Adenosine is a neurotransmitter implicated in sleep. The neuropeptides orexin A and orexin B are regulators of sleep that promote wakefulness and arise from the lateral hypothalamic neurons (Fig 1). But is there an interaction between the two? The following experiments may be one way to address this question.

Figure - Region of Interest

To test the effects of adenosine in the lateral hypothalamus (LHA), I’d first be interested in the synaptic effects of adenosine on LHA neurons. I’d generate orexin-GFP mice by crossing Orexin-cre;Rosa26-GFP mice. Then, I’d cut slices of LHA and patch the GFP neurons them using whole cell recordings/widefield epifluorescence. I’d look at sEPSCs and sIPCSs with bath application of adenosine. I’d bath apply AP5, CNQX and bicuculline to ask if these were glutamatergic/gabaergic synaptic potentials. Next, I’d focally delete adenosine (A1) receptor in LHA using an AAV injected into LHA (Scammell, Arrigoni et al. 2003). With these receptors ablated, I’d patch again and verify that the sEPSCs or sIPSCs frequency change was mediated by A1-receptors. After patching, I’d backfill with RFP to verify orexin expression. These experiments would instruct me as to the effect of adenosine on orexin producing LHA neurons.

To test the effects of adenosine under anesthesia in the whole brain, I’d use Fast-Scan Cyclic Voltammetry (FSCV), a technique which measures adenosine concentrations on a sub-second resolution (Swamy and Venton 2007). Sub-second temporal resolution is important so I can correlate neural activity (single units or LFPs) to adenosine concentration changes. When adenosine is near the electrode, it becomes oxidized at positive sweep and is reduced upon the negative sweep (Adamah-Biassi, Almonte et al. 2015). The transfer of electrons by the neurotransmitter is measured as current and is proportional to the amount of adenosine in the microenvironment (Swamy and Venton 2007). I would lower FSCV, laser optic fiber and tetrodes to LHA in mice that have astrocyte driven ChR2 (Perea, Yang et al. 2014). I would test for temporal correlations between firing rate changes, LFP spectral changes and adenosine concentration changes. To test loss of function, I would inhibit adenosine release in astrocytes with adenosine receptors (A1 or A2) and dominant negative SNARE (Florian, Vecsey et al. 2011, Orr, Hsiao et al. 2015) or injection of the AAV for A1-receptor blocking. Then I’d test for changes to units, LFP and adenosine to verify that receptors are required.

To test the effects of adenosine in freely behaving mice, I would use the same mice from the anesthesia section but train them to run on a trackball and head-fixed (Roseberry, Lee et al. 2016). Using tetrodes, light fiber and FSCV, I’d ask the question: does adenosine release by astrocytes induce sleep in mice? If so, how are the LHA neurons affected? Changes to LFP/unit activity? Are these neural response changes before or after adenosine has risen in the interstitial brain space? If adenosine activation is insufficient to induce sleep, I can still analyze the trackball data to see how their locomotion is impaired.

In summary, these experiments will allow us to identify the effect of adenosine on LHA neurons, a critical brain circuit for the regulation of wakefulness and sleep.

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