Nociceptive inputs to trigeminal nucleus caudalis neurons - implications for migraine

Migraine is a disabling and episodic brain disorder with high prevalence and complex pathophysiology. Trigeminal nociceptors and the trigeminal nucleus *caudalis* (TNC) are key brain structures for the integration and processing of craniofacial pain. Animal models suggest that sensitization of the trigeminovascular pathway plays a major role in the pathology of migraine, yet little is known about long-term changes in trigeminal afferents or their synapses in the TNC. We used mice expressing channelrhodopsin-YFP in TRPV1 lineage neurons (generated from TRPV1-Cre (B6.129-Trpv1tm1(cre)Bbm/J)) to investigate different forms of synaptic plasticity at nociceptive primary afferents projecting onto second order relay neurons within the TNC.

We found with immuno-labeling that these afferents mostly co-localize with CGRP-containing C- and Aδ-fibers. We also found that TRPV1 lineage neurons co-localized to a lesser extent with IB4-positive, non-peptidergic nociceptors in the trigeminal ganglion, thereby indicating that optical stimulation would activate a specific subset of primary afferents that are predominantly nociceptors.

Optical stimulation at the dorsolateral slice edge (473 nm, 0.4 - 1 msec) of neurons in laminae I-II in acutely prepared transverse TNC slices, evoked excitatory postsynaptic currents (EPSCs) and often polysynaptic activity. The neuronal population receiving direct optically-induced synaptic inputs in the TNC is very heterogeneous and is mainly composed of delayed- and tonic-firing neurons. Analysis of EPSC properties revealed that 65% of delayed-firing neurons and only 47% of tonic-firing neurons received optically-induced polysynaptic inputs. Investigation of the monosynaptic input kinetics showed that EPSCs in delayed-firing neurons show a faster decay time (5.06 ms ± 0.45) compared to tonic-firing neurons (9.34 ms ± 0.77, $p < 0.001$), which may indicate that nociceptors synapse more distally onto tonic- than onto delayed-firing neurons.

We then explored avenues to induce plasticity at these synapses. Using optical low-frequency stimulation (LFS, 1 Hz) we robustly induced long-term depression (LTD) of optically-evoked EPSCs ($n = 17$, 64% ± 9%, $p < 0.05$). Pre-incubation of brainstem slices with the NMDA receptor antagonist APV blocked depression at these synapses, indicating that NMDA receptors are required for LFS-induced LTD in the TNC. Next, we investigated the effect of acutely applied human migraine triggers on optically-evoked EPSCs. Bath application of the neuropeptide pituitary adenylate cyclase-activating peptide (PACAP) led to a robust depression of EPSCs at 16-20 min following drug infusion (PACAP: $n = 5$, 66% ± 8%, $p < 0.05$). Likewise, the nitric oxide

donors nitroglycerin (NTG) and sodium nitroprusside (SNP) depressed optically-evoked EPSCs at 16 - 20 min following drug application (NTG: $n = 8$, 73% ± 8%, $p < 0.05$; SNP: $n = 11$, 70% ± 7%, $p < 0.05$). As all triggers investigated here induced synaptic depression, future experiments will determine to what degree these forms of plasticity share common pathways.

To gain further mechanistic insights into alterations at nociceptive trigeminal synapses, we also plan to investigate changes in synaptic strength through whole-cell recordings of miniature EPSCs (mEPSCs) in nitroglycerin treated vs saline-injected animals. In the classic interpretation of this experiment changes in mEPSCs frequency reflect presynaptic changes through alterations in the probability of vesicle release whereas changes in mEPSCs amplitude represent modifications at the postsynaptic site, e.g. receptor density or phosphorylation. Importantly, these experiments will inform us about migraine-relevant mechanisms that are induced *in vivo* and it will help establishing further our slice model as *in vitro* model for migraine.

Our data show that depression at nociceptive afferent synapses is a prominent form of synaptic plasticity in the trigeminal nucleus caudalis, and is in part mediated through activation of NMDA receptors. This form of plasticity might also contribute to sensitization of the trigeminovascular pathway during migraine induced by PACAP, NTG or SNP. We hypothesize that reduced excitatory input onto inhibitory neurons could disinhibit projecting neurons, thereby yielding an increased net output to connected brain regions.