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Cocaine experience enhances thalamo-accumbens N-methyl-D-aspartate receptor function

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Abstract

BACKGROUND—Excitatory synaptic transmission in the nucleus accumbens (NAc) is a key biological substrate underlying behavioral responses to psychostimulants and susceptibility to relapse. Recent studies have demonstrated that cocaine induces changes in glutamatergic signaling at distinct inputs to the NAc. However, consequences of cocaine experience on synaptic transmission from the midline nuclei of the thalamus (mThal) to the NAc have yet to be reported.

METHODS—To examine synapses from specific NAc core inputs, we recorded light-evoked excitatory postsynaptic currents (EPSCs) following viral-mediated expression of channelrhodopsin-2 in the mThal, prefrontal cortex (PFC), or basolateral amygdala (BLA) from acute brain slices. To identify NAc medium spiny neuron (MSN) subtypes we utilized mice expressing tdTomato driven by the promoter for the dopamine receptor subtype 1 (D1). We recorded N-methyl-D-aspartate receptor (NMDAR) and α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPA) properties to evaluate synaptic adaptations induced by cocaine experience, a 5-day cocaine exposure followed by 2-weeks of abstinence.

RESULTS—We determine that excitatory inputs to the NAc core display differential NMDAR properties and cocaine experience uniquely alters AMPAR and NMDAR properties at mThal-D1(+), mThal-D1(–), and PFC-D1(+) synapses, but not PFC-D1(–) synapses. Finally, at mThal-D1(+) synapses, we demonstrate that cocaine enhances GluN2C/D function and NMDAR-dependent synaptic plasticity.

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CONCLUSIONS—Our results identify contrasting cocaine-induced AMPAR and NMDAR modifications at mThal- and PFC-NAc core synapses. These changes include an enhancement of NMDAR function and plasticity at mThal-D1(+) synapses. Incorporation of GluN2C/D-containing NMDARs most likely underlies these phenomena and represents a potential therapeutic target for psychostimulant use disorders.

Keywords

Addiction; Cocaine; Nucleus accumbens; NMDAR; Prefrontal cortex; Thalamus

INTRODUCTION

Addressing the persistent vulnerability of relapse to drug-seeking behavior remains an essential obstacle impeding the treatment of substance abuse disorders (1). The long-lasting urges and maladaptive behaviors conditioned by cocaine use are associated with changes in excitatory transmission throughout the mesolimbic dopamine system, including within the nucleus accumbens (NAc) (2; 3). Recent studies have supported the hypothesis that alterations in NAc α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPA) signaling are essential towards promoting maladaptive drug-associated behaviors (4–7).

Seminal findings by Thomas et al (8) demonstrated that AMPAR function in the NAc shell is altered by cocaine history. Since then, we and others have demonstrated circuit-specific, experience-dependent changes in NAc synaptic function by utilizing reporter mouse lines that label neuron populations and optogenetic approaches to drive specific excitatory afferents. The output neurons of the NAc, medium spiny neurons (MSNs), are largely distinguished by expression of dopamine receptor subtype 1 or 2 (D1 or D2). While both D1 (4; 7; 9–13) and D2 (10; 11; 14) NAc MSN subtypes have been shown to undergo drug-induced changes in AMPAR function, these changes occur in an experience-, subregion-, and input-specific manner. In contrast to AMPARs, drug-induced changes in NAc N-methyl-D-aspartate receptor (NMDAR) function have been less well documented. Following a short-term abstinence from cocaine exposure Huang et al, and others, have reported a transient increase in the expression of silent synapses (5; 6; 15–17), synapses that exhibit robust NMDAR-mediated excitatory postsynaptic currents (NMDAR EPSCs) but undetectable AMPAR-mediated EPSCs (AMPA EPSCs) (18; 19). These silent synapses are thought to be due in large part to the insertion of NMDARs containing the GluN2B subunit. Conversely, at 14 days following chronic amphetamine, decreased expression of GluN2B-containing NMDARs has been reported (20). Also, longer-lasting increases in NAc hyperpolarization-active GluN2C-containing NMDARs are implicated in alcohol seeking (21; 22), but their role in cocaine-related experience is unclear. Since NMDAR subunit composition can have a profound effect on synaptic function, the molecular stoichiometry of NMDARs in drug- and synapse-specific models warrants further investigation.

The NAc core and shell receive excitatory input from the prefrontal cortex (PFC), basolateral amygdala (BLA), and the ventral subiculum of the hippocampus (vSub) (23–25). Dysregulated plasticity of AMPARs has been identified as a substrate that promotes reward-

driven and addiction-like behaviors at NAc synapses from each of these inputs (4–7; 24). Projections from midline thalamic (mThal) nuclei also innervate the NAc (26; 27), yet whether drug experience alters these synapses remains to be shown.

The thalamus is described as a sensorimotor-cortical relay, and has received relatively little attention with regards to its capacity to modulate complex affective and cognitive behaviors (27). The thalamic paraventricular nucleus (PV) and paratenial nucleus (PT) receive inputs from multiple brain regions implicated in addiction-related behavior and project to the NAc (26; 28; 29). Thus the mThal nuclei are anatomically positioned to provide essential coordination of learned drug-induced behaviors. Indeed, lesion or pharmacological inhibition of the PV has been demonstrated to disrupt NAc-dependent behaviors including reinstatement of cocaine-seeking and expression of cocaine conditioned place preference (30–33). Therefore, to begin to address the role of mThal-NAc pathways in drug-related behaviors, we sought to test the hypothesis that cocaine exposure alters mThal-NAc AMPAR and NMDAR function.

To better understand mThal inputs to NAc core we compared AMPAR and NMDAR properties at mThal, PFC, and BLA synapses. We expressed channelrhodopsin-2 (ChR2) in bacterial artificial chromosome (BAC) transgenic D1-tdTomato mice and performed targeted, input-specific, whole-cell voltage-clamp recordings in acute slices of the NAc core. Synaptic NMDAR/AMPA ratios differed based on glutamatergic input. At mThal-D1(+) synapses, cocaine experience, defined as abstinence from 5 days of cocaine exposure, enhanced AMPAR function and altered NMDAR properties, while at mThal-D1(–) synapses the cocaine-induced changes were consistent with generation of silent synapses. In contrast to mThal-NAc synapses, inputs from the PFC to NAc core D1(+) MSNs, but not D1(–) MSNs, exhibited larger NMDAR/AMPA ratios following cocaine experience. Finally, we found that cocaine experience enhanced GluN2C/D function and unmasked NMDAR-dependent long-term depression (LTD) at mThal-D1(+) synapses. These results suggest that cocaine experience generates changes in NMDAR function at mThal-NAc core synapses and identifies these adaptations as targets with therapeutic potential for the treatment of psychiatric disorders associated with dysfunction of the reward system.

METHODS

Mice

Male BAC transgenic mice (8–11 weeks old) expressing tdTomato under the control of the *Drd1a* promoter (34; 35) were housed together in groups of 2–5 per cage on a 12/12-h light/dark cycle. Mice were used in accordance with the policies approved by the Institutional Animal Care and Use Committees (IACUC) at Vanderbilt University.

Stereotaxic injections

At 4–5 weeks of age, mice underwent stereotaxic surgery for viral-mediated gene transfer of channelrhodopsin-2 (ChR2). Mice were anesthetized with dexmetomidine-ketamine (0.5–80 mg/kg, intraperitoneal: *i.p.*). 250–400 nL injections of AAV5-CaMKII-ChR2-EYFP (UNC Viral Core) were delivered bilaterally as follows (relative to bregma): mThal [ML:

± 0.3 , AP: -1.2 , DV: -3.0], PFC [ML: ± 0.3 , AP: -1.75 , DV: -2.75], BLA [ML: ± 3.1 , AP: -1.25 , DV: -4.75]. Atipamazole (1.0 mg/kg, subcutaneous: *s.c.*) and ketoprofen (10 mg/kg, *s.c.*) were administered after surgery. Mice were sacrificed for electrophysiology 4–6 weeks following surgery.

Cocaine conditioning

Conditioning sessions began 2–3 weeks following surgery during the day phase of the light cycle. Following 2 habituation days, mice underwent 5 daily *i.p.* injections with cocaine (15 mg/kg) or saline (0.9%) (8; 14; 36). Injections were administered immediately prior to placing the mouse in an open field activity chamber (ENV-510, Med Associates). Overhead video recordings of mouse activity were analyzed with automated software (EthoVision XT, Noldus).

Electrophysiology

Parasagittal slices (250 μm) containing the NAc core were prepared from naïve mice or 10–14 days following the last cocaine/saline injection. Whole-cell voltage-clamp recordings and data analysis were performed as described (37). Briefly, D1(+) MSNs were identified visually by the presence of tdTomato. Neurons lacking any detectable tdTomato signal were defined as D1(–) MSNs. ChR2-dependent synaptic activity was driven using 470 nm LED illumination through the objective for 1–3 ms (pE-2, CoolLED). All recordings were made using a cesium-based internal solution and in the presence of picrotoxin. More detailed descriptions can be found in the supplemental information.

Data analysis

Data are expressed as mean $\pm$ SEM (N = number of animals; n = number of cells). For electrophysiology each data point represents the average from one cell. ANOVA or two-tailed Student's *t*-test were used. Post-tests employed Bonferroni corrections and $p < 0.05$ was considered significant.

Drugs

Cocaine HCl, picrotoxin, and d-cycloserine were purchased from Sigma-Aldrich. NBQX and D-AP5 were purchased from Tocris. CIQ was purchased from Brandt Labs.

RESULTS

Basal circuit-specific AMPAR and NMDAR properties of NAc core MSNs

To characterize synaptic AMPAR and NMDAR properties in the NAc core in a circuit-specific manner, we virally expressed ChR2 in the mThal, PFC, or BLA of D1-tdTomato marker mice. After 3 weeks robust expression of ChR2 was observed at the injection site and in the NAc (Figure 1A). Utilizing visualized whole-cell voltage-clamp techniques, we recorded light-evoked EPSCs at D1(+) and D1(–) MSNs. To compare AMPAR function across NAc core inputs, we elicited asynchronous EPSCs (asEPSCs) in low- Ca^{2+} aCSF supplemented with Sr^{2+} . asEPSCs are quantal-like events that reflect synaptic expression of AMPARs. The asEPSC amplitudes were similar across inputs onto D1(+) (Figure 1B1) and

D1(−) (Figure 1B2) MSNs. To better understand mThal-NAc core synaptic composition, we compared NMDAR/AMPA ratios to those recorded following PFC and BLA stimulation. The NMDAR-mediated component was recorded at 50 ms post onset (when the AMPAR component is minimal) and normalized to the AMPAR EPSC peak amplitude for each cell (14; 38). mThal synapses on D1(+) and D1(−) cell types exhibited smaller NMDAR/AMPA ratios relative to the PFC (mThal-D1(+): 0.325 ± 0.029 vs. PFC-D1(+): 0.564 ± 0.066 , $p < 0.01$, Figure 1C1; mThal-D1(−): 0.322 ± 0.029 vs. PFC-D1(−): 0.576 ± 0.042 , $p < 0.001$, Figure 1C2). D1(−) synapses from the BLA also exhibited smaller NMDAR/AMPA ratios relative to the PFC (BLA-D1(−): 0.372 ± 0.043 , $p < 0.01$).

As mentioned above, silent synapses are putative substrates for long-term potentiation that are characterized by the functional presence of NMDARs but not AMPARs (18; 19). Silent synapses are abundant in the developing brain, and recent work has demonstrated that cocaine exposure transiently increases silent synapses in the NAc (5; 6; 15–17). Therefore, we analyzed the ratio of the coefficient of variation of the NMDAR component relative to the AMPAR response (CV-N/CV-A), a measure inversely related to the proportion of silent synapses (15; 39). We found no difference in CV-N/CV-A across inputs onto D1(+) (Figure 1D1) or D1(−) MSNs (Figure 1D2). Analysis of CV measurements in isolation can be found in the supplemental text (Figure S1). Taken together, these data are consistent with the interpretation that glutamatergic inputs to the NAc core display basal differences in NMDAR function. This led us to assess whether cocaine experience would alter NMDAR function at these specific NAc core synapses.

Cocaine experience does not alter electrically-evoked NAc core AMPAR or NMDAR properties

In order to investigate consequences of cocaine experience on NAc synaptic properties we conditioned mice with 5-days of cocaine injections outside of the home cage. Repeated injections of cocaine induced robust increases in locomotor activity ($F = 27.46$, $df = 6$, $p < 0.001$, Figure 2A). Following a 10–14 day drug-free period, we performed targeted whole-cell recordings of electrically-evoked EPSCs in the NAc core. We observed no effects of this cocaine conditioning paradigm on NMDAR/AMPA ratios or properties within the NAc core on either D1(+) (Figure 2B1-E1) or D1(−) MSNs (Figure 2B2-E2).

Cocaine experience alters AMPAR and NMDAR function at mThal-D1(+) NAc core synapses

To investigate potential changes in excitatory drive specific to thalamic inputs following cocaine experience, we examined AMPAR and NMDAR function at mThal-D1(+) NAc core synapses (Figure 3A). We observed that cocaine experience increased the amplitude of AMPAR-mediated asEPSCs at these synapses relative to saline controls (sal: 23.9 ± 1.6 pA vs coc: 31.2 ± 2.6 pA, $p < 0.05$, Figure 3B). We expected that cocaine experience would result in a corresponding decrease in NMDAR/AMPA. However mThal-D1(+) NMDAR/AMPA at mThal-D1(+) from cocaine-treated mice was indistinguishable from saline controls (Figure 3C). Also, no change in CV-N/CV-A between the cocaine- and saline-treated groups was observed (Figure 3D). Given that we observed a cocaine-induced increase in AMPAR function, we hypothesized that cocaine treatment would be associated with a concomitant increase in NMDAR function at these synapses, and proceeded to

analyze pharmacologically-isolated NMDAR EPSCs (Figure 3E). The current-voltage relationship of NMDAR EPSCs is generally determined by the biophysical properties of GluN2 subunits expressed at the synapse (40). After normalizing to the peak NMDAR-EPSC at +30 mV, we observed that cocaine exposure increased the relative mThal-D1(+) NMDAR current passed at -30 mV (sal: -0.334 ± 0.057 vs coc: -0.465 ± 0.041 , $p < 0.05$). This change in the current-voltage relationship is consistent with the interpretation that cocaine experience altered the functional subunit stoichiometry of mThal-D1(+) NMDARs, potentially by increasing the expression of GluN2C isoforms (41). Overall, mice with a cocaine history exhibited a potentiation of synaptic strength/connectivity at mThal-D1(+) synapses, despite no observed change in the NMDAR/AMPA ratio.

NMDAR function at PFC-D1(+) NAc core synapses is increased following cocaine experience

To probe how cocaine experience affects cortical inputs to the NAc core, we examined AMPAR and NMDAR properties utilizing ChR2 expressed in axon terminals from the PFC (Figure 3F). In contrast to mThal inputs, we found no difference in asEPSC amplitude between cocaine- and saline-treated mice (Figure 3G). However, PFC-D1(+) NMDAR/AMPA ratio was greater following cocaine experience relative to the saline-treated group (sal: 0.388 ± 0.040 vs coc 0.550 ± 0.067 , $p < 0.05$, Figure 3H). To determine whether silent synapses contribute to the increase in NMDAR/AMPA ratio we analyzed CV-N/CV-A, but found no difference between cocaine and vehicle groups (Figure 3I). Finally, we examined NMDAR biophysical properties to address potential differences in subunit stoichiometry across groups. We also found no differences in current-voltage relationship (Figure 3J) suggesting that NMDAR subunit stoichiometry at PFC-D1(+) inputs is not modified by cocaine experience. Together these data indicate that at PFC-D1(+) NAc core synapses, cocaine experience yields an upregulation of NMDAR function without increasing silent synapses or altering subunit stoichiometry.

mThal projections to NAc core D1(-) MSNs exhibit functional properties consistent with more silent synapses following cocaine experience

In parallel to recordings from D1(+) MSNs, we assessed consequences of cocaine experience on AMPAR and NMDAR properties at mThal-D1(-) synapses (Figure 4A). The asEPSC amplitude did not differ between cocaine and saline treatment groups (Figure 4B). However unlike mThal-D1(+), NMDAR/AMPA at mThal-D1(-) synapses was larger in the cocaine-treated group (sal: 0.309 ± 0.045 vs coc: 0.422 ± 0.024 , $p < 0.05$, Figure 3C). Furthermore, we observed a much smaller CV-N/CV-A ratio following cocaine experience (sal: 1.224 ± 0.167 vs coc: 0.609 ± 0.050 , $p < 0.01$, Figure 4I). Cocaine experience did not alter mThal-D1(-) NMDAR stoichiometry as measured by current-voltage relationship (Figure 4E), however silent synapses are not known to be enriched in NMDARs with abnormal current-voltage relationships.

Cocaine experience does not alter PFC-D1(-) NAc core AMPAR or NMDAR properties

We also examined whether cocaine treatment induced changes in AMPAR or NMDAR function at PFC-D1(-) NAc core synapses. No differences in asEPSC amplitude, NMDAR/

AMPA ratiometric measures, or NMDAR current-voltage relationship at PFC-D1(–) synapses (Figure 4F–J) were observed following cocaine experience.

Functional upregulation of GluN2C/D at mThal-D1(+) synapses following cocaine experience

In order to investigate the cocaine-induced changes in NMDAR current-voltage relationship we utilized the GluN2C/2D positive allosteric modulator CIQ (42; 43). Washing on CIQ had no effect on mThal to D1 MSN AMPAR EPSC amplitude from either cocaine-treated mice or controls (Figure 5B), so we proceeded to assess isolated NMDAR currents in low-Mg²⁺ aCSF containing NBQX (Figure 5C). Application of CIQ did not affect mThal-D1(+) NMDAR-EPSCs from control animals ($96.0 \pm 9.2\%$, Figure 5D), but potentiated NMDAR currents at mThal-D1(+) synapses from cocaine-conditioned mice ($130.3 \pm 12.0\%$, $p < 0.05$ vs. sal). To confirm that the effect of CIQ occurs in standard, Mg²⁺-containing aCSF, we examined NMDAR/AMPA ratios in a separate set of slices (Figure 5E). In control D1 MSNs, mThal NMDAR/AMPA was not affected by the presence of CIQ (Figure 5F). However CIQ generated an increase in mThal NMDAR/AMPA in D1 MSNs from cocaine-conditioned animals, (aCSF: 0.394 ± 0.030 vs CIQ: 0.531 ± 0.044 , $p < 0.05$). To further investigate the contribution of GluN2C-containing NMDARs, we isolated NMDAR-EPSCs in the presence of d-cycloserine, a GluN2C-preferring agonist (22; 44; 45). Bath application of d-cycloserine abrogated the cocaine-induced alteration in NMDAR current-voltage relationship at mThal-D1(+) synapses (Figure S2). The findings from these convergent pharmacological experiments are consistent with the interpretation that cocaine exposure enhanced the function of GluN2C-containing NMDARs.

Cocaine experience unmask NMDAR-dependent LTD at mThal-D1(+) NAc core synapses

Synaptic plasticity is commonly accepted as a substrate for learning and memory. Metaplasticity is an adaptation that promotes greater dynamic change in synaptic strength and connectivity. After determining that cocaine alters the stoichiometry of mThal-D1(+) NMDARs, we sought to test their function by analyzing synaptically-induced NMDAR-dependent long-term plasticity (4; 6; 46). In slices from mThal-ChR2 mice, 1 Hz 470 nm light stimulation for 10 minutes (low frequency stimulation; LFS) did not decrease the EPSC amplitude in D1(+) NAc core MSNs (sal: $94.0 \pm 7.6\%$, Figures 6A–6D). However, LFS induced LTD of mThal-D1(+) EPSCs in slices from cocaine-conditioned mice (coc: $68.1 \pm 6.2\%$, $p < 0.05$ vs. sal). Bath application of D-AP5 during LFS blocked LTD in D1(+) MSNs from cocaine-treated animals ($92.6 \pm 10.7\%$, Figure S3), corroborating an NMDAR-dependent mechanism of LTD. Since we observed that cocaine exposure induced changes in NMDAR function at a moderately depolarized holding potential, we paired the LFS with depolarization and expected to observe enhanced NMDAR function. Indeed LFS paired with depolarization to -20 mV induced greater LTD at mThal-D1(+) synapses from cocaine-conditioned animals (sal: $83.9 \pm 9.1\%$ vs coc: $54.2 \pm 7.5\%$, $p < 0.05$, Figures 6E–G). These data provide further evidence that cocaine experience alters NMDAR function at mThal-D1(+) NAc core synapses and identify a circuit-based substrate underlying pathophysiological learning and memory processes.

DISCUSSION

In this report we investigated AMPAR and NMDAR function across several NAc synapses, and further demonstrated that cocaine experience exerts disparate outcomes on these circuits. We highlighted changes in excitatory synaptic transmission from the mThal to the NAc core, as this overlooked projection could yield important insight into the integration of sensorimotor information towards the execution motivated behaviors. As reported by MacAskill et al (47), AMPAR function at mThal-D1(+) and mThal-D1(−) is similar to the synapses from other inputs under basal conditions. However, our results suggest differences in NMDAR function across inputs to the NAc core. We proceeded to assess how cocaine exposure and abstinence altered NAc core NMDAR function in a circuit-specific manner.

Consistent with previous reports (8; 9), we found that cocaine exposure and withdrawal did not induce discernable changes in electrically-evoked AMPAR or NMDAR function within the NAc core. Notably, these results differ from Jedynak et al (13), who noted that the same cocaine conditioning paradigm enhanced AMPAR function in the NAc core as assessed by electrically-evoked AMPAR/NMDAR and miniature EPSC (mEPSC) amplitude. These discrepancies may be explained by technical differences, biology, or both. For one, Jedynak et al recorded peak isolated NMDAR currents at room temperature, whereas the values used in the present study reflect the late-phase dual component EPSC in a heated bath. As temperature is known to alter NMDAR decay kinetics (48), our methods were geared towards detecting differences in GluN2 subunit stoichiometry. Secondly, although asEPSCs and mEPSCs each reflect synaptic AMPAR function, nuanced differences between the measurements (exemplified by Thomas et al (8)) may explain the discrepancy in interpretations. From a biological standpoint, recordings made by Jedynak et al were performed in the medial NAc core (ML: 0.72–0.96 mm), whereas those from the present study were more lateral (ML: 1.0–1.25), and entirely dorsal to the anterior commissure. Relatively little attention has been paid to subregion-specific differences in NAc function (24; 49), but these subtle anatomical/physiological gradations merit further study. Notwithstanding these minor differences, the present data emphasize the importance of considering input specificity when interrogating NAc physiology. When isolating mThal-D1(+) NAc core synapses, we observed that cocaine exposure and abstinence enhanced AMPAR function without affecting ratiometric measurements. By augmenting AMPAR function at mThal-D1(+) synapses, a previous cocaine history is expected to promote future D1-MSN activation and associated reward-related behaviors (50), similar to inputs from the PFC, BLA, and vSub to the NAc shell (4–7).

In addition to increasing mThal-D1(+) AMPAR function, cocaine experience drove changes in NMDAR properties that enhanced their function. Specifically we found that following cocaine exposure, mThal-D1(+) NMDARs pass relatively more current at moderately hyperpolarized potentials and induce greater changes in downstream signaling. As the observed changes in NMDAR current-voltage relationship are consistent with the incorporation of GluN2C/D-containing NMDARs, we employed the GluN2C-preferring ligands, CIQ (42; 43) and d-cycloserine (44; 45) to assess NMDAR subunit stoichiometry. CIQ selectively potentiated mThal-D1(+) isolated NMDAR EPSCs and NMDAR/AMPA from cocaine-conditioned mice. Moreover, inclusion of d-cycloserine in the aCSF eliminated

the cocaine-induced difference in NMDAR current-voltage relationship. In the context of alcohol abuse, PFC-NAc core GluN2C function has been demonstrated to promote compulsive drug-seeking (21; 22). The present data provide the first evidence for the involvement of these NAc NMDAR receptors in psychostimulant exposure, and we speculate that the incorporation of GluN2C subunits at mThal-D1(+) synapses may be a permissive step towards the development of pathological drug-seeking behaviors. One interesting caveat is that d-cycloserine acts as an NMDAR partial antagonist when extracellular glycine concentrations are elevated (51–53), raising the possibility that NAc glycinergic transmission may be involved in the actions of cocaine (54). Nonetheless, the convergent biophysical and pharmacological data presented here support a role for the GluN2C subunit in the pathophysiological effects of cocaine experience. However in cell lines (40; 55) and in NAc MSNs following alcohol intake (21; 22), GluN2C subunits contribute to the EPSC at resting membrane potentials – a phenomenon we did not observe at mThal-D1(+) synapses. Instead our data resemble differences observed at thalamic inputs onto cortical principal neurons, which contributed greatly to the postsynaptic membrane potential and subsequent spiking (56). The difference in the current-voltage relationship suggests that cocaine experience enriches non-canonical GluN2C-containing receptors (i.e. heterotrimeric receptors) at mThal-D1(+) synapses. Testing this hypothesis requires tools not yet readily available (40; 57), but GluN2C-containing NMDARs represent an intriguing potential mediator of behaviors related to relapse.

At PFC-D1(+) synapses, we observed increased NMDAR/AMPA in cocaine treated groups without a consistent change in the AMPAR-mediated component as measured by asEPSC amplitude. Further unlike the mThal-D1(+) synapses, PFC-D1(+) synapses did not exhibit any changes in NMDAR current-voltage relationship following cocaine experience. These findings suggest that cocaine experience increases NMDAR expression without altering subunit stoichiometry. Alternatively, enhancement of the NMDAR-EPSC may have been generated by changes in the phosphorylation (58) and/or localization (59; 60) of the receptors. A recent report demonstrated that abstinence from cocaine self-administration enhanced relapse-preventing NMDAR-dependent LTD at prelimbic PFC-NAc core synapses (6). Our data provide an explanation whereby an increase in NMDAR function provided the cell type-specific molecular mechanism required for the light-induced reversal of cocaine craving. We also found that cocaine experience had no detectable effect on AMPAR or NMDAR function at PFC-D1(–) synapses. These data support continuing translational efforts towards restoring pathophysiological changes induced on D1-expressing MSNs, and emphasize that interactions between NMDARs and D1-MSN-specific proteins provide a target for developing pharmacological therapies for addiction-related disorders.

The current body of literature supports the view that the majority of drug-induced adaptive changes in synaptic function occur in D1(+) and not D1(–) MSNs in the NAc (50). To the best of our knowledge, only one report has illustrated that psychostimulant exposure induced a change in AMPAR or NMDAR function at D1(–)/D2(+) NAc core MSNs (10). However, experiments following ablation (61), transient inhibition (10), or activation (10; 62) of D2(+) NAc MSNs provide compelling evidence that these neurons do modulate the behavioral responses to psychostimulants. Our data indicate that excitatory synaptic transmission onto D1(–) MSNs is affected by cocaine experience, as we observed enhanced NMDAR/AMPA

at mThal-D1(–) synapses. This finding is fundamentally different from our other observations of enhanced NMDAR function: the change in mThal-D1(–) NMDAR/AMPA was accompanied by a decrease in CV-N/CV-A, suggestive of an increased proportion of silent synapses. Previous findings demonstrated that cocaine-generated silent synapses are important for persistent adaptations but exist themselves only temporarily (5; 6; 15; 16). Our data are the first to suggest that some populations of NAc silent synapses may persist beyond short-term abstinence. One intriguing hypothesis is that mThal-D1(–) silent synapses provide a substrate for subsequent strengthening of mThal-to-NAc core D1(–) MSN connectivity that might promote resilience to drug-seeking (10).

In the NAc, cocaine exposure has been demonstrated to alter excitatory synaptic strength following optogenetic activation of afferents from the PFC (4; 6; 7), BLA (5; 11; 47), and vSub (7; 11; 24). In some cases, long-term abstinence from cocaine exposure impairs the ability of NAc MSNs to undergo LTD (7; 8; 63; 64), whereas in other instances, enhanced plasticity has been observed (4–7). At mThal-D1(+) NAc core synapses, the metaplastic adaptations induced by cocaine facilitate GluN2C function and subsequent LTD of AMPAR. In sum, the cocaine-induced AMPAR and NMDAR changes at mThal-NAc core inputs are expected to contribute towards increased regulation of D1(+) MSNs by mThal and afford another mode of information processing that may contribute to the likelihood of reinstatement (65). As its expression is limited in the striatum and forebrain (40), modulation of GluN2C represents a potential novel treatment strategy for cocaine use disorders that may exert few off target effects. Additionally, because cocaine experience unmasks NMDAR-LTD at mThal-D1(+) NAc core synapses, a deep brain stimulation-like approach might be suitable for restoring synaptic function and generating beneficial behavioral outcomes without grossly affecting striatal circuitry. Despite being largely overlooked in recent years, the mThal-NAc circuit clearly merits further investigation for its involvement in the pathophysiology of addiction and other psychiatric disorders.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Basal α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic receptor (AMPA) and N-methyl-D-aspartate receptor (NMDAR) properties of nucleus accumbens core synapses. **(A)** Images of channelrhodopsin-2 (ChR2)-EYFP expression in D1-tdTomato brain slice following viral-mediated gene transfer directed at the midline nucleus of the thalamus (mThal). Wide field sagittal section (top) and high magnification merged confocal image (bottom) are displayed. **(B–D)** Input-specific AMPAR and NMDAR properties onto D1(+/-) neurons: **(B)** Amplitude of asynchronous excitatory postsynaptic currents (asEPSCs) at D1(+) **(B1)** and D1(-) **(B2)** synapses following stimulation of terminals from the mThal (light red/grey), prefrontal cortex (PFC) (medium red/grey) or basolateral amygdala (BLA) (dark red/grey) in the presence of Sr^{2+} . $n = 7\text{--}9/N = 4$ per group. **(C)** Ratio of NMDAR component relative to AMPAR EPSC while recording from D1(+) **(C1)** and D1(-) **(C2)** neurons. Representative input-specific EPSCs at -70 mV and $+40$ mV are displayed on the left. Inputs from mThal exhibited lower NMDAR/AMPA than from the PFC (**: $p < 0.01$; ***: $p < 0.001$, post-tests). $n = 10\text{--}13/N = 5\text{--}10$ per group. **(D)** Silent synapse coefficient of variation (CV) analysis. CV-NMDAR normalized to CV-AMPA at D1(+) **(D1)** and D1(-) **(D2)** synapses.

Figure 2.

Cocaine exposure and abstinence does not affect electrically-evoked nucleus accumbens core N-methyl-D-aspartate (NMDAR) or α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPA) function. (A) Top, schematic of behavioral conditioning and targeted approach. Slices were prepared 10–14 days after mice were conditioned with saline (filled circles) or cocaine (open circles) and excitatory postsynaptic currents (EPSCs) were elicited with a local electrode. Bottom, mice exhibited a sensitized locomotor response to cocaine (*: $p < 0.05$; ***: $p < 0.001$, post-test vs. day 1). $N = 20$ saline; 16 cocaine. (B) Amplitude of asynchronous EPSCs from both D1(+) (B1) and D1(-) (B2) neurons. $n = 7-9/N = 4-6$ per group. (C) Ratio of NMDAR component to AMPAR EPSC following while recording from D1(+) (C1) or D1(-) (C2) neurons. $n = 9-14/N = 7-9$ per group. (D) Coefficient of variation (CV)-NMDAR normalized to CV-AMPA in D1(+) (D1) and D1(-) (D2) cell types. (E) Isolated NMDAR current-voltage relationship from both D1(+) (E1) and D1(-) (E2) neurons. $n = 8-16/N = 5-8$ per group.

Figure 3.

Cocaine history exerts distinct effects on nucleus accumbens (NAc) core D1(+) N-methyl-D-aspartate (NMDAR) or α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPA) function based on input. (A) D1(+) neurons were targeted in slices prepared 10–14 days after mice were conditioned with saline (filled squares) or cocaine (open squares), and glutamate was released from the midline nuclei of the thalamus (mThal). (B) Cocaine conditioning increased the amplitude of asynchronous excitatory postsynaptic currents (asEPSCs) at mThal-D1(+) NAc core synapses ($p < 0.05$). $n/N = 6/4$ saline; $7/4$ cocaine. (C) Left, dual component EPSCs obtained at -70 mV and $+40$ mV in slices from mice conditioned with saline (black) or cocaine (grey) mice. The NMDAR component is normalized to the AMPAR EPSC. Scale bars denote 100 pA and 50 ms. Right, summary data of NMDAR/AMPA ratios. $n/N = 8/8$ saline; $10/10$ cocaine. (D) Coefficient of variation (CV)-NMDAR normalized to CV-AMPA. (E) Left, isolated NMDAR current-voltage relationship. Scale bars denote 50 pA and 50 ms. Right, mThal-D1(+) NAc core synapses exhibited an altered NMDAR current-voltage relationship following *in vivo* cocaine conditioning (**; $p < 0.01$). $n/N = 6/6$ saline; $9/7$ cocaine. (F) D1(+) neurons were targeted in slices prepared 10–14 days after mice were conditioned with saline (filled triangles) or cocaine (open triangles), and glutamate was released from the prefrontal cortex

(PFC). **(G)** asEPSC amplitude of PFC-D1(+) synapses. $n/N = 9/4$ saline; $8/4$ cocaine. **(H)** Left, dual component EPSCs obtained at -70 mV and $+40$ mV. Scale bars denote 100 pA and 50 ms. Right, cocaine exposure and abstinence increased NMDAR/AMPA relative to saline controls ($p < 0.05$). $n/N = 12/6$ saline; $9/5$ cocaine. **(I)** CV-NMDAR normalized to CV-AMPA. **(J)** Left, isolated NMDAR current-voltage relationship. Scale bars denote 50 pA and 50 ms. Right, Summary data of NMDAR current-voltage relationships. $n/N = 6/3$ saline; $4/3$ cocaine.

Figure 4.

Cocaine history alters midline nuclei of the thalamus (mThal)-D1(-) N-methyl-D-aspartate (NMDAR) function in nucleus accumbens (NAc) core. **(A)** D1(-) neurons were targeted in slices prepared 10–14 days after mice were conditioned with saline (filled squares) or cocaine (open squares), and glutamate was released from the midline nuclei of the thalamus (mThal). **(B)** Amplitude of asynchronous excitatory postsynaptic currents (asEPSCs) elicited in Sr^{2+} -replaced artificial cerebrospinal fluid (aCSF). $n/N = 7/4$ saline; $7/4$ cocaine. **(C)** Left, the NMDAR component is normalized to the α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPA) EPSC. Scale bars denote 100 pA and 50 ms. Right, cocaine exposure increased NMDAR/AMPA at mThal-D1(-) synapses ($p < 0.05$). $n/N = 8/8$ saline; $8/8$ cocaine. **(D)** Coefficient of variation (CV)-NMDAR normalized to CV-AMPA. Cocaine conditioning decreased CV-N/CV-A at mThal-D1(-) NAc core synapses ($p < 0.01$). **(E)** Left, isolated NMDAR current-voltage relationship. Scale bars denote 50 pA and 50 ms. Right, summary data of NMDAR current-voltage relationships. $n/N = 7/5$ saline; $7/4$ cocaine. **(F)** D1(-) neurons were targeted in slices prepared 10–14 days after mice were conditioned with saline (filled triangles) or cocaine (open triangles), and glutamate was released from the prefrontal cortex (PFC). **(G)** asEPSC amplitude of PFC-D1(-) synapses. $n/N = 9/4$ saline; $7/4$ cocaine. **(H)** Left, dual component EPSCs obtained at -70 mV and $+40$ mV. Scale bars denote 100 pA and 50 ms. Right, summary data of NMDAR/AMPA ratios. $n/N = 8/5$ saline; $14/8$ cocaine. **(I)** CV-NMDAR normalized to CV-AMPA. **(J)** Left,

isolated NMDAR current-voltage relationship. Scale bars denote 50 pA and 50 ms. Right, summary data of NMDAR current-voltage relationships. $n/N = 6/3$ saline; $5/3$ cocaine.

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Figure 5.

CIQ application reveals GluN2C/D function following cocaine experience. (A) Acute nucleus accumbens core slices were prepared 2 weeks after mice were conditioned for 5 days with saline or cocaine. D1(+) neurons were targeted and glutamate was released at terminals from the midline nuclei of the thalamus (mThal). (B), CIQ, a GluN2C/D positive allosteric modulator, did not alter EPSC amplitude after mice were treated with either saline (filled squares) or cocaine (open squares). $n/N = 4/3$ saline; $3/3$ cocaine. (C) Representative traces. Isolated N-methyl-D-aspartate receptor (NMDAR)-EPSCs were elicited in low-Mg²⁺ aCSF and NBQX. Scale bars denote 100 pA and 50 ms. (D) Bath application of CIQ potentiated mThal-D1(+) NMDAR-EPSCs following cocaine experience. $n/N = 5/3$ saline; $6/3$ cocaine. (E) Representative traces. The NMDAR component was normalized to the α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPA) EPSC in the presence of CIQ or aCSF. Scale bars denote 100 pA and 50 ms. (F) Left, CIQ did not affect mThal-D1(+) NMDAR/AMPA in control slices. $n/N = 5/3$ CSF; $7/3$ CIQ. Right, CIQ enhanced NMDAR/AMPA at mThal-D1(+) NAc core synapses following *in vivo* cocaine experience ($p < 0.05$). $n/N = 6/3$ CSF; $6/3$ CIQ.

Figure 6.

Cocaine exposure unmasks N-methyl-D-aspartate receptor (NMDAR)-dependent synaptic plasticity in nucleus accumbens core at midline nuclei of the thalamus (mThal) synapses on D1(+) neurons. (A) Top, schematic of behavioral conditioning and circuit-specific approach. Bottom, representative traces displaying low frequency stimulation (LFS: 1 Hz) of mThal-specific excitatory postsynaptic currents (EPSCs) at resting and depolarized holding potentials. (B) Representative long term depression (LTD) experiments following 10 minutes mThal-specific LFS. LFS exerted no effect on mThal-D1(+) EPSC amplitude in control cells (black) but induced LTD in D1(+) MSNs from cocaine-conditioned mice (grey). (C) Summary time course of NMDAR-LTD. $n/N = 7/7$ saline; $8/7$ cocaine. (D) Left, representative traces from the baseline (pre) and final 10 minutes of recording (post). Right, average EPSC amplitude during last 10 minutes. Cocaine exposure permits NMDAR-LTD ($p < 0.05$). (E) Representative LTD experiments. mThal-specific LFS was paired with depolarization. Paired LFS generated modest LTD in control D1(+) MSNs but greater LTD in cells from cocaine-conditioned animals. $n/N = 8/6$ saline; $6/5$ cocaine. (F) Time course of paired NMDAR-LTD. (G) Left, representative traces from the baseline and end of LTD recording. Right, average EPSC amplitude during last 10 minutes. Cocaine treatment enhanced LTD induction ($p < 0.05$).