SUPPLEMENTAL INFORMATION

Nucleus accumbens acetylcholine receptors modulate dopamine and motivation

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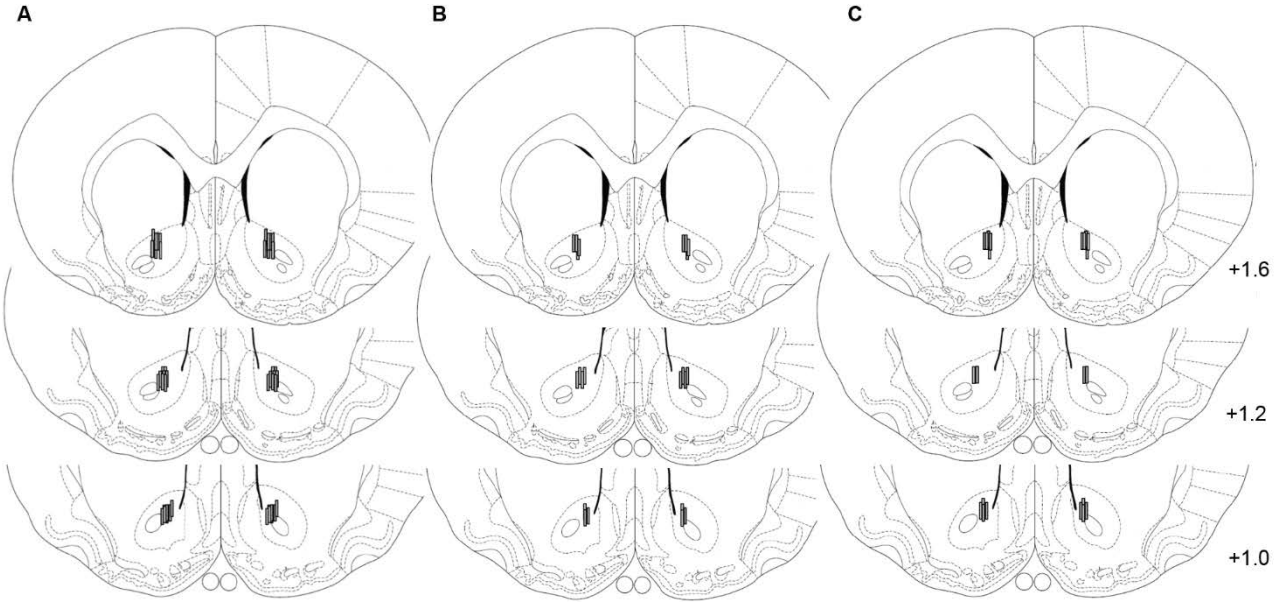
Supplemental Results

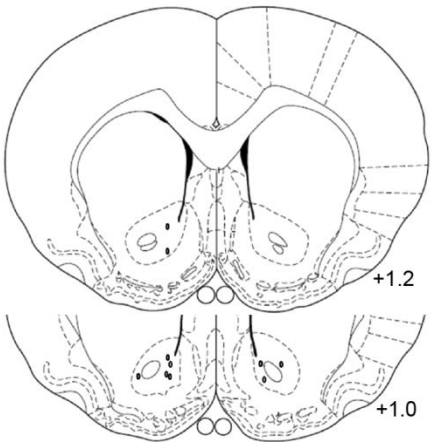
Training data.

By the end of Pavlovian training all rats entered the food-delivery port significantly more during the CS^+ probe period (at the CS^+ onset, but prior to reward delivery; Experiment 1: 30.24 entries/min, SEM= 2.52; Experiment 2: 25.57 entries/min, SEM= 2.95) relative to the baseline, CS-free period (Experiment 1: t_{23}=6.018, p<0.0001; Experiment 2: t_{14}=6.826, p<0.0001). At the end of instrumental training rats in Experiment 1 pressed at an average rate of 21.08 presses/min (SEM= 1.57) and in Experiment 2 at an average rate of 25.35 presses/min (4.61).

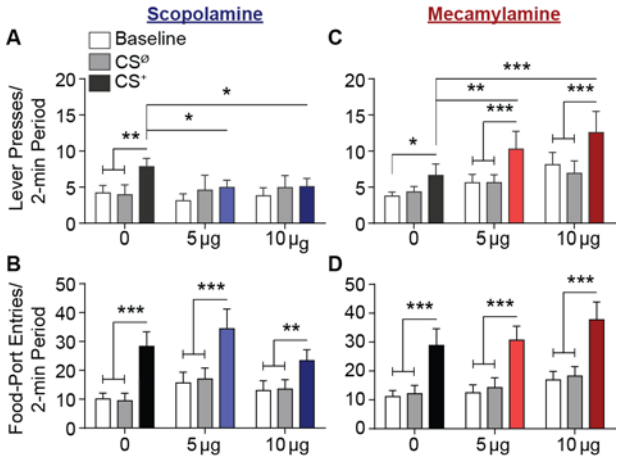
Supplemental Figures



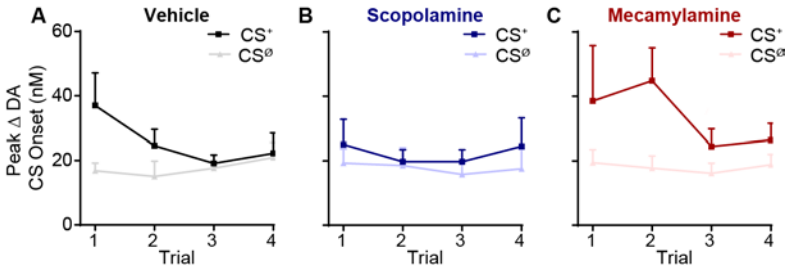
Supplemental Figure 1: Histological verification of NAc infusion sites for Experiment 1. Schematic representation of infusion sites in NAc core. Coronal section drawings taken from (Paxinos and Watson, 1998). Numbers to the lower right represent anterior-posterior distance (mm) from bregma. **A.** Experiment 1. **B.** Supplemental Experiment 1B (intra-NAc scopolamine multi-dose experiment). **C.** Supplemental Experiment 1C (intra-NAc mecamylamine multi-dose experiment).



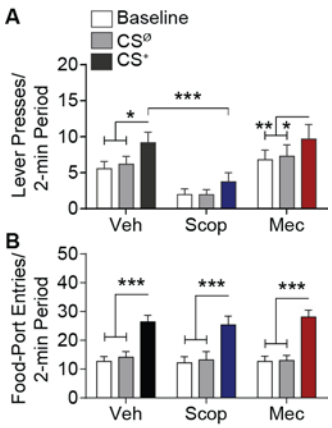
Supplemental Figure 2: Histological verification of NAc electrode placement for Experiment 2. Schematic representation of recording sites in NAc core. Coronal section drawings taken from (Paxinos *et al*, 1998). Numbers to the lower right represent anterior-posterior distance (mm) from bregma. Circles represent recording sites from electrode placement. Injector tip was always laterally positioned from electrode tip.



Supplemental Figure 3. Effect of intra-NAc scopolamine or mecamylamine at multiple doses on Pavlovian-to-instrumental transfer. A & B. Supplemental Experiment 1B. Rats (n=9) were trained and tested as described for Experiment 1 in the main text, with the exception that PIT tests were conducted following bilateral infusion of either 0 (ACSF-vehicle), 5, or 10 µg dose of scopolamine. **A.** The PIT effect. Number of lever presses during each 2-min period, averaged across trials compared between the CS-free (baseline), neutral stimulus ($CS^{Ø}$), and reward-predictive cue (CS^{+}) periods (main effect of CS period: $F_{2,16}$=3.85, p=0.04; Drug dose: $F_{2,16}$=0.80, p=0.47; Dose x CS period: $F_{4,32}$=1.64, p=0.19). **B.** Conditioned food-port approach responding. Number of entries into the food-delivery port during each 2-min CS period, averaged across trials compared between the baseline, $CS^{Ø}$, and CS^{+} periods (main effect of CS period: $F_{2,16}$=25.13, p<0.0001; Drug dose: $F_{2,16}$=1.148, p=0.34; Dose x CS period: $F_{4,32}$=1.490, p=0.23). **C & D.** Supplemental Experiment 1C. Rats (n=11) were trained and tested as described for Experiment 1 in the main text, with the exception that PIT testing was conducted following bilateral infusion of either 0, 5, or 10 µg dose of mecamylamine. **C.** The PIT effect. Number of lever presses during each 2-min period, averaged across trials compared between the baseline, $CS^{Ø}$, and CS^{+} periods (main effect of CS period: $F_{2,20}$=7.81, p=0.003; Drug dose: $F_{2,20}$=3.85, p=0.04; Dose x CS period: $F_{4,40}$=1.15, p=0.35). **D.** Conditioned food-port approach responding. Number entries into the food-delivery port during each 2-min CS period, averaged across trials compared between the baseline, $CS^{Ø}$, and CS^{+} periods (main effect of CS period: $F_{2,20}$=41.69, p<0.001; Drug dose: $F_{2,20}$=3.101, p=0.07; Dose x CS period: $F_{4.40}$=0.1803, p=0.95). In all cases the CS^{+} elevated food-port entries relative to the baseline and $CS^{Ø}$ periods (p<0.001). Error bars represent $\pm$1 SEM. *=p<0.05, **=p<0.01, ***=p<0.001.



Supplemental Figure 4. Time course of the effect of intra-NAc muscarinic and nicotinic acetylcholine receptor blockade on cue-evoked dopamine signaling during Pavlovian-to-instrumental transfer. Peak dopamine concentration change in the 30 s period following CS^{+} or $CS^{Ø}$ onset across each of the 4 trials for the PIT test following unilateral infusion of vehicle (A), scopolamine (B), or mecamylamine (C) into the extracellular space surrounding the recording electrode. Trials in which >10% of the data points had residual values in excess of the Qα were omitted from the analysis. These missing data points precluding our ability to run an appropriate within-subject analysis on these data. Although there was an apparent time-course effect on the peak CS^{+}-evoked dopamine response in the vehicle condition, likely due to the within-test extinction learning resulting from repeated CS^{+} presentations without accompanying reward, both scopolamine and mecamylamine appeared to have similar effects throughout the duration of the PIT test.



Supplemental Figure 5. Expression of Pavlovian-to-instrumental transfer following unilateral inactivation of nucleus accumbens muscarinic or nicotinic acetylcholine receptors. Rats in Experiment 2 were given 3 PIT tests, one each following unilateral infusion of vehicle (Veh), scopolamine (Scop; 10 µg), or mecamylamine (Mec; 10 µg) directly into the extracellular space surrounding a chronically-implanted carbon-fiber microelectrode, which was used to make FSCV dopamine measurements. **A.** Lever presses, averaged across trials, compared between the CS-free (baseline; average of all 2 min pre-CS periods after the first CS), neutral stimulus ($CS^{Ø}$), and reward-predictive cue (CS^{+}) periods (main effect of CS Period: $F_{2,28}$=6.39, p=0.005; unilateral Drug treatment: $F_{2,28}$=10.71, p=0.0004; CS period x Drug interaction: $F_{4,56}$=0.41, p=0.80). The CS^{+} significantly elevated responding above baseline levels for the vehicle (p<0.01) and mecamylamine (p<0.01) conditions, but only marginally significantly increased lever pressing following unilateral infusion of scopolamine (p=0.09). A one-way ANOVA on the PIT data following unilateral intra-NAc infusion of scopolamine did reveal a significant main effect of CS period (Baseline, $CS^{Ø}$, CS^{+} $F_{2,28}$=5.34, p=0.02), with a significant CS^{+}-induced elevation in lever pressing above both baseline and $CS^{Ø}$ periods (p<0.05, in both cases). Lever pressing during the CS^{+} was, however, lower following unilateral intra-NAc infusion of scopolamine, than following intra-NAc vehicle (p<0.001), indicating that, although this unilateral treatment did not abolish PIT (as was seen with bilateral infusion), it did attenuate it. Importantly however, the attenuating influence of this treatment on cue-evoked dopamine signaling was shown not to be a consequence of this attenuated in cue-evoked lever pressing. These two variables were not significantly correlated (Pearson r_{10}=-0.22, p=0.54) and there was no difference in the CS^{+}-evoked dopamine response on trials in which the subject made no lever presses relative to trials with lever press activity (Average CS^{+}-evoked dopamine response without lever presses: 21.35, SEM=3.02, with presses: 20.21, 2.46; t_{12}=0.28, p=0.79). **B.** Conditioned food-port approach responding. Number of entries into the food-delivery port during

each 2-min period, averaged across trials compared between the baseline, $CS^{\varnothing}$, and CS^{+} periods. Following each drug treatment the CS^{+} significantly elevated food-port entries relative to the $CS^{\varnothing}$ (main effect of CS period: $F_{2,28}=55.64$, $p<0.0001$; Drug: $F_{2,28}=0.10$, $p=0.91$; CS x Drug interaction: $F_{4,56}=0.65$, $p=0.63$).

References

Paxinos G, Watson C (1998). *The rat brain in stereotaxic coordinates*, 4th edn. Academic Press.