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

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Abstract

Background—While dopamine signaling in the nucleus accumbens (NAc) plays a well-established role in motivating cocaine use in early “non-addicted” stages, recent evidence suggests that other signaling pathways may be critical once addiction has developed. Given the importance of glutamatergic signaling in the NAc for drug-seeking and relapse, here we examined its role in motivating cocaine self-administration under conditions known to produce either a “non-addicted” or an “addicted” phenotype.

Methods—Following acquisition, male and female Sprague Dawley rats were given either short access (3 fixed-ratio 1 sessions, 20 infusions/day) or extended 24-hr access (10 days; 4 trials/hr; up to 96 infusions/day) to cocaine. Following a 14-day abstinence period, motivation for cocaine was assessed under a progressive-ratio schedule, and once stable, the effects of intra-NAc infusions of the glutamate AMPA/KA receptor antagonist CNQX (0.0, 0.01, 0.03, 0.1 µg/side) were determined. *As an additional measure for the development of an addicted phenotype, separate groups of rats were screened under an extinction/cue-induced reinstatement procedure following abstinence from short versus extended access self-administration.*

Results—Motivation for cocaine *and levels of extinction and reinstatement responding* were markedly higher following extended versus short access self-administration confirming the development of an addicted phenotype in the extended access group. CNQX dose-dependently reduced motivation for cocaine in the extended access group, but was without effect in the short access group.

Conclusions—These results suggest that the role of glutamatergic signaling in the NAc, though not essential for motivating cocaine use in “non-addicted” stages, becomes critical once addiction has developed.

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Keywords

cocaine; CNQX; extended access; nucleus accumbens; glutamate; motivation

INTRODUCTION

Despite its high prevalence, there is currently no FDA-approved treatment for cocaine addiction. Numerous pharmacotherapies have been examined, particularly antagonists of the dopamine reward system, yet despite compelling results from animal studies showing that these compounds markedly decrease cocaine self-administration (e.g., 1–4; for review see 5), their efficacy in humans has been modest and variable (6–8). It is important to note, however, that most preclinical studies have examined cocaine self-administration and its underlying neurobiology under short access conditions which results in low and stable levels of cocaine intake, and reveals dopaminergic signaling in the nucleus accumbens (NAc) as critical for motivating cocaine use (1–5,9–12). However, short access conditions do not capture critical features of cocaine addiction in humans, including compulsive use and an enhanced motivation to use the drug. Extended access procedures have been developed in animals that incorporate these characteristics (13–15), and may be useful for determining the underlying neurobiological mechanisms associated with the development of addiction. For example, rats given extended access to cocaine self-administer high levels of the drug in binge/abstinent patterns, and following abstinence, show an enhanced motivation to obtain the drug as assessed (14–15). This enhanced motivation for cocaine, as assessed under a progressive-ratio (PR) schedule, has been used to define the development of an addicted phenotype (14–15). Reinstatement *procedures* that assess levels of drug-seeking have also been used to probe for the development of an addicted phenotype (16). Importantly, the use of extended access conditions coupled with an abstinence period of 7 days or more appears to be necessary to induce this phenotype (for review see 14).

While few studies have compared the neurobiological mechanisms that motivate cocaine use following short versus extended access cocaine exposure, recent findings suggest that they are different. For example, we recently showed that while NAc dopamine D1-receptor antagonism dose-dependently reduced motivation for cocaine following short access self-administration, its effects were markedly diminished following extended access self-administration and the development of an addicted phenotype (15). These findings, together with findings showing that extended access cocaine self-administration produces a hypo-dopaminergic state in the NAc and leads to a decreased sensitivity of dopamine terminals in this brain region to cocaine (17), suggest that the role of dopaminergic signaling in motivation for cocaine becomes diminished following the development of an addicted phenotype. Although it is still possible that the role of dopamine D2-receptor signaling remains a prominent mechanism underlying motivation for cocaine with the development of an addicted phenotype (5,12,18–20), it is also possible that other signaling pathways may become increasingly recruited and drive the enhanced motivation to obtain cocaine.

One likely pathway that may underlie the enhanced motivation for cocaine characteristic of an addicted phenotype is glutamatergic signaling in the NAc. Although little information is

available on its role in motivation for cocaine, numerous studies have implicated glutamatergic signaling in cocaine-seeking and relapse vulnerability (for review see 21–23). While evidence suggests that NAc glutamatergic signaling is involved in mediating cocaine-seeking following both short and extended access self-administration (e.g., 24–27), it is possible that its role in motivating cocaine use differs between short versus extended access self-administration, with evidence suggesting that its role may increase following the development of an addicted phenotype. Specifically, results show that while acute cocaine does not alter glutamate release in the NAc, repeated administration decreases basal levels, and increases glutamate in response to subsequent cocaine (28–29). Additionally, several studies using behavioral sensitization models in which repeated cocaine injection results in increased behavioral responses to cocaine have documented changes in NAc AMPA receptors that include up-regulation in sensitized animals (30–31), and down-regulation following re-exposure to cocaine after an abstinence period (32–34). AMPA receptor signaling in the NAc has also been implicated in both cocaine and cue-induced reinstatement of cocaine-seeking following abstinence from self-administration (e.g. 35–36), and an upregulation of GluR1-containing AMPA receptors, which occurs in the core region of the NAc during prolonged abstinence from extended access cocaine self-administration, has been shown to mediate the progressive increase or “incubation” of cocaine-seeking over abstinence (23,37). Taken together, these findings suggest that the role of glutamatergic signaling, particularly AMPA-receptor signaling in the NAc, in motivating cocaine use may vary as a function of stage of the addiction process.

To address this possibility, in the present study we examined the effect of site-specific infusion of the AMPA/KA-receptor antagonist, CNQX, in the NAc on motivation for cocaine following abstinence from extended versus short access self-administration using the conditions shown to produce an “addicted” versus a “non-addicted” phenotype (15,38). As an additional measure for the development of an addicted phenotype, separate groups of rats were screened under an extinction/cue-induced reinstatement *procedure* following abstinence from short versus extended access self-administration. Based on the idea that the role of glutamate signaling in motivating cocaine use becomes more prominent with the development of addiction, we predicted that the effects of CNQX on motivation for cocaine would be more pronounced following abstinence from extended versus short access self-administration.

METHODS AND MATERIALS

Subjects

Adult male ($N=38$) and female ($N=48$) Sprague-Dawley rats were used in this study. Both males and females were included in order to increase the power of detecting a shift in the mechanisms of cocaine reinforcement by stage of addiction. Importantly, we recently showed that motivation for cocaine, measured following the same short and extended access conditions used here, does not differ between males and females (38; also see Figure 1). In order to facilitate rapid acquisition of cocaine self-administration, rats were first pre-trained to lever press for sucrose pellets using methods previously described. Further details on lever pre-training, as well as details on daily care, are provided in Supplementary Materials.

Procedures

The methods used for jugular catheterization and brain cannulation targeting the core region of the NAc were conducted as previously described (15) and are described in greater detail in Supplementary Materials. We targeted the core region of the NAc based on its important role in both early and late stage addiction (28,39), and because it is an important point of interaction between dopaminergic and glutamatergic signaling (for review see 40). The procedures used for acquisition of cocaine self-administration, short versus extended access self-administration, PR self-administration, and extinction/cue-induced reinstatement were conducted as previously described (15,38,41) and are described in greater detail in Supplementary Materials. Briefly, rats were trained to self-administer cocaine infusions (1.5 mg/kg) under a fixed-ratio 1 schedule with 20 maximum infusions/day, and once acquired, they were given either short access (3 fixed-ratio 1 sessions, 20 infusions/day; N=17) or extended 24-hr access (10 days; 4 trials/hr; up to 96 infusions/day; N=13). Motivation for cocaine (0.5 mg/kg/infusion) was assessed under a PR schedule beginning following a 14-day abstinence period. Cocaine-seeking was assessed under a within-session extinction/cue-induced reinstatement procedure in separate groups of rats following 14-days of abstinence from short (N=22) versus extended (N=24) access self-administration.

The effects of intra-NAc infusions of the glutamate AMPA/KA receptor antagonist CNQX (0.0, 0.01, 0.03, 0.1 µg/side) were determined beginning once responding under the PR schedule stabilized (defined as three consecutive sessions with no increasing or decreasing trend in the number of infusions obtained) using a within-subject design. Infusions (0.5 µl/side) were administered 15 minutes before the PR session, and at at least 3 consecutive days of stable PR sessions separated each test session. The order of dose presentation was counterbalanced across rats; however, due to challenges in maintaining catheter patency throughout this lengthy protocol, each *rat* received between 2 and 4 doses and at least 7 *rats* were tested at each dose. Further details on the procedures used for CNQX infusion, including justification for the doses selected and placement verification, are provided in the Supplementary Materials (also see Figure S1). The effect of CNQX on PR responding for sucrose was examined in separate groups of male (N=5) and female (N=5) rats using procedures similar to those used for short access cocaine self-administrations and are described in greater detail in Supplementary Materials. Data were analyzed using repeated measures ANOVA. Post-hoc comparisons to vehicle/control were made using the Dunnett t-test, and comparisons within each day/dose made using the Bonferroni corrected t-test. Statistical significance was set at $p < 0.05$. Further details are provided in Supplementary Materials.

RESULTS

Effect of Short versus Extended Access Cocaine Self-Administration on Subsequent Motivation for Cocaine *and Cocaine-Seeking*

During the 10-days of cocaine self-administration under the extended access discrete trials procedure, rats self-administered a high level of cocaine (average number of infusions were 68.9 ± 2.0 versus the set 20 infusion/day limit in the short access group), and as expected, females self-administered a greater number of infusions than did males (71.8 ± 2.0 versus

64.2±4.3, respectively; $t_{11}=4.2$, $p<0.05$). As expected, extended access rats maintained a markedly higher level of PR responding for cocaine following abstinence compared to short access controls (Figure 1; effect of access condition, $[F(1,26)=25.5, p<0.001]$), with significant differences between extended versus short access rats on all 3 PR sessions (Day 1, $t_{28}=6.7$, $p<0.001$, Day 2, $t_{28}=4.3$, $p<0.001$; Day 3, $t_{28}=4.3$, $p<0.001$). When PR responding was averaged over the three sessions, the mean number of infusions in the extended access group was 15.3±0.8 vs. 9.4±0.7 for the short access group; a difference of approximately 60%. Also as expected, males and females did not differ on levels of PR responding under either short or extended access conditions with results from the overall analysis, as well as analysis within each access group, revealing non-significant effects of sex (p 's>0.05).

Similar effects of short versus extended access cocaine self-administration were observed on levels of cocaine-seeking (Figure 2), and like the effects observed on PR responding for cocaine, the effects on extinction and reinstatement responding did not differ between males and females (p 's>0.05). *Specifically, rats in the extended access group responded at higher levels under extinction testing conditions* (Figure 2A and B; effect of access condition, $[F(1,41)=9.84, p<0.01]$), with a tendency for this effect to be most pronounced in the initial extinction sessions (session-by-access condition, $[F(1,41)=3.45, p=0.07]$). Although there was no difference between the groups during the last extinction session (Figure 2C; $p>0.05$), responding increased to a greater extent in the extended versus short access group from the last extinction session to the reinstatement session (session-by-access condition, $[F(1,41)=6.50, p<0.05]$). Subsequent analysis within the reinstatement session revealed that extended access rats responded at significantly higher levels in response to the cues formerly associated with cocaine (stimulus light and sound of pump activation) as compared to rats in the short access group (session-by-access condition, $[F(1,41)=6.57, p<0.05]$). Thus, motivation for cocaine, *as well as levels of cocaine-seeking under both extinction and reinstatement conditions*, was similar between the sexes under both short and extended access conditions, and both males and females showed a similar increase in both measures following extended access self-administration as compared to their short access counterparts. These findings confirm the development of an addicted phenotype in both males and females following abstinence from extended access self-administration.

Effect of CNQX on Motivation for Cocaine following Short versus Extended Access Self-Administration

CNQX infusion resulted in dose-dependent changes in PR responding within the extended access group, but was without effect in the short access group (Figure 3). The data are presented collapsed across males and females since the effects of CNQX were similar between the sexes under all conditions (p 's>0.05). Results from the repeated measures ANOVA comparing the number of infusions obtained on the day of CNQX treatment versus baseline revealed significant effects of day $[F(1,62)=12.1, p<0.01]$, access condition $[F(1,62)=6.3, p<0.05]$, day-by-dose $[F(3,62)=9.4, p<0.001]$, day-by-access condition $[F(1,62)=8.7, p<0.01]$, and day-by-dose-by-access condition $[F(3,62)=3.8, p<0.05]$. The average of the pretreatment baselines was significantly higher in the extended access group as compared short access group ($t_{28}=4.1$, $p<0.001$), confirming that the shift in motivation

for cocaine represents a lasting change. Although no significant effects of CNQX were observed within short access controls (Figure 3A; p 's > 0.05), analysis within the extended access group revealed a significant effect of day [$F(1,27)=7.4$, $p<0.05$], and day-by-dose [$F(3,27)=6.4$, $p<0.01$], with post-hoc comparison revealing significant decreases from baseline following the 0.03 ($p<0.05$) and 0.1 μg doses ($p<0.05$; Figure 3B). Similarly, an analysis of the effects of CNQX as percent change from baseline number of infusions revealed a significant dose-by-access condition [$F(3,62)=4.33$, $p<0.01$] (Figure 3C). Although no effect of dose was observed within the short access group ($p>0.05$), a significant effect was observed within the extended access group [$F(3,27)=6.7$, $p<0.01$], with posthoc comparison with vehicle revealing a significant effect for the 0.1 μg dose ($p<0.01$), and a trend for an effect at the 0.03 μg dose ($p=0.08$). However, a significant difference was observed for both the 0.03 and the 0.1 μg dose ($p<0.05$) in comparison to the lowest dose which tended to increase responding, though not significantly ($p>0.05$). Thus, while CNQX was without effect following short access self-administration, it dose-dependently reduced motivation for cocaine following extended access self-administration.

Effect of CNQX on Motivation for Sucrose Pellets

As a control for non-specific effects of CNQX treatment, we also measured its effect on PR responding for sucrose pellets (Figure 4). The data are presented collapsed across males and females since no difference was observed between the sexes for levels of PR responding and no differences were observed for the effects of CNQX (p 's > 0.05). The average level of baseline PR responding in sucrose self-administering *rats* was similar to that of cocaine self-administering *rats*, with levels of responding at an intermediate level between those of short and extended access cocaine groups (12.3 ± 0.9 for sucrose vs. 9.4 ± 0.7 for the short access group and 15.3 ± 0.8 for the extended access group). No significant effect of CNQX treatment on responding for sucrose was observed indicating that the observed effect of CNQX treatment was specific to PR responding for cocaine following extended access cocaine self-administration.

DISCUSSION

The goal of this study was to examine the role of glutamatergic receptor signaling in the NAc in motivation for cocaine using conditions known to produce an “addicted” versus a “non-addicted” phenotype. As expected, rats in the extended access group showed a markedly higher level of motivation for cocaine, *as well as higher levels of cocaine-seeking under both extinction and reinstatement conditions*, following abstinence as compared to short access controls thus confirming the development of an addicted phenotype in the extended access group. Notably, our results demonstrate that intra-accumbens infusion of CNQX produced differential effects on motivation for cocaine in the short versus extended access groups. While no effect of CNQX was observed in the short access group, it dose-dependently decreased motivation for cocaine in the extended access group. Similar effects were observed in both males and females indicating that the role of glutamate receptor signaling in mediating motivation for cocaine is similar between the sexes. No effect was observed on motivation for sucrose indicating that the effects of CNQX were selective for

cocaine. These data suggest that there is a shift in the role of NAc glutamatergic signaling in motivation for cocaine following the development of an addicted phenotype.

The most striking finding from this study is that intra-NAc infusion of CNQX produced differential effects in the short versus extended access groups. The increase in sensitivity to glutamate AMPA/KA receptor antagonism that we observed in extended compared to short access group is in accord with a growing number of studies indicating that neuro-adaptations in AMPA transmission may underlie the development of addiction-related behaviors (for review see 21). Our study, however, is the first to demonstrate that the role of NAc glutamatergic signaling in motivation for cocaine changes from short to extended access exposure with the development of an addicted phenotype. Motivation for cocaine, which is believed to be a sensitive and linear measures of its reinforcing effects (42), is believed to be mediated in large part via dopaminergic signaling in the NAc (5). While considerable evidence supports this conclusion for short access self-administration and during “non-addicted” stages, our results indicate that once addiction has developed, glutamatergic signaling in the NAc is critically involved. Our results also suggest that the increase in AMPA signaling that has been shown to mediate reinstatement and the incubation of cocaine craving over abstinence may also mediate motivation for cocaine following the development of an addicted phenotype.

One limitation of this study is that in addition to its effects at AMPA/KA receptors, CNQX also has antagonistic properties at the glycine site of the NMDA receptor (43–44). We therefore cannot rule out the contribution of NMDA receptors to the observed effects on motivation for cocaine. Moreover, it is possible that CNQX may act to decrease the general activation of accumbens neurons, thus reducing enhanced motivation for cocaine. Future studies using more selective glutamate receptor antagonists and other agents that enhance or inhibit synaptic transmission will be needed to address the question of the specific mechanism of action of CNQX. It should also be noted that GluR1-containing AMPA receptors are up-regulated during abstinence from extended access cocaine self-administration and down-regulated following a non-contingent cocaine challenge. This down-regulation of AMPA receptor surface expression has been shown to recover after an additional brief period of abstinence, suggesting a persistence of AMPA receptor up-regulation despite temporary decreases (39). The dynamics of AMPA receptor regulation has not been examined for the current procedure, i.e. following withdrawal from extended access and re-exposure to a contingent PR schedule. However, in addition to an increase in motivation for cocaine following abstinence, our extended access procedure, like other extended access procedures that produce up-regulation of calcium permeable AMPA receptors, results in heightened cocaine-seeking (See Figure 2). Our results are thus consistent with the idea that a persisting AMPA receptor up-regulation mediates long-lasting alterations in motivation for cocaine, and that CNQX acts on AMPA receptors to reduce motivation for cocaine.

Notably, these findings with glutamate receptor antagonism are opposite of our recent findings for the role of D1-receptor antagonism. Specifically, using the same short versus extended access *procedures* that were used here, we showed that while D1-receptor antagonism dose-dependently reduced motivation for cocaine following short access self-

administration, it was without effect following extended access self-administration (15). Together, these data suggest that there is a shift in the mechanisms underlying motivation for cocaine following the development of an addicted phenotype. While dopamine D1 receptor signaling in the NAc appears to be critical mediator of motivation for cocaine following short access self-administration, glutamate receptor signaling appears to be critical following extended access self-administration.

In summary, our findings suggest that the role of glutamatergic receptor signaling, though not essential in mediating motivation for cocaine in “non-addicted” stages, becomes critical once addiction has developed. These results have important implications for the development of treatments for addiction, and add to a growing body of literature suggesting that pharmacotherapies targeting glutamatergic transmission may be more effective than the dopamine-based therapies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Caine SB, Heinrichs SC, Coffin VL, Koob GF. Effects of the dopamine D-1 antagonist SCH 23390 microinjected into the accumbens, amygdala or striatum on cocaine self-administration in the rat. *Brain Res.* 1995; 692(1–2):47–56. [PubMed: 8548319]
2. Maldonado R, Robledo P, Chover AJ, Caine SB, Koob GF. D1 dopamine receptors in the nucleus accumbens modulate cocaine self-administration in the rat. *Pharmacol Biochem Behav.* 1993; 45(1):239–42. [PubMed: 8516365]
3. McGregor A, Roberts DC. Dopaminergic antagonism within the nucleus accumbens or the amygdala produces differential effects on the intravenous cocaine self-administration under fixed and progressive ratio schedules of reinforcement. *Brain Res.* 1993; 624:245–52. [PubMed: 8252397]
4. Robledo P, Maldonado-Lopez R, Koob GF. Role of dopamine receptors in the nucleus accumbens in the rewarding properties of cocaine. *Ann N Y Acad Sci.* 1992; 654:509–512. [PubMed: 1385935]
5. Pierce RC, Kumaresan V. The mesolimbic dopamine system: the final common pathway for the reinforcing effect of drugs of abuse? *Neurosci Biobehav Rev.* 2006; 30(2):215–38. [PubMed: 16099045]
6. Gorelick DA, Gardner EL, Xi ZX. Agents in development for the management of cocaine abuse. *Drugs.* 2004; 64(14):1547–73. [PubMed: 15233592]
7. Kleber HD. Pharmacologic treatments for heroin and cocaine dependence. *Am J Addict.* 2003; 2:S5–S18. [PubMed: 12857659]
8. Pierce RC, O'Brien CP, Kenny PJ, Vanderschuren LJ. Rational development of addiction pharmacotherapies: successes, failures, and prospects. *Cold Spring Harb Perspect Med.* 2012; 2(6):a012880. [PubMed: 22675669]
9. Roberts DC, Koob GF, Klonoff P, Fibiger HC. Extinction and recovery of cocaine self-administration following 6-hydroxydopamine lesions of the nucleus accumbens. *Pharmacol Biochem Behav.* 1980; 12:781–787. [PubMed: 7393973]

10. Di Ciano P, Coury A, Depoortere RY, Egilmez Y, Lane JD, Emmett-Oglesby MW, et al. Comparison of changes in extracellular dopamine concentrations in the nucleus accumbens during intravenous self-administration of cocaine or d-amphetamine. *Behav Pharmacol.* 1995; 6:311–322. [PubMed: 11224340]
11. Wise RA, Newton P, Leeb K, Burnette B, Pocock D, Justice JB Jr. Fluctuations in nucleus accumbens dopamine concentration during intravenous cocaine self-administration in rats. *Psychopharmacology.* 1995; 120:10–20. [PubMed: 7480530]
12. Anderson SM, Pierce RC. Cocaine-induced alterations in dopamine receptor signaling: implications for reinforcement and reinstatement. *Pharmacol Ther.* 2005; 106(3):389–403. [PubMed: 15922019]
13. Ahmed SH. The science of making drug-addicted animals. *Neuroscience.* 2012; 211:107–125. [PubMed: 21864653]
14. Roberts DC, Morgan D, Liu Y. How to make a rat addicted to cocaine. *Prog Neuropsychopharmacol Biol Psychiatry.* 2007; 31:1614–1624. [PubMed: 17888555]
15. Ramôa CP, Doyle SE, Lycas MD, Chernau AK, Lynch WJ. Diminished role of dopamine D1-receptor signaling with the development of an addicted phenotype in rats. *Bio Psychiatry.* 2013 in press.
16. Pacchioni AM, Gabriele A, See RE. Dorsal striatum mediation of cocaine-seeking after withdrawal from short or long daily access cocaine self-administration in rats. *Behav Brain Res.* 2011; 218(2): 296–300. [PubMed: 21167212]
17. Mateo Y, Lack CM, Morgan D, Roberts DC, Jones SR. Reduced dopamine terminal function and insensitivity to cocaine following cocaine binge self-administration and deprivation. *Neuropsychopharmacology.* 2005; 30:1455–1463. [PubMed: 15702135]
18. Ben-Shahar O, Keeley P, Cook M, Brake W, Joyce M, Nyffeler M, et al. Changes in levels of D1, D2, or NMDA receptors during withdrawal from brief or extended daily access to IV cocaine. *Brain Res.* 2007; 1131:220–228. [PubMed: 17161392]
19. Conrad KL, Ford K, Marinelli M, Wolf ME. Dopamine receptor expression and distribution dynamically change in the rat nucleus accumbens after withdrawal from cocaine self-administration. *Neuroscience.* 2010; 169(1):182–94. [PubMed: 20435100]
20. Park K, Volkow ND, Pan Y, Du C. Chronic cocaine dampens dopamine signaling during cocaine intoxication and unbalances D1 over D2 receptor signaling. *J Neurosci.* 2013; 33(40):15827–36. [PubMed: 24089490]
21. Pierce RC, Wolf ME. Psychostimulant-induced neuroadaptations in nucleus accumbens AMPA receptor transmission. *Cold Spring Harb Perspect Med.* 2013; 3(2):a012021. [PubMed: 23232118]
22. Kalivas PW, Volkow ND. New medications for drug addiction hiding in glutamatergic neuroplasticity. *Mol Psychiatry.* 2011; 16(10):974–86. [PubMed: 21519339]
23. Pickens CL, Airavaara M, Theberge F, Fanous S, Hope BT, Shaham Y. Neurobiology of the incubation of drug craving. *Trends Neurosci.* 2011; 34:411–420. [PubMed: 21764143]
24. Fischer KD, Houston AC, Rebec GV. Role of the major glutamate transporter GLT1 in nucleus accumbens core versus shell in cue-induced cocaine-seeking behavior. *J Neurosci.* 2013; 33(22): 9319–27. [PubMed: 23719800]
25. Ping A, Xi J, Prasad BM, Wang MH, Kruzich PJ. Contributions of nucleus accumbens core and shell GluR1 containing AMPA receptors in AMPA- and cocaine-primed reinstatement of cocaine-seeking behavior. *Brain Res.* 2008; 1215:173–82. [PubMed: 18486119]
26. Schmidt HD, Schassburger RL, Guercio LA, Pierce RC. Stimulation of mGluR5 in the accumbens shell promotes cocaine seeking by activating PKC gamma. *J Neurosci.* 2013; 33(35):14160–9. [PubMed: 23986250]
27. Torregrossa MM, Tang XC, Kalivas PW. The glutamatergic projection from the prefrontal cortex to the nucleus accumbens core is required for cocaine-induced decreases in ventral pallidal GABA. *Neurosci Lett.* 2008; 438(2):142–5. [PubMed: 18455875]
28. McFarland K, Lapish CC, Kalivas PW. Prefrontal glutamate release into the core of the nucleus accumbens mediates cocaine-induced reinstatement of drug-seeking behavior. *J Neurosci.* 2003; 23(8):3531–7. [PubMed: 12716962]

29. Miguéns M, Del Olmo N, Higuera-Matas A, Torres I, García-Lecumberri C, Ambrosio E. Glutamate and aspartate levels in the nucleus accumbens during cocaine self-administration and extinction: a time course microdialysis study. *Psychopharmacology*. 2008; 196(2):303–13. [PubMed: 17940751]
30. Boudreau AC, Wolf ME. Behavioral sensitization to cocaine is associated with increased AMPA receptor surface expression in the nucleus accumbens. *J Neurosci*. 2005; 25:9144–9151. [PubMed: 16207873]
31. Wolf ME, Ferrario CR. AMPA receptor plasticity in the nucleus accumbens after repeated exposure to cocaine. *Neurosci Biobehav Rev*. 2010; 35:185–211. [PubMed: 20109488]
32. Boudreau AC, Reimers JM, Milovanovic M, Wolf ME. Cell surface AMPA receptors in the rat nucleus accumbens increase during cocaine withdrawal but internalize after cocaine challenge in association with altered activation of mitogen-activated protein kinases. *J Neurosci*. 2007; 27:10621–10635. [PubMed: 17898233]
33. Ferrario CR, Li X, Wang X, Reimers JM, Uejima JL, Wolf ME. The role of glutamate receptor redistribution in locomotor sensitization to cocaine. *Neuropsychopharmacology*. 2010; 35:818–833. [PubMed: 19924109]
34. Thomas MJ, Beurrier C, Bonci A, Malenka RC. Long-term depression in the nucleus accumbens: A neural correlate of behavioral sensitization to cocaine. *Nat Neurosci*. 2001; 4:1217–1223. [PubMed: 11694884]
35. Backstrom P, Hyttia P. Involvement of AMPA/kainate, NMDA, and mGlu5 receptors in the nucleus accumbens core in cue-induced reinstatement of cocaine seeking in rats. *Psychopharmacology*. 2007; 192:571–580. [PubMed: 17347848]
36. Di Ciano P, Everitt BJ. Dissociable effects of antagonism of NMDA and AMPA/KA receptors in the nucleus accumbens core and shell on cocaine-seeking behavior. *Neuropsychopharmacology*. 2001; 25:341–360. [PubMed: 11522463]
37. Conrad KL, Tseng KY, Uejima JL, Reimers JM, Heng LJ, Shaham Y, et al. Formation of accumbens GluR2-lacking AMPA receptors mediates incubation of cocaine craving. *Nature*. 2008; 454:118–121. [PubMed: 18500330]
38. Ramôa CP, Doyle SE, Naim DW, Lynch WJ. Estradiol as a mechanism for sex differences in the development of an addicted phenotype following extended access cocaine self-administration. *Neuropsychopharmacology*. 2013; 38(9):1698–705. [PubMed: 23481437]
39. Ferrario CR, Gorny G, Crombag HS, Li Y, Kolb B, Robinson TE. Neural and behavioral plasticity associated with the transition from controlled to escalated cocaine use. *Biol Psychiatry*. 2005; 58:751–759. [PubMed: 16098484]
40. Kalivas PW, Volkow ND. The neural basis of addiction: A pathology of motivation and choice. *Am J Psychiatry*. 2005; 162:1403–1413. [PubMed: 16055761]
41. Lynch WJ, Piehl KB, Acosta G, Peterson AB, Hemby SE. Aerobic exercise attenuates reinstatement of cocaine-seeking behavior and associated neuroadaptations in the prefrontal cortex. *Biol Psychiatry*. 2010; 68:774–777. [PubMed: 20692647]
42. Arnold JM, Roberts DC. A critique of fixed and progressive ratio schedules used to examine the neural substrates of drug reinforcement. *Pharmacol Biochem Behav*. 1997; 57(3):441–7. [PubMed: 9218268]
43. Harris KM, Miller RJ. CNQX (6-cyano-7-nitroquinoxaline-2,3-dione) antagonizes NMDA-evoked [3H]GABA release from cultured cortical neurons via an inhibitory action at the strychnine-insensitive glycine site. *Brain Res*. 1989; 489:185–9. [PubMed: 2568152]
44. Mead AN, Stephens DN. CNQX but not NBQX prevents expression of amphetamine-induced place preference conditioning: a role for the glycine site of the NMDA receptor, but not AMPA receptors. *J Pharmacol Exp Ther*. 1999; 290:9–15. [PubMed: 10381753]

Figure 1. Effect of Short versus Extended Access Cocaine Self-Administration on Subsequent Motivation for Cocaine

Mean number ($\pm$ SEM) of cocaine infusions obtained and the corresponding final ratios completed during the first three stable PR sessions in females and males following short access (N=8 females, N=7 males) versus extended access (N=8 females, N=5 males) self-administration and a 14-day abstinence period. Asterisks indicate a significant effect of access condition ($p<0.05$).

Figure 2. Effect of Short versus Extended Access Cocaine Self-Administration on Subsequent Cocaine-Seeking

Number ($\pm$ SEM) of responses during each of the 6, 1-hr extinction sessions (A), totaled across all extinction sessions (B), and during the last extinction session versus the reinstatement session (C) in females and males following short access (N=13 females, N=9 males) versus extended access (N=12 females, N=12 males) self-administration and a 14-day abstinence period. A line/bracket and an asterisk indicate a significant effect of access condition ($p < 0.05$).

Figure 3. Effect of CNQX on Motivation for Cocaine following Short versus Extended Access Self-Administration

Mean number ($\pm$ SEM) of cocaine infusions obtained and the corresponding final ratios completed in the (A) short access versus the (B) extended access group at baseline and on the day of CNQX treatment (0, 0.01, 0.03, and 0.1 μ g/side). (C) Mean percent change from baseline in the number of infusions ($\pm$ SEM) obtained on the day of treatment for the short access versus the extended access groups. The dashed lines in panels A and B indicate average number of infusions obtained across all pre-treatment baselines, and the arrow with an asterisk indicates a significant effect of access group for these values. Asterisks in panel B represent a significant difference from pre-treatment baseline. The asterisk in panel C represents a significant difference from vehicle and the number sign indicates a significant difference from the 0.01 μ g dose. Each data point represents an N of 9–15 (short access) and 7–11 (extended access).

Figure 4. No Effect of CNQX on Motivation for Sucrose Pellets

Mean number ($\pm$ SEM) of sucrose pellets obtained and the corresponding final ratios completed at baseline (open bars) and on the day of CNQX treatment (filled bars; 0, 0.01, 0.03, and 0.1 µg/side). Each data point represents an N of 10.