Optogenetic induced extinction-like behavior in ethanol-withdrawn mice

Christine Cincotta[1]*, Nathen Murawski[1], Stephanie L. Grella[1] Monika Shpokayte[1], Olivia McKissick[1], Emily Doucette[1], Emily Merfeld[1], Kaitlyn Dorst[1], Steve Ramirez[1]
[1]Department of Psychological & Brain Science, Boston University, Boston, MA, USA

Abstract

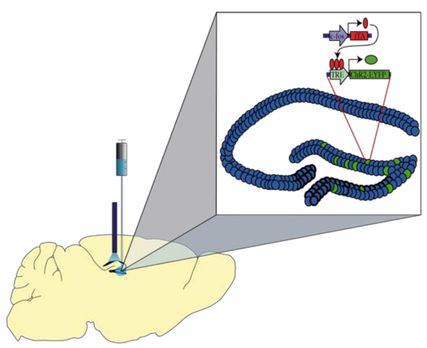
Withdrawal from chronic alcohol exposure impacts the brain's stress and memory systems, which may underlie individual susceptibility to drug seeking and stress-induced relapse. Preclinical studies demonstrate impaired fear memory processes in rodents withdrawn from alcohol, including abnormally heightened fear responses that are resilient to subsequent attenuation by extinction training. The underlying neural circuits mediating, and sufficient to intervene with, impaired extinction following alcohol withdrawal have remained elusive.

First, we demonstrated that mice withdrawn from chronic ethanol showed impaired fear extinction and heightened fear renewal. Next, we labeled ensembles in the dentate gyrus (DG) with an inducible and activity-dependent virus cocktail which allows expression of channelrhodopsin-2 (ChR2) in cells active during the formation of a contextual fear memory in mice withdrawn from chronic alcohol as well as saline control mice. Mice were placed into a distinct context and received chronic light stimulation in the DG, twice a day for five consecutive days. Chronic reactivation of fear ensembles led to context-specific reductions in fear responses in mice expressing ChR2.

This paradigm was successful in producing optogenetic induced extinction-like behavior effects in ethanol withdrawn mice. These results show how chronic reactivation of fear ensembles in the hippocampus may offer a means to facilitate extinction following withdrawal from chronic alcohol exposure. A mechanistic understanding of fear processes following drug withdrawal will aid in the development of therapies to attenuate stress-related cognitive dysfunction following drug withdrawal.

Methods

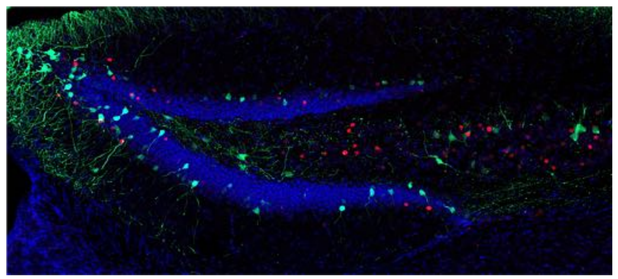
Activity-dependent Tagging of Hippocampal Cells



Cells are tagged in an activity dependent manner using an adeno-associated virus (AAV) controlled by doxycycline (dox) diet. When animals are "on dox," transcription of ChR2 is inhibited. When "off dox," tetracycline transactivator (tTa) binds to tetracycline response element (TRE), allowing *expression of ChR2 only in active (cFos+) cells.*

This activity-dependent system allows opening/closing of the labeling window. ChR2+ cells can be manipulated at later time point using optogenetic reactivation. Adult C57BL/6 males received bilateral microinjections of AAV9-cFos-tTa-TRE-ChR2-eYFP or AAV9-cFos-tTa-TRE-eYFP (control), targeted at the dorsal DG of the HPC.

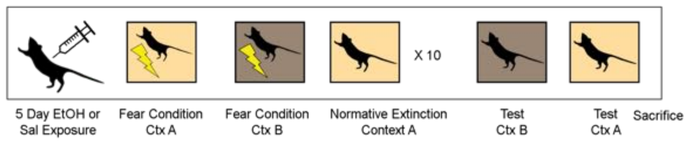
Verification of Viral Targeting



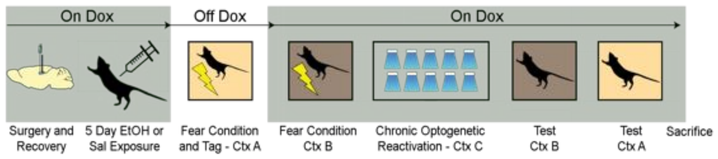
We used immunofluorescence and confocal microscopy to verify that the virus was accurately targeted to the dorsal DG.
Red: cFos Green: ChR2 Blue: DAPI
dDG Coordinates: AP -2,2, ML +/- 1.3, DV -2.0 (relative to Bregma)

Experimental Design

Normative Extinction

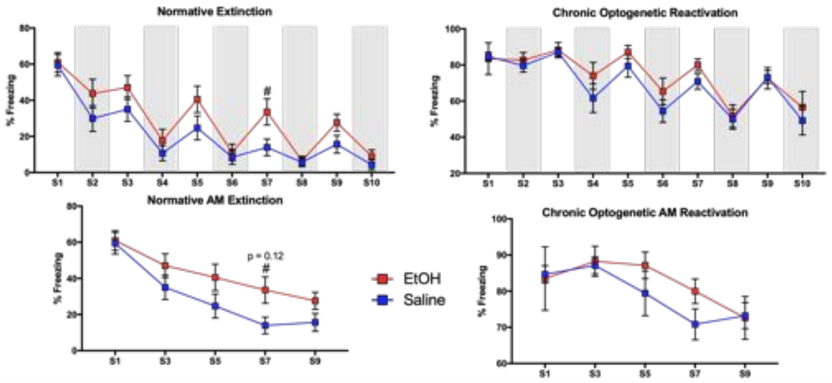


Chronic Optogenetic Reactivation

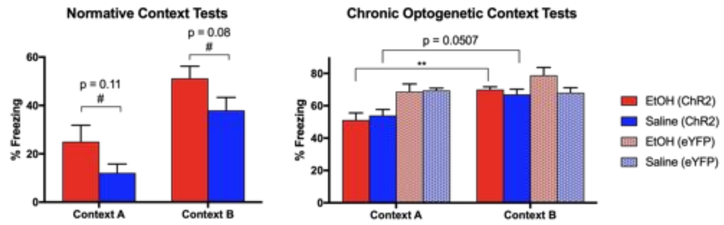


Ethanol Dose – 1.5 g/kg via intraperitoneal injection

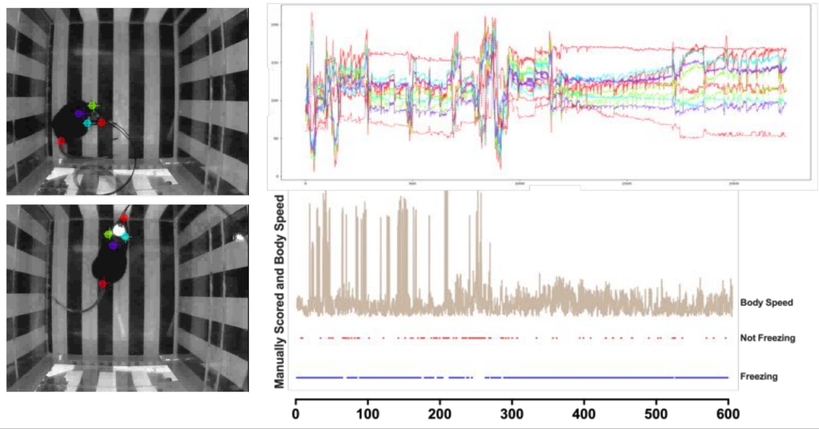
Normative Extinction vs. Chronic Optogenetic Extinction-Like Effects



Chronic optogenetic reactivation produces equivalent extinction-like effects in EtOH-WD and saline control mice

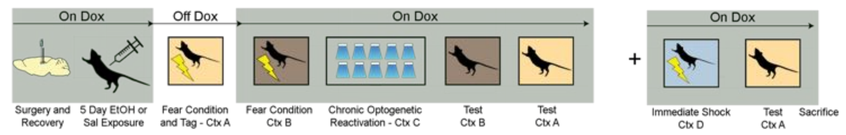


Future Direction - Automatic Tracking for Scoring with DeepLabCut

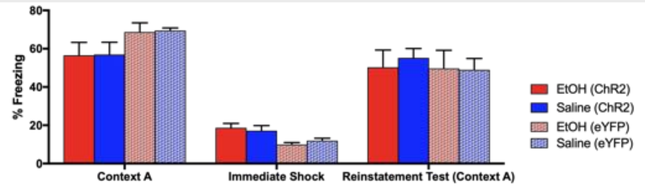


Investigating Reinstatement and Recovery

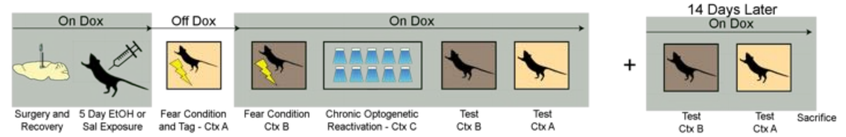
Chronic Reactivation and Immediate Shock



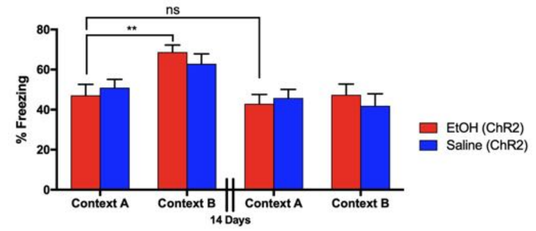
Immediate shock in a novel context does not produce reinstatement



Chronic Reactivation and Spontaneous Recovery



Extinction-like effects withstand spontaneous recovery time period



Conclusions

We demonstrate that chronic optogenetic reactivation of a tagged fear memory is effective in producing extinction-like behavioral effects in both ethanol-withdrawn and saline control adult C57BL/6 mice.

These extinction-like behavioral effects are resistant to reinstatement in both ethanol-withdrawn and saline control mice and withstand the two week testing period. After two weeks, we also see generalization to other contexts which have not been artificially extinguished.

Future Directions

Tag a withdrawal state to target cells that undergo withdrawal-related changes, and later inhibit these cells during fear learning and/or extinction. Viral targeting of cells active during withdrawal will further our understanding of the relationship between withdrawal-induced changes to these neurons and behavioral outcomes.

Withdrawal from a number of drugs of abuse – including alcohol, nicotine, heroin, cocaine – cause significant changes in brain stress systems. To achieve translatable findings, across a variety of drugs, we will examine potential fear extinction impairments and utilize our optogenetic approaches to mitigate those impairments following withdrawal from other drugs.

We hypothesize that common mechanisms link withdrawal- induced behavioral effects across drug classes and therefore a common therapy may be broadly efficacious.

Acknowledgements

Funding sources: NIH DP5, Brain and Behavior Foundation, Ludwig Family Foundation, Center for Memory and Brain at Boston University
Contributions: Mackenzie Mathis and Alexander Mathis

Contact

ccotta@bu.edu @crc_11797
www.theramirezgroup.org