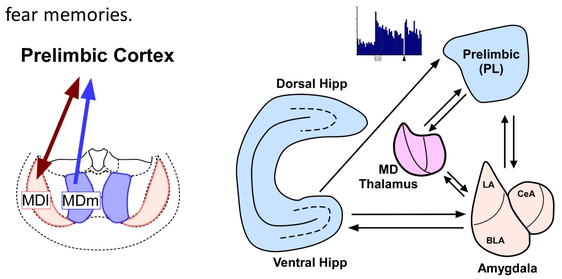
Prefrontal Cortical Output to the Mediodorsal Thalamus Encodes Trace Fear Conditioning

Grace Schamber, Matt LaViola, Kevin Grisales, Ahmed Awad, Matt R Herbst, Robert C Twining, Marieke R Gilmartin
Department of Biomedical Sciences, Marquette University, Milwaukee, WI

Introduction

The prelimbic cortex (PL) is essential for anticipating threat-predictive cues in trace fear conditioning. If disrupted, learning is impaired, suggesting a working memory role for PL in cued fear acquisition. It is unknown however, how this information is distributed downstream to support fear memory. Our recent work suggests that direct prelimbic output to the amygdala is important for learning, but fear acquisition can still occur in the absence of direct communication between the PL and amygdala (Kirry et al., 2020). Here we investigate the importance of the mediodorsal thalamus (MD) in trace fear conditioning. The MD is strongly interconnected with the PL and this connection is implicated in working memory. It is also connected to cognitive and emotional systems, which positions it as a potential node for integrating temporal and emotional information in memory. Using fiber photometry, optogenetics, and electrophysiology, we will examine the role of the MD in the acquisition and expression of episodic fear memories.

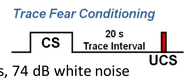


Methods

Animals: Male and Female Long-Evan rats (200-249 grams upon arrival)

Behavioral Training and Testing:

Trace Fear Conditioning



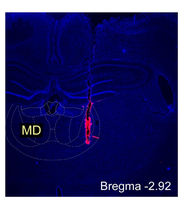
Trace Fear Conditioning: Rats received 6 pairings of 10s, 74 dB white noise conditioned stimulus (CS) and a 0.6 mA foot shock unconditioned stimulus (UCS) separated by a 20s trace interval. Intertrial interval was 240s +/- 20s.

CS and Contextual Memory Testing: 1 day after training, rats received 8 30-s CS presentations (ITI = 60s) in a novel chamber. 2-3 hours, later, rats were placed back in the original training chambers for 10 minutes to test fear to training context. In experiment 2, rats received a second CS and Context test 28-32 days after Test 1.

Extinction: In experiment 1, rats received 2 days of context extinction (53 minutes/day) followed by 3 days of CS extinction in the CS testing chamber (40 10-s CS presentations/day).

MD targeting
PL and MD show reciprocal connectivity, rostral MD.
- MDm cells provide input to PL
- MDl cells receive projections from PL

We targeted rostral MDl for injections of retrograde AAV



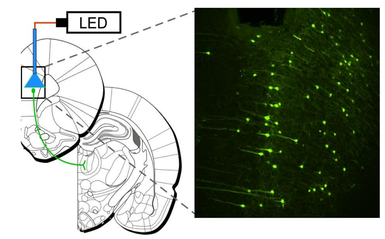
Experiment 1: Fiber photometry of PL-MD
n= 7 males, 8 females
virus in MD: rAAV-Retro CAG-CRE
virus in PL: AAV9-Syn-Flex-GCAMP6F-WPRE-SV40

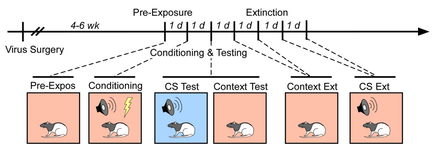
Experiment 2: Optogenetic silencing of PL-MD
n= 7 males, 9 females
virus in MD: 750 uL of retro pAAV-EF1a-Cre was injected into the MD
virus in PL: 750 uL of AAV9-CAG-Flex-ArchT-GFP or AAV9-CAG-Flex-GFP
Training began 4-6 weeks after virus injection

Experiment 3: Unit recording in MD
n= 9 males, 6 females
Trace or unpaired training
Analysis in progress

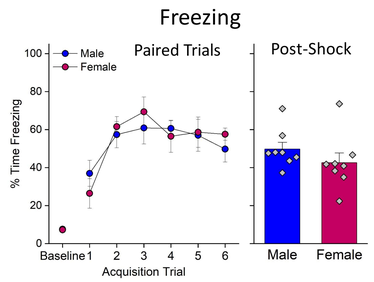
PL-MD Pathway Encodes Trace Fear Conditioning: Fiber Photometry

PL-MD viral targeting



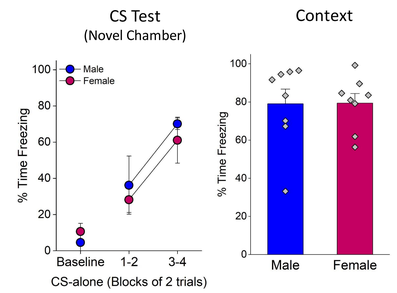


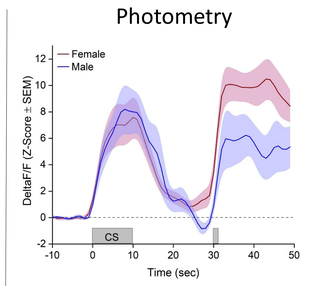
Trace Fear Acquisition



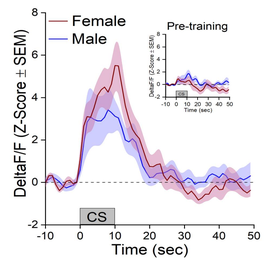
Males and females show similar acquisition (**above**) and retention (**below**) of trace fear conditioning. Females showed reduced contextual fear in the first 2 min of the context test (below right "Baseline"), but similar levels of freezing during the entire 15 min test (below middle). At the end of the context test rats received 4 CSs, with females showing less freezing compared with males.

Cued and Contextual Retention

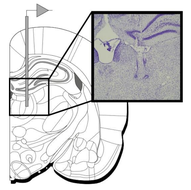




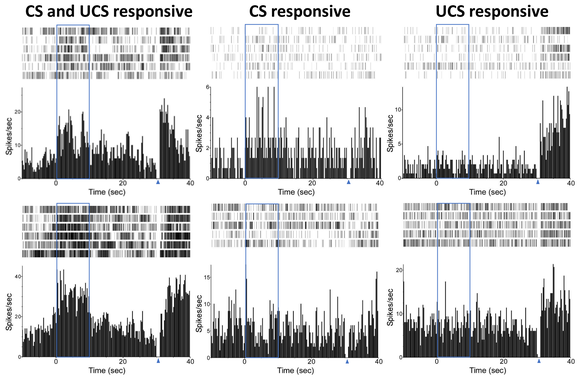
Males and females show similar changes in calcium activity (fiber photometry) in MD-projecting PL neurons to the CS during acquisition (**above**) and retention (**below**) of trace fear conditioning. Females showed an elevated response to the UCS.

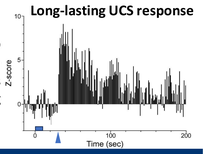


MD Unit Activity during TFC



Units recorded during trace fear conditioning show responding to the CS, UCS, or both. Some UCS responses are long-lasting.

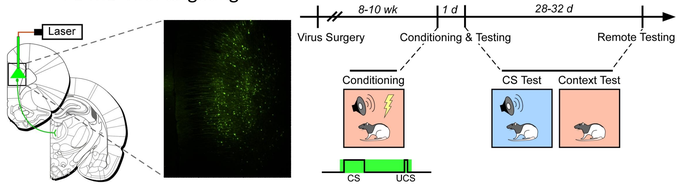




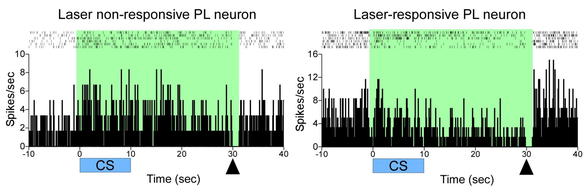
Above: Perievent histograms (250 ms bins) of example MD units representing different response profiles during trace fear conditioning. **Right**: Perievent histogram (z-score, 1s bins) showing an example of long-lasting UCS responding observed in some units. Arrowhead = UCS delivery.

Optogenetic Inhibition of PL to MD during TFC Alters Remote Fear

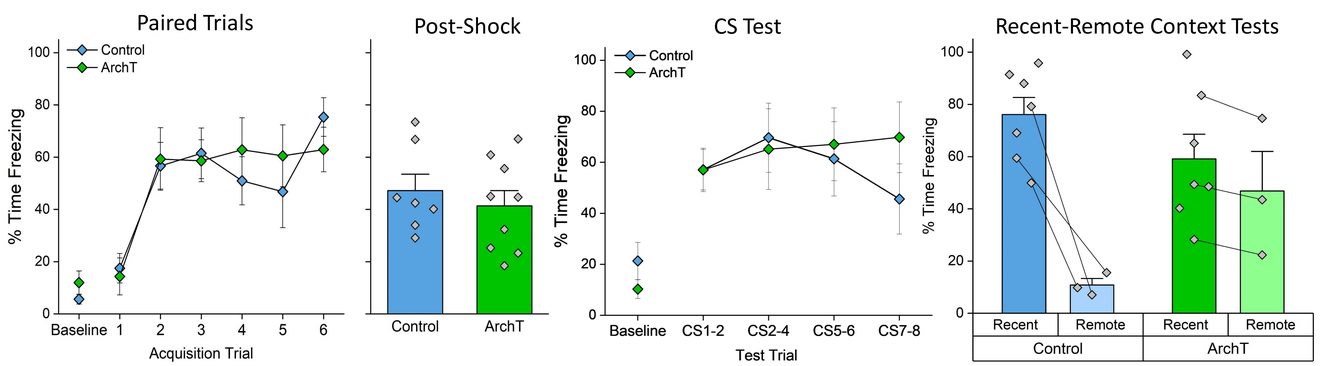
PL-MD viral targeting



Mixed-sex cohorts were trained with trace fear conditioning during which the PL-MD pathway was silenced or not during each training trial. Silencing did not affect the acquisition or retention of cued or contextual fear **(below).** However, PL-MD silencing during training led to maintained elevated fear to the context 30 days later **(below right).**



Delivery of laser light to the PL during trace fear conditioning trials reduced the firing rate in a subset of PL neurons. **Left**: Perievent histogram (100 ms bins) of a cue-sustaining neuron typical of PL that is not responsive to the laser. **Right**: Perievent histogram of a putative MD-projecting PL neuron that shows a reduction in firing at laser onset. Arrowhead = UCS delivery.



Conclusions

- PL-MD shows potentiated responding to a trace CS and robustly responds to the CS and UCS during training.
- PL-MD responding to threat-predictive cues diminishes with extinction.
- PL-MD activity during trace fear conditioning is not necessary for the acquisition of cued or contextual fear memories.
- Disruption of PL-MD activity during acquisition leads to a longer lasting contextual fear memory suggesting that PL-MD may normally serve to constrain the magnitude of fear in systems consolidation.
- These observations require further testing but suggest that PL-MD may influence the long-term storage of memory or the contextual control of memory retrieval as the memory ages.

Lab Information & Support

This work was supported by a Marquette University Regular Research Grant (MRG), the Charles E. Kubly Mental Health Research Center (MRG), and a Neurosurgery Research & Education Foundation fellowship (AA)

Gilmartin Lab:

Marquette Neuroscience PhD Program: