A Shift in the Role of Glutamatergic Signaling in the Nucleus Accumbens Core with the Development of an Addicted Phenotype

Supplemental Information

Supplementary Methods

Sexually mature male \((n = 38)\) and female \((n = 48)\) Sprague-Dawley rats, approximately 3 months old and weighing 280-310 grams (females) and 380-410 grams (males) at the start of the study, were used here. Upon arrival, rats were housed in individual operant chambers (Med-Associates, Inc., St. Albans, VT) in a temperature (20-22°C) and humidity (40-70%) controlled vivarium, and maintained on a 12-hour light/dark cycle (lights on 0700, off 1900). Rats had ad libitum access to food and water throughout the study. After an acclimation period of at least 3 days, rats were trained to lever press for sucrose pellets on a fixed-ratio 1 schedule. After two consecutive sessions during which 100 or more sucrose pellets were obtained, training was considered complete (2-3 days). The health of the rats was monitored daily, and rats were weighed three times per week. All procedures were approved by the University of Virginia Animal Care and Use Committee and were conducted in accordance with the guidelines set by the National Institutes of Health.

Surgery

Rats were anesthetized with a mixture of ketamine (40 mg/kg) and dexmedetomidine (0.2 mg/kg) and implanted with a silicone catheter into the right jugular vein and a 23-gauge bilateral stainless steel guide cannula into the nucleus accumbens (NAc) core, as previously described (1). Dummy cannulas (Plastics One, Roanoke, VA USA), were left in place throughout the
experiment to maintain patency and to prevent infection. Rats were allowed at least 5 days to recover from surgery before self-administration training began.

**Cocaine Self-Administration**

Rats were trained to self-administer cocaine under a fixed-ratio 1 schedule with a maximum of 20 infusions available per day, as previously described (1). A high dose of cocaine (1.5 mg/kg per infusion) was used to promote rapid acquisition of cocaine self-administration. Acquisition was defined as two consecutive daily sessions in which rats earned all 20 available infusions. All rats acquired rapidly under these high dose conditions (within 5 days) and rates of acquisition did not differ between groups.

Following acquisition, rats were randomly assigned to either a short or an extended access group using methods described previously (1-2). Rats in the short access group (n = 17) were maintained under the same fixed-ratio 1 schedule used for training for three additional consecutive sessions; whereas, rats in the extended access group (n = 13) were given 24-hour access to cocaine (1.5 mg/kg/infusion) for 10 days under a discrete trials procedure. Extended access self-administration trials were initiated every 15-minutes and were terminated after either a response on the left (cocaine-associated) lever or after 10 minutes had elapsed. Trials were limited to four per hour (e.g. 96 possible infusions per day) in order to prevent overdose. Responses on the right lever were recorded but not reinforced. After 10 consecutive days of the discrete trials procedure, rats were returned to the original fixed-ratio 1 schedule for 2 additional days in order to equilibrate levels of cocaine between rats prior to abstinence. At the end of both short and extended access self-administration procedures, all rats underwent 14 days of abstinence.
Progressive-Ratio (PR) Responding

Following abstinence, responding for cocaine was assessed under a PR schedule using methods previously described (1-2). A modest-to-high cocaine dose (0.5 mg/kg/infusion) was selected based on work showing that this dose enables the detection of a shift in motivation for cocaine between short and extended access self-administration (1-2). Under this schedule, the response requirement to obtain a cocaine infusion during the session increased progressively until rats no longer responded, typically within 2-3 hours. Breakpoints, a sensitive measure of motivation for cocaine and its reinforcing effects (3), were defined as the final ratio completed during each session, and were determined daily for each subject.

Extinction/Reinstatement Responding

Additional groups of rats were screened under an extinction/cue-induced reinstatement procedure following 14-days of abstinence from short ($n = 22$) versus extended ($n = 24$) access self-administration as an additional measure for the development of an addicted phenotype. This screen was conducted using a within-session extinction/reinstatement procedure consisting of a minimum of 6 1-hour extinction sessions followed by a 1-hour cue-induced reinstatement as described previously (4). Briefly, extinction sessions were separated by 5 min and continued for a minimum of 6 sessions until rats responded fewer than 15 times/session on the lever formerly associated with cocaine (generally 6 sessions). During these sessions, responding was recorded, but had no programmed consequence. The reinstatement session began 5-min after the last extinction session, and during this session, responding on the lever formerly associated with cocaine led to the delivery of the cues formerly associated with cocaine (stimulus light and the sound of the pump) under a fixed-ratio 1 schedule. These rats underwent the same experimental
procedures as described above for groups tested on PR responding following short versus extended access self-administration, except they did not undergo brain cannulation surgery.

**Site-Specific Infusions**

The effect of NAc infusions of the glutamate AMPA/KA receptor antagonist CNQX (0, 0.01, 0.03, 0.1 μg/side) were determined once PR responding had stabilized. Stability was defined as three consecutive sessions with no increasing or decreasing trend in the number of infusions obtained, and was achieved within 3-5 sessions in all rats. Doses, including a low dose expected to produce a sub-maximal effect, a moderately effective dose, and a high dose expected to produce a maximal effect on motivation for cocaine, were chosen based on previously published behavioral studies (5-6). Infusions were delivered via 1.0 ml glass Hamilton syringes housed in a KD Scientific infusion pump. The injection volume was 0.5 μl/side, administered over a period of 2 minutes, with infusion cannula left in position for an additional 2 minutes to prevent diffusion back up the cannula tract. Infusions were administered 15 minutes before the PR session, and at least 3 consecutive days of stable PR sessions separated each infusion test session. The order of dose presentation was counterbalanced across rats; however, due to challenges in maintaining catheter patency throughout this lengthy protocol, not all rats received all four doses. Each rat received at least 2 treatments and at least 7 rats were tested at each dose.

At the end of the experiment coronal brain sections (2000 μm) were stained with methylene blue and cannula placement for each subject was verified and plotted on histological reconstructions adapted from the atlas of Paxinos and Watson (7). Placement was found to lie outside the NAc core for two rats, and these rats were removed from the study and are not
included in the total numbers of subjects reported. For all other subjects, placement was within the NAc core (Figure S1).

**Sucrose Controls**

Additional male \((n = 5)\) and female \((n = 5)\) rats were trained to self-administer sucrose pellets using methods previously described (1). Briefly, responding was reinforced under on a fixed-ratio 1 schedule with training sessions run daily until at least 100 pellets were earned on 2 consecutive days. As with cocaine self-administering rats, following training, sucrose controls underwent 14 days of abstinence and were then placed on a PR schedule for sucrose pellets identical to that used for cocaine. Infusions of CNQX were administered when stable PR responding for sucrose was achieved, with stability criteria, infusions, and cannula placement procedures identical to those described above for cocaine (see Figure S1 for cannula placement). Sucrose controls were ad lib fed.

**Drugs**

Cocaine HCl, supplied by the National Institute on Drug Abuse (Research Triangle Park, NC), was dissolved to a concentration of 0.7 mg/ml in sterile heparinized saline. Cocaine solutions were delivered from syringes at a constant rate of 0.025 ml/s. The dose of cocaine/infusion was held constant across subjects while infusion duration varied according to body weight (1 s/100 g). CNQX (from Sigma Aldrich) was dissolved in water to appropriate concentrations.
Statistical Analysis

Statistical analyses were conducted using IBM SPSS Statistics (Version 20). A $t$-test was used to compare the average number of infusions obtained during the 10-day discrete trials period between extended access males versus females. Repeated measures analysis of variance (ANOVA) was used to compare the number of infusions obtained during the first 3 stable PR sessions with day as the within-subject factor and sex and access group as between subject factors. Differences in baseline PR performance on each day were analyzed using a $t$-test, controlling for family-wise error. Similar analyses were conducted to analyze the number of responses during the six 1-hour extinction sessions and to compare the number of responses during the last extinction session with those observed during the reinstatement session between access groups and sex. The effect of CNQX on PR responding for cocaine was examined by comparing the number of infusions obtained on the treatment day to baseline using repeated measures ANOVA. Baseline was determined prior to each treatment by taking the average number of cocaine infusions self-administered during the three stable PR sessions that preceded a test session. In order to control for demonstrated baseline differences between the two access groups, the effect of CNQX was also examined as percent change from baseline using univariate ANOVA. Subsequent within group and dose comparisons were analyzed using univariate ANOVA. Posthoc comparisons to vehicle/control were made using the Dunnett $t$-test, and comparisons within each dose were made using the Bonferroni $t$-test. The same analyses were used to compare the effect of CNQX on PR responding for sucrose. Statistical significance was set at $p < 0.05$. 
**Figure S1. Cannula placement verification.** Representation of coronal brain sections showing histological localization of infusion sites for rats in the (A) short access, (B) extended access and (C) sucrose control groups. Filled circles indicate individual cannula placements for females and open circles indicate placement for males. All micro-infusions were bilateral. Schematics adapted from the atlas of Paxinos and Watson (7) and reprinted with permission from Elsevier.
Supplemental References


