



A Proper Place for DREADD: Verification of Chemogenetic Surgery in Rats

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Introduction

- The pre-limbic cortex (PL) is responsible for guiding decision making, via its role in timing.¹
- The PL does not act alone, instead it projects via layer V pyramidal cells to the nucleus accumbens (NAc).² The NAc processes reward information and may be influenced in top-down control of PL.
- While previous research suggests that the PL is involved in temporal processing and guides choice behavior, there are no studies investigating the effects of the PL on time-based interventions to improve self-control. Interventions have previously demonstrated a promotion of self-control coupled with timing improvements and that may rely on this pathway.^{3,4}
- A pilot study was completed earlier to examine the hypothesis: inhibition of the PL will impair self-controlled decisions following intervention due to reductions in top-down regulation of the NAc.
- The experimental group (n=6) received an inhibitory DREADD (Designer Receptors Exclusively Activated by a Designer Drug) virus to decrease activity in the region while the SHAM group (n=6) received a sham control virus that did not effect activity. Then rats were given the option to press a lever and choose smaller, sooner (SS) rewards or larger, later (LL) rewards.
- The behavioral study found that the DREADD group made more impulsive (SS) choices, supporting the hypothesis (see figure 1).

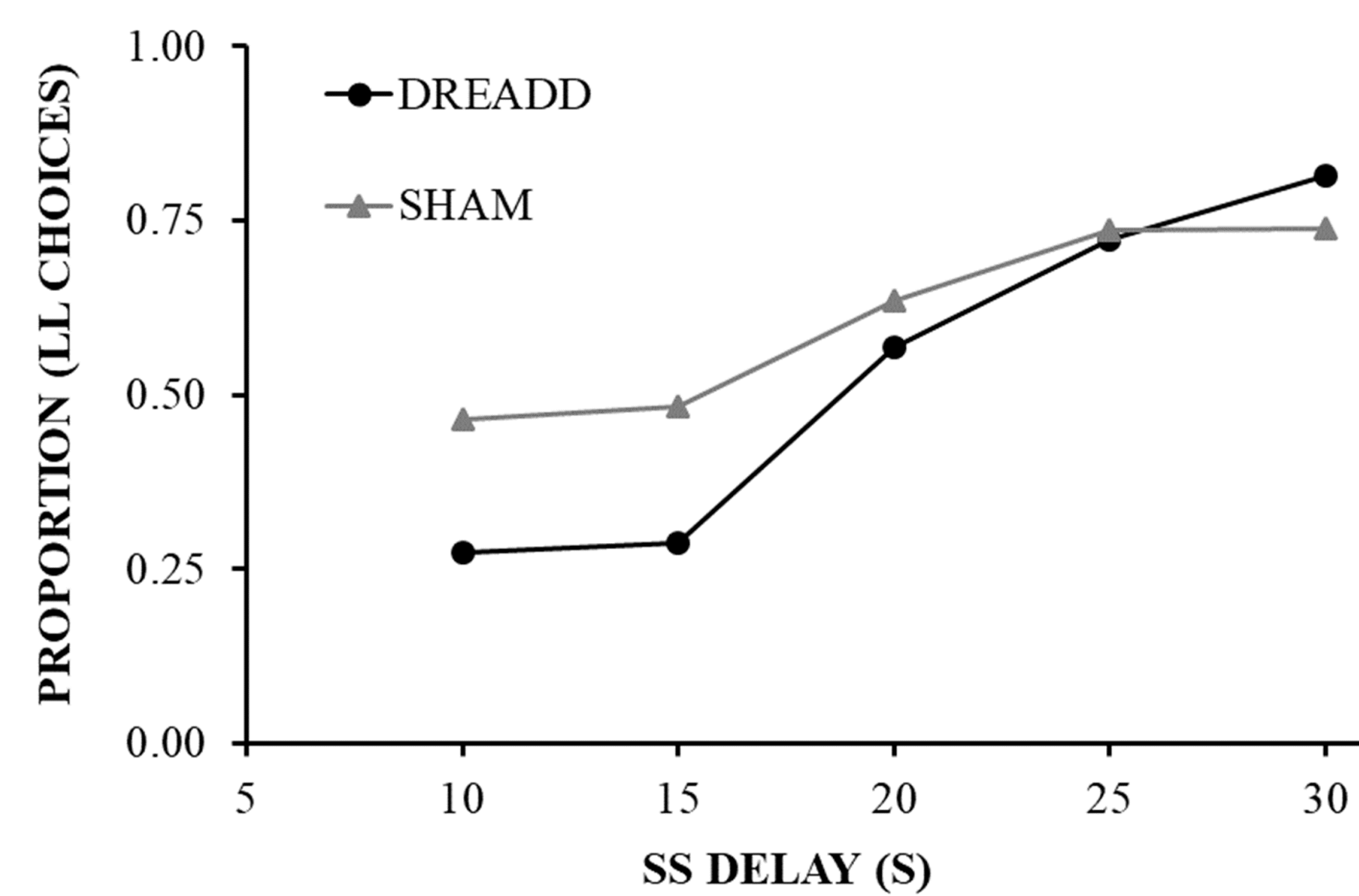


Figure 1 presents the larger-later (LL) choices as a function of smaller-sooner (SS) delay from all 12 rats (6 DREADD, 6 SHAM). Note that higher scores indicate more self control. The DREADD group made fewer LL choices at shorter SS delays, but as the SS delay increased both groups made similar choices.

- This study aimed to analyze the surgical placement and expression of the viruses in order to validate the data found in the above pilot study.

Methods

- Rats were euthanized and perfused transcardially. Tissue was fixed with 4% paraformaldehyde. Heads were decapitated and brains removed.
- Brains were soaked in paraformaldehyde for 24 hours and then sucrose for 48 hours. Brains were then frozen with dry ice.
- Brains were sectioned using the Rat Brain in Stereotaxic Coordinates Atlas (Paxinos & Watson) at 40µm thickness.⁵
- Slices were stained with DAPI (4',6-diamidino-2-phenylindole) as a counterstain and viewed under fluorescence (Green Fluorescent Protein for sham group, mCherry for the active DREADD).
- A surgical success had to show $\geq 60\%$ bilateral PL expression and no infralimbic (IL) or medial orbitofrontal cortex (mOFC) expression in the surrounding regions, as those regions have been shown to have antagonistic effects to the PL.⁶

Impulsive Choice Task Results: Hits

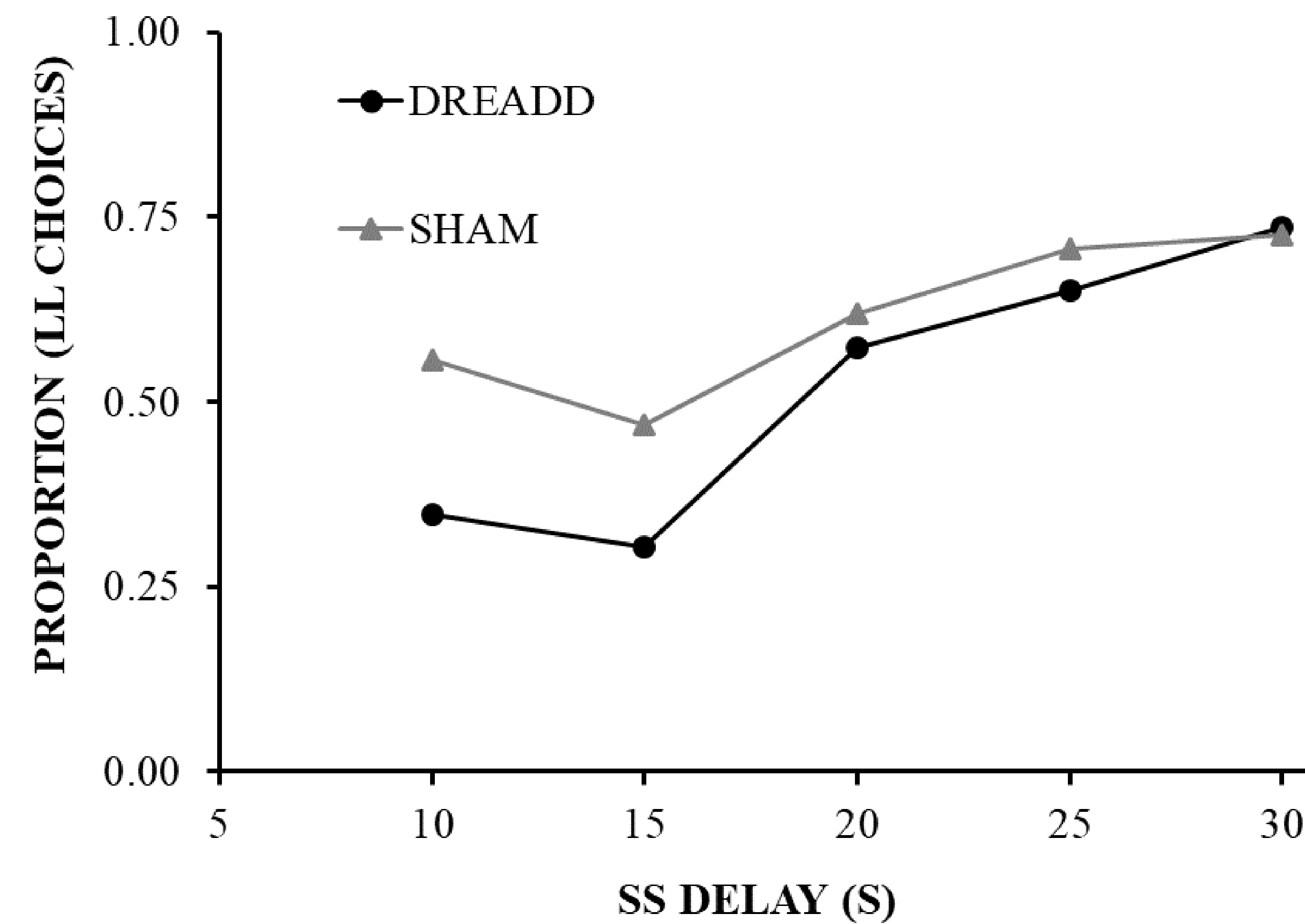
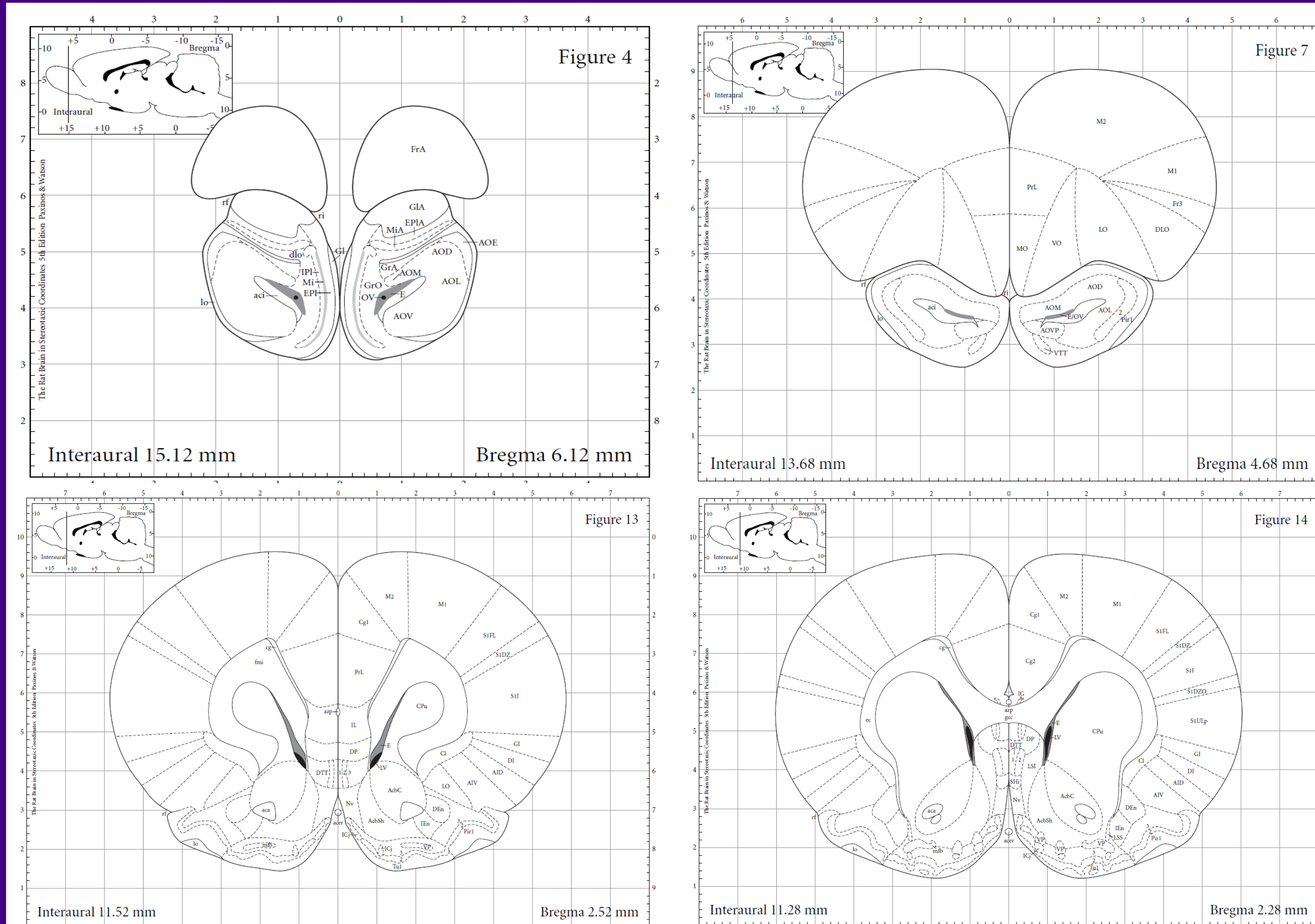


Figure 2 presents the behavioral data for the 5 rats that were considered surgical hits (3 SHAM, 2 DREADD). The DREADD rats made fewer LL choices at the shorter SS delays. This pattern mimics that in Figure 1.

Placement Locations



Figures 3A-D are taken from the rat atlas.⁵ Brain slicing generally went from 3A (top left, axis figure 4) to 3D (bottom right, axis figure 14). PL (labelled PrL on the rat axis) expression was analyzed from figure 3B (top right, axis figure 7) to figure 3C (bottom left, axis figure 13).

Placement Results

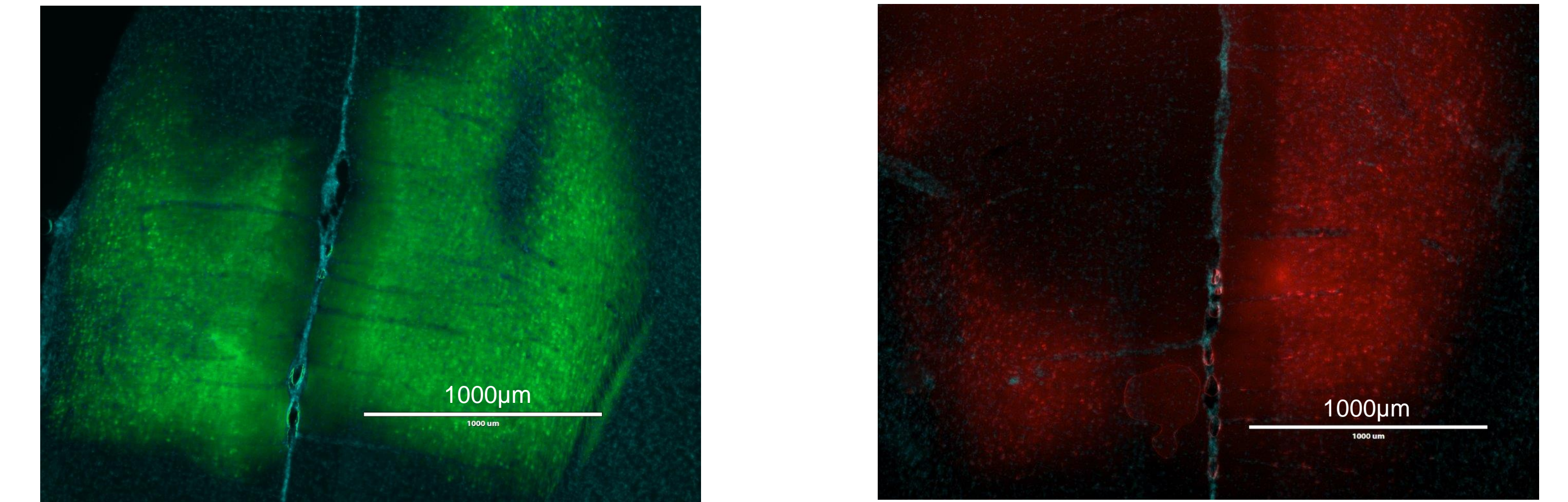


Figure 4A (above left) is a sham brain, specifically the PL, shown with Green Fluorescent Protein (GFP) that was considered a surgical success. Figure 4B (above right) is the same area in a DREADD brain shown with mCherry fluorescence that was considered a surgical failure due to weak expression.

Of the six sham brains, 3 were surgical hits (see Figure 3A), 1 had no expression, and 2 had significant expression in the IL. Of the six DREADD brains, 2 were surgical hits, 2 only had unilateral expression in the PL, 1 was correctly placed but did not cover at least 60% of the PL (see Figure 3B), and 1 showed no expression in the PL.

Discussion

- The pilot study found that the DREADDs group made fewer LL choices compared to the SHAM control, suggesting that the CNO inhibition of the PL may have blocked the intervention effects on choice.
- The 5 surgically successful rats also followed this trend and further support those findings.
- However, the small sample size of this pilot study coupled with some variability in surgical placements and expression levels indicates the need for further replication.
- The pilot study and this analysis suggest that the PL may be involved in timing interventions to promote self control, but more research is needed.

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Acknowledgements

We would like to acknowledge Robert Small and Cathryn Haas for their assistance in running the project and writing the poster introduction, respectively. This research was supported by the National Institutes of Health (Grants MH085739 and GM113109), the Boehringer Ingelheim Veterinary Scholars Program, and the KSU Veterinary Research Scholars Program.