INTRODUCTION

Post-traumatic stress disorder (PTSD) is characterized by overgeneralization of traumatic memories.

Generalization is the transfer of learned responses to stimuli that are similar to, but not the same as, the original stimuli.

We hypothesize that sparse neurons in the brain, known as 'neuronal ensembles,' may mediate fear generalization.

Our approach attempts to characterize these neuronal ensembles in mice using tamoxifen-dependent Cre recombinase expression (Cre-human estrogen receptor fusion protein construct, or CreERT2) driven by the Arc/arg3.1 (Arc) promoter.

EXPERIMENTAL DESIGN

METHODS

Subjects

- (B6.Cg-Tg(Arc-CreERT2)MRn/CdnyJ (JAX no. 022357) X B6.129X1-Itg(Rosa)26Ssa/J (JAX no. 008148) mice.
- ArcCreERT2 and ROSA positive mice were crossed. Offspring was genotyped using a PCR assay.

PCR

- DNA samples were collected via tail biopsy then incubated at 95°C overnight.
- Samples were mixed with ArcCre and ROSA "supermixes" and aliquoted into 20-μl tubes.
- Samples were placed in the thermocycler and allowed to run.
- Following thermocycling, samples were placed in an agarose gel and allowed to run for 20–30 minutes at 130 V.
- Samples were imaged to determine which were ROSA and Cre positive.

Fear Conditioning

- ArcCreERT2 X EYFP positive mice were dark housed for 24 hrs prior to behavioral testing.
- Mice injected with 4-OHPT to permanently genetically label cells during fear conditioning with Green Fluorescent Protein (GFP).
- 5 hours following 4-OHPT injection, mice were fear conditioned with 3 presentations of a 5-kHz auditory stimulus (CS; ~75 dB, 20s) followed by a footshock (US; 0.5s, 0.6 mA, 20–30P).

Imaging

- A Leica confocal microscope was used to image the EYFP and Arc expressing neurons in the medial prefrontal cortex.
- Resolution is 1024 x 1024 at 1x zoom. Images taken show a total of 32 z-stacks, at 0.79 microns per stack, and analysis width of 25.28 microns.

DISCUSSION AND FUTURE DIRECTIONS

- The refinement of the PCR protocol allows us to accurately identify ArcCre and EYFP positive mice.
- We tested different dilutions for the Arc and GFP primary and secondary antibodies to achieve optimal fluorescent imaging.
- The co-labeled neurons in Figure 2e are potentially part of a neuronal ensemble underlying the conditioned fear memory.
- These neurons give us an insight into how fear memory is formed and stored in the infralimbic cortex of the medial prefrontal cortex.
- Future studies will aim at characterizing neuronal ensembles underlying fear memory discrimination and generalization.
- In continuation with this project, we plan to test the same conditioning and recall assay on a remote group, 30 days after conditioning.

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RESULTS

Figure 1. Mice showed increase in freezing responses over time, CS+ conditioned stimulus. There is a significant relationship between CS freezing and time (F(2, 24) = 13.31, p = 0.001). N = 3 for 8Hz, N = 6 for 5Hz and N = 5 for 3Hz. All results are reported as mean ± SEM, with an alpha level of 0.05.

Figure 2. Visualizing active neurons using fluorescent imaging. Images taken using a Leica confocal microscope at 20x objective power. The female mouse brain shown was treated with 4-OHPT before fear conditioning. All areas shown are approximately 1.04 x 0.69 mm, and show an area of 1550 x 820 microns. (A) DAPI staining. Blue fluorescent DNA staining. (B) Arc expressing neurons which were activated shortly before brain was extracted, when mouse was reintroduced to auditory cues (C) GFP staining of EYFP expressing neurons active during cuing fear conditioning. (D) Merged channel of Arc and GFP show colocalized neurons active at both the original conditioning event and the recall event. Colocalized neurons are labeled with arrows, and are shown in detail B.

Figure 3. (A) 0 Hz conditioning. (B) 3 Hz conditioning. (C) 5 Hz conditioning. (D) 3 Hz recall. (E) 5 Hz recall.

Figure 4. Mice from the 5Hz group show a trend towards distinguishing auditory cues. There were no significant differences between groups due to low sample size, however there is a trend of reduced freezing in the 5Hz's novel group, indicating distinction between the two auditory cues. N = 2 for 8Hz, N = 6 for 5Hz and N = 5 for 3Hz. All results are reported as mean ± SEM, with an alpha level of 0.05.