Specific Aims

Chronic pain is a debilitating condition which severely impacts quality of life. Unfortunately, our current treatment options are limited by adverse effects, and the specific neural circuits that modulate pain remain incompletely understood. Specifically, descending modulation of pain through neurons in the **rostral ventromedial medulla (RVM)** occurs via ON-cells, which facilitate pain, and OFF-cells, which inhibit pain[1–4], but many details of this circuitry remain unclear. In this model, morphine, a mu-opioid receptor (MOR) agonist, is thought to suppress pain, at least in part, through two mechanisms: 1) direct inhibition of ON-cells, which express MOR[5–7] and 2) indirect activation of OFF-cells, via a mechanism of disinhibition[8,9]. The proportion of RVM spinal projections responsive to MOR is increased in persistent inflammatory pain states[10], and opioid-induced hyperalgesia is partially mediated by the RVM[11], suggesting these neurons underly a pro-nociceptive circuit. However, the precise role MOR+ neurons play in pain facilitation is unknown. Thus, there is a major gap in knowledge regarding the underlying circuitry by which the RVM facilitates pain and contributes to chronic pain states.

Until recently, we lacked the tools to clearly visualize and selectively manipulate MOR+ spinal projections *in vivo*. With the recent development of the Oprm1^{cre} mouse, MOR+ RVM spinal projection neurons can be selectively targeted and modulated for the first time, both *in vitro* and *in vivo,* to uncover the anatomy and physiology of these neurons and determine their role in acute pain behaviors and persistent pain states. Thus, we are now poised to **test the hypothesis that MOR-expressing RVM neurons that project to the spinal cord will innervate the dorsal horn (Aim 1), inhibit post-synaptic interneurons in the dorsal horn (Aim 2), and facilitate pain (Aim 3) (Figure 1).**

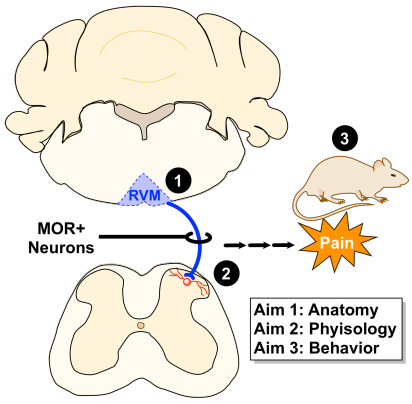

Figure 1: Hypothesis. spinally projecting MOR+ RVM neurons inhibit interneurons in the superficial dorsal horn to facilitate pain.

Aim 1: Determine the spinal targets and neurochemical phenotypes of MOR+ RVM neurons. To test the specific hypothesis that GABAergic MOR+ RVM neurons innervate the dorsal horn, I will trace these neurons with an adeno-associated virus (AAV) containing a fluorescent probe into the RVM of Oprm1^{cre} mice and will visualize fluorescent terminals throughout the neuraxis with immunohistochemistry (IHC). To determine the neurochemical phenotype of MOR+ RVM spinal projections, I will inject the retrograde neural tracer Fluoro-Gold into the dorsal horn and perform fluorescence *in situ* hybridization (FISH) in the RVM to co-stain with the cell type-specific markers Vgat, VGlut2, and Tph, and Oprm1 to determine which spinally projecting RVM neurons are GABAergic, glutamatergic, or serotonergic, and express MOR. Consistent with previous studies tracing RVM spinal projections, I expect to identify MOR+ GABAergic spinal projection neurons that synapse in laminae I and II.

Aim 2A: Identify the MOR+ RVM inputs received by interneurons in lamina I and II of the dorsal horn. In order to test the specific hypothesis that MOR+ RVM neurons inhibit dorsal horn interneurons, I will use optogenetics and the Oprm1^{cre} mouse to insert a cre-dependent channelrhodopsin (ChR2) into MOR+ RVM neurons. Then, I will use slice electrophysiology to quantify light-evoked inhibitory or excitatory post-synaptic currents (IPSCs or EPSCs) within dorsal horn interneurons. I expect to observe light-evoked IPSCs in dorsal horn interneurons, indicating inhibition. **Aim 2B: Explore the electrophysiological, neurochemical and morphological phenotype of dorsal horn interneurons receiving input from MOR+ RVM projections.** I using the preparation described in **Aim 2A**, I will characterize responsive interneurons by firing pattern and pharmacological responsivity. Post-hoc avidin-neurobiotin staining and confocal microscopy will be used to reconstruct the morphology of recorded cells. Consistent with inhibitory interneurons, I expect these interneurons to exhibit tonic firing and display an islet or central morphology.

Aim 3: Elucidate the functional role of MOR+ RVM spinal projections in behavioral assays of pain. To test the specific hypothesis that MOR+ RVM spinal projections facilitate pain, I will use a chemogenetic approach to selectively activate and inhibit MOR+ RVM spinal projections and measure the impact on nocifensive behavior evoked with chemical (capsaicin), mechanical (Von Frey), and thermal (Hargreaves) stimuli, as well as on a spared nerve injury (SNI) model of chronic neuropathic pain. I expect inhibition of MOR+ RVM spinal projections to attenuate chronic pain, while excitation of these neurons will exacerbate acute and SNI-induced chronic pain.

The results of these experiments will further validate (or potentially challenge) our current model of descending pain modulation by the RVM. Uncovering the underlying circuitry by which MOR+ RVM spinal projections facilitate pain will advance the pain field and pave the way for the development of novel pain therapeutics. Finally, the proposed experiments will provide me with excellent training in genetic techniques, electrophysiology, and behavioral paradigms which will enable me to achieve my long-term goal of becoming a physician-scientist.