Examining the Role of Cortical CGRP Neurons in Migraine

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Problem and Significance

According to the National Institute of Health, approximately 13 percent of adults in the United States suffer from migraines and it is the second most disabling disease only behind chronic back pain. Recent FDA approval of CGRP-targeted drugs are on the forefront of new migraine treatments. Despite their success, they only treat about half of people with migraine, and we do not understand if their site of action is peripheral or central. There remains a critical need to understand the different sites of CGRP action in migraine in hopes to understand the underlying biology of migraine and create more efficient CGRP-targeted therapeutics.

Since CGRP is both necessary and sufficient in migraine pathophysiology, our lab created a mouse model of migraine by injecting mice with CGRP and studying their subsequent responses to assess migraine-like symptoms. However, at present we are limited in understanding the circuitry of CGRP neurons in the brain.

Preliminary Work

I have done retro-orbital injections of a Cre-dependent reporter virus into mice with the Cre recombinase gene in the CGRP (*Calca*) locus. The retro-orbital method was chosen because it allows for brain-wide expression of the virus in Cre neurons. uDISCO clearing techniques, coupled with light sheet microscopy imaging, allowed us to examine expression of CGRP neurons throughout the whole, intact brain. We identified prominent expression in the following areas: cerebellar Purkinje cells, facial nucleus, oculomotor nucleus, somatosensory cortex, and visual cortex. Our lab's previous studies have shown that injection of CGRP into the posterior thalamus (PoT) causes light aversion in mice, a surrogate for photophobia in migraine.

Hypothesis and Specific Aims

We hypothesize that projections from the PoT to CGRP neurons in the visual cortex may play a role in the generation of migraine-like symptoms. This will be tested with the following specific aims:

- Aim 1: Examine whether optogenetic activation of neural projections from the posterior thalamus (PoT) to the visual cortex is sufficient to cause photophobia in mice.
- Aim 2: Determine whether optogenetic activation of CGRP-positive neurons within the visual cortex is sufficient to cause photophobia in mice.

Proposed Methods

To address Aim 1, I plan to inject a virus encoding channelrhodopsin 2 and the fluorescent reporter eYFP (AAV2-CaMKII-ChR2-eYFP) into the PoT of wild type mice. Preliminary and published data have shown that PoT fibers project to the visual cortex. Projection to the visual cortex from my injection site will be confirmed by uDISCO brain clearing and light sheet microscopy of the reporter. A fiber optic cannula will be inserted into the top layer of the visual cortex and fastened to the skull using screws and dental cement. Six weeks following viral injections and surgeries, I will begin running light aversion assays to determine whether activation of the PoT cortical projections causes light aversive behavior. During these assays, the fiber optic cannulas will be connected to a light source, which will stimulate the target area at 5 ms, 20 Hz bursts every other minute for 30 min.

To address Aim 2, I will use optogenetics to target CGRP-expressing neurons in the visual cortex, using the same optogenetic vector as in Aim 1. In this aim, a Cre-dependent optogenetic virus (AAV-EF1a-

DIO-ChR2-mCherry) will be injected into the visual cortex of the *Calca*-Cre mice. The fiber optic probe will be placed in the visual cortex. Six weeks following surgeries, I will begin running behavioral assays to measure light aversive behavior as in Aim 1.

For both aims, the primary behavioral assay will be aversion to dim light as a surrogate of photophobia. Following that assay, should time permit, cutaneous hypersensitivity (von Frey filaments), spontaneous pain (automated grimace assay), and anxiety (open field) will be measured. Overall, this project will couple our prior circuitry tracing with behavioral studies, which will help us better understand the neural architecture of areas involved in CGRP production underlying migraine.

References

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