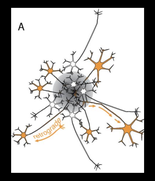
A Method for Investigating the Role of DH and mPFC Interactions in Episodic-Like Memory Consolidation

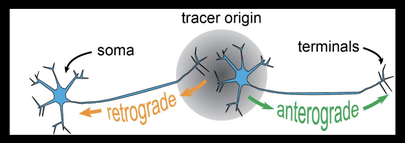
Lincoln, C.M., Schwabe, M.R., and Frick, K.M.
Department of Psychology, University of Wisconsin-Milwaukee, Milwaukee, WI

INTRODUCTION

- Concurrent activity in both the dorsal hippocampus (DH) and medial prefrontal cortex (mPFC) is necessary for the consolidation of spatial memory in mice, however, the mechanisms by which these two regions interact are not fully understood.
- **The goal** of this experiment is to investigate the role of these interactions in memory consolidation. We present preliminary work piloting a method to label and modulate activity of neurons projecting from DH to mPFC.
- First, we tested a retrograde Cre viral construct (pENN.AAVrg.hSyn.HI.eGFP-Cre.WPRE.SV40) that is taken up by axons and transported retrogradely to cell bodies of neurons.
- We then combined the retrograde Cre in the mPFC with Cre-dependent DREADD in the DH, which will allow us to inhibit these projections in future experiments.

Neuron





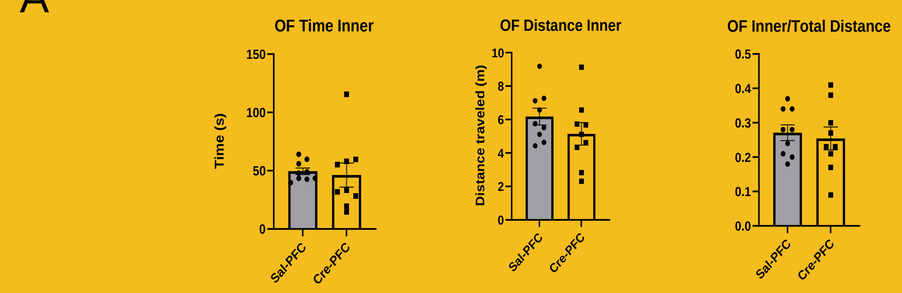
Haery, Leila. "Retrograde AAV: Making the Journey from Axon to Nucleus." Addgene Blog Share Science, May 2017, blog.addgene.org/retrograde-aav-making-the-journey-from-axon-to-nucleus?_ga=2.44789292.920932018.1581698503-1037618514.1559157252.

- Because Cre recombinase can affect cell health and, therefore, affect behavior, we tested Cre-infused mice, as well as control mice receiving saline in the PFC, in open field (OF), object recognition (OR) and object placement (OP) tasks to assess effects of Cre virus on anxiety, object recognition, and spatial memory.

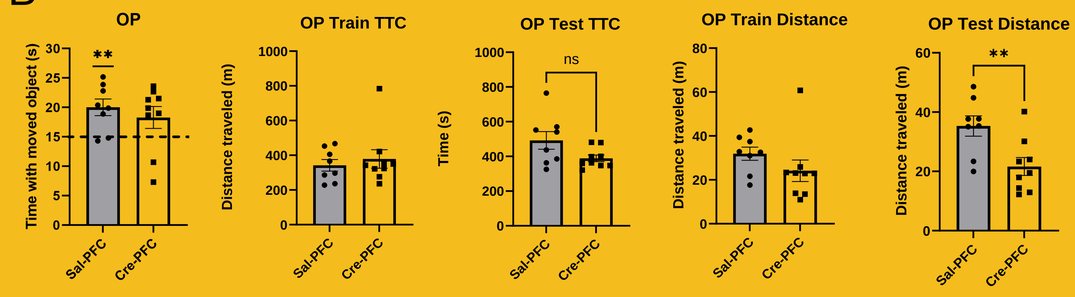
METHODS

- Female C57/B6 mice (n=20) received retro-Cre AAV (pENN.AAVrg.hSyn.HI.eGFP-Cre.WPRE.SV40) into the mPFC, or saline as a sham control, 0.2 microliters/hemisphere, and all animals received Cre-dependent KORD AAV (pAAV-hSyn-dF-HA-KORD-IRES-mCitrine) in the dorsal hippocampus and subiculum, 1.2 microliters/hemisphere, coordinates: AP: -1.7; ML +/- 1.0; DV -2.1 mm relative to Bregma.
- **Behavioral testing** began at 4 weeks based on initial work showing retro-Cre expression in the DH at 4 weeks. Immunohistochemistry (IHC) staining for HA-tag on the KOR DREADD confirmed expression in 2 animals euthanized at 4 weeks post-surgery.
- The mice were then placed in a box where they were exposed to 2 identical objects in each of the upper corners, and they were allowed 30s of exploration during the training session. 4 hours later the mice were placed back in the same box and allowed to accumulate 30s exploration, but the object that they spent the least time with was moved to the lower corner of the box, while the other remained in the same spot. Memory was assessed by preference for the novel object location, measured in time (s) spent with the moved object
- **Open Field:** On the first day of habituation, mice were recorded, and their movements scored using ANY-MAZE software. The object box was divided into a 5 × 5 grid of squares (12 cm × 12 cm). This grid included an outer zone (16 squares total) and a center zone (9 squares total). The time spent in the center zone, distance traveled into the center zone, distance in center zone relative to total distance traveled were quantified.
- **IHC:** Tissue was sectioned at 30 micron thickness using a cryostat. Sections were treated with 1% Sodium Borohydride ($NaBH_4$) for 15 min, and then rinsed 2x5 mins in PBS, blocked in 10% Normal Goat Serum (NGS, Biogenex), and incubated overnight at 4 ºC in HA-tag rabbit primary antibody (1:1000, Cell Signaling #3724). The next day, tissue was washed 3x5 min, incubated in Alexa-Fluor 594 goat anti-rabbit IgG secondary (1:500, ThermoFisher #A-11012) for 90 min at room temperature, washed 3x5 min, mounted onto glass slides, and coverslipped with an aqueous mounting medium containing DAPI (Santa Cruz). Tissue was protected from light at all times to protect fluorophores.

A



B



C

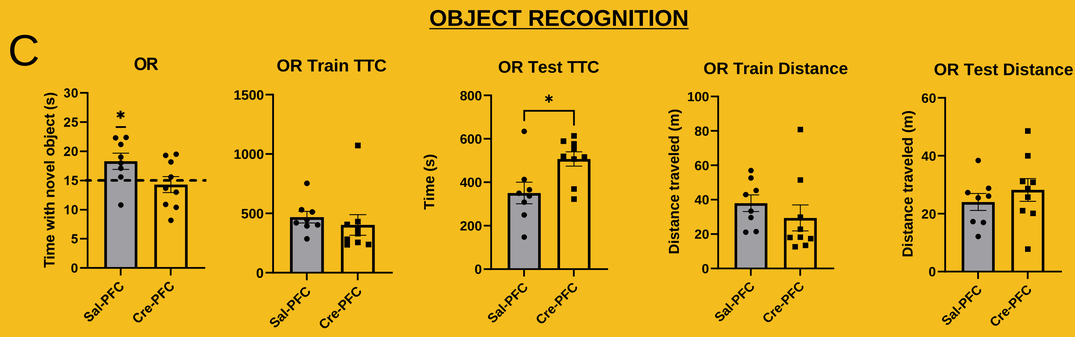


Figure 1. A. There were no significant differences in time spent in inner zone, distance traveled in inner zone, or inner/outer distance ratio in Open Field test. (two-sample t test) B. Saline Control mice spent significantly more time than chance with the moved object 4 h after training (**p<0.01), indicating intact memory, whereas the Cre group did not, indicating impaired memory. (**p<0.01). The Cre group traveled significantly less distance during the testing phase, but did not show any significant differences in time to accumulate 30s exploration during either phase or distance during training phase. Mean time spent with moved objects was compared to chance (15 s) using one-sample *t* tests and on other measures the groups were compared using a two-sample *t* test. C. Saline Control mice spent significantly more time than chance with the novel object 24 h after training, whereas the Cre group did not, indicating impaired memory (*p<0.05). Although Cre mice spent significantly more time to accumulate 30s exploration during test, there were no significant differences in time to accumulate during the training test or distance traveled during either phase (two-sample *t* test). N=8-9/group for all

Retrograde-Cre-eGFP and Cre-dependent DREADD Expression

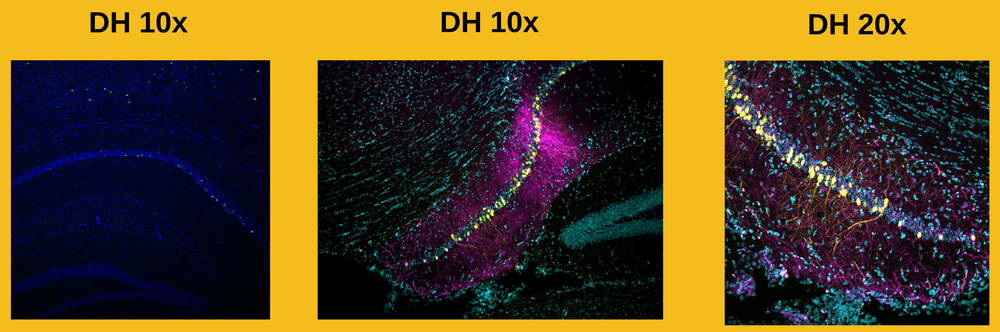


Figure 2. Representative expression of viral constructs in dorsal hippocampus. A) Expression of rg-Cre-eGFP AAV (green) in the CA1 layer of doral hippocampus (green) 4 weeks after injection into mPFC (blue = DAPI counterstain). B-C) Expression of Cre-dependent KORD (magenta) and rg-Cre-eGFP (yellow) in dorsal subiculum of hippocampus at 10x and 20x magnification (cyan = DAPI).

CONCLUSIONS

- We identified projections from DH to mPFC via an eGFP-labeled retrograde-Cre-AAV injected into mPFC.
- The double-viral method described here may interfere with episodic-like memory consolidation on its own, though these impairments do not seem to be due to trends in non-mnemonic aspects of the task.
- Reducing the amount of Cre-AAV (volume or concentration) may eliminate these memory effects and allow this method to be used in the study of memory consolidation.
- Future work will use this viral method to inactivate DH-mPFC projections to test their role in memory consolidation. The results from this and future studies will allow us to better understand the circuitry underlying memory formation and may lead to advances in treatments for alleviating memory dysfunction.

REFERENCE

Haery, Leila. "Retrograde AAV: Making the Journey from Axon to Nucleus." *Addgene Blog Share Science*, May 2017, blog.addgene.org/retrograde-aav-making-the-journey-from-axon-to-nucleus?_ga=2.44789292.920932018.1581698503-1037618514.1559157252.

ACKNOWLEDGEMENTS

Sponsored by National Institute of Mental Health grant R01MH107886 to KMF, Sigma Xi Grant in Aid of Research to MRS and the University of Wisconsin-Milwaukee Office of Undergraduate Research Support for Undergraduate Research Fellows award to CML.