Summary:

Sleep is a cornerstone of our physical and mental well-being. Beyond its apparent simplicity, sleep is a complex and multifaceted process that is tightly regulated by 2 processes: a circadian process that ensures sleep occurs at the proper time of the day and a homeostatic process that tracks the accumulation of sleep pressure while we remain awake. While the molecular mechanism of the circadian clock is currently well-established, the molecular underpinning of sleep pressure is largely unknown.

During sleep, brain electroencephalography (EEG) signals are dictated by high-amplitude slow waves (SW, ≤4 Hz). The spectral power of these SW, commonly referred to as SWA, accumulates with time being awake, peaking at the beginning of sleep and dissipating over the course of sleep. Notably, sleep loss is compensated for with a slight extension of total sleep time and a dramatic rise in SWA. Therefore, SWA is widely considered the most reliable in-vivo marker for sleep pressure and/or depth.

Although sleep is fundamentally manifested as a global behavior, sleep tenets are locally fine-tuned to satisfy homeostatic needs for individual brain networks. There is a large body of evidence across different species indicating that SWA is locally regulated in a use-dependent manner- cortical areas that experience more intense activity in wakefulness show higher SWA in subsequent sleep-, giving rise to the notion of local sleep homeostasis. Yet, the neuronal circuitry and molecular mechanism underlying this local sleep regulation is largely unknown.

Decades of research have shown that sleep SWA reflects rhythmic activities in thalamocortical ensembles. Nevertheless, a slab of cortex that is isolated from the rest of the brain or even a plate of cultured neurons exhibits some forms of SW (<1.5 Hz), indicating that the neocortex alone is sufficient for the generation and maintenance of SW. Taking these findings into consideration, I designed an in-vivo sleep assay that mainly focuses on cortical molecular mechanisms and circuitry involved in the regulation of local sleep SWA. In short, I unilaterally performed an artificial manipulation of a specific signaling pathway in the somatosensory cortex of mice while recording local field potentials (LFP) from the manipulation site as well as the contralateral hemisphere as a control. Utilizing this assay, I addressed different hypotheses that were long sought after as potential reasons for local and/or global sleep pressure including neuronal firing, astrocyte activity, core clock genes, and synaptic plasticity. Surprisingly, neuronal firing, driven by optogenetics or chemo-genetics, was not sufficient to induce subsequent upregulation of local sleep SWA. Moreover, astrocyte activity, driven by hM3Dq or hM4Di signaling, did not affect local sleep SWA dynamics. Similarly, knocking out the core cellular clock component *Bmal1* did not affect local SWA under baseline or sleep deprivation conditions. Interestingly, micro-infusion of brain-derived neurotrophic factor (BDNF) resulted in subsequent unihemispheric upregulation of sleep SWA in a dose-dependent manner. These effects of BDNF

were completely abolished when Tyrosine kinase B (TrkB) was blocked, indicating that BDNF-driven upregulation of local sleep SWA occurs through the activation of the TrkB receptor. Then, I utilized an optogenetic tool (opto-TrkB) that allows the activation of TrkB signaling with high spatial and temporal resolution. As hypothesized, activation of opto-TrkB led to subsequent unihemispheric upregulation of local SWA, which was largely mediated by cortical layer 5 pyramidal neurons. Furthermore, I demonstrated that the effects of BDNF/TrkB signaling on local sleep SWA was mediated through downstream activation of cAMP-response element-binding protein (CREB).

The second section of this thesis mainly addresses the role of cortical Gs-GPCR signaling in local and global sleep regulation. In brief, I observed that activation of cortical Gs-signaling, driven by rM3Ds, upregulated local sleep SWA. I have further activated Gs-signaling on multiple cortical regions and observed an increase in sleep duration. Together these findings provide mechanistic insights into the local regulation of sleep, and highlight BDNF/TrkB and Gs-GPCRs as potential therapeutics target for sleep disturbances.