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

Susan E. Doyle, Carolina Ramôa, Garrett Garber, Joshua Newman, Zeeshan Toor, and Wendy J. Lynch

Background: While dopamine signaling in the nucleus accumbens (NAc) plays a well-established role in motivating cocaine use in early nonaddicted 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 nonaddicted or an addicted phenotype.

Methods: Following acquisition, male and female Sprague Dawley rats were given either short access (three fixed-ratio 1 sessions, 20 infusions/day) or extended 24-hour access (10 days; 4 trials/hour; 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 alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionate/kainate receptor antagonist CNQX (0, .01, .03, .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-access versus extended-access self-administration.

Results: Motivation for cocaine and levels of extinction and reinstatement responding were markedly higher following extended-access 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 nonaddicted stages, becomes critical once addiction has developed.

Key Words: CNQX, cocaine, extended access, glutamate, motivation, nucleus accumbens

Despite its high prevalence, there is currently no Food and Drug Administration 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 result in low and stable levels of cocaine intake and reveal 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 (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-access 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 hypodopaminergic 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

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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-access and extended-access self-administration [e.g., (24–27)], it is possible that its role in motivating cocaine use differs between short-access 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 alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors that include upregulation in sensitized animals (30,31) and downregulation 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 glutamate receptor 1-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 kainate (KA)-receptor antagonist, CNQX (Sigma-Aldrich, St. Louis, Missouri), in the NAc on motivation for cocaine following abstinence from extended-access versus short-access self-administration using the conditions shown to produce an addicted versus a nonaddicted 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-access 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-access 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 male and female rats were included 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-access and extended-access conditions used here, does not differ between male and female rats [(38); also, see Figure 1]. To facilitate rapid acquisition of cocaine self-administration, rats were first pretrained to lever press for sucrose pellets using methods previously described. Further details on lever pretraining, as well as details on daily care, are provided in Supplement 1.

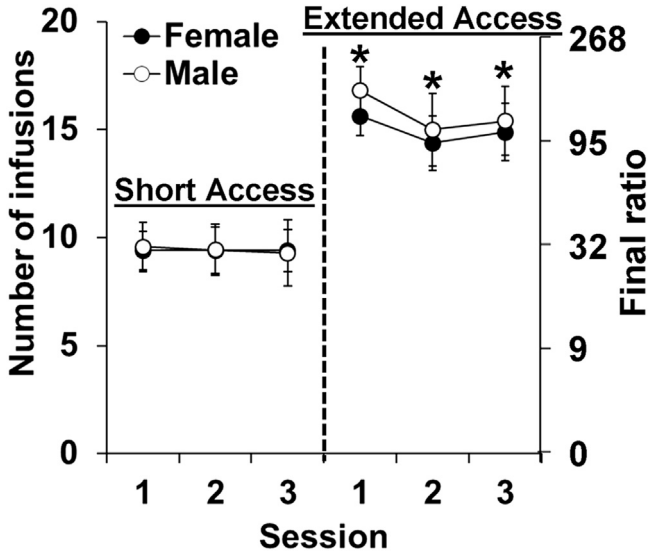

Figure 1. Effect of short-access vs. extended-access cocaine self-administration on subsequent motivation for cocaine. Mean number (±SEM) of cocaine infusions obtained and the corresponding final ratios completed during the first three stable progressive ratio sessions in female and male rats following short-access ($n = 8$ female rats, $n = 7$ male rats) vs. extended-access ($n = 8$ female rats, $n = 5$ male rats) self-administration and a 14-day abstinence period. Asterisks indicate a significant effect of access condition ($p < .05$).

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 Supplement 1. 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-access 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 Supplement 1. Briefly, rats were trained to self-administer cocaine infusions (1.5 mg/kg) under a fixed-ratio 1 schedule with 20 maximum infusions per day, and once acquired, they were given either short access (three fixed-ratio 1 sessions, 20 infusions/day; $n = 17$) or extended 24-hour access (10 days; 4 trials/hour; up to 96 infusions/day; $n = 13$). Motivation for cocaine (.5 mg/kg/infusion) was assessed under a PR schedule 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-access ($n = 22$) versus extended-access ($n = 24$) self-administration.

The effects of intra-NAc infusions of the glutamate AMPA/KA receptor antagonist CNQX (0, .01, .03, .1 μg/side) were determined once responses 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 (.5 uL/side) were administered 15 minutes before the PR session and 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 two and four doses and at least seven 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 Supplement 1 (also, see Figure S1 in Supplement 1). 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 Supplement 1. Data were analyzed using repeated measures analysis of variance. Post hoc comparisons with vehicle/control were made using the Dunnett t test, and comparisons within each day/dose were made using the Bonferroni corrected t test. Statistical significance was set at $p < .05$. Further details are provided in Supplement 1.

Results

Effect of Short-Access 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 was 68.9 ± 2.0 versus the set 20 infusions/day limit in the short-access group), and as expected, female rats self-administered a greater number of infusions than did male rats (71.8 ± 2.0 versus 64.2 ± 4.3, respectively; $t_{11} = 4.2$, $p < .05$). As expected, extended-access rats maintained a markedly higher level of PR responding for cocaine following abstinence compared with short-access control rats (Figure 1; effect of access condition, $F_{1,26} = 25.5$, $p < .001$), with significant differences between extended-access versus short-access rats on all three PR sessions (day 1, $t_{28} = 6.7$, $p < .001$; day 2, $t_{28} = 4.3$, $p < .001$; day 3, $t_{28} = 4.3$, $p < .001$). When PR responding was averaged over the three sessions, the mean number of infusions in the extended-access group was 15.3 ± .8 versus 9.4 ± .7 for the short-access group, a difference of approximately 60%. Also, as expected, male and female rats did not differ on levels of PR responding under either short-access or extended-access conditions, with results from the overall analysis, as well as analysis within each access group, revealing non-significant effects of sex ($ps > .05$).

Similar effects of short-access 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 male and female rats ($ps > .05$). Specifically, rats in the extended-access group responded at higher levels under extinction testing conditions (Figure 2A,B; effect of access condition, $F_{1,41} = 9.84$, $p < .01$), with a tendency for this effect to be most pronounced in the initial extinction sessions (session by access condition, $F_{1,41} = 3.45$, $p = .07$). Although there was no difference between the groups during the last extinction session (Figure 2C; $p > .05$), responding increased to a greater extent in the extended-access versus short-access group from the last extinction session to the reinstatement session (session by access condition, $F_{1,41} = 6.50$, $p < .05$). 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 with rats in the short-access group (session by access condition, $F_{1,41} = 6.57$, $p < .05$). 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-access and extended-access conditions, and both male and female rats showed a similar increase in both measures following extended-access self-administration as compared with their short-access counterparts. These findings confirm the development of an addicted phenotype in both male and female rats following abstinence from extended-access self-administration.

Effect of CNQX on Motivation for Cocaine Following Short-Access 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 male and female rats, since the effects of CNQX were similar between the sexes under all conditions ($ps > .05$). Results from the repeated measures analysis of variance comparing the number of infusions obtained on the day of CNQX

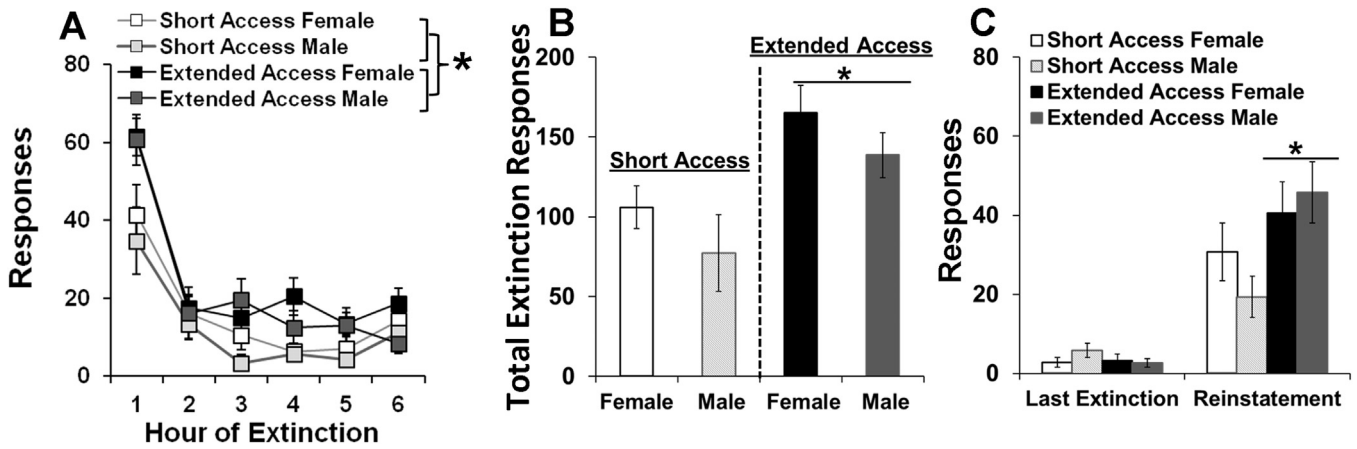

Figure 2. Effect of short-access vs. extended-access cocaine self-administration on subsequent cocaine seeking. Number (±SEM) of responses during each of the six 1-hour extinction sessions **(A)**, totaled across all extinction sessions **(B)**, and during the last extinction session vs. the reinstatement session **(C)** in female and male rats following short-access ($n = 13$ female rats, $n = 9$ male rats) vs. extended-access ($n = 12$ female rats, $n = 12$ male rats) self-administration and a 14-day abstinence period. A line/bracket and an asterisk indicate a significant effect of access condition ($p < .05$).

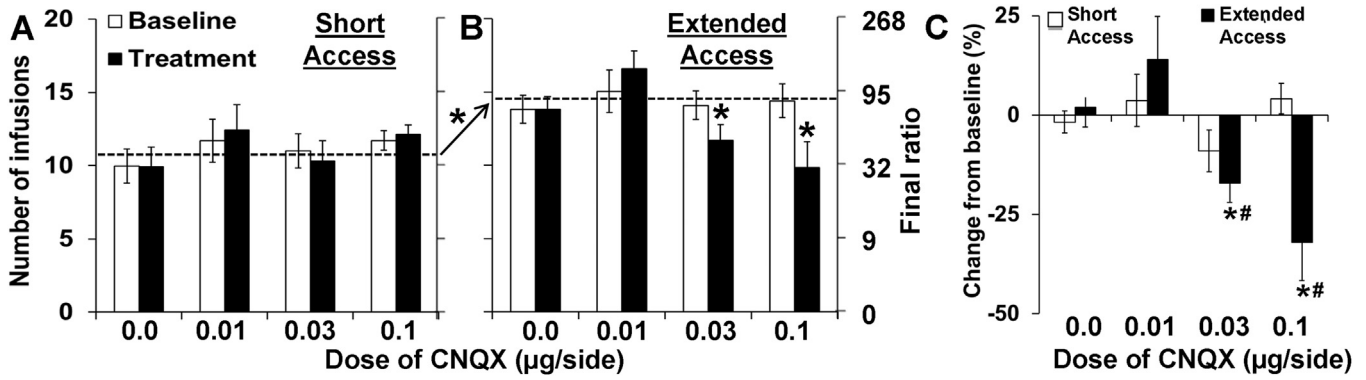

Figure 3. Effect of CNQX on motivation for cocaine following short-access vs. extended-access self-administration. Mean number (±SEM) of cocaine infusions obtained and the corresponding final ratios completed in the **(A)** short-access vs. the **(B)** extended-access group at baseline and on the day of CNQX treatment (0, .01, .03, and .1 μg/side). **(C)** Mean percent change from baseline in the number of infusions (±SEM) obtained on the day of treatment for the short-access vs. the extended-access groups. The dashed lines in panels **(A)** and **(B)** indicate average number of infusions obtained across all pretreatment 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 pretreatment baseline. The asterisk in panel **(C)** represents a significant difference from vehicle and the number sign indicates a significant difference from the .01 μg dose. Each data point represents an *n* of 9 to 15 (short access) and 7 to 11 (extended access).

treatment versus baseline revealed significant effects of day ($F_{1,62} = 12.1$, $p < .01$), access condition ($F_{1,62} = 6.3$, $p < .05$), day by dose ($F_{3,62} = 9.4$, $p < .001$), day by access condition ($F_{1,62} = 8.7$, $p < .01$), and day by dose by access condition ($F_{3,62} = 3.8$, $p < .05$). The average of the pretreatment baselines was significantly higher in the extended-access group as compared with the short-access group ($t_{28} = 4.1$, $p < .001$), confirming that the shift in motivation for cocaine represents a lasting change. Although no significant effects of CNQX were observed within short-access control rats (Figure 3A; $ps > .05$), analysis within the extended-access group revealed a significant effect of day ($F_{1,27} = 7.4$, $p < .05$) and day by dose ($F_{3,27} = 6.4$, $p < .01$), with post hoc comparison revealing significant decreases from baseline following the .03 μg and .1 μg doses ($p < .05$) (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 ($F_{3,62} = 4.33$, $p < .01$) (Figure 3C). Although no effect of dose was observed within the short-access group ($p > .05$), a significant effect was observed within the extended-access group ($F_{3,27} = 6.7$, $p < .01$), with post hoc comparison with vehicle revealing a significant effect for the .1 μg dose ($p < .01$) and a trend for an effect at the .03 μg dose ($p = .08$). However, a significant difference was observed for both the .03 μg and the .1 μg dose ($p < .05$) in comparison with the lowest dose, which tended to increase responding, though not significantly ($p > .05$). 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 nonspecific effects of CNQX treatment, we also measured its effect on PR responding for sucrose pellets (Figure 4). The data are presented collapsed across male and female rats, since no difference was observed between the sexes for levels of PR responding and no differences were observed for the effects of CNQX ($ps > .05$). 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-access and extended-access cocaine groups (12.3 ± .9 for sucrose versus 9.4 ± .7 for the short-access group and 15.3 ± .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 the 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 with short-access control rats, 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-access versus extended-access groups. While no effect of

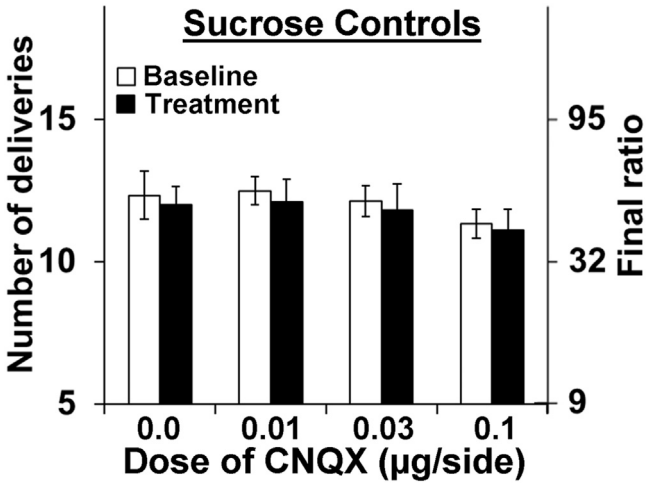

Figure 4. No effect of CNQX on motivation for sucrose pellets. Mean number (±SEM) of sucrose pellets obtained and the corresponding final ratios completed at baseline (open bars) and on the day of CNQX treatment (filled bars; 0, .01, .03, and .1 μg/side). Each data point represents an *n* of 10.

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 male and female rats, 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-access versus extended-access groups. The increase in sensitivity to glutamate AMPA/KA receptor antagonism that we observed in the extended-access compared with the short-access group is in accord with a growing number of studies indicating that neuroadaptations 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 measure 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 nonaddicted 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 *N*-methyl-D-aspartate receptor (43,44). We therefore cannot rule out the contribution of *N*-methyl-D-aspartate 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 glutamate receptor 1-containing AMPA receptors are upregulated during abstinence from extended-access cocaine self-administration and downregulated following a noncontingent cocaine challenge. This downregulation of AMPA receptor surface expression has been shown to recover after an additional brief period of abstinence, suggesting a persistence of AMPA receptor upregulation 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 upregulation of calcium permeable AMPA receptors, results in heightened cocaine seeking (Figure 2). Our results are thus consistent with the idea that a persisting AMPA receptor upregulation 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-access 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 a 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 nonaddicted 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 dopamine-based therapies.

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