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

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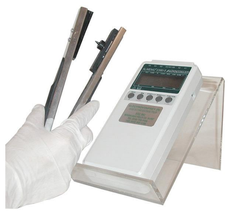
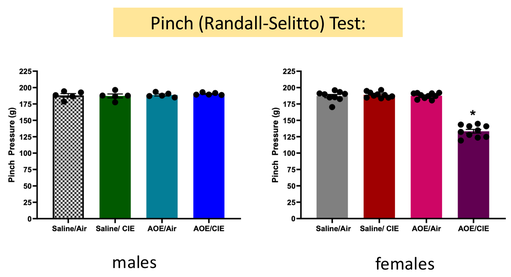
BACKGROUND

- Opioids such as oxycodone are commonly prescribed for pain and have high rates of misuse, even among adolescents.
- Adolescent opioid exposure (AOE) predisposes individuals to the development of substance use disorders (SUD) including alcohol use disorder (AUD) during adulthood.
- AOE and chronic alcohol use each lead to increased pain sensitivity (hyperalgesia) which is problematic because alcohol is often used to self-medicate due to its acute analgesic effects.
- The central amygdala (CeA) is involved in alcohol use, opioid use, and pain; however, **there is no published preclinical data illustrating the interaction of AOE and adult alcohol exposure on nociception and relevant brain circuitry.**
- Preliminary data shows AOE+CIE induces hyperalgesia in females





- **In this pilot study, we aim to address this gap by establishing a protocol for the use of whole-brain imaging to analyze three-dimensional cellular activation profiles of the CeA circuitry involved in pain for use in future studies investigating the intersection of adolescent oxycodone exposure and adult alcohol exposure.**

METHODS

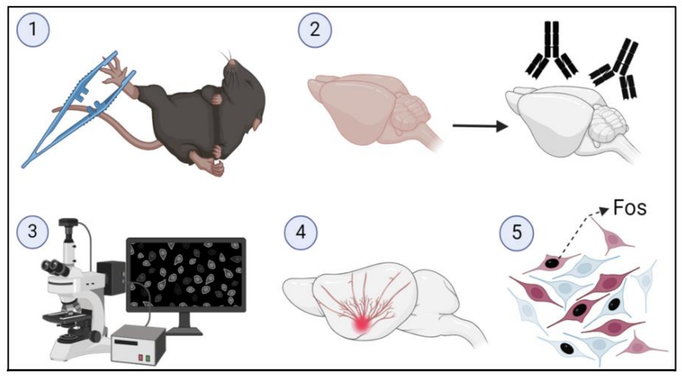
Animals: Adult female C57BL/6J mice.
Retroviral injection: Mice were bilaterally injected with retrograde td-Tomato virus (AAVrg-CAG-tdTomato, Addgene, 200nl/side) into the CeA.
Pinch (Randall-Selitto) Test: 4 wks after injection, mice were lightly anesthetized with isoflurane and a maximal cut-off of 200 g force was delivered to the plantar surface of the hind paws to prevent tissue damage. Five trials per animal were recorded and the average calculated.
Tissue Clearing: Brains were electrophoretically cleared and immunolabeled with the SmartBatch+ device from LifeCanvas Technologies (Cambridge, MA). Mice were transcardially perfused with PBS followed by 4% paraformaldehyde (PFA). Brains were removed and fixed in PFA for 24 h at 4°C prior to epoxy fixation via LifeCanvas' SHIELD protocol. Samples were incubated in SHIELD OFF solution for 3 days at 4°C then incubated in SHIELD ON Buffer for 24 h at 37°C. Next, brains were incubated in Delipidation Buffer for 24 h at 37°C. Samples were loaded into the SmartBatch+ device with fresh buffer and electrophoresis (40V, 1250mA, 42°C) proceeded for 48 h.
Immunolabeling: Brains were immunolabeled using the SmartBatch+ protocol. Once cleared, samples were incubated in 5% Donkey Serum in Antibody Blocking Solution at 37°C for 36 h followed by incubation in SmartBatch+ Primary Sample Buffer for 36 h. Brains were incubated with anti-c-fos primary antibody (Abcam rabbit monoclonal Anti-c-fos antibody [EPR20769], Cat# ab214672) 6 µg/brain for 1 day and Donkey Anti-Rabbit IgG (H+L), Highly Cross-Adsorbed-647 secondary (Biotium, cat # 20047) for 1 day.
Image Processing: Brains were index matched using EasyIndex solution prior to imaging. Images were acquired using light sheet microscopy. Scans were aligned to the Allen Brain Atlas through manual registration and cell counts generated. Cell counts were combined across brain regions and quantified by average fluorescence cell density (cells/mm3).

RESULTS

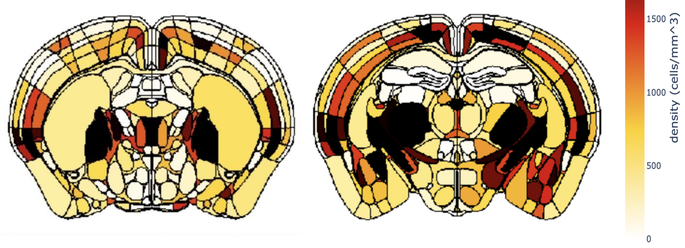
WHOLE-BRAIN IMAGING

CLEARING PROTOCOL:

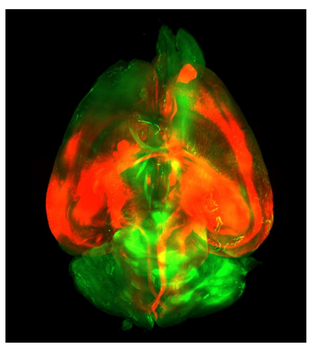
perfusion 24 hrs → SHIELD 4 d → delipidation (clearing) 30 hrs → immunolabeling 26 hrs → index matching 2 d → mounting and imaging <1 d



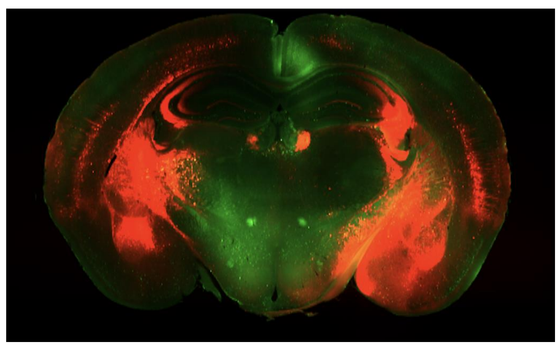
Workflow for brain clearing and c-fos mapping.



Distribution of co-labeled neurons represented as heatmaps.



Top-down view of whole brain.

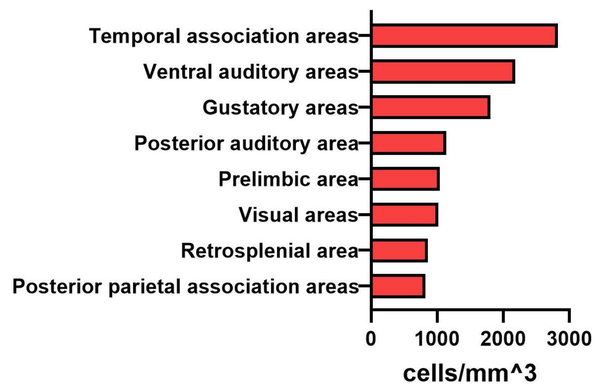


Coronal view taken at level of the amygdala.

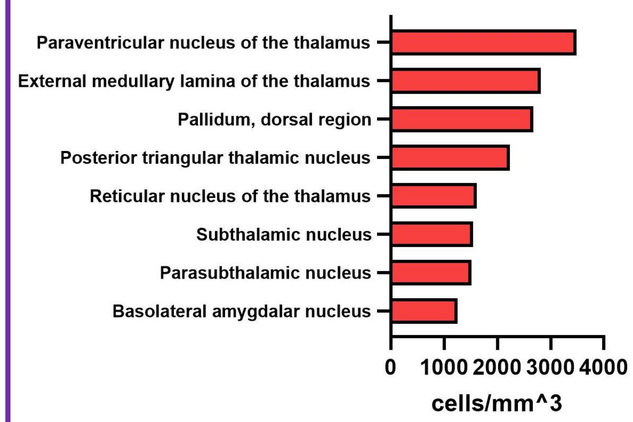
SCAN TO SEE 3D VIDEO

Cortical and subcortical regions expressing highest levels of co-labeled cells

Cortical regions: Number of tdTomato/cFos + cells



Subcortical regions: Number of tdTomato/cFos + cells



CONCLUSIONS

Retrograde viral injections into the central amygdala labeled known inputs and quantification of cellular activation profiles following a pain challenge provided an extensive list of regions to investigate in future projects. This study successfully established a protocol for clearing, immunolabeling, and imaging mouse brains for use in the lab.

FUTURE DIRECTIONS

- Future studies will explore a mouse model of adolescent oxycodone exposure and adult alcohol use to examine their combined behavioral effects and characterize changes to the neural circuitry involved.
- In addition to behavioral testing, brains will be cleared, immunolabeled, and imaged using the protocol established within this experiment.

ACKNOWLEDGEMENTS

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