

# Distinct Roles of the Anterior and Posterior Retrosplenial Cortices in Encoding, but not Retrieval, of Trace Fear Memory

Sydney Trask, Shane Pullins, & Fred J. Helmstetter  
University of Wisconsin-Milwaukee



## Introduction

The rodent retrosplenial cortex (RSC) makes critical contributions to learning and memory and is reciprocally connected with several brain structures important in these processes (e.g., hippocampus, entorhinal cortex)<sup>1,2</sup>.

Lesions of the complete RSC result in impairments in both the initial acquisition and later behavioral expression of the memory<sup>3</sup>.

Studies that have subdivided the RSC by anterior and posterior regions have found that disruptions in spatial/context learning depend on the pRSC<sup>4</sup>, with lesions of the aRSC often leaving this type of learning unaffected<sup>5,6</sup>.

We examined the contributions of retrosplenial subregions to trace fear conditioning, in which a conditional stimulus (CS) is followed by a stimulus-free trace interval that ends in an aversive unconditional stimulus (US). This type of fear conditioning allows for the separable study of "what" (stimulus-related information) and "where" (context-related information).

In the current experiments, the anterior or posterior RSC was temporarily and reversibly silenced using optogenetics either during fear memory acquisition (Experiment 1) or memory recall (Experiment 2) in order to assess their unique contributions to both trace fear memory formation and later retrieval.

## Procedure

### Surgery.

Animals were mounted on a stereotaxic apparatus and given infusions into either the anterior or posterior region of the retrosplenial cortex of either an active virus (AAV9-CAG-ArchT-GFP) that causes expression of a light-sensitive proton pump, that (when activated) causes neuronal silencing, or a control virus (AAV9-CAG-GFP). Following 6 weeks for virus expression, animals were implanted with an LED (Experiment 1) or bilateral optic fibers (Experiment 2) above the infusion site.

### Behavior.

**Training.** For all animals, trace conditioning consisted of 6 CS-UCS pairs in Context A. The CS was a 10-s 70dB white noise followed by a 20-s stimulus-free trace interval that coterminated with a 1-s 1.0 mA footshock.

**Retrieval/CS Testing.** Retrieval consisted of 4 30-s presentations of the white noise separated by a 60-s intertrial interval (ITI) in a distinct chamber, Context B. The first presentation occurred after a 60-s baseline period. Animals were removed from the chamber 60 s following the final CS presentation.

**Context Testing.** Animals were placed in the acquisition context for five minutes and freezing behavior was assessed.

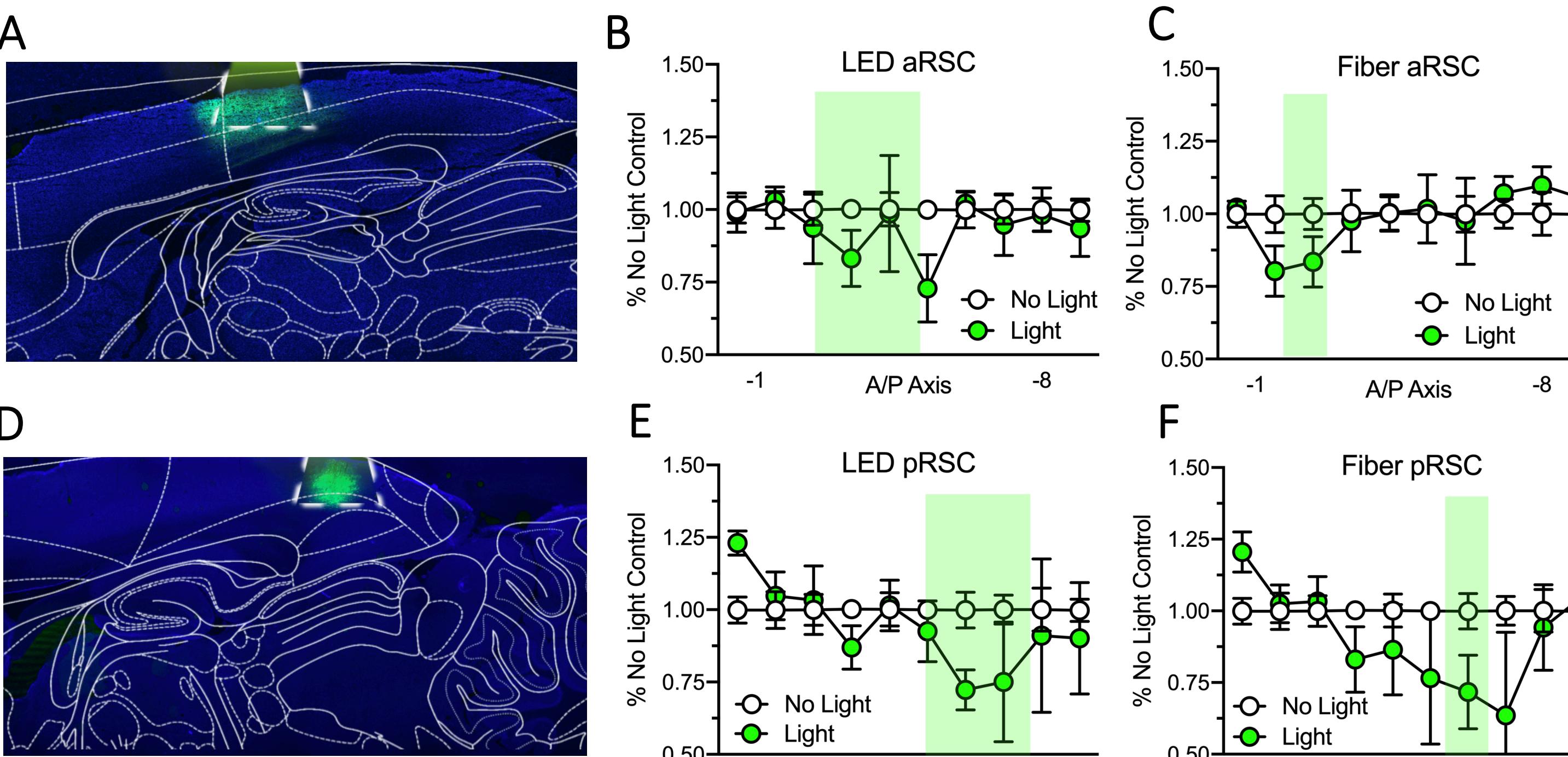
**Light Delivery.** Light was controlled via TTL pulses from a Med Associates computer (Med Associates, St. Albans, VT) at 520nm (green). The LED was illuminated for 32 total seconds such that it eclipsed the entirety of each CS presentation. Animals were connected to the LED light delivery by a freely moving patch cord that attached to either the LED or to the optic fibers secured to the skull.

## Immunofluorescence

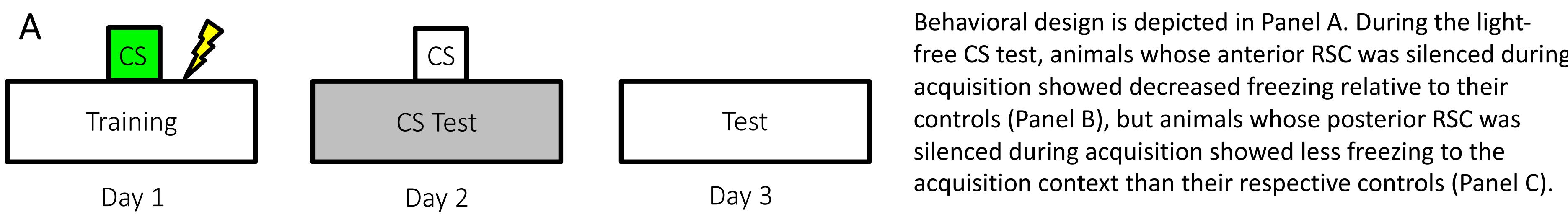
Animals were deeply anesthetized with isoflurane 60min following TFC. Brains were immediately removed and stored at -80°C until sliced in 20-micron sections and mounted onto charged slides. Slides were rehydrated in wash buffer (PBS + 0.05% Tween-20) and permeabilized (PBS + 0.3% Triton X) for 15-min and incubated in blocking solution (PBS + 0.7% NGS). Slides were then incubated in zif 268/EGR1 antibody (Cell Signaling, 1:500, #4153) solution (PBS + 0.3% Triton X + 5% NGS) overnight at 4 °C. The next day, slides were incubated in secondary antibody solution for 2 hours and rinsed with wash buffer, a DAPI counterstain was applied, and slides were cover slipped. Images were captured on the Olympus Fluoview FV1200 confocal microscope using a 20x objective lens. Serial z-stack images covered a depth of 4.55µm through five consecutive sections (0.91µm per section) and were acquired using Fluoview software (Olympus).

## Results and Discussion

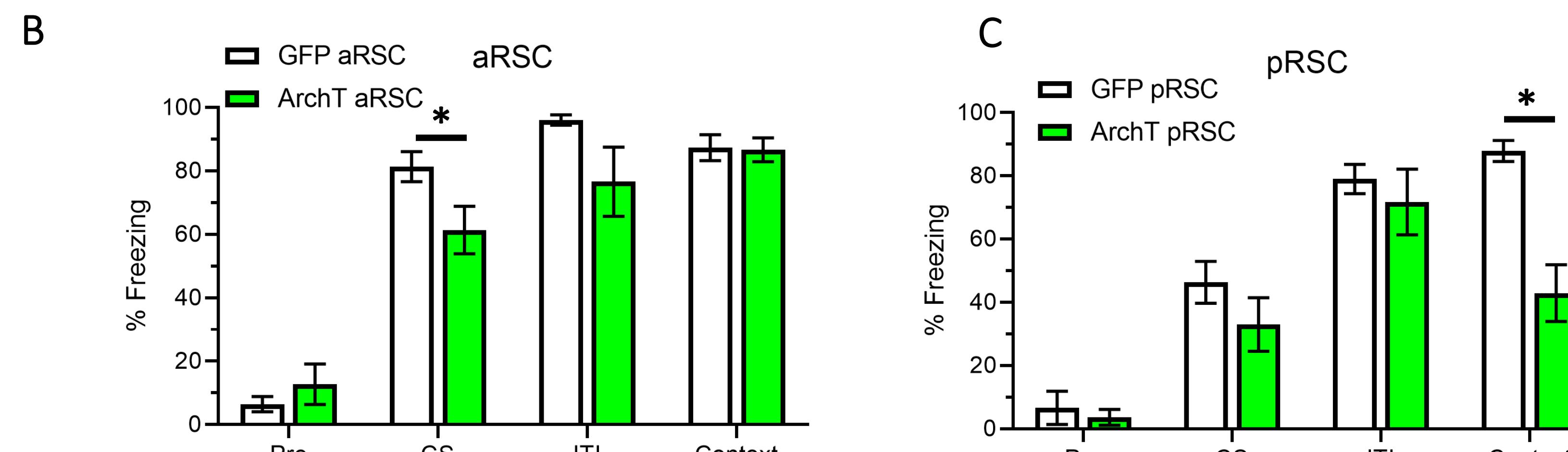
Anatomical reconstruction of ArchT expression in the anterior (Panel A) and posterior (Panel B) retrosplenial cortex (sagittal view). LED inactivation in the anterior (Panel C) and posterior (Panel D) let to decreases in zif 268 expression (expressed as a proportion of the control group) at a 60-min timepoint surrounding virus infusions. The same was true of fiber inactivation (Panels E and F). Green overlays (B, C, E, F) represent target virus infusion.



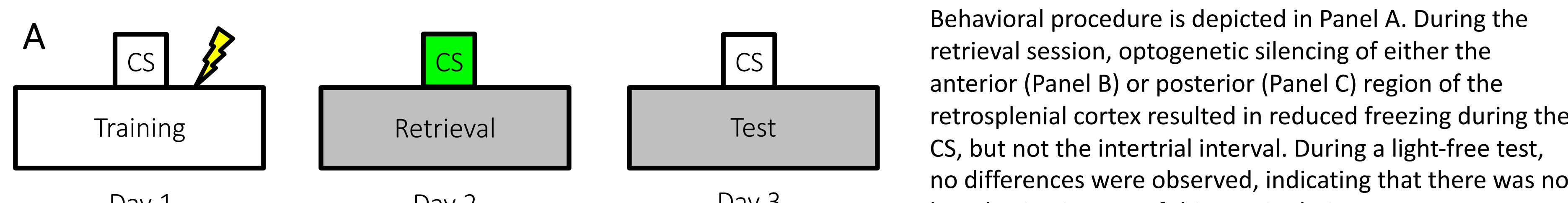
### Experiment 1. Inactivation of the aRSC during acquisition impairs later performance to the CS, but pRSC inactivation disrupts context freezing.



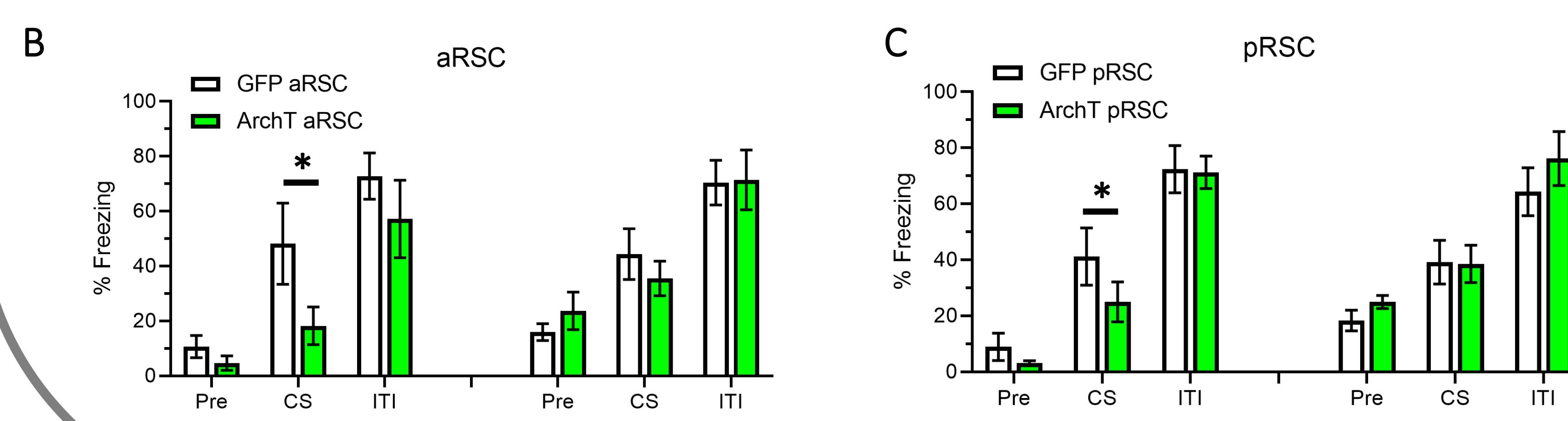
Behavioral design is depicted in Panel A. During the light-free CS test, animals whose anterior RSC was silenced during acquisition showed decreased freezing relative to their controls (Panel B), but animals whose posterior RSC was silenced during acquisition showed less freezing to the acquisition context than their respective controls (Panel C).



### Experiment 2. Inactivation of either the aRSC or pRSC during retrieval results in impaired fear memory recall.



Behavioral procedure is depicted in Panel A. During the retrieval session, optogenetic silencing of either the anterior (Panel B) or posterior (Panel C) region of the retrosplenial cortex resulted in reduced freezing during the CS, but not the intertrial interval. During a light-free test, no differences were observed, indicating that there was no long-lasting impact of this manipulation.



Optogenetic inhibition results in a region-specific decrease of neural activity.

The anterior retrosplenial cortex is needed for encoding of stimulus-related information and the posterior retrosplenial cortex is needed for encoding of context-related information in a trace fear conditioning paradigm. Both regions are needed for its retrieval.



SCAN ME

## References

- 1) Corcoran, K. A., Yamawaki, N., Leaderbrand, K., & Radulovic, J. (2018). Role of retrosplenial cortex in processing stress-related context memories. *Behavioral Neuroscience*, 132, 388-395.
- 2) Cowansage, K. K. (2018). Experience, memory, and the places they meet. *Behavioral Neuroscience*, 132, 409.
- 3) Keene, C. S., & Bucci, D. J. (2009). Damage to the retrosplenial cortex produces specific impairments in spatial working memory. *Neurobiology of Learning and Memory*, 91, 408-414.
- 4) Vann, S. D., Wilton, L. K., Muir, J. L., & Aggleton, J. P. (2003). Testing the importance of the caudal retrosplenial cortex for spatial memory in rats. *Behavioural Brain Research*, 140, 107-118.
- 5) Neave, N., Lloyd, S., Sahgal, A., & Aggleton, J. P. (1994). Lack of effect of lesions in the anterior cingulate cortex and retrosplenial cortex on certain tests of spatial memory in the rat. *Behavioural Brain Research*, 65, 89-101.
- 6) Vann, S. D., & Aggleton, J. P. (2004). Testing the importance of the retrosplenial guidance system: Effects of different sized retrosplenial cortex lesions on heading direction and spatial working memory. *Behavioural Brain Research*, 155, 97-108.

Contact: trask@uwm.edu

Supported by: NIHMH069558 (FJH) and F32MH120938 (ST)

