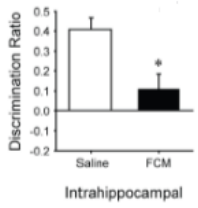
<student name> Undergraduate Research Grant Proposal

A. Project Description

Objective of research:
The objective of this study is to determine if hippocampal dopamine D1 receptors facilitate novel object recognition memory.

Background:
Mammalian spatial navigation and recognition memory are dependent on a circuit of interconnected brain regions in the medial temporal lobe, which includes the hippocampus (2). Lesions of the rodent hippocampus also impair non-spatial memory (2); however their effects on object recognition remains highly debated. Specifically, permanent hippocampal lesions spare object memory when rodents are tested in the spontaneous novel object recognition task (3-5). However, temporary hippocampal lesion pre-training impaired object recognition memory (6). Our lab has previously reported compelling studies demonstrate that an intact hippocampus is required during the encoding, consolidation and retrieval stages of object memory (7). These findings were obtained using a GABA-A agonist, muscimol, meant to temporarily inactivate the CA1 region of the dorsal hippocampus. Figure 1 illustrates that mice trained in a standard object recognition task are unable to discriminate between novel and familiar stimuli during a test session when under the influence of muscimol. These results indicate that a fully functional hippocampus is essential to the retrieval of object memory. Additionally, current theories propose that the phasic release of the neurotransmitter dopamine within the hippocampal formation facilitates the encoding of novel information. However, the precise circuitry by which dopamine influences hippocampal memory are yet to be determined. I propose that dopamine D1 receptors are also required for the retrieval of non-spatial object memory within the hippocampus.

Figure 1
Discrimination ratio (DR) for the novel object during a test session. Saline or fluorescent-conjugated muscimol microinfusions were conducted 45 min prior to the behavioral testing. A DR of 0 indicates chance performance.



* $P < 0.05$ vs. Saline vehicle; independent groups *t*-test

Methodology:
Mice. Male C57BL/6J mice (7-10 wk old; Jackson Labs) housed 4/cage with *ad libitum* access to food and water. All procedures were conducted in accordance with NIH guidelines and were approved by the FAU Institutional Animal Care and Use Committee.
Novel Object Recognition Task. Testing was conducted inside a square, high-walled arena (37.5 cm x 37.5 cm x 50 cm high) constructed of white acrylonitrile butadiene styrene. A video camera (93.8 cm above the arena floor), interfaced with a video tracking system (EthoVision 11

and XT, Noldus, Leesburg, VA), permitted manual scoring of individual behaviors as events and the automatic measurement of movement velocity, as well as cumulative distance traveled. Each NOR experiment consisted of three phases: two arena habituation sessions, one sample session (training), and one test session.

On the two days of arena habituation, each mouse will be placed into a clean polycarbonate cage. Five minutes later, the mouse will receive a 3 min mock microinfusion to habituate the mouse to the testing procedure. Next, the mouse will be transported in the polycarbonate cage into the testing room containing the arena. The mouse will be placed into the empty arena for 10 min, and then transported in the polycarbonate cage back to its home cage.

During the sample session, two identical objects (small plastic or metal toys) will placed in opposite corners of the arena (NE and SW), approximately 2.5 cm from the wall. The time spent exploring each object during the sample session will be scored using EthoVision XT nose point tracking software. Object exploration is defined as time spent with the head oriented toward and within 2-3 cm of the object. Previous studies in our lab have indicated that C57BL/6J mice allowed 38 s of exploration on one sample object, or 30 s on both objects (delineated as 38/30 s), exhibit strong novel object preference after a 24-h retention interval (8, 9). A maximum of 10 min was allotted for each mouse to accumulate 38/30 s of object exploration during the sample session. After reaching sample object exploration criterion, the mouse was immediately removed from the arena and transported in the polycarbonate cage back to its home cage.

Twenty-four h after the sample session, each mouse will be placed in a polycarbonate cage for five minutes and then given a microinfusion of SCH23390 or saline 15 min prior to being returned to the arena for a five-minute test session. During the test session, the arena will contain one object from the previous sample session (familiar) and one novel object. Object exploration during the test session will be manually scored using the EthoVision system's manual event counters while reviewing digital video files off-line. The placement of the novel and familiar object during the test sessions will be counterbalanced between the NE and SW corners of the testing arena across mice. After each session, the arena and objects will be thoroughly cleaned with 10% ethanol to remove odor cues that might guide object choice behaviors.

Intrahippocampal Cannulation and Microinfusion. Bilateral guide cannulae (Plastics One, Inc., Roanoke, VA) were implanted above the CA1 region of dorsal HPC (A/P - 2.0 mm, M/L ± 1.5 mm, D/V - 1.1 mm from bregma) (*1*). Mock infusions will be given each day for the 3 days prior to the actual intra-HPC microinfusion to habituate the mice to the microinfusion procedure. Mice will receive bilateral (0.35 µl/side, 0.334 µl/min) intra-HPC SCH23390 (4 µg/µl in 0.9% saline, Tocris) or saline 15 min prior to the test session.

Histology. Cannulae placements will be confirmed by post-mortem examination of Cresyl violet stained 50-µm coronal sections of brain tissue with light microscopic methods.

Data Analysis. To test the strength of object memory, we will analyze the preference ratio scores between vehicle and respective SCH23390 group using Student's *t*-tests. In order to verify that the future treatment groups exhibited equal motivation to explore both sample objects, latency to reach criterion will be measured and analyzed using Student's *t*-tests.

Anticipated Outcomes:

I expect that temporarily blocking hippocampal function, using SCH23390, object memory will be impaired. This finding woulf indicate that the dopamine D1 receptors found within the hippocampus are essential for the retrieval of nonspatial object memory.

B. Timeline: January – April 2015

Mid-January: Order mice and habituate to colony room.

February: Perform surgeries, allow mice to recover, run experiment, and euthanize.

March/April: Analyze data, perform histological verifications and prepare presentation.

April-August: Prepare publication.

C. Proposed Budget: *The following is a list of supplies that will be needed to conduct the proposed experiment. If awarded, the Undergraduate Research Grant would be used to cover part of the project costs. The mentor, Dr. x has agreed to cover the remainder of the project costs from his Departmental account.*

Guide Cannulae, *PlasticsOne*-$129.80
Dummies, *PlasticsOne*-$89
Screws (3 per mouse), *Small Parts*-$60
Dental acrylic-$30
Antibiotic-$10
PuraLube ointment, *CVS*-$2
Saline-$1.20
Buprenorphine-$2.80
Isoflurane-$18
Medical grade oxygen, *Airgas*-$18
Mice, *Jackson Laboratories*-$434
SCH23390, *Sigma-Aldrich*-$122.50
FAU Veterinary Services Mouse *Per Diem* (3 weeks)-$189

Total = $1106.30

The $500 allotted for the undergraduate research grant would be greatly appreciated in offsetting the total cost to run this experiment.

D. References:

1. K. B. J. Franklin, G. Paxinos, *The mouse brain in stereotaxic coordinates* (Academic Press, San Diego, CA, 2008).
2. L. R. Squire, C. E. Stark, R. E. Clark, The medial temporal lobe. *Annu Rev Neurosci* **27**, 279 (2004).
3. J. A. Ainge *et al.*, The role of the hippocampus in object recognition in rats: examination of the influence of task parameters and lesion size. *Behav Brain Res* **167**, 183 (2006).
4. G. R. Barker, E. C. Warburton, When is the hippocampus involved in recognition memory? *J Neurosci* **31**, 10721 (2011).
5. C. A. Duva *et al.*, Disruption of spatial but not object-recognition memory by neurotoxic lesions of the dorsal hippocampus in rats. *Behav Neurosci* **111**, 1184 (1997).
6. R. S. Hammond, L. E. Tull, R. W. Stackman, On the delay-dependent involvement of the hippocampus in object recognition memory. *Neurobiol Learn Mem* **82**, 26 (2004).
7. S.J. Cohen, A.H. Munchow, L.M. Rios, G. Zhang, H.N. Ásgeirsdóttir, R.W. Stackman, The rodent hippocampus is essential for nonspatial object memory. *Current Biology* **23**, 17 (2013).
8. Stackman, R.W., Hammond, R.S., Linardatos, E., Gerlach, A., Maylie, J., Adelman, J.P., Tzounopoulos, T. Small conductance Ca^{2+}-activated K^{+} channels modulate synaptic plasticity and memory encoding. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **22,** (2002).
9. Vick, K.A., Guidi, M., Stackman, R.W., Jr. In vivo pharmacological manipulation of small conductance Ca(2+)-activated K(+) channels influences motor behavior, object memory and fear conditioning. *Neuropharmacol* **58**, (2010).