

Animal Models of Narcolepsy

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Abstract The importance of orexin in the regulation of sleep/wakefulness states, especially in the maintenance of wakefulness, was initially revealed by animal studies which showed the relationship between orexin and narcolepsy. Familial narcolepsy of dogs was shown to be caused by functionally-null mutations in the orexin 2 receptor (*OX2R* or *hcrtr2*) gene (Lin et al. 1999). At the almost same time, orexin gene disruption in mice was shown to cause narcolepsy (Chemelli et al. 1999). Orexin neuron-ablated (*orexin/ataxin-3*-transgenic) mice, and *OX1R/OX2R* double-deficient mice were subsequently showed to have the same phenotype with *orexin*^{-/-} mice (Hara et al. 2001; Willie et al. 2003), characterized by behavioral arrests that are similar to cataplexy, occasional direct transitions to REM sleep from wakefulness, and highly fragmented sleep-wake cycles, all of which are important elements of narcolepsy. These phenotypes have strong parallels to the human narcolepsy. In this chapter, I will discuss animal models of narcolepsy.

1 Dog Narcolepsy

Narcolepsy has been well described in several mammalian species including dogs, mice and humans. Similar to human narcolepsy, main symptoms of dog narcolepsy are excessive daytime sleepiness (EDS) and cataplexy. Similar to that of humans, dog cataplexy is characterized by sudden muscle atonia without loss of consciousness. The animals usually remain alert and can follow movement with their eyes throughout the episode. The episodes typically last from several seconds to 30 min, often occurring when the dogs are running, eating, playing, excited, doing sexual activity. Food and play with other dogs are the well-documented paradigms used to trigger cataplexy. During cataplexy, dogs collapse onto its side or belly, and

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all physical movement ceases. Dogs usually come out of an episode in response to external stimuli, such as loud sounds. Occasionally, dogs fall asleep after attacks. Narcoleptic dogs sometimes suffer from sudden loss of consciousness or sleep attack, phenotypes that suggest excessive daytime sleepiness.

Like human cases, most dog narcolepsy is sporadic, and CSF orexin A levels are low or undetectable, suggesting that postnatal degeneration of orexin neurons due to similar pathophysiology as human cases. However, rare hereditary cases were found in Labrador retrievers, poodles, dachshunds, and Doberman pinschers.

Autosomal recessive transmission with full penetrance for canine narcolepsy was first established in Labrador retrievers and Doberman pinschers (Foutz et al. 1979). Although one of the predisposing genetic factors of human narcolepsy is a HLA-DQ allele, HLA-DQB1*0602, genetic linkage analysis had excluded the linkage between the dog narcolepsy and the canine major histocompatibility complex (Mignot et al. 1991).

In 1999, by screening BAC clones containing a genetic marker a team of Stanford University led by Mignot showed that loss of function mutations in *OX2R* (*hcrtr-2*) gene are responsible for familial narcolepsy-cataplexy in dogs, supporting the idea that deficiency in orexin (hypocretin) signaling might have an important role in pathophysiology of narcolepsy.

The *OX2R* gene of narcoleptic Dobermans contained a 226-bp insertion corresponding to a common canine short interspersed elements (SINE) repeat element located 35 bp upstream of the exon 4. The insertion of the SINE displaces a putative lariat branchpoint sequence located at position -40 through -46 upstream of the 3'-splice site. The mRNA potentially encodes a protein with 38 amino acids deletion within the fifth transmembrane domain followed by a frameshift, making the transcript functionally null. In narcoleptic Labrador retrievers, a separate line, G to A transition in the 5'-splice junction consensus sequence was found. Therefore, the exon 5 is spliced directly to exon 7, omitting exon 6. The C terminus of the *OX2R* of narcoleptic Labrador was truncated and did not include a seventh transmembrane domain. In both cases, these structural abnormalities of *OX2R* disrupt proper function of *OX2R*, suggesting the importance of *OX2R*-mediated signaling in the stabilization of wakefulness.

2 Rodent Narcolepsy

To date, several genetically-modified narcoleptic rodent models have been established, including *orexin*^{-/-} mice, *orexin-ataxin-3* mice/rats, *OX2R*-deficient mice, *OX1R/OX2R* -double knockout mice, and *orexin-tTA;TetO DTA* mice. Sleep/wakefulness states of these mice are similar, although each shows some distinguishing characteristics. In this section, I will firstly mention about their overall phenotypes, and then discuss about phenotypes of each model (Figs. 1 and 2).

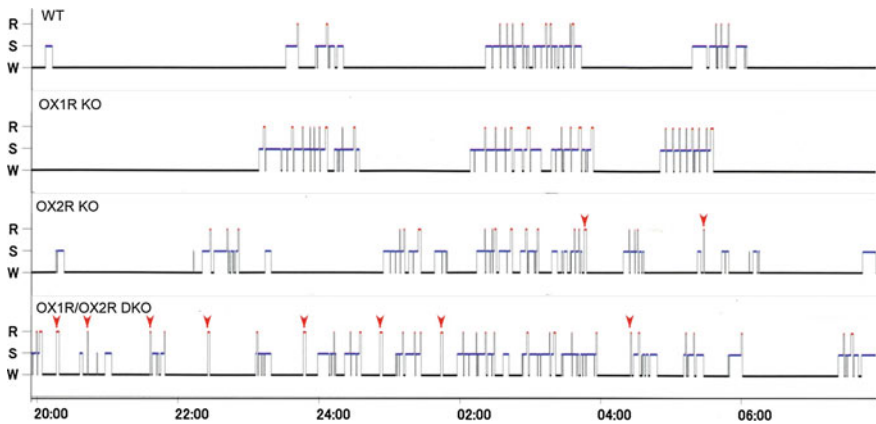


Fig. 1 Representative hypnograms of wild-type, $OX1R^{-/-}$, $OX2R^{-/-}$ and double receptor deficient mice over 12 h of the dark phase obtained by concatenating 16 s epoch EEG/EMG stage scores. The height of the *horizontal line above baseline* indicates the vigilance state of the mouse at the time. *Baseline*, *W* represents periods of wakefulness; *S* non-REM sleep; *R* REM sleep. *Arrowheads* highlight direct transitions from wakefulness to REM sleep

2.1 Phenotypes of Narcoleptic Mice

$Orexin^{-/-}$ mice, $orexin/ataxin-3$ mice, and $OX1R/OX2R$ double orexin receptor deficient ($OX1R^{-/-}; OX2R^{-/-}$) mice showed the almost identical sleep/wakefulness characteristics, including abrupt behavioral arrest (cataplexy) and fragmentation of sleep/wakefulness states. This section describes these phenotypes (Table 1).

These phenotypes caused by genetic modifications are strikingly similar to symptoms seen in human narcolepsy. However, it should be noted that familial transmission of human narcolepsy is very rare in humans, and even in these rare cases penetrance is far less than 100 %. No mutation has been found so far either in the *prepro-orexin* or *orexin receptor* genes of human narcolepsy patients, except an unusually severe, early onset case, which is associated with mutation in the *prepro-orexin* gene that impairs peptide trafficking and processing (Peyron et al. 2000).

Behavioral arrests (Cataplexy): These narcoleptic ($orexin^{-/-}$, $orexin/ataxin-3$, and $OX1R^{-/-}; OX2R^{-/-}$) mice exhibited substantial numbers of behavioral arrests during the dark periods. They were specifically recognized by the abrupt cessation of purposeful motor activity associated with sudden, sustained change in posture that was maintained throughout the episode, ending abruptly with complete resumption of purposeful motor activity. Characteristics of abrupt arrests were very different from those of quiet behavioral states or normal transitions into sleep. Each episode usually lasted for a short period, mostly less than a minute. Occasionally, gait disturbance lasting several seconds was observed immediately before episodes. Side-to-side rocking, without change in overall posture, frequently occurred several seconds after the start of the arrest.

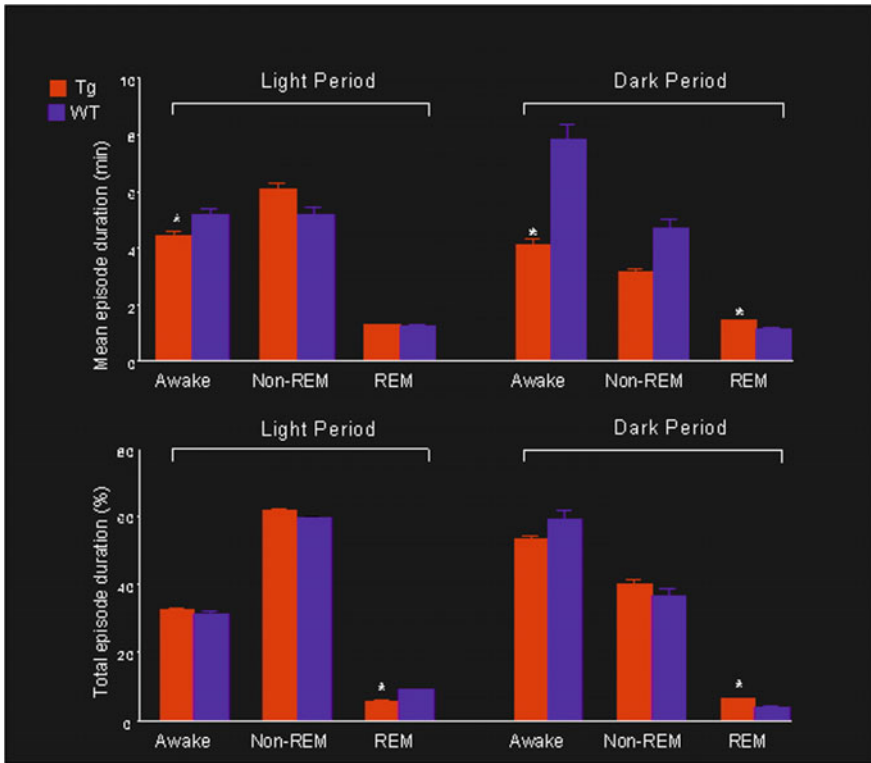


Fig. 2 Durations (*upper panels*) and total amounts (*lower panels*) of awake, non-REM sleep, and REM sleep states (means and SEM) for wild type and *orexin/ataxin-3* (Tg) mice in light and dark periods. Narcoleptic mice consistently show significantly increased REM sleep times during the dark phase compared to normal mice. *, $p < 0.05$

Detailed observations of behaviors during EEG/EMG recordings found that abrupt arrests in narcoleptic mice occurred at timing of the direct transitions from wakefulness to REM sleep or during pre-REM phase immediately after a waking period. (The pre-REM phase shows EEG with high-amplitude spindle oscillations superimposed on non-REM sleep background, and these spindles are observed only during the transition phase immediately prior to REM sleep in wild-type mice.) The direct or very rapid transitions from wakefulness to REM sleep are the most prominent characteristic of EEG/EMG in narcoleptic mice, which were observed almost exclusively in the dark phase, and were never observed in wild-type mice.

Abrupt arrests in *orexin*^{-/-} mice were ameliorated by systemic administration of clomipramine, an agent used for treatment of human cataplexy. While administration of caffeine, a psychostimulant used to treat excessive sleepiness in human narcolepsy, produce a mild exacerbation of abrupt arrest frequency.

These behavioral, electrophysiological, and pharmacological characteristics of abrupt arrests in narcoleptic mice suggest that these attacks are the counterpart of

Table 1 Phenotypes of rodent narcolepsy models produced by genetic engineering

	Sleep/wake state abnormality	Other phenotypes	Ref.
Prepro-orexin knockout	Cataplexy (+), sleep attacks (+) Sleep/wake fragmentation (severe)	Slight decrease in food intake, mild tendency for obesity (dependent on genetic background)	Chemelli et al. (1999); Hara et al. (2005)
OX1R knockout	Cataplexy (-), sleep attacks (+) Sleep/wake fragmentation	Impairment in emotional memory formation	Willie et al. (2001); Soya et al. (2013)
OX2R knockout	Cataplexy (+), sleep attacks (+) Sleep/wake fragmentation (severe)	ND	Willie et al. (2003)
Orexin/ataxin-3 mouse	Cataplexy (+), sleep attacks (+) Sleep/wake fragmentation (severe)	Decrease in food intake, mild tendency for obesity (dependent on genetic background), lack of food entrainable activity, lack of fasting-induced increase in wake time	Hara et al. (2001)
Orexin/ataxin-3 rat	Cataplexy (+), sleep attacks (+) Sleep/wake fragmentation (severe)	ND	Beuckmann et al. (2004)
orexin-tTA; TetO DTA mice	Cataplexy (+) (depending on numbers of orexin neurons ablated), sleep attacks (+) Sleep/wake fragmentation (severe)	Late onset obesity	Tabuchi et al. (2014)

cataplexy observed in human narcolepsy patients. Cataplexy has been well known to be triggered by strong emotions such as laughing, anger, fear, surprise, and excitement in human narcolepsy patients. In narcoleptic mice, abrupt arrests are very often preceded by purposeful motor activity such as excited ambulation, grooming, burrowing, and climbing. Palatable foods such as chocolates can also trigger cataplexy. The dramatic increase in the number of abrupt arrests noted in the group-housed mice as compared with the individually-housed littermates suggests that social interaction may enhance this attack. Chasing, tail biting, and social grooming were often observed to immediately precede the attacks in the group-housed mice.

Only approximately one-third of human patients experience full loss of muscle tone causing collapse to the floor with the majority having partial cataplexy

evidenced by jaw sagging, head bobbing, arm dropping, ptosis, or dysarthria (Parkes et al. 1974). Unambiguous full postural collapse was frequently observed in young *orexin*^{-/-} mice, while adults tended to collapse onto their ventral surface at odd angles, suggesting some residual muscle tone. Cataplexy is not always instantaneous in human patients but can progress over several seconds with some patients experiencing gait disturbance known as “zig-zag walking”. Similarly, gait ataxia immediately preceding almost one third of abrupt arrests in narcoleptic mice.

Sleepiness and sleep attack: One of the prominent features of sleep/wakefulness patterns in narcoleptic mice was shortened durations of both wakefulness and NREM sleep in the dark phase, causing increased fragmentation of sleep/wake cycle. The shortened durations of wakefulness suggests sleepiness, which is the cardinal symptom of narcolepsy. *OX2R*^{-/-} mice also showed sleep/wake fragmentation, while occurrence of REM sleep-related abnormalities was very rare as compared to *orexin*^{-/-}, *orexin/ataxin-3* or double orexin receptor knockout mice. This fragmentation was accompanied by statistically insignificant tendency toward reduced amounts of wakefulness and increased amounts of NREM sleep during the dark phase.

Presumptive excessive sleepiness in *orexin*^{-/-} mice was also shown by detailed analyses of “gradual arrests” in *orexin*^{-/-} and *OX2R*^{-/-} mice, which can be interpreted analogous to “sleep attacks” in human narcolepsy. Gradual arrests typically began during quiet wakefulness and could be easily distinguished from the normal onset of resting behavior by lack of stereotypic preparation for sleep (e.g., nesting and/or assumption of a curled or hunched posture, with limbs drawn under the body) and a characteristic ratchet-like “nodding” of the head over a period of several seconds, with a transition to a collapsed posture. Gradual arrests in both *orexin*^{-/-} and *OX2R*^{-/-} mice resemble the sleep attacks in human narcolepsy. Unlike cataplexy, the gradual arrests are not associated with strong emotions or muscle atonia, and are reduced by psychostimulants such as amphetamines, modafinil, and caffeine.

Systemic administration of caffeine dose-dependently suppressed gradual arrests, while administration of an anticataplectic agent clomipramine did not affect the frequency of gradual arrests in both *orexin*^{-/-} and *OX2R*^{-/-} mice.

EEG/EMG recordings with simultaneous video-capture further differentiated gradual arrests from abrupt ones. As described above, abrupt arrests were accompanied by direct transition from wakefulness to REM sleep in narcoleptic mice. In contrast, EEG/EMG correlates of gradual arrests in narcoleptic mice invariably revealed the onset of attenuated muscle tone, but not atonia, and an EEG transition from wakefulness to NREM sleep. Gradual arrests were occasionally accompanied by apparent automatic behavior, which is continuation of semipurposeful motor activity after the onset of light sleep such as stereotypic chewing of food.

2.2 Characteristic of Each Model

2.2.1 *Pepeo-Orexin Gene Knock Out (Orexin^{-/-}) Mice*

Conventional behavioral tests revealed that *orexin^{-/-}* mice did not show any overt abnormalities during the light period, except mild decrease in food intake (Chemelli et al. 1999). However, infrared video-recordings at the dark period to examine abnormalities on feeding behavior of *orexin^{-/-}* mice at night, when mice are normally most active, found frequent periods of behavioral arrest. No serum electrolyte imbalance or hypoglycemia was observed in *orexin^{-/-}* mice. Electroencephalograph/electromyographic (EEG/EMG) recordings from *orexin^{-/-}* mice showed no evidence of epileptic seizure during the attack. Instead, these EEG/EMG recordings revealed abnormal intrusions of REM sleep-like episodes into wakefulness and fragmentation of sleep/wakefulness cycle (Chemelli et al. 1999). Markedly reduced latency to REM sleep and increase in REM sleep during the dark phase were also observed. These characteristics of *orexin^{-/-}* mice were strikingly similar to characteristics human narcolepsy.

2.2.2 *Orexin-Ataxin-3 Mice*

Orexin levels in the CSF and postmortem brains of narcolepsy patients was dramatically decreased (Nishino et al. 2000; Peyron et al. 2000; Thannickal et al. 2000). However, in most cases of human narcolepsy, onset of symptoms occurs during adolescence. Based on the late onset, as well as a strong association of human narcolepsy with certain HLA alleles (Kadotani et al. 1998; Singh et al. 2013), it has been thought that narcolepsy result from acquired, selective autoimmune degeneration of orexin neurons.

In order to mimic the postnatal degeneration of orexin neurons in human narcolepsy, we generated transgenic mice (*orexin/ataxin-3* transgenic mice) in which orexin neurons are ablated by orexinergic-specific expression of a truncated Machado-Joseph disease gene (ataxin-3) fragment with expanded polyglutamine stretch as a toxic trans gene (Hara et al. 2001). Number of orexin neurons decreased after their birth, and at 12 weeks of age, over 99 % of orexin neurons were lost. *Orexin/ataxin-3* transgenic mice exhibited behavioral and electrophysiological defects that are essentially the same as those displayed by *orexin^{-/-}* mice, including cataplexy-like behavioral arrests, direct transitions from wakefulness to REM sleep, shortened latency to REM sleep, fragmentation of sleep/wakefulness states, and increases in REM sleep time and duration in the dark period. However, the behavioral arrests seen in *orexin/ataxin-3* transgenic mice typically began about 6 weeks of age, while arrests were observed in some *orexin^{-/-}* mice earlier than 3 weeks of age. Thus, *orexin/ataxin-3* transgenic mice have etiology and the course of disease similar to human narcolepsy. This model has been widely used as an useful narcoleptic model.

The fact that *orexin/ataxin-3* transgenic mice exhibited similar phenotype with that of *orexin*^{-/-} mice also revealed that orexin is the primarily important factor for the maintenance of the sleep/wakefulness state by orexin neurons, although orexin neurons produce other neurotransmitters such as glutamate, dynorphins, and neurtensin (Chou et al. 2001; Rosin et al. 2003; Furutani et al. 2013).

We utilized this mouse model of narcolepsy to rescue its narcoleptic phenotype in *orexin/ataxin-3* transgenic mice by genetic (overexpression of orexin peptides throughout the brain) and pharmacological (i.c.v. administration of orexin A) means (Mieda et al. 2004), demonstrating that mice retain the ability to respond to orexin neuropeptides even if they lack endogenous orexin neurons and that a temporally regulated and spatially targeted secretion of orexins is not necessary to prevent narcoleptic symptoms. This finding suggests that orexin receptor agonists would be of potential value for treating human narcolepsy.

2.2.3 *Orexin/Ataxin-3* Rats

Transgenic rats made with the same transgene as *orexin/ataxin-3* transgenic mice was also generated (*orexin/ataxin-3* transgenic rats) (Beuckmann et al. 2004). Similar to *orexin/ataxin-3* mice, number of orexin neurons was gradually reduced after their birth, and after 17 weeks of age orexin in the hypothalamus became undetectable. *Orexin/ataxin-3* transgenic rats showed a typical narcoleptic phenotype, with a decreased latency to REM sleep, increased REM sleep time in dark period, direct transitions from wakefulness to REM sleep, and a marked fragmentation of vigilance states. Brief episodes of muscle atonia and postural collapse resembling cataplexy were also observed while rats maintained the EEG characteristics of wakefulness, suggesting they were conscious during these episodes as human patients are during cataplexy.

2.2.4 OX2R-Deficient Mice

In infrared videotaping studies in the dark phase revealed that *OX2R*^{-/-} mice also exhibited behavioral arrests, or cataplexy (Willie et al. 2003). However, its frequency was much less than in *orexin*^{-/-} mice (31-fold lower frequency in *OX2R*^{-/-} mice as compared to *orexin*^{-/-} mice). *OX2R*^{-/-} mice appeared to exhibit different type behavioral arrests with onsets that were more gradual in nature (gradual arrests). *Orexin*^{-/-} mice and *orexin/ataxin-3* mice also found to exhibit the gradual arrests with the frequency similar to *OX2R*^{-/-} mice in addition to numbers of cataplexy-like, abrupt arrests.

OX2R^{-/-} mice and *orexin*^{-/-} mice are similarly affected by gradual arrests (“sleep attacks”) (Willie et al. 2003), but *OX2R*^{-/-} mice show a lower degree of disrupted wakefulness compared with double receptor-deficient (*OX1R*^{-/-}; *OX2R*^{-/-}) mice (Willie et al. 2003; Sakurai 2007; Hondo et al. 2010). In particular, *OX2R*^{-/-} mice are only mildly affected by cataplexy and direct transitions to REM

sleep from awake states, as compared with *orexin*^{-/-} mice and double receptor KO mice (Chemelli et al. 1999; Willie et al. 2003).

2.2.5 OX1R/OX2R Gene Double Knockout Mice

Orexin^{-/-} mice and double receptor-knockout mice have the virtually same phenotype (Willie et al. 2003). Considering the fact that *OX2R*^{-/-} mice are only mildly affected by cataplexy and direct transitions to REM sleep from awake states, these observations suggest that *OX2R* plays a pivotal role in maintain wakefulness, but *OX1R* has additional effects on sleep-wake regulation. The gating of REM sleep is likely to critically involve both receptors. The contributions of both receptors in the sleep/wakefulness states regulation are described in detail in our separate chapter (Mieda).

2.2.6 Orexin-TTA;TetO DTA Mice

In this model, diphtheria toxin A (DTA) was expressed in orexin neurons under control of the Tet-off system. Doxycycline removal from the diet of adult orexin-tTA;TetO DTA mice induce orexin neurodegeneration with 80 % cell loss after one week of tet-off condition, and resulted in apparent sleep/wakefulness fragmentation. However, cataplexy was only observed 14 d after the removal of doxycycline when less than 5 % of the orexin neurons remained. Cataplexy frequency increased for at least 11 weeks after doxycycline removal. These suggest that very low level of orexin could inhibit cataplexy. Body weight was reported to be increased without a change in food consumption, as in human narcolepsy and *orexin/ataxin-3* mice.

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