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Nitric oxide in the medial prefrontal cortex contributes to the acquisition of cocaine place preference and synaptic plasticity in the laterodorsal tegmental nucleus

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Abstract

Nitric oxide (NO), a gaseous neurotransmitter, is involved in a variety of brain functions, including drug addiction. Although previous studies have suggested that NO plays an important role in the development of cocaine addiction, the brain region(s) in which NO acts and how it contributes to cocaine addiction remain unclear. In this study, we examined these issues using a cocaine-induced conditioned place preference (CPP) paradigm and *ex vivo* electrophysiological recordings in rats. Specifically, we focused on the medial prefrontal cortex (mPFC) and laterodorsal tegmental nucleus (LDT), brain regions associated with cocaine CPP development and cocaine-induced plasticity. Intra-mPFC injection of the non-selective NO synthase (NOS) inhibitor L-NAME or the neuronal NOS (nNOS) selective inhibitor L-NPA during the conditioning phase disrupted cocaine CPP. Additionally, intra-mPFC injection of L-NPA prior to each cocaine injection prevented the induction of presynaptic plasticity, induced by repeated cocaine administration, in LDT cholinergic neurons. These findings indicate that NO generated in the mPFC contributes to the acquisition of cocaine CPP and the induction of neuroplasticity in LDT cholinergic neurons. Together with previous studies showing that NO induces membrane plasticity in mPFC neurons, that mPFC neurons project to the LDT, and that LDT activity is critical for the acquisition of cocaine CPP, the present findings suggest that NO-mediated neuroplasticity induced in the mPFC-LDT circuitry is critical for the development of cocaine addiction.

Keywords

nitric oxide synthase, laterodorsal tegmental nucleus; cocaine; dopamine; addiction; medial prefrontal cortex

1. Introduction

Nitric oxide (NO) is a gaseous neurotransmitter associated with a variety of physiological functions and with neuroplasticity related to learning and memory [1]. Additionally, NO has been reported to play an important role in addictive behaviors induced by cocaine [2]. A previous study revealed that systemic injection of an NO synthase (NOS) inhibitor attenuates cocaine-induced conditioned place preference (CPP) and that knockout of neuronal NOS (nNOS) abolishes development of cocaine CPP [3]. Although these findings indicate a critical role for NO produced by neuronal cells in cocaine addiction, the brain regions in which NO acts and how it contributes to the development of cocaine addiction remain to be determined.

The medial prefrontal cortex (mPFC) constitutes a part of the brain's reward circuitry and is associated with learning, memory, and decision making [4, 5, 6, 7]. Systemic cocaine administration increases the extracellular NO levels in the mPFC [8] and induces plasticity in the membrane properties of mPFC pyramidal cells in an NO-dependent manner [9, 10]. Additionally, lesion of the mPFC suppresses the expression of cocaine CPP [11]. The mPFC sends excitatory projections to the laterodorsal tegmental nucleus (LDT) [12, 13], which projects to the ventral tegmental area (VTA) [14, 15] and is critical for reward information processing as well as cocaine CPP development [16, 17, 18, 19]. We have previously reported that, in *ex vivo* electrophysiological recordings, repeated cocaine administration induces synaptic plasticity in LDT cholinergic neurons [16]. The plasticity in the LDT was inhibited when a NOS inhibitor was systemically administered prior to cocaine injection, suggesting an NO-dependent induction of plasticity. Moreover, inactivation of the mPFC inhibits the induction of LDT plasticity [16]. These findings suggest possible interactions among NOS activity in the mPFC, LDT plasticity, and cocaine CPP development. We hypothesize that NO released in the mPFC by cocaine administration activates mPFC neurons, leading to the induction of synaptic plasticity in the LDT and the development of cocaine CPP. Thus, in the present study, we address this question using a cocaine CPP paradigm with an intra-mPFC injection of NOS inhibitors

and *ex vivo* whole-cell patch-clamp recordings from LDT cholinergic neurons, which were obtained from rats that had received repeated cocaine administrations with an intra-mPFC injection of a NOS inhibitor.

2. Materials and methods

2.1. Animals

Male Sprague-Dawley rats weighing 180–260 g (8–10 weeks old) at the beginning of behavioral tests were maintained in a temperature-controlled (22 ± 1 °C) room under a 12-h light/dark cycle with food and water available *ad libitum*. All experiments were conducted in accordance with the National Institutes of Health guidelines and performed with the approval of the Institutional Animal Care and Use Committee at Hokkaido and Kanazawa University. All efforts were made to minimize the number and suffering of animals used in the experiments.

2.2. Drugs

Cocaine hydrochloride (Takeda Pharmaceutical, Osaka, Japan) was dissolved in saline. A NOS inhibitor Nω-Nitro-L-arginine methyl ester hydrochloride (L-NAME; Sigma-Aldrich, St. Louis, MO, USA) and nNOS inhibitor Nω-propyl-L-arginine (L-NPA; Tocris Bioscience, Bristol, UK) were dissolved in 0.1 M PBS (pH = 7.4). The doses of these drugs were determined on the basis of previous studies [20, 21]: L-NAME (100 nmol) and L-NPA (2 nmol).

2.3. Surgery and microinjection

Under sodium pentobarbital anesthesia (50 mg/kg, i.p.), rats were implanted bilaterally with 25-gauge stainless-steel guide cannulae (o.d., 0.5 mm; i.d., 0.22 mm) above the mPFC (3.0 mm rostral, 0.67 mm lateral, 4.0 mm ventral to bregma) [22]. After surgery, rats were housed individually in their home cages, allowed to recover for 6–9 days, and handled each day for three consecutive

days before the behavioral experiments. For microinjection, 33-gauge stainless-steel injection cannulae (o.d., 0.2 mm; i.d., 0.08 mm) were inserted bilaterally into the guide cannulae. The injection cannulae protruded 1.5 mm from the tip of the guide cannulae to reach the mPFC. Bilateral infusions were performed at a volume of 0.5 μL in each side at a rate of 0.5 μL/min and the injection cannulae were left in place for an additional 1 min after microinjection to prevent backflow.

2.4. CPP test

CPP tests were conducted as described previously [17, 18]. The CPP chambers consisted of two equally-sized compartments (30 × 30 × 30 cm) with distinct tactile and visual cues (one compartment had a black floor and walls with an equally spaced stainless-steel vertical grid on the floor, and the other had a white floor and walls with stainless-steel grid on the floor), which were separated by a removable partition. The CPP chambers were set in sound-attenuating boxes equipped with a ventilating fan. On days 1 (habituation) and 2 (pretest), rats freely explored the two compartments for 900 s, and the time spent in each compartment during the exploratory period and locomotor activity were measured using infrared sensors (Supermex, Muromachi Kikai, Tokyo, Japan), which were positioned on the top cover of each compartment. Rats that spent >80% (>720 s) of the total time (900 s) in one side on day 2 or showed a difference of >200 s in the time spent in one side between days 1 and 2 were eliminated from subsequent procedures. We used a bias-like protocol [23], in which the compartment where each rat spent less time on day 2 (pretest) was designated as their cocaine-paired compartment. On days 4–9 (conditioning), rats were given alternating injections of cocaine (20 mg/kg, i.p.) or saline (1 mL/kg, i.p.) and confined to one compartment for 30 min on six consecutive days. On cocaine-conditioning days, rats were given a bilateral intra-mPFC microinjection of L-NAME, L-NPA or vehicle 5 min before their cocaine injection. On day 11 (posttest), rats were allowed to explore the two compartments freely for 900 s, and the time spent in each compartment during the exploratory period and locomotor activity were measured. The CPP

scores were calculated by subtracting the time spent in the cocaine-paired compartment during the pretest from that during the posttest.

2.5. Histology

After the CPP tests, the brains were rapidly removed and frozen in powdered dry ice. Coronal sections (50 μm) of the mPFC were prepared using a cryostat, thaw-mounted onto slides, stained with thionin, and examined under a microscope.

2.6. Electrophysiology

Animal preparation

Sprague-Dawley rats weighing 80–140 g (3–6 weeks old) were randomly distributed to two groups that received alternating injections of saline (1.0 mL/kg, i.p.) or cocaine (20 mg/kg in saline, i.p.) once a day for six consecutive days in their home cage. Electrophysiological experiments were conducted on the next day.

Drug injection into the mPFC

Under sodium pentobarbital anesthesia (50 mg/kg, i.p.), rats were implanted bilaterally with 25-gauge stainless-steel guide cannulae (o.d., 0.5 mm; i.d., 0.22 mm) 1.0 mm above the mPFC (2.8 mm rostral, 0.65 mm lateral, 3.0 mm ventral to bregma). We previously found that this coordinate was appropriate to target the mPFC in juvenile rats [16]. After surgery, rats were housed individually in their home cage, allowed to recover for 2−8 days before injecting drugs. For microinjection, 33-gauge stainless steel injection cannulae (o.d., 0.2 mm, i.d., 0.08 mm) were inserted bilaterally into the guide cannulae. The injection cannulae protruded 1.0 mm from the tip of the guide cannulae to reach the mPFC. Five min before each cocaine injection, L-NPA (2 nmol/side) or vehicle was administrated bilaterally in a volume of 0.5 μL/side at a rate of 0.5 μL/min, and the injection

cannulae were left in place for an additional 1 min after microinjection to prevent backflow. To confirm the placements of drug injection histologically, 1% Cresyl Violet solution dissolved in saline (0.5 µL/side) was injected into the mPFC just before the rats were decapitated. After removing the rostral part of the brain during the slice preparation as described below, coronal sections (50 µm thick) including the mPFC were prepared with a cryostat, thaw-mounted onto slides, stained with thionine, and examined under a microscope.

Slice preparation and electrophysiology

The drug-treated rats were anesthetized with sodium pentobarbital and decapitated. The brains were submerged in ice-cold modified Ringer's solution containing (in mM) choline chloride, 125; KCl, 4.0; NaH_2PO_4, 1.25; $MgCl_2$, 7.0; $CaCl_2$, 0.5; $NaHCO_3$, 26; glucose, 20; ascorbate, 1.0; and pyruvate, 3.0; and bubbled with 95% O_2–5% CO_2 (pH 7.4). Parasagittal slices (250 µm thick) of the LDT were cut with a Microslicer (VT1200S, Leica Microsystems, Wetzlar, Germany) and incubated at 32–34°C for 15-30 min in standard Ringer's solution containing (in mM) NaCl, 125; KCl, 2.5; NaH_2PO_4, 1.25; $MgCl_2$ 1.0; $CaCl_2$, 2.0; $NaHCO_3$, 26; and glucose, 25; and bubbled with 95% O_2–5% CO_2 (pH 7.4) and then transferred to standard Ringer's solution at room temperature. Slices were mounted in a recording chamber on an upright microscope (BX-51WI, Olympus, Tokyo, Japan) and continuously superfused with the standard Ringer's solution at a flow rate of 2–2.5 mL/min. Whole-cell voltage-clamp recordings were obtained from LDT neurons by visual control of patch pipettes, which were prepared from borosilicate glass capillaries and were filled with an internal solution containing (in mM) Cs-gluconate, 150; CsCl, 5.0; $MgCl_2$, 2.0; Na_2ATP, 4.0; Na_3GTP, 0.3; EGTA, 10; HEPES, 10; and QX-314, 2–3 (pH 7.3 with CsOH). To stain the recorded neurons, biocytin (2–3 mg/mL; Sigma) was dissolved in the solution. The resistance of the electrodes was 3–8 MΩ in the Ringer's solutions. The actual membrane potentials were corrected by the liquid junction potential of -10 mV. All recordings were performed at 32–34°C. Electrical stimulation was applied as a cathodal

square-wave pulse of 200 μs duration with an intensity of up to 200 μA using a glass electrode filled with normal Ringer's solution, which was located ~400 μm dorsorostral to the recorded neurons. To calculate paired pluse ratios (PPRs) of evoked EPSCs, membrane potentials were held at -70 mV in the presence of gabazine (10 μM; Sigma) and strychnine (10 μM; Sigma) and two repetitive stimuli with inter-stimulus intervals of 50 ms were delivered. A minimum of 20 evoked EPSCs were collected for analysis. Membrane input and series resistances were monitored by applying a hyperpolarizing pulse (-5 mV, 30 ms) through the patch-clamp electrode. Recordings where either parameter altered by >20% during the course of the recording were excluded from the analyses.

Histochemistry

After recordings, slices were fixed in 4% paraformaldehyde in 0.1 M PBS (pH 7.4) overnight at 4°C and then stored in 0.1 M PBS. Cholinergic neurons were stained using NADPH-diaphorase histochemistry, a marker of mesopontine cholinergic neurons [24]. The slices were incubated in a solution containing β-NADPH (1 mg/mL) and nitroblue tetrazolium (0.1 mg/mL) in 0.1 M PBS containing 0.3% Triton X-100 (pH 7.4) for 1 h at 37°C. After rinsing three times for 10 min in 0.05 M PBS, intracellularly infused biocytin was then visualized by incubating the slices with streptavidin-conjugated Alexa Fluor 594 (5 μg/mL; Molecular Probes, Eugene, USA) in 0.05 M PBS for 2 h at room temperature. After rinsing, the slices were mounted on glass slides, dehydrated and coverslipped. Alexa Fluor and NADPH-diaphorase positive neurons were identified under a microscope equipped with both fluorescence and bright-field optics (BX-60, Olympus) and only double-labeled cells were determined as cholinergic neurons (Fig. 1). We recorded from a total of 39 neurons. Among them, 28 neurons were identified as cholinergic neurons *post hoc* and data from those neurons were analyzed.

2.7. Statistical analyses

Data are expressed as means ± S.E.M. and were compared using student's *t*-test when comparing only two groups or one-way or two-way analysis of variance (ANOVA) followed by the Holm-Sidak *post hoc* test when comparing more than two groups.

3. Results

3.1. Inhibition of NOS in the mPFC disturbs the acquisition of cocaine CPP

We first investigated whether NO in the mPFC is involved in the acquisition of cocaine CPP. For this, we bilaterally microinjected the non-selective NOS inhibitor L-NAME (100 nmol/side) or vehicle into the mPFC before each cocaine conditioning session (Fig. 2A). In the intra-mPFC vehicle-injected rats, the time spent in the cocaine-paired compartment during the posttest was 493.3 ± 40.7 s, which was significantly longer than the time during the pretest (267.8 ± 31.4 s, $n = 7$, $t_6 = 6.618$, $P = 0.0006$, paired *t*-test), resulting in a remarkable CPP score (220.5 ± 30.8 s; Fig. 2B). On the other hand, in the intra-mPFC L-NAME-injected rats, no significant difference was observed in the time spent in the cocaine-paired compartment between the pretest and posttest (pretest: 380.1 ± 3.6 s, posttest: 430.9 ± 25.3 s, $n = 7$, $t_6 = 2.157$, $P = 0.074$, paired *t*-test), leading to a CPP score of 50.7 ± 25.4 s (Fig. 2B). Accordingly, the CPP scores in rats that were treated with L-NAME were significantly smaller than in those treated with vehicle ($F_{2,17} = 10.83$, $t_{17} = 4.507$, $P = 0.0009$, one-way ANOVA with *post hoc* Holm-Sidak test; Fig. 2B).

We further examined whether the intra-mPFC L-NAME injection itself induces place aversion or preference. To test this, cocaine was substituted with saline during the conditioning period, i.e., saline was injected every conditioning day (six days of saline injections), and L-NAME was microinjected into the mPFC before confinement in one compartment every other day (three days of L-NAME injections) in a separate group of rats ($n = 5$; Fig. 2C). Under this condition, there was no significant difference between the time spent in the L-NAME injection-paired compartment during

the pretest (346.0 ± 21.7 s) and posttest (424.7 ± 42.9 s) sessions (n = 5, t_4 = 2.108, P = 0.103, paired t-test; Fig. 2D), indicating the induction of neither place aversion nor preference by intra-mPFC L-NAME injection. These results suggest that NO generated in the mPFC is involved in the acquisition of cocaine CPP.

3.2. Inhibition of nNOS in the mPFC disturbs the acquisition of cocaine CPP

Next, we addressed whether nNOS is involved in the acquisition of cocaine CPP by injecting the selective nNOS inhibitor L-NPA (2 nmol/side) into the mPFC prior to each cocaine conditioning session (Fig. 2A). In the intra-mPFC L-NPA-injected rats, a small but significant difference was observed in the time spent in the cocaine-paired compartment between the pretest and posttest (pretest: 336.5 ± 25.1 s, posttest: 432.1 ± 40.8 s, n = 6, t_5 = 3.596, P = 0.0156, paired t-test), leading to a CPP score of 95.6 ± 26.6 s (Fig. 2B). Accordingly, L-NPA injection significantly decreased CPP scores compared to vehicle injection ($F_{2,17}$ = 10.83, t_{17} = 3.185, P = 0.0108, one-way ANOVA with *post hoc* Holm-Sidak test; Fig. 2B).

We also examined whether intra-mPFC L-NPA injection itself induces place aversion or preference by performing the same experiment as with L-NAME in a separate group of rats (n = 6; Fig. 2C). The time spent in the intra-mPFC L-NPA microinjection-paired compartment during the pretest (352.8 ± 16.4 s) was not significantly different from that during the posttest (406.0 ± 60.7 s, n = 6, t_5 = 1.083, P = 0.328, paired t-test; Fig. 2D), confirming that intra-mPFC L-NPA injection does not induce place aversion or preference. Taken together, these findings indicate that nNOS in the mPFC is crucial for acquiring cocaine CPP.

3.3. mPFC nNOS activity is necessary for inducing presynaptic plasticity in LDT cholinergic neurons

We have previously reported that systemic L-NAME injection suppresses the presynaptic plasticity

induced by repeated cocaine administration in LDT cholinergic neurons [16]. As the mPFC is one of the major sources of glutamatergic inputs to the LDT [12, 13], we investigated a possible contribution of mPFC nNOS activity in the induction of LDT plasticity. To address this, we used four different injection paradigms. In the first and second paradigms, cocaine and saline were injected on alternating days for six days. In these paradigms, vehicle or L-NPA was microinjected into the mPFC 5 min prior to each cocaine injection. In the third and fourth paradigms, saline was injected every day for six days. In these paradigms, vehicle or L-NPA was microinjected into the mPFC 5 min prior to each saline injection every other day (total of three injections) (Fig. 3A, B). As shown in Fig. 3C and D, repeated cocaine administration reduced the PPR, indicating an increased release probability. A two-way ANOVA revealed a significant mPFC treatment × cocaine interaction ($F_{1,24}$ = 6.330, P = 0.0190). A *post hoc* Holm-Sidak test showed that cocaine treatment significantly reduced the PPRs as compared to saline treatment (vehicle + saline, 1.32 ± 0.01, n = 6; vehicle + cocaine, 1.08 ± 0.04, n = 8, P = 0.001) and that the intra-mPFC L-NPA injection recovered the cocaine-induced reduction of PPRs (L-NPA + cocaine, 1.33 ± 0.02, n = 7, P = 0.0004). We confirmed that the L-NPA itself had no effect on PPRs (L-NPA + saline, 1.36 ± 0.06, n = 7, P = 0.5318). Thus, these results indicate that nNOS activity in the mPFC is necessary for inducing presynaptic plasticity in LDT cholinergic neurons.

4. Discussion

The main findings of the present study were that: (1) intra-mPFC injection of the non-selective NOS inhibitor L-NAME or the selective nNOS inhibitor L-NPA inhibits cocaine CPP acquisition; and (2) intra-mPFC injection of L-NPA inhibits the induction of presynaptic plasticity in LDT cholinergic neurons induced by repeated cocaine administration. To the best of our knowledge, this is the first direct evidence showing that NO generated by nNOS in the mPFC plays a crucial role in both the

acquisition of cocaine CPP and the induction of neuroplasticity in the LDT.

Although previous studies have demonstrated that NOS activation is involved in cocaine CPP [3, 25], the brain region(s) in which NO acts and how it contributes to cocaine CPP development were not fully understood. Our data indicate that both L-NAME and L-NPA injections into the mPFC before cocaine injection significantly reduce cocaine CPP scores, indicating that NO exerts its effects at least partly in the mPFC and contributes to the acquisition of cocaine CPP, i.e., the association between cocaine reward and environmental context. However, given that the NO-dependency of cocaine CPP is influenced by many factors, such as the procedure of conditioning, conditioning apparatus, strain, species, and sex [26], the role of NO may not be limited to the acquisition of cocaine CPP. Indeed, NO has also been implicated in memory reconsolidation in cocaine CPP [25]. Additionally, it has been reported that NO in the nucleus accumbens contributes to the acquisition and expression of morphine-induced CPP [27]. These data suggest that NO in other brain areas may be associated with addictive drug-induced CPP formation.

Repeated cocaine administration induces membrane plasticity in mPFC pyramidal neurons in an NO-dependent manner [9, 10], although it remains unknown whether nNOS activation in the mPFC is involved in the induction of the plasticity. The extracellular level of NO has been reported to increase in the mPFC after cocaine administration [8], suggesting a possible contribution of nNOS activity in the mPFC to the induction of membrane plasticity. At present, it is unclear whether this plasticity in the mPFC is associated with the acquisition of cocaine CPP. However, considering that the membrane plasticity enhances excitation of mPFC neurons [10], that mPFC excitation is involved in cocaine CPP acquisition [28], and that systemic administration of a NOS inhibitor disrupts both the induction of the mPFC membrane plasticity [10] and the development of cocaine CPP [3], the plasticity in the mPFC is likely to be associated with cocaine CPP acquisition.

In the present study, we confirmed the induction of presynaptic plasticity in LDT cholinergic neurons with the change in the PPRs of EPSCs after repeated cocaine administration. Given that

LDT receives glutamatergic inputs from the mPFC [12, 13], it is likely that the activation of the mPFC is important for the induction of LDT plasticity. Indeed, we have previously shown that inactivation of mPFC neurons prior to cocaine administration prevents the LDT plasticity induced by repeated cocaine exposure [16]. However, this does not necessarily mean that the synapses that exhibit plasticity are formed between mPFC-derived axon terminals and LDT cholinergic neurons: although we stimulated the dorsorostral portion of the LDT, in which mPFC fibers have been suggested to be passing [12, 29], our stimulating electrode would have stimulated not only mPFC axons but also excitatory fibers from other brain regions. Thus, further studies using an optogenetic technique to selectively stimulate mPFC-derived fibers would be necessary to clarify which axons exhibit the presynaptic plasticity. In any case, we also found that systemic administration of a NOS inhibitor before cocaine injection also inhibits the induction of presynaptic plasticity in the LDT [16]. Additionally, we showed in the current study that nNOS inhibition in the mPFC prevents the induction of synaptic plasticity in LDT cholinergic neurons. Although the precise molecular mechanisms underlying the induction of LDT plasticity are unclear, these findings suggest that cocaine-induced NO production in the mPFC induces membrane plasticity in mPFC pyramidal neurons which, in turn, results in the potentiation of glutamatergic transmission to LDT cholinergic neurons. However, it should be noted that recent studies have suggested direct excitation of mPFC neurons by NO. NO mediates M_3 muscarinic receptor-dependent activation of mPFC neurons [30] and designer receptors exclusively activated by designer drugs (DREADD)-induced excitation of mPFC neurons [31]. Thus, the cocaine-induced NO release itself may activate mPFC neurons, contributing to the induction of synaptic plasticity in LDT cholinergic neurons and the acquisition of cocaine CPP, irrespective of inducing NO-dependent membrane plasticity in mPFC neurons.

We have previously reported that repeated cocaine administration enhances membrane excitability in LDT cholinergic neurons via an increase in persistent sodium conductance [18]. This membrane plasticity, together with the synaptic plasticity that depends on NO production in the

mPFC, may lead to excitation of LDT cholinergic neurons. Considering that activity of LDT cholinergic neurons is required for cocaine CPP acquisition [17], it is likely that these cocaine-induced cooperative adaptations in the mPFC and LDT may play a critical role in the acquisition of cocaine reward memory.

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Conflict of interest

The authors declare no conflict of interest.

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Figure Legends

Fig. 1. Photomicrographs of a representative recorded LDT cholinergic neuron. (A) A low magnification photomicrograph showing NADPH-diaphorase positive neurons in the LDT. (B) A high magnification of (A). Arrow indicates the recorded neuron that is NADPH-diaphorase positive. (C) A fluorescent image of (B) after biocytin staining.

Fig. 2. Inhibition of NOS in the mPFC disturbs the acquisition of cocaine CPP. (A) The placements of the tips of the microinjection cannulae for vehicle (white circles, n = 7), L-NAME (gray circles, n = 7), and L-NPA (black circles, n = 6) injections into the mPFC. The numbers inside each panel represent the approximate AP distance (mm) from bregma. (B) Summary graph of CPP scores. * P < 0.05, ***P < 0.001, one-way ANOVA with *post hoc* Holm Sidak test. (C) The placements of the tips of the microinjection cannulae for L-NAME (gray circles, n = 5) and L-NPA (black circles, n = 6) injections into the mPFC. The numbers inside each panel represent the approximate AP distance (mm) from bregma. (D) The columns show the time spent in the L-NAME- (n = 5) or L-NPA-paired (n = 6) side during the pretest (white columns) and posttest (gray or black column) sessions.

Fig. 3. Inhibition of nNOS in the mPFC prevents cocaine-induced synaptic plasticity in LDT cholinergic neurons. (A) Schedule of drug administration and whole-cell recordings. (B) The placements of the tips of the microinjection cannulae for the vehicle + saline (white circles), L-NPA + saline (black circles), vehicle + cocaine (white triangles), and L-NPA + cocaine (black triangles) groups. The numbers inside each panel represent the approximate AP distance (mm) from bregma. (C) Representative traces of evoked EPSCs recorded from LDT cholinergic neurons, obtained after vehicle or L-NPA injection and followed by saline administration (top row), and vehicle or L-NPA injection followed by cocaine administration (bottom row). (D) Summary graph showing the effects

of vehicle or L-NPA pretreatment on cocaine-induced PPR changes (vehicle + saline, $n = 6$ from four rats; L-NPA + saline, $n = 7$ from four rats; vehicle + cocaine, $n = 8$ from three rats; L-NPA + cocaine, $n = 7$ from four rats). ***$P < 0.001$, two-way ANOVA with *post hoc* Holm Sidak test.

Fig. 1

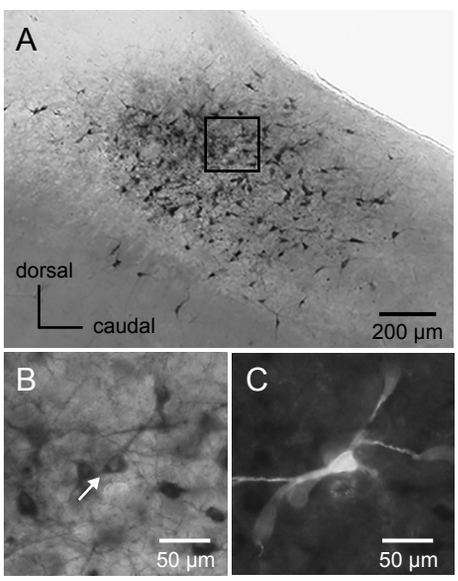
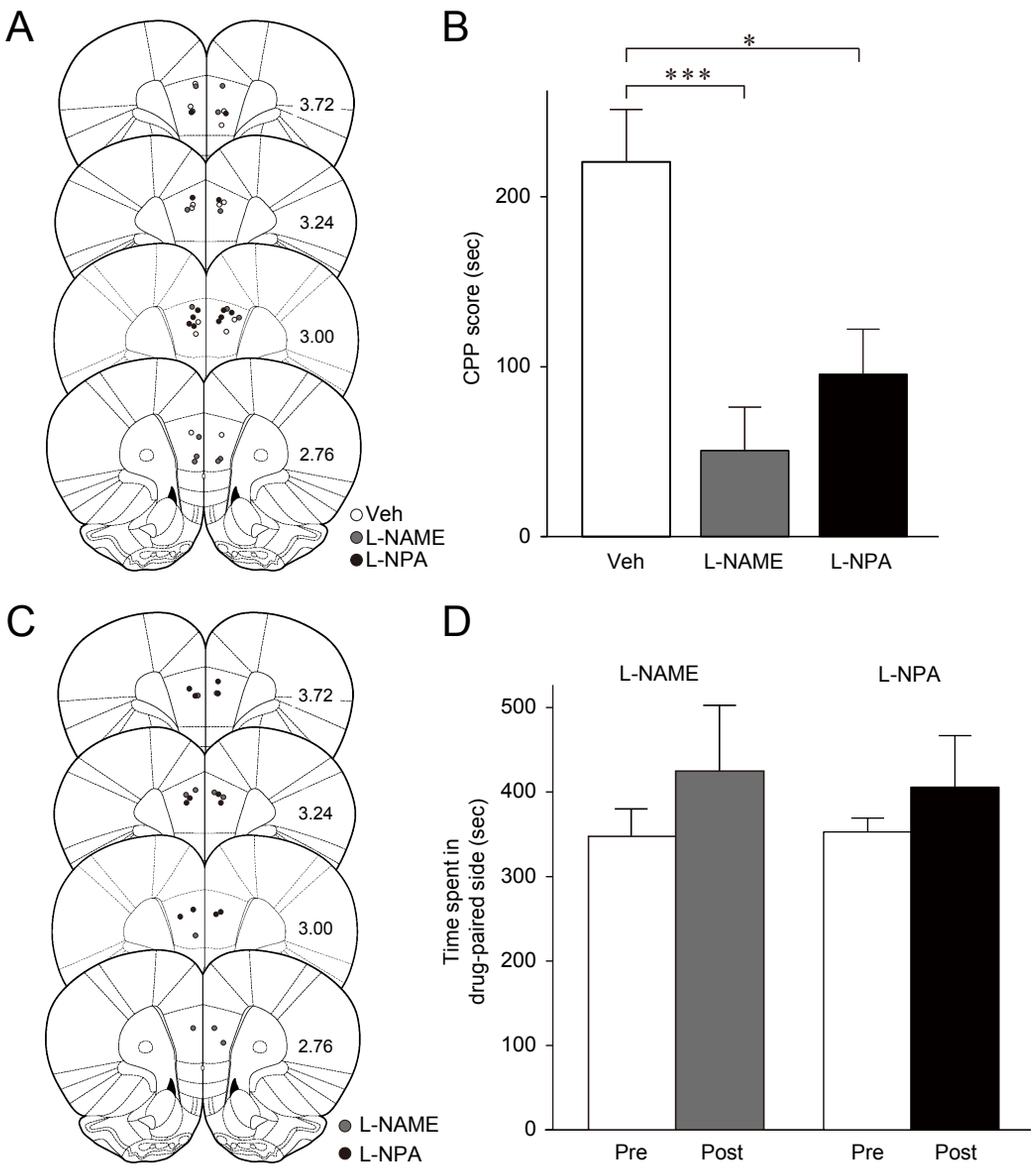


Fig. 2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3.24
3.00
2.76
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L-NPA
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L-NPA
C
3.72
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3.00
2.76
L-NAME
L-NPA
D
L-NAME
L-NPA
Time spent in drug-paired side (sec)
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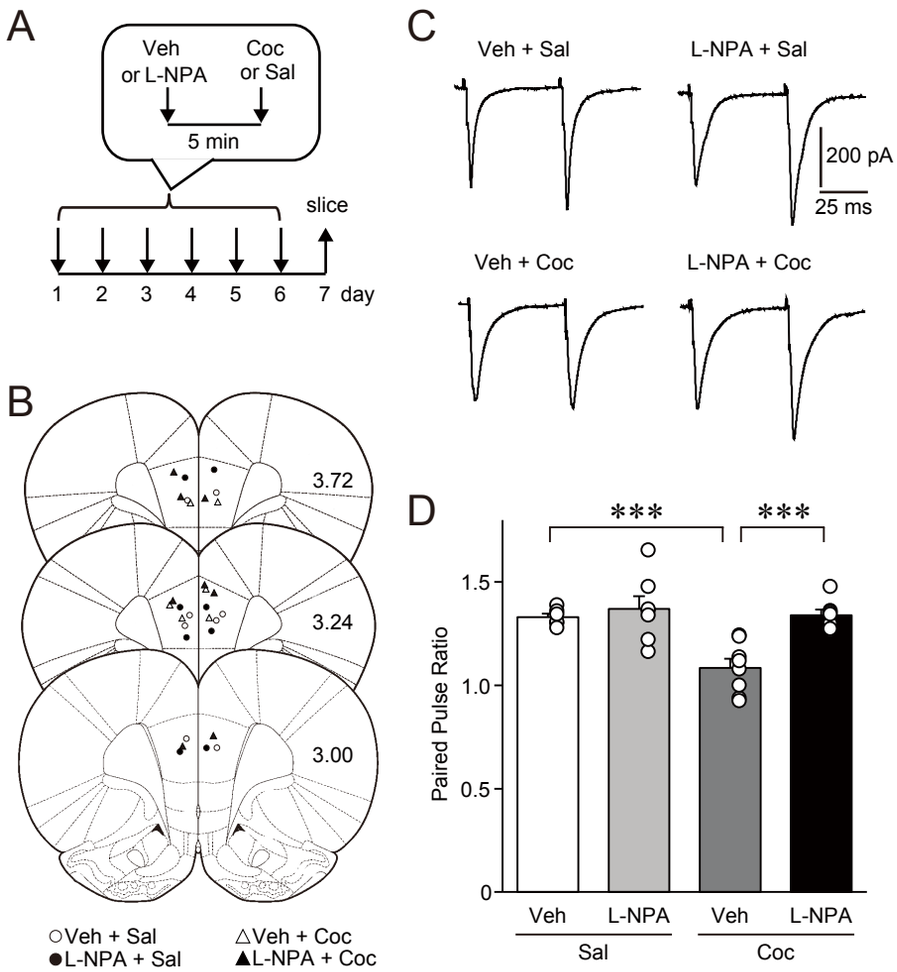
400
300
200
100
0
Pre
Post
Pre
Post

Fig. 3


A
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or L-NPA
Coc
or Sal
5 min
slice
1 2 3 4 5 6 7 day
B
3.72
3.24
3.00
○Veh + Sal
●L-NPA + Sal
△Veh + Coc
▲L-NPA + Coc
C
Veh + Sal
L-NPA + Sal
200 pA
25 ms
Veh + Coc
L-NPA + Coc
D
***
***
Paired Pulse Ratio
1.5
1.0
0.5
0
Veh
L-NPA
Veh
L-NPA
Sal
Coc