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# NMDA and dopamine interactions in the nucleus accumbens modulate cortical acetylcholine release

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## Abstract

The nucleus accumbens (NAC) plays a key role in directing appropriate motor output following the presentation of behaviorally relevant stimuli. As such, we postulate that accumbens efferents also participate in the modulation of neuronal circuits regulating attentional processes directed toward the identification and selection of these stimuli. In this study, *N*-methyl-D-aspartate (NMDA) and D1 ligands were perfused into the shell region of the NAC of awake rats. Cortical cholinergic transmission, a mediator of attentional processes, was measured via microdialysis probes inserted into the prefrontal cortex (PFC). NMDA perfusions (150 or 250 μM) into NAC resulted in significant increases in acetylcholine (ACh) efflux in PFC (150–200% above baseline levels). Co-administration of the D1 antagonist SCH-23390 (150 μM) markedly attenuated (by approx. 70%) ACh efflux following perfusions of 150 μM NMDA but not following 250 μM NMDA, suggesting that D1 receptor activity contributes to the ability of the lower but not the higher concentration of NMDA to increase cortical ACh release. Collectively, these data reveal a positive modulation of NMDA receptors by D1 receptors in NAC that is expressed *trans*-synaptically at the level of cortical transmission. This modulation may underlie the coordinated linking of attentional processes and motor output following exposure to salient and behaviorally relevant stimuli.

## Introduction

The nucleus accumbens (NAC) has long been recognized in its role as an interface between limbic input and motor output (Mogenson *et al*., 1980; Mogenson & Yang, 1991; Pennartz *et al*., 1994; Kelley, 1999). Consistent with this role, more recent functional characterizations of the NAC have focused on the selection of appropriate behaviors in response to novel (Rebec *et al*., 1997; Legault & Wise, 1999) and discriminative cues with incentive salience (Berridge & Robinson, 1998; Neigh *et al*., 2004; Nicola *et al*., 2004a,b; Yun *et al*., 2004a,b). This linking of motor responses to cues that predict reward is a central feature of motivated behavior.

Recent studies have focused on the role of the NAC in directing the activity of various ensembles of projection neurons to direct motor responses following the presentation of cues that predict reward (Schultz *et al*., 2003; Deadwyler *et al*., 2004; Ghitza *et al*., 2004). Far less effort, however, has been directed toward the NAC's modulation of neuronal circuits contributing to the attentional processing of these discriminative cues. Through experience animals become biased to direct their limited attentional resources toward the identification, selection and processing of stimuli that are novel or have acquired incentive salience (Franken, 2003; Sarter *et al*., 2003; Dalley *et al*., 2004a).

The basal forebrain cortical cholinergic system has been repeatedly demonstrated to play a key role in the mediation of attentional processing. Administration of muscarinic antagonists results in performance deficits on tasks that require attentional processing in humans (Mauri *et al*., 1994; Koller *et al*., 2003) and laboratory animals (Chudasama *et al*., 2004; Chen *et al*., 2004). Selective lesions of cholinergic inputs to cortex impair the performance of tasks that explicitly tax sustained and visuo-spatial attention in laboratory animals (McGaughy *et al*., 1996; Dalley *et al*., 2004b). Moreover, performance of these tasks is sufficient to increase the release of acetylcholine (ACh) in cortical regions (prefrontal and parietal) known to play key roles in the mediation of attentional processing (Himmelheber *et al*., 2000; Passetti *et al*., 2000; Dalley *et al*., 2001; Arnold *et al*., 2002). Recently, we have demonstrated that the magnitude of this stimulation in cortical ACh release is proportional to the degree of cognitive effort exerted in the performance of a sustained attention task (Kozak *et al*., 2005). Finally, a growing number of studies have reported improvements in attentional processing following nicotinic receptor agonists that, among other things, increase cortical cholinergic activity (Hahn *et al*., 2003; Kumari *et al*., 2003).

The NAC is one of several input structures that play a key role in regulating the excitability of the basal forebrain and hence cortical ACh release. Anatomically, γ-aminobutyric acid (GABA)ergic projections from the NAC terminate on cholinergic neurons within the basal forebrain (Zaborszky & Cullinan, 1992), and $GABA_A$ receptor activity within basal forebrain affects cortical ACh release (Moore *et al*., 1992). Our laboratory has conducted a series of studies demonstrating the effect of various transmitter manipulations in NAC on cortical ACh release. First, perfusion of *N*-methyl-D-aspartate (NMDA), AMPA or non-selective glutamate antagonists into the shell regions of the NAC was sufficient to stimulate ACh release in prefrontal cortex (PFC; Neigh-McCandless *et al*., 2002). Second, perfusion of D2, but not D1, antagonists into the NAC shell was sufficient to block the stimulation of cortical ACh release seen following systemic administration of a benzodiazepine-selective partial inverse agonist (Moore *et al*., 1999). Finally, performance in a task in which consumption of a mildly aversive liquid became a

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discriminative cue for access to a highly palatable cheese treat markedly increased cortical ACh release, and this stimulated release was attenuated following perfusion of tetrodotoxin (TTX) into the shell region of the NAC (Neigh *et al*., 2004).

The convergence of glutamatergic and dopaminergic inputs on medium spiny GABAergic projections of NAC provides a chemoanatomical substrate for understanding the linking of discriminative cues with motor output. Glutamate inputs from PFC, amygdala and hippocampus are involved in distinguishing between different discriminative cues (Schoenbaum *et al*., 1998; 1999). Dopaminergic inputs from ventral tegmental area (VTA) are activated following novel or salient stimuli (Legault & Wise, 1999; Rebec *et al*., 1997), particularly those cues that predict reward (Schultz *et al*., 2003; Nicola *et al*., 2004a,b). The interaction of these inputs has been best conveyed in theories speculating that dopamine selectively gates the corticolimbic inputs to NAC medium spiny neurons and results in the biasing toward certain behavioral responses at the expense of others (Pennartz *et al*., 1994; Nicola *et al*., 2004a,b; Yun *et al*., 2004a,b).

Guided by recent demonstrations of NAC glutamate–dopamine gating of cue-evoked goal-directed behavior (Yun *et al*., 2004a,b) and the clear anatomical and functional links between NAC efferents and the basal forebrain, we tested the hypothesis that NAC glutamate and dopamine receptor activity would modulate cortical ACh release. Such modulation would direct the recruitment of attentional resources, necessary for the coordinated interplay between discriminative cues in the environment and the appropriate behavioral response. More specifically, we determined whether NMDA perfusions into the NAC shell would affect cortical ACh release and, if so, whether D1 receptor activity positively modulates this effect as has been shown at several other levels of analysis (Snyder *et al*., 1998; O'Donnell, 1999; Vezina & Kim, 1999; Goto & O'Donnell, 2001b). In these experiments, different concentrations of NMDA were perfused into the shell region of the NAC, and changes in cortical ACh efflux were measured. The modulation of this effect by D1 receptors was assessed by studying the effects of NMDA co-administered with the D1 antagonist SCH-23390.

## Materials and methods

### *Subjects*

Male Fisher 344/Brown Norway F1 rats (Harlan Sprague–Dawley, Indianapolis, IN, USA) weighing between 300 and 400 g were utilized for all studies. Animals were maintained in a temperature- and humidity-controlled room on a 12 : 12 h light : dark cycle (lights on: 06.30 h) and individually housed in plastic cages lined with corn cob bedding (Harlan Teklad, Madison, WI, USA). Animals had access to food and water *ad libitum*. All procedures involving animals were approved by The Ohio State University Institutional Animal Care and Use Committee in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

Three days prior to surgery, animals were acclimated to the microdialysis testing environment in clear plastic bowls (35 cm height × 38 cm diameter; CMA, Stockholm, Sweden) lined with corn cob bedding. Animals remained in the bowls for a minimum of 4 h/day, and returned to their home cages at the end of each acclimation period.

### *Surgery*

Following their 3 days of acclimation, animals were anesthetized using isoflurane gas (2%, 0.6 L/min, $O_2$ delivery) and unilaterally implanted with microdialysis guide cannulae (0.38 mm o.d.; Sci Pro, Sanborn, NY, USA) into the medial prefrontal cortex (mPFC; in mm from bregma: AP +4.2, ML ±0.6, DV −0.6 with the guide tip at 20 ° rostral) and ipsilateral NAC shell (NAC; in mm from bregma: AP +1.3, ML ±1.0, DV −5.8). The PFC was selected because its role in the top-down regulation of attentional processing (see Sarter *et al*., 2001) suggests that it should be affected by motivational factors mediated by mesolimbic regions such as the NAC. The shell region of the NAC was selected because it (as opposed to the NAC core) projects directly to the cholinergic-rich areas of the basal forebrain (Zaborszky & Cullinan, 1992). Coordinates were determined from the atlas of Paxinos & Watson (1998). Hemispheres were counterbalanced in each experiment. Cannulae were inserted with dummy stylets to prevent occlusion, and were fixed using dental cement and three stainless steel skull screws. Animals received a prophylactic dose of the antibiotic amoxicillin (100 mg/kg) subcutaneously, and the surgical site was swabbed with a topical antibiotic ointment (lidocaine, 5%). Animals were allowed to recover for 3 days following surgery while continuing to be acclimated daily to the testing environment.

### *General microdialysis procedures*

Microdialysis sessions were conducted using repeated perfusions, with each animal receiving three different pharmacological manipulations, one every other day. This repeated testing paradigm has the advantage of decreasing variability among treatment conditions because each subject is able to serve as his own control. Furthermore, it allows for paradigms such as dose–response analyses, as well as agonist–antagonist interactions to be studied in the same animal. The procedure has been repeatedly validated by demonstrating that basal cortical ACh efflux does not significantly change over repeated dialysis sessions, and that the effects of behavioral, pharmacological or sensory manipulations on ACh levels do not interact with dialysis sessions (Bruno *et al*., 1999; Nelson *et al*., 2002).

On the fourth day following surgery, animals were brought to the testing environment and allowed to acclimate for 30 min prior to insertion of microdialysis probes. Following the 30-min acclimation period, stylets were removed and probes (Sci Pro, 0.2 mm o.d., 3 mm membrane tip for mPFC, 0.2 mm o.d., 2.0 mm membrane tip for NAC) were inserted into each guide.

Probes were continuously perfused with artificial cerebral spinal fluid (aCSF) (containing, in mM: NaCl, 166.5; $NaHCO_3$, 27.5; KCl, 2.4; $CaCl_2$, 1.2; $Na_2SO_4$, 0.5; $KH_2PO_4$, 0.5; glucose, 1.0, pH 6.8) at a flow rate of 1.25 μL/min. This perfusion medium did not contain an acetylcholinesterase inhibitor. A 3-h washout period was observed after probe insertion to allow ACh efflux to reach a stable baseline that was maximally sensitive to TTX before beginning collections (Moore *et al*., 1992). Three distinct experiments were conducted using this general paradigm; they are described in detail as follows.

The concentrations of NMDA and SCH-23390 utilized in these microdialysis experiments were selected from a review of several studies in the literature. Local perfusions of NMDA, via reverse dialysis, in concentrations of 100–500 μM NMDA produced concentration-dependent changes in basal and stress-induced dopamine release in PFC (Del Arco & Mora, 2001; Lorrain *et al*., 2003). Thus, we chose to study two concentrations of NMDA, 150 and 250 μM, which were in a range shown to have effects on forebrain neurochemistry. Likewise, intrahippocampal perfusions of SCH-23390 (100–250 μM) attenuated NMDA-induced locomotion and dopamine release (Zornoza *et al*., 2005), whereas intrastriatal perfusions of SCH-23390 (10 μM) attenuated NMDA-induced increases in glutamate release in substantia nigra (Marti *et al*., 2002). Thus, our

selection of 150 μM SCH-23390 was within the range of concentrations known to attenuate certain actions of NMDA.

### *Experiment 1: the effects of a D1 antagonist on the lower concentration of NMDA*

Animals ($n = 8$) were tested three times, with a different pharmacological manipulation (vehicle–aCSF, 150 μM NMDA, or 150 μM SCH-23390 + 150 μM NMDA) administered, in counterbalanced order, every other day. Following the 3-h washout period, four baseline samples were collected; 15-min collection intervals were observed with all time points. Following the four baseline collections, the syringe was switched from aCSF to one containing aCSF + the D1 antagonist, SCH-23390 (aCSF was perfused for an additional two collections in the vehicle and NMDA sessions). Following a 15-min washout, two additional collections of either aCSF or aCSF + antagonist were taken. The syringe was then switched to one containing aCSF + drug (150 μM NMDA, 150 μM SCH-23390 + 150 μM NMDA, or aCSF vehicle). Following a 15-min washout, four additional collections were taken before switching the syringe back to one containing only aCSF. This perfusion lasted a total of 60 min, including an initial 15-min washout period. Following the last collection, probes were removed and stylets were reinserted before returning animals to their home cages. A separate group of rats ($n = 4$) was tested once and served as timed-controls for the effects of the D1 antagonist over the entire interval in which NMDA would have been perfused. In this group, SCH-23390 (150 μM) was perfused after the fourth baseline collection until the time at which drugs are typically removed and aCSF was re-introduced.

### *Experiment 2: the effects of a D1 antagonist on the higher concentration of NMDA*

Animals ($n = 9$) were tested three times, with a different pharmacological manipulation (vehicle–aCSF, 250 μM NMDA, or 150 μM SCH-23390 + 250 μM NMDA) administered, in counterbalanced order, every other day. Following the 3-h washout period, four baseline samples were collected; 15-min collection intervals were observed with all time points. Following the four baseline collections, the syringe was switched from aCSF to one containing aCSF + the antagonist, SCH-23390 (aCSF was perfused for an additional two collections in the vehicle and NMDA sessions). Following a 15-min washout, two additional collections of either aCSF or aCSF + antagonist were taken. The syringe was then switched to one containing aCSF + drug (150 μM NMDA, 150 μM SCH-23390 + 150 μM NMDA, or aCSF vehicle). Following a 15-min washout, four additional collections were taken before switching back to one containing only aCSF. This perfusion lasted a total of 60 min, including an initial 15-min washout period. Following the last collection, probes were removed and stylets were reinserted before returning animals to their home cages. A separate group of rats ($n = 4$) was tested once and served as timed-controls for the effects of the D1 antagonist over the entire interval in which NMDA would have been perfused. In this group, SCH-23390 (150 μM) was perfused after the fourth baseline collection until the time at which drugs are typically removed and aCSF was re-introduced.

### *HPLC analysis*

Dialysis samples were stored at −80 °C until analysed using high-performance liquid chromatography (HPLC) with electrochemical detection. A volume of 15 μL of each sample was injected by an autosampler (ESA, Chelmsford, MA, USA). ACh and choline were separated by a C-18 polymer column (ESA; 250 × 3 mm) using a sodium phosphate mobile phase (in mM: $Na_2HPO_4$, 100.0; TMACl, 0.5; 1-octanesulphonic acid, 2.0; 0.005% microbicide reagent MB, pH = 8.0; flow rate of 0.5 mL/min). A pre-column immobilized enzyme reactor (IMER; ESA) was utilized to hydrolyse choline, allowing for greater separation between choline and ACh peaks. ACh and choline were then hydrolysed post-column by an additional enzyme reactor (ESA), converted to $H_2O_2$ (Potter *et al*., 1983), and quantified using a peroxidase-wired ceramic glassy carbon electrode (Model #5041 microdialysis analytical cell, ESA), with an applied potential of −200 mV (Huang *et al*., 1995). The detection limit under these conditions was approximately 1.0 fmol/15 μL injection.

### *Histology*

Following the final microdialysis session, animals were given an overdose of sodium pentobarbital and transcardially perfused with 0.9% heparinized saline followed by 10% formalin. Brains were removed and stored in 10% formalin for 24 h then transferred to a 30% sucrose solution for at least 3 days. Brains were sectioned using a cryostat; 50-μm sections were mounted on slides, stained using Cresyl violet, and examined under a light microscope. Subjects whose probe placements were located outside of either the mPFC or NAC shell were excluded from further analysis.

### *Data analysis*

For each experiment, changes in basal ACh efflux (fmol/15 μL) across sessions and treatment groups were analysed using one-way repeated-measures analyses of variance (ANOVAs). Basal efflux was then defined as the mean of the four baseline collections, and subsequent data were expressed as percent change from that mean baseline. Statistical analyses of drug effects were conducted using a two-way, within-subjects ANOVA with drug GROUP and TIME as within-subjects measures. Two-way ANOVAs were utilized to examine differences between specific treatment groups where appropriate. One-way ANOVAs were also used to determine the time that post-drug ACh efflux levels returned to baseline where appropriate. A minimum number of *t*-tests was also utilized to examine differences between drug conditions at specific time points. Significance was defined as $P < 0.05$, and the Huynh–Feldt correction was utilized to reduce Type 1 errors associated with repeated-measures ANOVAs (Vasey & Thayer, 1987). All statistical tests were preformed using SPSS for windows (version 12.0).

## Results

### *Guide cannulae placements*

Figure 1 shows representative placements of these areas. Any animals whose probe placements fell outside the mPFC and/or the NAC shell were excluded from further analysis.

### *Experiment 1: the effects of a D1 antagonist on a moderate concentration of NMDA*

This experiment examined the contribution of D1 receptor activity to the ability of a moderate concentration of NMDA to stimulate cortical ACh release. Figure 2 illustrates the effects of aCSF vehicle, 150 μM

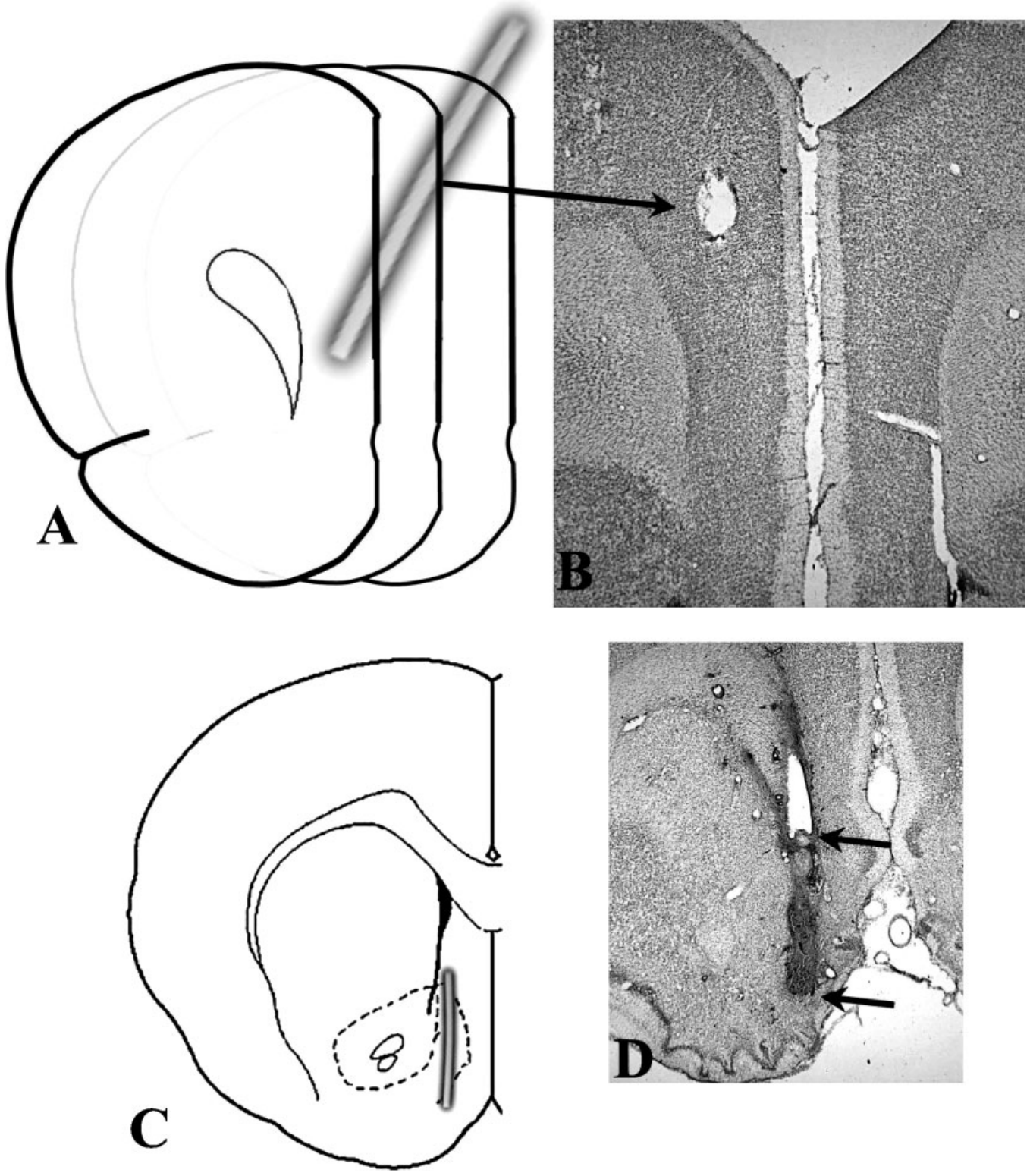


FIG. 1. (A) A schematic representation of a mPFC probe placement. Guides were implanted so that when probes were inserted, the membrane tip (3.0 mm) was located at: AP +4.2, ML ±0.6, DV −0.6 from dura at 20 ° rostral. (B) A photomicrograph from one of the subjects that is representative of the mPFC placement illustrated in (A). The arrow indicates the end of the guide cannula; the probe extends 3 mm beyond that point. (C) A schematic representation of a NAC shell probe placement. Guides were implanted so that when probes were inserted (2.0 mm active tip) the membrane tip was located at AP +1.3, ML ±1.0, DV −5.8 from dura matter. (D) A photomicrograph from a NAC shell placement. The area between the arrows represents the active membrane tip. All of the above coordinates were calculated according to Paxinos & Watson (1998).

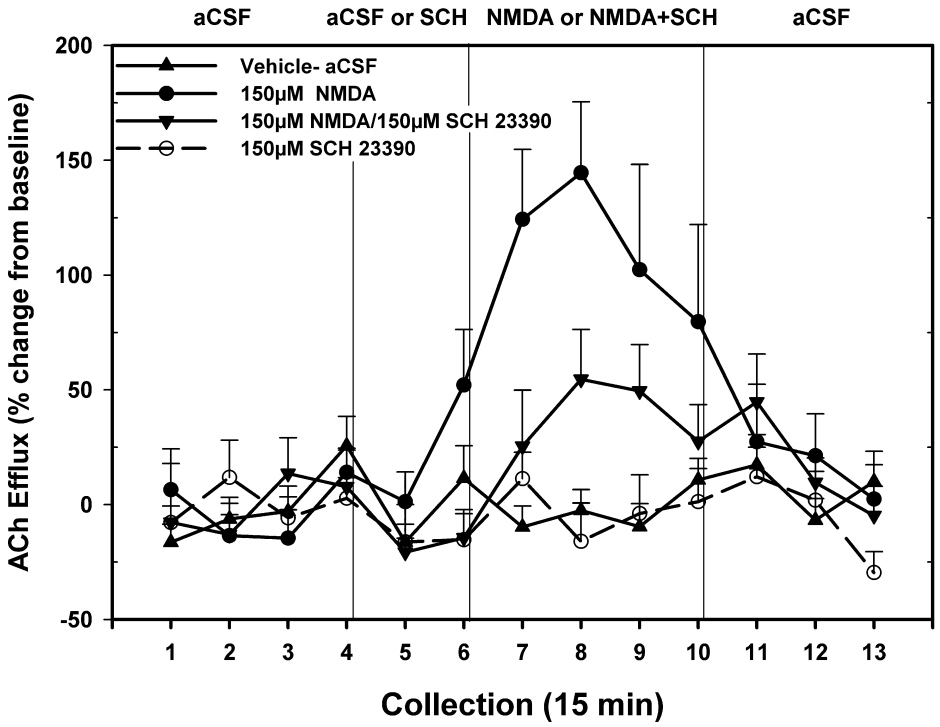


FIG. 2. Mean (± SEM) acetylcholine (ACh) efflux in the mPFC of animals ($n = 8$) receiving, in counterbalanced order, vehicle (artificial cerebrospinal fluid, aCSF), a moderate concentration of *N*-methyl-D-aspartate (NMDA) alone (150 μM), and co-administration of a D1 antagonist SCH-23390 + NMDA into the NAC shell during three separate dialysis sessions. Following baseline collections (collections 1–4), vehicle or the antagonist was administered for 30 min (collections 5–6). This was followed by administration of aCSF + drug (vehicle, NMDA, or SCH-23390 + NMDA) for 60 min (collections 7–10). Upon conclusion of the 60-min perfusion, aCSF alone was perfused for 45 min until the end of the dialysis period (collections 11–13). NMDA alone significantly increased ACh efflux above vehicle, and co-administration of the D1 antagonist SCH-23390 + NMDA partially, though significantly, attenuated the effect of the moderate concentration of NMDA. A separate group of animals was tested as time-controls for the prolonged effects of perfusion of SCH-23390 (dotted line). It is clear that the extended perfusion of the D1 antagonist had no effect on cortical ACh efflux, indicating that the effect of SCH-23390 was in interaction with the administration of NMDA.

NMDA and 150 μM SCH-23390 + 150 μM NMDA on cortical ACh efflux ($n = 8$). Basal levels of efflux were stable over the three dialysis SESSIONs ($F_{2,16} = 3.244$, $P = 0.07$), and across the three treatment GROUPs ($F_{2,16} = 0.546$, $P = 0.591$), as revealed by one-way ANOVAs. Basal levels of ACh (mean ± SEM, fmol/15 μL) were 6.9 ± 2.6, 4.4 ± 1.2 and 5.9 ± 1.5 for aCSF, 150 μM NMDA, and 150 μM SCH-23390 + 150 μM NMDA sessions, respectively. Given that basal levels of ACh efflux did not differ over session or group, all subsequent values were expressed and analysed as a percent change from session baseline. Intra-NAC perfusions of drugs led to differential effects on cortical ACh efflux (GROUP, $F_{2,14} = 8.652$, $P = 0.005$). These effects varied across collection interval (TIME, $F_{12,84} = 5.958$, $P < 0.001$; GROUP × TIME, $F_{24,168} = 3.231$, $P = 0.001$). Considering the overall main effects comparing all three treatment groups, a series of smaller two-way ANOVAs was conducted to look for differences between pairs of treatment groups. Administration of 150 μM NMDA significantly increased ACh levels above the vehicle aCSF session (GROUP, $F_{1,7} = 11.655$, $P = 0.011$; TIME, $F_{12,84} = 4.853$, $P < 0.001$; GROUP × TIME, $F_{12,84} = 4.591$, $P < 0.001$). NMDA was significantly higher than aCSF within the first 15 min of perfusion (collection 7, $t_7 = -4.705$, $P = 0.002$), and levels became comparable between the two treatment groups by the end of the 60-min perfusion period (collection 10, $t_7 = -1.470$, $P = 0.185$). Moreover, ACh levels from collection 10, in both treatment groups, were no longer elevated above their respective baseline values.

Co-administration of a D1 antagonist attenuated the NMDA-induced increases in ACh, as efflux following 150 μM NMDA differed significantly from that seen following 150 μM SCH-23390 + 150 μM NMDA (GROUP, $F_{1,7} = 7.025$, $P = 0.033$; TIME, $F_{12,84} = 6.197$, $P < 0.001$; GROUP × TIME, $F_{12,84} = 2.289$, $P = 0.038$). The D1 antagonist suppressed NMDA-induced levels during collection 7 and 8 (both $P < 0.05$). The D1 attenuation of the NMDA effect is also supported by the observation that 150 μM SCH-23390 + 150 μM NMDA did not differ from the vehicle aCSF session (GROUP, $F_{1,7} = 3.395$, $P = 0.108$), although there was an effect of TIME ($F_{12,84} = 2.751$, $P = 0.003$) and a GROUP–TIME interaction ($F_{12,84} = 2.320$, $P = 0.013$). The D1 receptor-mediated attenuation seen following co-administration of NMDA + D1 antagonist was not complete. Administration of NMDA + SCH-23390 yielded significant increases, above the aCSF vehicle session, during collections 8 and 9 (corresponding to 30 and 45 min of double drug perfusion). ACh efflux following perfusion of the antagonist alone (collections 5 and 6) did not differ from any of the baseline points (all $P > 0.05$), indicating that perfusion of D1 antagonist alone did not affect ACh release. Finally, in a separate group of animals ($n = 4$) we determined the effects of SCH-23390 perfusions throughout the period during which NMDA + SCH-23390 were perfused. There was no significant deviation of ACh efflux from the aCSF control condition as the mean (SEM) efflux (% change from baseline) was −6.5 ± 5.3 ($F_{12} = 0.641$, $P = 0.793$). These data are represented as dotted lines in Figs 2 and 3.

### *Experiment 2: the effects of a D1 antagonist on a higher concentration of NMDA*

This experiment determined whether D1 receptor activity contributed to the ability of a *higher* concentration of NMDA to stimulate cortical

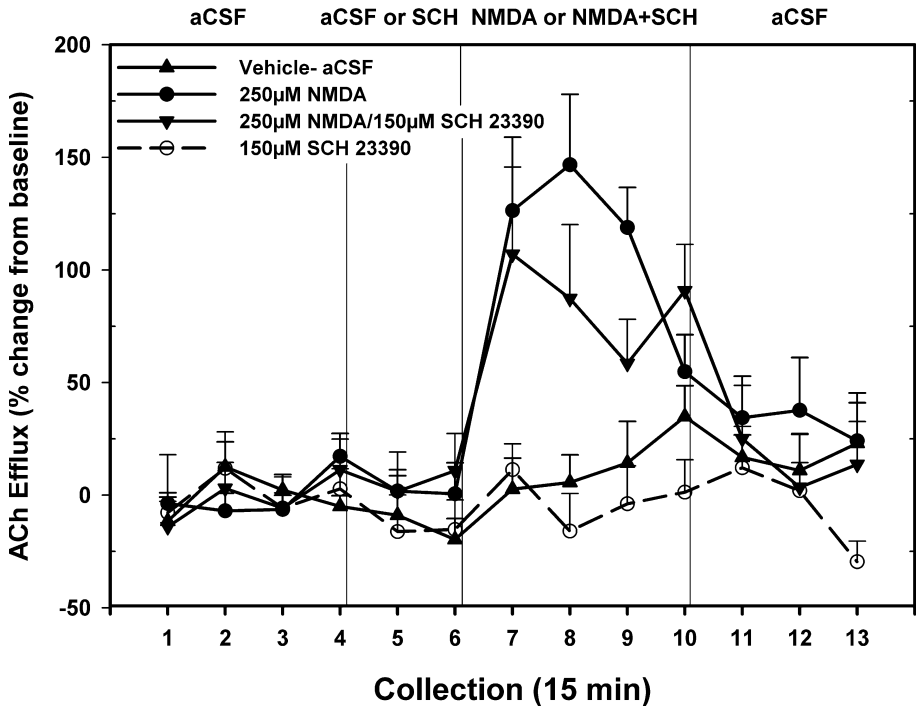


FIG. 3. Mean (± SEM) acetylcholine (ACh) efflux in the mPFC of animals ($n = 9$) receiving, in counterbalanced order, vehicle (artificial cerebrospinal fluid, aCSF), a high concentration of *N*-methyl-D-aspartate (NMDA) alone (250 μM), and co-administration of a D1 antagonist SCH-23390 + NMDA into the NAC shell during three separate dialysis sessions. Following baseline collections (collections 1–4), vehicle or the antagonist was administered for 30 min (collections 5–6). This was followed by administration of aCSF + drug (vehicle, NMDA, or SCH-23390 + NMDA) for 60 min (collections 7–10). Upon conclusion of the 60-min perfusion, aCSF alone was perfused for 45 min until the end of the dialysis period (collections 11–13). NMDA alone significantly increased ACh efflux above vehicle. Unlike the case with 150 μM NMDA, co-administration of the D1 antagonist SCH-23390 + 250 μM NMDA did not attenuate the effect of the high concentration of NMDA. A separate group of animals was tested as time-controls for the prolonged effects of perfusion of SCH-23390 (dotted line). It is clear that the extended perfusion of the D1 antagonist had no effect on cortical ACh efflux.

ACh efflux as it does following a more moderate concentration of NMDA (Experiment1). Figure 3 illustrates the effects of 250 μM NMDA and 150 μM SCH-23390 + 250 μM NMDA on cortical ACh efflux ($n = 9$). Basal levels of ACh efflux were stable over both SESSIONs ($F_{2,16} = 0.052$, $P = 0.950$), and treatment GROUPs ($F_{2,16} = 3.089$, $P = 0.073$), as shown by a one-way ANOVA. Basal levels of ACh (mean ± SEM, fmol/15 μL) were 7.1 ± 1.7, 4.9 ± 0.8 and 10.3 ± 1.9 for aCSF, 250 μM NMDA, and the 150 μM SCH-23390 + 250 μM NMDA sessions, respectively. Given that basal levels of ACh efflux did not differ over session or group, all values were expressed as a percent change from session baseline. An overall analysis revealed significant differences among treatment groups and collection intervals (GROUP, $F_{2,16} = 13.986$, $P < 0.001$; TIME, $F_{12,96} = 9.674$, $P < 0.001$; GROUP × TIME, $F_{24,192} = 2.987$, $P < 0.001$).

A series of smaller two-way ANOVAs was conducted to look for differences between pairs of treatment groups. Administration of 250 μM NMDA significantly elevated ACh levels above aCSF levels (GROUP, $F_{1,8} = 33.164$, $P < 0.001$; TIME, $F_{12,96} = 8.533$, $P < 0.001$; GROUP × TIME, $F_{12,96} = 5.522$, $P < 0.001$). Pairwise comparisons revealed NMDA-induced increases in ACh efflux during collections 7, 8 and 9 (all $P < 0.05$). As was the case with the lower concentration, ACh levels fell to control aCSF values despite the continued perfusion of NMDA during collection 10.

Three analyses support the conclusion that co-perfusion of the D1 antagonist did not attenuate the ability of the higher concentration of NMDA to stimulate ACh efflux. First, overall ACh efflux from NMDA alone did not differ from efflux induced by administration of the NMDA + SCH-23390 (GROUP, $F_{1,8} = 3.883$, $P = 0.084$). Second, administration of 150 μM SCH-23390 + 250 μM NMDA significantly elevated ACh efflux above aCSF levels (GROUP, $F_{1,8} = 8.405$, $P = 0.020$). Levels from both 250 μM NMDA alone and 250 μM NMDA + 150 μM SCH-23390 were significantly higher than those following aCSF beginning at the first post-drug time point (collection 7, both $P < 0.01$), but did not differ from one another ($t_8 = 0.494$, $P = 0.635$). By the end of the 60-min perfusion period, there were no significant differences among any of the drug treatments (collection 10, all $P > 0.05$). Finally, administration of NMDA + SCH-23390 (TIME, $F_{12,96} = 4.048$, $P = 0.001$) yielded significant increases relative to baseline values in this group (all $P < 0.05$). Importantly, perfusion of the antagonist alone (collections 5 and 6) yielded no increases above any of the baseline points (all $P > 0.05$), indicating the antagonist alone did not differentially influence ACh release.

## Discussion

The aim of these studies was to test the capacity of D1 receptors in the NAC to positively modulate local glutamate activity based on the degree of NMDA receptor activation, and to determine whether this modulation could be shown *trans*-synaptically at the level of ACh release in mPFC. Several major findings can be reported. First, intra-accumbens administration of either 150 μM or 250 μM concentration of NMDA significantly elevated ACh efflux above baseline in the mPFC. Second, the D1 receptor antagonist SCH-23390 significantly, though not completely, attenuated the effect of the lower concentration of NMDA, supporting the hypothesis of D1 receptor activity as a

positive modulator of NMDA function. Third, the D1 antagonist was not able to attenuate ACh efflux induced by the higher concentration of NMDA, indicating that the contribution of D1 receptor activity may interact with the level of NMDA receptor activation to stimulate cortical ACh release. The following discussion will address several issues raised by these experiments.

### *Intra-accumbens NMDA-induced increases in cortical ACh*

Our present studies illustrate that increases in NMDA receptor activity in the shell of the NAC are sufficient to increase ACh distally in the mPFC. Interestingly, there were no differences between the effects of 150 μM and 250 μM concentrations of NMDA; administration of each resulted in ACh increases approximately 150% above baseline. This may reflect a similar degree of receptor activation produced by the two concentrations. It is possible that the lower NMDA concentration saturates available receptors or other transduction mechanisms. Given that we are measuring *trans*-synaptic effects of NMDA receptor stimulation, it is also possible that excitatory inputs to basal forebrain (or locally within cortex) are similar following the perfusion of each concentration of NMDA, and that these inputs, rather than the degree of NMDA receptor activity, are limiting the amount of ACh released. Pilot studies in our laboratory have revealed that perfusions of 75 μM NMDA are insufficient to stimulate ACh efflux. We are currently determining whether concentrations between 75 and 150 μM NMDA will yield an elevation of ACh release that is significant but less than that seen following 150 or 250 μM NMDA.

The NMDA-induced increase in cortical ACh release was, unexpectedly, seen under baseline conditions. This effect stands in contrast to previous experiments with NMDA infusions into basal forebrain in which stimulation of cortical ACh release only occurred following concomitant activation with a behavioral stimulus (Fadel *et al*., 2001). NMDA receptor activation is both ligand and voltage dependent, which suggests that additional sources are providing local depolarization in the NAC following NMDA perfusions. NMDA receptor activation has been shown to be dependent on depolarization by AMPA and metabotropic glutamate receptors (Hu & White, 1998; Taverna & Pennartz, 2003), though there may be influences other than glutamate impacting NMDA activity as well. For example, intra-accumbens activation of DA receptors increases glutamate transmission (Dalia *et al*., 1998), and this may serve as a source of necessary depolarization.

The neuronal circuitry underlying the ability of NMDA receptors in NAC to stimulate ACh release in PFC is presently unknown. *A priori*, there are several possibilities. As discussed in the Introduction, NMDA (and dopamine) receptors modulate the firing rates of GABAergic projections, which in turn regulate the excitability of basal forebrain neurons (Mogenson *et al*., 1983). Basal forebrain neurons can then regulate cortical ACh release directly via cholinergic projections from substantia innominata to the entire neocortical mantle (Zaborszky *et al*., 1999; Semba, 2000). The basal forebrain can also affect cortical ACh release indirectly via a ventral pallidal–thalamocortical circuit (Maurice *et al*., 1997). Local increases in AMPA receptor activity have recently been shown to increase ACh release in prefrontal and posterior parietal cortex (Nelson *et al*., 2005), and this glutamate receptor activity could arise, in part, from thalamic inputs. NAC efferents could also indirectly affect cortical ACh release via recurrent projections to the VTA. The VTA could then, in turn, influence basal forebrain excitability via dopaminergic projections to PFC (Seamans & Yang, 2004). In this regard, local D1 receptor activity has recently been shown to modulate ACh efflux in PFC (Laplante *et al*., 2004). We are currently conducting experiments to assess the relative contributions of basal forebrain and VTA in the stimulatory affects of accumbens NMDA on cortical ACh efflux. We are also conducting control experiments to determine whether diffusion of NMDA out of the shell to other regions of the NAC or dorsal striatum would be sufficient to stimulate cortical ACh release, although the efferent projections of the shell to both the basal forebrain and the VTA make perfusions in this area the likely locus of action.

### *Antagonism of D1 receptors interacts with the concentration of NMDA*

Although there may be a lack of dose responsivity between the two concentrations of NMDA, differences are seen when considering each concentration's interaction with the D1 antagonist. Differential interactions between NMDA and SCH-23390 were very clear, as administration of SCH-23390 significantly attenuated the NMDA-induced increase from the lower concentration of NMDA, but had no effect on ACh efflux following the higher concentration of NMDA. Local NMDA receptor activity has been shown to stimulate dopamine release within NAC (Grace, 1991; Floresco *et al*., 2001; Howland *et al*., 2002), and this may contribute to the ability of D1 receptor activity to modulate the effects of NMDA on cortical ACh release. While the mechanisms remain to be specified, our present data suggest that the higher concentration of NMDA was sufficient to stimulate NAC efferents regulating ACh release, whereas the lower concentration required some positive modulation by D1 receptors in order to achieve much of its impact on cortical ACh release. It remains possible, however, that a higher concentration of SCH-23390 might be required to attenuate the effects of 250 μM NMDA. However, 100 μM of SCH-23390 has been recently shown to attenuate the ability of perfused NMDA (250 μM) to stimulate locomotor behavior and local dopamine release (Zornoza *et al*., 2005). It also remains possible, although not likely, that the effects of SCH-23390 reflected the drug's ability to affect 5HT receptors (Alburges *et al*., 1992; Millan *et al*., 2001). A test of this hypothesis will require the use of a more selective D1 receptor antagonist.

The exact mechanisms underlying the cooperativity between D1 receptor activity and the lower concentration of NMDA in modulating cortical ACh release are yet to be determined. It is certainly possible that this cooperative interaction begins at the level of common NAC efferents. NAC medium spiny projection neurons contain ionotropic (NMDA, AMPA/kainate) and metabotropic glutamate receptors, as well as D1, D2, D3 and D4 receptors (Jongen-Relo *et al*., 1995; Tarazi *et al*., 1998; Lu *et al*., 1999). These inputs converge in close proximity to one another on the dendrites of NAC medium spiny projection neurons (Sesack & Pickel, 1990). Such a dense innervation allows for rich and complex interactions between glutamate and dopamine in the NAC. The cooperative modulation seen in this experiment is consistent with a number of other studies that have characterized NAC D1 and NMDA interactions at several levels of analysis. At the level of receptor-linked transduction mechanisms, D1 receptors regulate the affinity of the NMDA receptor by increasing the phosphorylation and decreasing the dephosphorylation (via a PKA/DARP-32/PP-1 pathway) of the NR1 subunit of the NMDA receptor (Snyder *et al*., 1998). D1 receptors have also been shown to regulate the trafficking of NMDA receptors in striatum (Dunah & Standaert, 2001) and also AMPA receptors in NAC (Chao *et al*., 2002). In hippocampus, there is evidence of an even more direct D1–NMDA receptor interaction as D1 receptors are involved in the inhibition of NMDA-mediated excito-

toxicity via actions on NMDA-gated currents (Lee *et al*., 2002). At the level of target cell excitability, D1 receptors potentiate NMDA-mediated excitation of medium spiny projection neurons in dorsal striatum (see Cepeda & Levine, 1998 for review) and in NAC (Goto & O'Donnell, 2001a,b; West & Grace, 2002; Charara & Grace, 2003; O'Donnell, 2003). Finally, positive interactions between D1 and NMDA receptors have been demonstrated at the level of locomotor activity, instrumental responding and conditioned reinforcement (Burns *et al*., 1994; Smith-Roe & Kelley, 2000).

### *The complexities of accumbens glutamate receptor mediation of cortical ACh release*

The ability of NMDA receptor *activation* to increase cortical ACh efflux is surprising given a recent report from our laboratory demonstrating that *blockade* of ionotropic glutamate receptors (NMDA and/or AMPA/kainate) in the NAC increased ACh release in PFC (Neigh-McCandless *et al*., 2002). Importantly, these iGluR antagonists were perfused through similar probes directed at identical coordinates as in the present study. Thus, the seemingly contradictory results do not likely reflect methodological differences. An inspection of the literature reveals other reports of apparent paradoxical neurochemical and behavioral effects of local administration of NMDA and NMDA antagonists. For example, intra-NAC infusions of both NMDA (Svensson *et al*., 1994) and NMDA antagonists (Ault & Werling, 1999) have been reported to increase local dopamine release. At the behavioral level, locomotor activity is enhanced following intra-NAC infusions of either NMDA (Svensson *et al*., 1994; David *et al*., 2004) or NMDA antagonists (Kelley & Throne, 1992; David *et al*., 2004).

The puzzling results of our experiments on NMDA mediation of cortical ACh release, and the above studies on dopamine release or locomotor activity, are only contradictory if one presumes that the infused or perfused drugs are acting at precisely the same receptors. While possible, the likelihood that multiple receptors (subtypes with different NR2 compositions) located on several populations of NAC neurons (e.g. interneurons, medium spiny projection neurons to basal forebrain or to VTA) are differentially stimulated by bolus or perfused administration of NMDA agonists and antagonists is considerable. With respect to our finding that both NMDA and CPP stimulate cortical ACh release, we are currently testing the hypothesis that NMDA agonists and antagonists exert similar effects on cortical cholinergic transmission via their influence on different populations of NAC interneurons or efferents that directly or indirectly regulate cortical ACh release (see above).

### *Functional implications*

The NAC has long been shown to be critical as an integrator of limbic and motor information (Kelley, 1999), in reward circuits (see Deadwyler *et al*., 2004) and in the attribution of incentive salience (Berridge & Robinson, 1998). In these roles, it seems appropriate that information processing within the NAC influences both motor output and cognitive processes. The ability of NAC efferents to modulate motor output has been well documented over the past several decades (for review, see Tzschentke & Schmidt, 2000). In addition, the convergence of excitatory information from cortex, hippocampus and amygdala in NAC has also become the focus of recent studies (Groenewegen *et al*., 1999; O'Donnell, 1999; Grace, 2000). It is vital that this convergence of information then modulates the detection and selection of subsequent stimuli for processing, and this involves the recruitment of attentional resources. The ability of dopaminergic and glutamatergic transmission in NAC to modulate cortical ACh release may be a chemoanatomical representation of such recruitment.

In conclusion, the present studies demonstrate that D1 receptors are stimulated following perfusion of NMDA into the NAC and that this stimulation contributes significantly to the ability of certain concentrations of NMDA to increase cortical ACh release. As such, these findings contribute to a large body of data revealing a positive modulation of NMDA receptors by D1 receptors in NAC. The novelty of the present study is the demonstration that this modulation is expressed *trans*-synaptically at the level of cholinergic transmission within the PFC. Further research is required to uncover the complexities of these interactions, and to determine the contribution of these interactions to the allocation of attention and the processing of behaviorally relevant stimuli.

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## Abbreviations

ACh, acetylcholine; aCSF, artificial cerebrospinal fluid; GABA, γ-aminobutyric acid; HPLC, high-performance liquid chromatography; NAC, nucleus accumbens; NMDA, *N*-methyl-D-aspartate; PFC, prefrontal cortex; TTX, tetrodotoxin; VTA, ventral tegmental area.

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