

Optogenetic manipulation of orexin neuronal activity affects sleep/wakefulness state in mice

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Orexin/hypocretin is a neuropeptide produced in the neurons sparsely distributed in the lateral hypothalamic area. The mice lack prepro-orexin or orexin-producing neurons (orexin neurons) show phenotypes, a fragmentation of sleep/wakefulness and sudden attack of muscle weakness. These symptoms are characteristic in human sleep disorder, narcolepsy. These suggest that orexin neurons have an important role in the maintenance of arousal.

To study physiological significance of orexin neuronal activity on maintenance of wakefulness, optogenetic manipulation of orexin neurons was incorporated. We previously generated *orexin/halorhodopsin* transgenic mice which enable inhibit orexin neuronal activity *in vivo*. However, halorhodopsin-induced inhibition did not last for more than one minute, probably due to desensitization.

In present study, to archive long lasting silencing of orexin neuronal activity *in vivo*, *orexin/archaeorhodopsin-3* transgenic mice were generated. In these mice brain, the orexin neurons specifically express green light-driven proton pump, archaeorhodopsin-3. Slice patch clamp analyses of orexin neurons showed that green light illumination completely ceased firing of archaeorhodopsin-3-expressing orexin neurons during illumination for up to one hour. In the early dark period (active period), one hour continuous silencing of orexin neuronal activity *in vivo* induced an increase in total time in slow wave sleep. Although inhibition of orexin neurons for one hour induced a fragmentation of sleep/wakefulness state, cataplexy was not observed. On the other hand, light illumination had no effect on sleep/wakefulness pattern in the light period (an inactive period).

These results suggest that orexin neuronal activity plays an important role in the maintenance of wakefulness especially in the active phase (in the early dark period).