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Rewiring brain circuits to block cataplexy in murine models of narcolepsy

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Narcolepsy was first identified almost 130 years ago, but it was only 15 years ago that it was identified as a neurodegenerative disease linked to a loss of orexin neurons in the brain. It is unclear what causes the orexin neurons to die, but our strategy has been to place the gene for orexin into surrogate neurons in the validated mouse models of narcolepsy, and test whether it can block narcolepsy symptoms, such as cataplexy. In both the orexin knockout and the orexin–ataxin-3 mouse models of narcolepsy we have found that cataplexy can be blocked if the surrogate neurons are part of the circuit responsible for cataplexy. We have also determined that the orexin gene can be inserted into surrogate neurons in the amygdala to block emotion-induced cataplexy. Through the use of optogenetics we anticipate that it will be possible to preemptively block cataplexy.

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Gene therapy is being used to treat neurodegenerative diseases (for review see Refs. [1,2]). In a phase I study eight patients with mild Alzheimers were given *ex-vivo* NGF gene therapy and their rate of cognitive decline was slowed [3]. Currently, phase II trials are in progress. Motor functions were improved in six patients with Parkinson's disease that were treated with AAV mediated delivery of aromatic L-amino acid decarboxylase (AADC) gene for synthesis of dopamine and serotonin [4].

Genetic methods can be used to treat narcolepsy since the disease is linked to loss of the orexin neurons that are localized in only one place in the brain [5,6]. Therefore, it is feasible to test the hypothesis that orexin gene insertion into surviving neurons is able to decrease narcoleptic symptoms. We have focused on cataplexy, an important distinguishing symptom of narcolepsy, and demonstrated in two mouse models that cataplexy can be improved by orexin gene transfer [7,8]. In this review, we discuss the use of genetically engineered methods as a neurobiological tool to understand the neural circuitry underlying narcoleptic symptoms. We recognize that pharmacological agents such as modafinil (Provigil) or sodium oxybate (Xyrem) are prescribed to patients with narcolepsy. We agree that the pharmacological approach is economical, easily distributed and accepted by patients. However, the current drugs of choice treat only some of the symptoms, and they lack specificity since they bathe the entire body. It is preferable to use pharmacological agents that act at specific sites and the gene transfer method can serve as a tool to identify such sites. We envision that with the aid of optogenetics and pharmacogenetics it will be feasible to identify the neurons that trigger the cataplexy attacks and then proceed to blocking the attacks from being triggered by emotions.

Orexins (hypocretins) and linkage with narcolepsy

The neuropeptides hypocretins (HCRT), also known as orexins, were linked to narcolepsy through forward [9] and reverse [10] genetic approaches. Subsequently, post-mortem examination of brain tissue revealed loss of the orexin neurons in the narcoleptic patients [5,6]. It is now abundantly clear that narcolepsy is a neurodegenerative disease, since other markers that colocalize with HCRT are also absent in humans with narcolepsy [11,12].

Validated mouse models exist that clearly show that narcoleptic symptoms develop with deletion of the orexin peptide (orexin knockout) [10], the two orexin receptors [13], or the death of the orexin-containing neurons [14,15••]. In the newest mouse model [15••] the timing of the death of the orexin neurons can be controlled through the application of doxycycline. In the presence of doxycycline the orexin neurons remain intact, but once doxycycline administration (via drinking water) is stopped the diphtheria toxin accumulates in the orexin neurons, and within 7 days 80% of hypocretin neurons die. This model is best at mimicking the degeneration of the orexin neurons in human narcolepsy.

The orexin neurons are localized only in the perifornical and lateral hypothalamus [16] area from where they project widely throughout the CNS, with especially heavy innervation to neurons implicated in arousal.

Therefore, it is not surprising that sleep attacks and sleepiness occur with loss of the orexin neurons. Cataplexy is another symptom of narcolepsy that is usually triggered by strong emotions [17]. The orexin neurons project to the pontine areas implicated in maintaining muscle tone during waking [16], and loss of the orexin innervation destabilizes the circuit.

Since the underlying neurons that make and secrete orexin have died, it is necessary to identify surrogates that will accomplish this task. The important question is to identify suitable surrogate neurons. The orexin neurons project to the entire brain and spinal cord but it is not known which target site controls some or all of the narcoleptic symptoms [16]. Replacing the orexin could restore normal sleep–wake function, but which function? Narcolepsy is characterized by excessive daytime sleepiness, sleep fragmentation, sleep attacks, SOREMPs (sleep-onset REM periods), and cataplexy. Would transfer of the orexin gene rescue all of these, or are some more sensitive and easily affected by the presence of the peptide?

When we began our studies [7,8] we considered these issues and began by first placing the orexin gene in surrogate neurons of the lateral hypothalamus since the orexin neurons are localized only in this region from where they innervate various targets.

Orexin gene transfer into surrogate neurons in the hypothalamus and pons

Our initial approach was to use the surviving neurons in the perifornical area as surrogates because they are likely to have the same neuronal connectivity as the neurons that died. Therefore, we first tested our hypothesis in the orexin knockout mice [7]. In these mice only the orexin gene has been deleted but the underlying network is intact compared to the hypocretin–ataxin mice where the underlying neurons have died. We used a herpes-simplex virus-1 (HSV-1) to transfer the gene for orexin into surrogate neurons [7]. Our *in-vitro* and *in-vivo* tests demonstrated that the virus successfully transferred the orexin gene into neurons. Moreover, an ELISA assay confirmed that the peptide was detected in the CSF of mice given the gene transfer. During the four day life-span of the vector the incidence of cataplexy declined by 60%, and the levels of REM sleep during the second half of night were similar to levels in wild-type mice indicating that narcoleptic sleep–wake behavior in orexin knockout mice can be improved by targeted gene transfer.

In the next study [8] we used the orexin–ataxin-3 mice and a recombinant adenoassociated (rAAV) virus inserted the orexin gene into lateral hypothalamic neurons. The advantage of the rAAV was that it increased the lifespan of expression of HCRT to at least three weeks, compared to the four-day active cycle of the HSV-1 that was used in our initial study. We also inserted the orexin gene into neurons containing melanin concentrating hormone. The MCH neurons lie alongside the orexin neurons and project to the same targets as the orexin neurons. However, they are a separate population [16]. The MCH neurons are active during sleep [18] whereas the orexin neurons are active in waking [19,20]. Thus, the MCH neurons promote sleep whereas the orexin neurons promote arousal. Optogenetic activation of the MCH neurons induces sleep in both mice [21] and rats [22•] indicating that these neurons may induce sleep across mammals. Optogenetic activation of the orexin neurons induces arousal [23]. In the orexin–ataxin-3 mice the orexin neurons die while the MCH neurons are still present [14]. Therefore, we hypothesized that placing the orexin gene into the MCH neurons should block narcoleptic symptoms. However, we found that insertion of the orexin gene into MCH neurons did not affect cataplexy whereas insertion of orexin gene into other adjacent neurons significantly decreased cataplexy. We were initially puzzled why placing the orexin gene into MCH neurons did not work. However, the MCH neurons are not active in waking and therefore cannot release orexin to block cataplexy. We also found that using the striatal neurons as surrogates had no effect since these neurons are not part of the sleep network. Therefore, from this study we concluded that one cannot simply place the orexin gene anywhere in the brain; for it to be effective the orexin gene has to be placed in a neuron that is connected to the circuit regulating cataplexy, and the neuron also has to be active during cataplexy.

In the third study [24], the orexin gene was placed in the dorsal pons, an area that has a rich history in regulating REM sleep and motor control. The results indicated that orexin gene transfer into the pontine neurons was also effective in decreasing cataplexy. We also found that it increased length of waking bouts at night, albeit modestly. In our pontine study we did not target the orexin into phenotypic specific neurons in the pons. However, another group combined optogenetics and DREADD to provide a better cellular resolution of the phenotype of neurons in the pons involved in cataplexy [25••]. They showed that the orexin input onto serotonin neurons reduces cataplexy, while orexin input onto LC neurons maintains waking [25••].

Other groups have shown that there are brain sites where waking can be improved. For instance, one group injected the orexin gene into more ventral regions of the lateral hypothalamus in orexin–ataxin-3 mice and it modestly improved waking but not cataplexy [26]. Another group [27] used mice with a mutation in the hypocretin-2 receptor (orexin-2R). Canine narcolepsy is linked to a mutation in the hypocretin-2 receptor [9] and mice with deletion of this receptor also show narcoleptic symptoms [13]. Mochizuki *et al.*, [27] normalized the hypocretin-2