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Animal Models of Narcolepsy

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Abstract

Narcolepsy is a debilitating sleep disorder with excessive daytime sleepiness and cataplexy as its two major symptoms. Although this disease was first described about one century ago, an animal model was not available until the 1970s. With the establishment of the Stanford canine narcolepsy colony, researchers were able to conduct multiple neurochemical studies to explore the pathophysiology of this disease. It was concluded that there was an imbalance between monoaminergic and cholinergic systems in canine narcolepsy. In 1999, two independent studies revealed that orexin neurotransmission deficiency was pivotal to the development of narcolepsy with cataplexy. This scientific leap fueled the generation of several genetically engineered mouse and rat models of narcolepsy. To facilitate further research, it is imperative that researchers reach a consensus concerning the evaluation of narcoleptic behavioral and EEG phenomenology in these models.

Keywords

Narcolepsy; Cataplexy; Sleep; EEG; Animal model; Rodent; Canine; REM

INTRODUCTION

Although narcolepsy symptoms were first described by the German psychiatrist Carl Friedrich Otto Westphal in 1877 [1] and the French word *narcolepsie* was coined in 1880 by the physician Jean-Baptiste-Édouard Gélinau [2], an animal model of this disease did not become available for almost a century. In the 1970s, though, a canine model of narcolepsy was established [3]. In 1998, orexins (also known as hypocretins) and their receptors were discovered [4, 5]. Orexins are peptides that are produced mainly in the perifornical region of the hypothalamus (PFH). Two isoforms of orexins (orexin-A and -B, or hypocretin-1 and hypocretin-2) are derived from proteolytic cleavage of a precursor peptide (preproorexin, or preprohypocretin) and exert their actions through two types of G-protein-coupled receptors (OxR1 and OxR2, or Hctr1 and Hctr2) [4, 5]. Subsequently, it was found that a deficiency of the orexin system plays a key role in narcolepsy [6–8]. Since then, multiple genetically engineered rodent models of narcolepsy have become available [9–11]. In this review, we will discuss narcolepsy in animals, especially various canine and rodent models of narcolepsy. A summary of these findings is provided in Table 1. These models have helped elucidate the pathophysiology of narcolepsy and develop more effective treatment of this disease.

NARCOLEPSY IN HUMAN BEINGS

Etiology

Narcolepsy is a debilitating sleep disorder that affects approximately 0.05% of the general population, with most cases first appearing during adolescence [12]. The prevalence of narcolepsy is 10 times higher among first-degree relatives than in the general population, indicating this disorder has a genetic predisposition. On the other hand, a pair of monozygotic twins was found to be discordant for narcolepsy, suggesting a strong environmental influence [12]. It is now generally accepted that narcolepsy is most likely caused by a loss of orexin neurons in the hypothalamus in genetically susceptible individuals with detrimental environmental exposure [13].

The majority (90%) of narcoleptic patients with cataplexy are positive for a specific class II human leukocyte antigen (HLA) allele, HLA-DQB1*0602, compared to about 30% of the general population. HLA genes (also known as human major histocompatibility complex genes, MHC) are essential for antigen processing by the immune system and many HLA alleles are biomarkers of autoimmune diseases. This strong HLA allele association, along with other epidemiological characters such as adolescence onset and genetic predisposition, led to the belief that an autoimmune-mediated mechanism is the most probable etiology for narcolepsy [14]. However, despite many years of research, neither autoimmune activation nor antibodies against the orexin system have been found in narcoleptic patients [15]. Recently, the search for genes associated with narcolepsy susceptibility was expanded beyond the HLA region and two genes, CPT1B, which regulates long-chain fatty-acid β oxidation, and CHKB, which is involved in phosphatidylcholine biosynthesis, have been suggested to be possible candidates [16].

Symptoms

As discussed by Yoss and Daly in 1957 [17], many patients with narcolepsy have a classic tetrad of symptoms. The first is the universal symptom of narcolepsy, excessive daytime sleepiness (EDS, a general feeling of sleepiness throughout the day with episodes of an irresistible urge to sleep). The second is the most specific symptom of this disorder, cataplexy (sudden muscle atonia during wakefulness triggered by emotions, usually laughing and joking). Two other symptoms are auxiliary and are not essential for the diagnosis. They are sleep paralysis (inability to move or to speak during transitions between sleep and wakefulness) and hypnagogic hallucinations (dream-like experience occurring at sleep onset).

Although the major manifestations of narcolepsy occur during wakefulness in day time, 95% of narcoleptic patients have disturbed night-time sleep as well, including prolonged non-rapid eye movement (NREM) sleep/ rapid eye movement (REM) sleep ultradian cycle, sleep fragmentation, REM sleep dissociation events, increased periodic leg movements (PLM), as well as co-morbidities such as obstructive sleep apnea (OSAS), REM sleep behavior disorder (RBD) and sleep-related eating disorder (SRED)/nocturnal eating syndrome (NES) [18].

Finally, it should be noted that although cataplexy is pathognomonic for narcolepsy, it is also extremely variable in its presentation. A complete attack usually affects all antigravity muscles bilaterally and causes the individual to collapse. Sometimes an attack can be limited, affecting only facial muscles or arms or legs, causing slurred speech, facial muscle flickering, jaw tremor, head or jaw dropping, dropping of objects, and/or buckling of the knees. Most attacks last from a few seconds to less than 10 minutes and the occurrence frequency can vary from less than one episode per year to several episodes per day [12,19]. Cataplexy is often associated with waking EEG [20]. However, especially in long attacks, a

REM sleep like EEG pattern (4–7.5 Hz, in contrast to regular 8–13 Hz waking alpha activity) can be seen [21].

Diagnosis

According to the 2nd edition of the International Classification of Sleep Disorders (ICSD-2, 2005), diagnosis of narcolepsy can be divided into 3 categories; narcolepsy with cataplexy, narcolepsy without cataplexy, and narcolepsy due to another underlying medical condition. The major criteria for narcolepsy with cataplexy are 1) The patient has a complaint of EDS occurring almost daily for at least 3 months and 2) A definite history of cataplexy is present. In addition, the hypersomnia is not due to other identified medical causes.

The diagnosis of narcolepsy is confirmed by nocturnal polysomnography followed by a multiple sleep latency test (MSLT). The MSLT is an objective test of sleepiness in a sleep-inducing environment [22]. It has been used in the diagnosis of narcolepsy and idiopathic hypersomnia. The most common clinical version of the MSLT is comprised of 5 nap opportunities during the daytime, scheduled 2 h apart, starting at 1.5–3 h after awakening. At the beginning of the test, the patient is encouraged to fall asleep and the bedroom lights are turned off. Each test session is terminated 20 min after lights-out if there is no sleep or 15 min after the first epoch of sleep. Sleep latency is defined as the elapsed time from lights-out to the onset of sleep. REM sleep latency is defined as the time from the beginning of sleep onset to the beginning of the first REM sleep epoch. If REM sleep latency is shorter than 15 min, the epoch is defined as a sleep onset REM (SOREM) period. An abnormal MSLT indicative of narcolepsy is defined as a mean sleep latency less than or equal to 8 min and two or more SOREM periods following a minimally sufficient nocturnal sleep (minimum 6 h) during the night prior to the test.

If a patient does not have typical cataplexy but meets all the other criteria listed above, then a diagnosis of narcolepsy without cataplexy can be made. In a case in which a patient meets the diagnostic criteria in one of the above two categories accompanied by a significant underlying medical or neurological disorder which may account for the EDS, then a diagnosis of the 3rd category, narcolepsy due to a medical condition is made.

In the event that a MSLT test is not feasible or results are dubious, detection of low levels of orexin-A (less than 110ng/L, or a third of mean control values) in the cerebrospinal fluid (CSF) can also indicate narcolepsy with cataplexy or narcolepsy due to a medical condition. However, CSF orexin-A measurement is not advised in the diagnosis of narcolepsy without cataplexy since the majority of these patients do not have CSF orexin deficiency [23, 24].

Treatment

Currently, there is no cure for narcolepsy. Some treatments are available, and new treatments are being developed. Among them, pharmacological treatment is generally necessary [25]. Traditionally, amphetamine-like CNS stimulants have been used to combat EDS and tricyclic antidepressants such as imipramine have been used to treat cataplexy. Later, nonamphetamine CNS stimulants such as modafinil (provigil™) were also used to combat EDS. Currently, sodium oxybate (the sodium salt of gamma-hydroxybutyrate, GHB, a natural metabolite of GABA) is a leading treatment option for narcolepsy, as it is effective in treating EDS, cataplexy and disturbed nocturnal sleep in narcoleptic patients [25].

Canine Models of Narcolepsy

Narcolepsy has been reported in many domestic animals, including the horse, the sheep, and the bull [26–30]. The subjects of the first non-human narcolepsy report were a dog and a cat in 1973 [31]. Contemporaneously, a female toy poodle named Monique was studied by

researchers at Stanford University. Monique developed cataplectic attacks when she was approximately 4 to 5 months old. The attacks were often partial, involving only neck and hind legs, but at times developed into full collapse. Emotional experiences, such as the presentation of food, water, or a plaything, were likely triggers. Polygraphic recordings revealed normal EEG and sleep stages. However, multiple SOREM periods were noted. A breeding program was soon started but unfortunately offspring were all asymptomatic.

By 1979, at least 15 breeds were identified to have canine narcolepsy with cataplexy. Two of them, Doberman pinschers and Labrador retrievers, have inheritable versions of this disorder. Pedigree analysis indicated an autosomal recessive mode of transmission with full penetrance [32]. Thus, it became clear that there are both familial and sporadic forms of canine narcolepsy. The familial cases generally have an early onset (1 to 5 months old) with relatively mild symptoms fully developed by 6 months. In contrast, sporadic cases have a more varied onset time (7 weeks to 7 years) and sometimes much more severe symptoms [33, 34]. These findings paved the way for the successful establishment of the Stanford canine narcolepsy colony. Between 1976 and 1995, nearly 500 dogs were bred and Doberman pinschers accounted for 80% of them [35].

As food is a very powerful emotional stimulus in dogs, cataplexy is usually evaluated by the highly standardized food-elicited cataplexy test (FECT). In this test, 12 pieces of wet canned dog food (1 cm³) are placed on the ground in a semi-circular pattern at 30 cm intervals. The dogs have previously been trained to eat all pieces of food in order. In a test, a normal dog will eat all the food in approximately 10 sec., whereas a narcoleptic dog will have several partial or complete cataplectic attacks before eating all the food. An observer records the number of attacks and the duration required to complete the task [36]. Another less standardized but more sensitive test, especially in young canines, is called the play-elicited cataplexy test (PECT). During the PECT, two or more dogs are brought into a test room and are allowed to freely interact with each other and with the toys provided. Play behavior, including playful fighting and use of play objects, elicited multiple cataplectic attacks. Occurrences and durations of all episodes of cataplexy are recorded [34, 36].

A detailed analysis of cataplexy in dogs with polygraphic recordings revealed 3 distinct stages. The initial stage had muscle atonia, waking-like EEG and visual tracking. The second stage resembled REM sleep with hippocampal theta activity. The final stage was characterized by EEG with mixed frequency and amplitude before a transition into wakefulness or sleep [37].

Early sleep recordings in a few affected dogs displayed normal EEG and normal distribution of various vigilance stages with the exception of reduced REM sleep when SOREM periods were scored as cataplexy [38, 39]. A subsequent study demonstrated that total sleep time (both NREM and REM sleep) was similar between narcoleptic dogs and their controls. However, the narcoleptic dogs revealed a substantial increase in the time spent in the drowsy state, suggesting these dogs had excessive sleepiness [40]. Narcoleptic dogs were also found to be relatively inactive during daytime and did not show a clear rest/activity pattern, similar to that of human narcoleptics [41]. Moreover, marked sleep fragmentation was observed in narcoleptic dogs [42]. Interestingly, mimicking the human MSLT, a canine MSLT was designed and performed in narcoleptics and their controls. The test consists of five consecutive 1h sessions. In the first half of each session the lights are on and the dogs are not allowed to sleep, then the lights are turned off to allow the dogs to sleep [43]. Narcoleptic dogs were found to have shorter sleep latency and a higher frequency of SOREM periods (defined as REM sleep occurring within 15 min of lights off), compared to normal dogs of the same breed. On the other hand, both narcoleptic and control dogs had the

same 30 min REM sleep cyclicity, indicating ultradian rhythmicity is not altered in narcoleptic dogs [43].

The search for a gene that is responsible in familial canine narcolepsy, designated as canarc-1, turned into a monumental project. A large number of backcrosses were performed in order to have both heterozygous and homozygous offspring for genetic linkage analysis. Since there are strong associations between narcolepsy and MHC class II HLA in humans, investigators initially attempted to seek an association between the MHC class II dog leukocyte antigen (DLA-D) and canine narcolepsy. However, no linkage between canarc-1 and canine MHC was found [44]. A canine genetic library was built using DNA fragments from heterozygous Doberman pinschers to cover the entire canine genome, including the mutated gene [45]. Based on previous linkage analysis, genome library clones containing a genetic marker near the canarc-1 gene were isolated and sequenced. Newly obtained sequence data were analyzed and a region of conserved synteny between human chromosome 6 and canine chromosome 12 was found. Armed with this knowledge, additional clones that contain the canine homolog were identified using the available human sequence data.

Gradually, the critical region for canine narcolepsy was flanked and cloned. Only one known gene, OxR2, was discovered to be present in the critical region [7]. OxR2 was at that time a newly described G-protein coupled receptor [4, 5]. Sequence analysis indicated a short interspersed nucleotide element insertion between exon 3 and 4 of the OxR2 gene in Doberman pinschers. In Labrador retrievers, a single G to A transition was found between exon 6 and 7. Both of these mutations, although different, caused exon skipping and nonfunctional receptors [7]. Further research in a Dachshund family exhibiting narcolepsy identified a single nucleotide mis-sense mutation, which leads to loss of ligand binding. In contrast, there are no genetic mutations identified in sporadic narcoleptic dogs [46]. However, those dogs had undetectable level of orexin in the CSF, in contrast with normal orexin levels in adult narcoleptic Dobermans [47, 48].

It should be noted that genetic mutation alone may not account for the full symptomatic development of narcolepsy. In Doberman pinschers carrying the OxR2 mutation, concomitant to disease onset at around 1 month of age, there was a significant increase in microglial expression of MHC class II molecules [49], as well as signs of neuronal degeneration in the amygdala, basal forebrain and other brain regions [50]. In addition, there were higher CSF orexin levels 4 days after birth, but the level declined to the normal range at the time when the narcoleptic symptoms first occurred [51]. Treating these narcoleptic dogs with immunosuppressive and anti-inflammatory agents starting within 3 weeks after birth doubled the time to disease onset and reduced the time spent in cataplexy by 90% [52].

Recent studies on the downstream molecular alterations in narcoleptic Dobermans revealed that the brain mRNA levels of two neuropeptide precursor molecules, Tachykinin precursor 1 (TAC1) and Proenkephalin (PENK), as well as the suppressor of cytokine signaling 2 (SOCS2), were decreased when compared to levels in their heterozygous siblings. The difference was particularly pronounced in the amygdala [53]. The amygdala plays a critical role in processing emotional stimuli and heavily interconnects with brainstem sleep circuits [54]. The tachykinin peptide family includes Substance P and it has been shown that substance P afferents from the central nucleus of the amygdala project to brain regions that might generate muscle atonia, e.g., the parvocellular reticular nuclei of the medulla [55]. On the other hand, the release of endogenous opioids, such as the enkephalins, is involved in the regulation of positive emotion in humans, a frequent trigger of cataplexy [56]. Moreover, cytokines have been implicated in both physiological and pathological sleep [57]. Taken

together, these studies suggest that multiple molecular abnormalities may exist in the narcoleptic dogs.

Rodent Models of Narcolepsy

In the same time frame during which the OxR2 mutation was found in narcoleptic canines, a study in orexin knockout (KO) mice revealed that they had brief periods of peculiar behavior arrest during the dark period. Specifically, these episodes were defined as “*the abrupt cessation of purposeful motor activity associated with a sudden, sustained change in posture that was maintained throughout the episode, ending abruptly with complete resumption of purposeful motor activity.*” [6] The narcoleptic attacks were usually preceded by ambulating and grooming and followed by eating and drinking. Subsequent polygraphic sleep recording revealed that these behavioral arrests corresponded to EEG/EMG patterns similar to REM sleep or NREM sleep with spindles, a pattern indicative of transition to REM sleep. These mice also had more fragmented NREM sleep and increased REM sleep during the dark period. In addition, REM sleep latency was decreased along with multiple sleep onset REM (SOREM) periods containing direct transitions from wakefulness into REM sleep [6].

Although it is still early to draw a firm conclusion, it seems that rodent cataplexy can be triggered by social interaction and/or positive emotions. The cataplectic episodes occurred in orexin KO mice at a younger age (starting from 4 weeks) if group housed than if individually housed (starting from 6 weeks) [6]. Interestingly, juvenile rats make 50 kHz ultrasonic vocalizations (USV) when they play or when they are “tickled” by researchers. This chirping USV has been thought to be a homolog of human laughter [58]. Since human cataplexy is commonly triggered with laughter [59], it is intriguing that USV episodes were found to precipitate cataplexy in orexin KO mice [60]. Other triggers of rodent cataplexy have been evaluated. For example, it was reported that murine cataplexy can be triggered by anticipation of highly palatable food [61]. Another strong trigger of cataplexy may be locomotor activity. It was found that wheel running doubled the amount of cataplexy in orexin KO mice [62].

A detailed sleep EEG study confirmed that orexin KO mice had fragmented sleep and wakefulness, although amounts were normal when compared with wild type littermates [63]. There was also a slight increase in REM sleep during the dark (active) period when cataplexy (operationally defined as atonia with REM sleep-like EEG spectra that is immediately preceded and followed by active wake) was excluded. In addition, this study found that orexin KO mice had intact circadian control of sleep, normal sleep homeostasis and an unchanged amount of wakefulness. However, an important theoretical consideration is that REM like phenomena (cataplexy) could also be viewed as an alteration in the diurnal distribution of REM components. A developmental study revealed that infant orexin KO mice and WT mice had similar sleep patterns in the first 3 weeks. However, WT mice consolidated their sleep as they aged while orexin KO mice failed to do so [64].

Later studies in both orexin and OxR2 KO mice found that, in addition to the abrupt arrests usually preceded by purposeful motor activity, another type of gradual behavioral arrests may occur, usually preceded by quiet wakefulness. These gradual arrests were accompanied by EEG/EMG signals characteristic of NREM sleep. Caffeine suppressed gradual arrests while the anticataplectic drug clompiramine suppressed abrupt arrests [11]. Therefore, two classic symptoms of human narcolepsy, cataplexy and EDS (sleep attacks), are closely mimicked in orexin KO and OxR2 KO mice. It is worth noting that OxR2 KO mice were only mildly affected and rarely had abrupt cataplectic arrests, which is consistent with mild symptoms exhibited in narcoleptic Dobermans.

Early reports indicated that OX1R KO did not appear to have cataplectic attacks and only had moderate sleep fragmentation. On the other hand, orexin receptor double receptor KO mice had the same phenotype as orexin null mice [65, 66]. In another preliminary study, the expression of both orexin receptors was prevented by the use of transcriptional blocking sequences. Although these KO mice had severely fragmented sleep and could not maintain sustained periods of wakefulness during the dark period compared to WT littermates, no apparent cataplexy was found [67]. It is difficult to speculate as to the underlying mechanisms responsible for the differences between these genetic models since these studies have not yet been published in peer reviewed journals.

It is well known that the use of constitutive knockout animals has the potential confound of developmental compensation. As a result, other approaches have also been taken to inactivate the orexin system in rodents. For example, since narcolepsy with cataplexy is thought to be a disease of orexin neurotransmission deficiency, it is natural to assume inhibiting either one or both orexin receptor(s) will lead to cataplexy. However, a selective OX1R antagonist (SB-334867) reversed the effects of orexin-A on REM sleep but did not induce cataplexy [68]. More recently, a dual orexin receptor antagonist (ACT-078573), which has approximately equal selectivity to both receptors (the concentrations of antagonist required for IC₅₀ were 16nM for the OX1R receptor and 15 nM for the OX2R), increased sleep, particularly REM sleep, but did not induce cataplexy in rats and dogs [69].

Administration into the rat lateral hypothalamus of orexin-B conjugated to the ribosome-inactivating toxic protein saporin eliminated up to about 90% of orexin neurons, but also caused significant loss of neighboring neuronal cells such as those containing melanin-concentrating hormone (MCH) [70]. This lesion induced an increase in NREM and REM sleep in the dark period but a decrease of REM sleep in the light period. In addition, multiple SOREMs (defined as a REM sleep episode that occurred after 2 min or more of wakefulness with less than 2 min of an intervening episode of NREM sleep) were observed [70]. Together, this approach showed promise in that relative specific elimination of orexin neurons might be achieved by precise microinjection and adjusting the doses of the toxin. However, although multiple sleep disturbances similar to human narcolepsy were observed, the concurrent loss of other non-orexin neurons make it a less optimal model of human narcolepsy [70, 71].

Human narcolepsy is caused by the loss of about 90% of orexin neurons [72] together with concomitant loss of dynorphin and activity-regulated pentraxin (NARP) signaling from those neurons [73, 74]. Therefore, a model of selective loss of orexin neurons is more appropriate than just orexin gene KO alone. To address this, orexin neuron ablation has been successfully achieved in both mice and rats by expressing a cytotoxic transgene, the N-terminal truncated cDNA for human ataxin-3, in orexin neurons [10, 75]. This transgene induced apoptosis and orexin neurons were ablated by the age of 15 weeks in mice. Orexin/ataxin3 mice displayed the same kind of behavioral arrests, sleep fragmentation and direct wake to REM sleep transitions as seen in orexin KO mice. In addition, a late onset obesity starting from 12–15 weeks of age was observed despite reduced food consumption during the dark period at 8–10 weeks of age [10]. This obesity could be caused by sleep fragmentation-induced suppression of activity and energy expenditure in these mice [76]. However, the development of obesity was only seen in orexin/ataxin-3 mice that did not have a pure C57/BL6J genetic background or in those fed with high-fat food. In contrast, the orexin KO mice did not develop obesity regardless of whether they had a pure C57/BL6J background or not [77]. An additional study confirmed that orexin-ataxin3 transgenic mice with mixed genetic background were obese [78]. Furthermore, it was found that both female orexin-KO mice and mice tended to be heavier than wild type mice and this was associated with higher serum leptin levels [78]. These studies highlighted the importance of an array of

complex factors, such as sex, genetic background, and environment, in shaping the phenotype of narcoleptic animals.

The same transgenic technique was used to generate orexin/ataxin-3 rats. Similar to transgenic mice, these rats had an almost complete loss of orexin-positive neurons at 17 weeks of age. In orexin/ataxin-3 rats, sleep was fragmented and wakefulness was decreased during the dark (active) period, especially near the dark-light transition time. REM sleep was increased during the dark period and decreased during the light (inactive) period. Furthermore, REM sleep latency was shortened and frequent SOREM periods were seen. Interestingly, brief episodes of muscle atonia and postural collapse were observed, resembling cataplexy while rats maintained the EEG characteristics of wakefulness [75], a feature often seen in human and canine narcolepsy. A recent study confirmed orexin-ataxin3-rats had a similar polysomnographic profile [79]. In addition, a dramatic decline of CSF orexin levels (approximately 74%) occurred between weeks 2 and 4. More importantly, the orexin levels in target areas such as the cortex and the brain stem of the transgenic rats were less than 1% of the baseline levels in WT rats. These rats had residual orexin neurons in the lateral hypothalamus and 6h of sleep deprivation almost doubled the CSF orexin levels, although the levels were still much lower than those basal levels in the WT rats.

Another promising approach to investigate narcolepsy in the rodent model is to knockdown the level of orexin or their receptors *in vivo* by using antisense, and more recently, RNA interference (RNAi) technology. The effects of RNAi are highly selective and reversible. In addition, RNAi has the advantage of having a fast onset (24–48 h), whereas chemical lesion or postnatal neurodegeneration takes days or weeks to achieve its full effect. A study using microdialysis perfusion of OxR2 antisense into the pontine reticular formation (PRF) of rats for 3 days revealed an increase of REM sleep and episodes of cataplectic behavior [80]. Microinjection of short interfering RNAs (siRNA) targeting prepro-orexin mRNA into the rat perifornical hypothalamus suppressed prepro-orexin mRNA expression by 60% and induced a persistent increase in REM sleep during the dark (active) period in rats. Cataplexy-like episodes were also observed in some of these animals. Wakefulness and NREM sleep were unaffected [81]. Similarly, microinjection of OxR1 siRNA into the locus coeruleus also increased REM sleep during the dark period [82]. Although the effects of siRNA treatment are transient, siRNA can be effectively employed to complement other loss-of-function approaches. Moreover, a long-term knockdown is technically feasible using viral based RNA interference.

Neurochemical Alterations in Animal Models of Narcolepsy

Narcolepsy is a disorder with multiple REM sleep-related abnormalities and cataplexy is characterized by REM-like muscle atonia. Since a reciprocal interaction of monoaminergic and cholinergic neurotransmission plays an essential role in REM sleep generation [83], it is logical to assume an imbalance between monoaminergic and cholinergic system exists in narcolepsy. Studies in narcoleptic animals support this idea.

Initial research into the neurochemical profile of narcoleptic canines focused on adrenergic and serotonergic uptake inhibitors since these were used to treat human cataplexy [84, 85]. A systematic study of the effects of various monoamine uptake blockers or release enhancers on canine cataplexy found that all compounds affecting noradrenergic systems suppressed cataplexy at low doses, whereas compounds affecting serotonergic and dopaminergic transmissions were either inactive or partially active at high doses [86]. Most tricyclic antidepressants are metabolized into desmethyl metabolites which are more potent for adrenergic than serotonergic inhibition. When the anticataplectic effects of five antidepressants (amitryptiline, clomipramine, fluoxetine, imipramine and zimelidine) and their respective desmethyl metabolites were compared, the metabolites were found to be

more effective. Furthermore, the anticonvulsant potency of these compounds was positively correlated to the *in vitro* adrenergic uptake inhibition but was negatively correlated with serotonergic uptake inhibition [87]. These results suggest a predominant involvement of the adrenergic system in the control of cataplexy, yet the participation of the 5-HT system cannot be completely ruled out. For example, 5-HT_{1A} agonists can significantly suppress cataplexy and their potency in doing so correlates with their *in vitro* affinities to the canine central 5-HT_{1A} receptor [88]. In an *in vitro* study, it was also found that orexin A excited serotonergic dorsal raphe neurons in the rat [89], suggesting that downregulation of the serotonin system may contribute to narcoleptic symptoms.

Treatment of narcoleptic dogs with prazosin, a selective α_1 -adrenergic receptor antagonist, increased canine cataplexy whereas treatment with the α_1 agonist, methoxamine, ameliorated it [90, 91]. Subsequent studies revealed that this effect was mediated by the α_{1b} adrenergic receptor subtype, and the receptor is upregulated in the amygdala of narcoleptic animals [92–94]. On the other hand, α_2 -adrenergic receptor antagonists suppressed cataplexy whereas a group of α_2 agonists aggravated cataplexy [95]. Receptor binding studies showed an increase of α_2 receptors in the locus coeruleus [96]. Together, these data implicate a norepinephrine neurotransmission deficiency in the canine narcolepsy model that is accompanied by an upregulation of postsynaptic α_1 receptors in the amygdala and presynaptic α_2 autoreceptors in the locus coeruleus. This is consistent with anatomical studies showing a dense innervation of OxR1 positive neurons in the locus coeruleus and *in vitro* excitatory effects of orexin on them [97].

Concentrations of CSF dopamine metabolites in narcoleptic poodles were lower than their normal controls [98]. In two separate studies, samples from discrete brain regions of narcoleptic Doberman pinschers and breed-matched controls were assayed for the content of monoamines and their metabolites. A significantly higher concentration of dopamine and its major intracellular metabolite, DOPAC (3,4-dihydroxyphenylacetic acid), was observed in the amygdala [99, 100]. In line with this, concentrations of dopamine D₂ receptors in the amygdala were significantly higher in narcoleptic animals [101]. The results indicate a localized reduction of dopamine release.

Systemic administration of dopaminergic D₂/D₃ antagonists such as quinpirole suppressed cataplexy without affecting REM sleep while D₂/D₃ agonists aggravated cataplexy [102, 103]. Local perfusion of quinpirole into the dopaminergic ventral tegmental area (VTA), substantia nigra (SN), or diencephalic dopaminergic cell groups significantly aggravates cataplexy [104–106], while perfusion of a D₂/D₃ antagonist reduced cataplexy. On the other hand, VTA perfusion of quinpirole significantly increased sleep in narcoleptic Dobermans, while SN perfusion did not significantly modify sleep [104, 105]. These different roles in regulating sleep by VTA and SN neurons could be caused by broad activation of both dopaminergic and nondopaminergic neurons by orexins in the VTA and selective activation of GABAergic neurons in the SN [107, 108].

In control and narcoleptic OxR2 mutant dogs, amphetamine-like stimulants and modafinil were able to dose-dependently increase wakefulness. Their *in vivo* potencies strongly correlated with their *in vitro* affinities to the DA transporter, but not to the NE transporter [109–112]. It was found that modafinil more effectively increased wakefulness time in orexin KO than in WT mice [113]. Together, these data indicate CNS stimulants promote wakefulness *via* increased DA neurotransmission, independent of the orexin system.

Histaminergic neurons in the tuberomammillary nucleus are thought to play an important role in promoting wakefulness [114]. Orexins excite these neurons [115], predominately *via* OxR₂ [116] and Orexin A was unable to promote wakefulness in histamine H₁ receptor KO

mice [117]. Histamine content was decreased in the cortex and thalamus in OxR2 mutated narcoleptic Dobermans [118]. This result is consistent with the notion that low brain histamine level induces drowsiness. Recent studies reported low CSF histamine levels in human narcoleptic patients [119, 120].

Although cataplexy has many features that resemble REM sleep such as REM atonia, extra cellular single-unit recordings in narcoleptic Dobermans revealed a distinct neuronal activity profile as compared to REM sleep. For example, most cells in the medial medulla and the medial mesopontine region of the narcoleptic dog were silent in cataplexy but were active in REM sleep [121–123]. It is known that during REM sleep, monoaminergic neurons in the locus coeruleus, the dorsal raphe, and the tuberomammillary nucleus are silent. During cataplexy, neurons in the locus coeruleus cease activity [124]. Drugs that precipitate cataplexy decreased locus coeruleus discharge rate [124]. Serotonergic neurons in the dorsal raphe reduced their discharge rate to the level seen in NREM sleep [125]. On the other hand, histaminergic neurons maintained waking level activity during cataplexy [126]. A recent study in orexin KO mice found that most neurons in rostral pons were less active during cataplexy than during REM sleep [127].

Interestingly, prior to and during cataplexy in the amygdala of narcoleptic dogs, a group of sleep active cells increased their discharge rate, while another group of wake active cells reduced firing [128]. The $\alpha 1$ blocker prazosin, at doses which increased the frequency of cataplectic attacks, increased discharge in a subgroup of the cataplexy active cells and in a number of other amygdala cells, indicating that prazosin may modulate cataplexy by its action on amygdala cells or their afferents.

The cholinergic system was also found early on to be involved in canine narcolepsy. An acetylcholinesterase inhibitor, physostigmine, significantly increased the amount of cataplexy. This effect may be partially mediated by the cholinergic basal forebrain [121, 123]. Alternatively, enhancement of the biosynthesis of acetylcholine by intracerebroventricular perfusion of methyl-B12 or choline aggravated cataplexy [129]. Further testing showed that a muscarinic agonist had the same effect but nicotine was ineffective. Not surprisingly, muscarinic antagonists were able to reduce the amount of cataplexy [130]. Furthermore, muscarinic receptor density, likely of the M2 subtype, which is involved in REM sleep generation [131], was elevated in the brain stem (nucleus reticularis gigantocellularis) of narcoleptic doberman pinschers [132, 133]. Acetylcholine (ACh) levels in the pontine reticular formation were increased by about 50% in narcoleptic Doberman pinschers after four repeated sessions of FECT, which induced cataplexy [134]. Direct perfusion of carbachol or a M2 agonist into the pontine reticular formation dose-dependently increased cataplexy. This increase was quickly reversed by atropine or a M2 antagonist [135, 136]. Similarly, microinjection of carbachol, but not a M1 agonist, into the basal forebrain also triggered cataplexy, and injection of atropine had the opposite effect [137]. Direct perfusion of carbachol or a M2 agonist into the basal forebrain produced a dose-dependent increase in cataplexy. This increase was blocked by M2 antagonists [138]. Interestingly, it was found that in otherwise asymptomatic Dobermans that are heterozygous for the OxR2 mutation, weekly administration of compounds that act on cholinergic or monoaminergic systems, such as physostigmine, prazosin and quinpirole, induced cataplexy [139].

The postulate of anatomical abnormalities of the cholinergic system in narcoleptic dogs has been hard to prove. A preliminary study found the number of cholinergic neurons in the brainstem laterodorsal tegmental (LDT) and pedunculopontine nuclei (PPN) of narcoleptic canines was increased [140]. However, a comprehensive examination of the number and organization of cholinergic and catecholaminergic cells in mesopontine regions failed to

reveal any significant differences between normal and narcoleptic dogs [141]. In addition, the ratio of cholinergic to catecholaminergic cells was identical in the two groups.

Although most of the above pharmacological studies have been performed in OxR2 mutant dogs, these results were supported by newly conducted studies in mice. For example, administration of a D2/D3 antagonist suppressed cataplexy in KO mice while a D2/D3 agonist aggravated it [142, 143]. Moreover, REM sleep or other vigilance states were not affected. In orexin receptor double KO mice, physostigmine significantly increased cataplexy-like behavior, whereas atropine, a muscarinic antagonist, decreased the number of arrests. Terazosin, an alpha-1 receptor antagonist, increased the number of partial arrests [144].

Some other compounds that do not directly affect monoaminergic or cholinergic systems have been studied. For example, intravenous administration of prostaglandin E2 or its lipophilic derivative induced a dose-dependent reduction of cataplexy [145]. However, CSF levels of prostaglandins are not modified in these narcoleptic dogs when compared to heterozygous or control dogs [146]. Thyrotropin-releasing hormone (TRH) and its analogs were also able to reduce cataplexy in canine narcolepsy [147, 148]. Interestingly, thalidomide, both a hypnotic and an immune modulator, aggravated canine cataplexy [149].

Other sleep related receptors were found to be unaltered in the brain of narcoleptic dogs, including GABA_A receptors [150] and adenosine A1 and A2 receptors [151].

In summary, narcoleptic animals have become indispensable tools for understanding the neurochemical mechanism of narcolepsy. A general picture of suppressed monoaminergic tone and (relatively) increased cholinergic tone emerged as the prominent pathophysiological feature of this disorder.

Treatment Progress, Current Issues and Future Directions

The availability of animal models provides multiple ways to test the mechanisms underlying the current treatments, as well as to explore new therapeutic possibilities. For example, recently, a histamine H3 autoreceptor antagonist, tiprolisant, was found to increase wakefulness and decrease both the number and the duration of SOREM periods *via* activation of histaminergic and noradrenergic systems in orexin null mice [152]. Other H3 antagonists were also found to reduce cataplexy in Doberman dogs [153].

Orexin supplement therapy was tried in narcoleptic dogs with varying degrees of success. An early report demonstrated that systemic administration (3 µg/kg) of orexin-A significantly reduced cataplexy in narcoleptic Dobermans [154]. However, this finding was not replicated using either systemic or central administration of orexins [155]. On the other hand, systemic administration of orexin-A at very high doses (>48 µg/kg) suppressed cataplexy in a dose dependent manner [156]. However, in a case report, intrathecal perfusion of orexin-A for 2 months did not relieve cataplexy in a narcoleptic Weimaraner. In mice, central administration (intracerebral ventricular, icv) of orexin-A into orexin/ataxin-3 mice resulted in increased wakefulness and reduced REM sleep, combined with a suppression of cataplectic episodes [157]. These results indicate that orexin neuron-ablated mice retain the ability to respond to orexin. In support of this, it was found that in orexin/ataxin-3 mice, sporadic narcoleptic dogs, and narcoleptic patients, there is only a modest reduction in orexin receptor mRNA levels, primarily of OxR1 in cortex. In addition, in OxR2 mutated narcoleptic dogs, OxR1 expression was normal [158].

Another promising line of research has been to replace orexin either through neuronal transplantation or genetic engineering. In one study, orexin neurons derived from 10 days

old rat pups were grafted into the pons of adult rats (60 days old). While the majority of the grafted neurons did not survive over 36 days, the surviving neurons (6%) had the same morphological features as normal mature orexin neurons [159]. Chronic overproduction of orexin peptides from an ectopically expressed transgene prevented the development of the narcolepsy-cataplexy syndrome in mice [157]. Recently, it has been demonstrated that transferring a HSV-1 vector that carries the mouse preproorexin gene into the lateral hypothalamus (LH) of orexin knockout mice can decrease the incidence of cataplexy by 60% [160]. In another extraordinary technical advance, it has been shown to be feasible to manipulate the activity of orexin neurons *in vivo* using light by genetically expressing channelrhodopsin-2 (light gated ion channels) in these cells [161].

Although great progress has been made by studying orexin deficiency in rodents, some significant obstacles remain. For example, most past studies did not employ both polysomnographic as well as video recordings that are essential in evaluating cataplexy. Traditionally, EMG electrodes are implanted in nuchal muscles in rodents. As human narcolepsy often starts with buckling of the knees, and 80% of cataplectic attacks in Dobermans begin in the hind legs [162], EMG recordings from the rodent hind legs will probably enable researchers to detect more cataplectic attacks, as well as allowing the study of PLMs (periodic leg movements) associated with narcolepsy. The first report of murine narcolepsy scored vigilance states in 20s epochs while most sleep labs use epochs 10s or shorter. Given the rapid transitions between behavioral states in rodents and the nature of sleep fragmentation in narcoleptic animals, an even shorter scoring epoch probably should be utilized, especially if new models of narcolepsy are developed in which the rodents are less severely affected. Finally, although it appeared that orexin null mice remained conscious during cataplectic attacks (escape attempt in response to the tip of a pen waved in front of a mouse) [11] no systematic research has been done to verify this, such as examining eye tracking in cataplectic mice. This leaves it difficult to distinguish between a cataplectic attack and a direct SOREM episode.

A recent encouraging development is a consensus definition of murine cataplexy developed by the International Working Group on Rodent Models of Narcolepsy [163]. The group defined murine cataplexy as an abrupt episode of nuchal atonia lasting at least 10 seconds, preceded by at least 40 seconds of wakefulness. The episode should be accompanied by prominent EEG theta activity and behavioral immobility. However, as the group acknowledged, this definition may overlook partial cataplexy, and there is no standard definition of theta activity among sleep labs [163].

While cataplexy is pathognomonic for narcolepsy, we should not lose sight of the fact that many narcoleptic patients develop this symptom at a later stage or do not have cataplexy at all. Thus, an ideal model of animal narcolepsy should be able to mimic the full clinical spectrum of human narcolepsy with or without cataplexy. The MSLT is an important tool in clinical diagnosis of narcolepsy, and a corresponding test should be applied in rodent models of narcolepsy. A murine MSLT test has been developed [164]. Recently, our lab further refined this procedure and extended it to rats (rat multiple sleep latencies test, rMSLT) [165]. The rMSLT consisted of 5 min wakefulness induced by sensory stimulation followed by 25 min of freedom to sleep. This procedure is repeated 6 times in a 3 h time period to minimize the amount of sleep lost due to the testing procedure. Our method provides an objective and minimally intrusive measurement of sleepiness in rodents that closely resembles a similar clinical test used in patients [165]. This method is predicted to open doors to investigate potential new models of narcolepsy, and provide an objective measure for evaluation of treatment effects. One remaining issue is to establish clear criteria for normal REM sleep latency and SOREM in rodent MSLT tests. One report defined SOREM as a REM sleep episode that occurred after 2 min or more of wakefulness with less than 2

min of an intervening episode of NREM sleep [70]. This definition needs to be adapted or modified by the sleep community in general.

Some narcoleptic symptoms such as hypnagogic hallucinations will be difficult to verify in an animal model. On the other hand, we have made great strides in identifying two major symptoms of narcolepsy, EDS and cataplexy, in both canine and rodent animal models. In addition, some secondary symptoms and signs of human narcolepsy are also seen in narcoleptic animals. For example, narcoleptic dogs were found to have periodic leg movements during sleep [166]. Core body temperature of orexin KO mice is about 0.6 degrees Celsius higher than in WT mice [167], which correlates well with human data [168]. In the narcoleptic canine, there was a moderate (18%) increase in heart rate before cataplexy onset, which then returned to baseline levels following the attack [169], similar to sympathetic changes seen in narcoleptic patients [170]. The study of these animal models has provided a trove of data to shed light on the pathophysiology of this disease as well as the mechanisms of normal sleep and wakefulness.

It should also be noted that cataplexy like behavior is not only associated with orexin deficiency. For example, a strain of myelin mutant rat, known as taiep (an acronym of the major symptoms of this mutant strain, which includes tremor, ataxia, immobility, epilepsy, and paralysis) was found to have immobility episodes induced by gripping [171]. These immobility episodes are associated with REM like EEG [172]. Similar to results obtained from pharmacological manipulations in narcoleptic dogs, selective $\alpha 1$ - or $\alpha 2$ -adrenergic receptor antagonist induced immobility episodes whereas 5-HT₁ agonist and $\alpha 2$ antagonist decreased the frequency and duration of these episodes [173–175].

Given that the orexin system is conserved in mammals and narcolepsy has been found in multiple species, it is likely more animal models will be developed in the future. For example, a narcolepsy model in squirrel monkeys will be very useful since primates have consolidated wakefulness similar to humans. It has been reported that Wistar-Kyoto (WKY) rats (an animal model of depression) had sleep fragmentation and a 50% increase in REM sleep during the light period [176]. Interestingly, the WKY rats also have 15% less orexinergic neurons compared to the parent Wistar strain [177]. Currently, only sporadic narcoleptic dogs offer us an opportunity to study the complicated interactions between genetic and environmental factors that lead to orexin deficiency. This line of research would be greatly accelerated if naturally occurring narcoleptic rodents could be found. On the other end of the spectrum, the zebrafish, a poikilotherm vertebrate, has orexin cells very similar to those in mammals but has only one type of orexin receptor. Overexpression of orexin in zebrafish larvae increased and consolidated wakefulness [178]. However, no cataplexy-like behavior was observed in adult zebrafish with orexin receptor mutation [179]. Furthermore, with the accumulation of experimental data, a mathematical model of narcolepsy is possible [180]. Finally, with the rapid advancement of genetic techniques, we will be able to temporally and spatially control the activity of the orexin system in the near future, thus likely generating an array of different phenotypes that reflect the wide spectrum of disease severity in humans.

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ABBREVIATIONS

CSF Cerebrospinal fluid

EDS	Excessive daytime sleepiness
EEG	Electroencephalogram
EMG	Electromyogram
FECT	Food-elicited cataplexy test
HLA	Human leukocyte antigen allele
KO	Knockout
MHC	Major histocompatibility complex
MSLT	Multiple sleep latency test
NE	Norepinephrine
NREM	Non-rapid eye movement
OxR1	Orexin/hypocretin type I receptor
OxR2	Orexin/hypocretin type II receptor
PECT	Play-elicited cataplexy test
PLM	Periodic leg movements
REM	Rapid eye movement
RNAi	RNA interference
siRNA	Short interfering RNAs
SOREM	Sleep onset REM sleep
WT	Wild type

REFERENCES

- Westphal C. Eigentümliche mit einschlafen verbundene anfälle. Arch. Psychiatr. Nervenkr. 1877; 7:631–635.
- Gelineau J. De la narcolepsie. Gaz. Hôp. (Paris). 1880; 55:635–637.
- Mitler, MM. Toward an Animal Model of Narcolepsy-Cataplexy. In: Guilleminault, C.; Dement, W.; Passouant, P.; Weitzman, E., editors. Narcolepsy. Holliswood, NY: Spectrum Publications; 1976. p. 387-409.
- de Lecea L, Kilduff TS, Peyron C, Gao X, Foye PE, Danielson PE, Fukuhara C, Battenberg EL, Gautvik VT, Bartlett FS, Frankel WN, van den Pol AN, Bloom FE, Gautvik KM, Sutcliffe JG. The hypocretins: hypothalamus-specific peptides with neuroexcitatory activity. Proc. Natl. Acad. Sci. USA. 1998; 95:322–327. [PubMed: 9419374]
- Sakurai T, Amemiya A, Ishii M, Matsuzaki I, Chemelli RM, Tanaka H, Williams SC, Richardson JA, Kozlowski GP, Wilson S, Arch JR, Buckingham RE, Haynes AC, Carr SA, Annan RS, McNulty DE, Liu WS, Terrett JA, Elshourbagy NA, Bergsma DJ, Yanagisawa M. Orexins and orexin receptors: a family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behavior. Cell. 1998; 92:573–585. [PubMed: 9491897]
- Chemelli RM, Willie JT, Sinton CM, Elmquist JK, Scammell T, Lee C, Richardson JA, Williams SC, Xiong Y, Kisanuki Y, Fitch TE, Nakazato M, Hammer RE, Saper CB, Yanagisawa M. Narcolepsy in orexin knockout mice: molecular genetics of sleep regulation. Cell. 1999; 98:437–451. [PubMed: 10481909]
- Lin L, Faraco J, Li R, Kadotani H, Rogers W, Lin X, Qiu X, de Jong PJ, Nishino S, Mignot E. The sleep disorder canine narcolepsy is caused by a mutation in the hypocretin (orexin) receptor 2 gene. Cell. 1999; 98:365–376. [PubMed: 10458611]

8. Nishino S, Ripley B, Overeem S, Lammers GJ, Mignot E. Hypocretin (orexin) deficiency in human narcolepsy. *Lancet*. 2000; 355:39–40. [PubMed: 10615891]
9. Beuckmann CT, Yanagisawa M. Orexins: from neuropeptides to energy homeostasis and sleep/wake regulation. *J. Mol. Med.* 2002; 80:329–342. [PubMed: 12072908]
10. Hara J, Beuckmann CT, Nambu T, Willie JT, Chemelli RM, Sinton CM, Sugiyama F, Yagami K, Goto K, Yanagisawa M, Sakurai T. Genetic ablation of orexin neurons in mice results in narcolepsy, hypophagia, and obesity. *Neuron*. 2001; 30:345–354. [PubMed: 11394998]
11. Willie JT, Chemelli RM, Sinton CM, Tokita S, Williams SC, Kisanuki YY, Marcus JN, Lee C, Elmquist JK, Kohlmeier KA, Leonard CS, Richardson JA, Hammer RE, Yanagisawa M. Distinct narcolepsy syndromes in Orexin receptor-2 and Orexin null mice: molecular genetic dissection of Non-REM and REM sleep regulatory processes. *Neuron*. 2003; 38:715–730. [PubMed: 12797957]
12. Dauvilliers Y, Arnulf I, Mignot E. Narcolepsy with cataplexy. *Lancet*. 2007; 369:499–511. [PubMed: 17292770]
13. Longstreth WT Jr, Koepsell TD, Ton TG, Hendrickson AF, van Belle G. The epidemiology of narcolepsy. *Sleep*. 2007; 30:13–26. [PubMed: 17310860]
14. Lin L, Hungs M, Mignot E. Narcolepsy and the HLA region. *J. Neuroimmunol.* 2001; 117:9–20. [PubMed: 11431000]
15. Overeem S, Black JL III, Lammers GJ. Narcolepsy: immunological aspects. *Sleep Med. Rev.* 2008; 12:95–107. [PubMed: 18291691]
16. Miyagawa T, Kawashima M, Nishida N, Ohashi J, Kimura R, Fujimoto A, Shimada M, Morishita S, Shigeta T, Lin L, Hong SC, Faraco J, Shin YK, Jeong JH, Okazaki Y, Tsuji S, Honda M, Honda Y, Mignot E, Tokunaga K. Variant between CPT1B and CHKB associated with susceptibility to narcolepsy. *Nat. Genet.* 2008; 40:1324–1328. [PubMed: 18820697]
17. Yoss RE, Daly DD. Criteria for the diagnosis of the narcoleptic syndrome. *Proc. Staff. Meet. Mayo Clin.* 1957; 32:320–328. [PubMed: 13441766]
18. Plazzi G, Serra L, Ferri R. Nocturnal aspects of narcolepsy with cataplexy. *Sleep Med. Rev.* 2008; 12:109–128. [PubMed: 18282773]
19. Nishino S. Clinical and neurobiological aspects of narcolepsy. *Sleep Med.* 2007; 8:373–399. [PubMed: 17470414]
20. Rubboli G, d'Orsi G, Zaniboni A, Gardella E, Zamagni M, Rizzi R, Meletti S, Valzania F, Tropeani A, Tassinari CA. A video-polygraphic analysis of the cataplectic attack. *Clin. Neurophysiol.* 2000; 111(Suppl 2):S120–S128. [PubMed: 10996565]
21. Dyken ME, Yamada T, Lin-Dyken DC, Seaba P, Yeh M. Diagnosing narcolepsy through the simultaneous clinical and electrophysiologic analysis of cataplexy. *Arch. Neurol.* 1996; 53:456–460. [PubMed: 8624222]
22. Carskadon MA, Dement WC, Mitler MM, Roth T, Westbrook PR, Keenan S. Guidelines for the multiple sleep latency test (MSLT): a standard measure of sleepiness. *Sleep*. 1986; 9:519–524. [PubMed: 3809866]
23. Bourgin P, Zeitzer JM, Mignot E. CSF hypocretin-1 assessment in sleep and neurological disorders. *Lancet Neurol.* 2008; 7:649–662. [PubMed: 18565458]
24. Nishino S, Ripley B, Overeem S, Lammers GJ, Mignot E. Hypocretin (orexin) deficiency in human narcolepsy. *Lancet*. 2000; 355:39–40. [PubMed: 10615891]
25. Billiard M. Narcolepsy: current treatment options and future approaches. *Neuropsychiatr. Dis. Treat.* 2008; 4:557–566. [PubMed: 18830438]
26. White EC, De Lahunta A. Narcolepsy in a ram lamb. *Vet. Rec.* 2001; 149:156–157. [PubMed: 11517987]
27. Lunn DP, Cuddon PA, Shaftoe S, Archer RM. Familial occurrence of narcolepsy in miniature horses. *Equine Vet. J.* 1993; 25:483–487. [PubMed: 7903939]
28. Strain GM, Olcott BM, Archer RM, McClintock BK. Narcolepsy in a Brahman bull. *J. Am. Vet. Med. Assoc.* 1984; 185:538–541. [PubMed: 6541218]
29. Dreifuss FE, Flynn DV. Narcolepsy in a horse. *J. Am. Vet. Med. Assoc.* 1984; 184:131–132. [PubMed: 6538191]

30. Sweeney CR, Hendricks JC, Beech J, Morrison AR. Narcolepsy in a horse. *J. Am. Vet. Med. Assoc.* 1983;183, 126–128.
31. Knecht CD, Oliver JE, Redding R, Selcer R, Johnson G. Narcolepsy in a dog and a cat. *J. Am. Vet. Med. Assoc.* 1973; 162:1052–1053. [PubMed: 4736237]
32. Foutz AS, Mitler MM, Cavalli-Sforza LL, Dement WC. Genetic factors in canine narcolepsy. *Sleep.* 1979; 1:413–421. [PubMed: 574310]
33. Baker TL, Foutz AS, McNeerney V, Mitler MM, Dement WC. Canine model of narcolepsy: genetic and developmental determinants. *Exp. Neurol.* 1982; 75:729–742. [PubMed: 7199479]
34. Riehl J, Nishino S, Cederberg R, Dement WC, Mignot E. Development of cataplexy in genetically narcoleptic Dobermans. *Exp. Neurol.* 1998; 152:292–302. [PubMed: 9710528]
35. Cederberg R, Nishino S, Dement WC, Mignot E. Breeding history of the Stanford colony of narcoleptic dogs. *Vet. Rec.* 1998; 142:31–36. [PubMed: 9481825]
36. Nishino S, Mignot E. Pharmacological aspects of human and canine narcolepsy. *Prog. Neurobiol.* 1997; 52:27–78. [PubMed: 9185233]
37. Kushida CA, Baker TL, Dement WC. Electroencephalographic correlates of cataplectic attacks in narcoleptic canines. *Electroencephalogr. Clin. Neurophysiol.* 1985; 61:61–70. [PubMed: 2408864]
38. Lucas EA, Foutz AS, Dement WC, Mitler MM. Sleep cycle organization in narcoleptic and normal dogs. *Physiol. Behav.* 1979; 23:737–743. [PubMed: 228334]
39. Mitler MM, Dement WC. Sleep studies on canine narcolepsy: pattern and cycle comparisons between affected and normal dogs. *Electroencephalogr. Clin. Neurophysiol.* 1977; 43:691–699. [PubMed: 72649]
40. Kaitin KI, Kilduff TS, Dement WC. Evidence for excessive sleepiness in canine narcoleptics. *Electroencephalogr. Clin. Neurophysiol.* 1986; 64:447–454. [PubMed: 2428595]
41. Nishino S, Tafti M, Sampathkumaran R, Dement WC, Mignot E. Circadian distribution of rest/activity in narcoleptic and control dogs: assessment with ambulatory activity monitoring. *J. Sleep Res.* 1997; 6:120–127. [PubMed: 9377531]
42. Kaitin KI, Kilduff TS, Dement WC. Sleep fragmentation in canine narcolepsy. *Sleep.* 1986; 9:116–119. [PubMed: 3704433]
43. Nishino S, Riehl J, Hong J, Kwan M, Reid M, Mignot E. Is narcolepsy a REM sleep disorder? Analysis of sleep abnormalities in narcoleptic Dobermans. *Neurosci. Res.* 2000; 38:437–446. [PubMed: 11164570]
44. Mignot E, Wang C, Rattazzi C, Gaiser C, Lovett M, Guilleminault C, Dement WC, Grumet FC. Genetic linkage of autosomal recessive canine narcolepsy with a mu immunoglobulin heavy-chain switch-like segment. *Proc. Natl. Acad. Sci. USA.* 1991; 88:3475–3478. [PubMed: 1673032]
45. Li R, Mignot E, Faraco J, Kadotani H, Cantanese J, Zhao B, Lin X, Hinton L, Ostrander EA, Patterson DF, de Jong PJ. Construction and characterization of an eightfold redundant dog genomic bacterial artificial chromosome library. *Genomics.* 1999; 58:9–17. [PubMed: 10331940]
46. Hungs M, Fan J, Lin L, Lin X, Maki RA, Mignot E. Identification and functional analysis of mutations in the hypocretin (orexin) genes of narcoleptic canines. *Genome Res.* 2001; 11:531–539. [PubMed: 11282968]
47. Ripley B, Fujiki N, Okura M, Mignot E, Nishino S. Hypocretin levels in sporadic and familial cases of canine narcolepsy. *Neurobiol. Dis.* 2001; 8:525–534. [PubMed: 11442359]
48. Wu MF, John J, Maidment N, Lam HA, Siegel JM. Hypocretin release in normal and narcoleptic dogs after food and sleep deprivation, eating, and movement. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 2002; 283:R1079–R1086. [PubMed: 12376401]
49. Tafti M, Nishino S, Aldrich MS, Liao W, Dement WC, Mignot E. Major histocompatibility class II molecules in the CNS: increased microglial expression at the onset of narcolepsy in canine model. *J. Neurosci.* 1996; 16:4588–4595. [PubMed: 8764647]
50. Siegel JM, Nienhuis R, Gulyani S, Ouyang S, Wu MF, Mignot E, Switzer RC, McMurry G, Cornford M. Neuronal degeneration in canine narcolepsy. *J. Neurosci.* 1999; 19:248–257. [PubMed: 9870955]
51. John J, Wu MF, Maidment NT, Lam HA, Boehmer LN, Patton M, Siegel JM. Developmental changes in CSF hypocretin-1 (orexin-A) levels in normal and genetically narcoleptic Doberman pinschers. *J. Physiol.* 2004; 560:587–592. [PubMed: 15308685]

52. Boehmer LN, Wu MF, John J, Siegel JM. Treatment with immunosuppressive and anti-inflammatory agents delays onset of canine genetic narcolepsy and reduces symptom severity. *Exp. Neurol.* 2004; 188:292–299. [PubMed: 15246829]
53. Lindberg J, Saetre P, Nishino S, Mignot E, Jazin E. Reduced expression of TAC1, PENK and SOCS2 in Hcrtr-2 mutated narcoleptic dog brain. *BMC Neurosci.* 2007; 8:34. [PubMed: 17521418]
54. Morrison AR, Sanford LD, Ross RJ. The amygdala: a critical modulator of sensory influence on sleep. *Biol. Signals Recept.* 2000; 9:283–296. [PubMed: 11025335]
55. Fort P, Luppi PH, Jouvet M. Afferents to the nucleus reticularis parvicellularis of the cat medulla oblongata: a tract-tracing study with cholera toxin B subunit. *J. Comp. Neurol.* 1994; 342:603–618. [PubMed: 7518846]
56. Koeppe MJ, Hammers A, Lawrence AD, Asselin MC, Grasby PM, Bench CJ. Evidence for endogenous opioid release in the amygdala during positive emotion. *Neuroimage.* 2009; 44:252–256. [PubMed: 18809501]
57. Kapsimalis F, Basta M, Varouchakis G, Gourgoulialis K, Vgontzas A, Kryger M. Cytokines and pathological sleep. *Sleep Med.* 2008; 9:603–614. [PubMed: 18024171]
58. Panksepp J. Neuroevolutionary sources of laughter and social joy: modeling primal human laughter in laboratory rats. *Behav. Brain Res.* 2007; 182:231–244. [PubMed: 17363075]
59. Overeem S, Lammers GJ, van Dijk JG. Weak with laughter. *Lancet.* 1999; 354:838. [PubMed: 10485735]
60. Burgess C, Rudchenko A, Takkala P, Peever JH, Yeomans J. Ultrasonic vocalizations promote cataplexy in hypocretin/orexin knockout mice. *Soc. Neurosci. Abstr.* 2008; 38:784–786.
61. Clark EL, Baumann CR, Cano G, Scammell TE, Mochizuki T. Feeding-elicited cataplexy in orexin knockout mice. *Neuroscience.* 2009 in Press.
62. Espana RA, McCormack SL, Mochizuki T, Scammell TE. Running promotes wakefulness and increases cataplexy in orexin knockout mice. *Sleep.* 2007; 30:1417–1425. [PubMed: 18041476]
63. Mochizuki T, Crocker A, McCormack S, Yanagisawa M, Sakurai T, Scammell TE. Behavioral state instability in orexin knock-out mice. *J. Neurosci.* 2004; 24:6291–6300. [PubMed: 15254084]
64. Blumberg MS, Coleman CM, Johnson ED, Shaw C. Developmental divergence of sleep-wake patterns in orexin knockout and wild-type mice. *Eur. J. Neurosci.* 2007; 25:512–518. [PubMed: 17284193]
65. Kisanuki YY, Chemelli RM, Tokita S, Willie JT, Sinton CM, Yanagisawa M. Behavioral and polysomnographic characterization of orexin-1 receptor and orexin-2 receptor double knockout mice. *Sleep.* 2001; 24:A22.
66. Willie JT, Chemelli RM, Sinton CM, Yanagisawa M. To eat or to sleep? Orexin in the regulation of feeding and wakefulness. *Annu. Rev. Neurosci.* 2001; 24:429–458. [PubMed: 11283317]
67. Alexandre C, Mochizuki T, Arrigoni E, Clark E, Marcus JN, Elmquist JK, Lowell BB, Scammell TE. Orexin acts in the basal forebrain to stabilize wakefulness. *Soc. Neurosci. Abstr.* 2008; 38:285.5.
68. Smith MI, Piper DC, Duxon MS, Upton N. Evidence implicating a role for orexin-1 receptor modulation of paradoxical sleep in the rat. *Neurosci. Lett.* 2003; 341:256–258. [PubMed: 12697296]
69. Brisbare-Roch C, Dingemanse J, Koberstein R, Hoeber P, Aissaoui H, Flores S, Mueller C, Nayler O, van Gerven J, de Haas SL, Hess P, Qiu C, Buchmann S, Scherz M, Weller T, Fischli W, Clozel M, Jenck F. Promotion of sleep by targeting the orexin system in rats, dogs and humans. *Nat. Med.* 2007; 13:150–155. [PubMed: 17259994]
70. Gerashchenko D, Blanco-Centurion C, Greco MA, Shiromani PJ. Effects of lateral hypothalamic lesion with the neurotoxin hypocretin-2-saporin on sleep in Long-Evans rats. *Neuroscience.* 2003; 116:223–235. [PubMed: 12535955]
71. Gerashchenko D, Kohls MD, Greco M, Waleh NS, Salin-Pascual R, Kilduff TS, Lappi DA, Shiromani PJ. Hypocretin-2-saporin lesions of the lateral hypothalamus produce narcoleptic-like sleep behavior in the rat. *J. Neurosci.* 2001; 21:7273–7283. [PubMed: 11549737]
72. Peyron C, Faraco J, Rogers W, Ripley B, Overeem S, Charnay Y, Nevsimalova S, Aldrich M, Reynolds D, Albin R, Li R, Hungs M, Pedrazzoli M, Padigaru M, Kucherlapati M, Fan J, Maki R,

- Lammers GJ, Bouras C, Kucherlapati R, Nishino S, Mignot E. A mutation in a case of early onset narcolepsy and a generalized absence of hypocretin peptides in human narcoleptic brains. *Nat. Med.* 2000; 6:991–997. [PubMed: 10973318]
73. Blouin AM, Thannickal TC, Worley PF, Baraban JM, Reti IM, Siegel JM. Narp immunostaining of human hypocretin (orexin) neurons: loss in narcolepsy. *Neurology.* 2005; 65:1189–1192. [PubMed: 16135770]
 74. Crocker A, Espana RA, Papadopoulou M, Saper CB, Faraco J, Sakurai T, Honda M, Mignot E, Scammell TE. Concomitant loss of dynorphin, NARP, and orexin in narcolepsy. *Neurology.* 2005; 65:1184–1188. [PubMed: 16247044]
 75. Beuckmann CT, Sinton CM, Williams SC, Richardson JA, Hammer RE, Sakurai T, Yanagisawa M. Expression of a poly-glutamine-ataxin-3 transgene in orexin neurons induces narcolepsy-cataplexy in the rat. *J. Neurosci.* 2004; 24:4469–4477. [PubMed: 15128861]
 76. Zhang S, Zeitzer JM, Sakurai T, Nishino S, Mignot E. Sleep/wake fragmentation disrupts metabolism in a mouse model of narcolepsy. *J. physiol.* 2007; 581:649–663. [PubMed: 17379635]
 77. Hara J, Yanagisawa M, Sakurai T. Difference in obesity phenotype between orexin-knockout mice and orexin neuron-deficient mice with same genetic background and environmental conditions. *Neurosci. Lett.* 2005; 380:239–242. [PubMed: 15862893]
 78. Fujiki N, Yoshida Y, Zhang S, Sakurai T, Yanagisawa M, Nishino S. Sex difference in body weight gain and leptin signaling in hypocretin/orexin deficient mouse models. *Peptides.* 2006; 27:2326–2331. [PubMed: 16626839]
 79. Zhang S, Lin L, Kaur S, Thankachan S, Blanco-Centurion C, Yanagisawa M, Mignot E, Shiromani PJ. The development of hypocretin (orexin) deficiency in hypocretin/ataxin-3 transgenic rats. *Neuroscience.* 2007; 148:34–43. [PubMed: 17618058]
 80. Thakkar MM, Ramesh V, Cape EG, Winston S, Strecker RE, McCarley RW. REM sleep enhancement and behavioral cataplexy following orexin (hypocretin)-II receptor antisense perfusion in the pontine reticular formation. *Sleep Res. Online.* 1999; 2:112–120. [PubMed: 11382892]
 81. Chen L, Thakkar MM, Winston S, Bolortuya Y, Basheer R, McCarley RW. REM sleep changes in rats induced by siRNA-mediated orexin knockdown. *Eur. J. Neurosci.* 2006; 24:2039–2048. [PubMed: 17067300]
 82. Chen L, Bolortuya Y, Basheer R, McCarley RW. Sleep Changes induced by siRNA knockdown of orexin type 1 receptor in locus coeruleus. *Soc. Neurosci. Abs.* 2008; 38:384.6.
 83. McCarley RW. Neurobiology of REM and NREM sleep. *Sleep Med.* 2007; 8:302–330. [PubMed: 17468046]
 84. Foutz AS, Delashaw JB Jr, Guilleminault C, Dement WC. Monoaminergic mechanisms and experimental cataplexy. *Ann. Neurol.* 1981; 10:369–376. [PubMed: 6976152]
 85. Babcock DA, Narver EL, Dement WC, Mitler MM. Effects of imipramine, chlorimipramine, and fluoxetine on cataplexy in dogs. *Pharmacol. Biochem. Behav.* 1976; 5:599–602. [PubMed: 1035802]
 86. Mignot E, Renaud A, Nishino S, Arrigoni J, Guilleminault C, Dement WC. Canine cataplexy is preferentially controlled by adrenergic mechanisms: evidence using monoamine selective uptake inhibitors and release enhancers. *Psychopharmacology (Berl.).* 1993; 113:76–82. [PubMed: 7862832]
 87. Nishino S, Arrigoni J, Shelton J, Dement WC, Mignot E. Desmethyl metabolites of serotonergic uptake inhibitors are more potent for suppressing canine cataplexy than their parent compounds. *Sleep.* 1993; 16:706–712. [PubMed: 8165384]
 88. Nishino S, Shelton J, Renaud A, Dement WC, Mignot E. Effect of 5-HT1A receptor agonists and antagonists on canine cataplexy. *J. Pharmacol. Exp. Ther.* 1995; 272:1170–1175. [PubMed: 7891329]
 89. Brown RE, Sergeeva O, Eriksson KS, Haas HL. Orexin A excites serotonergic neurons in the dorsal raphe nucleus of the rat. *Neuropharmacology.* 2001; 40:457–459. [PubMed: 11166339]
 90. Mignot E, Guilleminault C, Bowersox S, Rappaport A, Dement WC. Role of central alpha-1 adrenoceptors in canine narcolepsy. *J. Clin. Invest.* 1988; 82:885–894. [PubMed: 2843574]

91. Mignot E, Guilleminault C, Bowersox S, Rappaport A, Dement WC. Effect of alpha 1-adrenoceptors blockade with prazosin in canine narcolepsy. *Brain Res.* 1988; 444:184–188. [PubMed: 2834022]
92. Mignot E, Guilleminault C, Bowersox S, Frusthofer B, Nishino S, Maddaluno J, Ciaranello R, Dement WC. Central alpha 1 adrenoceptor subtypes in narcolepsy-cataplexy: a disorder of REM sleep. *Brain Res.* 1989; 490:186–191. [PubMed: 2569353]
93. Renaud A, Nishino S, Dement WC, Guilleminault C, Mignot E. Effects of SDZ NVI-085, a putative subtype-selective alpha 1-agonist, on canine cataplexy, a disorder of rapid eye movement sleep. *Eur. J. Pharmacol.* 1991; 205:11–16. [PubMed: 1687464]
94. Nishino S, Frusthofer B, Arrigoni J, Guilleminault C, Dement WC, Mignot E. Further characterization of the alpha-1 receptor subtype involved in the control of cataplexy in canine narcolepsy. *J. Pharmacol. Exp. Ther.* 1993; 264:1079–1084. [PubMed: 8095546]
95. Nishino S, Haak L, Shepherd H, Guilleminault C, Sakai T, Dement WC, Mignot E. Effects of central alpha-2 adrenergic compounds on canine narcolepsy, a disorder of rapid eye movement sleep. *J. Pharmacol. Exp. Ther.* 1990; 253:1145–1152. [PubMed: 1972749]
96. Frusthofer B, Mignot E, Bowersox S, Nishino S, Dement WC, Guilleminault C. Canine narcolepsy is associated with an elevated number of alpha 2-receptors in the locus coeruleus. *Brain Res.* 1989; 500:209–214. [PubMed: 2557958]
97. Horvath TL, Peyron C, Diano S, Ivanov A, Ston-Jones G, Kilduff TS, van den Pol AN. Hypocretin (orexin) activation and synaptic innervation of the locus coeruleus noradrenergic system. *J. Comp. Neurol.* 1999; 415:145–159. [PubMed: 10545156]
98. Faull KF, Barchas JD, Foutz AS, Dement WC, Holman RB. Monoamine metabolite concentrations in the cerebrospinal fluid of normal and narcoleptic dogs. *Brain Res.* 1982; 242:137–143. [PubMed: 6179569]
99. Miller JD, Faull KF, Bowersox SS, Dement WC. CNS monoamines and their metabolites in canine narcolepsy: a replication study. *Brain Res.* 1990; 509:169–171. [PubMed: 1689603]
100. Mefford IN, Baker TL, Boehme R, Foutz AS, Ciaranello RD, Barchas JD, Dement WC. Narcolepsy: biogenic amine deficits in an animal model. *Science.* 1983; 220:629–632. [PubMed: 6188216]
101. Bowersox SS, Kilduff TS, Faull KF, Zeller-DeAmicis L, Dement WC, Ciaranello RD. Brain dopamine receptor levels elevated in canine narcolepsy. *Brain Res.* 1987; 402:44–48. [PubMed: 3828787]
102. Nishino S, Arrigoni J, Valtier D, Miller JD, Guilleminault C, Dement WC, Mignot E. Dopamine D2 mechanisms in canine narcolepsy. *J. Neurosci.* 1991; 11:2666–2671. [PubMed: 1831837]
103. Okura M, Riehl J, Mignot E, Nishino S. Sulpiride, a D2/D3 blocker, reduces cataplexy but not REM sleep in canine narcolepsy. *Neuropsychopharmacology.* 2000; 23:528–538. [PubMed: 11027918]
104. Reid MS, Tafti M, Nishino S, Sampathkumaran R, Siegel JM, Mignot E. Local administration of dopaminergic drugs into the ventral tegmental area modulates cataplexy in the narcoleptic canine. *Brain Res.* 1996; 733:83–100. [PubMed: 8891251]
105. Honda K, Riehl J, Mignot E, Nishino S. Dopamine D3 agonists into the substantia nigra aggravate cataplexy but do not modify sleep. *Neuroreport.* 1999; 10:3717–3724. [PubMed: 10619672]
106. Okura M, Fujiki N, Kita I, Honda K, Yoshida Y, Mignot E, Nishino S. The roles of midbrain and diencephalic dopamine cell groups in the regulation of cataplexy in narcoleptic Dobermans. *Neurobiol. Dis.* 2004; 16:274–282. [PubMed: 15207284]
107. Korotkova TM, Eriksson KS, Haas HL, Brown RE. Selective excitation of GABAergic neurons in the substantia nigra of the rat by orexin/hypocretin *in vitro*. *Regul. Pept.* 2002; 104:83–89. [PubMed: 11830281]
108. Korotkova TM, Sergeeva OA, Eriksson KS, Haas HL, Brown RE. Excitation of ventral tegmental area dopaminergic and nondopaminergic neurons by orexins/hypocretins. *J. Neurosci.* 2003; 23:7–11. [PubMed: 12514194]

109. Shelton J, Nishino S, Vaught J, Dement WC, Mignot E. Comparative effects of modafinil and amphetamine on daytime sleepiness and cataplexy of narcoleptic dogs. *Sleep*. 1995; 18:817–826. [PubMed: 8746387]
110. Kanbayashi T, Honda K, Kodama T, Mignot E, Nishino S. Implication of dopaminergic mechanisms in the wake-promoting effects of amphetamine: a study of D- and L-derivatives in canine narcolepsy. *Neuroscience*. 2000; 99:651–659. [PubMed: 10974428]
111. Nishino S, Mao J, Sampathkumaran R, Shelton J. Increased dopaminergic transmission mediates the wake-promoting effects of CNS stimulants. *Sleep Res*. Online. 1998; 1:49–61. [PubMed: 11382857]
112. Wisor JP, Nishino S, Sora I, Uhl GH, Mignot E, Edgar DM. Dopaminergic role in stimulant-induced wakefulness. *J. Neurosci*. 2001; 21:1787–1794. [PubMed: 11222668]
113. Willie JT, Renthal W, Chemelli RM, Miller MS, Scammell TE, Yanagisawa M, Sinton CM. Modafinil more effectively induces wakefulness in orexin-null mice than in wild-type littermates. *Neuroscience*. 2005; 130:983–995. [PubMed: 15652995]
114. Brown RE, Stevens DR, Haas HL. The physiology of brain histamine. *Prog. Neurobiol*. 2001; 63:637–672. [PubMed: 11164999]
115. Eriksson KS, Sergeeva O, Brown RE, Haas HL. Orexin/hypocretin excites the histaminergic neurons of the tuberomammillary nucleus. *J. Neurosci*. 2001; 21:9273–9279. [PubMed: 11717361]
116. Yamanaka A, Tsujino N, Funahashi H, Honda K, Guan JL, Wang QP, Tominaga M, Goto K, Shioda S, Sakurai T. Orexins activate histaminergic neurons *via* the orexin 2 receptor. *Biochem. Biophys. Res. Commun*. 2002; 290:1237–1245. [PubMed: 11811995]
117. Huang ZL, Qu WM, Li WD, Mochizuki T, Eguchi N, Watanabe T, Urade Y, Hayaishi O. Arousal effect of orexin A depends on activation of the histaminergic system. *Proc. Natl. Acad. Sci. USA*. 2001; 98:9965–9970. [PubMed: 11493714]
118. Nishino S, Fujiki N, Ripley B, Sakurai E, Kato M, Watanabe T, Mignot E, Yanai K. Decreased brain histamine content in hypocretin/orexin receptor-2 mutated narcoleptic dogs. *Neurosci. Lett*. 2001; 313:125–128. [PubMed: 11682143]
119. Nishino S, Sakurai E, Nevsimalova S, Yoshida Y, Watanabe T, Yanai K, Mignot E. Decreased CSF histamine in narcolepsy with and without Low CSF hypocretin-1 in comparison to healthy controls. *Sleep*. 2009; 32:175–180. [PubMed: 19238804]
120. Kanbayashi T, Kodama T, Kondo H, Satoh S, Inoue Y, Chiba S, Shimizu T, Nishino S. CSF histamine contents in narcolepsy, idiopathic hypersomnia and obstructive sleep apnea syndrome. *Sleep*. 2009; 32:181–187. [PubMed: 19238805]
121. Siegel JM, Nienhuis R, Fahringer HM, Paul R, Shiromani P, Dement WC, Mignot E, Chiu C. Neuronal activity in narcolepsy: identification of cataplexy-related cells in the medial medulla. *Science*. 1991; 252:1315–1318. [PubMed: 1925546]
122. Siegel JM, Nienhuis R, Fahringer HM, Chiu C, Dement WC, Mignot E, Lufkin R. Activity of medial mesopontine units during cataplexy and sleep-waking states in the narcoleptic dog. *J. Neurosci*. 1992; 12:1640–1646. [PubMed: 1578258]
123. Nishino S, Honda K, Riehl J, Okura M, Mignot E. Neuronal activity in the cholinceptive basal forebrain of freely moving narcoleptic dobermans. *Neuroreport*. 1998; 9:3653–3661. [PubMed: 9858375]
124. Wu MF, Gulyani SA, Yau E, Mignot E, Phan B, Siegel JM. Locus coeruleus neurons: cessation of activity during cataplexy. *Neuroscience*. 1999; 91:1389–1399. [PubMed: 10391445]
125. Wu MF, John J, Boehmer LN, Yau D, Nguyen GB, Siegel JM. Activity of dorsal raphe cells across the sleep-waking cycle and during cataplexy in narcoleptic dogs. *J. physiol*. 2004; 554:202–215. [PubMed: 14678502]
126. John J, Wu MF, Boehmer LN, Siegel JM. Cataplexy-active neurons in the hypothalamus: implications for the role of histamine in sleep and waking behavior. *Neuron*. 2004; 42:619–634. [PubMed: 15157423]
127. Thankachan S, Kaur S, Shiromani PJ. Activity of pontine neurons during sleep and cataplexy in hypocretin knock-out mice. *J. Neurosci*. 2009; 29:1580–1585. [PubMed: 19193905]

128. Gulyani S, Wu MF, Nienhuis R, John J, Siegel JM. Cataplexy-related neurons in the amygdala of the narcoleptic dog. *Neuroscience*. 2002; 112:355–365. [PubMed: 12044453]
129. Honda K, Riehl J, Inoue S, Mignot E, Nishino S. Central administration of vitamin B12 aggravates cataplexy in canine narcolepsy. *Neuroreport*. 1997; 8:3861–3865. [PubMed: 9462456]
130. Delashaw JB Jr, Foutz AS, Guilleminault C, Dement WC. Cholinergic mechanisms and cataplexy in dogs. *Exp. Neurol*. 1979; 66:745–757. [PubMed: 573697]
131. Coleman CG, Lydic R, Baghdoyan HA. M2 muscarinic receptors in pontine reticular formation of C57BL/6J mouse contribute to rapid eye movement sleep generation. *Neuroscience*. 2004; 126:821–830. [PubMed: 15207317]
132. Kilduff TS, Bowersox SS, Kaitin KI, Baker TL, Ciaranello RD, Dement WC. Muscarinic cholinergic receptors and the canine model of narcolepsy. *Sleep*. 1986; 9:102–106. [PubMed: 3704431]
133. Boehme RE, Baker TL, Mefford IN, Barchas JD, Dement WC, Ciaranello RD. Narcolepsy: cholinergic receptor changes in an animal model. *Life Sci*. 1984; 34:1825–1828. [PubMed: 6539848]
134. Reid MS, Siegel JM, Dement WC, Mignot E. Cholinergic mechanisms in canine narcolepsy--II. Acetylcholine release in the pontine reticular formation is enhanced during cataplexy. *Neuroscience*. 1994; 59:523–530. [PubMed: 8008206]
135. Reid MS, Tafti M, Geary JN, Nishino S, Siegel JM, Dement WC, Mignot E. Cholinergic mechanisms in canine narcolepsy-I: modulation of cataplexy *via* local drug administration into the pontine reticular formation. *Neuroscience*. 1994; 59:511–522. [PubMed: 8008205]
136. Reid MS, Tafti M, Nishino S, Siegel JM, Dement WC, Mignot E. Cholinergic regulation of cataplexy in canine narcolepsy in the pontine reticular formation is mediated by M2 muscarinic receptors. *Sleep*. 1994; 17:424–435. [PubMed: 7991953]
137. Nishino S, Tafti M, Reid MS, Shelton J, Siegel JM, Dement WC, Mignot E. Muscle atonia is triggered by cholinergic stimulation of the basal forebrain: implication for the pathophysiology of canine narcolepsy. *J. Neurosci*. 1995; 15:4806–4814. [PubMed: 7623112]
138. Reid MS, Nishino S, Tafti M, Siegel JM, Dement WC, Mignot E. Neuropharmacological characterization of basal forebrain cholinergic stimulated cataplexy in narcoleptic canines. *Exp. Neurol*. 1998; 151:89–104. [PubMed: 9582257]
139. Mignot E, Nishino S, Sharp LH, Arrigoni J, Siegel JM, Reid MS, Edgar DM, Ciaranello RD, Dement WC. Heterozygosity at the canarc-1 locus can confer susceptibility for narcolepsy: induction of cataplexy in heterozygous asymptomatic dogs after administration of a combination of drugs acting on monoaminergic and cholinergic systems. *J. Neurosci*. 1993; 13:1057–1064. [PubMed: 8095066]
140. Nitz DL, Andersen A, Fahringer H, Nienhuis R, Mignot E, Siegel J. Altered distribution of cholinergic cells in the narcoleptic dog. *Neuroreport*. 1995; 6:1521–1524. [PubMed: 7579139]
141. Tafti M, Nishino S, Liao W, Dement WC, Mignot E. Mesopontine organization of cholinergic and catecholaminergic cell groups in the normal and narcoleptic dog. *J. Comp. Neurol*. 1997; 379:185–197. [PubMed: 9050784]
142. Kantor S, Clark EL, Scammell TE. The role of dopamine in modulation of cataplexy. *Soc. Neurosci. Abs*. 2008; 38:384.15.
143. Burgess C, Tse G, Peever J. Dopaminergic modulation of cataplexy in hypocretin-null mice. *Sleep*. 2008; 31:A5.
144. Kalogiannis M, Hsu E, Leonard CS. Video characterization and neurochemical modulation of behavioral arrests in double orexin receptor knockout (DKO) mice. *Soc. Neurosci. Abs*. 2008; 38:586.21.
145. Nishino S, Mignot E, Fruhstorfer B, Dement WC, Hayaishi O. Prostaglandin E2 and its methyl ester reduce cataplexy in canine narcolepsy. *Proc. Natl. Acad. Sci. USA*. 1989; 86:2483–2487. [PubMed: 2928344]
146. Nishino S, Mignot E, Kilduff TS, Sakai T, Hayaishi O, Dement WC. Prostaglandin E2 levels in cerebrospinal fluid of normal and narcoleptic dogs. *Biol. Psychiatry*. 1990; 28:904–910. [PubMed: 2268692]

147. Riehl J, Honda K, Kwan M, Hong J, Mignot E, Nishino S. Chronic oral administration of CG-3703, a thyrotropin releasing hormone analog, increases wake and decreases cataplexy in canine narcolepsy. *Neuropsychopharmacology*. 2000; 23:34–45. [PubMed: 10869884]
148. Nishino S, Arrigoni J, Shelton J, Kanbayashi T, Dement WC, Mignot E. Effects of thyrotropin-releasing hormone and its analogs on daytime sleepiness and cataplexy in canine narcolepsy. *J. Neurosci*. 1997; 17:6401–6408. [PubMed: 9236248]
149. Kanbayashi T, Nishino S, Tafti M, Hishikawa Y, Dement WC, Mignot E. Thalidomide, a hypnotic with immune modulating properties, increases cataplexy in canine narcolepsy. *Neuroreport*. 1996; 7:1881–1886. [PubMed: 8905685]
150. Bowersox SS, Kilduff TS, Kaitin KI, Dement WC, Ciaranello RD. Brain benzodiazepine receptor characteristics in canine narcolepsy. *Sleep*. 1986; 9:111–115. [PubMed: 3010425]
151. Hawkins M, O'Connor S, Radulovacki M, Bowersox S, Mignot E, Dement W. Radioligand binding to adenosine receptors and adenosine uptake sites in different brain regions of normal and narcoleptic dogs. *Pharmacol. Biochem. Behav*. 1991; 38:1–6. [PubMed: 1901994]
152. Lin JS, Dauvilliers Y, Arnulf I, Bastuji H, Anaclet C, Parmentier R, Kocher L, Yanagisawa M, Leher P, Ligneau X, Perrin D, Robert P, Roux M, Lecomte JM, Schwartz JC. An inverse agonist of the histamine H(3) receptor improves wakefulness in narcolepsy: studies in orexin-/- mice and patients. *Neurobiol. Dis*. 2008; 30:74–83. [PubMed: 18295497]
153. Bonaventure P, Letavic M, Dugovic C, Wilson S, Aluisio L, Pudiak C, Lord B, Mazur C, Kamme F, Nishino S, Carruthers N, Lovenberg T. Histamine H3 receptor antagonists: from target identification to drug leads. *Biochem. Pharmacol*. 2007; 73:1084–1096. [PubMed: 17129577]
154. John J, Wu MF, Siegel JM. Systemic administration of hypocretin-1 reduces cataplexy and normalizes sleep and waking durations in narcoleptic dogs. *Sleep Res. Online*. 2000; 3:23–28. [PubMed: 11382896]
155. Schatzberg SJ, Cutter-Schatzberg K, Nydam D, Barrett J, Penn R, Flanders J, deLahunta A, Lin L, Mignot E. The effect of hypocretin replacement therapy in a 3-year-old Weimaraner with narcolepsy. *J. Vet. Intern. Med*. 2004; 18:586–588. [PubMed: 15320603]
156. Siegel JM. Hypocretin administration as a treatment for human narcolepsy. *Sleep*. 2003; 26:932–933. [PubMed: 14746368]
157. Mieda M, Willie JT, Hara J, Sinton CM, Sakurai T, Yanagisawa M. Orexin peptides prevent cataplexy and improve wakefulness in an orexin neuron-ablated model of narcolepsy in mice. *Proc. Natl. Acad. Sci. USA*. 2004; 101:4649–4654. [PubMed: 15070772]
158. Mishima K, Fujiki N, Yoshida Y, Sakurai T, Honda M, Mignot E, Nishino S. Hypocretin receptor expression in canine and murine narcolepsy models and in hypocretin-ligand deficient human narcolepsy. *Sleep*. 2008; 31:1119–1126. [PubMed: 18714784]
159. Arias-Carrion O, Drucker-Colin R, Murillo-Rodriguez E. Survival rates through time of hypocretin grafted neurons within their projection site. *Neurosci. Lett*. 2006; 404:93–97. [PubMed: 16762505]
160. Liu M, Thankachan S, Kaur S, Begum S, Blanco-Centurion C, Sakurai T, Yanagisawa M, Neve R, Shiromani PJ. Orexin (hypocretin) gene transfer diminishes narcoleptic sleep behavior in mice. *Eur. J. Neurosci*. 2008; 28:1382–1393. [PubMed: 18973565]
161. Adamantidis AR, Zhang F, Aravanis AM, Deisseroth K, de Lecea L. Neural substrates of awakening probed with optogenetic control of hypocretin neurons. *Nature*. 2007; 450:420–424. [PubMed: 17943086]
162. Fujiki N, Morris L, Mignot E, Nishino S. Analysis of onset location, laterality and propagation of cataplexy in canine narcolepsy. *Psychiatry Clin. Neurosci*. 2002; 56:275–276. [PubMed: 12047593]
163. Scammell TE, Willie JT, Guilleminault C, Siegel JM. A consensus definition of cataplexy in mouse models of narcolepsy. *Sleep*. 2009; 32:111–116. [PubMed: 19189786]
164. Veasey SC, Yeou-Jey H, Thayer P, Fenik P. Murine Multiple Sleep Latency Test: phenotyping sleep propensity in mice. *Sleep*. 2004; 27:388–393. [PubMed: 15164889]
165. McKenna JT, Cordeira JW, Christie MA, Tartar JL, McCoy JG, Lee E, McCarley RW, Strecker RE. Assessing sleepiness in the rat: a multiple sleep latencies test compared to polysomnographic measures of sleepiness. *J. Sleep Res*. 2008; 17:365–375. [PubMed: 18823428]

166. Okura M, Fujiki N, Ripley B, Takahashi S, Amitai N, Mignot E, Nishino S. Narcoleptic canines display periodic leg movements during sleep. *Psychiatry Clin. Neurosci.* 2001; 55:243–244. [PubMed: 11422858]
167. Mochizuki T, Klerman EB, Sakurai T, Scammell TE. Elevated body temperature during sleep in orexin knockout mice. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 2006; 291:R533–R540. [PubMed: 16556901]
168. Mosko SS, Holowach JB, Sassin JF. The 24-hour rhythm of core temperature in narcolepsy. *Sleep.* 1983; 6:137–146. [PubMed: 6878983]
169. Siegel JM, Tomaszewski KS, Fahringer H, Cave G, Kilduff T, Dement WC. Heart rate and blood pressure changes during sleep-waking cycles and cataplexy in narcoleptic dogs. *Am. J. physiol.* 1989; 256:H111–H119. [PubMed: 2912173]
170. Donadio V, Plazzi G, Vandi S, Franceschini C, Karlsson T, Montagna P, Vetrugno R, Bugiardi E, Mignot E, Liguori R. Sympathetic and cardiovascular activity during cataplexy in narcolepsy. *J. Sleep Res.* 2008; 17:458–463. [PubMed: 19021855]
171. Holmgren B, Urba-Holmgren R, Riboni L, Vega-Saenz de Miera EC. Sprague Dawley rat mutant with tremor, ataxia, tonic immobility episodes, epilepsy and paralysis. *Lab. Anim. Sci.* 1989; 39:226–228. [PubMed: 2724922]
172. Prieto GJ, Urba-Holmgren R, Holmgren B. Sleep and EEG disturbances in a rat neurological mutant (taiep) with immobility episodes: a model of narcolepsy-cataplexy. *Electroencephalogr. Clin. Neurophysiol.* 1991; 79:141–147. [PubMed: 1713828]
173. Cortes MD, Rias-Montano JA, Eguibar JR. Prazosin increases immobility episodes in taiep rats without changes in the properties of alpha1 receptors. *Neurosci. Lett.* 2007; 412:159–162. [PubMed: 17194536]
174. Eguibar JR, Cortes MC, Valencia J, Rias-Montano JA. alpha2 adrenoceptors are involved in the regulation of the gripping-induced immobility episodes in taiep rats. *Synapse.* 2006; 60:362–370. [PubMed: 16838363]
175. Ita ML, Cortes MC, Valencia J, Eguibar JR. Activation of serotonin 5-HT(1)-receptors decreased gripping-induced immobility episodes in taiep rats. *Neurosci. Lett.* 2009; 449:147–150. [PubMed: 18996171]
176. Dugovic C, Solberg LC, Redei E, van Reeth O, Turek FW. Sleep in the Wistar-Kyoto rat, a putative genetic animal model for depression. *Neuroreport.* 2000; 11:627–631. [PubMed: 10718326]
177. Allard JS, Tizabi Y, Shaffery JP, Manaye K. Effects of rapid eye movement sleep deprivation on hypocretin neurons in the hypothalamus of a rat model of depression. *Neuropeptides.* 2007; 41:329–337. [PubMed: 17590434]
178. Prober DA, Rihel J, Onah AA, Sung RJ, Schier AF. Hypocretin/orexin overexpression induces an insomnia-like phenotype in zebrafish. *J. Neurosci.* 2006; 26:13400–13410. [PubMed: 17182791]
179. Yokogawa T, Marin W, Faraco J, Pezeron G, Appelbaum L, Zhang J, Rosa F, Mourrain P, Mignot E. Characterization of sleep in zebrafish and insomnia in hypocretin receptor mutants. *PLoS Biol.* 2007; 5:e277. [PubMed: 17941721]
180. Behn DCG, Kopell N, Brown EN, Mochizuki T, Scammell TE. Delayed orexin signaling consolidates wakefulness and sleep: physiology and modeling. *J. Neurophysiol.* 2008; 99:3090–3103. [PubMed: 18417630]

Table 1

Summary of Current and Potential Animal Models of Narcolepsy

Model	Pathophysiology	Characteristics
<i>Canine: familial</i>	Loss of Ox2R function	<ul style="list-style-type: none"> - narcolepsy is hereditary. - cataplexy can be induced and measured using FECT and PECT.
<i>Canine: sporadic</i>	Unknown, likely loss of orexin neurons	<ul style="list-style-type: none"> - narcoleptic symptoms are usually more severe.
<i>Rodent (mouse): Orexin KO</i>	Loss of orexin ligand	<ul style="list-style-type: none"> - have SOREM/cataplectic attacks. - unlike in most human cases, orexin neurons are intact despite loss of ligand.
<i>Rodent (mouse): Orexin receptor KO</i>	Loss of one or both orexin receptors	<ul style="list-style-type: none"> - double receptor KO is equivalent to ligand KO. - single receptor KO has much milder symptoms.
<i>Rodent (mouse/rat) : Orexin/ataxin-3 transgenic animals</i>	Complete post-natal degeneration of orexin neurons induced by a neurotoxic peptide at 15–17 weeks	<ul style="list-style-type: none"> - have SOREM/cataplectic attacks. - best model to mimic loss of orexin neurons observed in most human cases. - difficult to breed.
<i>Rodent (rat): antisense/RNA interference against orexin or its receptors</i>	Transient inhibition of the gene expression of orexin or its receptors	<ul style="list-style-type: none"> - knockdown but not KO. - increase in REM, infrequent cataplectic attacks. - increase in REM, infrequent cataplectic attacks.
<i>Rodent (rat): chemical lesion</i>	Orexin-B conjugated saporin injected into the lateral hypothalamus	<ul style="list-style-type: none"> - destroys both orexin and non-orexin cells. - multiple SOREMs.
<i>All species: orexin antagonist</i>	Antagonist against both orexin receptors	<ul style="list-style-type: none"> - no cataplexy. - increase sleep, especially REM sleep in rats, dogs, and humans.