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"Use of Brain Clearing and Labeling to Evaluate Activity of CeA Inputs in Response to a Pain Challenge in C57BL/6J Mice"

Background: Opioids such as oxycodone are commonly prescribed for pain and have high rates of misuse, even among adolescents. Studies show that adolescent opioid use increases the risk of substance use disorders, including alcohol use disorder, during adulthood. Adolescent opioid use and chronic alcohol use each lead to increased pain sensitivity, or hyperalgesia, which is problematic as alcohol is often used to self-medicate due to its acute analgesic effects. It is known that the central amygdala (CeA) is involved in alcohol use, opioid use, and pain; however, there is no published preclinical data illustrating the interaction of adolescent opioid use and adult alcohol exposure on nociception and relevant brain circuitry. In this pilot study, we aim to address this gap by establishing a protocol for the use of whole-brain imaging to analyze 3D cellular activation profiles of the CeA circuitry involved in pain for use in future studies investigating adolescent oxycodone exposure and adult alcohol exposure.

Hypothesis: We hypothesize that a pain challenge will activate specific CeA inputs including the parabrachial nucleus, basolateral amygdala, bed nucleus of the stria terminalis, hippocampus, and prefrontal cortex. It is possible that these inputs may differ by sex.

Methods: Adult female C57BL/6J mice received bilateral retroviral injections of tdTomato into the CeA. After 4-5 weeks, subjects underwent 5 trials of pinch testing with a maximal force of 200 g delivered to the plantar surface of the hind paws. After 90 minutes, brains were collected, cleared, and immunolabeled using LifeCanvas Technologies' SmartBatch+ device. Samples will be imaged via light sheet microscopy and c-fos activation profiles within CeA inputs will be quantified as a measure of cellular activation.

Expected Results and Conclusions: It is predicted that the retrograde virus into the CeA will label known inputs and that those involved in pain (PBN, BLA, mPFC) will exhibit enhanced c-fos expression following a pain challenge. This study will establish a brain clearing protocol for the lab and provide critical information about CeA inputs.

Future Directions: Future studies will explore a mouse model of adolescent oxycodone exposure and adult alcohol use to examine their combined behavioral effects and changes to the neural circuitry involved. Brains will be cleared, immunolabeled, and imaged using the protocol established within this experiment. These findings will provide the groundwork for a future proposal that aims to identify and rescue the neural plasticity underlying opioid-alcohol interactions on behavior.