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Differential regulation of ionotropic glutamate receptor subunits following cocaine self-administration

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Abstract

Previous examination of binge cocaine self-administration and 2 week withdrawal from cocaine self-administration on ionotropic glutamate receptor subunit (iGluRs) protein levels revealed significant alterations in iGluR protein levels that differed between the mesocorticolimbic and nigrostriatal pathways. The present study was undertaken to extend the examination of cocaine-induced alterations in iGluR protein expression by assessing the effects of acute withdrawal (15–16 h) from limited access cocaine self-administration (8 h/day, 15 days). Western blotting was used to compare levels of iGluR protein expression (NR1–3B, GluR1–7, KA2) in the mesolimbic (ventral tegmental area, VTA; nucleus accumbens, NAc; and prefrontal cortex, PFC) and nigrostriatal pathways (substantia nigra, SN and dorsal caudate–putamen, CPu). Within the mesolimbic pathway, reductions were observed in NR1 and GluR5 immunoreactivity in the VTA although no significant alterations were observed in any iGluR subunits in the NAc. In the PFC, NR1 was significantly upregulated while GluR2/3, GluR4, GluR5, GluR6/7, and KA2 were decreased. Within the nigrostriatal pathway, NR1, NR2A, NR2B, GluR1, GluR6/7 and KA2 were increased in the dorsal CPu, whereas no significant changes were observed in the SN. The results demonstrate region- and pathway-specific alterations in iGluR subunit expression following limited cocaine self-administration and suggest the importance for the activation of pathways that are substrates of the reinforcing and motoric effects of cocaine.

Keywords

Mesocorticolimbic; Nigrostriatal; Cocaine; Self-administration; Glutamate; Reinforcement

1. Introduction

Neuropharmacological evidence indicates that the functional integrity of the mesocorticolimbic dopamine pathway is essential for the reinforcing effects of cocaine [8,26] and that biochemical processes within this pathway are significantly regulated by cocaine administration [39]. The mesocorticolimbic pathway originates in the ventral tegmental area (VTA) and projects to several forebrain regions, most notably the nucleus accumbens (NAc) and prefrontal cortex (PFC). In contrast, the nigrostriatal dopamine pathway, which originates in the substantia nigra (SN) and projects primarily to caudate–putamen (CPu), does not appear to be involved in the reinforcing effects of cocaine [27].

Cocaine administration induces neuroadaptive changes in various biochemical pathways in the mesocorticolimbic pathway [38,39]. Significant attention has focused on genes and

proteins associated with dopaminergic neurotransmission including a generalized upregulation of the cAMP pathway [2,6,37,40,47,49,54] and activator protein 1 family members [15,21,23,41,45]. In addition, alterations in glutamatergic function are known to be involved in synaptic plasticity, learning, and memory and more recent evidence indicates involvement of glutamate transmission in the VTA and NAc in the mediation of the neurochemical effects of cocaine and neuroadaptations induced by cocaine administration. The effects of glutamate are mediated in part by ionotropic glutamate receptors which are comprised of assemblies of subunits classified as *N*-methyl-D-aspartate (NMDA; NR1, NR2A–D, NR3), ($\pm$)- α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA; GluR1–4), and kainate (GluR5–7, KA1–2) receptor subunits based on their pharmacological characteristics and sequence information [5,22]. AMPA and kainate receptors contribute to fast neurotransmission and all three iGluR subtypes are thought to play roles in LTP induced by cocaine [55]. Since subunit composition determines the functional properties of ionotropic glutamate receptors [5], alterations in the expression of specific subunits within the mesocorticolimbic pathway may induce changes in the excitability of dopamine transmission underlying long-term biochemical and behavioral effects of cocaine.

Previous studies have shown that cocaine administration increased extracellular glutamate concentrations in the NAc and VTA [25,46,48] and produced behavioral sensitization [46]. Specifically, cocaine administration leads to increased responsivity of glutamate receptors in the NAc and VTA [55,59,64] which are mediated in part by increased AMPA and NMDA receptor expression in the VTA [12,30,59,64], as expression returns to control levels following withdrawal [7,31]. Indeed, GluR1 and NR1 protein levels were increased in the VTA following chronic cocaine administration [12] and remained elevated following 1 day but not 3 weeks of withdrawal in rats that manifest behavioral sensitization to cocaine [7]. In contrast, others have reported elevated VTA NR1 protein levels after protracted withdrawal periods (3 and 14 days; [30] and no change in VTA GluR1 levels following 16–24 h of withdrawal [31]). Similarly, we have demonstrated significant upregulation of specific iGluR subunits in the VTA and NAc of cocaine overdose victims and rhesus monkeys with cocaine self-administration histories [20,52].

The role of ionotropic glutamate receptor alterations in cocaine-induced behavioral sensitization is well documented; however, the effect of cocaine self-administration on alterations in the abundance of glutamate receptor subunits is less clear. The majority of studies evaluating the effects of cocaine on mRNA and protein expression have employed experimenter-administered cocaine and evaluated the effects following acute or chronic withdrawal. These studies have advanced understanding of the neurobiological basis of sensitization, learning, and memory, but do not address the role of iGluR subunits in cocaine reinforcement inasmuch as several studies indicate pronounced biochemical differences between the contingent and noncontingent administration of drugs [16–18,34]. Recently, we examined alterations in iGluR subunit expression as a function of binge cocaine self-administration and following 2 weeks of abstinence from this binge. Western blotting was used to compare levels of iGluR protein expression (NR1–3B, GluR1–7, KA2) in the VTA, SN, NAc, dorsal CPu, and PFC of rats. Selective alterations of ionotropic glutamate receptor subtypes were associated with binge cocaine self-administration and withdrawal in a region-specific manner. For example, in the SN and VTA, alterations in iGluR protein levels compared to controls occurred only following binge access, whereas in the dorsal CPu and PFC, iGluR alterations compared to controls occurred with binge access and following withdrawal. In the NAc, GluR2/3 levels were increased following withdrawal compared to binge access and were the only changes observed in this region [53].

The goal of the present study was to determine if the effects of limited access cocaine self-administration on iGluR subunit protein expression were similar to the effects observed

following binge and/or withdrawal. Differences may explain differential excitability of dopaminergic regions following different histories of cocaine self-administration and provide a temporal profile of such changes within pathways known to be involved in cocaine reinforcement. Furthermore, region-specific regulation of iGluRs may provide a novel mechanism by which cocaine self-administration exerts long-term effects on brain function.

2. Materials and methods

2.1. Subjects

Male Sprague–Dawley rats (60–90 days; 225–275 g; Charles River, Wilmington, MA) were housed individually in operant conditioning cages in a temperature-controlled vivarium on a 12 h reversed light–dark cycle (lights on: 8:00 p.m.) with food and water available ad libitum throughout the experiment. The number of subjects required was derived from the power function for determination of sample size for analysis of variance according to the following parameters: number of groups = 2; least detectable difference = 4; standard deviation = 2; power = 0.9; level of significance = 0.05 yielding a sample population of 7/group. Least detectable difference and standard deviation are based on the PI's experience in self-administration studies in rats.

2.2. Intravenous catheterization

Rats were anesthetized with halothane and implanted with chronic indwelling venous catheters, as described previously [2,17–19]. Catheters were inserted into the right jugular vein, terminating just outside the right atrium and anchored to muscle near the point of entry into the vein. The distal end of the catheter was guided subcutaneously to exit above the scapulae through a Teflon shoulder harness. The harness provided a point of attachment for a spring leash connected to a single channel swivel at the opposing end. The catheter was threaded through the leash for attachment to the swivel where the fixed end of the swivel was connected to a syringe by polyethylene tubing. Infusions were administered by a motor driven syringe pump controlled by a computer. Infusions of methohexital (100 μ l; 10 mg/kg, i.v.) were administered to assess catheter patency, as needed. Health of the rats was monitored thrice daily by the experimenter and biweekly by institutional veterinarians according to the guidelines issued by the Emory University Institutional Animal Care and Use Committee and the National Institute of Health.

2.3. Self-administration procedures

Following surgery, subjects were housed in standard operant conditioning chambers (24.5 $\times$ 23.5 $\times$ 21 cm) containing a retractable lever and a stimulus light mounted directly above the lever. The chambers were enclosed in sound-attenuating boxes containing an exhaust fan, a house light, a tone source, and a water bottle. A motor driven syringe pump was located on the side of this external chamber. Extraneous noise was masked by the exhaust fan. Immediately following surgery, rats were placed in their respective chambers where they received infusions of heparinized 0.9% bacteriostatic saline (1.7 U/ml; 200 μ l/30 min) for 48 h. On the following day, the self-administration procedure was initiated.

Responding was engendered and maintained initially under a fixed ratio 1 (FR1): 20-s time-out schedule of reinforcement (0.5 mg/infusion; 200 μ l/infusion; 6.2 s/infusion) during 8 h self-administration sessions (dark phase of the light cycle). Upon completion of the response requirement, a cocaine infusion was delivered and a 20-s time-out was in effect. During the time-out, the lever light was extinguished, the house light was illuminated, and a tone was generated. The end of the time-out was signaled by illumination of the lever light and the house light and tone were extinguished. During the time-out, responses were recorded but

had no scheduled consequence. IBM compatible computers were used for session programming and data collection. The ration was gradually increased to FR5 over 7 days. From days 8–22, rats self-administered cocaine under 8 h limited access conditions (FR5). Rats assigned to the control group did not receive catheter implants similar to our previous study [53]. Control rats were not implanted in order to minimize any discomfort associated with surgery. Rats were sacrificed 15–16 h following the end of the self-administration. The rationale for the 16 h delay in sacrificing the rats was based on the attempt to study changes in iGluR subunit protein expression and on historical precedent. Sacrifice immediately after self-administration (while drug is still on-board) likely reveals post-translational modifications (i.e. phosphorylation) initiated by second messengers activated by monoamine receptors. In the literature, two time points are typically examined, 1–3 days of withdrawal and 2 – 3 weeks of withdrawal [58,61,62]—time points for transient induction-related changes to diminish.

2.4. Tissue preparation and Western blot analysis

Rats were anesthetized with halothane and intracardially perfused with phosphate-buffered saline (pH 7.2). Brains were removed and sectioned on ice in the coronal plane using a brain matrix. Areas of interest were dissected immediately on ice-cooled aluminum plates from 1-mm slices (approximately +3.2 to +2.2, PFC; +1.7 to 0.7, NAc; 0.48 to –0.4, dorsal CPu; –5.2 to –6.2, SN/VTA; all measures relative to bregma; Fig. 1 [43]) and immediately frozen at –80 °C in Eppendorf tubes.

Due to the size of the VTA and SN, two samples were pooled such that $n = 4$ for the limited access and control groups for these regions. Tissue samples were homogenized in 10 mM HEPES, 10 mM NaCl, 1 mM KH_2PO_4 , 5 mM NaHCO_3 , 1 mM CaCl_2 , 0.5 mM MgCl_2 , 5 mM EDTA, and the following protease inhibitors (PI): 1 mM phenyl-methylsulfonylfluoride, 10 mM benzamidine, 10 $\mu\text{g/ml}$ aprotinin, 10 $\mu\text{g/ml}$ leupeptin, and 1 $\mu\text{g/ml}$ pepstatin and centrifuged using a Beckman Coulter SW55Ti swinging bucket rotor at $5333 \times g$ for 5 min. Supernatant (cytosol and crude membrane) was removed and centrifuged at $59,255 \times g$ for 30 min at 4 °C and the pure cytosolic supernatant was removed and stored at –80 °C. The pellet containing the crude plasma membrane was re-suspended in 20 mM Tris–HCl, 1 mM EDTA (pH 8.0), and 300 mM sucrose with PI and centrifuged at $5333 \times g$ for 5 min. This procedure was repeated twice and the pellet was re-suspended in phosphate-buffered saline and stored at –80 °C (crude plasma membrane fraction). The pellet from the initial centrifugation was re-suspended in 10 mM Tris (pH 7.5), 300 mM sucrose, 1 mM EDTA (pH 8.0), 0.1% NP40 and PI and centrifuged at $2370 \times g$ for 5 min at 4 °C. The supernatant was discarded and the pellet was re-suspended in the buffer and washed three times before re-suspension in the PI buffer and storage of samples at –80 °C (nuclear fraction), as described previously [52].

Protein concentrations were calculated using the bicinochonic acid protein assay kit (Pierce, Rockford, IL) and diluted in Laemmli sample buffer to achieve equivalent final protein concentrations. Five micrograms of the membrane fraction was loaded into 10% sodium dodecyl sulfate-polyacrylamide gel, electrophoresed and transferred to nitrocellulose by electroblotting (30 V, overnight at 4 °C) in $1 \times$ transfer buffer (Bio-Rad, Richmond, CA). Nitrocellulose membranes were blocked in 0.5% w/v nonfat dry milk and 0.1% v/v Tween 20 in phosphate-buffered saline (pH 7.4, 0.12 M) for 1 h at room temperature prior to being incubated with primary antibodies in blocking buffer (Bio-Rad) overnight at 4 °C followed by secondary antibody for 1 h at room temperature. Protein bands were visualized on a Kodak XAR-5 film with enhanced chemiluminescence (ECL plus, Amersham Pharmacia Biotech). Primary antibodies, source, epitope, species, dilution, and cross-reactivity are noted in Table 1. The selection of antibodies was based on commercial availability, specificity, and those used in our lab, previously [20,52,53]. The present study provides the

most extensive assessment of alterations in iGluR subunit protein levels as a function of cocaine administration to date.

Equal protein loading was confirmed by stripping the blots and re-probing them with a monoclonal α -tubulin antibody (Upstate Biotechnology Cell Signaling Systems, Waltham, MA; 1:5000 v/v) followed by incubation with secondary antibody and visualization as described above. No significant differences were detected in α -tubulin abundance between the groups for any of the blots, indicating that any differences in ionotropic glutamate receptor abundance between the groups were not due to unequal loading of protein in the gels. Protein abundances were calculated by optical densitometry with a Scan Jet 2200 C and imported into NIH Image 1.61 software for analysis. Film background was subtracted from the optical density values to give the optical density value for each subject. All assays were conducted under conditions in which densitometric signal intensity was linear with protein concentration as determined by preliminary experiments. Data were analyzed using Student's *t* tests and were expressed graphically as percent of control levels (mean $\pm$ SEM). Null hypotheses were rejected when $P < 0.05$.

3. Results

3.1. Behavioral data

Cocaine engendered and maintained rates of self-administration observed previously under similar limited access, fixed ratio schedules of reinforcement in rats (Fig. 2) [1,2,17–19,33]. There was no statistically significant effect of self-administration session on number of infusions [$F(13,111) = 1.224$; $P = 0.274$].

3.2. Mesolimbic regions

Analysis revealed significantly decreased levels of NR1 ($t = 3.850$, $df = 12$, $P = 0.002$) and GluR5 immunoreactivity in the VTA ($t = 2.799$, $df = 12$, $P = 0.016$) of rats self-administering cocaine. No significant changes were observed for any of the iGluR subunits studied in the NAc. In the PFC, cocaine self-administration induced a significant increase in NR1 ($t = 2.739$, $df = 14$, $P = 0.016$; Fig. 3), whereas significant decreases were observed for GluR2/3 ($t = -2.573$, $df = 14$, $P < 0.022$), GluR4 ($t = 4.244$, $df = 14$, $P < 0.001$; Fig. 5), GluR5 ($t = 5.815$, $df = 14$, $P < 0.001$), GluR6/7 ($t = 5.043$, $df = 14$, $P < 0.001$), and KA2 ($t = 3.970$, $df = 14$, $P = 0.001$; Fig. 6) immunoreactivity in this region. Representative immunoblots of NR1 from PFC and dorsal CPu are depicted in Fig. 4.

3.3. Nigrostriatal regions

In the dorsal CPu, cocaine self-administration induced significant upregulation of NR1 ($t = -2.749$, $df = 12$, $P = 0.018$), NMDAR2A ($t = -2.419$, $df = 12$, $P = 0.032$), NMDAR2B ($t = -2.704$, $df = 12$, $P = 0.019$; Fig. 3), GluR1 ($t = -2.428$, $df = 12$, $P = 0.032$; Fig. 5), GluR6/7 ($t = -2.664$, $df = 12$, $P = 0.021$), and KA2 ($t = -3.479$, $df = 12$, $P = 0.005$; Fig. 6). Cocaine self-administration did not induce significant alterations in any of the iGluR subunits examined in this study.

4. Discussion

In the present study, Western blot analysis was used to examine the expression of iGluR protein subunits following limited access cocaine self-administration in the mesocorticolimbic and nigrostriatal dopamine pathways. Results of this study provide a comprehensive assessment of iGluR subunit protein alterations in mesocorticolimbic brain regions associated with cocaine reinforcement (VTA, NAc, and PFC) compared with regions in the nigrostriatal pathway (SN and dorsal CPu) and demonstrate that cocaine self-

administration alters specific iGluR protein subunits in a region- and pathway-specific manner. In addition, these findings compliment a previous study assessing binge cocaine self-administration and withdrawal in rats [53], thus allowing a preliminary timeline of various transitional states in the addiction process and a comparative metric for changes observed in cocaine overdose victims and nonhuman primate models [20,52].

Previously, we evaluated alterations in protein expression of iGluRs following binge (8 h/day, 15 days; 24 h access, days 16–21) cocaine self-administration and 2 weeks of abstinence following the binge self-administration [53]. In light of the present findings, these studies have provided novel insight into the coordinate regulation of iGluR subunits during critical transitional phases of cocaine self-administration and indicate that the changes are both regionally and pathway-specific. Examined in conjunction with our previous study [53], alterations in NMDA subunits were affected by limited exposure or during withdrawal, but not as a function of binge access. For example, NR1 was decreased in the VTA and increased in the PFC following limited access, but returned to baseline levels during binge and withdrawal suggesting that the present changes represent transient alteration [53]. In contrast, other studies have reported increased NR1 protein levels in the VTA and NAc following *experimenter-administered* cocaine [7,12] and increased NAc NR1 levels following 1 and 90 days of withdrawal of cocaine self-administration [32]. The lack of observed change in the NAc in the present study may be due to the short withdrawal time (16 h) examined or the amount of cocaine exposure. From a functional perspective, NR1 subunits are the requisite subunit for NMDA receptor function. Phosphorylation of the NR1 subunit leads to increased Ca^{2+} permeability which in turn is necessary for long-term synaptic changes such as LTP. Within the mesolimbic system, decreased NR1 in the VTA may represent a compensatory mechanism to offset the increased responsivity of VTA NMDA receptor stimulation to cocaine reported by others [55], thereby limiting the excitability of dopamine neurons in this region. Although NR1 was not decreased during binge self-administration, the kainate subunits GluR5 and GluR6/7 were decreased in the VTA which may provide an alternate means to suppress dopaminergic excitability in this region.

In the PFC, we propose that elevated NR1 levels and corresponding decreased GluR2/3 levels lead to increased Ca^{2+} permeability in neurons in this region and thus represent an initial step in long-term synaptic changes associated with cocaine. Whereas NR1 levels return to baseline during binge and withdrawal, NR3A is decreased compared to control levels during the withdrawal phase—an effect that likely leads to increased Ca^{2+} permeability and hyperexcitability [44,53]. Decreased GluR2 levels following limited access compliment the effects of elevated NR1 levels in the PFC mentioned above given that GluR2 subunits exist in the edited form in vivo which confers decreased cation permeability [13,24]. Therefore, increased excitability of PFC during limited access and withdrawal are likely mediated by the regulation of separate subunits—yielding similar functional endpoints. Selective targeting of NR1 and GluR2 subunits also influences Ca^{2+} permeability within various cellular fields of specific cell types. Future studies examining mRNA and proteins assessments in discrete cellular populations of the PFC and other regions involved in the effects of cocaine are warranted.

Interestingly, none of the AMPA subunits studied were significantly different from control levels in the NAc in the present study corroborating previous findings [7,12]. However, a study employing self-administration had demonstrated a significant upregulation of GluR1 1 and 90 days of withdrawal and upregulation of GluR2 1 and 30 days of withdrawal [32]. Extending these findings, Sutton and colleagues demonstrated that rats with a previous history of cocaine self-administration exhibited significant increases in the levels of GluR1 and GluR2/3 in the NAc following 1 week of extinction training and 1 week of extinction

training plus 3 days of withdrawal, but not after 1, 7, or 10 days of withdrawal [51]. These intriguing data indicate that the plasticity of AMPA receptors in the NAc is associated with the behavioral history of the subjects (extinction) rather than simply the removal of drug availability.

Cocaine exposure exerts appreciably different effects on the regulation of iGluR subunit abundances within the nigrostriatal pathway. Elevations in dorsal CPu NR1 and NR2B levels following limited access, as well as following binge access and withdrawal [53], indicate induction of protein levels during early cocaine exposure that persist through the transitional phases studied. In contrast, increased NR1 in SN following binge access only likely indicates a conditional alteration due to elevated drug intake leading to increased excitability of SN dopamine neurons and corresponding increased motoric activation. Likewise, only binge access induced elevations in NR3A and 3 B levels. Speculation on the functional consequence is tenuous given the paucity of physiological information for these subunits.

The activation of NMDA subunits in the nigrostriatal pathway is complemented by similar elevations in GluR1 levels in the dorsal CPu during limited access and withdrawal while these elevations were suppressed during binge access [53]. Similarly, GluR4 levels in the dorsal CPu were increased slightly during limited access, returned to baseline levels during binge access, and elevated again following withdrawal. GluR2/3 follows a similar pattern of regulation with the exception that binge access significantly *decreased* protein levels. The reasons for binge induced suppression of AMPA subunits in this region are not readily apparent but warrant further examination. Previous studies using experimenter-administered cocaine failed to observe any significant alterations in AMPA subunits in the nigrostriatal pathway following chronic cocaine administration or withdrawal [7,12].

Limited access cocaine self-administration also induced a biphasic effect on dorsal CPu kainate receptor subunit levels with increased GluR6/7 and KA2, no change during binge access, and a significant upregulation of all kainate subunits during withdrawal [53]. Unlike NMDA and AMPA receptor subunits, the physiological function of kainate receptors remains unclear; however, they appear to be important for controlling Ca^{2+} influx through the kainate ionophore in different pathological states, such as cocaine addiction [42]. Interestingly, this is the first report examining alterations in kainate subunits following cocaine self-administration.

Given the biochemical and structural similarities of the mesocorticolimbic and nigrostriatal pathways, it is interesting that cocaine self-administration history would differentially regulate iGluR levels between the two pathways. A possible explanation for the regional specificity of such changes would be the structural and functional anatomy of the two pathways. With regard to glutamatergic input to the regions under study, the VTA and SN receive inputs from the amygdala, hypothalamus, and frontal and orbitofrontal cortical regions [50,56,57], while the SN receives additional projections from the subthalamic nucleus [3]. Glutamatergic afferents to the NAc and dorsal CPu are markedly different. Regions projecting to the NAc include the amygdala [35,63], intermediodorsal, paraventricular and parataenial thalamic nuclei [3,4], and meso- and allocortical regions [14]. In the dorsal CPu, glutamatergic afferents arise from midline and intralaminar thalamic nuclei [3,4] and the sensorimotor, visual, and auditory cortices [11,14,36]. The PFC receives glutamatergic input from the intralaminar and mediodorsal thalamic nuclei [9,10,28] and the amygdala [10,29]. From a functional perspective, the mesocorticolimbic pathway is generally considered a primary substrate for the reinforcing effects of abuse drugs, while the nigrostriatal pathway is generally considered to be involved in mediating the locomotor and stereotypy activating effects of the drugs [27,60]. The method of dissection may have

contributed also to the observed differences inasmuch as the dorsal aspect of the dorsal CPu and lateral aspects of the SN were dissected to prevent overlap with the NAc and VTA, respectively. Immunocytochemistry may provide a more detailed understanding of the anatomical disparities of iGluR protein levels between the two pathways following cocaine administration; however, improvements in anatomical resolution would be gained at the expense of the ability to quantify such changes.

The present study demonstrated the regional and subunit-specific changes in iGluR protein expression following limited access cocaine self-administration. Differences between the present data and previous rat studies will require further investigation including varying limited access and alternate binge-withdrawal conditions. While additional studies are needed to establish causal relationships between changes in iGluR protein expression and function, the present results in conjunction with our previous efforts [53] provide a significant addition to the knowledge of altered glutamatergic function by evaluation of such changes during critical transition phases of cocaine abuse. Alterations in iGluR subunit abundance may be related to behaviors associated with sensitization, increased anxiety, and other enduring effects and likely reflect important mechanisms by which cocaine exerts long-term differential effects on the mesocorticolimbic and nigrostriatal pathways. Examination of post-transcriptional and post-translational modification of these subunits is warranted in order to further understand how such cocaine-induced neuroadaptations may relate to further drug intake and abuse.

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Abbreviations

AMPA	(±) alpha-amino-3-hydroxy-5-methylisoxazole-4-propionate
iGluR	ionotropic glutamate receptor subunit
SN	substantia nigra
VTA	ventral tegmental area
NAc	nucleus accumbens
NMDA	<i>N</i> -methyl-D-aspartate
PFC	prefrontal cortex
CPu	caudate-putamen

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Fig. 1. Schematic of representative regions used for tissue dissection. Shaded areas represent dissected areas, including PFC, NAc, dorsal CPu, VTA, and SN. Values represent mm from bregma [43].

Fig. 2.

Mean number of infusions/session during cocaine self-administration at FR5. Responding was engendered and maintained by intravenous cocaine infusions (0.5 mg/infusion/8 h session) under an FR5 schedule of reinforcement for 14 days. Symbols represent mean number of infusions $\pm$ SEM. The mean total intake for the self-administration sessions under FR5 was 423 ± 29.3 mg.

Fig. 3.

Comparisons of NMDA receptor subunit protein levels in VTA, NAc, PFC, SN, and dorsal CPu following limited access to cocaine self-administration. Data are mean $\pm$ SEM percent of values for control rats in each region. * Indicates $P < 0.05$. The availability of sufficient tissue limited the number of NMDA subunit antibodies assessed in the VTA and SN.

Fig. 4. Representative immunoblot of NR1 protein levels following limited access cocaine self-administration. Protein levels in the (A) PFC and (B) dorsal CPu were significantly increased following cocaine self-administration compared to controls. Data were analyzed by quantitative densitometry. Refer to Fig. 3 for graphic representation.

Fig. 5.

Comparisons of AMPA receptor subunit protein levels in VTA, NAc, PFC, SN, and dorsal CPu following limited access to cocaine self-administration. Data are mean $\pm$ SEM percent of values for control rats in each region. * Indicates $P < 0.05$.

Fig. 6.

Comparisons of kainate receptor subunit protein levels in VTA, NAc, PFC, SN, and dorsal CPU following limited access to cocaine self-administration. Data are mean $\pm$ SEM percent of values for control rats in each region. * Indicates $P < 0.05$.

Table 1

Description of antibodies used in the present study

Antibody	Epitope	Source	Species	Dilution	Cross-reactivity
NR1	Amino acids 660–811	Chemicon (mab363)	Ms	1:500	No cross-reactivity with NMDAR2–5
NR2A	Amino acids 1265–1464 of mouse NR2A	Upstate (07–632)	Rb	1:1000	No cross-reactivity noted
NR2B	C-terminal 20 amino acids of mouse NR2B	Upstate (06–600)	Rb	1:1000	No cross-reactivity noted
NR3A	Peptide corresponding to amino acids 1098–1113 (SRKTELEEYQKTNRTC) of rat NR3A	Upstate (07–356)	Rb	1:500	No cross-reactivity noted
NR3B	Amino acids 916–930 of mouse NR3B	Upstate (07–351)	Rb	1:1000	No cross-reactivity noted
GluR1	Amino acids 276–287 of rat GluR1	Upstate (06–600)	Rb	1:1000	No cross-reactivity noted
GluR2/3	Amino acids 864–883 of rat GluR2	Upstate (07–598)	Rb	1:1000	GluR2 and GluR3
GluR4	21 residue synthetic peptide (KHTGTAIRQSSGLAVIASDLP) corresponding to the C-terminal of G	Upstate (06–308)	Rb	1:1000	No cross-reactivity noted
GluR5	Peptide corresponding to amino acids 937–949 (CHQRRTQRKETVA) of rat GluR5-2C isoform precursor	Upstate (06–600)	Rb	1:1000	Cross-reacts with rat GluR5-2 and GluR5-2B/GluR5-1, no reactivity with rat isoform GluR5-2A
GluR6/7	16 residue synthetic peptide (KHTFNDRRRLPGKETMA) corresponding to the C-terminus of rat GluR6	Upstate (06–600)	Rb	1:1000	GluR6 and GluR7
KA2	21 residue synthetic peptide (KTSPPRPRPGPTGPRELTEHE) corresponding to the C-terminus of rat KA2	Upstate (06–600)	Rb	1:1000	No cross-reactivity noted