IN VIVO EFFECTS OF OREXIN ON SLEEP-WAKE STATES IN MICE: THE ROLE OF CORTICAL LAYER 6B

E. Meijer[1,2], *M. Mueller*[1], *L. Krone*[1,3], *A. Hoerder-Suabedissen*[1], *T. Yamagata*[1,4], *V. Vyazovskiy*[1], *Z. Molnár*[1]
[1]Oxford University, Oxford, United Kingdom, [2]University Medical Centre Utrecht, Utrecht, Netherlands, [3]University of Bern, Bern, Switzerland, [4]Toho University, Tokyo, Japan

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Background: Orexin (hypocretin) is considered one of the main wake-promoting neuromodulators in mammals. Orexin receptors are expressed widely throughout the brain, including the cortex, yet the direct cortical effects of orexin in mediating behavioural states are not fully understood. It has been demonstrated in vitro that both pyramidal neurons and interneurons in layer 6b of the cortex can be activated by orexin (Bayer et al, 2004; Wenger-Combremont et al 2016). In this study, we investigated the in vivo effects of orexin on sleep-wake states in wild-type mice and compared this with animals having a non-functional subset of neurons in cortical layer 6b.
Methods: We used the Drd1a-Cre;TdTom;Snap25fl/fl mouse model, wherein Cre expressing Drd1a cells are predominantly localized in layer 6b and express a truncated variant of Snap25. This abolishes synaptic transmission from around birth, resulting in the 'chronically layer 6b silenced' mouse. We implanted n=8 layer-6b silenced and n=5 littermate control adult male mice with a frontal and an occipital EEG electrode referenced to the cerebellum, neck EMG electrodes, and an intracerebroventricular canula. At light onset, we infused orexin A (0.6 nmol) or vehicle in a counterbalanced design. We then left animals undisturbed and continuously recorded EEG for 24 hours.
Results: As expected, orexin increased the time spent in spontaneous wakefulness in the first 3 hours after infusion (vehicle, controls 62.6 ± 7.13 min vs layer 6b silenced 58.9 ± 7.23 min, orexin A controls 99.4 ± 9.87 min vs layer 6b silenced 118 ± 4.95 min) and this effect did not differ between genotypes (two-way ANOVA, $F(1,6)=2.320$, $p=0.1785$). Moreover, orexin increased the duration of spontaneous wakefulness episodes (vehicle, controls 19.8 ± 4.92 vs layer 6b silenced 10.6 ±1.40 min, orexin A controls 25.8 ± 5.39 vs layer 6b silenced 39.7 ± 7.53 min). There was a larger increase in wake duration following orexin administration in layer 6b silenced animals (two-way ANOVA, $F(1,11)=6.176$, $p=0.0303$).
Conclusions: Orexin administration leads to an increased average duration of wakefulness episodes, and chronically silencing layer 6b of the cortex seems to enhance this effect. This is the first study to investigate the effects of orexin on layer 6b in vivo. Further data collection is ongoing.