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Current Opinion in Neurobiology

Circuit mechanisms of sleepiness and cataplexy in narcolepsy

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Narcolepsy is a debilitating sleep disorder caused by loss of orexin neurons in the lateral hypothalamus. Excessive daytime sleepiness and cataplexy are the major complaints in narcolepsy, and are associated with impaired quality of life. Although it is unclear how orexin loss causes sleepiness and cataplexy, animal models have been instrumental in identifying the neurobiological underpinnings of narcolepsy because they reliably recapitulate disease symptoms. Current evidence indicates that orexin cell loss causes sleepiness and cataplexy by destabilizing the ability of the circuits that initiate and sustain normal levels of arousal and motor activity. This review highlights the latest research concerning the normal function of the orexin system and how its dysfunction causes narcolepsy symptoms.

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Current Opinion in Neurobiology 2017, **44**:50–58

This review comes from a themed issue on **Neurobiology of sleep**

Edited by **Yang Dan** and **Thomas Kilduff**

For a complete overview see the Issue and the Editorial

Available online 23rd March 2017

http://dx.doi.org/10.1016/j.conb.2017.02.010

Introduction

Narcolepsy is a debilitating sleep disorder that can impair a person's ability to work, socialize, and drive safely. It is estimated that people with narcolepsy experience a quality of life that is as poor or worse than patients with Parkinson's disease, epilepsy or sleep apnea [1,2]. Narcolepsy affects 0.02–0.07% of North Americans [3,4] and is caused by loss of orexin (hypocretin) neurons in the lateral hypothalamus [5–7]. The cause of orexin cell death is unknown, but multiple lines of evidence suggest that an autoimmune disturbance is involved [8–11]. However, recent evidence challenges this view [12•].

Narcolepsy is characterized by relentless sleepiness, disturbed sleep, cataplexy, sleep paralysis, sleep-onset REM sleep periods, and hypnagogic hallucinations [13]. Excessive sleepiness is usually the presenting symptom, with cataplexy developing over the next few months and persisting thereafter [14]. Cataplexy and sleepiness are the major complaints in narcolepsy, and are associated with difficulty executing daily activities, socializing, and maintaining personal relationships.

Although orexin cell loss underlies narcolepsy, it is unclear how orexin loss leads to disease symptomatology. This review will highlight the most recent experimental data describing the normal biological function of the orexin system and how orexin loss leads to sleepiness and cataplexy in narcolepsy. Our working hypothesis is that the orexin system normally functions to stabilize the circuits that initiate and sustain both arousal and motor activity, and that its loss in narcolepsy destabilizes these circuits to cause sleepiness and cataplexy.

Models of narcolepsy

Animal models have been an invaluable resource for both identifying the cause of narcolepsy and determining the mechanisms that underlie its major symptoms. Narcoleptic dogs were the first animal model of this disorder and were pivotal in showing that a mutation in the orexin-2 receptor causes canine narcolepsy [15], and this initial discovery led to the eventual observation that orexin cell loss underlies human narcolepsy [6,7].

Mouse models have also been useful in showing that loss of the orexin ligand, loss of orexin cells or mutation of orexin receptors can trigger narcolepsy symptoms (Table 1) [16–19], and they have been instrumental in identifying orexin's role in normal physiology. For example, orexin knockout mice have been used to show that the orexin system participates in stabilizing sleep–wake behaviour, regulating metabolism and feeding, and facilitating behaviours associated with motivation, reward and addiction [20–22].

One of the newest and most useful narcolepsy models are mice in which orexin cells can be conditionally ablated. Tabuchi *et al.* used the Tet-off system to control diphtheria toxin A accumulation in orexin neurons, thereby allowing for temporal neuron degeneration [23••]. They found that normal sleep architecture is disrupted after 80% of orexin neurons die, but that cataplexy only developed once 95% of cells are lost. This model has therefore

Table 1

Behavioural and metabolic symptoms of mouse models of narcolepsy

Strain	Sleepiness	Cataplexy	Metabolic
Preproorexin knockout [16]	Hypersomnia Fragmented wake, NREM, REM Decrease wake time in dark period Increased NREM sleep Sudden onset REM periods Decreased REM sleep onset Increased REM sleep time	Yes	Reduced activity Increased weight
OX_1R knockout [17]	Mild NREM fragmentation Moderate increase in REM sleep	No	–
OX_2R knockout [18]	Hypersomnia Fragmented wake, NREM Moderate REM fragmentation Decreased wake time in dark period Increased NREM sleep time Decreased latency to REM onset	Yes	–
OX_1R and *OX_2R* double knockout [17]	Hypersomnia Decreased wake time in dark period Decreased REM sleep onset Increased REM sleep time	No	–
Orexin/ataxin-3 [19]	Hypersomnia Fragmented wake, NREM, REM Decrease wake in dark period Sudden onset REM periods Decreased REM sleep onset Increased REM sleep time Decreased intervals between REM sleep episodes	Yes	Disrupted metabolism Decreased locomotion, feeding, drinking, energy expenditure Increased weight-obesity
Orexin-DTA [23••]	Hypersomnia Fragmented wake, NREM, REM Decreased wake in dark period Increased REM sleep time Decreased REM sleep onset	Yes	Increased weight Decreased drinking, locomotion
CD8 T-orexin [25••]	Sleep attacks	Yes	–

become an important resource for correlating the magnitude and timing of orexin cell loss with the onset of candidate disease symptoms.

Although evidence suggests that narcolepsy is of autoimmune origin [24•], no previous animal model has effectively shown that immunological factors contribute to the loss of orexin neurons. Recently, Bernard-Valnet *et al.* showed that CD8T cell-mediated death of orexin neurons in engineered mice can lead to both disturbed sleep and cataplexy [25••]. This new mouse model represents an important advance in how autoimmune factors contribute to orexin death in narcolepsy because it demonstrates the potential role of cytotoxic CD8T cells as final effectors of the immunopathological process in narcolepsy.

Models of narcolepsy have also been valuable for identifying the mechanisms of action of pharmacological treatments for narcolepsy. Both tricyclic antidepressants and sodium oxybate are effective in reducing cataplexy, but their mechanisms of action remain speculative. Studies in narcoleptic mice have been useful in showing that tricyclic antidepressants may reduce muscle paralysis in cataplexy by increasing noradrenergic tone onto skeletal motoneurons [26], and that sodium oxybate appears to reduce cataplexy by a $GABA_B$ receptor mediated mechanism [27•].

Mechanisms underlying sleepiness in narcolepsy

Persistent daytime sleepiness is a defining feature of narcolepsy. It is unclear how orexin cell loss causes this troublesome symptom, but there are several possible explanations. Because most people with narcolepsy also have disturbed nighttime sleep, one possibility is that sleepiness stems from poor nighttime sleep quality. Indeed, many people with narcolepsy report that their cataplexy is worsened when they are sleep deprived, suggesting that poor sleep quality can exacerbate daytime symptoms. However, clinical studies show that nighttime sleep quality is poorly correlated with cataplexy and

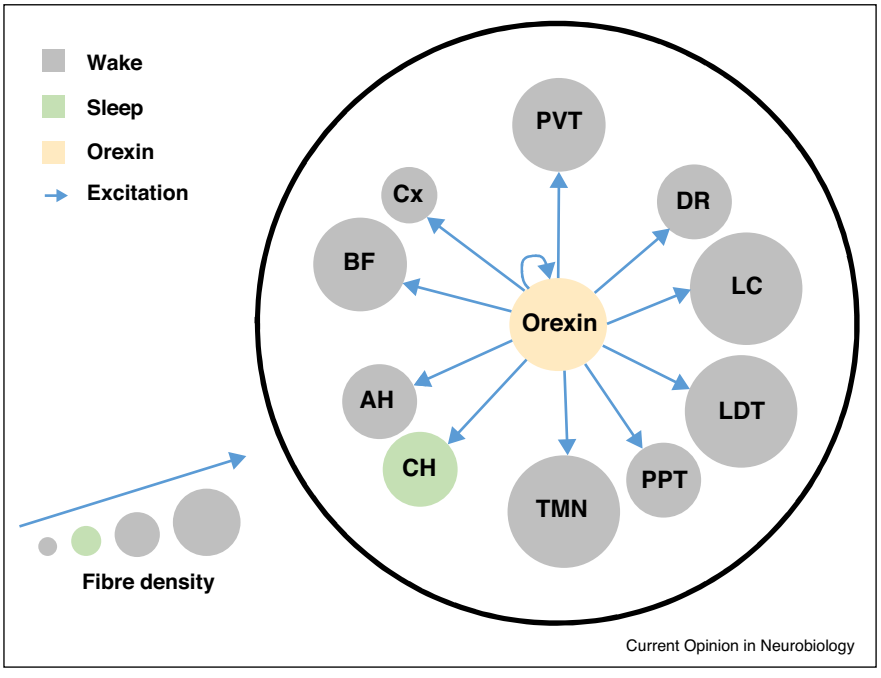
daytime sleepiness [28]. Also, most narcoleptics typically report feeling well rested in the morning or after a daytime nap, suggesting that their sleepiness is not caused by poor sleep. However, sodium oxybate is an effective treatment for sleepiness in narcolepsy, and data suggest that it functions by reducing sleep fragmentation and improving slow-wave sleep quality [29], indicating that some degree of daytime sleepiness could result from poor sleep quality.

Another explanation for sleepiness is that orexin cell loss destabilizes the circuits that promote arousal. Multiple lines of anatomical, electrophysiological and behavioral data support the idea that the orexin system functions to promote and stabilize wakefulness. Orexin cells are predominantly active during wakefulness [30–32]. Orexin release is highest during wakefulness, particularly at wake onset, whereas it is lowest during sleep and at sleep onset [30,33]. Also, orexin cells project to and activate wake-promoting cell groups (Figure 1), including the locus coeruleus (LC) [34], tuberomammillary nucleus (TMN), dorsal raphe (DR), laterodorsal tegmentum/pedunculopontine nucleus (LDT/PPT) [35], and basal forebrain (BF) [35–37], suggesting that they facilitate arousal by stimulating arousal-promoting circuitry.

Optogenetic activation of orexin cells promotes arousal from sleep [38], while optogenetic inhibition increases slow-wave sleep [39]. However, these arousal-promoting effects are lost when noradrenergic cells in the LC are inactivated [40]. Orexin cells may also engage wakefulness by activating histaminergic cells in the TMN [41,42], serotonergic cells in DR [43,44] and cholinergic cells in the LDT/PPT [45,46•]. For example, increasing orexin receptor levels in these nuclei consolidates sleep–wake architecture in narcoleptic mice. These results suggest that orexin normally functions to activate wake-promoting circuits (Figure 1), and that orexin loss causes sleepiness and sleep fragmentation by reducing excitatory drive to the circuits that support arousal.

Two other important considerations concerning mechanisms of sleepiness are that wake-promoting circuits project to and inhibit sleep-inhibiting circuitry (*e.g.*, ventrolateral preoptic area) [47], and that orexin cells themselves have a feed-forward auto-excitatory mechanism that maintains their activity once they become active [48]. Therefore, orexin cell death could function to destabilize arousal and produce sleepiness by reducing their sustained auto-excitatory drive to wake-promoting circuits and by reducing inhibition of the circuits that support sleep (Figure 1).

Figure 1



A schematic representation of the connections between orexin neurons and sleep–wake circuitry. Orexin neurons in the lateral hypothalamus project to and modulate the activity of both sleep- and wake-promoting circuits, but the magnitude of their innervation varies between different regions. Orexin neurons densely innervate wake-promoting cells in the locus coeruleus (LC), tuberomammillary nucleus (TMN), laterodorsal tegmentum (LDT) and basal forebrain (BF). They also send dense projections to the paraventricular thalamus (PVT). Orexin neurons moderately innervate the dorsal raphe (DR), pedunculopontine nucleus (PPT), anterior hypothalamus (AH), and cortex (Cx). Moderate levels of orexinergic innervation have also been identified in NREM sleep-promoting regions in the caudal hypothalamus (CH). In addition, orexin cells project to and excite local glutamate neurons in the LH in an auto-excitatory fashion.

Mechanisms underlying cataplexy

Although excessive sleepiness is the most common symptom in narcolepsy, the pathogenomic symptom of this disorder is cataplexy. Cataplexy only occurs in 70% of narcoleptics [49] and it is at this symptom where diagnosis of narcolepsy diverges. Type-1 narcolepsy refers to patients with both excessive sleepiness and cataplexy, whereas type-2 narcolepsy presents with excessive sleepiness alone [50]. Cataplexy is incapacitating because it leaves the affected individual awake, but either fully or partially paralyzed. The mechanism responsible for muscle paralysis during cataplexy is unknown, but is hypothesized to result from intrusion of REM sleep paralysis (atonia) into wakefulness [50–52]. Although cataplexy can occur spontaneously, it is typically triggered by strong positive emotions such as laughter or delight [49,50, 52,53], suggesting that brain structures that mediate emotional context are altered in narcolepsy.

A longstanding hypothesis in sleep medicine is that cataplexy results from intrusion of REM sleep paralysis into wakefulness [50,51,54,55]. This hypothesis stems from the fact that most narcolepsy symptoms result from disturbances in REM sleep phenomena [56]. Narcoleptics frequently transition directly into REM sleep, which is exceedingly rare in healthy people [55,57]. They also experience hypnagogic hallucinations [58,59] and sleep paralysis [58,60,61]. Like cataplexy, sleep paralysis resembles the muscle paralysis of REM sleep.

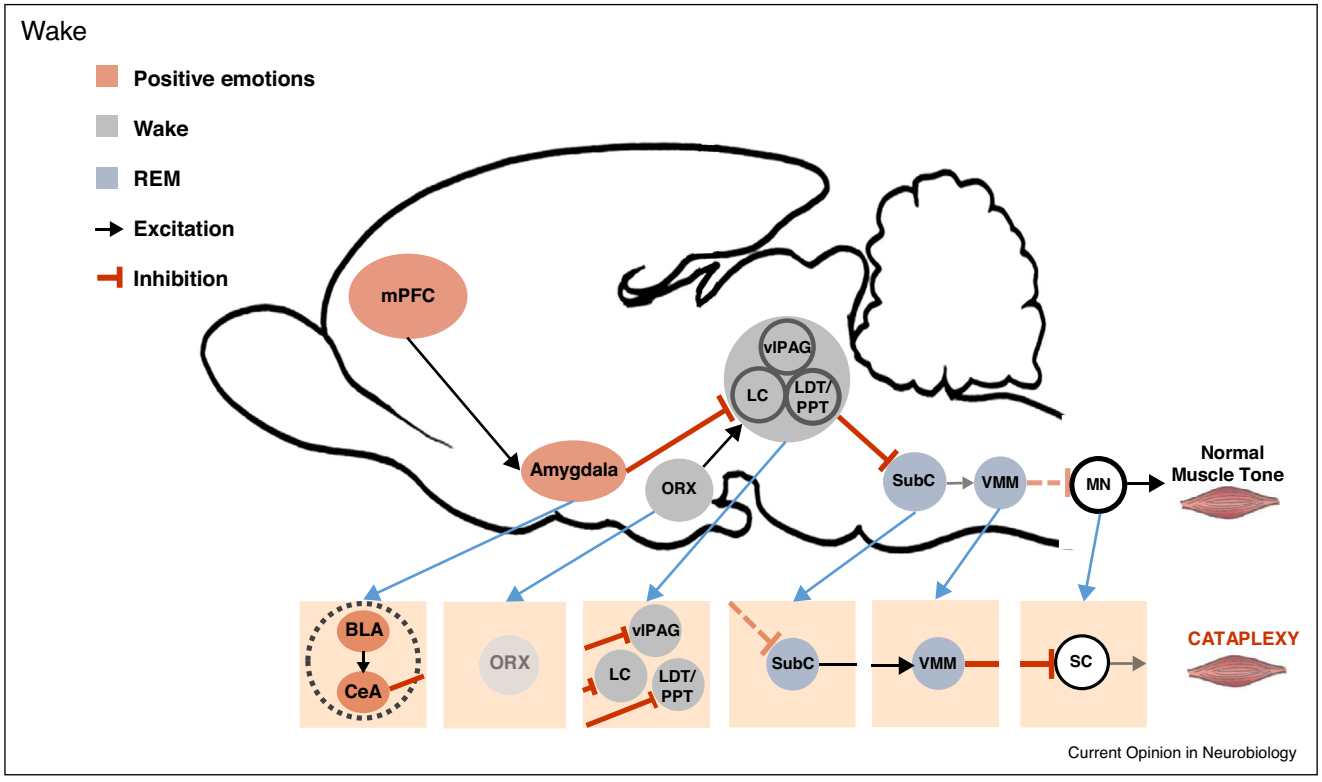
Different lines of evidence indicate that cataplexy and REM sleep paralysis share a common neural mechanism. Tricyclic antidepressants which are used to alleviate cataplexy also suppress REM sleep [62]. Rapid withdrawal from these drugs causes large rebounds in both cataplexy and REM sleep [63,64]. Deep tendon and monosynaptic H-reflexes are also absent during both cataplexy and REM sleep [65,66]. Functional imaging studies show that brainstem regions that are active during REM sleep are also active during cataplexy [67–69]. *In vivo* unit recordings show that REM sleep circuitry behaves similarly during both REM sleep and cataplexy. In narcoleptic dogs, cells in the LC abruptly stop firing during both REM sleep and cataplexy [70], and cells in the ventromedial medulla (a region that promotes REM sleep paralysis) increase their activity during both REM sleep and cataplexy [71]. Although REM sleep and cataplexy share many commonalities the most striking difference between these behaviours is consciousness, where environmental awareness is present in cataplexy but absent in REM sleep.

It is hypothesized that cataplexy is triggered by inappropriate recruitment of the circuits that generate REM sleep paralysis because skeletal muscle paralysis/weakness is a defining feature of both REM sleep and cataplexy. REM sleep paralysis is generated by a two-part circuit located in the brainstem [72]. The subcoeruleus nucleus (SubC) represents the core of this circuit (Figure 2) and multiple studies show that REM paralysis can either be produced or eliminated by manipulating SubC function. Chemical stimulation of SubC cells generates muscle paralysis in cats and rats [73–75], whereas, lesions of the SubC abolish REM sleep paralysis in behaving cats, rats, and mice [76–79]. Glutamate cells in the SubC are likely responsible for producing REM atonia because they are active during REM sleep [80,81••] and lesions of glutamatergic SubC cells eliminates REM atonia [79]. Cataplexy may be triggered by SubC cells because cataplexy attacks are increased in orexin knockout mice following chemogenetic activation of the SubC and cataplexy-like attacks are induced in wild-type mice following SubC activation [82]. Together, these observations suggest that cataplexy may result from pathological recruitment of the core circuit that causes REM sleep paralysis, and that muscle paralysis in REM sleep and cataplexy stem from a common neural mechanism.

Glutamatergic cells in the SubC induce REM sleep paralysis by projecting to and activating GABA and glycine neurons in the ventromedial medulla (VMM), which in turn induces muscle atonia by inhibiting skeletal motoneurons (Figure 2). VMM lesions abolish REM paralysis and VMM stimulation triggers muscle paralysis in both awake and decerebrate animals [73,75]. However, recent evidence suggests that GABA cells in this region may also be essential for initiating and maintaining the state of REM sleep [83••]. VMM cells are active during REM sleep paralysis and cataplexy [71] and electrical stimulation of GIG neurons triggers brief lapses in muscle tone in awake cats [73], suggesting that cataplexy may be mediated by a REM sleep mechanism (Figure 2). However, circuits that do not directly control REM sleep may also be involved. For example, serotonin cells in the DR, which play a debatable role in REM sleep control [84], also influence cataplexy. Restoration of orexin receptors onto DR neurons in mice lacking orexin receptors can decrease cataplectic attacks in this model of narcolepsy [85•].

Although cataplexy can occur spontaneously, it is typically associated with strong positive emotions such as laughter, joking, or elation [86,87]. Less frequently, it is associated with negative emotions such as fear, anger, or frustration [86]. In animal models of narcolepsy, cataplexy is elicited by conditions associated with positive value. In narcoleptic dogs, cataplexy is triggered by palatable foods, play or sex [71,88], and, in narcoleptic mice, cataplexy is increased by rewarding stimuli such as social reunion, running wheels, and palatable food [53,89–91]. The strong association between positive emotions and cataplexy suggests the involvement of circuits that encode emotion content.

Figure 2



Circuit mechanisms controlling REM sleep paralysis (atonia) and cataplexy. The subcoeruleus nucleus (SubC) and ventromedial medulla (VMM) in the brainstem constitute the core REM sleep circuit generating muscle paralysis. When glutamate neurons in the SubC switch-on they activate GABA and glycine neurons in the VMM such that they trigger REM sleep atonia by hyperpolarizing skeletal motor neurons (MN). In narcolepsy, positive emotions activate cortical areas such as the medial prefrontal cortex (mPFC), which innervate the basolateral area (BLA) neurons that project to GABA neurons in the central nucleus (CeA). GABA CeA neurons then inhibit the locus coeruleus (LC), laterodorsal tegmentum/ pedunculopontine nuclei (LDT/PPT), and ventrolateral periaqueductal grey (vlPAG), which in turn disinhibits the SubC, thereby allowing it to activate the VMM to produce motor neuron inhibition and hence muscle paralysis during cataplexy. But, in non-narcoleptics, positive emotions are unable to trigger muscle paralysis because CeA-mediated inhibition of the LC, LDT/PPT, and vlPAG is counterbalanced by excitatory orexin inputs, which prevent positive emotions from accessing the circuits (*i.e.*, SubC-VMM) that trigger muscle paralysis.

The amygdala not only underlies the processing of emotions, but is also associated with REM sleep regulation [92], and could therefore underlie cataplexy. Although the amygdala is traditionally associated with negative emotions, recent studies demonstrate its involvement in processing positive emotions and rewarding stimuli [92]. The link between the amygdala and cataplexy is supported by imaging studies showing that it is active during cataplexy [67], and unit recordings in narcoleptic dogs which show that cell activity within the central nucleus of the amygdala (CeA) is tightly linked to cataplexy, with CeA cells switching on when cataplexy begins and off when it ends [93]. Importantly, in orexin knockout mice, lesions of the CeA markedly reduce cataplexy and AAV-driven expression of orexin within the amygdala suppresses cataplexy [94•], suggesting that the amygdala is involved in promoting cataplexy.

Cells in the CeA may function as a 'relay center' between the cortical structures that interpret emotional stimuli and the brainstem circuits that generate motor paralysis during cataplexy (Figure 2). This idea stems from the fact that rewarding conditions activate the medial prefrontal cortex (mPFC), which innervates circuits within the amygdala [91,95,96]. Connections between the mPFC and CeA are integral in promoting cataplexy because removing either of them suppresses cataplexy in orexin knockout mice [91,97]. GABA cells are the primary extrinsic pathway from the CeA [98–100] and they innervate the LC, lateral pontine tegmentum (LPT), and ventrolateral periaqueductal grey (vlPAG), which collectively function to facilitate waking muscle tone by silencing atonia-generating regions in the SubC. Indeed, lesions of the LPT/vlPAG [76,101] and reduced noradrenergic release from the LC [40] can trigger cataplexy-like attacks in awake rodents. Positive emotions may therefore elicit cataplexy by activating the mPFC, which switches on GABA CeA cells that inhibit the LC, LPT, and vlPAG thereby generating muscle paralysis by disinhibiting the SubC (Figure 2). In healthy people, strong

positive emotions do not initiate muscle paralysis because orexin cells excite the LC, LPT, and vlPAG, which prevents disinhibition of SubC cells. However, in narcolepsy, orexin cell loss upsets this balance, so that GABA CeA cells are unopposed in inhibiting the LC, LPT and vlPAG, thus creating a circuit environment conducive to muscle atonia and hence cataplexy (Figure 2).

Conclusions

Here, we examined the evidence that the orexin system normally functions to stabilize the circuits that initiate and sustain arousal and motor activity, and that orexin loss in narcolepsy destabilizes these circuits to cause sleepiness and cataplexy. We provided an overview of the various animal models (Table 1) that have been used to show how orexin cells are lost in narcolepsy and how their loss triggers sleepiness and cataplexy.

We highlighted that orexin cells project to and activate numerous wake-promoting cell groups, including the LC, TMN, DR, LDT/PPT and BF, which suggests that orexin cells may normally function to facilitate arousal by stimulating and coordinating the activity of these arousal-promoting circuits (Figure 1). We also pointed out that orexin cells possess an auto-excitatory mechanism that sustains their activity once active and that orexin cell death in narcolepsy leads to destabilization of arousal-promoting circuits. These arousal circuits are then no longer able to reliably consolidate arousal, which leads to fragmented sleep–wake behaviours and sleepiness.

We also suggested that the muscle paralysis in cataplexy stems from pathological recruitment of the circuits that normally induce REM sleep paralysis (Figure 2). We also argued that positive emotions, which normally trigger cataplexy, result from activation of the GABA cells in the CeA, inhibit cells in the LPT, vlPAG and LC, which normally function to support waking muscle tone. Inhibition of these cell groups leads to disinhibition of the SubC and activation of the medullary circuits (*i.e.*, VMM) that triggers muscle paralysis (Figure 2).

Although not discussed in detail, additional brain circuits also mediate cataplexy. Cells in the zona incerta, LDT, and dorsal pons have been shown to suppress cataplexy [91,102–104]. In addition, noradrenergic, serotonergic, and dopaminergic systems also appear to contribute to cataplexy as their manipulation influences cataplexy [85,105–109]. In addition, the trace amine-associated receptor-1 has recently been identified as a novel therapeutic target for narcolepsy, as agonism of this receptor increases wakefulness and suppresses cataplexy (SW Black *et al.*, unpublished). While multiple brain circuits, including GABA cells in the CeA, are involved in controlling cataplexy, it remains unclear how they communicate with and influence one another. Understanding how these systems function together represents a major challenge in identifying circuit mechanisms of cataplexy in narcolepsy.

Acknowledgements

Funding was provided to JP from the National Science and Engineering Research Council and the Canadian Institutes of Health Research.

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- •• of outstanding interest

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