Cocaine Self-Administration Abolishes Associative Neural Encoding in the Nucleus Accumbens Necessary for Higher-Order Learning

Supplemental Information

Supplemental Methods

Animals

Male Sprague-Dawley rats (*n* = 19; Charles River Laboratories) weighing ~300-350 g at the beginning of the experiment were used. Rats were individually housed, given ad libitum access to food and water except during self-administration testing (water deprivation to 25 ml water/d) or Pavlovian conditioning (food deprivation to 15 g chow/d). Rats were assigned to self-administer either cocaine (*n* = 8 with a 0.33 mg/inf and *n* = 5 with a 0.16 mg/inf), or water with yoked saline (0.9%) infusions (*n* = 3); naïve animals did not receive intrajugular catheters or self-administration testing (*n* = 6). Animals in the saline and naïve groups showed no differences in behavior (Figure S1) and were subsequently grouped together as a control group. Testing procedures were conducted in accordance with the University of North Carolina at Chapel Hill Institutional Care and Use Committee.

Surgery

All surgical procedures were conducted under aseptic conditions using established procedures (1-3). For self-administration, rats were implanted with an indwelling catheter in the jugular vein as described previously (4). For electrophysiology, rats received stereotaxic bilateral implantation of 8-wire arrays (2 x 4 50 μm dia Teflon-coated stainless steel wires spaced 500 μm apart; NM Labs, Denison, TX) into the core in one hemisphere (AP: +1.8 mm, ML: ± 1.4 mm, DV -6.2 mm relative to Bregma) and the shell of the contralateral hemisphere (AP: +1.8 mm, ML: ± 0.8 mm, DV -6.2 mm), as described previously (3).

Behavior

Self-administration proceeded as previously described in chambers that were distinct from those used for later Pavlovian conditioning [see (3) for details]. Briefly, for cocaine administration, rats performed daily 2 h sessions during which each lever press resulted in intravenous cocaine delivery, retraction of the lever, and delivery of an intermittent 1200 Hz tone coupled with the illumination of the houselight for 20 s. After the houselight and tone terminated, the lever extended back into the chamber. To control for self-administration experience, controls received IV vehicle (0.9% saline) infusion, which were received on a yoked scheduled with a paired cocaine-administering animal. To maintain similar motivated instrumental experience between controls and cocaine-administering rats, presses on the lever for controls resulted in the same stimuli (retracted lever, illuminated house light, intermittent tone for 20 s), and the delivery of a small bolus of water (0.05 mls) to a foodcup as a reinforcer.

Pavlovian First- and Second-Order Conditioning

For Pavlovian conditioning, rats were run in behavioral chambers that were easily discriminable from the cocaine-administration context as described previously (3). Behavior was assessed by entries into the foodcup during relevant periods using infrared beams (MED Associates) positioned across the cup.

For first-order conditioning, rats received 10 sessions of Pavlovian light-food pairing, one session per day on consecutive days. Each session consisted of fourteen 10-s presentations each of a flashing cue light or a solid cue light, one serving as the CS+ and the other serving as the CS- for each subject. For the CS+ cue, 12 presentations were immediately followed with the delivery of three sucrose pellets, while the other two CS+ presentations were non-reinforced. The

use of non-reinforced trials was done to both slow down conditioning and allow for more robust and stable performance during second-order conditioning which occurs under extinction. None of the fourteen presentations of the CS- were reinforced. Cues were separated by a 3 ± 1.2 min intertrial interval. Following first-order conditioning, all rats were trained on three days of second-order conditioning. On a pre-conditioning day, rats were pre-exposed to the novel auditory stimuli to reduce any unconditioned responding to 10-s presentations of either a solid 1500 Hz tone (n = 3) or white noise stimuli (n = 3), neither of which were reinforced with food. On the three days of second-order conditioning, one 10-s auditory cue (e.g., noise: SOC+) was immediately followed by a 10-s CS+ light cue (n = 18; here called the FOC+, to differentiate it from CS+, which is when the light is presented and followed by food), while the other 10-s auditory cue (e.g. tone; SOC-) was immediately followed by the CS- light cue (FOC-; n = 17). Importantly, neither SOC/FOC pairings were reinforced with food. Further, at the beginning of each of these sessions, rats received six CS presentations (three each of the CS+ and CS-) and a further six CS only "reminders" (three each CS+ and CS-) pseudorandomly distributed throughout the session which were reinforced on the same schedule as during first-order conditioning, and was done to maintain performance against a background of extinction for the SOC cues.

Behavioral Data Analysis

For behavioral scoring, we compared the number of foodcup entries during a 10 s effect period (cue, reward) to the 10 s baseline bin immediately prior to cue onset for each subject on each day. Data were normalized in both first-order and second-order sessions by subtracting the baseline from the epoch in question (i.e., cues and reward period). For some analyses, we

employed a simple elevation score as a behavioral index of performance, using the average number of foodcup entries in a session for a subject during the average baseline, CS+ and CS- epochs:

$$Beh_{(FOC)} = \frac{(CS_+ - (CS- + Baseline)^{-½})}{(CS_+ + (CS- + Baseline)^{-½})}$$

This index was employed to show the degree of discrimination between the CS+ effect period compared to the control baseline and CS-, such that if a given subject shows poor discrimination on a session (i.e., when there is no discrimination between cues), then the index should be near 0, whereas when foodcup entries are almost exclusive to the CS+ period, then this index would approach 1. We employed the same index to assess performance on the SOC sessions:

$$Beh_{(SOC)} = \frac{(SOC_+ - (SOC- + Baseline)^{-½})}{(SOC_+ + (SOC- + Baseline)^{-½})}$$

Electrophysiology

Electrophysiological procedures have been detailed previously (3). Briefly, rats were connected with to a harness via two 8-channel Omnetics connectors wired to a unity-gain headstage (Plexon Inc). The cable was connected on the other end to a commutator (Crist), allowing the rat to freely move throughout the test chamber during recording sessions. Neural activity was captured using commercially available software (Plexon, Inc), and spikes were sorted using principal components analysis on waveforms. Units were analyzed in relation to behavioral markers using NeuroExplorer software (Nex Technologies).

Neural Analysis

Cells that showed significant differences in firing rate (either excitation or inhibition) following the onset of a behavioral event were considered phasic for that event. Significant differences were calculated by performing a 2-way analysis of variance (ANOVA) for each cell using the mean firing rate for each trial over in each 10 s bin (baseline, cue period, and reward [post-cue] period) for each cue presentation, as is routinely done in this lab and elsewhere (3, 5). Phasic cells showed a significant interaction between cue type (i.e., CS+ vs CS- for first-order sessions or SOC+ vs SOC- for second-order) X bin (baseline bin, cue bin, reward bin). Critically, each analysis performed was independently run on a single cell in a single recording session; as such, each analysis was considered independent and strictly controlled for multiple comparisons. Post-hoc analysis using Tukey's honestly significant difference (HSD) for these cells was used to determine the nature of the interaction. Phasic cells showed no difference in baseline, but a significant difference between CS+ and CS- (or SOC+ and SOC-) in the effect bin; any cells with a significant difference between the baselines (<1% cells recorded) were excluded from analysis. As such, s*elective* cells showed significantly different firing during the CS+ than both the CS- and baseline (similarly for the SOC+ different than SOC- and baselines) while *non-phasic* cells showed no differences between either cue or baseline bins. Cells showing variable dynamic responses (i.e., significant excitations and inhibitions during the same cue period) were excluded from analysis. These accounted for 1.4% of all recorded neurons, and thus constituted a small minority of recorded neurons. Reward-selective cells were defined as non-cue-selective neurons that displayed significantly different firing during reward receipt compared to baseline and the temporally-matched bin following the CS- cue.

Encoding was assessed as the percent of cells encoding a particular event (e.g., CS+) out of the total number of recorded cells for each subject within a region (core or shell) on a given session. For first-order conditioning, cells were sampled on day 1 (no learning), day 5 (moderate learning) and day 10 (accurate performance). For second-order sessions, all three days were analyzed. For analysis, ANOVAs were performed using these averages for each subject on each session for each region (core or shell) by treatment group (control vs cocaine) and day (either day 1, 5 and 10 [first –order], or day 1, 2 and 3 [second-order]).

In some analyses, neural encoding was correlated with behavior using the percent cells that encoded a particular event on each session (e.g., CS+ selective) and the behavioral index for that session (see above). We used days 1, 5 and 10 for first-order conditioning and days 1-3 for second-order condition for this analysis.

Histology

At the completion of all behavioral testing, rats were heavily anaesthetized and transcardially perfused with 3% potassium ferricyanide in 10% formalin, as detailed previously [e.g., (6)]. Histological placement of electrode positions is shown in Figure S1.

Statistical Tests

Significance between groups was tested using ANOVAs. Post-hoc tests for individual pairwise differences were performed using Tukey's HSD to control for multiple comparisons with Statistica software (StatSoft, Tulsa, OK); all post-hoc tests reflect the *p*-value of those comparisons. Population data was analyzed using χ^2 with Fisher's Exact Test (GraphPad). Correlations were done with Prism (GraphPad).

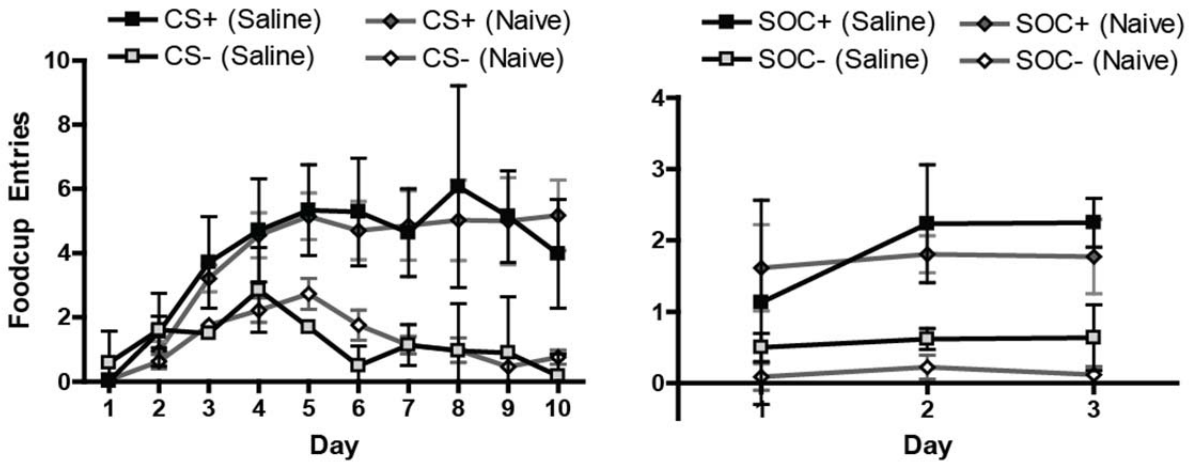

Figure S1. Comparison of saline-treated and naïve rats in the control groups. At no point were there significant differences in behavior during first-order (left) or second-order (right) sessions.

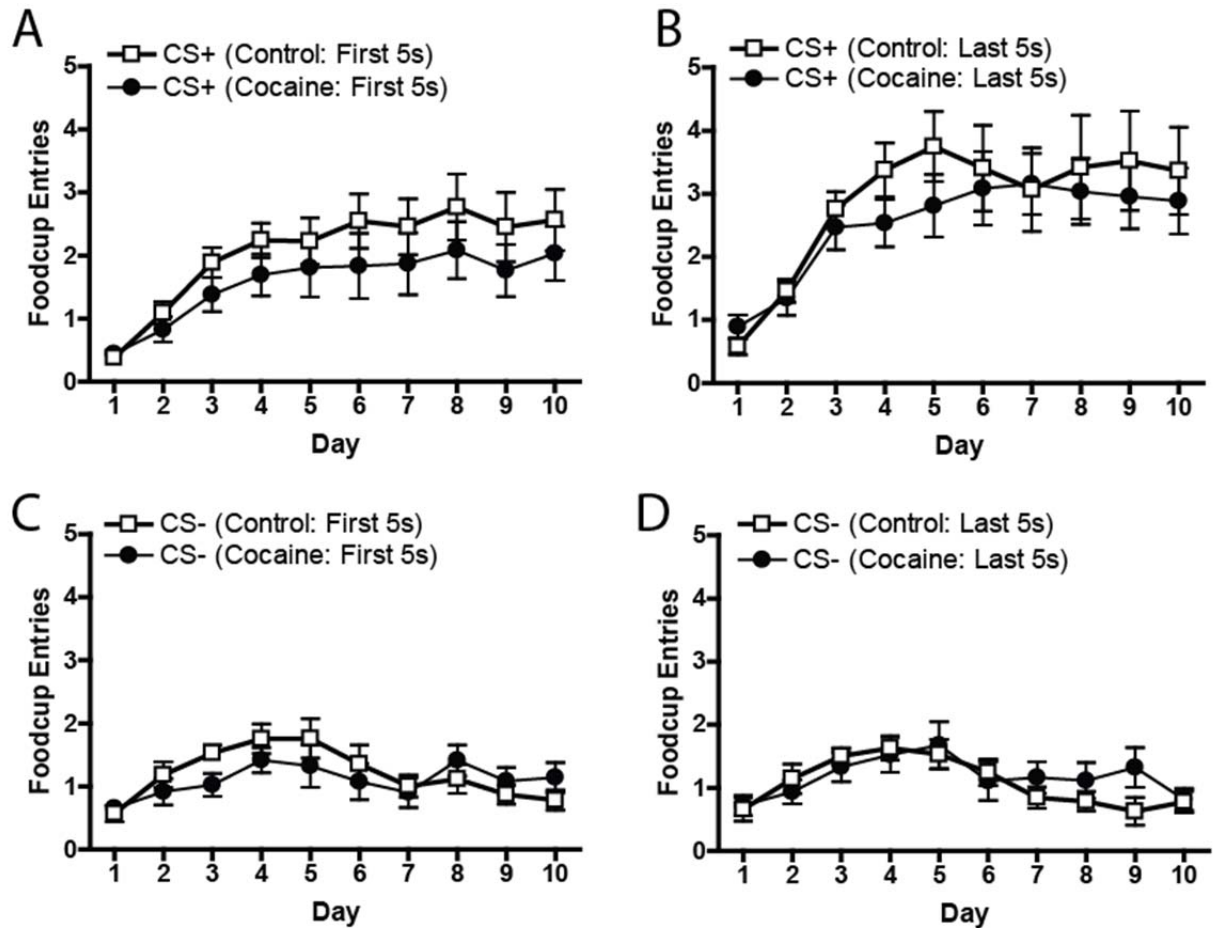

Figure S2. Previous work has suggested that during Pavlovian conditioning, rats initially spend more time approaching the cue as an autoshaped response, but closer to reward delivery shift to responding at the goal foodcup. To address this possibility, we examined foodcup behavior during the first versus second half of the cues. We found a significant interaction of cue period (first half vs second half of cue) X cue type (CS+ vs CS-), $F_{(2,34)} = 37.28$, $p < 0.0001$). Further, this effect changed as learning proceeded across days, cue period X cue type X day, $F_{(18,306)} = 2.57$, $p < 0.001$. Responding was significantly higher in the 2nd half of the CS+ presentation than the 1st half as early as day 3 ($p < 0.0001$), and persisted on each subsequent day of behavior. Indeed, by the last two days of training, foodcup entries during the first half were no longer different from baseline, while entries remained highly elevated in the 2nd half. However this analysis again found no effect of drug or any interactions of drug by any other factor.

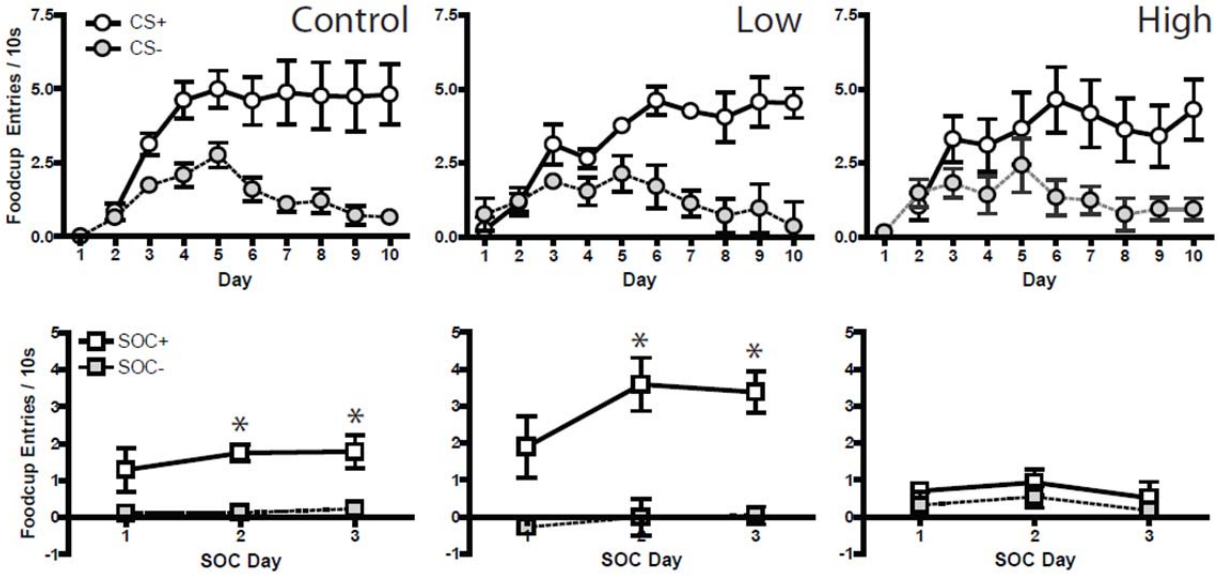

Figure S3. Behavioral performance for rats with different levels of cocaine experience. During first-order conditioning (top row), rats in all groups, regardless of drug treatment, showed successful discrimination between the CS+ and CS-. However, during second-order conditioning (bottom row), drug-naive controls and rats with low exposure (<7 d) to cocaine showed successful second-order conditioning, while rats with high cocaine experience (avg: 14 d) failed to show significant differences between SOC cues. *$p < 0.05$ vs SOC-.

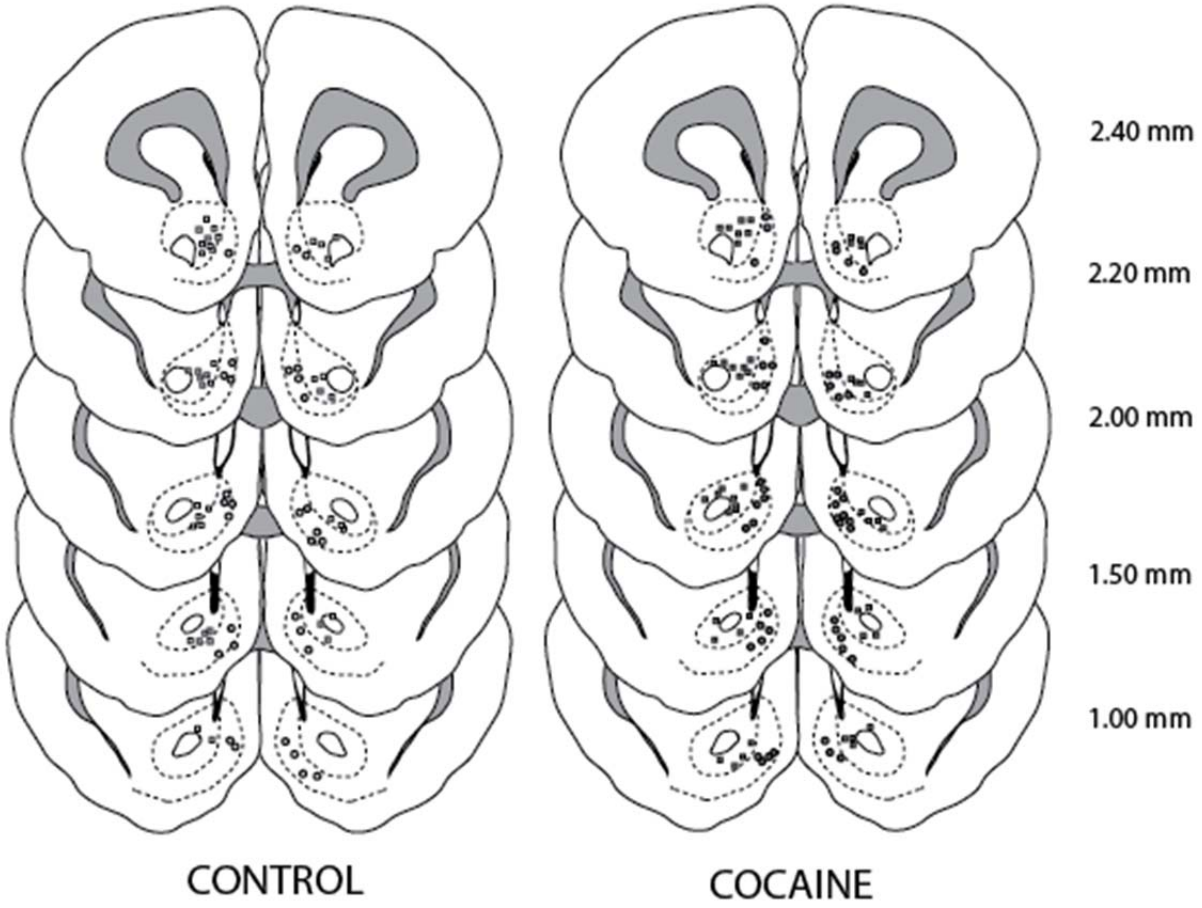

Figure S4. Histological verification of recording array wires in the nucleus accumbens core and shell. Sections on the left column show the locations of array wires from which neural data were collected from control subjects (white squares: core; white circles: shell). Sections on the right are locations of wires with neural data in cocaine-administering subjects (gray squares: core; gray circles: shell). Numbers at far right show locations of each section anterior to Bregma. Brain images and core/shell boundaries derived directly from photomicrographs of a representative subject.

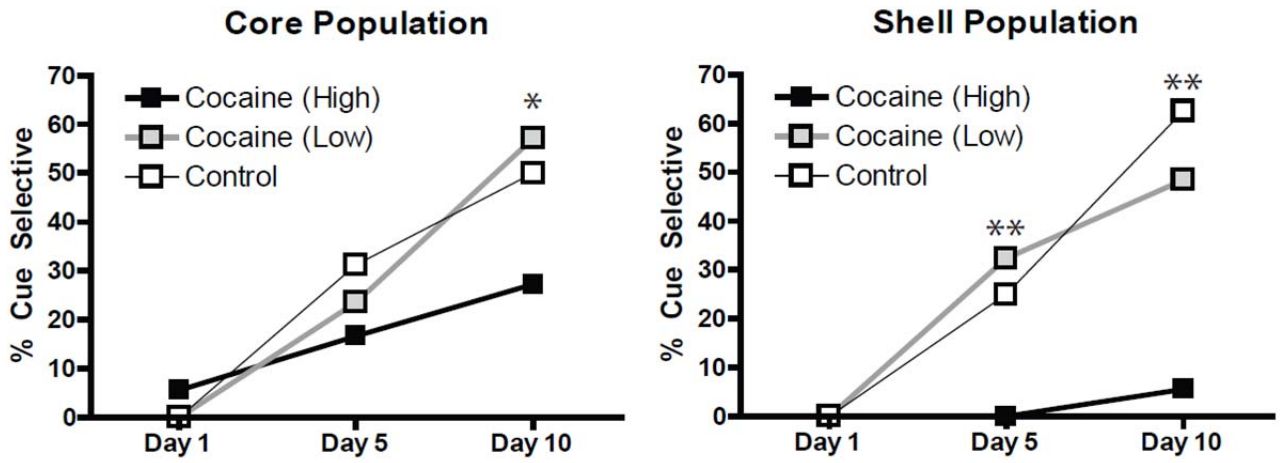

Figure S5. Population averages of cue selectivity in the core and shell of subjects. In the drug-treated groups, rats had either high exposure (avg: 14 d) or low exposure (less than 7 d) to self-administration, or controls who had none. Populations were the percent of total cue-selective neurons out of the total populations of neurons recorded that day in each group. Only the High Cocaine (but not Low Cocaine) group showed significantly decreased less cue encoding as learning progressed compared to controls, mirroring the effect seen in the subject-by-subject data. Indeed, controls and low-cocaine exposure (<7 d) rats showed nearly identical rates of cue-selective encoding in both core (day 10: $\chi^2 = 0.29$, $p = 0.59$; left) and shell neurons (day 10: $\chi^2 = 0.03$, $p = 0.86$; right), which were both greater than in the high cocaine exposure group (core day 10: $\chi^2 = 4.31$, $p = 0.038$ vs High; shell day 10: $\chi^2 = 9.035$, $p = 0.003$), again indicating the role of repeated cocaine access in this effect. Notably there were no differences between Controls and Low Cocaine groups on any day. *$p < 0.05$ Control and Low vs High; **$p < 0.01$, Control vs High, Low vs High (comparisons using χ^2).

Supplemental References

1. Carelli RM, King VC, Hampson RE, Deadwyler SA (1993): Firing patterns of nucleus accumbens neurons during cocaine self-administration in rats. *Brain Res* 626: 14-22.
2. Hollander JA, Carelli RM (2005): Abstinence from cocaine self-administration heightens neural encoding of goal-directed behaviors in the accumbens. *Neuropsychopharmacology* 30: 1464-74.
3. Saddoris MP, Stamatakis A, Carelli RM (2011): Neural correlates of Pavlovian-to-instrumental transfer in the nucleus accumbens shell are selectively potentiated following cocaine self-administration. *Eur J Neurosci* 33: 2274-87.
4. Carelli RM, Ijames SG, Crumling AJ (2000): Evidence that separate neural circuits in the nucleus accumbens encode cocaine versus "natural" (water and food) reward. *J Neurosci* 20: 4255-66.
5. Saddoris MP, Gallagher M, Schoenbaum G (2005): Rapid associative encoding in basolateral amygdala depends on connections with orbitofrontal cortex. *Neuron* 46: 321-31.
6. Wheeler RA, Twining RC, Jones JL, Slater JM, Grigson PS, Carelli RM (2008): Behavioral and electrophysiological indices of negative affect predict cocaine self-administration. *Neuron* 57: 774-85.