

Different Adaptations in AMPA Receptor Transmission in the Nucleus Accumbens after Short vs Long Access Cocaine Self-Administration Regimens

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Ca²⁺-permeable AMPA receptors (CP-AMPA) accumulate in the nucleus accumbens (NAc) after ~1 month of withdrawal from a long-access cocaine self-administration regimen (6 h/d, 10d). This is functionally significant because CP-AMPA mediates the 'incubated' cue-induced cocaine craving produced by this regimen. Our present goal was to determine if other commonly employed cocaine self-administration regimens also elicit CP-AMPA accumulation. We compared four regimens, named according to whether sessions were short-access (ShA, 2 h) or long-access (LgA, 6 h) and the total number of sessions: LgA/10d (already shown to elicit CP-AMPA accumulation), ShA/11d, ShA/20-24d, and LgA/20-24d. In the latter regimens, rats began with 10 days of ShA and then entered a differential phase (10–14 days) in which ShA sessions either continued or switched to LgA. Controls self-administered saline. After >40 days of withdrawal, whole-cell patch-clamp recordings were performed in NAc core medium spiny neurons to assess the contribution of CP-AMPA transmission, based on the magnitude of synaptic suppression elicited by bath application of the selective CP-AMPA antagonist naspm (100 μM). Naspm produced a non-significant (~10%) attenuation of electrically evoked local excitatory postsynaptic current in the saline and ShA groups. By contrast, a significant naspm-induced synaptic attenuation (25–30%) was observed in both the LgA groups. Further analyses indicate that this emergence of CP-AMPA transmission in the LgA groups is associated with increased baseline responsiveness of MSN to excitatory drive. Together with data on cocaine infusions in each group, our results show that CP-AMPA accumulation and enhanced glutamate transmission is associated with longer sessions (6 h), rather than the number of sessions or cocaine infusions.

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INTRODUCTION

Medium spiny neurons (MSN), the output neurons of the nucleus accumbens (NAc), are excited primarily by AMPA-type glutamate receptors (AMPA). Studies in animal models have shown that AMPA transmission in the NAc is required for cocaine seeking, suggesting AMPA plasticity as a key control point for craving and relapse (Kalivas and

Volkow, 2005; Wolf and Ferrario, 2010). Many different cocaine regimens have been used to study cocaine-induced adaptations in AMPA transmission in the NAc. Broadly speaking, they can be classified as non-contingent administration (cocaine is delivered by the experimenter), short-access self-administration (ShA; multiple sessions of 1–2 h/day), or long-access self-administration (LgA; multiple sessions of 4–6 h/day). There is evidence that regimens classified in this manner lead to different adaptations in the NAc as well as different behavioral outcomes (Wolf, 2010). Given the centrality of AMPA transmission to NAc output, it would be helpful to know whether and how basic properties of AMPA transmission are altered after different types of regimens, particularly ShA regimens which have been most widely utilized.

One important way to classify AMPA is based on whether or not the tetrameric AMPA includes the GluA2 subunit. GluA2-containing AMPA are Ca²⁺-impermeable (CI-AMPA), whereas those lacking GluA2 are Ca²⁺-permeable (CP-AMPA). CP-AMPA show higher

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single channel conductance and exhibit inward rectification due to voltage-dependent block by endogenous polyamines (Isaac *et al*, 2007; Lee, 2012). In the NAc of drug-naive rats or rats treated with non-contingent (experimenter-administered) cocaine, CI-AMPA receptors account for the vast majority of excitatory synaptic transmission in the NAc (Boudreau *et al*, 2007; Kourrich *et al*, 2007; Conrad *et al*, 2008; Ghasemzadeh *et al*, 2009; Ferrario *et al*, 2010; McCutcheon *et al*, 2011b). By contrast, CP-AMPA receptors accumulate in the core and shell subregions of the adult rat NAc after ~1 month of withdrawal from a particular LgA regimen (6 h/day for 10 days) that leads to withdrawal-dependent intensification ('incubation') of cue-induced cocaine craving (Conrad *et al*, 2008; Ferrario *et al*, 2011; McCutcheon *et al*, 2011a,b; Wolf and Tseng, 2012). Importantly, studies conducted after ~6 weeks of withdrawal from this regimen showed that intra-NAc core injection of the selective CP-AMPA receptor antagonist naspam blocked the expression of incubation of cue-induced cocaine craving (Conrad *et al*, 2008). Thus, CP-AMPA receptors, which account for ~30% of evoked EPSC amplitude at this withdrawal time, mediate the expression of incubation after prolonged withdrawal (Conrad *et al*, 2008). CP-AMPA receptors also accumulate in the mouse NAc after a similar regimen in association with enhanced cocaine craving (Mameli *et al*, 2009). From the standpoint of synaptic transmission, the presence of CP-AMPA receptors is expected to have important consequences, such as providing a route for Ca^{2+} entry other than NMDARs and thus altering the 'rules' for induction of synaptic plasticity (eg, Mameli *et al*, 2011).

The goal of the present study was to determine whether CP-AMPA receptors accumulate in the NAc after withdrawal from several cocaine self-administration regimens that have been widely used in recent years. This is important for two major reasons. First, as already noted above, it will help to clarify the state of AMPA transmission in the NAc, a critical determinant of NAc output, in a broad range of published studies that have examined cocaine-induced adaptations in the NAc. Second, we are presently engaged in developing strategies to reduce CP-AMPA transmission and thereby reduce cue-induced cocaine craving (McCutcheon *et al*, 2011b; Loweth *et al*, 2013). It is important to know the circumstances in which these strategies may have utility.

We compared four regimens and named them according to duration of each session (ShA, 2 h; LgA, 6 h) and the total number of sessions. First, we used our standard incubation regimen (6 h/day for 10 days; Conrad *et al*, 2008), hereafter termed LgA/10d. We also assessed a ShA regimen that has been widely used (2 h/day for 10–12 days; eg, Moussawi *et al*, 2009; Knackstedt *et al*, 2010; Wiggins *et al*, 2011; Trantham-Davidson *et al*, 2012), hereafter termed ShA/11d. Finally, we included classical ShA and LgA regimens (Ahmed and Koob, 1998; Ahmed and Koob, 1999; Ahmed, 2011), which lead to different behavioral and cellular adaptations as addressed in the Discussion. The last two groups begin with a pre-training phase (~10 days of ShA sessions) that is designed to avoid confounds associated with differential initial learning in the groups; then the ShA group remains at 2 h/day for another 10–14 days, while the LgA group switches to 6 h/day for 10–14 days. These two groups are termed ShA/20-24d and LgA/20-24d, respectively. After >40 days of withdrawal, a period sufficient to

enable CP-AMPA receptor accumulation following our standard incubation regimen (LgA/10d), we used patch-clamp recordings to determine the contribution of CP-AMPA receptors to excitatory synaptic transmission onto MSN of the NAc core. Comparing ShA and LgA groups enabled us to determine the effect of session duration on AMPA receptor plasticity. Furthermore, the inclusion of two ShA groups with different numbers of sessions (ShA/11d and ShA/20-24d) and two LgA groups with different numbers of sessions (LgA/10d and LgA/20-24d) enabled us to determine whether the number of sessions also influences the nature of AMPA receptor plasticity.

MATERIALS AND METHODS

All experimental procedures were performed according to the USPHS Guide for Care and Use of Laboratory Animals and were approved by the Rosalind Franklin University of Medicine and Science Institutional Animal Care and Use Committee.

Animals and Cocaine Self-Administration

Our procedures for animal preparation and cocaine self-administration have been described in detail previously (Conrad *et al*, 2008; Ferrario *et al*, 2011; McCutcheon *et al*, 2011a,b). Briefly, adult male Sprague-Dawley rats (250–275 g on arrival; Harlan, Indianapolis, IN) were housed singly in standard rat cages (lights on at 1900 hours, off at 0700 hours; food and water available *ad libitum*). After a week of acclimatization to the animal colony, rats were implanted with a jugular catheter under isoflurane anesthesia. Immediately after surgery and every day after surgery, the catheters were flushed with sterile saline solution to ensure patency of the catheter. After 5–7 days of recovery, rats began self-administration training in operant chambers (MED Associates, St Albans, VT) using different regimens described in the following section. All self-administration sessions were conducted during the dark cycle. Nose-poking in the inactive hole had no consequences, whereas nose-poking in the active hole delivered an infusion of saline or cocaine (0.5 mg/kg in a 100 μ l/kg volume over 3 s), paired with a 20 s light cue inside the nose-poke hole. Catheter patency was verified by flushing with saline before and after each self-administration session.

Cocaine Regimens

Background on each cocaine regimen is provided in the Introduction and Discussion sections. Rats in the ShA/11d group self-administered cocaine for 2 h/d for 11 days. Rats in the LgA/10d group self-administered cocaine for 6 h/day for 10 days. LgA/20-24d and ShA/20-24d rats underwent an initial phase of training in which they self-administered cocaine for 10 days (2 h/day). These rats then entered a differential phase during which LgA/20-24d rats self-administered cocaine for 6 h/day for 10–14 additional days, while ShA/20-24d rats continued to self-administer cocaine for 2 h/day during that same period (approximately half of these rats had a 10-day differential phase, while the other half had a 14-day differential phase). Controls self-administered saline according to either the ShA/20-24d or LgA/20-24d regimen. We did not use yoked controls

because yoked delivery of cocaine is aversive and decreases subsequent motivation to obtain cocaine (Twining *et al*, 2009). After the last training session, rats were returned to their home cages for at least 40 days before electrophysiological recordings were performed.

Electrophysiology

All drugs and reagents were obtained from Sigma-Aldrich. Rats (459 ± 20 g at the time of recording) were taken directly from their home cages and anesthetized with chloral hydrate (400–600 mg/kg, i.p.). Brains were rapidly removed and coronal slices (300 μ m) were cut using a vibrating microtome (VT1200S; Leica, Wetzlar, Germany) in ice-cold cutting solution (in mM: 122.5 NaCl, 20 glucose, 25 NaHCO₃, 2.5 KCl, 0.5 CaCl₂, 3 MgCl₂, 1 NaH₂PO₄, 1 ascorbic acid) constantly oxygenated with 95% O₂-5% CO₂. Slices were then transferred to a warm (32–34 °C) incubation chamber containing 95% O₂-5% CO₂ oxygenated artificial CSF (aCSF; in mM: 122.5 NaCl, 20 glucose, 25 NaHCO₃, 2.5 KCl, 0.5 CaCl₂, 3 MgCl₂, 1 NaH₂PO₄, 1 ascorbic acid) for at least 1 h after slicing. For recordings, CaCl₂ was increased to 2.5 mM, MgCl₂ was reduced to 1 mM and 0.1 mM picrotoxin was added. All recordings were conducted at 32–34 °C. Patch pipettes (6–8 M Ω) were pulled from 1.5 mm borosilicate glass capillaries (World Precision Instruments, Sarasota, FL) with a horizontal puller (P-97, Sutter Instruments, Novato, CA, USA) and filled with a potassium-based solution (in mM: 140 KCl, 10 HEPES, 2 MgCl₂, 5 NaATP, 0.6 NaGTP). MSNs in the NAc core were visualized using infrared (IR)-differential interference contrast video microscopy with a 40 \times water-immersion objective (Olympus BX51-WI, Olympus America, Center Valley, PA) connected to an IR-sensitive CCD camera and displayed on a monitor. Electrophysiologically, MSNs were identified by their characteristic slow membrane potential ramp to subthreshold somatic depolarizing current steps. Whole-cell patch-clamp recordings were conducted with a computer-controlled Axo-patch 200B amplifier, digitized with Digidata 1322A at a sampling rate of 10 kHz, and acquired using pClamp 9.2 (Molecular Devices). The liquid junction potential was not corrected and electrode potentials were adjusted to zero before obtaining the whole-cell configuration. As previously reported (McCutcheon *et al*, 2011a,b), a bipolar tungsten-stimulating electrode placed \sim 300 μ m from the recording site was used to elicit EPSCs (0.2 ms pulse, every 20–30 s). The intensity of stimulation was chosen from the minimum amount of current required to elicit a synaptic response with <15% variability in amplitude during the first 10 min of recording. Only neurons that continued to exhibit this synaptic response reliability during the subsequent 15 min of baseline recording were included in the analysis. NaspM was then bath-applied for at least 10 min to neurons that met this criterion, during which time EPSCs were monitored as described above. Analyses for input–output data were conducted in cells for which a stimulation titration was performed. Briefly, current intensity of the stimulating electrode was increased in 25 μ A steps starting at 50 μ A. Following the identification of a synaptic response, stepwise titration was continued until a suitable and consistent EPSC was achieved, at least six steps (150 μ A) above rheobase.

Statistical Analysis

Data from the LgA and ShA groups were analyzed using two-way repeated-measures ANOVA with between-factor experimental groups and within-factor sessions of self-administration. In all electrophysiological recordings, naspM-induced changes in EPSC amplitude relative to baseline values were assessed using paired *t*-tests. The magnitude of naspM sensitivity across the different experimental groups was further compared using one-way ANOVA. Data are expressed as mean \pm SEM. Effects were considered significant when $p < 0.05$.

RESULTS

Rats were trained to self-administer cocaine or saline according to one of the four regimens described in Methods. All cocaine rats acquired self-administration as indicated by preference for the active hole over the inactive hole on all training days ($*p < 0.05$, *t*-tests comparing nose-pokes in active and inactive holes for each group; data not shown). The number of infusions on each training day is shown in Figure 1a–c for cocaine groups that were trained with the ShA/11d regimen, the LgA/10d (incubation) regimen, and the ShA/20-24d or LgA/20-24d regimens, respectively. Saline controls, run in parallel with the ShA/20-24d and LgA/20-24d cocaine groups, are shown in Figure 1c. Infusions during each 2 h session were similar for cocaine rats in the ShA/11d and ShA/20-24d groups, as expected (compare panels a and c in Figure 1). During the differential phase, when the LgA/20-24d group switched to 6 h/day sessions while the ShA/20-24d group remained at 2 h/day, the number of infusions increased markedly in the LgA/20-24d group (Figure 1c) to levels significantly higher than the ShA/20-24d group ($F_{(1,9)} = 101.3$, $p < 0.01$). We limited the number of sessions for LgA/20-24d rats during their differential phase (10–14 sessions) so that they would be comparable with the number of 6 h/day sessions in the Lg/10d group (10 sessions). No significant escalation of intake was observed during the differential phase for the LgA/20-24d group ($F_{(9,81)} = 1.95$, $p = 0.06$), although there was a trend towards more infusions on the final training day (two-tailed, paired *t*-test, infusions on differential day 1 *vs* differential day 10, $p = 0.08$). Similar trends were observed when only the first hour of self-administration was analyzed for the LgA/20-24d group (data not shown). The number of infusions per session was similar in the LgA/10d group and the LgA/20-24d group during its differential phase (compare panels b and c in Figure 1). The total number of infusions over all sessions for each experimental group is shown in Table 1. This is an accurate measure of cocaine intake because cocaine concentration in the syringe was the same for all the groups and delivery volume was adjusted for the weight of each rat.

After completion of the self-administration regimen, rats were returned to home cages for at least 40 days before electrophysiological analysis. This withdrawal period was selected because it is sufficient for CP-AMPA accumulation to occur in the NAc following completion of our standard incubation regimen, here termed LgA/10d (Wolf and Tseng, 2012). After withdrawal, slices were prepared and whole-cell patch-clamp recordings were conducted in

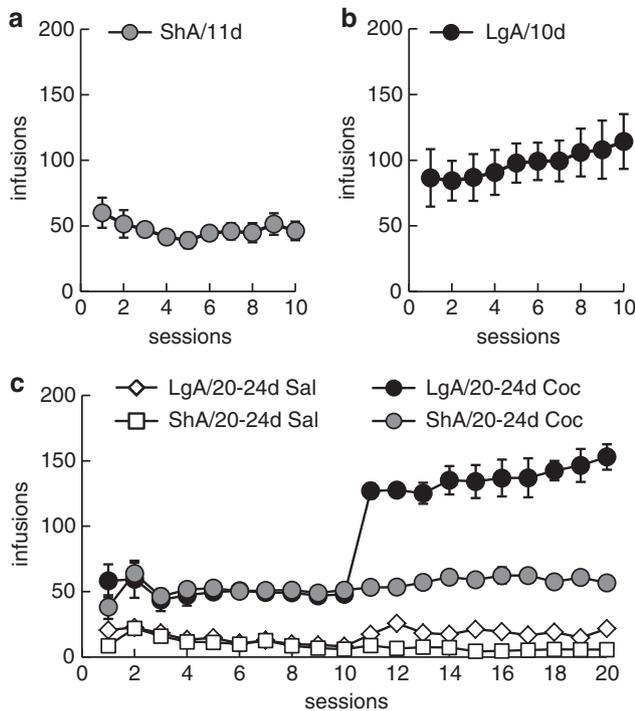


Figure 1 Rats were trained with four different self-administration regimens. Data depict mean \pm SEM number of infusions per session. For all rats receiving cocaine, the cocaine dose was 0.5 mg/kg/infusion. (a) In the ShA/11d paradigm ($n = 6$ cocaine), rats self-administered for 2 h/day over 11 days. Infusions on day 11 are not shown but were nearly identical to day 10. (b) In the incubation paradigm ($n = 7$ cocaine), rats self-administered for 6 h/day over 10 days. (c) In the LgA/20-24d ($n = 6$ cocaine, $n = 7$ saline) and ShA/20-24d ($n = 7$ cocaine, $n = 7$ saline) regimens, rats self-administered for 2 h/day over 10 days during an initial phase, and then entered a differential phase in which they were either switched to 6 h/day or maintained at 2 h/day (LgA/20-24d and ShA/20-24d, respectively). Although some rats self-administered for slightly longer than the number of days presented here (eg, about half of the LgA/20-24d rats had a differential phase lasting 14 days rather than 10 days), infusions are shown only for those training days when data for all rats in the group are available (eg, differential days 1–10 for the LgA/20-24d group).

MSN of the NAc core. In previous studies, we have shown that the LgA/10d regimen leads to CP-AMPA accumulation in all nearly MSN sampled in the core (eg, McCutcheon *et al*, 2011b), which reduces concerns about whether different populations of MSN were sampled in the different experimental groups. To compare relative levels of CP-AMPA-mediated transmission in MSN synapses from each treatment group, we measured changes in the amplitude of electrically evoked EPSC following bath application of naspam (100 μ M, 10 min). Saline controls from the ShA/20-24d and LgA/20-24d groups were combined because they did not differ. As expected from our previous studies (Conrad *et al*, 2008; Ferrario *et al*, 2011; McCutcheon *et al*, 2011a,b), naspam produced only a modest, non-significant reduction of the evoked EPSC amplitude in saline controls ($10.1 \pm 0.05\%$, $p = 0.37$; $n = 7$ cells/5 rats; Figure 2a). This appears to reflect a small population of CP-AMPA receptors based on experiments in which the effect of bath application of vehicle (aCSF alone) was examined. Over the same 10-min time-period, the rundown determined in this manner

Table 1 Total Number of Infusions Over All Self-Administration (SA) Sessions

| SA regimen | Infusions (mean \pm SEM) |
|----------------|----------------------------|
| ShA/20-24d Sal | 173 \pm 23 |
| LgA/20-24d Sal | 325 \pm 52 |
| ShA/11d Coc | 471 \pm 43 |
| ShA/20-24d Coc | 1088 \pm 58 |
| LgA/20-24d Coc | 1806 \pm 125 |
| LgA/10d Coc | 973 \pm 161 |

($5.9 \pm 0.03\%$, $p = 0.17$; $n = 6$ cells/6 rats) was somewhat smaller than the percentage of reduction produced by naspam in the saline group (Figure 2f), although the difference was not statistically significant. Similar to results in saline controls, naspam produced a non-significant reduction of EPSC amplitude in slices from rats that self-administered cocaine under ShA conditions (ShA/11d rats: $13.4 \pm 0.04\%$, $p = 0.66$; $n = 5$ cells/4 rats; ShA/20-24d rats: $12.1 \pm 0.05\%$, $p = 0.43$, $n = 7$ cells/6 rats; Figure 2b–c). By contrast, after prolonged withdrawal from the LgA/20-24d regimen, naspam produced a significant reduction of the evoked EPSC amplitude (23.8 ± 0.05 , $p < 0.05$; $n = 8$ cells/5 rats; Figure 2d) that was comparable with that observed in the LgA/10d group ($28.8 \pm 0.04\%$, $p < 0.05$; $n = 10$ cells/7 rats; Figure 2e). Thus, as summarized in Figure 2f, CP-AMPA-mediated synaptic transmission in the NAc core increased after LgA regimens but not ShA regimens.

We next determined whether the accumulation of CP-AMPA is associated with an enhanced baseline glutamatergic transmission at NAc core MSN synapses. For this purpose, we assessed the magnitude of EPSC amplitude increase in response to synaptic stimulation as a function of current input above rheobase intensity. Here, rheobase is defined as the minimal synaptic stimulation current required to elicit an EPSC. For this analysis, the LgA/10d and LgA/20-24d groups were combined as they showed a similar pattern of response across a range of stimulation intensities above rheobase (group \times intensity interaction, $F_{(2,27)} = 0.1$, $p = 0.91$, two-way ANOVA). Similarly, data from both the ShA groups were pooled as the pattern of the evoked response was indistinguishable from each other (group \times intensity interaction, $F_{(2,24)} = 0.02$, $p = 0.98$, two-way ANOVA). Overall, we found that MSNs recorded from the LgA groups ($n = 11$ cells/7 rats) exhibited an increased responsiveness to synaptic stimulation when compared with the ShA groups ($n = 10$ cells/7 rats) and saline controls ($n = 7$ cells/4 rats; main group effect $F_{(2,75)} = 8.87$, $p < 0.001$, two-way ANOVA; Figure 3). From the three ranges of current intensity tested, a marked increase in EPSC amplitude response was observed at 50 and 100 μ A above rheobase in the LgA groups. By contrast, the pattern of EPSC response obtained from the ShA groups was indistinguishable from that recorded in saline controls. Together, these results indicate that baseline glutamatergic synaptic transmission onto NAc MSNs becomes strengthened only following LgA regimens, concurrently with the accumulation of CP-AMPA.

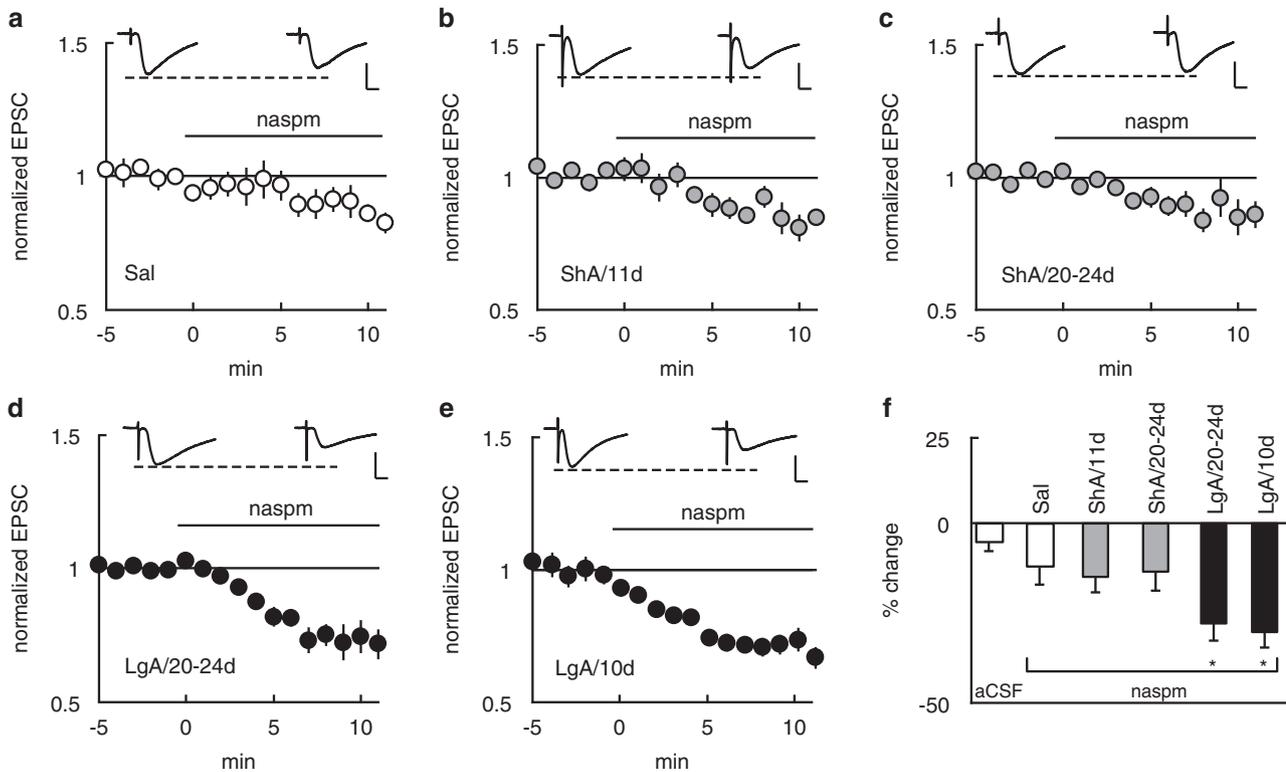


Figure 2 Naspm significantly decreases nucleus accumbens (NAc) excitatory synaptic transmission in LgA/10d (incubation) and LgA/20-24d paradigms only, indicating a significant contribution of CP-AMPA receptors to excitatory synaptic transmission after LgA but not ShA cocaine self-administration. Rats self-administered cocaine or saline as shown in Figure 1. Recordings of NAc MSN were performed after 40–79 days of withdrawal in home cages, with each experimental group sampled at both shorter and longer withdrawal times within this range. (a) Saline (Sal) controls, $n = 7$ cells (5 rats). Saline controls from ShA and LgA/20-24d groups were combined because they did not differ. (b) ShA/11d cocaine rats, $n = 5$ cells (4 rats). (c) ShA/20-24d cocaine rats, $n = 7$ cells (6 rats). (d) LgA/20-24d cocaine rats, $n = 8$ cells (5 rats). (e) LgA/10d (incubation) cocaine rats, $n = 10$ cells (7 rats). (f) Summary of percentage of reduction in evoked EPSC amplitude after naspm in the experimental groups shown in panels a–e, as well as a control group in which bath application of vehicle (aCSF without naspm) was performed at $t = 0$ min (aCSF group). * $p < 0.05$ vs baseline (t -tests), and vs aCSF, Sal, ShA/11d, ShA/20-24d groups (Fisher's LSD *post-hoc* test after significant one-way ANOVA, main treatment effect $F(5,39) = 7.23$, $P < 0.00005$).

Taken together, our behavioral and electrophysiological data indicate that session duration, rather than the number of sessions, was the important variable in determining the nature of AMPAR plasticity, as the LgA/10d and ShA/11d groups underwent approximately the same number of self-administration sessions yet only the LgA/10d group showed CP-AMPA accumulation (Figure 2). Nor did overall cocaine exposure, expressed as the total number of cocaine infusions (Table 1), appear to be a critical variable. Thus, the number of infusions was very similar in the LgA/10d and ShA/20-24d groups, yet only the LgA/10d group showed CP-AMPA accumulation. Furthermore, the ShA/20-24d group had many more infusions than the ShA/11d group, yet neither group exhibited CP-AMPA accumulation.

DISCUSSION

In the NAc of drug-naïve rats, most AMPARs are GluA1A2- or GluA2A3-containing, although a minority population of CP-AMPA receptors is detected with biochemical and electrophysiological techniques (Boudreau *et al*, 2007; Conrad *et al*, 2008; Reimers *et al*, 2011). In agreement with these previous results, we found that the selective CP-AMPA antagonist

naspm produced only a small, not statistically significant $\sim 10\%$ reduction of the evoked EPSC amplitude in MSN of the NAc core recorded after > 40 days of withdrawal from saline self-administration. Similar non-significant reductions were observed after ShA cocaine self-administration, indicating that the level of CP-AMPA-mediated synaptic transmission in the NAc core following ShA regimens is indistinguishable from saline controls. By contrast, CP-AMPA receptors accounted for $\sim 25\text{--}30\%$ of the evoked EPSC after LgA sessions (6 h/day), regardless of whether these occur as part of a classical LgA/20-24d regimen or according to the regimen typically used to elicit incubation of cocaine craving (LgA/10d). Our results in the LgA/10d group replicate our previous reports that naspm decreases evoked EPSC amplitude by $\sim 25\text{--}30\%$ after the same incubation regimen (Conrad *et al*, 2008; McCutcheon *et al*, 2011a). These results, together with data on total cocaine infusions in each group, indicate that CP-AMPA accumulation depends on longer sessions (6 h vs 2 h), rather than the total number of sessions or the total amount of cocaine intake. Furthermore, our electrophysiological analysis demonstrated that CP-AMPA accumulation was associated with an overall enhancement of MSN baseline responsiveness to excitatory stimulation.

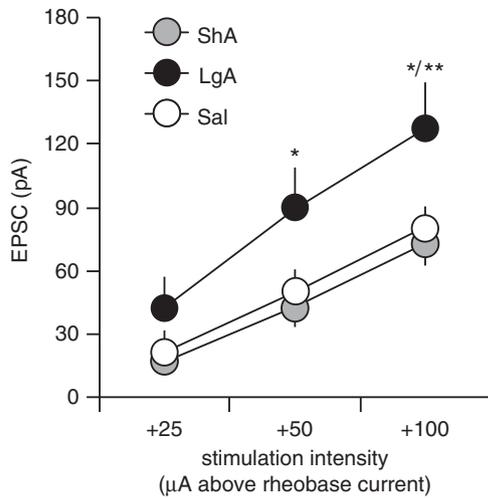


Figure 3 Baseline glutamatergic transmission at NAc excitatory synapses is enhanced after LgA regimens only. A two-way ANOVA revealed a significant main effect of group ($F_{(2,75)} = 8.87$, $p < 0.001$; LgA: $n = 11$ cells, 7 rats; ShA: $n = 10$ cells, 7 rats; saline controls: $n = 7$ cells, 4 rats). Note that the evoked EPSC amplitude was significantly enhanced in MSNs recorded from the LgA group as compared with the ShA group at $+50 \mu\text{A}$ ($*p < 0.05$, Fisher's LSD *post-hoc* test). Additionally, evoked EPSC amplitude was significantly enhanced after LgA cocaine self-administration as compared with ShA cocaine or saline self-administration at $+100 \mu\text{A}$ ($*p < 0.05$ vs saline, $**p < 0.01$ vs ShA, Fisher's LSD *post-hoc* test).

AMPA Plasticity after ShA Cocaine Self-Administration

A major finding from our study is that ShA cocaine self-administration, which has been very widely used to study cocaine-induced neuroadaptations in the NAc, does not lead to CP-AMPA accumulation. However, our negative results do not preclude upregulation of CI-AMPA after ShA cocaine self-administration. Indeed, a recent study found increased miniature EPSC amplitude and AMPA/NMDA ratios in the NAc shell after 3–4 weeks, but not 1–2 days, of abstinence from a ShA regimen (Ortinski *et al*, 2012). In light of our findings, it seems most likely that these were CI-AMPA, although an important caveat is that our studies were performed in NAc core and at a significantly longer withdrawal time (40–79 days). However, there is an indirect support for AMPA upregulation in the core after ShA cocaine self-administration. Thus, long-term potentiation (LTP) but not long-term depression (LTD) was impaired in the NAc core on withdrawal day 21 after a ShA regimen (Knackstedt *et al*, 2010). This could reflect occlusion of LTP by previous AMPA upregulation, although there are many caveats associated with inferring AMPA levels from changes in the ability to elicit LTP or LTD (Wolf, 2010; Wolf and Ferrario, 2010). Regardless of whether CI-AMPA upregulation occurs in NAc core after 3–4 weeks abstinence, it may not be present at the later withdrawals examined here, based on the fact that input-output studies in Figure 3 did not reveal a difference between the ShA and saline groups. However, we cannot rule out the possibility of concurrent adaptations in the ShA groups that masked the consequences of CI-AMPA upregulation.

Why does ShA cocaine self-administration fail to increase CP-AMPA-mediated synaptic transmission? An obvious

difference between ShA and LgA regimens is the total amount of cocaine intake (see Table 1). However, as noted in the introductory paragraph of the Discussion, this explanation is not supported by our data, as the LgA/10d and the ShA/20–24d groups had very similar numbers of cocaine infusions but only the former exhibited CP-AMPA accumulation. Thus, in the present study, the duration of each self-administration session was the most important factor in determining the nature of AMPA plasticity. Presumably, the longer activation of monoamine receptors that results from a longer duration session leads to more persistent activation of signaling pathways. There are many ways that this could be envisioned to ‘flip a switch’ (ie, CP-AMPA accumulates, drug seeking becomes compulsive, and so on); for example, persistent signaling pathway activation may be more likely to alter gene expression. It should be noted that the voluntary and learning aspects of cocaine self-administration are also important as we have shown that non-contingent cocaine exposure does not lead to CP-AMPA accumulation in the NAc of adult rats (McCutcheon *et al*, 2011b).

Functional Significance of CP-AMPA Accumulation after LgA Regimens

The functional role of CP-AMPA has already been studied in the LgA/10d regimen that leads to incubation of cocaine craving. Incubation describes the progressive intensification of cue-induced craving during abstinence, measured as responding on the previously active lever or hole during an extinction test. Craving begins to incubate during the first week of withdrawal, increases progressively for ~2 months, and remains elevated even 6 months after the last self-administration session (Lu *et al*, 2004; Pickens *et al*, 2011). By contrast, the rise in CP-AMPA levels is first detected after 3–4 weeks of withdrawal (Wolf and Tseng, 2012), and the ability of naspam to block incubated cocaine craving was demonstrated on withdrawal day 45 (Conrad *et al*, 2008). Putting these findings together, other neuroadaptations must account for the initial phase of incubation, which is not surprising given that incubation involves multiple brain regions and cellular changes (Pickens *et al*, 2011). However, once CP-AMPA are in place, their stimulation is required for the expression of incubation (Conrad *et al*, 2008). We have hypothesized that the synaptic incorporation of CP-AMPA enhances the responsiveness of NAc neurons to glutamate inputs from cortical and limbic regions, due to their larger single channel conductance (Conrad *et al*, 2008; Wolf and Tseng, 2012). Support for this hypothesis is provided by the present input-output studies showing enhanced evoked EPSC amplitude in MSN from the LgA groups but not the ShA groups compared with saline controls. In addition, the Ca^{2+} permeability of CP-AMPA is likely to qualitatively alter AMPA signaling within the MSN. Thus, when cocaine-associated cues are presented after prolonged withdrawal from cocaine, and glutamate is released in the NAc, our results predict that the MSN will respond more robustly, leading to ‘incubated’ cue-induced cocaine seeking. The failure of ShA regimens to elicit CP-AMPA accumulation may explain why cue-induced seeking is greater in LgA than in ShA rats in tests conducted after 30–47 days of withdrawal (Lu *et al*, 2004; Ferrario *et al*, 2005).

Is there any relationship of CP-AMPA receptors to other behavioral changes that follow LgA cocaine self-administration? It is well established that animals with LgA cocaine exposure demonstrate addiction-like behaviors not present in ShA animals, namely: (1) increased motivation for cocaine, (2) persistence of drug self-administration despite adverse consequences, and (3) augmented cocaine-, stress-, or cue-induced reinstatement following a period of extinction training (eg, Ahmed and Koob, 1998; Ahmed and Koob, 1999; Paterson and Markou, 2003; Deroche-Gamonet *et al*, 2004; Mantsch *et al*, 2004; Vanderschuren and Everitt, 2004; Ferrario *et al*, 2005; Kippin *et al*, 2006; Knackstedt and Kalivas, 2007; Mantsch *et al*, 2008; Ben-Shahar *et al*, 2008; reviewed by Ahmed, 2011). In most instances, these augmented behaviors were demonstrated within the first week or two after discontinuing LgA cocaine self-administration, a time period when CP-AMPA receptor levels have not yet increased (Wolf and Tseng, 2012). Thus, CP-AMPA receptors cannot be responsible for these augmented behaviors. Instead, it is more reasonable to propose that animals that develop these augmented behaviors subsequently exhibit elevated CP-AMPA receptor levels. Once this occurs, the CP-AMPA receptors, which we have already demonstrated to underlie incubated cue-induced cocaine seeking (Conrad *et al*, 2008), may also contribute to other delayed behavioral changes that follow LgA cocaine self-administration. For example, after 30 days of abstinence, rats resume cocaine taking at an even higher level than occurred during their previous LgA training sessions (Ahmed and Koob, 1998). This could be related to increased activation of MSN due to the presence of CP-AMPA receptors, as discussed in the previous paragraph. It will be important to further characterize long-term behavioral consequences of LgA cocaine self-administration and the role of AMPA receptor plasticity in the NAc and other regions in such consequences.

Absence of Significant Escalation in LgA/20-24d and LgA/11d Cocaine Groups

Escalation to heavy drug use is a core diagnostic criterion for addiction; likewise, escalation of intake occurs in animals during LgA cocaine self-administration and is generally associated with other behaviors (see Discussion in the previous section) that model compulsive drug seeking and taking (Ahmed, 2011). We did not observe statistically significant escalation of cocaine intake in our LgA/20-24d group, which experienced 10–14 LgA sessions. In fact, while escalation is a reliable phenomenon under many conditions, there are other studies that have failed to detect it (Ahmed, 2011). Based on previous results (Mantsch *et al*, 2004; Kippin *et al*, 2006), it is likely that our use of Sprague-Dawley rats and a relatively low cocaine dose contributed to minimal escalation in our study. The timing of escalation also varies between studies. Sometimes, it is detected over the first 3–5 sessions (eg, Figure 1 of Ahmed and Koob, 1998; Figure 2 of Ben-Shahar *et al*, 2009), but it can require more sessions (19 sessions in Figure 3 of Ahmed and Koob, 1998). There are many procedural differences between these studies and ours; a particularly relevant difference may be that rats lever-pressed for cocaine in the previous studies, whereas we trained rats to nose-poke for cocaine. Nose-pokes are easier

to learn, which may work against the detection of escalation during early self-administration sessions.

We also failed to observe escalation in our LgA/10d group. We have run many identical groups over the past 5 years in our studies of incubation of cocaine craving (Conrad *et al*, 2008; Ferrario *et al*, 2011; McCutcheon *et al*, 2011a,b; additional unpublished studies). Sometimes, we observed escalation in the course of 10 sessions (eg, Ferrario *et al*, 2011). However, sometimes we and others have failed to observe escalation with this regimen (eg, Conrad *et al*, 2008; Knackstedt and Kalivas, 2007). Yet all incubation rats exhibit CP-AMPA receptors in the NAc core when analyzed after a month or so of withdrawal. Therefore, we conclude that LgA cocaine exposure can result in incubation of cocaine craving and synaptic accumulation of CP-AMPA receptors independent of whether we detect escalation over the course of 10 training sessions. Previous studies have shown that escalation can be dissociated from augmented cocaine- and cue-induced reinstatement after LgA cocaine self-administration (Kippin *et al*, 2006; Knackstedt and Kalivas, 2007), and compulsive drug seeking (eg, persistence despite aversive consequences) has been demonstrated after different types of LgA regimens where escalation was not tested (Deroche-Gamonet *et al*, 2004; Vanderschuren and Everitt, 2004). Together, these findings underscore the complexity of the relationships between different behavioral adaptations observed following different cocaine regimens.

Therapeutic Implications

In rats, the cocaine exposure required for the synaptic accumulation of CP-AMPA receptors in NAc synapses is not extreme (6 h/day \times 10 days and \sim 1 month of withdrawal). If a similar phenomenon occurs in humans, it may become important in situations where drug use is terminated for a month or two due to hospitalization or incarceration (Reichel and Bevins, 2009). After this period of abstinence, new CP-AMPA receptors could increase the likelihood of relapse by mediating intensified cue-induced cocaine craving. We are presently exploring mGluR1-based strategies for removing CP-AMPA receptors from NAc synapses and reducing cue-induced cocaine craving (McCutcheon *et al*, 2011a; Loweth *et al*, 2013).

Studies of incubation provide insight specifically into how to counteract cue-induced craving after prolonged withdrawal. Other regimens, including ShA regimens that do not lead to CP-AMPA receptor accumulation, provide insight into strategies for decreasing addictive behavior under different conditions. The most striking example concerns the drug N-acetylcysteine, which reduces cocaine-related behaviors after non-contingent cocaine exposure and ShA cocaine self-administration and has shown promise in clinical trials (Mardikian *et al*, 2007; Amen *et al*, 2011; Olive *et al*, 2012).

CONCLUSIONS

A significant increase in CP-AMPA receptor levels was exhibited by MSN in the NAc core of rats after $>$ 40 days of withdrawal from LgA (6 h) cocaine self-administration sessions but not after ShA (2 h) sessions. Based on earlier work showing that 3–4 weeks of withdrawal is required for CP-AMPA receptor accumulation, these high conductance AMPA receptors are likely

to mediate delayed behavioral changes that follow LgA cocaine self-administration. Furthermore, the accumulation of CP-AMPA receptors (detected with naspm) was associated with enhanced baseline responsiveness of MSN to excitatory stimulation (detected by analysis of input-output relationships). By determining the experimental conditions that lead to CP-AMPA accumulation, our results help evaluate the conditions under which CP-AMPA-based therapeutic intervention might be valuable. Furthermore, our results enable conclusions to be drawn about the state of AMPAR transmission in the NAc in many studies in the literature that utilize the ShA and LgA models evaluated herein.

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DISCLOSURE

The authors declare no conflict of interest.

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