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ACTIVATING ASTROCYTES WITH CHEMOGENETICS ACCENTUATES THEIR COMPLEX ROLE IN SPATIAL WORKING MEMORY

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INTRODUCTION

Astrocytes are a type of glial cell within the central nervous system that have a pivotal role in maintaining the blood brain barrier, helping metabolic processes and supporting synaptic transmission.[1] Previous research has indicated a role for astrocytes in memory.[2-4] We employed a chemogenetic technique (Designer Receptors Exclusively Activated by Designer Drugs, or DREADDs) to specifically increase intracellular calcium level and activate hippocampal astrocytes in Long Evans rats during spatial working memory (SWM) using a delayed spontaneous alternation task.[5,6] Our hypothesis is that activating astrocytes will increase their supportive functions, hence improve working memory (measured by percent spontaneous alternation). Previous research has used clozapine-N-oxide (CNO) as an agonist for the DREADDs receptor, however this has been shown to metabolize to clozapine, which can have effects on a variety of receptors.[7] In the current experiment we use Compound 21 (C21) which was purported to have less effects on endogenous receptors as it does not metabolize to clozapine, however whether C21 itself has effects similar to clozapine has been debated.[7,8]

METHODOLOGY

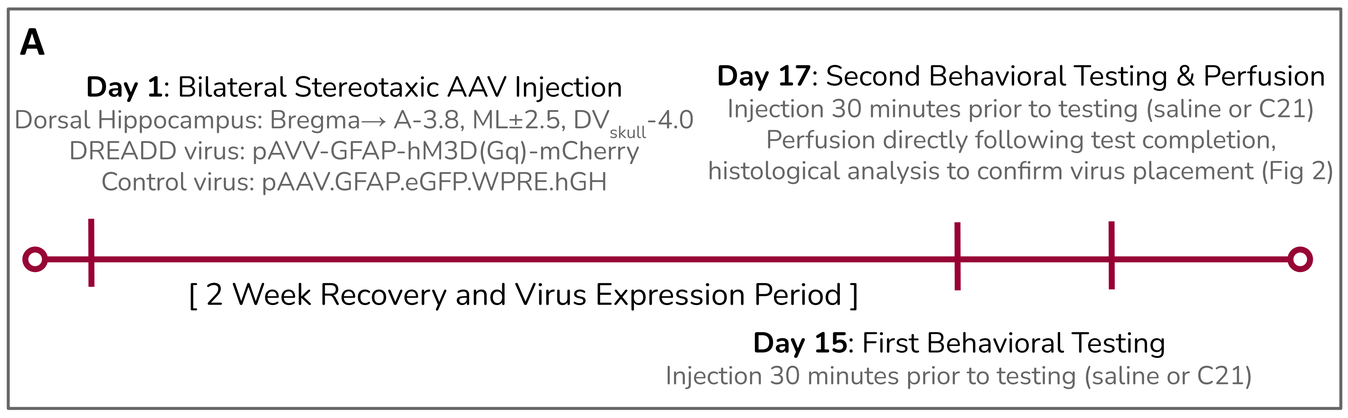

Fig 1. Experimental Design. (**A**) All Long Evans rats received either a saline control or compound 21 (C21, 1 mg/kg) Gq agonist on testing days and were counterbalanced to account for testing bias. Female rats also received vaginal smears 10 minutes prior to testing and immediately after testing. (**B**) Male (n=13) and female (n=9) rats were tested for spatial working memory using a 20-minute 4-arm delayed spontaneous alternation task with visual cues that were changed each test. Upon arm entry, a barrier was introduced for 20 seconds. Arm entries were manually recorded and alternation scores were calculated based on whether the rat entered all four arms within five entries.

HISTOLOGY

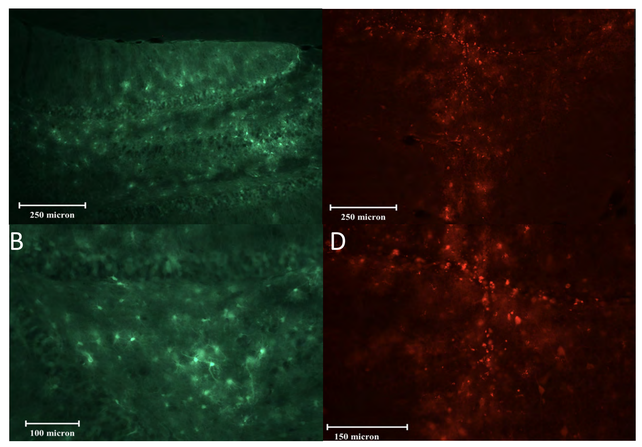

Fig 2. Dorsal Hippocampus Expression. (**A & C**) Expression at 100x magnification (A: control/eGFP, C: DREADD/mCherry. (**B & D**) Expression with astrocyte morphology 200x magnification.

RESULTS

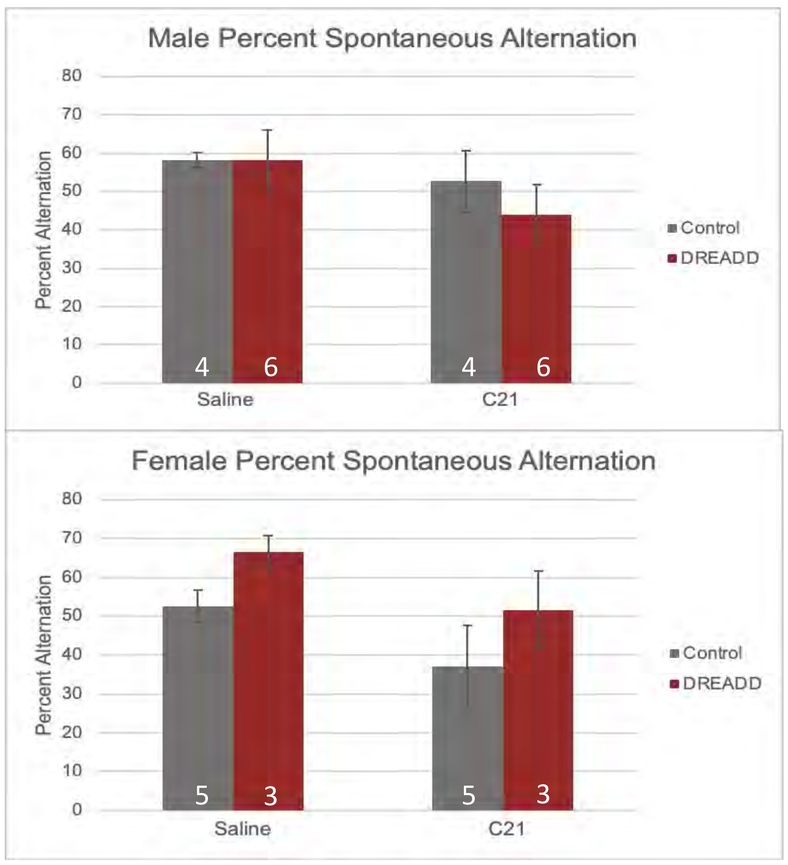

Fig 3. Overall, we saw a significant reduction in percent spontaneous alternation performance with C21 injection compared to saline ($F_{1,14}$=8.80, p=0.01). Males alone did not have a significant difference between the two injections. Females had a strong trend of impaired performance with C21 ($F_{1,6}$=5.45, p=0.058). In a previous study where we looked at viral injections in the prelimbic cortex, there was a similar trend of reduced performance with C21 in control animals ($F_{1,21}$=3.38, p=0.08).

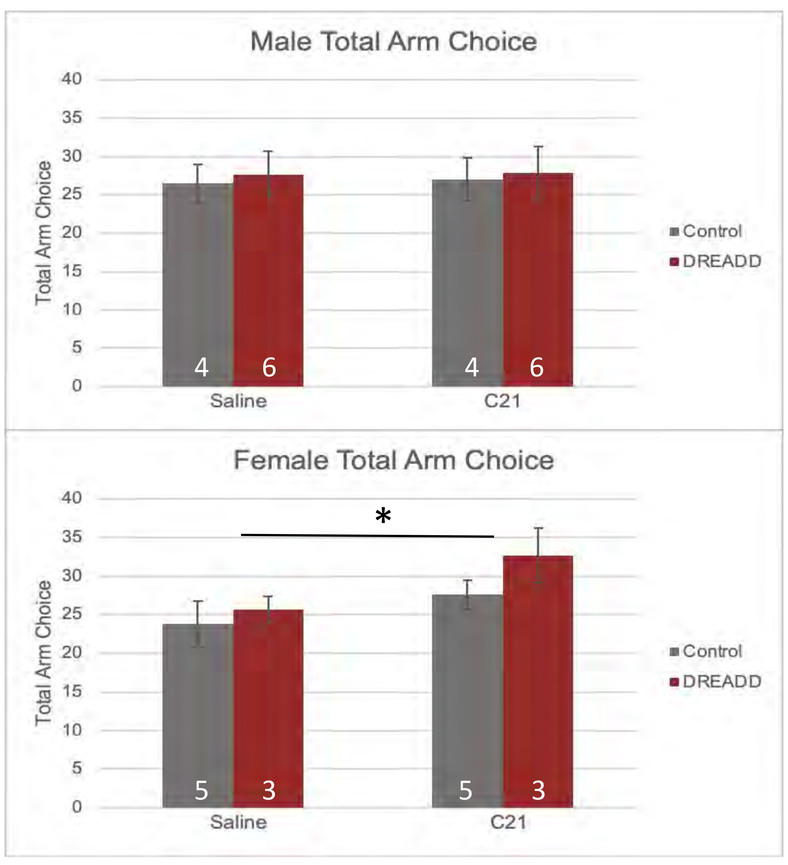

Fig 4. There was an overall increase in arm choices with the C21 injection ($F_{1,14}$=7.49, p=0.016). In addition, we saw an interaction between the administration of C21 and sex ($F_{1,14}$=5.85 p=0.03). Further analysis showed no effect of C21 on arm entries in males (p>0.05). However, females showed a significant increase in arm choices with C21 ($F_{1,6}$=7.51, p=0.034).

DISCUSSION & FUTURE DIRECTIONS

- From the current data, it is likely that C21 alone is having effects on behavior:
  - We found significant increase in the total amount of arm choices in the females with C21.
  - There is an overall trend for a decrease in spontaneous alternation with C21.
  - We had seen a similar trend in a previous study with control viral injections in the prelimbic cortex (PrL). However, in the PrL DREADDs activation in males improved spatial working memory.
- In the future we plan to increase our sample sizes in the hopes that this will clarify some of the effects.
- We will assess the extent of the viral expression. If there is a relationship between the number of astrocytes expressing the DREADDs virus and behavioral outcomes, this may inform how we can modify the study design to improve the effectiveness of the DREADDs activation. Specifically, if increases in viral expression correlate with behavioral effects it may allow us use larger viral injections and reduce the dose of C21. Using CNO is also possible, however it is unclear that the side effects of CNO would be an improvement.[7]
- We plan to explore the sex differences further by 1) assessing estrous phase to see if estrogens are interacting with behavior and astrocyte activation[9] 2) looking at colocalization of astrocyte activation markers to see if there are effects of the DREADDs virus on astrocyte function and whether this dissociates by sex.

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