Long time silencing of orexin/hypocretin neuronal activity using optogenetics in archaerhodopsin-3-expressing transgenic mice

Tomomi Tsunematsu, Makoto Tominaga, Edward S. Boyden, Thomas S. Kilduff, Akihiro Yamanaka

Orexin 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, cataplexy. All these symptoms are characteristic in human sleep disorder, narcolepsy. Additionally, orexin neurons are specifically degenerated in the narcoleptic patients. These suggest that orexin neurons have an important role in the regulation of sleep/wakefulness, especially in the maintenance of arousal.

To study physiological significance of orexin neuronal activity on maintenance of waking state, optogenetics was incorporated. We previously generated *orexin/halorhodopsin* transgenic mice which enable us to inhibit orexin neuronal activity *in vivo*. Acute silencing of orexin neurons during the daytime (inactive period) induced slow wave sleep. However, halorhodopsin-induced inhibition did not last for more than one minute, probably due to desensitization.

In this study, to archive long lasting silencing of orexin neuronal activity *in vivo*, *orexin/archaerhodopsin-3* transgenic mice were generated. In these mice brain, the orexin neurons specifically express green light-driven proton pump, archaerhodopsin-3. Immunohistochemical analyses confirmed specific expression of archaerhodopsin-3 in the orexin neurons. About 80% of orexin-immunoreactive neurons express archaerhodopsin-3 in these transgenic mice brain. No ectopic expression was observed throughout the brain. The function of archaerhodopsin-3 is confirmed by *in vitro* electrophysiological experiments. Slice patch clamp analyses of orexin neurons showed that archaerhodopsin-3 expression did not affect basic membrane properties of orexin neurons. However, under loose-cell attached mode, green light (549 ± 7.5 nm, 20 mW) illumination completely ceased firing of archaerhodopsin-3-expressing orexin neurons during illumination for up to one hour.

These results suggest that an optical silencing of orexin neuronal activity using archaerhodopsin-3 is a powerful tool to investigate the regulatory mechanisms sleep/wakefulness *in vivo*.