Morphology and Function of CRH-Expressing Central Amygdala Neurons: The Potential Role in Pain and Pain Related Behavior

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Overview

Chronic pain affects 100 million Americans, causing serious problems in various aspects of life. Current pain therapeutics have delayed and incomplete efficacy, creating a need to obtain experimental evidence for novel pain treatment approaches. The amygdala, an almond-shaped brain area in the medial temporal lobe, plays a key role in the emotional-affective dimension of pain. The CeA (central nucleus of the amygdala) encompasses the main output pathways of the amygdala and projects to pain modulatory systems through forebrain and brainstem connections. The CeA can be divided into a lateral (CeL) and a medial sub region (CeM). The CeL and CeM can project to the midbrain periaqueductal gray (PAG), which is a key brain structure in descending pain modulation. Corticotropin-releasing hormone (CRH) is a peptide hormone involved in the stress response. Emerging evidence has demonstrated that CRH-expressing CeA neurons are involved in pain modulation.

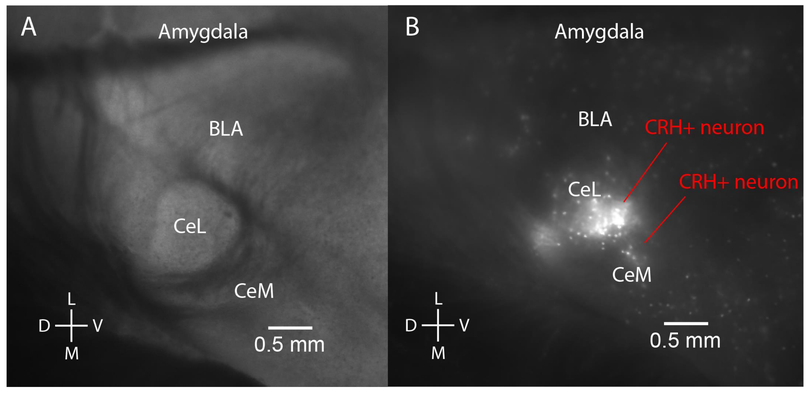

Figure 1: CRH expression in Central Nucleus of Amygdala sub regions

Objectives

Our preliminary studies using CRH-TdToM Cre mice revealed that CRH is expressed in both CeL and CeM sub region. However, the differences in electrical properties, morphology and function in the CRH-expression CeL and CeM neurons and how these attributes contribute to pain modulation remain unknown.

Methods

Part I: Through whole-cell patch-clamp, Complete Freund's Adjuvant-induced inflammatory (CFA) pain model, CRH Cre transgenic mouse lines, and confocal imaging, we aim to determine the compositions of neuron populations located in the CeL and CeM of mice. The first step of imaging involves harvesting 300 µm thick brain sections of the CRH+ Cre mice through brain slicing vibratome and bubbled in Artificial Cerebrospinal Solution (ASCF) to prevent cell death and to incubate during patching.

Continued: Slices are then patch clamped with a 3-4 MΩ pipette to record action potentials of selected CeL and CeM neurons, which are then filled with biocytin for imaging. Following patching, slices are preserved in PFA/PB solution to terminate any ongoing biochemical reactions. Solutions are rinsed with PB daily until mounting. Prior to mounting, a protocol entailing the rinsing and fixing of slices is replicated to ensure the preserving of neurons. Slices are exposed to biocytin conjugate, Alex Streptavidin 488, to allow for fluorescence of selected Amygdala cells. Slices are finally mounted onto a well constructed of slide covers to prevent cell damage and sealed.

Part II: Mounted slices are imaged with Nikon A1R confocal microscope to guarantee the finest resolution images. When imaged, specific biomarkers are selected, such as EGFP and TRITC to enable fluorescence of biocytin (Green) and CRH (Red). Images are then superimposed utilizing Z stack and Z intensity correction to yield a maximum exposure overlay, allowing us to interpret neuron morphology and function.

Results

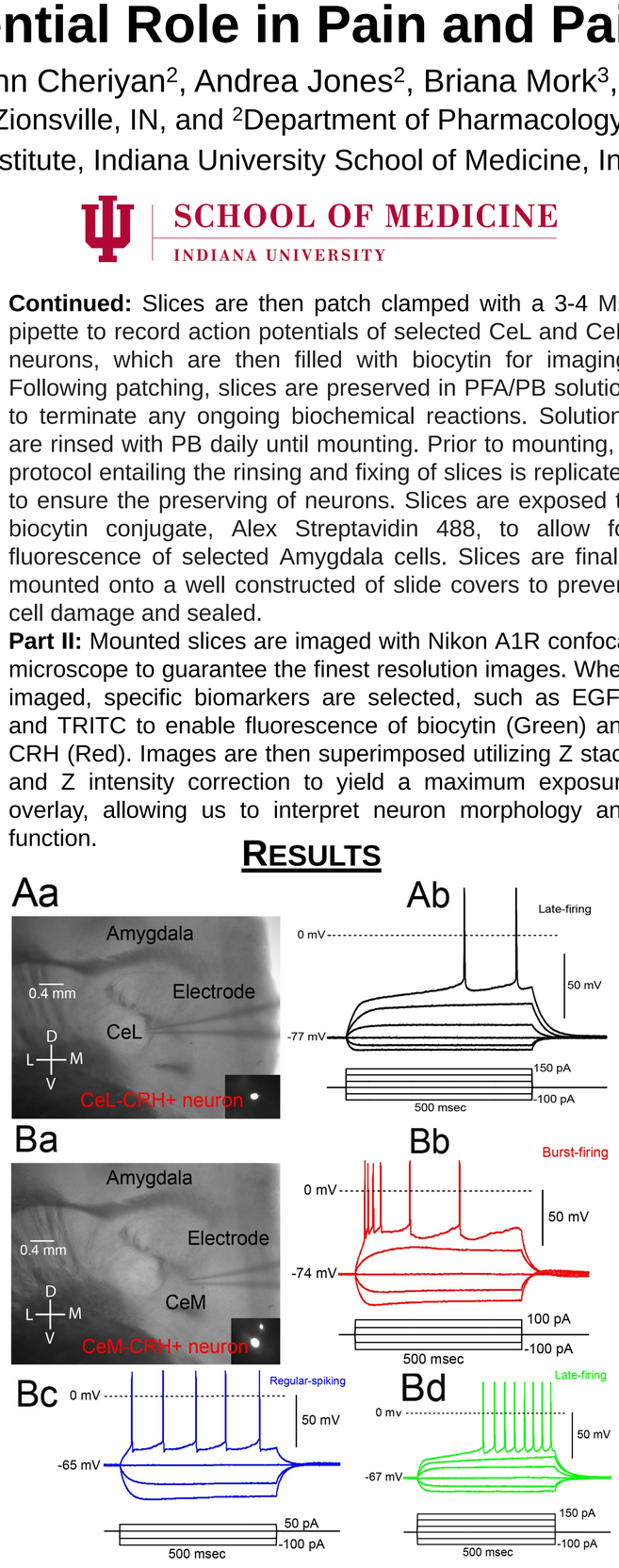

Figure 2: Patch clamp site and electrophysiological recordings

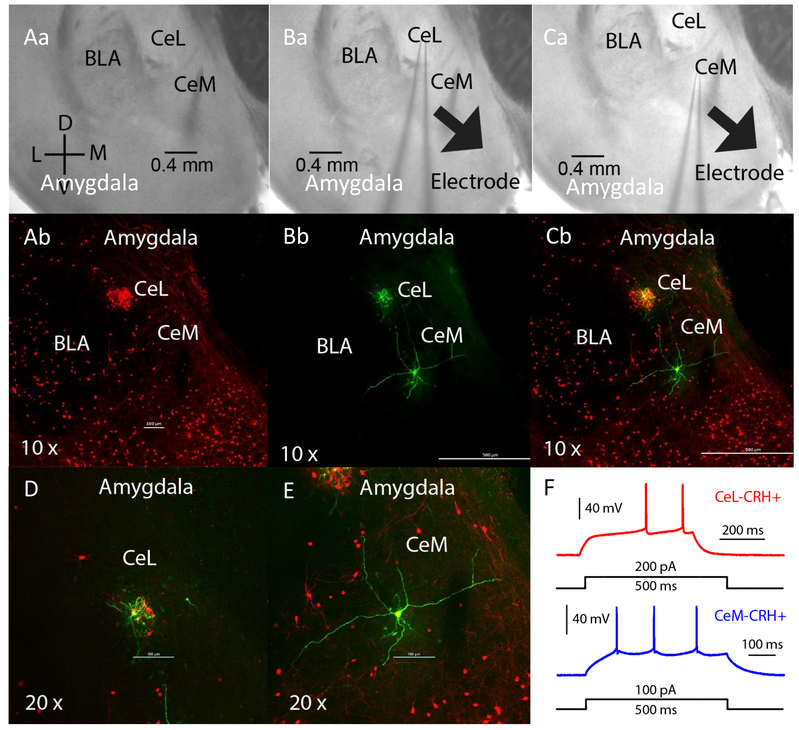

Figure 3: Morphological and electrophysiological characteristics

Summary

- The Central Lateral Amygdala is constituted of intrinsically and morphologically homogenous CRH neurons that likely store fear memories
- The Central Medial Amygdala CRH neurons are intrinsically and morphologically heterogeneous and may regulate fear response
- CeL CRH neurons have short, dense dendroidal connections
- CeM CRH neurons have lengthy axons, reaching distant structures

Future Work

Because our research is currently ongoing, we will continue to image new slices, as well as behavioral tests on mice of different genders. More Cre mice will later be injected and sacrificed to validate the results of our experiment and to analyze changes in morphology following pain model construction.

Acknowledgements

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