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Endogenous Dopamine and Endocannabinoid Signaling Mediate Cocaine-Induced Reversal of AMPAR Synaptic Potentiation in the Nucleus Accumbens Shell

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Endogenous Dopamine and Endocannabinoid Signaling Mediate Cocaine-induced Reversal of AMPAR Synaptic Potentiation in the Nucleus Accumbens Shell

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Keywords

AMPA receptor, Cocaine, Dopamine, Endocannabinoid, Nucleus accumbens, Synaptic plasticity

Abstract

Repeated exposure to drugs of abuse alters the structure and function of neural circuits mediating reward, generating maladaptive plasticity in circuits critical for motivated behavior. Within meso-corticolimbic dopamine circuitry, repeated exposure to cocaine induces progressive alterations in AMPAR-mediated glutamatergic synaptic transmission. During a 10–14 day period of abstinence from cocaine, AMPAR signaling is potentiated at synapses on nucleus accumbens (NAc) medium spiny neurons (MSNs), promoting a state of heightened synaptic excitability. Re-exposure to cocaine during abstinence, however, rapidly reverses and depotentiates enhanced AMPAR signaling. To understand how re-exposure to cocaine alters AMPAR synaptic transmission, we investigated the roles of dopamine and endocannabinoid (eCB) signaling in modifying synaptic strength in the NAc shell. Using patch-clamp recordings from NAc slices prepared after 10–14 days of abstinence from repeated cocaine, we found that AMPAR-mediated depotentiation is rapidly induced in the NAc shell within 20 min of cocaine re-exposure *ex vivo*, and persists for up to five days before synapses return to levels of potentiation observed during abstinence. In cocaine-treated animals, global dopamine receptor activation was both necessary and sufficient for the cocaine-evoked depotentiation of AMPAR synaptic function. Additionally, we identified that CB1 receptors are engaged by endogenous endocannabinoids (eCBs) during re-exposure to cocaine *ex vivo.* Overall, these results indicate the central role that dopamine and eCB signaling mechanisms play in modulating cocaine-induced AMPAR plasticity in the NAc shell.

1. Introduction

Progressive functional changes in neural circuitry mediating learning and motivation are known to underlie key behavioral hallmarks of drug addiction (Hyman et al., 2006, Thomas et al., 2008, Volkow and Morales, 2015). A critical component of mesocorticolimbic circuitry governing reward-related learning and motivated behavior, the nucleus accumbens (NAc) is a structure composed principally of medium spiny neurons (MSNs) that receive converging input from midbrain dopaminergic afferents and glutamatergic projections from cortical and limbic areas. Extensive experimental evidence indicates that drugs of abuse engage endogenous mechanisms of neuronal plasticity to drive persistent changes in synaptic transmission at NAc MSNs (Kauer and Malenka, 2007, Lüscher and Malenka, 2011). This maladaptive form of plasticity has been well-demonstrated to underlie the development and persistence of drug-seeking behavior in animal models of addiction (Wolf, 2016).

Accumulating evidence indicates that experience-dependent alterations in glutamate transmission in the NAc, such as those produced by repeated exposure to cocaine, involve alterations in postsynaptic signaling mediated by AMPA-type receptors (Pierce and Wolf, 2013, Wolf and Ferrario, 2010). In the NAc shell, repeated cocaine exposure results in a progressive increase in MSN AMPAR signaling during abstinence that heightens sensitivity to glutamatergic input, and is a key factor in the development and persistence of addiction-related behavior such as behavioral sensitization, craving, and relapse (Boudreau and Wolf, 2005, Conrad et al., 2008, Kourrich et al., 2007, Pascoli et al., 2014, Pascoli et al., 2011, Terrier et al., 2016). Conversely, additional studies citing both biochemical (Boudreau et al., 2007, Ferrario et al., 2009) and physiological evidence (Kourrich et al., 2007; et al., 2011) have shown

that exposure to relapse-inducing stimuli such as stress or a cocaine challenge injection reverses (depotentiates) cocaine-dependent increases in NAc shell AMPAR signaling, suggesting that this reversal of plasticity may play a role in driving this behavior. While the ability of relapse-inducing stimuli to bidirectionally modify NAc AMPAR signaling has been demonstrated on numerous occasions, the cellular mechanisms underlying this plasticity remain unclear.

Within the striatum, pre- and postsynaptic neuromodulatory signaling plays a critical role in gating changes in MSN glutamatergic synaptic strength (Lerner and Kreitzer, 2011). Prominent among these modulators is dopamine (DA), which is known to regulate MSN intrinsic and synaptic excitability (Nicola et al., 2000, Tritsch and Sabatini, 2012). Moreover, activation of DA D1 or D2 receptors in the NAc shell reinstates cocaine seeking (Schmidt and Pierce, 2006), whereas activation of D1Rs during abstinence normalizes cocaine-induced increases in AMPAR-mediated synaptic transmission (Ortinski et al., 2012), highlighting a potential role for DA receptors in the depotentiation of AMPAR synaptic strength observed in the NAc shell following cocaine re-exposure.

Similar to the striatal DA system, neurotransmission mediated by the endocannabinoid (eCB) system powerfully modulates synaptic efficacy at NAc glutamatergic synapses (Lovinger and Mathur, 2012, Piet et al., 2011, Robbe et al., 2003). Induction of eCB signaling requires post-synaptic release of eCBs which then signal retrogradely at pre-synaptic cannabinoid type 1 (CB1) receptors, depressing neurotransmitter release (Hoffman and Lupica, 2001, Kano et al., 2009). Throughout the striatum and other brain regions, eCB signaling mediates various forms of short-term and long-term synaptic depression, comprising a dominant mechanism for reducing synaptic strength at excitatory synapses (Lüscher and Huber, 2010). Recent evidence implicating NAc CB1 signaling in cocaine-primed behavioral sensitization and reinstatement (McReynolds et al., 2016, Ramiro-Fuentes and Fernandez-Espejo, 2011, Xi et al., 2006) makes this system a prime candidate in modulating cocaine-dependent reversal of AMPAR signaling in the NAc; however, our understanding of how repeated cocaine engages and alters eCB-mediated synaptic plasticity in the NAc remains relatively incomplete. The present study investigated the temporal dynamics and potential cellular mechanisms underlying cocaine-induced depotentiation of AMPAR signaling in the NAc, focusing on potential roles for dopamine and eCB signaling using *in vivo* and *ex vivo* approaches.

2. Materials and methods

2.1. Animals

Adult male (P49-70) C57BL/6 mice (Jackson Laboratories, Bar Harbor, Maine, USA) were used. All animals were group-housed in a temperature- and humidity-controlled environment on a 12 h light/12 h dark cycle, with food and water available *ad libitum.* All experiments were approved by the Institutional Animal Care and Use Committee at the University of Minnesota.

2.2. Behavioral sensitization

Prior to testing, mice were habituated to experimenter handling, *i.p*. injections, and the testing environment (individual activity boxes, 8.5 × 17.5 x 9 in) over two days. On five consecutive testing

days, mice were habituated to the testing environment for 30 min (min) before receiving an intraperitoneal injection of either cocaine (15 mg/kg) or saline. Mice were immediately placed back into the testing environment, and their activity was monitored for 90 min using a video-based tracking system (Any-Maze, Stoelting, WI, USA). Animals were returned to their home cages at the end of each testing period. Following the last day of testing, animals remained in their home cages in the colony for 10–14 days before electrophysiology experiments were performed. For *in vivo* drug challenge experiments only, mice were handled and injected with saline during the last 2 days of the 10–14 day abstinence period to prevent any potential effects of stress during the challenge experiment. Mice were habituated to the testing environment for 30 min before receiving a challenge injection of cocaine (15 mg/kg) or saline, followed by activity monitoring for 90 min. Electrophysiology experiments took place within 24 h (h) or 5 days (d) following the challenge injection.

2.3. Slice electrophysiology

Following 10–14 days of abstinence from cocaine treatment *or* either 24 h or 5 d after an *in vivo* cocaine challenge injection, mice were anesthetized with isoflurane, decapitated, and the brain rapidly removed. Parasagittal slices (240 μm) containing the nucleus accumbens shell were prepared in 2–4 °C sucrose-containing artificial cerebrospinal fluid (ACSF) saturated with 95% O_2/5% CO_2 and composed of (in mM): 75 sucrose, 87 NaCl, 2.5 KCl, 1.25 NaH_2PO_4, 26 $NaHCO_3$, 11 glucose, 7 $MgSO_4$, 0.5 $CaCl_2$, and 3 sodium ascorbate (Britt et al., 2012). Slices rested for at least 30 min in a holding chamber at room temperature containing standard ACSF composed of (in mM): 119 NaCl, 2.5 KCl, 1.0 NaH_2PO_4, 26.2 $NaHCO_3$, 11 glucose, 1.3 $MgSO_4$, 2.5 $CaCl_2$, and 1 sodium ascorbate. During recording, standard ACSF was used with the exception of the sodium ascorbate. Additionally, picrotoxin (100 μM) and lidocaine (0.7 μM) were included to block $GABA_A$-mediated inhibitory transmission and action potentials, respectively, during recording. Neurons in the NAc shell were visualized on an upright microscope using infrared differential interference contrast (IR-DIC) optics. MSNs were identified by their morphology and hyperpolarized resting membrane potential (−70 to −80 mV). To assess excitatory synaptic transmission, cells were voltage-clamped at −80 mV using an Axon Instruments MultiClamp 700A (Molecular Devices, Sunnyvale, CA, USA). Patch pipettes (3–5 MΩ) were filled with a cesium-gluconate-based internal solution containing (in mM): 117 cesium gluconate, 2.8 NaCl, 20 HEPES, 0.4 EGTA, 5 tetraethylammonium-Cl, 2 MgATP, and 0.3 MgGTP (pH 7.2–7.4). Data were filtered at 2 kHz, digitized at 5 kHz, and collected using custom Igor Pro software (Wavemetrics, Lake Oswego, OR, USA). A depolarizing step (4 mV, 100 ms) was generated at the beginning of each sweep to monitor series resistance (10–40 MΩ) and input resistance (>500 MΩ). MiniAnalysis software (Synaptosoft, Decatur, GA, USA) was used offline to analyze mEPSC amplitude, frequency, rise time, and decay time. To determine the rise time and decay time, two exponentials were fit to the rise time or decay time of mEPSCs using the MiniAnalysis curve-fitting function, and the time constant was calculated between 10 and 90% of peak.

2.4. Ex vivo drug application

Drugs were prepared in a stock solution and added at the final concentration to the ACSF perfusing the slices. A between-cell experimental design was utilized in which slices were bathed in the drug or

control ACSF prior to recording synaptic activity. During these experiments, slices were exposed to one of the following conditions: 1) no challenge (exposure to ACSF only); 2) *ex vivo* cocaine challenge (10 μM; 10 min); 3) *ex vivo* bath application of flupenthixol (non-specific dopamine receptor antagonist; 20 μM; 10 min) *or* SR141716A (CB1 antagonist/inverse agonist; 1 μM; 10 min) alone; 4) *ex vivo* bath application of flupenthixol *or* SR141716A alone (10 min) followed by flupenthixol *or* SR141716A in the presence of 10 μM cocaine (10 min); or 5) *ex vivo* bath application of apomorphine (non-specific dopamine receptor agonist; 1 μM; 10 min) or WIN 55,212-2 (CB1 receptor agonist; 1 μM; 20 min) alone. Following the timed *ex vivo* drug exposure(s), the perfusion was switched back to standard ACSF for recording. Recordings took place up to 1 h following *ex vivo* drug exposure, and the time elapsed between drug exposure and beginning a recording was noted to determine whether time following drug exposure was a factor in synaptic plasticity.

2.5. Drugs

Apomorphine, flupenthixol, SR141716A, and WIN 55,212-2 were purchased from Tocris Bioscience (Bristol, United Kingdom). Lidocaine and picrotoxin were purchased from Sigma Aldrich (St. Louis, MO, USA). Cocaine was obtained from Boynton Health Services Pharmacy (University of Minnesota, Minneapolis, MN, USA). Drugs were prepared in a stock solution at 100–1000 times the desired concentration and added into standard ACSF at the final concentration. SR141716A stock was dissolved in DMSO, and WIN 55,212-2 stock was dissolved in ethanol. The final concentrations of DMSO or ethanol were <0.01%.

2.6. Statistical analysis

All data are presented as group mean ± SEM. Statistical significance was assessed with a Student's t-test, one-way ANOVA, or two-way ANOVA using JMP Pro (SAS, Cary, NC, USA) and GraphPad Prism (GraphPad Software, La Jolla, CA, USA). Tukey HSD *post hoc* tests were used for pairwise comparisons where appropriate. The threshold for significance was $p < 0.05$.

3. Results

3.1. Depotentiation of AMPAR synaptic transmission induced by cocaine re-exposure is rapidly induced and temporally constrained

Previous studies examining cocaine-induced AMPAR plasticity in the NAc have shown that the ability of intra-NAc AMPA to induce locomotor activity is decreased 24 h but not 6 d following a cocaine challenge (Bachtell and Self, 2008), indicating that cocaine challenge-induced plasticity in the NAc is transient in nature. To investigate the duration of AMPAR synaptic plasticity in the NAc shell, we performed *ex vivo* recordings of mEPSCs, a direct measure of synaptic AMPAR function, 24 h or 5 d following a cocaine challenge injection (given on day 10–14 of abstinence). Twenty-four hours following cocaine challenge, mEPSC amplitude (picoamperes of current, pA) and frequency (events per second, Hz) were significantly reduced compared to cocaine-treated animals receiving a saline challenge [amplitude (Fig. 1C-left): Sal (11.29 ± 0.40); Coc (14.50 ± 0.97); Coc-coc 24h (10.84 ± 0.42);

$F_{(3,29)}$ = 7.72, p = 0.0006); frequency (Fig. 1C-right): Sal (3.80 ± 0.44); Coc (5.52 ± 0.30); Coc-coc 24h (3.55 ± 0.28); $F_{(3,29)}$ = 6.04, p = 0.0026]. However, 5 d following *in vivo* cocaine challenge, neither mEPSC amplitude (14.60 ± 0.89) nor frequency (5.93 ± 0.80) were significantly different from cocaine-treated controls receiving no challenge injection. Additionally, no differences in AMPAR mEPSC rise time kinetics [Sal (3.26 ± 0.35); Coc (2.70 ± 0.14); Coc-coc 24h (3.33 ± 0.10); Coc-coc 5d (3.00 ± 0.10); $F_{(3,20)}$ = 0.73, p = 0.55) or decay time kinetics [Sal (3.24 ± 0.19); Coc (3.73 ± 0.15); Coc-coc 24h (3.93 ± 0.30); Coc-coc 5d (3.93 ± 0.21); $F_{(3,20)}$ = 2.31, p = 0.11] were observed.

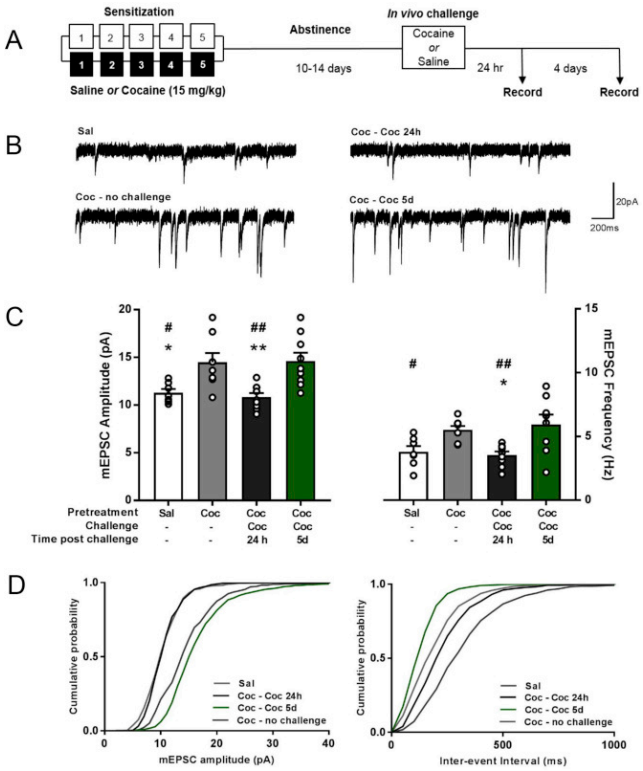

Fig. 1. **Cocaine-induced potentiation of AMPAR synaptic transmission in the NAc shell is reversed by *in vivo* cocaine challenge and persists for up to 4 days post challenge**.

(A) Experimental timeline. Following 5 days of saline or cocaine (5 mg/kg; i.p.) injections and a 10–14 d abstinence period, animals received a single challenge injection of cocaine. Electrophysiological recordings were performed either during abstinence, 24 h following a challenge injection, or 5 d following a challenge injection. (B) Representative miniature excitatory postsynaptic current (mEPSC) traces from NAc shell neurons from saline + no challenge (Sal), cocaine + no challenge (Coc - no challenge), cocaine + cocaine challenge at 24h (Coc – 24h), and cocaine + cocaine challenge at 5d (Coc – 5d). (C) Mean mEPSC amplitude (pA; left) and frequency (Hz; right) in the NAc shell from saline + no challenge (Sal, white; n = 7, N = 3), cocaine + no challenge (Coc, gray; n = 8, N = 5), cocaine + cocaine challenge at 24h (Coc-coc 24h, black; n = 9, N = 3); cocaine + cocaine challenge at 5d (Coc-coc 5d, green; n = 9, N = 3). (D) Cumulative probability distributions of mEPSC amplitude (pA; left) and inter-event interval (ms; right) from saline + no challenge (Sal, light gray), cocaine + no challenge (Coc-sal, medium gray), cocaine + cocaine challenge at 24h (Coc-coc 24h, black), cocaine + cocaine challenge at

5d (Coc-coc 5d, green). All data are presented as mean ± SEM. n, number of cells; N, number of animals. *p ≤ 0.05 vs. Coc, **p ≤ 0.01 vs Coc; #p ≤ 0.05 vs Coc-coc 5d, ##p ≤ 0.01 vs Coc-coc 5d.

Studies investigating cocaine-induced reversal of synaptic potentiation in the NAc have characteristically observed synaptic plasticity at 2–24 h following *in vivo* cocaine challenge injection, leaving open the question of how rapidly plasticity is induced by cocaine re-exposure (Bachtell and Self, 2008, Boudreau et al., 2007, Kourrich et al., 2007, Rothwell et al., 2011). To examine this, we utilized a recently developed *ex vivo* model of cocaine re-exposure which our lab has shown reverses the increase in NAc shell AMPAR transmission produced by repeated *in vivo* cocaine exposure (Jedynak et al., 2016). Following bath application of cocaine, recordings of mEPSCs were performed in the NAc shell for up to 1 h, with cells binned into 20-, 40-, and 60-min groups based on the time elapsed between cocaine challenge and recording. Consistent with previous studies (Jedynak et al., 2016, Kourrich et al., 2007), the amplitude and frequency of mEPSCs were significantly elevated in MSNs from cocaine-treated animals in the NAc shell, indicating potentiation of AMPAR synaptic transmission during abstinence [amplitude (Fig. 2C-left): Coc (13.65 ± 0.38); frequency (Fig. 2C-right): Coc (6.8 ± 0.58)]. Following *ex vivo* challenge at all time intervals, we observed a significant decrease in mEPSC amplitude and frequency, with a ∼30% reduction in AMPAR-mediated signaling observed equally at all three post-challenge timepoints [amplitude (Fig. 2C-left): 20 min (9.62 ± 0.40); 40 min (10.30 ± 0.65); 60 min (10.46 ± 0.60); $F_{(3,46)} = 14.36$, $p < 0.0001$; frequency (Fig. 2C-right): 20 min (3.95 ± 0.49); 40 min (3.44 ± 0.22); 60 min (3.44 ± 0.37); $F_{(3,46)} = 9.03$, $p < 0.0001$]. No differences in rise time kinetics (milliseconds, ms) [Coc (3.29 ± 0.08); 20 min (3.53 ± 0.19); 40 min (3.60 ± 0.20); 60 min (3.58 ± 0.23); $F_{(3,32)} = 1.21$, $p = 0.32$) or decay time kinetics (milliseconds, ms) [Coc (4.11 ± 0.15); 20 min (4.12 ± 0.21); 40 min (3.72 ± 0.38); 60 min (4.24 ± 0.36); $F_{(3,34)} = 0.62$, $p = 0.61$] of AMPAR mEPSCs were observed. Taken together, these results demonstrate that in cocaine-experienced animals, re-exposure to cocaine after a period of abstinence reverses the cocaine-elicited potentiation of AMPAR-mediated signaling on a rapid timescale (less than 20 min), but the reduction in AMPAR synaptic transmission is constrained to a time window of 1–5 days before synapses return to a potentiated state.

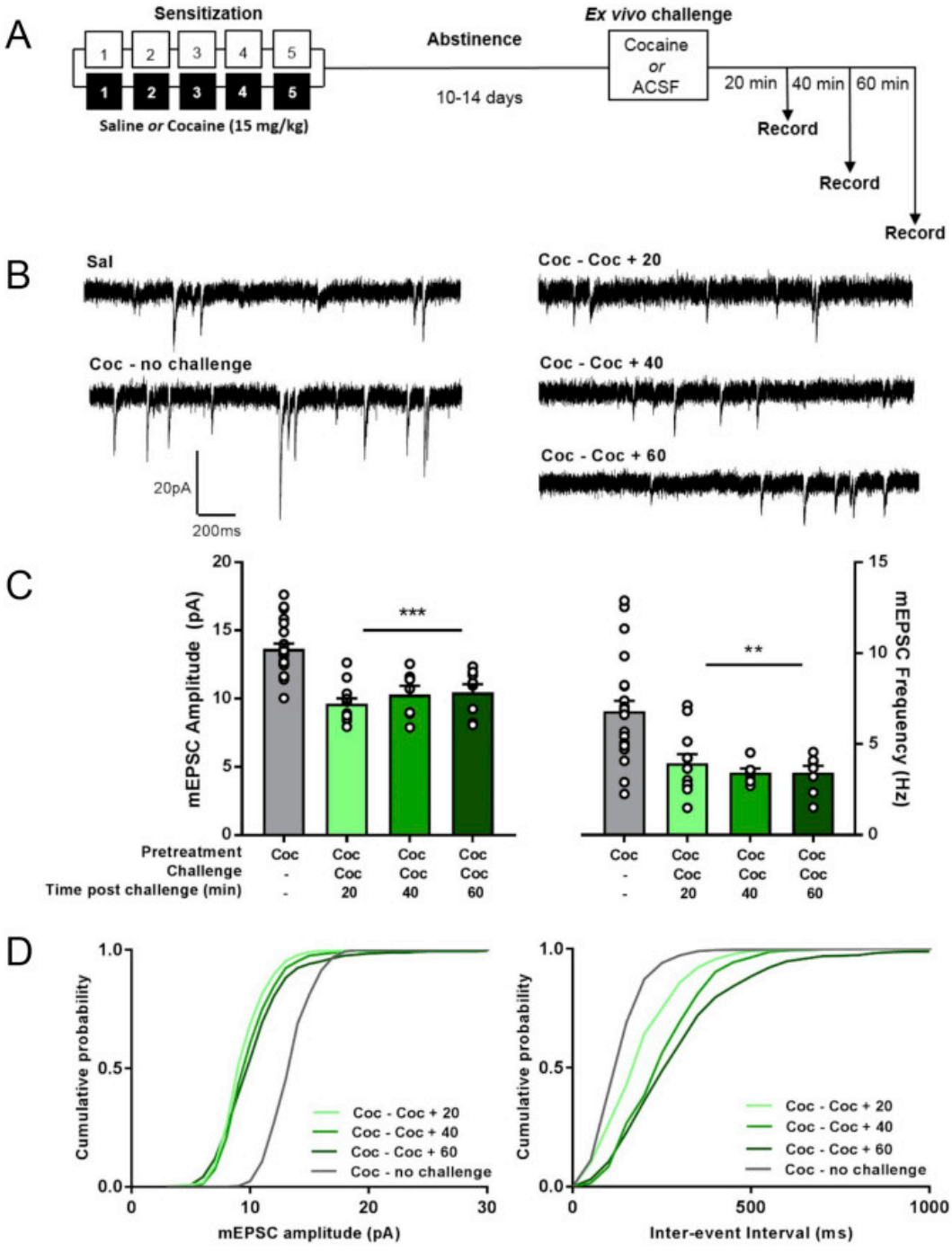

Fig. 2. ***Ex vivo* cocaine re-exposure induces depotentiation of AMPAR signaling in the NAc shell that is maximally expressed within 20 min post challenge**.

(A) Experimental timeline. Following 10–14 days of abstinence from repeated saline or cocaine (5 mg/kg; i.p.) injections, electrophysiological recordings were performed in acute slices receiving 1) no bath challenge (ACSF only) or 2) *ex vivo* cocaine (10 µM; 10 min), with recordings taking place at 20, 40, or 60 min following the initial 10 min bath challenge. (B) Representative miniature excitatory

postsynaptic current (mEPSC) traces from NAc shell neurons from saline + no challenge (Sal), cocaine + no challenge (Coc - no challenge), cocaine + cocaine challenge at 20 min (Coc-coc + 20), cocaine + cocaine challenge at 40 min (Coc-coc + 40), and cocaine + cocaine challenge at 60 min (Coc-coc + 60). (C) Mean mEPSC amplitude (pA; left) and frequency (Hz; right) in the NAc shell from cocaine + no challenge (Coc, gray; n = 23, N = 19), cocaine + cocaine 20 min post challenge (Coc-coc 20, light green; n = 7, N = 7), cocaine + cocaine 40 min post challenge (Coc-coc 40, medium green; n = 6, N = 6), cocaine + cocaine 60 min post challenge (Coc-coc 60, dark green; n = 6, N = 6). (D) Cumulative probability distributions of mEPSC amplitude (pA; left) and inter-event interval (ms; right) from cocaine + no challenge (Coc-sal, gray), cocaine + cocaine 20 min post challenge (Coc-coc + 20, light green), cocaine + cocaine 40 min post challenge (Coc-coc + 40, medium green), cocaine + cocaine 60 min post challenge (Coc-coc + 60, dark green). All data are presented as mean ± SEM. n, number of cells; N, number of animals. **p ≤ 0.01 vs. Coc, ***p ≤ 0.001 vs Coc.

3.2. Dopamine receptor activation is necessary and sufficient for cocaine-induced synaptic depotentiation

In the striatum, dopamine plays a critical role in regulating the induction of synaptic plasticity by modulating excitatory synaptic transmission through several mechanisms, including modification of post-synaptic neurotransmitter receptors (O'Donnell, 2003, Surmeier et al., 2007, Tritsch and Sabatini, 2012). Several studies indicate that dopamine receptor activation promotes increased surface expression of MSN AMPARs (Snyder et al., 2000, Wolf, 2010). Thus, we hypothesized that dopamine receptor activation provoked by cocaine re-exposure might participate in the reversal of enhanced AMPAR synaptic transmission in the NAc shell. To examine this, we applied the non-specific dopamine antagonist flupenthixol *ex vivo* prior to cocaine challenge to determine whether dopamine receptor activation is necessary for the induction of AMPAR synaptic depotentiation. A non-specific dopamine receptor antagonist was chosen for these studies, as our goal was to assess AMPAR function in NAc MSN subpopulations as a whole, rather than differentiate between effects that may selectively occur on MSNs expressing select DA receptor sub-types. As previously demonstrated, abstinence (10–14 d) from repeated cocaine potentiated mEPSC amplitude, which was reversed by *ex vivo* cocaine bath challenge [Fig. 3C-left: Sal (10.19 ± 0.53); Coc (13.09 ± 0.46); Coc-coc (10.04 ± 0.40); $F_{(4,37)} = 10.39$, $p < 0.0001$]. Similarly, mEPSC frequency was increased during withdrawal from cocaine but reversed by *ex vivo* cocaine challenge [Fig. 3C-right: Sal (4.08 ± 0.53); Coc (6.87 ± 0.84); Coc-coc (4.23 ± 0.42); $F_{(4,37)} = 4.59$, $p = 0.0041$]. However, bath application of flupenthixol (20 μM) prior to *ex vivo* cocaine challenge blocked the cocaine-induced reduction in mEPSC amplitude (12.42 ± 0.70) and frequency (6.410 ± 0.86). Exposure to flupenthixol alone had no baseline effects on mEPSC amplitude or frequency in saline-treated (amplitude: 10.53 ± 0.89; frequency: 4.81 ± 0.76) or cocaine-treated animals (amplitude: 12.08 ± 0.64; frequency: 5.36 ± 0.67). In addition, there were no differences in the rise time kinetics [Sal (3.28 ± 0.14); Coc (3.18 ± 0.08); Coc-coc (3.61 ± 0.19); Coc-Flp-coc (3.31 ± 0.13); $F_{(4,34)} = 1.70$, $p = 0.17$) or decay time kinetics [Sal (3.62 ± 0.22); Coc (3.89 ± 0.16); Coc-coc (3.79 ± 0.27); Coc-Flp-coc (4.17 ± 0.23); $F_{(4,34)} = 0.80$, $p = 0.53$] of AMPAR mEPSCs evident among the groups.

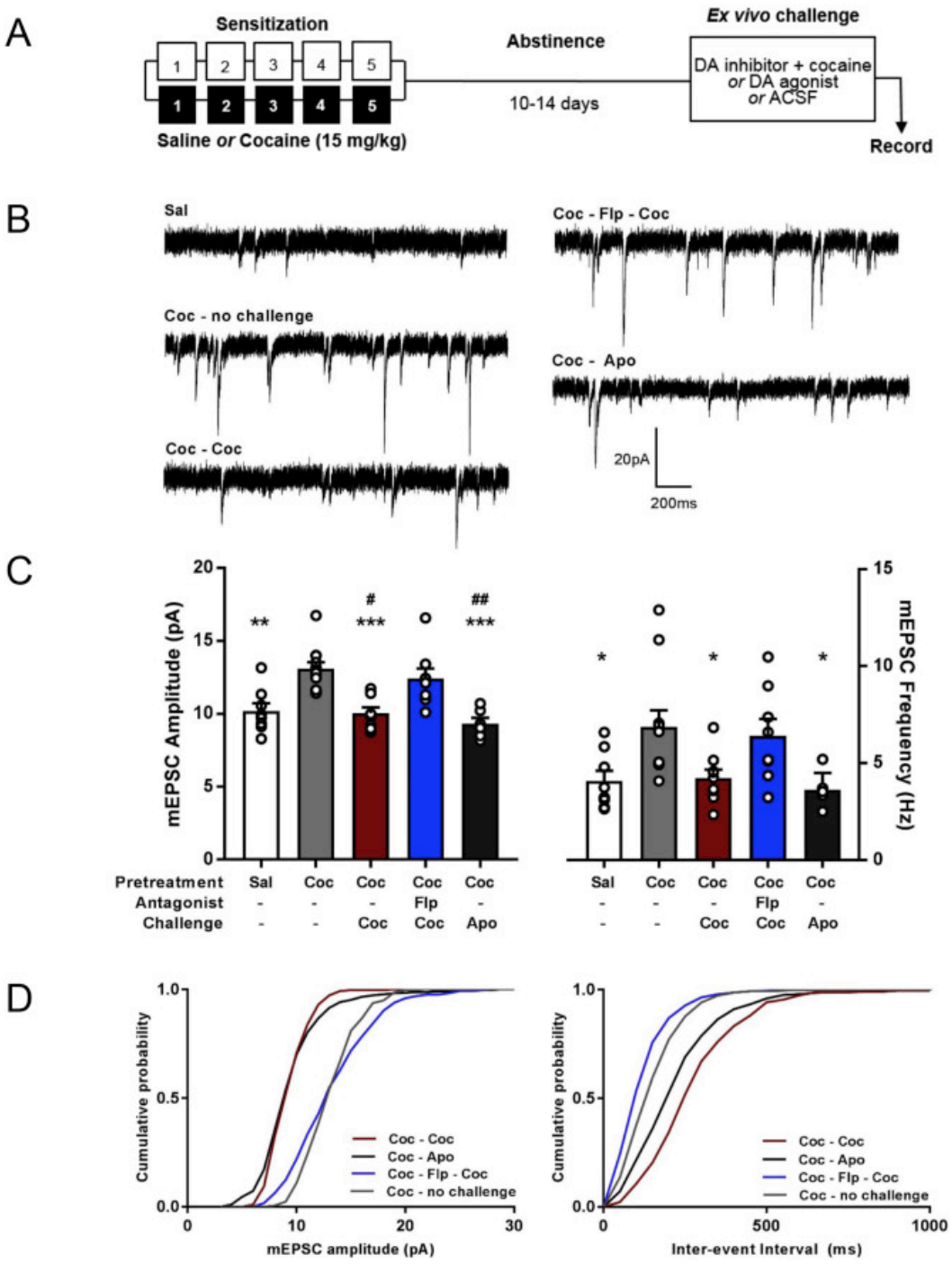

Fig. 3. **Dopamine receptor activation is required for depotentiation of NAc shell AMPAR signaling induced by *ex vivo* cocaine re-exposure**.

(A) Experimental timeline. Following 10–14 days of abstinence from repeated saline or cocaine (5 mg/kg; i.p.) injections, electrophysiological recordings were performed in acute slices receiving 1) no bath challenge (ACSF only); 2) *ex vivo* cocaine challenge (10 µM; 10 min); 3) *ex vivo* bath application of the dopamine receptor antagonist flupenthixol (20 µM; 10 min) followed by 10 µM

cocaine + flupenthixol (10 min); or 4) *ex vivo* bath application of the dopamine receptor agonist apomorphine (1 μM). (B) Representative miniature excitatory postsynaptic current (mEPSC) traces from NAc shell neurons from saline + no challenge (Sal), cocaine + no challenge (Coc - no challenge), cocaine + cocaine challenge (Coc-coc), cocaine + flupenthixol/cocaine (Coc-Flp-coc), cocaine + apomorphine (Coc-Apo). C) Mean mEPSC amplitude (pA; left) and frequency (Hz; right) in the NAc shell from saline + no challenge (Sal, white; n = 8; N = 6), cocaine + no challenge (Coc, gray; n = 10, N = 11), cocaine + cocaine challenge (Coc-coc, red; n = 9, N = 7), cocaine + flupenthixol/cocaine (Coc-Flp-coc, blue; n = 7, N = 5), cocaine + apomorphine (Coc-Apo, black; n = 6, N = 4). (D) Cumulative probability distributions of mEPSC amplitude (pA; left) and inter-event interval (ms; right) from cocaine + no challenge (Coc-sal, gray), cocaine + cocaine challenge (Coc-coc, red), cocaine + flupenthixol/coc (Coc-Flp-coc, blue), cocaine + apomorphine (Coc-Apo, black). All data are presented as mean ± SEM. n, number of cells; N, number of animals. *p ≤ 0.05 vs. Coc, **p ≤ 0.01 vs. Coc, ***p ≤ 0.001 vs Coc; #p ≤ 0.06 vs. Coc-Flp-coc, ##p ≤ 0.01 vs. Coc-Flp-coc.

Given that activation of DA receptors has been shown to mediate the induction of striatal LTD (Calabresi et al., 2007, Kreitzer and Malenka, 2005), we next investigated whether dopamine receptor activation is sufficient to drive reductions in AMPAR signaling produced by cocaine and subsequent abstinence. *Ex vivo* application of the non-specific dopamine receptor agonist apomorphine (1 μM) reduced mEPSC amplitude (Fig. 3C-left; 9.33 ± 0.40) and frequency (Fig. 3C-right; 3.61 ± 0.36) compared to cocaine + no challenge controls. Apomorphine application in saline animals had no effect on baseline mEPSC amplitude (9.62 ± 0.63) or frequency (3.37 ± 0.61). AMPAR mEPSC rise time (3.56 ± 0.21) and decay time kinetics (3.91 ± 0.26) were unchanged by apomorphine. Taken together, these data demonstrate that dopamine receptor activation is both necessary and sufficient for AMPAR synaptic depotentiation elicited by re-exposure to cocaine, demonstrating a central role for dopamine receptors in mediating this form of plasticity.

3.3. Activation of CB1 receptors drives cocaine-induced synaptic depotentiation

Within the NAc, endogenous cannabinoids play a central role in modulating glutamatergic synaptic transmission (Lüscher and Huber, 2010, Robbe et al., 2002). Located predominantly on axon terminals contacting MSNs, activation of CB1 receptors leads to suppression of presynaptic transmitter release following activation of postsynaptic signaling (Hoffman and Lupica, 2001, Robbe et al., 2001, Robbe et al., 2003). To test whether CB1 receptor activation mediates cocaine-induced reductions in AMPAR synaptic transmission, we bath applied the CB1 antagonist/inverse agonist SR141716A during an *ex vivo* cocaine challenge. While bath application of SR141716A (1 μM) in the presence of *ex vivo* cocaine produced a modest blockade of the cocaine-induced reductions in mEPSC amplitude (Fig. 4C-right; Sal (10.32 ± 0.35); Coc (14.17 ± 0.59); Coc-coc (9.76 ± 0.52); $F_{(4,48)} = 11.31$, $p < 0.0001$), it robustly blocked the decrease in mEPSC frequency (Fig. 4C-right; Sal (3.50 ± 0.38); Coc (6.73 ± 0.82); Coc-coc (3.26 ± 0.28); Coc-SR-coc (6.36 ± 0.62); $F_{(4,48)} = 6.73$, $p = 0.0002$). No changes in AMPAR rise time kinetics [Sal (3.75 ± 0.17); Coc (3.30 ± 0.09); Coc-coc (3.53 ± 0.17); Coc-SR-coc (3.18 ± 0.16) or decay time kinetics [Sal (5.10 ± 0.32); Coc (4.36 ± 0.23); Coc-coc (4.33 ± 0.27); Coc-SR-coc (4.01 ± 0.26) were observed. Bath application of SR141716A slightly elevated baseline mEPSC frequency in both saline and cocaine pre-treated animals, but this effect was not significant [Sal-SR (5.09 ± 1.12); Coc-SR (7.91 ± 1.36), not shown].

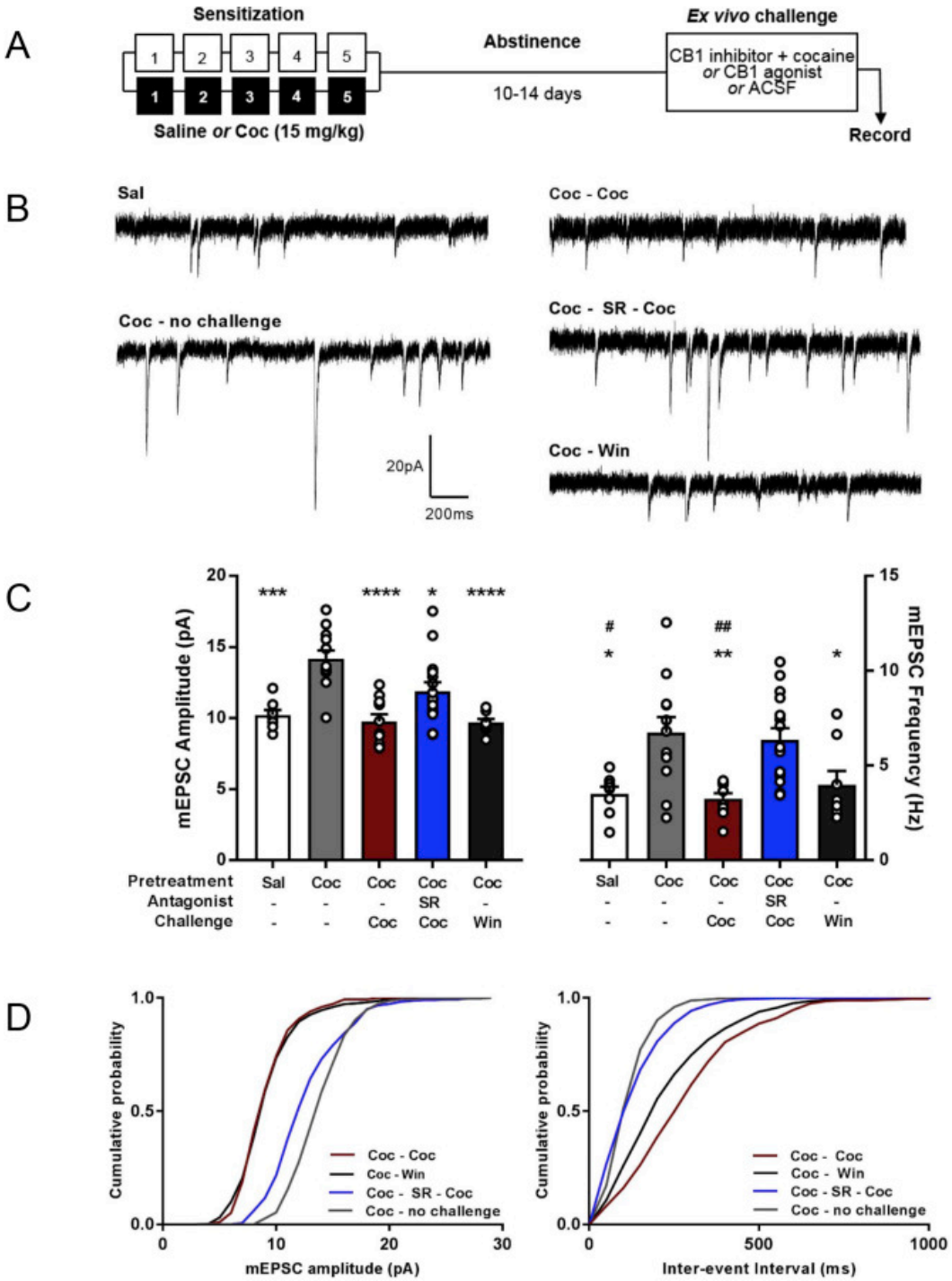

Fig. 4. **Activation of CB1 receptors is required for reversal of NAc shell AMPAR potentiation induced by *ex vivo* cocaine re-exposure**.

(A) Experimental timeline. Following 10–14 days of abstinence from repeated saline or cocaine (5 mg/kg; i.p.) injections, electrophysiological recordings were performed in acute slices receiving 1) no bath challenge (ACSF only); 2) *ex vivo* cocaine challenge (10 µM; 10 min); 3) *ex vivo* bath application of

the CB1 receptor antagonist SR141716A (1 µM; 10 min) followed by 10 µM cocaine + SR141716A (10 min); or 4) *ex vivo* bath application of CB1 receptor agonist WIN 55,212-2 (1 µM). (B) Representative miniature excitatory postsynaptic current (mEPSC) traces from NAc shell neurons from saline + no challenge (Sal), cocaine + no challenge (Coc - no challenge), cocaine + cocaine challenge (Coc-coc), cocaine + SR141716A/cocaine (Coc-SR-coc), cocaine + WIN 55,212-2 (Coc-Win). C) Mean mEPSC amplitude (pA; left) and frequency (Hz; right) in the NAc shell from saline + no challenge (Sal, white; n = 8, N = 7), cocaine + no challenge (Coc, gray; n = 12, n = 9), cocaine + cocaine challenge (Coc-coc, red; n = 10, N = 6), cocaine + SR141716A/cocaine (Coc-SR-coc, blue; n = 15, N = 9), cocaine + WIN 55,212-2 (Coc-Win, black; n = 8, N = 4). (D) Cumulative probability distributions of mEPSC amplitude (pA; left) and inter-event interval (ms; right) from cocaine + no challenge (Coc-sal, gray), cocaine + cocaine challenge (Coc-coc, red), cocaine + SR141716A/coc (Coc-SR-coc, blue), cocaine + WIN 55,212-2 (Coc-Win, black). All data are presented as mean ± SEM. n, number of cells; N, number of animals. *p ≤ 0.05 vs. Coc, **p ≤ 0.01 vs. Coc, ***p ≤ 0.001 vs Coc, ****p ≤ 0.0001; #p ≤ 0.06 vs. Coc-SR-coc, ##p ≤ 0.01 vs. Coc-SR-coc.

We next asked whether activation of CB1 receptors is sufficient to induce synaptic depotentiation in the NAc shell. While several studies have reported that activation of CB1 receptors in the striatum is sufficient to induce eCB-mediated LTD (Kreitzer and Malenka, 2005, Robbe et al., 2001), it is not clear whether these mechanisms remain intact or are altered following cocaine exposure. To investigate this, we substituted the *ex vivo* cocaine challenge with bath application of the CB1 receptor agonist WIN 55,212-2 *ex vivo.* Application of WIN 55,212-2 (1 µM) was sufficient to induce depotentiation of mEPSC amplitude (Fig. 4C-left; 9.69 ± 0.25) and frequency (Fig. 4C-right; 3.99 ± 0.73) in slices from animals abstinent from cocaine, without altering basal mEPSC amplitude (10.38 ± 0.72) or frequency (3.38 ± 0.51) in saline-treated animals. No changes in mEPSC rise time (3.72 ± 0.23) or decay time (4.76 ± 0.27) kinetics were detected. Our results demonstrate that activating CB1 receptors during abstinence from repeated cocaine is sufficient to depotentiate AMPAR-mediated synaptic transmission in the NAc.

4. Discussion

The current study investigated the temporal and mechanistic profile of bidirectional AMPAR plasticity in the NAc shell following re-exposure to cocaine during abstinence. We demonstrate that 1) enhanced AMPAR signaling produced by repeated cocaine is rapidly reversed by *in vivo* and *ex vivo* cocaine re-exposure and persists up to 4 d post challenge injection, and 2) that both endogenous dopamine and eCB signaling play necessary roles in the cocaine challenge-induced depotentiation of AMPAR synaptic transmission.

4.1. Cocaine-induced reversal of AMPAR synaptic strength is temporally constrained

Data from our lab and others has established that a single cocaine re-exposure during late abstinence reverses the enhanced AMPAR synaptic potentiation that develops during a period of abstinence from repeated cocaine, demonstrating the ability of cocaine to bidirectionally modulate synaptic strength at

NAc MSNs (Jedynak et al., 2016, Kourrich et al., 2007, Pascoli et al., 2011, Rothwell et al., 2011). However, the temporal dynamics of this plasticity were not well-defined.

Numerous studies have demonstrated that repeated exposure to cocaine promotes a time-dependent enhancement of AMPAR plasticity in the NAc that progresses as withdrawal becomes more protracted (Boudreau and Wolf, 2005, Conrad et al., 2008, Kourrich et al., 2007, Pascoli et al., 2014, Terrier et al., 2016). Our data indicate that, unlike progressive increases in AMPAR signaling, re-exposure to cocaine produces rapid-onset plasticity that culminates in reversal of potentiated AMPAR signaling and is maximally expressed within 20 min, as further reductions are not observed at 60 min or 24 h. However, within 5 days of the challenge injection, AMPAR synaptic transmission returns to a pre-challenge state that is indistinguishable from MSNs from animals in abstinence from cocaine, suggesting that synapses undergoing AMPAR-mediated depotentiation by cocaine re-exposure are effectively "repotentiated." Consistent with this, previous work has shown that the ability of an intra-NAc infusion of AMPA to augment locomotor activity is abolished within 24 h, but not 6 d following cocaine challenge (Bachtell and Self, 2008), and NAc surface expression of AMPARs is decreased 24 h after cocaine challenge but returns to pre-challenge levels of expression after 7 d abstinence (Ferrario et al., 2009).

Within the striatum, short-term and long-lasting reductions in excitatory synaptic strength are commonly expressed through rapid internalization of AMPARs (Lüscher and Huber, 2010, Snyder et al., 2001). The opposing alterations in AMPAR trafficking may simply serve as a homeostatic regulatory mechanism counteracting shifts in glutamatergic signaling induced by cocaine exposure. For example, synaptic expression of AMPARs may reflect a compensatory response to decreased extracellular glutamate and synaptic excitability that develops during extended abstinence from cocaine(Baker et al., 2003, Kourrich and Thomas, 2009, Kourrich et al., 2007), while removal of AMPARs from the synapse might offset the rapid increase in glutamate elicited by re-exposure to cocaine(Kalivas et al., 2005). Although the functional relevance of bidirectional alterations in AMPAR plasticity is unclear, these findings highlight the existence of multiple forms of plasticity at NAc synapses that likely serve to regulate and update synaptic strength as a consequence of varied drug experiences that may underlie relapse susceptibility.

4.2. Dopamine modulation of enhanced AMPAR synaptic transmission

Exposure to cocaine *in vivo* (Di Chiara and Imperato, 1988) and *ex vivo* (Hoffmann et al., 2012, Kelly and Wightman, 1987) increases the synaptic availability of dopamine within the NAc. In the present study, the ability of both DA receptor antagonists to block cocaine-induced short-term plasticity and receptor agonists to suppress excitatory signaling in a manner indistinguishable from *ex vivo* or *in vivo* cocaine (Jedynak et al., 2016) demonstrates that transient activation of endogenous dopamine signaling in the NAc shell is both necessary and sufficient for cocaine challenge-induced suppression of AMPAR plasticity. Notably, it is unclear exactly how cocaine augments endogenous DA in our *ex vivo* conditions; however, spontaneous DA transients mediated by exocytotic release events have been observed in striatal slice preparations (Beckstead et al., 2004, Gantz et al., 2013, Zhou et al., 2001), and acute *ex vivo* cocaine has been shown to increase the frequency and amplitude of dopamine transients in the NAc through activation of nicotinic receptors, indicating the involvement of cholinergic interneurons (Yorgason et al., 2017).

The effects of dopamine receptor activity on AMPAR signaling in the NAc are likely to be varied and complex, given that dopamine receptor activation is capable of bidirectionally modifying excitatory synaptic strength depending on the dopamine receptor subtype and location, MSN cell subtype, level of synaptic activity, as well as previous drug experience (Tritsch and Sabatini, 2012, Wang et al., 2012). Previous work has shown that cooperative activation of dopamine D1 and D2 receptors in the NAc shell is necessary for cocaine-primed reinstatement, while individual blockade of either receptor subtype disrupts this behavior (Anderson et al., 2005, Anderson et al., 2003, Bachtell et al., 2005, Schmidt et al., 2006), suggesting that increased extracellular dopamine elicited by cocaine might participate in modifying excitatory synaptic strength in the NAc shell during cocaine re-exposure. Because data from this study and previous work (Kourrich et al., 2007, Jedynak et al., 2016; et al., 2011) were collected from pooled populations of MSNs, it is difficult to determine not only the DA receptor sub-type involved but also the exact locus of the cocaine-dependent suppression of excitatory signaling.

Numerous studies have demonstrated that cocaine-induced changes in excitatory signaling occur almost exclusively at D1-MSN synapses (Kim et al., 2011, Lobo and Nestler, 2011, Pascoli et al., 2014, Terrier et al., 2016), suggesting that observed reductions in synaptic signaling occur in D1-MSNs. However, it is unlikely that this involves direct activation of DA receptors on D1R-MSNs, as D1R stimulation has been shown to increase AMPAR surface expression and AMPAR-mediated currents in MSNs (Chao et al., 2002, Price et al., 1999, Sun et al., 2008). Conversely, activation of presynaptic D1 receptors is known to reduce excitatory postsynaptic potentials in striatal MSNs, indicating that any effects of D1R activation are likely localized to presynaptic signaling (Harvey and Lacey, 1996, Nicola and Malenka, 1997).

Another intriguing possibility is that reductions in AMPAR signaling reflect suppression of baseline excitatory signaling primarily at D2R-MSN, rather than reversal of plasticity at D1R-MSN synapses – presumably through direct activation of D2R signaling. In agreement with this, D2R activation reduces basal and agonist-induced AMPAR signaling in striatal MSNs (André et al., 2010, Hernández-Echeagaray et al., 2004, Håkansson et al., 2006, Snyder et al., 2000). Moreover, in agreement with a role for suppressed excitatory signaling in driving addiction-related behavior, recent findings suggest that cocaine-induced reinstatement is associated with a reduction in excitatory signaling specifically in NAc shell D2R-expressing MSNs (Ortinski et al., 2015). This reduction at D2-MSN synapses would theoretically shift the ratio of excitatory drive at D1R-versus D2R-MSN circuits in favor of D1R signaling – a possibility that fits well with the ventral striatal D1 pathway in behavior and reward, as well as emerging theories on circuit-based plasticity responsible for relapse (Dobi et al., 2011, Graziane et al., 2016, Hearing et al., 2016, Kim et al., 2011, Lobo and Nestler, 2011, Ortinski et al., 2015, Ortinski et al., 2012, Pascoli et al., 2011, Smith et al., 2013, Suska et al., 2013). Regardless, our findings indicate that endogenous dopamine signaling plays a critical role in modifying NAc synapses potentiated by previous cocaine experience, and may provide a mechanism for developing therapeutic strategies.

4.3. CB1 receptors mediate synaptic depotentiation in the NAc shell

Within the striatum, eCB signaling is a critical regulator of synaptic activity and plasticity, facilitating synaptic depression at glutamatergic synapses on MSNs through CB1 receptor-mediated suppression

of excitatory transmission (Gerdeman et al., 2002, Kreitzer and Malenka, 2007, Robbe et al., 2002). Furthermore, accumulating evidence indicates a critical role for eCB signaling in reward-related behavior and facilitation of excitatory synaptic plasticity in the striatum (Wiskerke et al., 2008, Zlebnik and Cheer, 2016). Blocking CB1 receptors reduces the rewarding and reinforcing properties of cocaine (Orio et al., 2009, De Vries et al., 2001, Xi et al., 2006), indicating that CB1 activity potently regulates behavioral sensitivity to cocaine. Given the fundamental role that eCBs play in dampening excitatory signaling throughout the striatum, eCB signaling mechanisms might be engaged by cocaine re-exposure to mediate cocaine-induced depotentiation of synaptic strength in the NAc shell. While evidence of the impact of repeated cocaine on eCB-mediated signaling and synaptic plasticity in the NAc is still relatively limited, several studies previously suggested that eCB-mediated plasticity in the striatum is impaired by acute cocaine (Fourgeaud et al., 2004, Grueter et al., 2010). However, more recent work demonstrated that CB1 signaling remains functionally intact following repeated cocaine (McCutcheon et al., 2011, Ortinski et al., 2012). In the present study, we observed that application of the CB1 receptor antagonist/inverse agonist SR141716A prevented the cocaine-induced reduction in mEPSC frequency while partially rescuing the challenge-induced reduction in mEPSC amplitude. Additionally, we found that activation of CB1 receptors with the agonist WIN 55,212-2 was sufficient to depotentiate both mEPSC amplitude and frequency. These findings confirm that CB1 signaling remains functionally intact following repeated cocaine and is capable of modulating glutamatergic synaptic plasticity in the NAc shell.

To our knowledge, our data are the first to report that CB1 signaling modifies post-synaptic AMPAR signaling during protracted abstinence from cocaine. The ability of the CB1 agonist WIN 55,212-2 to induce alterations in both mEPSC frequency and amplitude is surprising given the pre-synaptic localization of these receptors on glutamatergic afferents in the NAc. However, there is some evidence that WIN 55,212-2 is capable of depressing mEPSC amplitude and frequency in the NAc under basal (non-drug) conditions (Hoffman and Lupica, 2001). Additionally, endogenous and exogenous cannabinoid ligands have been shown to alter post-synaptic signaling properties by modulating a number of ionic conductances (Deadwyler et al., 1995, Mackie et al., 1995, Schweitzer, 2000) or other non-CB1 targets such as post-synaptic TRPV1 receptors (Grueter et al., 2010). Furthermore, it is relatively unknown how repeated cocaine exposure influences these complex eCB signaling mechanisms. Additional studies will be needed to elucidate how CB1 receptor activation is capable of broadly modulating both pre- and post-synaptic glutamatergic transmission in the NAc following experience with cocaine.

Given that striatal CB1 signaling plays a central role in synaptic depression at glutamatergic synapses, our data suggests that cocaine re-exposure during abstinence engages similar CB1 signaling mechanisms that promote a reduction in glutamatergic synaptic transmission at NAc MSN synapses. CB1 signaling in the NAc therefore might participate in reducing excitatory synaptic strength by interacting with a number of potential cellular mechanisms. A substantial body of evidence has demonstrated that eCB-mediated depression of glutamatergic transmission in the striatum commonly requires activation of Group I mGluRs (mGluR1 and mGluR5) (Lüscher and Huber, 2010). Activation of post-synaptic mGluR1/5 on NAc MSNs promotes the release of eCBs, which activate pre-synaptic CB1 receptors to decrease pre-synaptic release probability and promote synaptic depression (Castillo et al., 2012, Robbe et al., 2002). Enhanced glutamatergic synaptic transmission in the NAc elicited by re-

exposure to cocaine (McFarland et al., 2003, Park et al., 2002) may therefore activate mGluR1/5 and initiate eCB signaling, promoting a reduction in synaptic strength. Previously, our lab demonstrated that mGluR5 signaling in the NAc shell is necessary for cocaine-induced AMPAR synaptic depotentiation (Jedynak et al., 2016). Our data here showing that synaptic depotentiation requires CB1 receptor activity in the NAc shell could reflect serial activation of mGluR5 and CB1 receptors that participate in reducing glutamatergic synaptic transmission in the NAc shell following cocaine re-exposure.

Dopamine D2 receptors have been shown to cooperate with Group I mGluRs to promote eCB signaling and excitatory synaptic depression in the striatum (Kreitzer and Malenka, 2005, Yin and Lovinger, 2006). Glutamatergic synapses onto D2 receptor-expressing MSNs in the striatum preferentially express eCB-mediated LTD compared to D1-MSNs (Grueter et al., 2010, Kreitzer and Malenka, 2007), and several studies have reported a requirement for D2 receptor activation in striatal eCB-LTD (Kreitzer and Malenka, 2007, Shen et al., 2008, Wang et al., 2006). A potential explanation for this phenomenon is that D2 receptor activation facilitates eCB production in the striatum (Centonze et al., 2004, Giuffrida et al., 1999, Patel et al., 2003, Wang et al., 2006), and therefore may cooperate with Group I mGluRs to promote eCB-mediated synaptic depression. Increased synaptic availability of dopamine elicited by cocaine re-exposure may therefore promote eCB signaling preferentially through D2 receptor activation in MSNs of the NAc shell. While additional studies are needed to determine the cell type- and receptor type-specific mechanisms by which cocaine re-exposure engages both dopamine and eCB signaling mechanisms to modify glutamatergic synaptic plasticity, our findings demonstrate that the eCB system participates in attenuating potentiated AMPAR synaptic transmission during re-exposure to cocaine. Given that increasing clinical evidence suggests that an interaction between both the dopamine and eCB systems is involved in the pathophysiology of numerous limbic-based disorders involving the striatum, (García et al., 2016, Kuepper et al., 2010, Parolaro and Rubino, 2008, Pisani et al., 2011), understanding the complex interactions between these two systems may be critical in developing future therapeutic strategies for relapse.

5. Conclusion

These studies broadly demonstrate that dopamine and endocannabinoid signaling in the NAc can have diverse effects on AMPAR-mediated synaptic function depending on previous psychostimulant experience. Here, we examined mechanisms underlying the depotentiation of AMPAR-mediated synaptic transmission in the NAc shell evoked by cocaine re-exposure, finding that plasticity is rapidly induced and persists for up several days before returning to “repotentiated” levels, suggesting that synapses altered by cocaine exposure remain plastic and are capable of undergoing further modifications by re-exposure to cocaine. Both dopamine and eCBs, critical modulators of synaptic plasticity in the NAc, contribute to AMPAR synaptic depotentiation, indicating that cocaine re-exposure engages multiple signaling pathways in the NAc that cooperate in modifying excitatory synaptic strength. Alterations in glutamatergic synaptic transmission induced by experience with cocaine may therefore profoundly alter the responsiveness of MSNs to future stimulation by dopamine, modifying dopamine's capacity to effectively gate activity and plasticity at glutamatergic synapses. Additionally, changes in dopamine receptor expression and signaling during abstinence or extinction (Anderson and

Pierce, 2005) may modify how dopamine and glutamate receptor signaling mechanisms converge and interact at an intracellular level. Endocannabinoid signaling may additionally modulate MSN responsiveness to alterations in glutamatergic signaling induced by cocaine experience. Further studies will be needed to determine the cellular signaling factors mediating the complex effects of these critical neuromodulators on cocaine-induced AMPAR plasticity.

These signaling mechanisms may gate the induction of plasticity at inputs to the NAc by converging on common factors that drive alterations in MSN excitability and plasticity and ultimately drive addiction-related behavior, providing targetable mechanisms for developing therapeutic strategies.

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References

S. Anderson, A. Bari, C. Pierce **Administration of the D1-like dopamine receptor antagonist SCH-23390 into the medial nucleus accumbens shell attenuates cocaine priming-induced reinstatement of drug-seeking behavior in rats**, Psychopharmacology, 168 (2003), pp. 132-138

S. Anderson, H. Schmidt, C. Pierce **Administration of the D2 dopamine receptor antagonist sulpiride into the shell, but not the core, of the nucleus accumbens attenuates cocaine priming-induced reinstatement of drug seeking**, Neuropsychopharmacology, 31 (2005), pp. 1452-1461

S. Anderson, R.C. Pierce **Cocaine-induced alterations in dopamine receptor signaling: implications for reinforcement and reinstatement**, Pharmacol. Ther., 106 (2005), pp. 389-403

V.M.M. André, C. Cepeda, D.M. Cummings, E.L. Jocoy, Y.E. Fisher, X. William Yang, M.S. Levine, **Dopamine modulation of excitatory currents in the striatum is dictated by the expression of D1 or D2 receptors and modified by endocannabinoids**Eur. J. Neurosci., 31 (2010), pp. 14-28

R. Bachtell, D. Self **Renewed cocaine exposure produces transient alterations in nucleus accumbens AMPA receptor-mediated behavior**, J. Neurosci., 28 (2008), pp. 12808-12814

R.K. Bachtell, K. Whisler, D. Karanian, D.W. Self **Effects of intra-nucleus accumbens shell administration of dopamine agonists and antagonists on cocaine-taking and cocaine-seeking behaviors in the rat**, Psychopharmacol. (Berl.), 183 (2005), pp. 41-53

D. Baker, K. McFarland, R. Lake, H. Shen, X.-C. Tang, S. Toda, P. Kalivas **Neuroadaptations in cystine-glutamate exchange underlie cocaine relapse**, Nat. Neurosci., 6 (2003), pp. 743-749

M. Beckstead, D. Grandy, K. Wickman, J. Williams **Vesicular dopamine release elicits an inhibitory postsynaptic current in midbrain dopamine neurons**, Neuron, 42 (2004), pp. 939-946

A. Boudreau, M. Wolf **Behavioral sensitization to cocaine is associated with increased AMPA receptor surface expression in the nucleus accumbens**, J. Neurosci., 25 (2005), pp. 9144-9151

A.C. Boudreau, J.M. Reimers, M. Milovanovic, M.E. Wolf **Cell surface AMPA receptors in the rat nucleus accumbens increase during cocaine withdrawal but internalize after cocaine challenge in association with altered activation of mitogen-activated protein kinases**, J. Neurosci., 27 (2007), pp. 10621-10635

J. Britt, F. Benaliouad, R. McDevitt, G. Stuber, R. Wise, A. Bonci **Synaptic and behavioral profile of multiple glutamatergic inputs to the nucleus accumbens**, Neuron, 76 (2012), pp. 790-803

P. Calabresi, B. Picconi, A. Tozzi, M. Filippo **Dopamine-mediated regulation of corticostriatal synaptic plasticity**, Trends Neurosci., 30 (2007), pp. 211-219

P.E. Castillo, T.J. Younts, A.E. Chavez, Y. Hashimotodani, Neuron, 76 (1) (2012), pp. 70-81

D. Centonze, N. Battista, S. Rossi, N.B. Mercuri, A. Finazzi-Agrò, G. Bernardi, P. Calabresi, M. Maccarrone **A critical interaction between dopamine D2 receptors and endocannabinoids mediates the effects of cocaine on striatal gabaergic Transmission**, Neuropsychopharmacology, 29 (2004), pp. 1488-1497

S. Chao, M. Ariano, D. Peterson, M. Wolf **D1 dopamine receptor stimulation increases GluR1 surface expression in nucleus accumbens neurons**, J. Neurochem., 83 (2002), pp. 704-712

G. Di Chiara, A. Imperato **Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats**, Proc. Natl. Acad. Sci. U.S.A., 85 (1988), pp. 5274-5278

K.L. Conrad, K.Y. Tseng, J.L. Uejima, J.M. Reimers, L.-J.J. Heng, Y. Shaham, M. Marinelli, M.E. Wolf **Formation of accumbens GluR2-lacking AMPA receptors mediates incubation of cocaine craving**, Nature, 454 (2008), pp. 118-121

Deadwyler, Hampson, Mu, Whyte, Childers **Cannabinoids modulate voltage sensitive potassium A-current in hippocampal neurons via a cAMP-dependent process**, J. Pharmacol. Exp. Ther., 273 (1995), pp. 734-743

A. Dobi, G. Seabold, C. Christensen, R. Bock, V. Alvarez **Cocaine-induced plasticity in the nucleus accumbens is cell specific and develops without prolonged withdrawal**, J. Neurosci., 31 (2011), pp. 1895-1904

C.R. Ferrario, X. Li, X. Wang, J.M. Reimers, J.L. Uejima, M.E. Wolf **The role of glutamate receptor redistribution in locomotor sensitization to cocaine**, 35 (2009), pp. 818-833

L. Fourgeaud, S. Mato, D. Bouchet, A. Hémar, P.F. Worley, O.J. Manzoni **A single in vivo exposure to cocaine abolishes endocannabinoid-mediated long-term depression in the nucleus accumbens**, J. Neurosci., 24 (2004), pp. 6939-6945

S. Gantz, J. Bunzow, J. Williams **Spontaneous inhibitory synaptic currents mediated by a G protein-coupled receptor**, Neuron, 78 (2013), pp. 807-812

C. García, C. Palomo-Garo, Y. Gómez-Gálvez, J. Fernández-Ruiz **Cannabinoid-dopamine interactions in the physiology and physiopathology of the basal ganglia**, Br. J. Pharmacol., 173 (2016), pp. 2069-2079

G.L. Gerdeman, J. Ronesi, D.M. Lovinger **Postsynaptic endocannabinoid release is critical to long-term depression in the striatum**, Nat. Neurosci., 5 (2002), pp. 446-451

A. Giuffrida, L.H. Parsons, T.M. Kerr, F de Fonseca, M. Navarro **Dopamine activation of endogenous cannabinoid signaling in dorsal striatum**, Nat. Neurosci., 2 (1999), pp. 358-363

N.M. Graziane, S. Sun, W.J. Wright, D. Jang, Z. Liu, Y.H. Huang, E.J. Nestler, Y.T. Wang, O.M. Schlüter, Y. Dong **Opposing mechanisms mediate morphine- and cocaine-induced generation of silent synapses**, Nat. Neurosci., 19 (2016), pp. 915-925

B.A. Grueter, G. Brasnjo, R.C. Malenka **Postsynaptic TRPV1 triggers cell type-specific long-term depression in the nucleus accumbens**, Nat. Neurosci., 13 (2010), pp. 1519-1525

Harvey, Lacey **Endogenous and exogenous dopamine depress EPSCs in rat nucleus accumbens in vitro via D1 receptors activation**, J. Physiol., 492 (1996), pp. 143-154

M.C. Hearing, J. Jedynak, S.R. Ebner, A. Ingebretson, A.J. Asp, R.A. Fischer, C. Schmidt, E.B. Larson, M.J. Thomas **Reversal of morphine-induced cell-type-specific synaptic plasticity in the nucleus accumbens shell blocks reinstatement**, Proc. Natl. Acad. Sci. U.S.A., 113 (2016), pp. 757-762

E. Hernández-Echeagaray, A. Starling, C. Cepeda, M. Levine **Modulation of AMPA currents by D2 dopamine receptors in striatal medium-sized spiny neurons: are dendrites necessary?**, Eur. J. Neurosci., 19 (2004), pp. 2455-2463

Hoffman, Lupica **Direct actions of cannabinoids on synaptic transmission in the nucleus accumbens: a comparison with opioids**, J. Neurophysiol., 85 (2001), pp. 72-83

H. Hoffmann, R. Nadal, M. Vignes, J. Ortiz **Chronic cocaine self-administration modulates ERK1/2 and CREB responses to dopamine receptor agonists in striatal slices**, Addict. Biol., 17 (2012), pp. 565-575

S. Hyman, R. Malenka, E. Nestler **Neural mechanisms of addiction: the role of reward-related learning and memory**, Annu. Rev. Neurosci., 29 (2006), pp. 565-598

K. Håkansson, S. Galdi, J. Hendrick, G. Snyder, P. Greengard, G. Fisone **Regulation of phosphorylation of the GluR1 AMPA receptor by dopamine D2 receptors**, J. Neurochem., 96 (2006), pp. 482-488

J. Jedynak, M. Hearing, A. Ingebretson, S.R. Ebner, M. Kelly, R.A. Fischer, S. Kourrich, M.J. Thomas **Cocaine and amphetamine induce overlapping but distinct patterns of AMPAR plasticity in nucleus accumbens medium spiny neurons**, Neuropsychopharmacology, 41 (2016), pp. 464-476

P.W. Kalivas, N. Volkow, J. Seamans **Unmanageable motivation in addiction: a pathology in prefrontal-accumbens glutamate transmission**, Neuron, 45 (2005), pp. 647-650

M. Kano, T. Ohno-Shosaku, Y. Hashimotodani, M. Uchigashima, M. Watanabe **Endocannabinoid-mediated control of synaptic transmission**, Physiol. Rev., 89 (2009), pp. 309-380

J.A. Kauer, R.C. Malenka **Synaptic plasticity and addiction**, Nat. Rev. Neurosci., 8 (2007), pp. 844-858

R. Kelly, M. Wightman **Detection of dopamine overflow and diffusion with voltammetry in slices of rat brain**, Brain Res., 423 (1987), pp. 79-87

J. Kim, B.-H. Park, J. Lee, S. Park, J.-H. Kim **Cell type-specific alterations in the nucleus accumbens by repeated exposures to cocaine**, Biol. Psychiat, 69 (2011), pp. 1026-1034

S. Kourrich, P.E. Rothwell, J.R. Klug, M.J. Thomas **Cocaine experience controls bidirectional synaptic plasticity in the nucleus accumbens**, J. Neurosci., 27 (2007), pp. 7921-7928

S. Kourrich, M. Thomas **Similar neurons, opposite adaptations: psychostimulant experience differentially alters firing properties in accumbens core versus shell**, J. Neurosci., 29 (2009), pp. 12275-12283

A. Kreitzer, R. Malenka **Dopamine modulation of state-dependent endocannabinoid release and long-term depression in the striatum**, J. Neurosci., 25 (2005), pp. 10537-10545

A. Kreitzer, R. Malenka **Endocannabinoid-mediated rescue of striatal LTD and motor deficits in Parkinson's disease models**, Nature, 445 (2007), pp. 643-647

R. Kuepper, P. Morrison, J. Os, R. van Murray, G. Kenis, C. Henquet **Does dopamine mediate the psychosis-inducing effects of cannabis? A review and integration of findings across disciplines**, Schizophrenia Res., 121 (2010), pp. 107-117

T. Lerner, A. Kreitzer **Neuromodulatory control of striatal plasticity and behavior**, Curr. Opin. Neurobiol., 21 (2011), pp. 322-327

M.K. Lobo, E.J. Nestler **The striatal balancing act in drug addiction: distinct roles of direct and indirect pathway medium spiny neurons**, Front. Neuroanat., 5 (2011), p. 41

D. Lovinger, B. Mathur **Endocannabinoids in striatal plasticity**, Park Relat. Disord., 18 (2012), pp. S132-S134

C. Lüscher, K. Huber **Group 1 mGluR-dependent synaptic long-term depression: mechanisms and implications for circuitry and disease**, Neuron, 65 (2010), pp. 445-459

C. Lüscher, R.C. Malenka **Drug-evoked synaptic plasticity in addiction: from molecular changes to circuit remodeling**, Neuron, 69 (2011), pp. 650-663

Mackie, Lai, Westenbroek, Mitchell **Cannabinoids activate an inwardly rectifying potassium conductance and inhibit Q-type calcium currents in AtT20 cells transfected with rat brain cannabinoid receptor**, J. Neurosci. Official J. Soc. Neurosci., 15 (1995), pp. 6552-6561

J.E. McCutcheon, J.A. Loweth, K.A. Ford, M. Marinelli, M.E. Wolf, K.Y. Tseng **Group I mGluR activation reverses cocaine-induced accumulation of calcium-permeable AMPA receptors in nucleus accumbens synapses via a protein kinase C-dependent mechanism**, J. Neurosci., 31 (2011), pp. 14536-14541

K. McFarland, C.C. Lapish, P.W. Kalivas **Prefrontal glutamate release into the core of the nucleus accumbens mediates cocaine-induced reinstatement of drug-seeking behavior**, J. Neurosci. Official J. Soc. Neurosci., 23 (2003), pp. 3531-3537

J. McReynolds, E. Doncheck, O. Vranjkovic, G. Ganzman, D. Baker, C. Hillard, J. Mantsch **CB1 receptor antagonism blocks stress-potentiated reinstatement of cocaine seeking in rats**, Psychopharmacology, 233 (2016), pp. 99-109

Nicola, Malenka **Dopamine depresses excitatory and inhibitory synaptic transmission by distinct mechanisms in the nucleus accumbens**, J. Neurosci. Official J. Soc. Neurosci., 17 (1997), pp. 5697-5710

Nicola, Surmeier, Malenka **Dopaminergic modulation of neuronal excitability in the striatum and nucleus accumbens**, Annu. Rev. Neurosci., 23 (2000), pp. 185-215

L. Orio, S. Edwards, O. George, L. Parsons, G. Koob **A role for the endocannabinoid system in the increased motivation for cocaine in extended-access conditions**, J. Neurosci., 29 (2009), pp. 4846-4857

P.I. Ortinski, L.A. Briand, R.C. Pierce, H.D. Schmidt **Cocaine-seeking is associated with PKC-dependent reduction of excitatory signaling in accumbens shell D2 dopamine receptor-expressing neurons**, Neuropharmacology, 92 (2015), pp. 80-89

P.I. Ortinski, F.M. Vassoler, G.C. Carlson, R.C. Pierce **Temporally dependent changes in cocaine-induced synaptic plasticity in the nucleus accumbens shell are reversed by D1-like dopamine receptor stimulation**, Neuropsychopharmacology, 37 (2012), pp. 1671-1682

P. O'Donnell **Dopamine gating of forebrain neural ensembles**, Eur. J. Neurosci., 17 (2003), pp. 429-435

W.-K. Park, Bari, Jey, Anderson, Spealman, Rowlett, Pierce **Cocaine administered into the medial prefrontal cortex reinstates cocaine-seeking behavior by increasing AMPA receptor-mediated glutamate transmission in the nucleus accumbens**, J. Neurosci. Official J. Soc. Neurosci., 22 (2002), pp. 2916-2925

Parolaro, Rubino **The role of the endogenous cannabinoid system in drug addiction**, Drug news Perspect., 21 (2008), pp. 149-157

V. Pascoli, J. Terrier, J. Espallergues, E. Valjent, E.C. O'Connor, C. Lüscher **Contrasting forms of cocaine-evoked plasticity control components of relapse**, Nature, 509 (2014), pp. 459-464

V. Pascoli, M. Turiault, C. Lüscher **Reversal of cocaine-evoked synaptic potentiation resets drug-induced adaptive behavior**, Nature, 481 (2011), pp. 71-75

S. Patel, D. Rademacher, C. Hillard **Differential regulation of the endocannabinoids anandamide and 2-arachidonylglycerol within the limbic forebrain by dopamine receptor activity**, J. Pharmacol. Exp. Ther., 306 (2003), pp. 880-888

C.R. Pierce, M.E. Wolf **Psychostimulant-induced neuroadaptations in nucleus accumbens**, AMPA Recept. Transm., 3 (2013), Article a012021

R. Piet, A. Garenne, F. Farrugia, G. Masson, G. Le Marsicano, P. Chavis, O.J. Manzoni **State-dependent, bidirectional modulation of neural network activity by endocannabinoids**, J. Neurosci., 31 (2011), pp. 16591-16596

V. Pisani, G. Madeo, A. Tassone, G. Sciamanna, M. Maccarrone, P. Stanzione, A. Pisani **Homeostatic changes of the endocannabinoid system in Parkinson's disease**, Mov. Disord., 26 (2011), pp. 216-222

C. Price, P. Kim, L. Raymond **D1 dopamine receptor-induced cyclic AMP-dependent protein kinase phosphorylation and potentiation of striatal glutamate receptors**, J. Neurochem., 73 (1999), pp. 2441-2446

S. Ramiro-Fuentes, E. Fernandez-Espejo **Sensitization to cocaine is inhibited after intra-accumbal GR103691 or rimonabant, but it is enhanced after co-infusion indicating functional interaction between accumbens D3 and CB1 receptors**, Psychopharmacology, 214 (2011), pp. 949-959

Robbe, Alonso, Duchamp, Bockaert, Manzoni **Localization and mechanisms of action of cannabinoid receptors at the glutamatergic synapses of the mouse nucleus accumbens**, J. Neurosci. Off. J. Soc. Neurosci., 21 (2001), pp. 109-116

D. Robbe, G. Alonso, O. Manzoni **Exogenous and endogenous cannabinoids control synaptic transmission in mice nucleus accumbens**, Ann. N. Y. Acad. Sci., 1003 (2003), pp. 212-225

D. Robbe, M. Kopf, A. Remaury, J. Bockaert, O. Manzoni **Endogenous cannabinoids mediate long-term synaptic depression in the nucleus accumbens**, Proc. Natl. Acad. Sci., 99 (2002), pp. 8384-8388

P. Rothwell, S. Kourrich, M. Thomas **Synaptic adaptations in the nucleus accumbens caused by experiences linked to relapse**, Biol. Psychiat, 69 (2011), pp. 1124-1126

H. Schmidt, S. Anderson, C. Pierce **Stimulation of D1-like or D2 dopamine receptors in the shell, but not the core, of the nucleus accumbens reinstates cocaine-seeking behaviour in the rat**, Eur. J. Neurosci., 23 (2006), pp. 219-228

H. Schmidt, C. Pierce **Systemic administration of a dopamine, but not a serotonin or norepinephrine, transporter inhibitor reinstates cocaine seeking in the rat**, Behav. Brain Res., 175 (2006), pp. 189-194

Schweitzer **Cannabinoids decrease the K(+) M-current in hippocampal CA1 neurons**, J. Neurosci. Official J. Soc. Neurosci., 20 (2000), pp. 51-58

W. Shen, M. Flajolet, P. Greengard, J. Surmeier **Dichotomous dopaminergic control of striatal synaptic plasticity**, Science, 321 (2008), pp. 848-851

R.J. Smith, M.K. Lobo, S. Spencer, P.W. Kalivas **Cocaine-induced adaptations in D1 and D2 accumbens projection neurons (a dichotomy not necessarily synonymous with direct and indirect pathways)**, Curr. Opin. Neurobiol., 23 (2013), pp. 546-552

Snyder, Allen, Fienberg, Valle, Huganir, Nairn, Greengard **Regulation of phosphorylation of the GluR1 AMPA receptor in the neostriatum by dopamine and psychostimulants in vivo**, J. Neurosci. Official J. Soc. Neurosci., 20 (2000), pp. 4480-4488

E. Snyder, B. Philpot, K. Huber, X. Dong, J. Fallon, M. Bear **Internalization of ionotropic glutamate receptors in response to mGluR activation**, Nat. Neurosci., 4 (2001), pp. 1079-1085

X. Sun, M. Milovanovic, Y. Zhao, M.E. Wolf **Acute and chronic dopamine receptor stimulation modulates AMPA receptor trafficking in nucleus accumbens neurons cocultured with prefrontal cortex neurons**, J. Neurosci., 28 (2008), pp. 4216-4230

J. Surmeier, J. Ding, M. Day, Z. Wang, W. Shen **D1 and D2 dopamine-receptor modulation of striatal glutamatergic signaling in striatal medium spiny neurons**, Trends Neurosci., 30 (2007), p. 228235

A. Suska, B. Lee, Y. Huang, Y. Dong, O. Schlüter **Selective presynaptic enhancement of the prefrontal cortex to nucleus accumbens pathway by cocaine**, Proc. Natl. Acad. Sci., 110 (2013), pp. 713-718

J. Terrier, C. Lüscher, V. Pascoli **Cell-type specific insertion of GluA2-lacking AMPARs with cocaine exposure leading to sensitization, cue-induced seeking, and incubation of craving**, Neuropsychopharmacology, 41 (2016), pp. 1779-1789

Thomas, Kalivas, Shaham **Neuroplasticity in the mesolimbic dopamine system and cocaine addiction**, Br. J. Pharmacol., 154 (2008), pp. 327-342

N.X. Tritsch, B.L. Sabatini **Dopaminergic modulation of synaptic transmission in cortex and striatum**, 76 (2012), pp. 33-50

N. Volkow, M. Morales **The brain on drugs: from reward to addiction**, Cell, 162 (2015), pp. 712-725

T.J. De Vries, Y. Shaham, J.R. Homberg, H. Crombag, K. Schuurman, J. Dieben, L.J. Vanderschuren, A.N. Schoffelmeer **A cannabinoid mechanism in relapse to cocaine seeking**, Nat. Med., 7 (2001), pp. 1151-1154

W. Wang, D. Dever, J. Lowe, G. Storey, A. Bhansali, E. Eck, I. Nitulescu, J. Weimer, N. Bamford **Regulation of prefrontal excitatory neurotransmission by dopamine in the nucleus accumbens core**, J. Physiol., 590 (2012), pp. 3743-3769

Z. Wang, L. Kai, M. Day, J. Ronesi, H. Yin, J. Ding, T. Tkatch, D. Lovinger, J. Surmeier **Dopaminergic control of corticostriatal long-term synaptic depression in medium spiny neurons is mediated by cholinergic interneurons**, Neuron, 50 (2006), pp. 443-452

J. Wiskerke, T. Pattij, A.N. Schoffelmeer, T.J. De Vries **The role of CB1 receptors in psychostimulant addiction**, Addict. Biol., 13 (2008), pp. 225-238

M. Wolf **Regulation of AMPA receptor trafficking in the nucleus accumbens by dopamine and cocaine**, Neurotox. Res., 18 (2010), pp. 393-409

M. Wolf **Synaptic mechanisms underlying persistent cocaine craving**, Nat. Rev. Neurosci., 17 (2016), pp. 351-365

M. Wolf, C. Ferrario **AMPA receptor plasticity in the nucleus accumbens after repeated exposure to cocaine**, Neurosci. Biobehav. Rev., 35 (2010), pp. 185-211

Z.-X. Xi, J. Gilbert, X.-Q. Peng, A. Pak, X. Li, E. Gardner **Cannabinoid CB1 receptor antagonist AM251 inhibits cocaine-primed relapse in rats: role of glutamate in the nucleus accumbens**, J. Neurosci., 26 (2006), pp. 8531-8536

H. Yin, D. Lovinger **Frequency-specific and D2 receptor-mediated inhibition of glutamate release by retrograde endocannabinoid signaling**, P Natl. Acad. Sci. U. S. A., 103 (2006), pp. 8251-8256

J. Yorgason, D. Zeppenfeld, J. Williams **Cholinergic interneurons underlie spontaneous dopamine release in nucleus accumbens**, J. Neurosci., 37 (2017), pp. 2086-2096

F.-M. Zhou, Y. Liang, J. Dani **Endogenous nicotinic cholinergic activity regulates dopamine release in the striatum**, Nat. Neurosci., 4 (2001), pp. 1224-1229

N.E. Zlebnik, J.F. Cheer **Drug-induced alterations of endocannabinoid-mediated plasticity in brain reward regions**, J. Neurosci., 36 (2016), pp. 10230-10238