Artificial Reactivation of a Cocaine-Associated Engram in the Dorsal Dentate Gyrus Attenuates Cocaine Prime-Induced Reinstatement of Conditioned Place Preference

Brody Morgan V., Edwards L. Hazel, Papanikolaou Lola Fay, Arora Sonia A., Wade William F., Stratmann Alexander, Wilson Melissa R., McAnespie Molly M., Asgarali Hussain K., Chatterjee Prajit, Culter Mikayla, Kantz Alex, Grella, Stephanie L.

Department of Psychology, Program in Neuroscience, Loyola University Chicago, Chicago, IL

INTRODUCTION

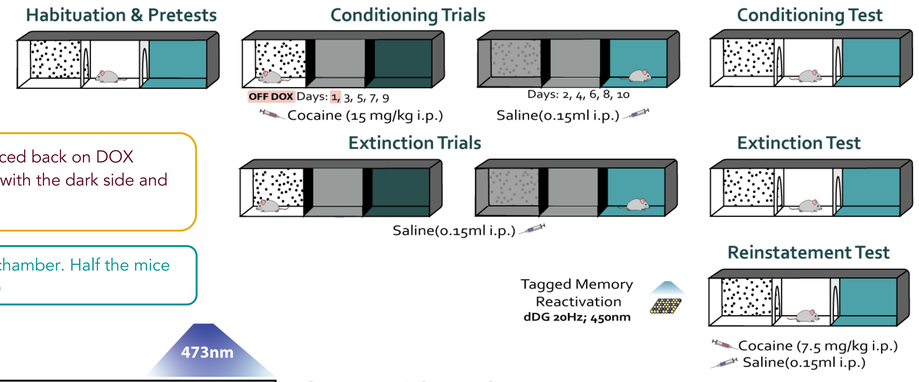
- Substance abuse is characterized by a continual propensity to relapse[1]
- Relapse-prevention strategies following abstinence, focus on reducing cravings
- In rodent models, cravings are studied by examining drug-seeking behavior[2]
- Factors precipitating drug-seeking include exposure to drug-related cues, the drug itself, and stress[3-4]
- One factor not yet directly investigated is the contribution of drug-related memories to drug seeking behavior
- Contextual memory traces (engrams) are stored in the hippocampus[5]
- In mice, we can genetically tag and manipulate engrams, such that we can reactivate them with light post-encoding[6]
- To investigate the role of drug-related memories in reinstatement, we tagged dorsal dentate gyrus (dDG) hippocampal cells involved in encoding a cocaine-related memory[7] (1st conditioning session / exposure to cocaine) using a doxycycline (DOX) inducible, Tet-tag system to express channelrhodopsin-2 (ChR2) driven by the c-Fos promoter, in male and female c57BL/6 mice
- Conditioned place preference (CPP) can be used to study rewarding aspect of drugs, and specifically, the reinstatement model has been used to study relapse[8]
- Using CPP, we assess whether a cocaine-tagged experience could be used in place of cocaine to reinstate preference thus exploring whether reinstatement could be primed via the memory of the drug in comparison to the drug itself
- Mice underwent cocaine (15 mg/kg, i.p.)[7,9] CPP training
- Preference for the drug-paired side was then extinguished and reinstated using a priming injection of cocaine (7.5mg/kg)[7,9] (or saline), optical reactivation of the tagged cocaine-related memory (20Hz, ChR2 or eYFP)[7] , or both
- We found that artificially reactivating a cocaine engram concurrently with cocaine administration, blocked reinstatement
- These data suggest that such reactivation may confer protective effects, potentially reducing relapse risk, and therefore have therapeutic significance
- Building on what we have previously shown with fear memories[10], we hypothesize that artificial reactivation of these memories, may lead to extinction-like effects

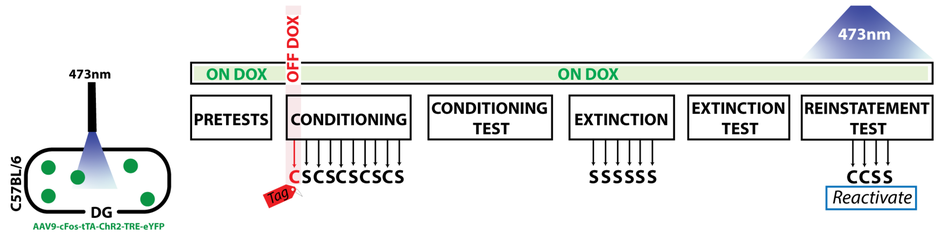
EXPERIMENTAL DESIGN

Mice initially preferred dark side; therefore, we used a *biased design:* pairing the light side with cocaine

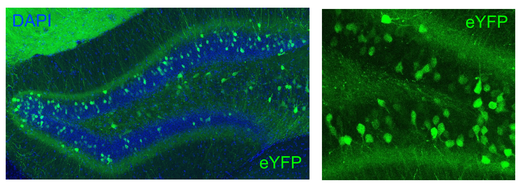
1st cocaine conditioning session (D1) was tagged off DOX. Mice were immediately placed back on DOX after this session. Mice then received alternating days of saline (D2, 4, 6, 8, 10) paired with the dark side and cocaine (D3, 5, 7, 9) paired with the light side

For reinstatement, reactivation of the tagged cocaine memory took place in the CPP chamber. Half the mice received saline, and half received cocaine; all received light stimulation (20Hz, 450nm)



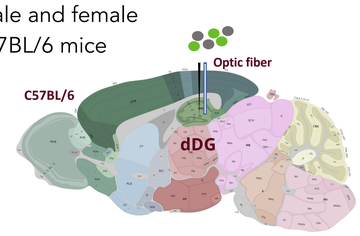


Labeling of dDG Neurons Involved in a Cocaine Experience



AAV9-c-Fos-tTA-TRE(ChR2)-eYFP (tag cocaine memory)

- Male and female
- c57BL/6 mice



Injections of an adeno-associated virus (AAV) were targeted to the dDG such that mice would express the tetracycline transactivator (tTA) protein driven by the c-Fos promoter, encoding the light sensitive opsin, channelrhodopsin-2 (ChR2) fused to the fluorescent reporter eYFP under the control of the tetracycline response element (TRE)

CPP MEASURES

Measured with Noldus Ethovision:

<u>Locomotion:</u> Mean speed / Mean distance travelled

<u>Preference Ratio (%):</u>

Calculated during test as:

Time on Side A / (Time on Side A + Time on Side B)

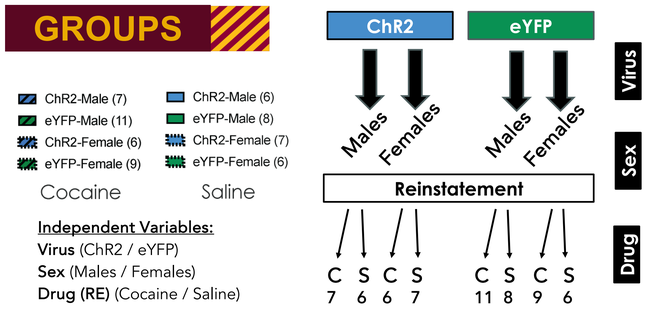
- For Pre-Test Preference Ratio, we used the mean of all 3 tests to establish initial preference
- *White: Time spent on white side / (Time on white + Time spent on black)*
- *Black: Time spent on black side / (Time on white + Time spent on black)*

<u>Preference Score (%):</u>

Calculated during test as:

%Time on drug paired side - %Time on saline-paired side

GROUPS



<u>Independent Variables:</u>

Virus (ChR2 / eYFP)

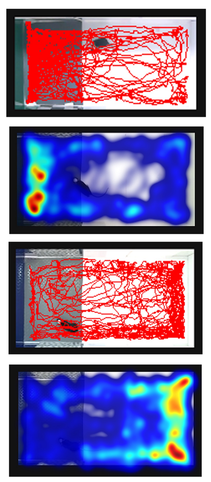
Sex (Males / Females)

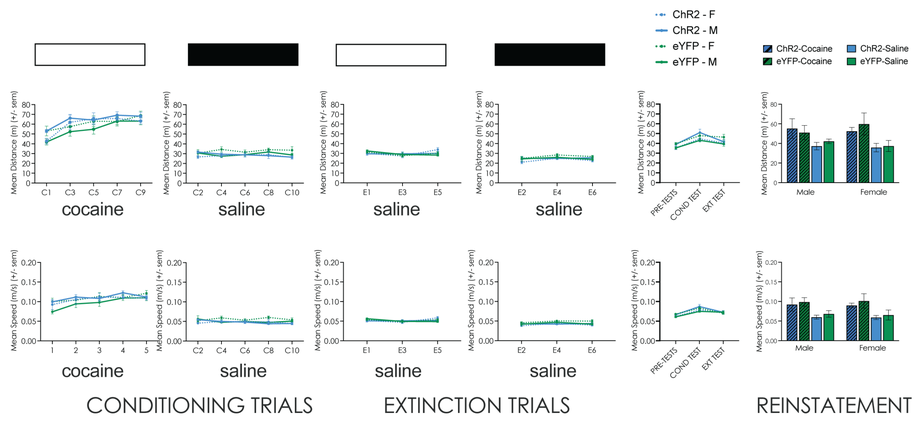
Drug (RE) (Cocaine / Saline)

RESULTS

LOCOMOTION

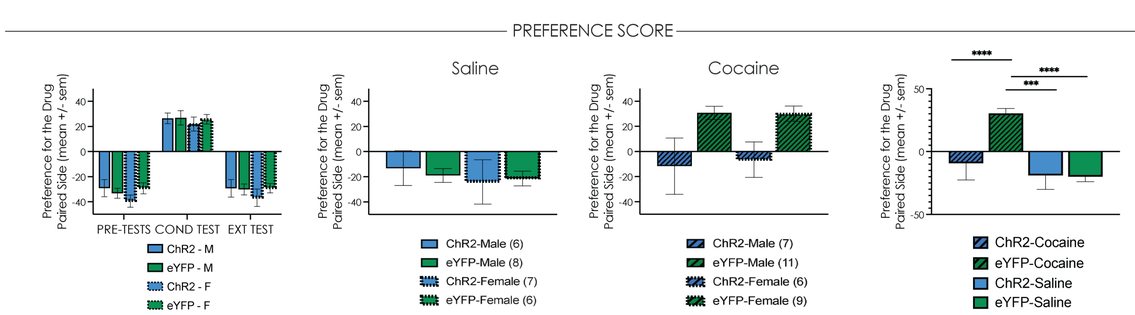
TRACKING AND HEAT MAPS





Cocaine induces locomotor sensitization. Mice traverse more distance at higher speeds when given cocaine vs. saline. Artificial reactivation of a tagged dDG cocaine engram (1st exposure) in doesn't induce locomotor effects nor does it interfere with the effects locomotor effects of cocaine.

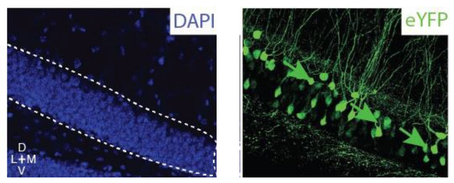
PREFERENCE SCORE



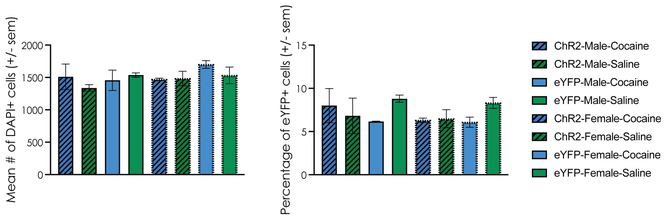
Artificial reactivation of a cocaine-related memory (1st exposure) did not reinstate place preference similar to the drug. In fact, when mice were given the drug and reactivation of the memory, it drug prime-induced place preference was blocked. We saw no sex differences.

HISTOLOGY

CELL COUNTS



A higher proportion of cells were labeled with the eYFP virus compared to ChR2. However, this did not significantly affect our results.



Engram size was similar across groups at around 7% of DAPI labeled dDG cells

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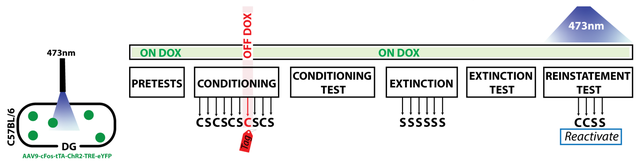
CONCLUSIONS & FUTURE DIRECTIONS

- Cocaine increased locomotion during conditioning. Sensitization was observed from the first session to the last. Upon reinstatement, locomotion was slightly higher than normal in saline mice (perhaps due to the presence of the patch cord). Generally speaking, reactivation of the tagged 1st exposure to cocaine did not induce locomotion nor did it interfere with the effects of the drug during reinstatement. There was a dissociation between preference and locomotion suggesting that this aspect of cocaine is not what drives preference.
- In eYFP mice, a cocaine priming injection induced reinstatement of CPP, as expected
- We saw no sex differences in any measure
- Artificial reactivation of the 1st exposure to cocaine did not reinstate place preference for the light side. In fact, when administered cocaine, it conferred protective effects, as it attenuated reinstatement. This may be due to extinction-like effects. Another possible explanation is that the initial exposure was associated with some aversive property (e.g., stress, anxiety, fear), which became encoded. Thus, we plan to tag the 4th exposure to capture sensitization effects and compare. We also predict distinct effects in the ventral vs. dorsal DG, consistent with prior research.



Tag 4th exposure - compare 1st and 4th exposure. Compare ventral vs. dorsal DG.

REFERENCES

1. Deroche-Gamonet V, Belin D, Piazza PV. (2004). *Science.* 305(5686):1014-7. 2. Li X, Caprioli D, Marchant NJ (2014). Recent updates on incubation of drug craving: a mini review. *Addict Biol.* 20(5): 872-6. 3. Shaham Y, Shalev U, Lu L, de Wit H, Stewart J. (2003). The reinstatement model of drug relapse: history, methodology and major findings. *Psychopharmacol(Berl).* 168(1-2):3-20. 4. Erb S, Shaham Y, & Stewart J. (1996). Stress reinstates cocaine-seeking behavior after prolonged extinction and a drug-free period. *Psychopharmacol (Berl).* 128, 408–412. 5. Semon RW, The Mneme. (Cornell University Library, 1921). 6. Reijmers LG, Perkins BL, Matsuo N, & Mayford M. (2007). Localization of a stable neural correlate of associative memory. *Science,* 317(5842): 1230-1233. 7. Grella SL, Fortin AH, Ruesch E, Bladon Reynolds LF et al. (2022). Reactivating hippocampal-mediated memories during reconsolidation to disrupt fear. *Nat Comms, 13*(1), 4733. 8. McKendrick G, Graziane NM. (2020). Drug-induced conditioned place preference and its practical use in substance use disorder research. *Front Behav Neurosci.* 29;14:582147. 9. Zhou Y, Zhu H, Liu Z, Chen X, Su X, et al. (2019). A ventral CA1 to nucleus accumbens core engram circuit mediates conditioned place preference for cocaine. *Nat Neurosci,* 22(12): 1986–1999. 10. Chen BK, Murawski NJ, Cincotta C, McKissick O, Finkelstein A, et al. (2019). Artificially enhancing and suppressing hippocampus-mediated memories. *Curr Biol.* 2019 Jun 3;29(11):1885-1894.e4.

grella_lab

@sgrella.bsky.social

MNEMELAB
Memory & Neuromodulatory Mechanisms Lab