Brown Institute for Brain Science

Brown University

Association of Migraine Disorders Progress Report

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Migraine-related plasticity at central synapses of TRPV1 trigeminal nociceptors

Impact of the AMD support:

The team and the Brown Institute for Brain Science are grateful for the AMD support, which:

- Advanced our understanding of basic neural circuits implicated in migraines;
- Catalyzed the development of a mouse model for the use of optogenetics to interrogate the synapses that may be critical in the initiation and or maintenance of migraines;
- Promoted a unique collaboration that applied multiple methodologies to investigate migraine from genes to behavior; and
- Developed the career of a highly talented young scientist who will develop his own independent research program in the study of migraine.

Overview of the team:

Funding from the Association of Migraine Disorders catalyzed collaborations across four laboratories with expertise investigating the source of migraines. This expertise ranged from genes to synapses to neural networks to behavior:

- Julie Kauer emerged as the scientific leader of the team, having the required deep expertise in synaptic plasticity and having the skills to measure events at synapses in the trigeminal nucleus—a critical region implicated in migraines.
- Diane Lipscombe contributed mouse models to enable recording from this regions of the brain using optogenetic methods and, importantly, permitting behavioral assays.
- Carl Saab collaborated with Chris Moore to record larger populations of neurons in the cortex in response to optical activation of peripheral sensory terminal in skin and dura of the brain.

Critically, the team was able to recruit an outstanding postdoctoral associate who has led this project. Bruno Pradier joined Brown because of his ability to work across these four laboratories, gaining a unique combination of skills to establish his own independent research program in migraine disorders.

Relevance of this project to the fellow's research career objectives:

Dr. Pradier' long-term goal is to become an independent neuroscientist within academia, and this project significantly contributed to my research career objectives in several aspects.

- First, he received intensive training in electrophysiological methods, and he deepened his knowledge of synaptic physiology.

- Second, the use of optogenetic models has become state of the art in dissecting neuronal circuits of brain disorders. The present project helped him integrate these tools into his existing repertoire of behavioral techniques and his newly acquired skills in neurophysiological recordings.
- Finally, pursuing postdoctoral research in the vibrant working environment of Brown University strengthened his professional skills in scientific communication and also expanded his research network.

Overview of the project

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 this pathway plays a major role in the pathology of migraine, yet surprisingly, little is known about long-term changes in trigeminal afferents or their synapses in the TNC. One potential mechanism of sensitization of the TNC could be changes in synaptic plasticity of nociceptive afferents. Given its importance in migraine and the small number of physiological studies of the TNC, this study aimed at the investigation of migraine-related plasticity at primary nociceptive afferent synapses onto second-order relay neurons in acute TNC slices.

Process

We employed an optogenetic mouse line (channelrhodopsin-YFP expressed in TRPV1 lineage neurons) to selectively stimulate primary nociceptive afferents and to study sensitization of these fibers in vitro. We found with immuno-labeling that TRPV1 lineage afferents mostly co-localize with CGRP-containing C- and Aδ-fibers (Figure 1), thereby indicating that light stimulation would only activate a specific subset of primary afferents predominantly designated to the transmission of nociception.

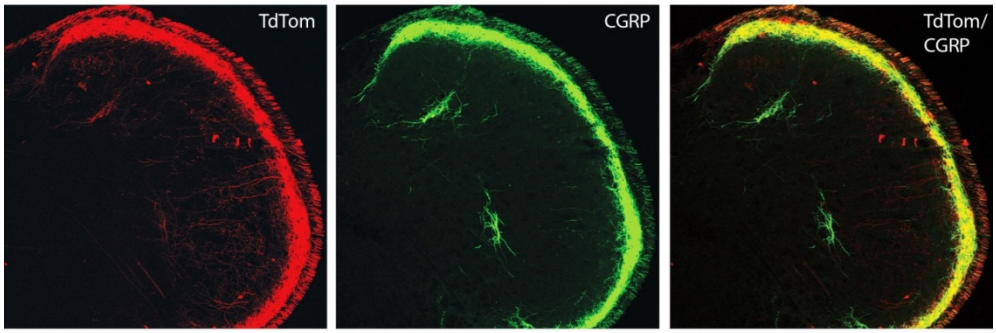

Figure 1. Expression of TRPV1/TdTom and CGRP in the trigeminal nucleus caudalis. *Both expression patterns of TRPV1/TdTom (red) and CGRP (green) in the TNC show large overlap.*

Light stimulation at the slice edge (473 nm, 0.4 - 1 msec) evoked excitatory postsynaptic currents (EPSCs) and often polysynaptic activity in neurons in laminae I-II in acutely prepared transverse TNC slices. Bath applying the *pituitary adenylate cyclase activating peptide* (PACAP), a neuropeptide that induces migraine in humans and sensitizes the trigeminal pathway in mice (10min, 10nM, $n = 5$, $66\% \pm 8\%$ at 10 - 20 min after washout, $p < 0.05$) robustly induced long-term

depression of optically-evoked EPSCs (Figure 2A). Similar effects were observed using optic low-frequency stimulation (LFS, 1 Hz, n = 11, 65% ± 14%, $p < 0.05$) (Figure 2B).

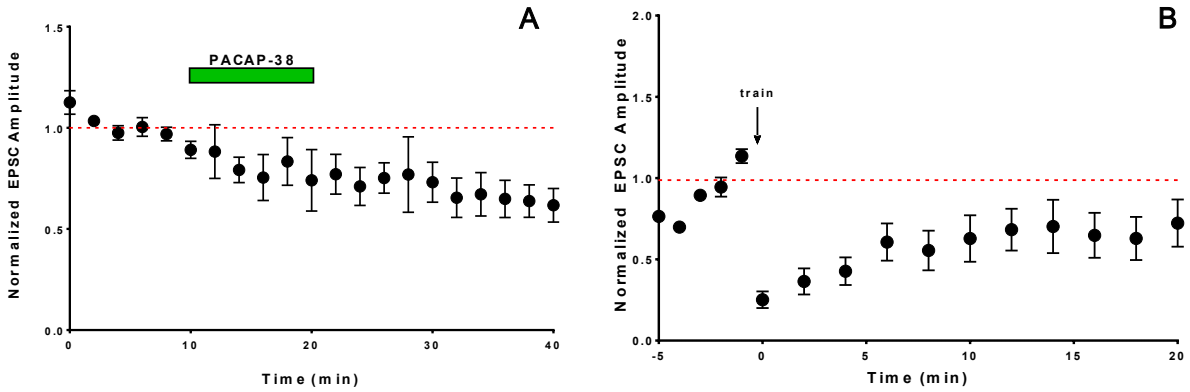

Figure 2. Synaptic depression in TNC cells of lamina I/II following bath application of PACAP or optic low-frequency stimulation*. (A) PACAP induced depression of light-evoked EPSCs (n = 6, 58% ± 13%, $p < 0.05$). (B) Optically induced LTD following a 1 Hz train (n = 11, 65% ± 14%, $p < 0.05$).*

Conclusion

Thus far, our data demonstrate depression of synaptic transmission as a form of persistent plasticity at primary nociceptive afferent synapses. We hypothesize that reduced excitatory input onto TNC inhibitory neurons could disinhibit projecting neurons, thereby yielding an increased net output to downstream brain regions processing pain signals. These results suggest that persistent synaptic changes at these synapses might play a role in migraine pathophysiology.

Communicating project findings

This past summer, we presented our data at an international conference on pain mechanisms and therapeutics in Europe, which were well received by the scientific community. A part of the study will also be presented at the annual meeting of the Society for Neuroscience in November 2016.

In the future, we expect that we will make a contribution in assessing the role of pharmacological migraine triggers as modifiers of synaptic plasticity in the TNC. These findings may suggest new treatment strategies and may lead towards a better understanding of the disease.

We will continue to present our data at international conferences. We also plan to publish original articles in international, peer-reviewed journals.