Final report

NN 125601: Mechanisms of pathological thalamocortical oscillations

In this project we studied the neuronal mechanisms of pathological oscillations in various rodent models of absence epilepsy by performing electrophysiological and optogenetic expeiments in various brain areas. We published a review article on the topic of intrinsic contributions of rhythmic thalamic activity and its relation to neuronal plasticity in the journal **Nature Reviews Neuroscience** (Crunelli, Lőrincz et al. 2018). The ideas outlined here are important for future experiments studying the link between rhythmic thalamic activity, plasticity and epilepsy. We summarized the mechanisms of absence seizure generation in the cortico-thalamo-cortical system and basal ganglia and how this is linked to various comorbidities of absence seizures in the journal **Brain** (Crunelli, Lőrincz et al. 2020). We have outlined our current knowledge and possible future directions of research concerning the balance between cortical inhibition and excitation in a book chapter in **Jasper's Basic Mechanisms of the Epilepsies** (Lőrincz et al. 2022).

In the thalamus it was widely disputed whether the intrinsic cellular properties of thalamocortical neurons (i.e. low threshold calcium spike mediated burst firing) or network interactions (i.e. cortico-thalamo-cortical synaptic communication) generate spike and wave discharges, the cellular substrates of absence epilepsy. In a collaboration with Prof. Crunelli's group (Cardiff University) we performed electrophysiological recordings from somatotopically aligned cortical and thalamic sites, pharmacological blockade of thalamic T-type Ca^{2+} channels in rodent models of absence epilepsy and modelling and found that the intrinsic thalamic bursting in the primary somatosensory thalamus is not necessary for the generation of absence seizures. Instead, cortically derived feed-forward inhibition in the thalamus is necess plays a crucial role in the generation of these pathological events. These results were published in the journal **Nature Neuroscience** (McCafferty et al. 2018).

We next studied the role of thalamic and cortical I_h currents mediated by HCN channels in absence epilepsy. The role of these channels have been debated with some results suggesting they have an anti-epileptic role. We performed *in vivo* and *in vitro* pharmacological experiments in combination with virally mediated genetic ablation of HCN channels and found that thalamic HCN channels are pro-epileptic. These results were published in the **Journal of Neuroscience** (David et al. 2018). Although HCN channels are important in absence epilepsy their clinical relevance is restricted by the fact that HCN channel antagonists are brain inpenetrant. We tested the effects of the HCN channel blocker, Ivabradine administered in such a way that it can cross the blood-brain barrier when administered systemically and fond that it can block absence seizures in rodent models. We next tested the cellular effect of Ivabradine and found that it reduces the "sag" potential generated by HCN channels in cortical and thalamic neurons. These results were published in the journal **Epilepsia** (Iacone … Lőrincz, 2021)

It is known that inducing rhythmic rebound burst firing by specific optogenetic inhibition of thalamic is sufficient (although not necessary) for the initiation of absence seizures in rodent models. We tested whether direct depolarization of thalamic neurons in vivo (leading to tonic firing, but not burst firing) can induce seizures. We found that rhythmic tonic firing can induce burst firing further confirming our previous experiments (McCafferty et al. 2018). Importantly, the induction of seizures was highly brain state dependent with quiet wakefulness being the most likely and slow wave sleep the most unlikely state to generate seizures by direct channelrhodopsin mediated depolarization of thalamocortical neurons. Because depolarization of these neurons during sleep leads to burst firing these results further confirm that thalamic bursting is not the key player in generating absence seizures. These results were submitted for publication to the journal **Epilepsia** (Taylor ... Lőrincz, 2022).

We have previously shown that the long-range reciprocal inhibitory interaction between the lateral hypothalamus (LH) and the dorsal raphe nucleus (DRN) is important for promoting wakefulness (Gazea ... Lőrincz, 2021). We have now shown that the LH also sends direct glutamatergic inputs to DRN serotonergic neurons. Importantly, LH neurons decrease their firing during absence

seizures. These results were published in the journal **International Journal of Molecular Sciences** (Sere ... Lőrincz, 2021).

We developed a head restrained rat preparation to study the membrane potential and action potential firing of identified cortical neurons during absence seizures. We found that contrary to the experiments performed under anesthesia, neurons in the awake cortex show extreme heterogeneity during seizures. Specifically, pyramidal neurons were either characterized by an ictal increase in their mean firing rate or in the minority of the cases ictal and interictal firing rates did not differ. In contrast, regular firing interneurons and fast spiking basket cells in the infragranular layers of the somatosensory cortex could either increase, decrease or maintain the same ictal and interictal firing rates. These results will be submitted for publication to the journal **Neurobiology of Disease** by October 2022 (Sere ... Lőrincz, 2022).

We next studied the thalamic mechanisms of absence seizure generalization in behaving GAERS rats. Combining simultaneous multiple single unit recordings, pharmacological inactivation and optogenetics in higher order thalamic nuclei we found that the generalization of the absence seizures is affected by higher order thalamic nuclei. Specifically, inactivating the posterior or the lateral posterior thalamic nuclei resulted in an increase in the onset delay of the seizures in cortical areas distal to the initiation site, the primary somatosensory cortex. Specific stimulation of thalamocortical neurons in higher order thalamic nuclei could alter the generalization of seizures by suppressing seizures in the visual cortex. Neural dynamics of higher order nuclei single units revealed two groups of putative excitatory neurons. All groups exhibited a switch from tonic to burst firing before onset of seizures but had different dynamics during the seizures. The results of these experiments converge on the conclusion that higher order thalamic nuclei are utilized at seizure onset and contribute to cortical synchrony throughout. These results will be submitted for publication to the journal **Neurobiology of Disease** (Atherton ... Lőrincz, 2022) by the end of September 2022.

The striatum is important in coordinating goal directed behaviors. The ictal activity of different striatal neurons has not been studied extensively in rodent models. We recorded the activity of various morphologically identified striatal neurons in the Stargazer mouse model of absence

epilepsy and found extreme ictal heterogeneity between the activity of medium spiny neurons and various other striatal interneurons. These results could have important implications for the ictogenesis of absence seizures and also for revealing the neuronal mechanisms of some comorbidities of absence seizures. These results will be submitted for publication to the journal **Epilepsia** (Zsigri … Lőrincz, 2022) by the end of November 2022.

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