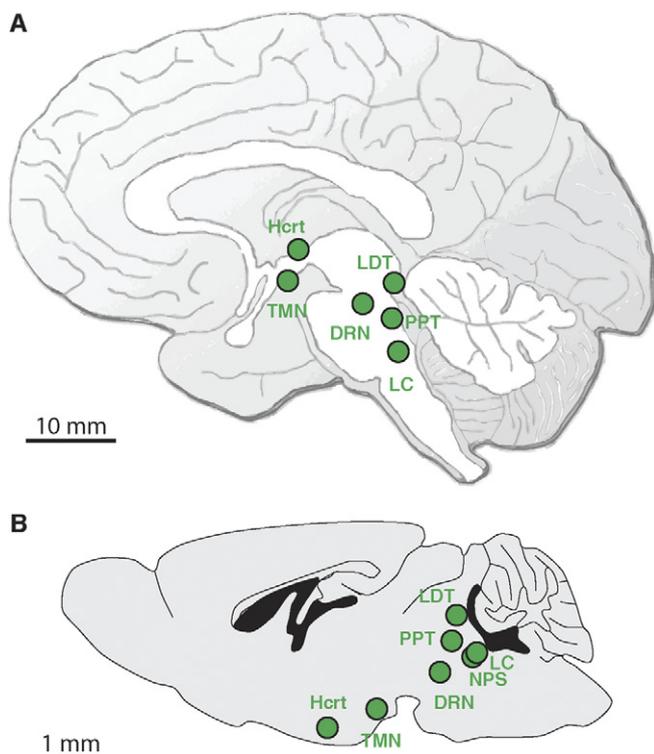
1. The hypocretin (Hcrt)-expressing neurons in the lateral hypothalamus
2. The noradrenergic locus coeruleus (LC)-expressing neurons in the brainstem
3. The neuropeptide S neurons in the brainstem
4. The serotonergic dorsal raphe nuclei in the brainstem
5. The histaminergic tuberomammillary nucleus (TMN) in the posterior hypothalamus
6. The cholinergic pedunculopontine (PPT) and laterodorsal tegmental nuclei in the midbrain, as well as cholinergic neurons in the basal forebrain
7. Glutamatergic and gamma-aminobutyric acidergic neurons located in the above-mentioned nuclei and brain areas

Activation of these systems not only promotes wakefulness but also engages other arousal-related behavioral outputs such as reward seeking, sexual activity, flight-or-fight responses, etc. Interestingly, specific behavioral outputs are not necessarily consistent from one arousal system to another. For example, activation of the LC-norepinephrine system increases arousal and can cause anxiety-like behaviors (8). In contrast, the neuropeptide S system also increases arousal but decreases anxiety (9). Thus, to support such diverse behaviors, these arousal systems must be fine-tuned based on their specific afferent and efferent connections into extremely specific circuits to regulate specific arousal-related behaviors.

How do each of these arousal systems specifically affect wakefulness and arousal? How do they functionally interact to promote and maintain particular arousal states in specific contexts? We and others have recently begun to address these questions, especially focusing on the Hcrt and LC systems. Here, we summarize recent optogenetic experiments that test the hypothesis that Hcrt and LC neurons cause arousal state transitions and maintenance. First, we briefly highlight and summarize previous reports about these systems using traditional genetic and pharmacological techniques. Next, we integrate our own findings using optogenetic probes to selectively stimulate or inhibit these systems in freely moving mice. Finally, we discuss unresolved questions and speculate on future anatomical and functional dissections of arousal circuits.

### The Hypocretin System

Hypocretins (10) are a pair of secreted neuropeptides, hypocretin-1 (Hcrt1) and hypocretin-2 (Hcrt2), that are cleaved from the same genetic precursor. These peptides are exclusively expressed



**Figure 1.** Schematic representation of the evolutionary conserved arousal systems in the human (A) and mouse (B) brains. These systems include the hypocretin (Hcrt) neurons (lateral hypothalamus), the noradrenergic neurons (locus coeruleus [LC]), the neuropeptide S (NPS) neurons (brainstem), the serotonergic neurons (dorsal raphe nuclei [DRN]), the histaminergic neurons (tuberomammillary nucleus [TMN] in the posterior hypothalamus), the cholinergic neurons (pedunculopontine [PPT] and laterodorsal tegmental [LDT] nuclei in the midbrain, as well as basal forebrain). Local glutamatergic and gamma-aminobutyric acid neurons located in the vicinity of these arousal systems are not represented.

by a population of glutamatergic neurons located in the lateral hypothalamus (Figure 2A) and bind with different affinities to two Hcrt receptors, hypocretin receptor 1 and hypocretin receptor 2 (Hcrt-r2) (11). These receptors are located on postsynaptic terminals in a pattern consistent with the efferent projections of Hcrt neurons.

Hypocretin neurons show high discharge activity during arousal elicited by environmental stimuli (e.g., an auditory stimulus) and behavior accompanied with a strong locomotor activity (e.g., goal-oriented behaviors) (12–14). These recordings suggest that in an awake state, Hcrt neurons may participate in the neurobiological mechanisms underlying alertness and in the increase in arousal observed during various goal-oriented behaviors. Hypocretin neurons are generally silent during quiet wakefulness, nonrapid eye movement (NREM) sleep, and rapid eye movement (REM) sleep and are reactivated during REM sleep-to-wake transitions.

Studies that block or suppress Hcrt signaling demonstrate that Hcrt neurons are necessary for maintaining wakefulness or emerging from anesthesia in mice, rats, dogs, and humans (15). The most compelling loss-of-function evidence comes from the link between Hcrt deficiency and the symptoms of narcolepsy (16,17). Narcoleptic patients with cataplexy have nondetectable or barely detectable levels of Hcrt in the cerebrospinal fluid and an absence of Hcrt gene transcripts in the hypothalamus. Doberman narcoleptic dogs bear a mutation in Hcrt-r2, and all genetically engineered rodents with

either a deletion of Hcrt, Hcrt-r2, or Hcrt cells present behavioral arrests that resemble cataplexy, the hallmark of narcolepsy (17).

Intracerebroventricular infusion of Hcrt peptides or Hcrt agonists causes an increase in the time spent awake and a decrease in NREM and REM sleep (15). Stereotactic injections of the peptide in the LC, LTD, basal forebrain, and lateral hypothalamus each also cause increased wakefulness and locomotor activity accompanied by a marked reduction in NREM and REM sleep. Interestingly, Hcrt administration can reverse behavioral attacks in narcoleptic dogs. More recently, genetic disinhibition of Hcrt neurons using a selective gamma-aminobutyric acid type B receptor gene deletion only in Hcrt neurons induced severe fragmentation of sleep/wake states during both the light and dark periods without showing an abnormality in total sleep/wake durations or signs of cataplexy (18). Although one should not expect the occurrence of cataplexy upon disinhibition of Hcrt neurons, it is puzzling that the total duration of sleep/wake duration remains unchanged in those conditions. A possible explanation for this sleep/wake fragmentation comes from the persistent genetic disinhibition of Hcrt neurons and thus the increased transitions from sleep to wakefulness. This is somewhat consistent with our optogenetic studies (see below).

### The Locus Coeruleus/Norepinephrine System

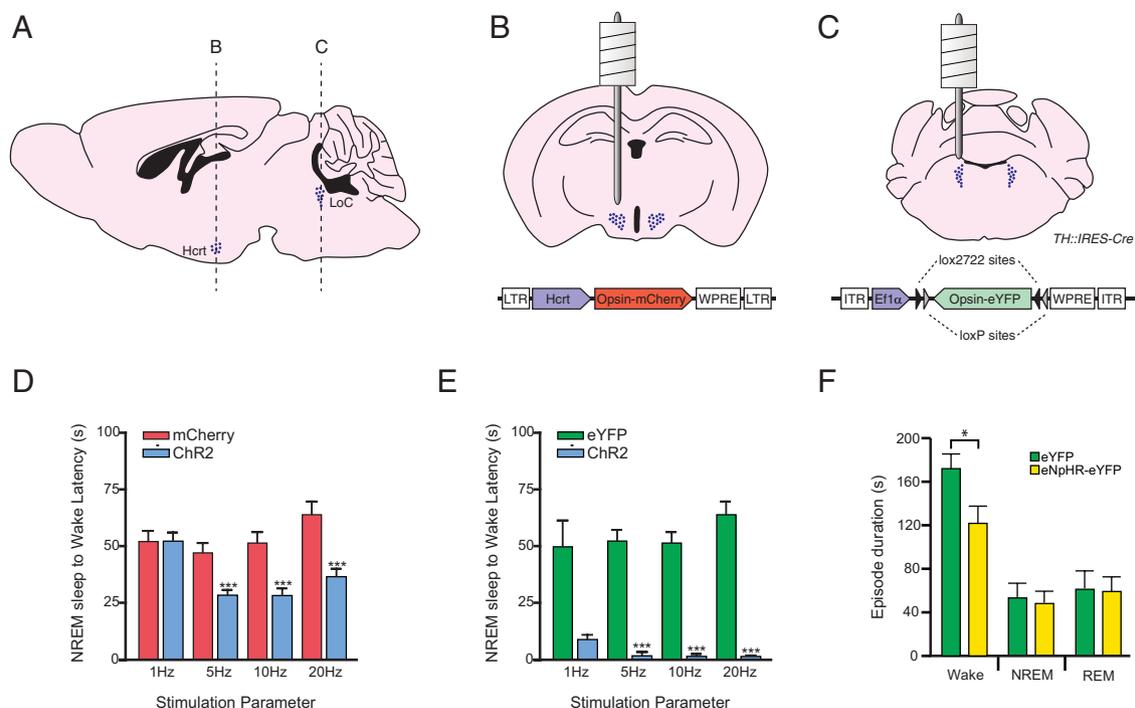
The LC is located adjacent to the fourth ventricle in the brainstem and synthesizes the monoamine norepinephrine (NE) (19–22). At least four other cell populations produce norepinephrine (the A1, A2, A5, and A7 cell groups), but the LC produces approximately 50% of the brain's total NE and is the only source to the cortex (19–22). There are many functional NE receptors located throughout the brain, with  $\alpha 1$  and  $\beta$  receptors usually causing excitatory postsynaptic potentials and  $\alpha 2$  receptors usually causing inhibitory postsynaptic potentials (19–22). Alpha-2 receptors are densely found on LC neurons themselves and serve as inhibitory autoreceptors to suppress intrinsic activity.

Recordings in awake, behaving animals show that LC neurons fire tonically at 1 Hz to 3 Hz during awake states, fire less during NREM sleep, and are virtually silent during REM sleep (23,24). The LC also fires phasically in short bursts of 8 Hz to 10 Hz during the presentation of salient stimuli that prolong wake states (23,25). Like Hcrt neurons, alterations in discharge rate precede changes in sleep-to-wake transitions (23,24).

Interestingly, physical lesions of the LC do not elicit consistent changes in cortical EEG or behavioral indices of arousal (26–28). Genetic ablation of dopamine beta-hydroxylase, an enzyme required for NE synthesis, also does not disrupt sleep-wake states (29). However, central injections of pharmacological antagonists of  $\alpha 1$  and  $\beta$  noradrenergic receptors or agonists of inhibitory  $\alpha 2$  autoreceptors have substantial sedative effects (30). In contrast, central administration of NE directly into the ventricles or forebrain promotes wakefulness (31,32). Stimulation of neurons in the LC using local microinjections of the cholinergic agonist bethanechol produces rapid activation of the forebrain EEG in halothane-anesthetized rats (33). Recently, the LC/NE system was shown to be critical for maintaining the increased membrane potential of cortical neurons in awake compared with sleep states (34). Taken together, these studies imply that the LC/NE system desynchronizes cortical activity and increases cortical membrane potential to increase arousal.

### Acute Control of Arousal Using Optogenetics

Experimental evidence reviewed in the previous sections consistently suggests that both the Hcrt and NE systems represent important arousal circuits of the brain. However, it remains un-



**Figure 2.** Optogenetic modulation of hypocretin (Hcrt) and noradrenaline neurons in vivo. Schematic drawings representing the anatomical location of Hcrt- and norepinephrine (NE)-producing neurons in sagittal (A) and coronal (B, C) sections through the mouse brain. (B) and (C) (top panels) show the stereotaxic placement of cannula guide for deep brain light delivery through optical fibers in freely moving animals. Note that the tip of the optical fiber has a short projection from the cannula guide to target the entire Hcrt or noradrenaline fields. (B) and (C) (bottom panels) represent the backbone of the virus vector used to genetically target Hcrt and NE neurons. (D) Optogenetic stimulation at high frequencies (>5 Hz) of Hcrt neurons increase the probability of nonrapid eye movement (NREM) sleep-to-wake transitions (modified from Adamantidis *et al.* [36]). (E) Optogenetic stimulation of locus coeruleus-NE neurons at all frequencies increases the probability of NREM sleep-to-wake transitions (modified from Carter *et al.* [38]). (F) The duration of individual wake, NREM, and rapid eye movement (REM) episodes during 1 hour photoinhibition during the active period. \* $p < .05$ , \*\*\* $p < .0001$ , Student *t* test between transduced animals. Chr2, channelrhodopsin-2; EF1 $\alpha$ , elongation factor 1 $\alpha$ ; eNpHR, enhanced halorhodopsin; eYFP, enhanced yellow fluorescent protein; IRES, internal ribosome entry site; ITR, inverted terminal repeat; LoC, locus coeruleus; LTR, long terminal repeat; TH, tyrosine hydroxylase; WPRE, woodchuck posttranscriptional regulatory element.

known whether 1) these systems are permissive or sufficient to promote or maintain arousal; 2) if Hcrt and NE neurons have defined patterns of discharge (e.g., spike frequency) for arousal control; and 3) if selective activation of these systems is causally associated with increased arousal. In this section, we summarize the main advantage of the optogenetic technology to dissect neural circuits controlling arousal, as illustrated by recent studies.

Selectively stimulating or inhibiting specific cell populations without affecting surrounding cells or fibers of passage has been difficult using traditional pharmacologic, electrical, and physical techniques (35). Therefore, we recently applied optogenetic techniques to reversibly and selectively manipulate the activity of Hcrt (36,37) and LC (38) neurons in freely moving animals to probe their function in sleep and wakefulness. Optogenetics is a recent technology in which a genetically encoded neuromodulatory probe is expressed in a specific cell type of interest and then activated by a specific wavelength of light (39–48). For example, channelrhodopsin-2 (Chr2) is a nonspecific cation channel that depolarizes neurons upon blue light illumination; halorhodopsin (NpHR) is a chloride pump that hyperpolarizes neurons in response to yellow light. To deliver these probes to Hcrt or LC neurons, we used lentiviral or adeno-associated viral gene delivery tools, respectively, under the control of cell-type specific promoters (Figure 2B,C) (36,37). To deliver light to the brain regions containing the Hcrt- or NE-expressing cells, we place fiber optic cables inside surgically implanted cannulae in the brain (Figure 2A–C) (36). Further information

about optogenetic technology can be found in many other excellent reviews (49–58).

We first genetically delivered Chr2 to Hcrt neurons and demonstrated millisecond-precise stimulation in vitro (36). The high temporal and spatial precision of stimulation allowed us to mimic the physiological range of hypocretin neuronal spikes (1 Hz to 30 Hz) and thus overcome the limitations of previous techniques. Indeed, parameters of the optogenetic stimulation we used were based on actual frequency analysis of Hcrt (12,59) and LC (23,25) neurons in vivo. We found that direct optical stimulation of Hcrt neurons increased the probability of transitions to wakefulness from either NREM or REM sleep (Figure 2D). Interestingly, photostimulation using 5 to 20 Hz light pulse trains reduced the latency to wakefulness, whereas 1 Hz trains did not. We also showed that the effects of stimulating Hcrt neurons could be blocked by injection of a hypocretin receptor 1 antagonist or by genetic deletion of the Hcrt gene (36). These results demonstrated a causal link between Hcrt neuron activation and sleep-to-wake transitions, as suggested by previous single unit recording studies. They also showed that Hcrt release from Hcrt-expressing neurons is necessary for the wake-promoting properties of these neurons.

A recent pharmacogenetic study confirmed these results. Sasaki *et al.* (60) used a designer receptors exclusively activated by designer drugs approach to activate and suppress Hcrt neural activity. Designer receptors exclusively activated by designer drugs technology allows bimodal modulation of neural activity with temporal

resolution of several hours (61). Sasaki *et al.* (60) found that activation of Hcrt neural activity increased wakefulness, while suppression of Hcrt activity promoted NREM. The latter result was corroborated by inhibition of Hcrt neurons using NpHR (62).

We further showed that Hcrt-mediated sleep-to-wake transitions are blocked by sleep pressure caused by sleep deprivation (63). Furthermore, downstream arousal centers such as the LC and TMN increase activity as measured by c-Fos expression in response to Hcrt stimulation, as previously suggested (64). Because the behavioral effects of Hcrt stimulation are not mediated by the histamine system in those experimental conditions (37), we focused our experimental investigations on the noradrenergic LC as an alternative target of the Hcrt system (64) for optogenetic manipulation.

Optogenetic stimulation of LC neurons caused immediate sleep-to-wake transitions from both NREM and REM sleep (38). Interestingly, the effects of LC stimulation occurred within seconds of the onset of stimulation, while the effects of Hcrt stimulation took an average of 20 to 30 seconds to cause awakenings (Figure 2D vs. 2E). Stimulation of LC neurons during wakefulness increased locomotor activity and the total time spent awake. Inhibition of LC neurons with NpHR decreased the duration of wake episodes but did not block sleep-to-wake transitions when animals were asleep (Figure 2F). Taken together, these results demonstrate that LC activity is sufficient to promote wakefulness from sleep and general locomotor arousal but is not necessary for animals to wake from sleep, probably due to lack of activity (or disinhibition) in other arousal systems.

It is interesting that acute stimulation of Hcrt neurons causes sleep-to-wake transitions over an average time period of 10 to 30 seconds, while acute stimulation of LC neurons causes sleep-to-wake transitions in less than 5 seconds. There are at least three possible reasons for this difference. First, the Hcrt study was the first to use *in vivo* optogenetics through unilateral stimulation, while the LC study uses bilateral stimulation. Bilateral stimulation of Hcrt neurons reduces latency of sleep-to-wake transitions even more, although to a less dramatic effect than NE (A.A., unpublished data; 2010). Second, the effect of Hcrt neurotransmission on downstream targets (mediated by the dynamics of Hcrt receptors and interactions with other ion channels) may not be as potent as the effect of NE neurotransmission. Third, Hcrt neurons are farther upstream in the neural circuitry of arousal, with the LC, TMN, and other populations (dorsal raphe, pedunculopontine tegmentum-PPT) acting as the main effectors in a putative hierarchy (65), suggesting the importance of Hcrt activation of not only the LC neurons but also the dorsal raphe and the PPT. Finally, perhaps the effects of photostimulation of ChR2-transduced neurons in Hcrt and LC neurons are different due to the different gene delivery methods used to target each structure. We used a lentivirus carrying an endogenous 3.1 kilobase Hcrt promoter to target Hcrt neurons and an adeno-associated virus using a strong elongation factor 1 $\alpha$  promoter, in combination with a tyrosine hydroxylase::Cre knockin mouse, to target LC neurons. Expression of reporter genes is substantially higher in the LC than in Hcrt neurons, indicating a much lower expression of ChR2 in Hcrt cells. It is unclear whether this reduced expression results in reduced effects of photostimulation.

It is also interesting that relatively long periods of photostimulation over 1 to 4 hours caused differential effects on arousal between Hcrt and LC neurons. Long-term stimulation of Hcrt neurons increased sleep-to-wake transitions but did not increase the total duration of wakefulness (36,37). In contrast, long-term stimulation of LC neurons significantly reduced the total duration of wakefulness with an increase in locomotor behavior (38). One explanation is that Hcrt neurons play a more specialized role in regulating the

boundaries between sleep-wake transitions based on hypothalamic-related functional inputs (circadian rhythms, metabolic status and associated stress response, and homeostatic sleep pressure), while LC neurons have a greater impact on increasing cortical membrane potential and desynchronizing the cortical EEG to promote wakefulness, attention, or hyperarousal.

An important question for future investigation is whether Hcrt and LC neurons promote wakefulness in parallel or participate in the same arousal circuit. For example, Hcrt neurons project strongly to the LC. It is likely that Hcrt neurotransmission is relayed by the LC to the cortical microcircuitry, where NE induces desynchronization and raises the membrane potential of individual neurons.

### Aroused, not Just Awake

The ability to target and selectively manipulate Hcrt and LC neurons allows the opportunity to study these nuclei in different contexts of hyperarousal. For example, in addition to promoting wakefulness, Hcrt neurons have also been implicated in rodent models of food intake, addiction, stress, attention, and male sexual arousal (15). Locus coeruleus neurons have been implicated in many similar behaviors including stress, addiction, attention, learning and memory, and depression (20). These studies beg the question, which stands for most modulatory systems of the brain: how can these arousal systems play a role in such a wide spectrum of behaviors? How are these circuits anatomically and functionally organized to modulate the diverse forms of arousal?

One possibility is that Hcrt and LC neurons are organized into subpopulations such that each module projects to different downstream structures and therefore mediates different functions. For example, Harris and Aston-Jones (66) proposed that Hcrt neurons are functionally organized such that reward and cue processing are mediated by the lateral Hcrt field by projecting to the ventral tegmental area, while drug-related stress or aversive behaviors are mediated by dorsomedian and perifornical Hcrt neurons (66,67). However, such a dichotomy has not been verified anatomically (65,68). In addition, although subpopulations of Hcrt neurons exhibit distinct electrophysiological properties and can be differentiated on their integration of metabolic changes (e.g., glucose) (69–71), this functional dichotomy has not been matched with anatomical territories of the lateral hypothalamus. Recent studies have also found that Hcrt neuron response to NE *in vitro* varies with previously experienced sleep pressure (72,73). Furthermore, the functional characterization of Hcrt neurons using immunohistochemical detection of the immediate early gene c-Fos after behavioral challenges has not presented evidence for functional subpopulations of neurons (74–76). As already documented in zebrafish and mice (19), exciting new circuit-labeling tools such as rainbow technology (77) may help to anatomically or functionally reconstruct the connectivity of individual Hcrt neurons in intact mammalian systems, which may ultimately reveal precise anatomical features of these neurons.

An alternative hypothesis to explain how arousal systems may differentially affect arousal-related behaviors is through different release of peptides and neuromodulators depending on specific patterns of activity. For example, it is possible that Hcrt neurons may release different contents from synaptic vesicles during synaptic neurotransmission depending on phasic or tonic activity. In addition to Hcrt, these neurons secrete glutamate, dynorphin, and likely other transmitters/modulators (78). Thus, the vesicular content from Hcrt-containing terminals includes small vesicles with glutamate and large, dense core vesicles with Hcrt and other modulators. Small vesicles are thought to be released upon slow tonic

firing of the cell, whereas the release of large vesicles happens upon firing of the cell in burst mode. Although this hypothesis has never been tested in regard to the Hcrt system, it is reasonable to propose that low-frequency firing of Hcrt neurons (as it occurs during quiet wakefulness) may induce the release of glutamate. In contrast, burst firing of the neurons during sleep-to-wake transitions or goal-oriented behaviors may preferentially provoke the release of neuropeptide-containing vesicles (e.g., hypocretins, dynorphin, and others). Interestingly, similar mechanisms have been observed for other peptidergic neurons (79–82); however, it remains unknown whether such mechanisms have significant behavioral consequences.

In addition, the composition of vesicular glutamate transporters in modulatory systems of the brain, such as the serotonergic system, and the vesicular synergy—a process for enhanced packaging and the release of the primary transmitter—have been recently highlighted as possible mechanisms of synapse specialization (83). Thus, it is possible that the local composition of vesicular glutamate transporters in the Hcrt terminals may condition the vesicular composition of the Hcrt terminals and thus its synaptic transmission. This may define new anatomical terminals territories in which Hcrt neurons would differentially affect postsynaptic cells, depending on the firing mode of the cells and the targeted brain area. Although this is purely speculative, it may diversify the functional repertoire of Hcrt and NE neuron modulation by releasing a combination of different transmitters that would result in a regionalized, yet highly specific, postsynaptic response and possibly behavioral output as well. Optogenetics makes it possible to stimulate arousal systems with different frequency patterns and examine the output at different synapses, and this will be an important opportunity for future study.

### Arousal in Neuropsychiatric Disease

Changes in arousal states and thresholds are at the core of most neuropsychiatric disorders, including depression, anxiety, schizophrenia, and posttraumatic stress disorder. The control of arousal in the mammalian brain results from a balance between inhibitory and excitatory networks highly organized on both temporal and spatial scales. Subtle imbalance in the temporal dynamics of arousal circuits impede the parameters of the sleep-wake cycle, which frequently results in altered cognitive functions (84–86). Hyperarousal is associated with schizophrenia, addiction, generalized anxiety disorder (3), and posttraumatic stress disorder, while hypoarousal correlates with depression, aggressiveness, and attention-deficit/hyperactivity disorder (4,5).

The boundary between hyperarousal and hypoarousal in a given psychiatric disorder might be less clear, as it is the case in narcolepsy. Narcolepsy is characterized by a cluster of distinct symptoms, including hypnagogic hallucinations, sleepiness, sleep paralysis, and cataplexy. Cataplexy is complete loss of skeletal muscle tone during awake or alert states upon athletic activity, laughter, anger, or other rapid emotions trigger (87). Thus, human narcoleptic patients exhibit both hyperarousal during the night (fragmented sleep) and hypoarousal during the day (inability to maintain long wake periods) (87). Although the underlying mechanism of cataplexy remains unknown, increasing experimental evidence using a wide variety of tools has identified the Hcrt system as necessary for proper sleep-wake cycle and muscle tone (15,88). In addition to the lack of Hcrt function, NE neurons in the LC cease firing during cataplexy (89), which supports a role for NE in maintaining appropriate muscle tone, as well as arousal, during goal-oriented behaviors in mammals.

Although the Hcrt and NE systems modulate arousal with different kinetics (as discussed above), our optogenetic approach suggests they are systems linked with hyperarousal. Our results are in agreement with the hypothesis that Hcrt and NE neurons are necessary to maintain appropriate arousal levels during strong positive or negative emotions (e.g., stress, joy, laughter) (90) that typically trigger cataplexy when the Hcrt system is not functional (87). The role of the NE neurons in narcolepsy remains unclear; however, the lack of a functional Hcrt-NE link to transfer emotion or motivational state into hyperarousal might explain some of the cataplexy symptoms, as suggested by our recent studies (37,38).

In addition to sleep, Hcrt neurons have recently been shown to tune motivational states related to food intake (91–93), sexual behavior (94), drug intake (67,95–97), stress (98), and depressive-like behaviors (99,100). These studies induced hypoarousal by decreasing Hcrt tone. In contrast, NE transmission has been shown to be crucial for attention and cognitive function (21). Activation of the NE system increases arousal and causes anxiety-like behaviors (8). Although a causal role for imbalance of Hcrt or NE activity and hypoarousal or hyperarousal is strongly suggested by the literature, it remains unknown whether dysfunction of these systems is responsible for the hypoarousal or hyperarousal states associated with neuropsychiatric conditions. Which comes first is debatable and requires extended preclinical investigation.

### Conclusions

Optogenetics has allowed us to make major advances in our understanding of the Hcrt and LC/NE systems, and this technology should be used to dissect other arousal systems as well. Due to the multitasking properties of the Hcrt and NE systems in a wide variety of brain functions, it is conceptually and experimentally difficult to separate the arousal from the motivational consequence upon activation of those systems. Optogenetic tools now provide a better alternative for controlling neural circuits *in vivo* to examining sleep/wake boundaries. In future research, it will be important to determine the mechanisms by which each arousal system affects hypoarousal and hyperarousal based on anatomical projections, synaptic neurotransmission, and the frequency patterns of stimulation. Such experiments will undoubtedly shine light on how neural circuits share such functions in the normal and the pathological brain.

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