

# **Diazepam reverses increased anxiety-like behavior, social behavior deficit and dopamine dysregulation following withdrawal from acute amphetamine**

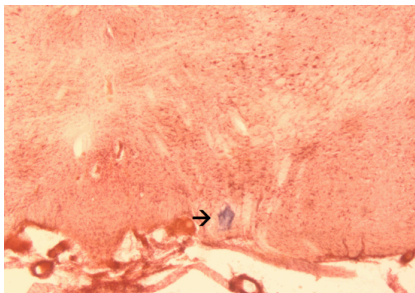
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## **Supplementary Materials and Methods**

### **Histology/Placement Verification**

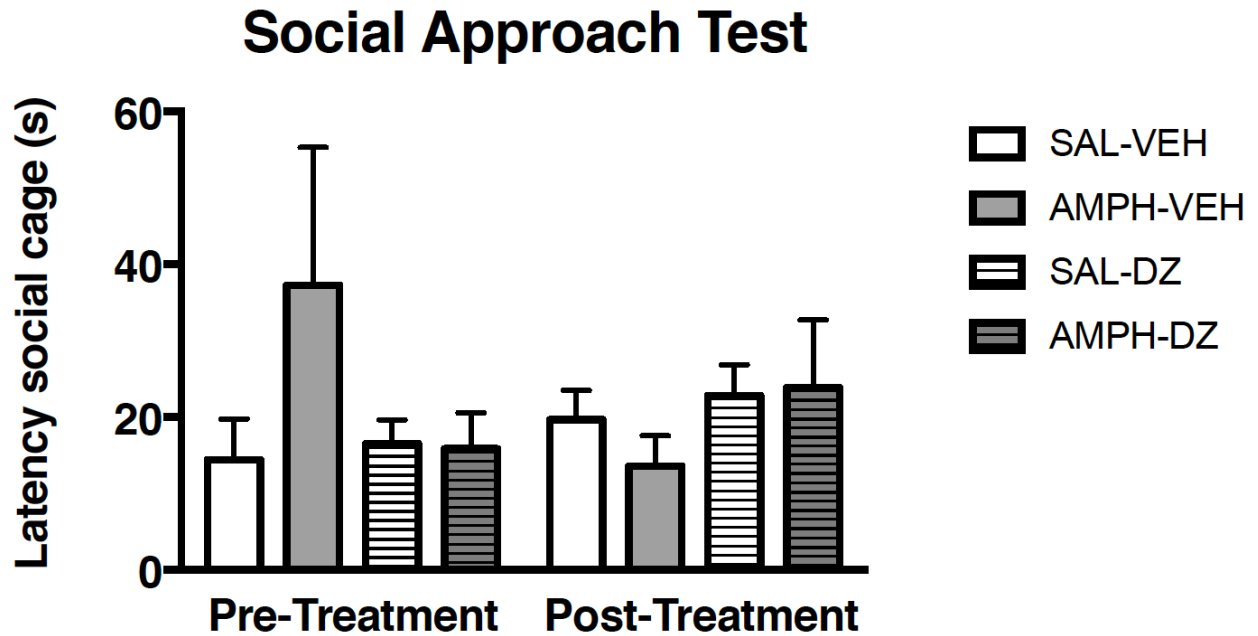
Electrode placement was verified via electrophoretic ejection of Chicago Sky Blue dye (Sigma) at the last recording site. Rats were euthanized with a lethal dose of chloral hydrate (additional 400mg/kg i.p.) and brains were removed. Tissue was fixed in 8% paraformaldehyde for at least 48 hours and then transferred to a 25% sucrose solution for cryoprotection until sectioning. Once saturated, brains were frozen and sliced coronally at 60  $\mu$ m (Cryostar NX50, ThermoScientific) and mounted on gelatin-coated slides. Tissue was stained with a combination of neutral red and cresyl violet. Animals were required to have a minimum of 6 tracks located within 0.4mm of target coordinates to be included in the study. All recorded DA neurons were located -5.0 to -6.12 mm from bregma. Only rats with verified track/electrode placement were included in the data analysis. 6 animals were excluded from the study due to electrode misplacement or insufficient number of tracks.



**Figure S1 Representative histology placement.** A representative pontamine sky blue deposit in which the final location of the last track was marked by electrophoretic ejection of dye for histological verification (arrow indicates final location of electrode).

## Supplementary Results

At baseline (i.e. pretreatment), no difference in latency to contact (i.e. sniff) the social cage was found across treatment groups [one-way ANOVA: ( $F_{3,26} = 1.13$ ,  $p = 0.36$ )]. No differences were found post-treatment [one-way ANOVA: ( $F_{3,26} = 0.71$ ,  $p < 0.05$ )].



**Figure S2 Latency to contact social cage following acute AMPH withdrawal.** Rats exhibited comparable latencies to contact/sniff the social cage across treatment groups on both testing days.