

Direct Actions of Cannabinoids on Synaptic Transmission in the Nucleus Accumbens: A Comparison With Opioids

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Received 15 June 2000; accepted in final form 15 September 2000

Hoffman, Alexander F. and Carl R. Lupica. Direct actions of cannabinoids on synaptic transmission in the nucleus accumbens: a comparison with opioids. *J Neurophysiol* 85: 72–83, 2001. The nucleus accumbens (NAc) represents a critical site for the rewarding and addictive properties of several classes of abused drugs. The medium spiny GABAergic projection neurons (MSNs) in the NAc receive innervation from intrinsic GABAergic interneurons and glutamatergic innervation from extrinsic sources. Both GABA and glutamate release onto MSNs are inhibited by drugs of abuse, suggesting that this action may contribute to their rewarding properties. To investigate the actions of cannabinoids in the NAc, we performed whole cell recordings from MSNs located in the shell region in rat brain slices. The cannabinoid agonist WIN 55,212-2 (1 μ M) had no effect on the resting membrane potential, input resistance, or whole cell conductance, suggesting no direct postsynaptic effects. Evoked glutamatergic excitatory postsynaptic currents (EPSCs) were inhibited to a much greater extent by [Tyr-D-Ala², N-CH₃-Phe⁴, Gly-ol-enkephalin] (DAMGO, ~35%) than by WIN 55,212-2 (<20%), and an analysis of miniature EPSCs suggested that the effects of DAMGO were presynaptic, whereas those of WIN 55,212-2 were postsynaptic. However, electrically evoked GABAergic inhibitory postsynaptic currents (eIPSCs), were reduced by WIN 55,212-2 in every neuron tested (EC₅₀ = 123 nM; 60% maximal inhibition), and the inhibition of IPSCs by WIN 55,212-2 was completely antagonized by the CB1 receptor antagonist SR141716A (1 μ M). In contrast eIPSCs were inhibited in ~50% of MSNs by the μ/δ opioid agonist D-Ala²-methionine²-enkephalinamide and were completely unaffected by a selective μ -opioid receptor agonist (DAMGO). WIN 55,212-2 also increased paired-pulse facilitation of the eIPSCs and did not alter the amplitudes of tetrodotoxin-resistant miniature IPSCs, suggesting a presynaptic action. Taken together, these data suggest that cannabinoids and opioids differentially modulate inhibitory and excitatory synaptic transmission in the NAc and that the abuse liability of marijuana may be related to the direct actions of cannabinoids in this structure.

INTRODUCTION

The nucleus accumbens/ventral striatum (NAc) represents a critical site for mediating the rewarding and/or addictive properties of several classes of abused drugs, including ethanol, opioids, psychomotor stimulants, and marijuana (Gardner and Vorel 1998; Koob 1992; Koob et al. 1998; Wise 1996; Wise and Bozarth 1987). It is generally appreciated that all of these drugs augment extracellular dopamine levels in the NAc and that this action contributes to their rewarding properties (Di

Chiara and Imperato 1988; Koob 1992; Koob et al. 1998; Wise and Bozarth 1987). However, recent evidence also suggests that many drugs of abuse have dopamine-independent interactions with NAc neuronal circuitry (Carlezon and Wise 1996; Chieng and Williams 1998; Koob 1992; Martin et al. 1997; Yuan et al. 1992).

The majority (>90%) of neurons within the NAc are GABAergic medium spiny neurons (MSNs) (Groenewegen et al. 1991) that send their output to several brain structures, including the ventral pallidum (Chang and Kitai 1985) and the ventral tegmental area (VTA) (Steffensen et al. 1998). These cells receive dopaminergic input from the VTA and glutamatergic inputs from the hippocampus, amygdala, and prefrontal cortex (Christie et al. 1985, 1987; Pennartz and Kitai 1991). Previous work has suggested that the synaptic inhibition of striatal MSNs occurs via axon collaterals of the MSNs themselves (Park et al. 1980; Wilson and Groves 1980). However, more recent studies provide strong evidence that GABAergic inhibition is mediated by intrinsic interneurons that comprise only a small fraction of the striatal/NAc neuronal population (i.e., 3–5%) but provide extensive innervation of medium spiny output neurons (Jaeger et al. 1994; Kawaguchi et al. 1995; Koos and Tepper 1999).

Many commonly abused drugs, including opioids, psychomotor stimulants and phencyclidine (PCP) are self-administered into the NAc by animals (Carlezon and Wise 1996; McBride et al. 1999) and inhibit fast amino acid-mediated synaptic transmission in this structure (Chieng and Williams 1998; Harvey and Lacey 1996, 1997; Martin et al. 1997; Nicola and Malenka 1997). These two observations suggest that at least part of the rewarding effects of these drugs may be due to their direct effects on synaptic transmission in the NAc. In addition, since dopamine itself inhibits both GABAergic and glutamatergic synaptic inputs to NAc MSNs (Harvey and Lacey 1996, 1997; Nicola and Malenka 1997; Pennartz et al. 1992), it is possible that the direct effects of these drugs on NAc circuitry, and their indirect effects via an increase in NAc dopamine levels may contribute to the rewarding properties of these drugs. Based on these data, we hypothesize that to the extent that the inhibition of synaptic transmission in the NAc reflects the rewarding properties of drugs of abuse, other drugs

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that similarly modulate synaptic transmission in the NAc will also exhibit rewarding properties.

Cannabis sativa (marijuana) is a drug possessing pharmacological properties that sustain its use in humans (Abood and Martin 1992). Although marijuana has been used for centuries, it is only recently that its actions on neuronal circuitry have begun to be understood. Its active constituents, termed cannabinoids, activate various cellular effectors via interaction with G-protein-coupled receptors classified as CB1 and CB2 (Ameri 1999; Howlett 1995; Pertwee 1997). Whereas CB2 receptor distribution is restricted to peripheral sites, the CB1 receptor is found extensively throughout the mammalian CNS (Pertwee 1997). Cannabinoids have been shown to inhibit the release of several neurotransmitters, including glutamate (Misner and Sullivan 1999; Shen et al. 1996; Takahashi and Linden 2000) and GABA (Chan et al. 1998; Hoffman and Lupica 2000; Vaughan et al. 1999, 2000) in a variety of brain areas. In addition to these actions, cannabinoids increase the activity of midbrain dopamine neurons that project to the NAc (French 1997), increasing dopamine levels in this structure (Chen et al. 1990). Although the NAc contains a moderately high-density of CB1 receptors (Herkenham et al. 1991; Tsou et al. 1998), the actions of these drugs in this structure remain largely unknown. Therefore in the present study we have examined the consequences of cannabinoid receptor activation on the physiology of MSNs in the shell region of the NAc and have compared these effects to those generated by opioid receptor activation.

METHODS

All protocols were conducted under National Institutes of Health Guidelines using the handbook "Animals in Research" and were approved by the Institutional Animal Care and Use Committee (National Institute on Drug Abuse, Intramural Research Program, Baltimore, MD).

Slice preparation

Male Sprague-Dawley rats (Charles River Labs, Raleigh, NC), 14–30 days old, were killed by decapitation, and their brains were rapidly removed and placed in ice-cold oxygenated artificial cerebral spinal fluid (ACSF; see following text). The brain was then blocked in a coronal plane ~3 mm anterior to, and 5 mm posterior to Bregma using a razor blade. The posterior end of the tissue block was then glued to the stage of a vibrating tissue slicer (Technical Products International, St. Louis, MO) using cyanoacrylate. A midsagittal cut was then made with a scalpel blade to separate the two hemispheres, and coronal brain slices were cut at 300- μ m nominal thickness. The slices were then transferred to a beaker containing ACSF, aerated with 95% O₂-5% CO₂ at room temperature, where they were stored for ≥ 90 min before recordings. Slices were transferred to a recording chamber (~250 μ l volume) that was integrated into the stage of an upright microscope (Carl Zeiss Instruments, Germany). The slices were held in place by a circular platinum-iridium wire and were continuously superfused with oxygenated artificial cerebrospinal fluid (ACSF) at a rate of 2 ml/min, at room temperature (~23°C). Control ACSF consisted of (in mM) 126 NaCl, 3.0 KCl, 1.5 MgCl₂, 2.4 CaCl₂, 1.2 NaH₂PO₄, 11.0 glucose, and 26 NaHCO₃ and was saturated with 95% O₂ and 5% CO₂.

Localization of MSNs

All MSNs included in this study were located within the shell region of the NAc, contained in slices taken from ~1.6 to 0.7 mm

anterior to Bregma (Paxinos and Watson 1986). The anterior commissure and the islands of Calleja were used as landmarks for locating the shell region of the NAc. Thus recordings were made from MSNs found ~300–500 μ m medial to the anterior commissure and 100–1200 μ m dorsal to the islands of Calleja (Chieng and Williams 1998; Paxinos and Watson 1986). Also in some slices, the major islands of Calleja could be readily distinguished, and in these cases, recordings were confined to an area lateral to this structure. MSNs were visually distinguished from interneurons in the NAc by the comparative size of their somata (Chieng and Williams 1998) using a fixed stage upright microscope (Carl Zeiss Instruments, Germany) equipped with differential interference contrast optics and infrared illumination (DIC-IR) (Dodt and Zieglansberger 1990; Miller et al. 1997; Svoboda et al. 1999). In addition, electrophysiological criteria (resting membrane potentials = –75 to –85 mV; absence of the H current, and absence of spontaneous firing) were also used to distinguish these neurons (Chieng and Williams 1998; Uchimura et al. 1990).

Whole cell recording

Whole cell patch-clamp recordings of NAc neurons were performed using methods adapted from those described previously (Lupica 1995; Miller et al. 1997). Signals were acquired using an Axoclamp-2A, or an Axopatch 200A amplifier (Axon Instruments, Burlingame, CA) and electrodes pulled from thick-walled borosilicate capillary tubing (0.75 mm ID, 1.5 mm OD, Sutter Instrument, Novato, CA). Voltage or current steps, used for monitoring series resistance, and for construction of current-voltage (*I*-*V*) curves, were generated using a Master-8 (AMPI, Jerusalem, Israel). Series resistance was monitored continuously using small (10 mV), hyperpolarizing voltage steps (200 ms), and only cells demonstrating <20 M Ω series resistance were used in these experiments. In most cases, the series resistance did not change appreciably during the recording period. However, when the series resistance increased, there was a noticeable decrease in whole cell conductance and a sudden and sustained decrease in the holding current. When this occurred the cell was not used in further analyses.

Excitatory postsynaptic currents (EPSCs) were recorded using whole cell electrodes (5–7 M Ω) filled with the following solution (in mM): 125.0 K⁺-gluconate, 10.0 KCl, 10.0 HEPES, 1.0 EGTA, 0.1 CaCl₂, 2.0 Mg²⁺-ATP, and 0.2 Na⁺-GTP; adjusted to pH 7.2–7.4 with 1 M KOH (270–280 mosM). During EPSC recordings, neurons were voltage clamped at –80 to –90 mV, and GABA_A inhibitory postsynaptic currents (IPSCs) were blocked by adding picrotoxin (100 μ M) to the ACSF. IPSCs were recorded in cells voltage clamped at –70 to –90 mV using whole cell electrodes filled with the following solution (in mM): 125.0 CsCl, 10.0 HEPES, 1.0 EGTA, 0.1 CaCl₂, 2.0 Mg²⁺-ATP, and 0.2 Na⁺-GTP and the quaternary lidocaine derivative QX-314, 2, pH 7.2–7.4. During IPSC recordings, glutamatergic EPSCs were blocked by adding the glutamate receptor antagonists 6,7-dinitroquinoxaline-2,3-dione (DNQX, 10 μ M) and D(-)-2-amino-5-phosphonopentanoic acid (APV, 40 μ M) to the ACSF.

Miniature IPSCs and EPSCs (mIPSCs, mEPSCs) were amplified 5- to 100-fold, filtered at 1–3 kHz and either recorded to videotape or directly to the hard drive of a personal computer for later analysis. Epochs of 1–3 min of data were digitized at 4–10 kHz using a National Instruments (Austin, TX) Lab PC 1200 A/D converter and the Strathclyde electrophysiology software package (courtesy of Dr. John Dempster, Strathclyde University, Glasgow, UK, <http://innovol.sibs.strath.ac.uk/physpharm>). The frequency, amplitudes, and kinetic properties of these currents were then analyzed using the Mini Analysis software package (v4.3, Synaptosoft, Leonia, NJ, <http://www.synaptosoft.com>). Average mIPSCs/mEPSCs were generated by aligning individual events by rise time, and a peak to decay single exponential fit was applied to each average using the formula

$$y = AI * \exp(-x/\tau) + \text{Baseline}$$

where *AI* is the peak amplitude and τ is the time constant for decay.

Evoked postsynaptic currents were generated using a bipolar tungsten stimulating electrode placed within 100–150 μm of the recording electrode. Whole cell access was monitored using voltage (or current) step pulses (-10 – 20 mV, 200 ms) delivered after each stimulus using the Master-8 pulse generator. Stimulation (0.1-ms pulse duration) was delivered at 15- to 30-s intervals using an optically isolated constant current unit (AMPI) and the timer. Paired-pulse stimulation was performed by delivering the same stimulus at either 150 ms (IPSC) or 100 ms (EPSC) inter-pulse intervals. In each experiment, stimulation intensity was adjusted to evoke a submaximal response (50–400 μA). Analyses of drug effects on evoked synaptic responses, membrane potential, and input resistance were performed using PC-based software (Neuropro, R. C. Electronics, Goleta, CA, or the Strathclyde program, WCP v3.05).

Chemicals

Drugs were obtained from the following sources: tetrodotoxin (TTX), 6,7-dinitroquinoxaline-2,3-dione (DNQX), picrotoxin, ruthenium red, bicuculline methiodide, dopamine, [Tyr-D-Ala², N-CH₃-Phe⁴, Gly-ol-enkephalin] (DAMGO), and D-Ala²-methionine²-enkephalinamide (DALA), naloxone (Sigma, St. Louis, MO); APV, (RS)-baclofen, and WIN 55,212-2 (Tocris Cookson, Ballwin, MO). SR141716A was obtained from the National Institute on Drug Abuse drug-supply system. WIN 55,212-2 and SR141716A were prepared as concentrated (10 mM) stock solutions in DMSO. Final (bath) concentrations were $<0.01\%$ DMSO. All drugs were made up at either 50 or 100 times the desired final concentration in deionized water and then added to the flow of the superfusion medium using a calibrated syringe pump (Razel Scientific Instruments, Stamford, CT).

Statistical analysis

Group data are presented as the mean \pm SE in all cases. Drug-induced changes in cumulative mEPSC and mIPSC amplitude and inter-event interval distributions were analyzed for statistical significance using the Kolmogorov-Smirnov test (Mini Analysis v4.3), with a conservative critical probability level of $P < 0.01$. All other statistical tests, including t -tests and ANOVAs, were performed using a critical probability of $P < 0.05$ (Prism version 3.0, GraphPad Software, San Diego, CA). Post hoc analysis (Newman-Keuls test) was performed only when an ANOVA yielded a significant ($P < 0.05$) main effect.

RESULTS

Classification of neurons

Previous studies have demonstrated that the GABAergic MSNs are the most numerous neurons in the NAC and that these cells can be electrophysiologically distinguished from the smaller population of interneurons (Chieng and Williams 1998; Uchimura et al. 1990). All of the neurons included in this study were classified as MSNs based on the lack of spontaneous firing, relatively hyperpolarized resting membrane potentials (-75 to -85 mV), and the absence of a membrane sag associated with the hyperpolarization-activated cation current (I_h). The MSNs were further distinguished from interneurons because their somata are much smaller when visualized using DIC-IR microscopy (Chieng and Williams 1998).

Effects of WIN 55,212-2 on postsynaptic properties of NAC neurons

Although there is ample evidence to suggest that CB1 receptors are localized to presynaptic terminals within the CNS,

several studies have demonstrated postsynaptic effects of cannabinoids on a variety of membrane conductances (Deadwyler et al. 1995; Mackie et al. 1995; Schweitzer 2000). To determine whether cannabinoids alter the postsynaptic properties of MSNs, we examined the effect of the cannabinoid agonist WIN 55,212-2 on the passive membrane properties of these cells in current clamp. Under control conditions, the mean resting membrane potential of these cells ($n = 11$) was -83.5 ± 2.6 mV and the membrane input resistance was 187 ± 25 M Ω . However, superfusion of WIN 55,212-2 (1 μM) for a period of time sufficient to achieve maximal effects on synaptic responses (see following text) did not significantly affect these parameters (resting membrane potential, -85.1 ± 2.7 mV, input resistance, 183 ± 16 M Ω , $P > 0.05$, paired Student's 2-tailed t -test). Furthermore, in cells voltage-clamped at -60 mV, WIN 55,212-2 had no effect on either the steady-state conductance, measured using a series of hyperpolarizing voltage steps ($n = 4$; Fig. 1), or on holding current (control, 60 ± 14 pA, WIN 55,212-2, 69 ± 9 pA, $n = 4$, $P > 0.05$, 2-tailed Student's paired t -test). Thus WIN 55,212-2 did not alter either the resting membrane properties, or whole cell conductance in these MSNs.

CB1 and μ -opioid modulation of evoked EPSCs in NAC neurons

Previous studies have demonstrated that CBs inhibit glutamatergic transmission in several brain regions, including the hippocampus (Misner and Sullivan 1999; Shen et al. 1996), cerebellum (Takahashi and Linden 2000), and substantia nigra

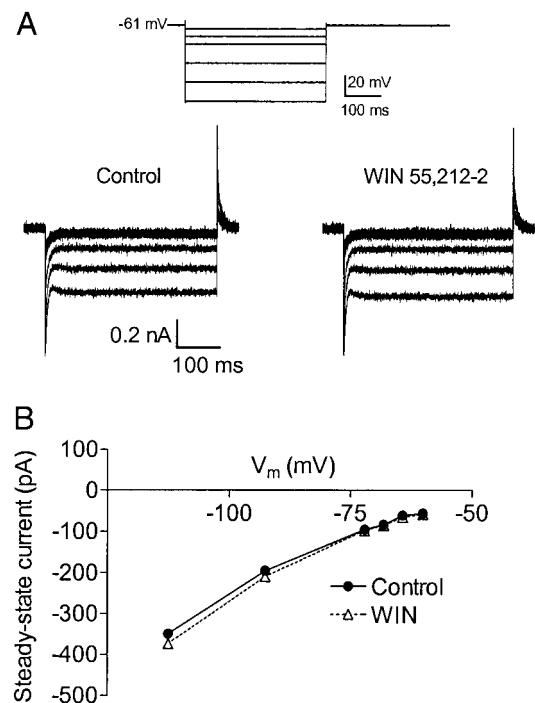


FIG. 1. Absence of WIN 55,212-2 effects on membrane conductance in medium spiny neurons (MSNs). A: current responses to voltage steps in a single MSN located in the shell region of the NAC prior to and at the end of a 15-min application of the cannabinoid agonist WIN 55,212-2 (1 μM). B: steady-state current-voltage relationship for the responses shown in A. WIN 55,212-2 had no effect on membrane conductance, holding current, or membrane potential in any of the cells tested ($n = 11$).

(Szabo et al. 2000). Thus to determine whether glutamatergic inputs to the NAc were modulated by cannabinoid receptors we isolated evoked EPSCs (evEPSCs) by electrically stimulating the NAc under conditions where GABA_A-receptor activity was eliminated by addition of picrotoxin (100 μ M). As shown in Fig. 2, the inward currents elicited under these conditions were largely mediated by non-*N*-methyl-D-aspartate (NMDA) glutamate receptors because they were nearly eliminated ($12 \pm 2\%$ of control, $n = 5$) by 10 μ M DNQX. The μ -opioid agonist DAMGO (1 μ M) reversibly inhibited the evEPSCs ($64 \pm 7\%$ of control, $P < 0.05$, $n = 8$, paired *t*-test; Fig. 2). However, in contrast to the opioid effect, the cannabinoid agonist WIN 55,212-2 (1 μ M) caused a smaller inhibition of these evEPSCs (Fig. 2, $82 \pm 6\%$ of control, $P < 0.05$, $n = 10$, paired *t*-test), that was only partially blocked by the CB1 antagonist SR141716A (Rinaldi-Carmona et al. 1994) (1 μ M, $93 \pm 5\%$ of control, $P > 0.05$, $n = 4$; paired *t*-test). Together, these data suggest that μ -opioid receptors inhibit glutamatergic synaptic transmission in the NAc to a greater extent than CB1 receptors.

Because paired-pulse facilitation is a presynaptic phenomenon (Mennerick and Zorumski 1995), pharmacological agents that alter this process are thought to act presynaptically (Chieng and Williams 1998; Jiang et al. 2000). To determine whether the inhibition of evoked EPSCs by either CB1 or μ -opioid receptors was due to a presynaptic interaction, we examined their effects on paired evEPSCs using identical stimuli, delivered at a 100-ms inter-pulse interval. The control average ratio of the second response to the first (EPSC2/EPSC1) was 1.13 ± 0.05 ($n = 9$; Fig. 3). At the end of a 10-

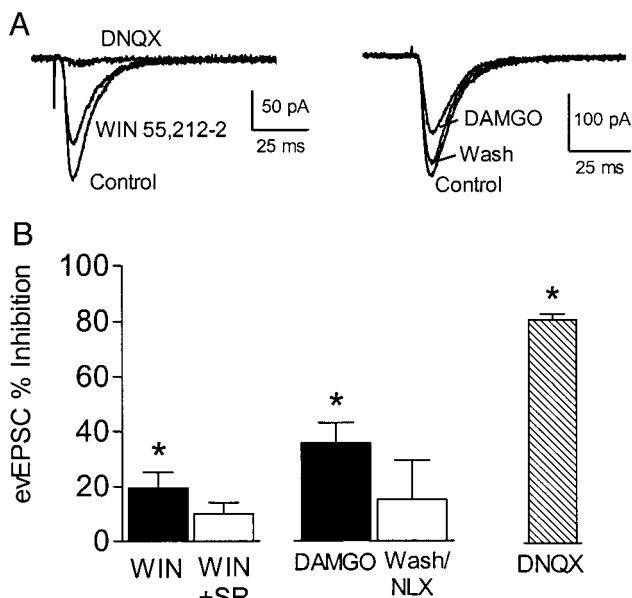


FIG. 2. Effects of opioid and cannabinoid agonists on evoked excitatory postsynaptic currents (evEPSCs) in NAc MSNs. *A*: effects of WIN 55,212-2 (1 μ M) and [Tyr-D-Ala², N-CH₃-Phe⁴, Gly-ol-enkephalin] (DAMGO, 1 μ M) on averaged (10 sweeps) evEPSC responses recorded from different MSNs. These evEPSCs were isolated by addition of 100 μ M picrotoxin to the artificial cerebrospinal fluid (ACSF). *B*: mean effects of WIN 55,212-2 (WIN, $n = 10$), WIN 55,212-2 + SR141716A (WIN + SR, $n = 4$), DAMGO ($n = 8$), wash/naloxone (NLX, 5 μ M, $n = 5$), and 6,7-dinitroquinoxaline-2,3-dione (DNQX, $n = 5$) on evEPSCs. * $P < 0.05$ vs. control, 1-way ANOVA. Note that the evEPSC was significantly reduced by WIN 55,212-2 and DAMGO and that it was completely eliminated by DNQX (10 μ M).

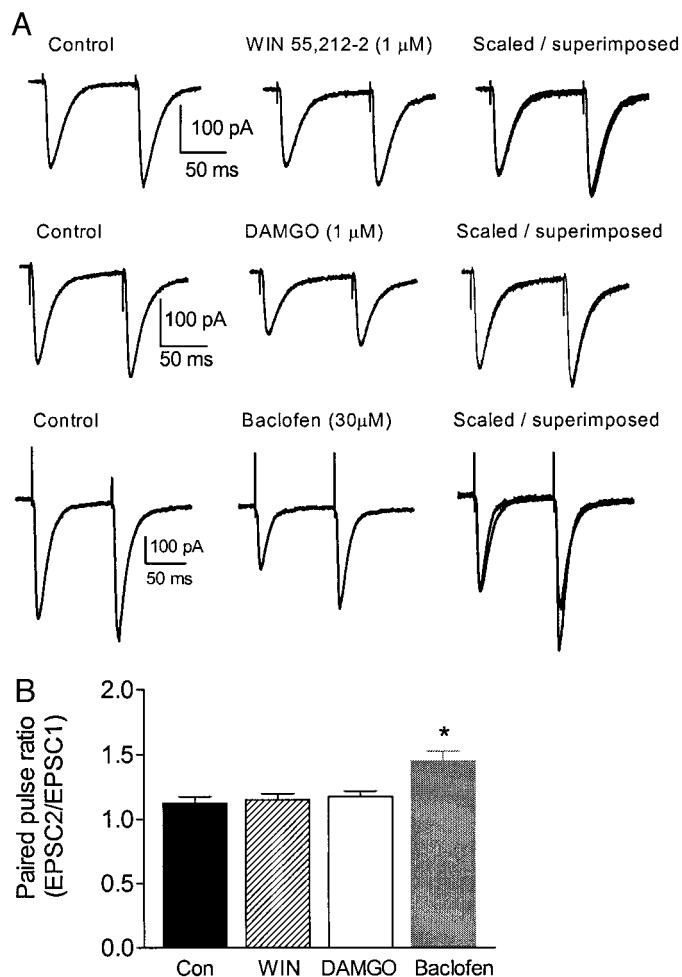


FIG. 3. Effects of WIN 55,212-2 (1 μ M), DAMGO (1 μ M), and baclofen (30 μ M) on paired evEPSC responses elicited using a 100-ms inter-pulse interval. *A*: averaged traces, representing 10 sweeps, obtained during the indicated condition in 3 different MSNs. *B*: mean effects of WIN 55,212-2 ($n = 9$), DAMGO ($n = 9$), and baclofen ($n = 5$) on paired evEPSC responses. Although the 2nd evEPSC of the pair (EPSC2) was facilitated compared with the 1st (EPSC1), neither WIN 55,212-2 nor DAMGO significantly altered this level of facilitation ($P > 0.05$, Student's paired 2-tailed *t*-test). However, baclofen significantly enhanced the level of facilitation (* $P < 0.05$, Student's paired 2-tailed *t*-test).

to 15-min application of WIN 55,212-2 (1 μ M), this ratio was not significantly changed (1.15 ± 0.05 , $P = 0.65$, 2-tailed paired Student's *t*-test; Fig. 3). Similarly, the μ agonist DAMGO (1 μ M) did not significantly effect the paired-pulse ratio (control, 1.18 ± 0.04 ; DAMGO, 1.17 ± 0.04 , $n = 9$, $P = 0.89$, paired Student's *t*-test; Fig. 3).

To determine whether the lack of changes in paired-pulse ratios was characteristic of our slice preparation, we also examined the effects of the GABA_B receptor agonist, baclofen, and dopamine on these paired evEPSC responses. In direct contrast to the effects of the cannabinoid and the opioid agonist, both baclofen (30 μ M; Fig. 3) and dopamine (30 μ M) reversibly and significantly increased the paired evEPSC ratio (ratio values: control = 1.21 ± 0.11 , baclofen = 1.45 ± 0.13 , $P = 0.03$, $n = 5$; control = 1.26 ± 0.09 , dopamine = 1.48 ± 0.13 , wash = 1.12 ± 0.11 , $P = 0.02$, $n = 5$; repeated-measures ANOVA and Newman-Keuls post hoc test) Thus both baclofen and dopamine increased paired evEPSC ratios, whereas the

cannabinoid and opioid agonists had no effect on this parameter.

CB1 and μ -opioid modulation of mEPSCs in NAc neurons

The preceding results suggest that both WIN 55,212-2 and DAMGO did not inhibit excitatory transmission in NAc MSNs through a presynaptic mechanism. However, previous work has suggested that DAMGO acts both pre- and postsynaptically to alter glutamatergic transmission in these cells (Martin et al. 1997), and the mechanism of the cannabinoid effect is unknown. Thus to explore these possibilities further, we examined the effects of WIN 55,212-2 and DAMGO on miniature, action potential-independent EPSCs (mEPSCs) in the presence of the Na^+ channel blocker TTX (500 nM). Miniature EPSCs were recorded in MSNs voltage clamped at -80 to -90 mV using whole cell electrodes containing K^+ -gluconate, in ACSF containing picrotoxin (100 μM). These responses are thought to reflect the quantal release of glutamate acting at non-NMDA receptors (Nicola and Malenka 1997). The mEPSCs recorded under these conditions ($n = 10$) exhibited an average frequency of 1.7 ± 0.2 Hz, an average amplitude of 22.9 ± 1.6 pA, a decay time constant of 8.3 ± 0.4 ms, and were greatly reduced in frequency and amplitude by DNQX (10 μM ; Fig. 5A). These data therefore suggest that the majority of these events were mediated by glutamate activating non-NMDA receptors. The cannabinoid agonist WIN 55,212-2 (1 μM , $n = 6$) produced a small but significant reduction in the average amplitude (paired Student's t -test, $P = 0.01$, Fig. 4) of these mEPSCs but did not significantly alter the frequency (Student's paired t -test, $P = 0.09$) or the average decay time constant (control, 7.7 ± 0.3 ms, WIN 55,212-2, 8.9 ± 0.7 ms, $P = 0.07$, paired Student's t -test) of these events. In contrast, the μ -opioid agonist DAMGO (1 μM , $n = 4$) significantly reduced the mean mEPSC frequency ($P < 0.05$, paired Student's t -test, Fig. 5) but did not alter mean mEPSC amplitude ($P > 0.05$, paired Student's t -test, Fig. 5) or the average decay time constant (control, 9.4 ± 0.6 ms, DAMGO, 10 ± 0.5 ms, $P = 0.19$, paired Student's t -test). In addition, the effect of DAMGO on mEPSC frequency was reversed by application of the opioid receptor antagonist, naloxone (NLX, 5 μM , Fig. 5). These data suggest that the cannabinoid receptor agonist WIN 55,212-2 produced a small reduction of glutamatergic transmission through a postsynaptic mechanism while DAMGO likely acted via a presynaptic mechanism.

CB1 and opioid receptor effects on evoked IPSCs in NAc neurons

There is ample evidence that cannabinoids inhibit GABAergic neurotransmission in other brain areas (Chan et al. 1998; Hoffman and Lupica 2000; Szabo et al. 1999; Vaughan et al. 1999, 2000). Therefore we examined whether GABAergic IPSCs were also modulated by cannabinoids in the NAc and compared these effects to those of opioid receptor activation. In these experiments, MSNs were voltage-clamped at -80 to -90 mV using whole cell electrodes containing CsCl, with APV (40 μM) and DNQX (10 μM) included in the superfusion medium to block glutamatergic transmission. Under these conditions, electrical stimulation of the slice produced large inward currents that were completely blocked by the GABA_A

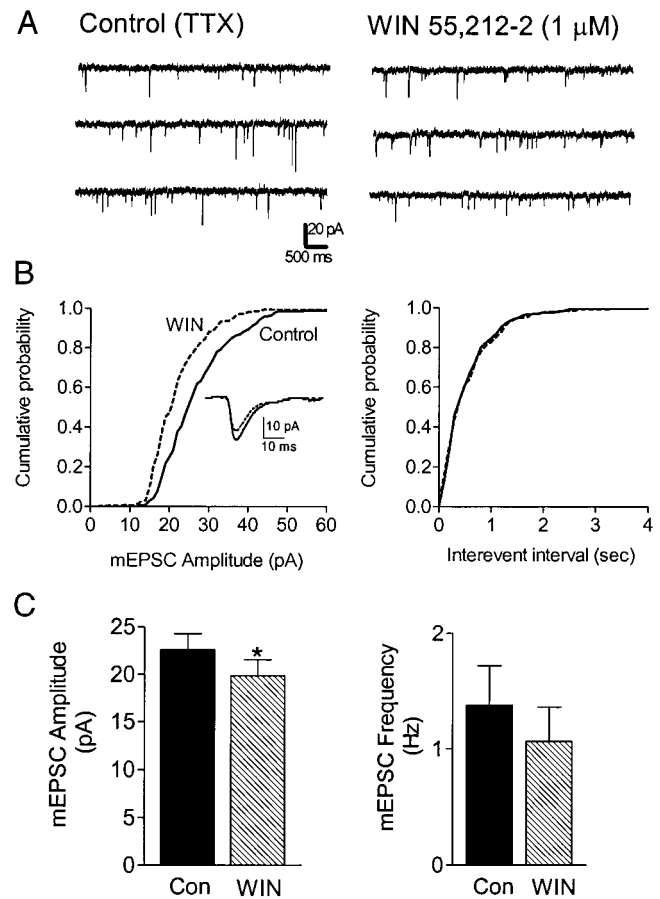


FIG. 4. Effects of WIN 55,212-2 (1 μM) on miniature EPSCs (mEPSCs) recorded during superfusion with TTX (0.5 μM). A: raw traces showing mEPSCs during a 2-min control epoch and during the 10th to 12th min of WIN 55,212-2 application in a single MSN. B: cumulative probability distributions for mEPSC amplitudes and inter-event intervals during control and WIN 55,212-2 epochs taken from the same cell as shown in A. Inset: averaged mEPSC during the control period ($n = 236$ mEPSCs) and during WIN 55,212-2 ($n = 227$) application (---). Note the decrease in mEPSC amplitude in the presence of WIN 55,212-2 that was consistent with the significant change in the cumulative amplitude distribution [$P < 0.01$, Kolmogorov-Smirnov (K-S) test]. C: mean effects of WIN 55,212-2 on mEPSC amplitude and frequency ($n = 6$). Note that WIN 55,212-2 significantly inhibited mEPSC amplitude without altering mEPSC frequency (paired Student's t -test, $*P < 0.05$).

antagonist bicuculline methiodide (BMI, 20 μM , Fig. 6). As shown in Fig. 6A, WIN 55,212-2 (1 μM) caused a time-dependent decrease in the evIPSC amplitude that was maximal at 8–10 min into the agonist application. This inhibition of evIPSCs was seen in every MSN examined. As we have previously reported, the highly lipophilic nature of cannabinoid ligands results in a relatively long period of time to achieve equilibrium in the tissue, and precludes washout during these experiments (Hoffman and Lupica 2000). Therefore to ensure cannabinoid receptor mediation of these effects, we pretreated several slices with the selective CB1 receptor antagonist SR141716A (1 μM) for 10–15 min prior to WIN 55,212-2 application. As previously reported in the hippocampus (Hoffman and Lupica 2000), the CB1 antagonist had no effect by itself on the evIPSC ($93 \pm 8\%$ of control, $P > 0.05$, 1 sample t -test) but completely blocked the expected inhibition during WIN 55,212-2 application (Fig. 6, B and C). In a further attempt to establish whether the effects of WIN 55,212-2 were

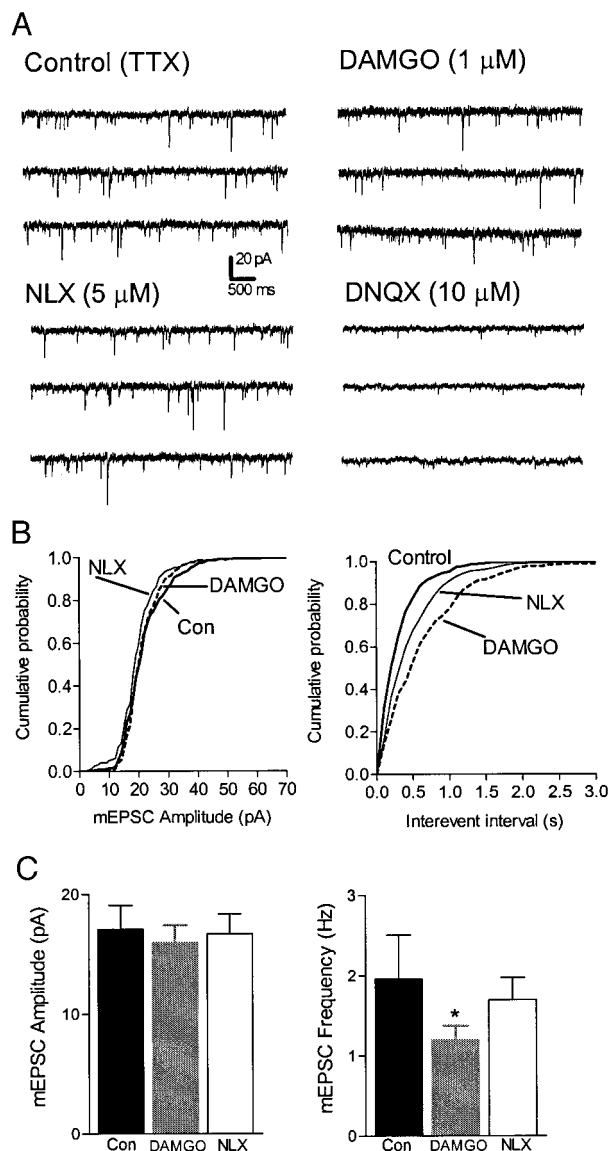


FIG. 5. Effects of the μ -opioid agonist DAMGO ($1 \mu\text{M}$) on mEPSCs recorded during superfusion with TTX ($0.5 \mu\text{M}$). **A**: raw traces showing mEPSCs during 2-min epochs recorded, sequentially, under control conditions (in TTX), during DAMGO application, during DAMGO + NLX application, and during application of DNQX. **B**: cumulative probability distributions for mEPSC amplitudes and inter-event intervals during the indicated drug conditions. In this cell, DAMGO significantly decreased the mEPSC frequency ($P < 0.01$, K-S test), but did not alter mEPSC amplitude. **C**: mean effects of DAMGO on mEPSC amplitude and frequency ($n = 4$). Note that DAMGO significantly inhibited mEPSC frequency, without altering mEPSC amplitude (1-way ANOVA followed by Newman-Keuls post hoc analysis, $*P < 0.05$), and that NLX ($5 \mu\text{M}$) reversed this effect.

receptor mediated and to compare its effects in the NAc with those in other systems, we constructed a concentration-response curve using the cumulative administration of WIN 55,212-2 (10 nM – $5 \mu\text{M}$) while measuring evIPSCs in NAc neurons. As shown in Fig. 7, WIN 55,212-2 inhibited the evoked IPSC in a concentration-dependent fashion, with an estimated EC_{50} of 123 nM .

In contrast to the robust inhibition of evIPSCs by the cannabinoid agonist, the μ -opioid agonist DAMGO ($1 \mu\text{M}$) had no effect on these responses (Fig. 6, **A** and **B**), suggesting that these receptors do not modulate GABA release onto MSNs in

the NAc. Because DAMGO is a highly selective μ -opioid receptor agonist (Goldstein and Naidu 1989), we also examined the possible modulation of evIPSCs with the nonselective μ/δ opioid agonist DALA. In contrast to the effects of DAMGO, DALA ($5 \mu\text{M}$) significantly reduced evIPSCs recorded in NAc MSNs ($69 \pm 8\%$ of control, $P < 0.05$, repeated measures ANOVA, $n = 11$, Fig. 8). However, it was found that, unlike the activation of CB1 receptors in which virtually all evIPSCs were inhibited, only a fraction ($\sim 50\%$) of these responses were significantly inhibited by DALA. Thus the effects of DALA on evIPSCs could be further divided into two groups: those in which DALA had a large effect ($45 \pm 6\%$ of

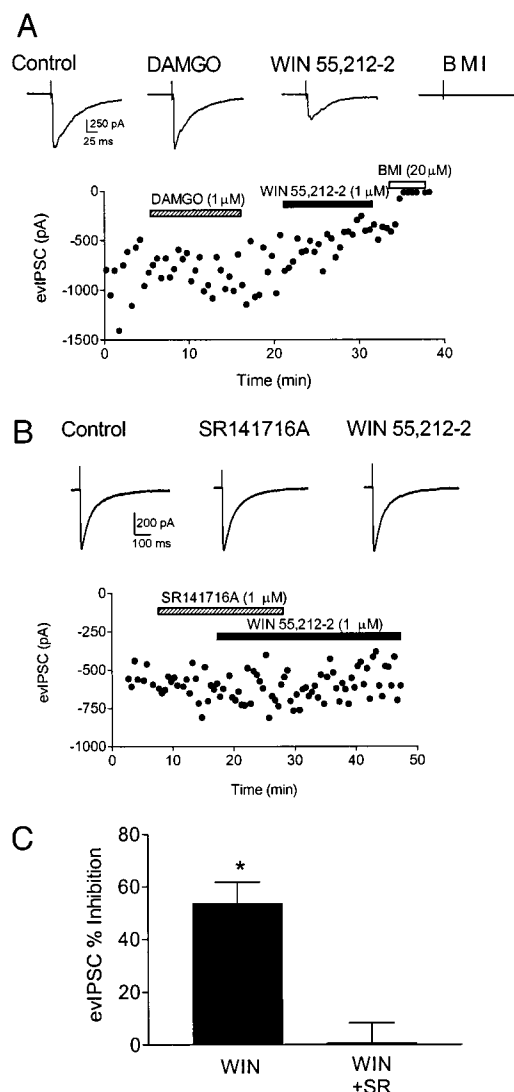


FIG. 6. Differential effects of WIN 55,212-2 and DAMGO on evoked inhibitory postsynaptic currents (evIPSCs) in NAc MSNs. **A**: effects of DAMGO ($1 \mu\text{M}$) and WIN 55,212-2 ($1 \mu\text{M}$) on evIPSCs in the same MSN. Note that WIN 55,212-2 inhibited the evIPSC, whereas DAMGO had no effect and that the evIPSC was completely blocked by the GABA $_A$ antagonist bicuculline methiodide (BMI; $20 \mu\text{M}$). **B**: the selective CB1 antagonist SR141716A ($1 \mu\text{M}$) completely blocked the inhibition of the evIPSC by WIN 55,212-2. Averaged traces (10 sweeps) were obtained during the control period, 15 min after beginning SR141716A application and 10 min after beginning application of WIN 55,212-2. **C**: mean effects of WIN 55,212-2 on evIPSCs, and the antagonism of the effect of the cannabinoid agonist by SR141716A. Note that SR141716A completely blocked the effect of the cannabinoid agonist ($*P < 0.05$ vs. control, 1-way ANOVA).

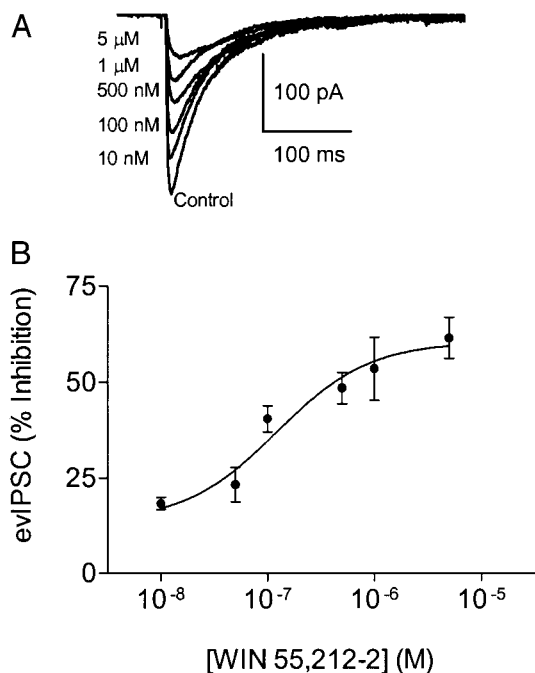


FIG. 7. Concentration response relationship for the inhibitory effect of WIN 55,212-2 on evIPSCs. *A*: averaged waveforms showing a concentration response for the inhibition of the evIPSCs in a single MSN. *B*: concentration-response relationship for WIN 55,212-2. Each point represents the average (\pm SE) for 4–12 individual cells. The EC_{50} for this effect was 123 nM.

control, $n = 5$) and those in which DALA had a very small effect ($89 \pm 8\%$ of control, $n = 6$). Therefore it appears that while virtually all evIPSCs were inhibited by WIN 55,212-2, the effects of opioid receptor activation were much more variable, ranging from no significant response for the selective μ agonist, DAMGO, to a significant inhibition of evIPSCs with

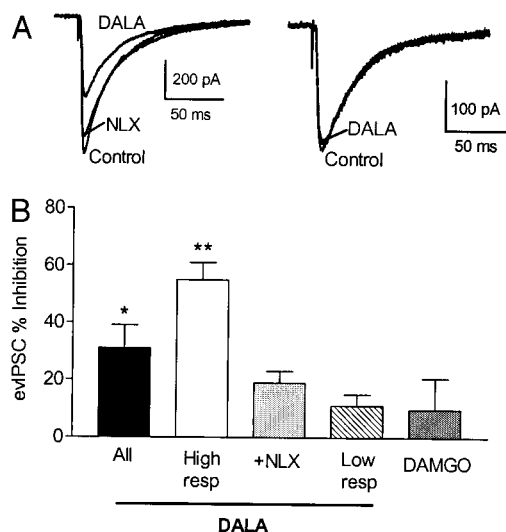


FIG. 8. Inhibition of evIPSCs in a subpopulation of NAc neurons by the μ/δ agonist D-Ala²-methionine²-enkephalinamide (DALA). *A*: averaged traces (10 sweeps) from a single experiment obtained during the control period, during DALA application (1 μ M), and during subsequent application of NLX (5 μ M). In a 2nd cell, *right*, DALA had no observable effect on the evIPSC. *B*: mean effects of 1 μ M DALA on all cells ($n = 11$) and on the subpopulations of cells that showed either robust inhibition (High resp, $n = 5$) or little inhibition (Low resp, $n = 6$) in response to DALA. NLX reversed the effect of DALA in the responsive cells (NLX, $n = 5$, $P > 0.05$ vs. control). In addition, the μ -agonist DAMGO (1 μ M) had no significant effect on the evIPSCs in any of the cells tested ($n = 8$). * $P < 0.05$, ** $P < 0.01$ vs. control, 1-way ANOVA.

the nonselective opioid agonist DALA in a subpopulation of MSNs.

In an effort to establish whether the observed inhibition of GABAergic transmission reflected pre- or postsynaptic actions, we examined the effects of WIN 55,212-2 and DALA on paired evIPSCs elicited with stimuli separated by 150 ms. In the absence of the agonist, the second evIPSC of the pair was inhibited relative to the first, indicating a modest paired-pulse depression (IPSC2/IPSC1 = 0.84 ± 0.05 , $n = 10$). However, during WIN 55,212-2 (1 μ M) application, IPSC2 was, on average, significantly larger than evIPSC1 (IPSC2/IPSC1 = 1.2 ± 0.11 , $P < 0.05$ vs. control, paired Student's t -test; Fig. 9), suggesting a presynaptic site of WIN 55,212-2 action. Similarly, the opioid agonist DALA (5 μ M) significantly increased the paired evIPSC ratio to 1.5 ± 0.25 ($n = 5$, $P < 0.05$, repeated-measures ANOVA, Newman-Keuls) in the group of neurons that was highly sensitive to DALA, and this effect was reversed by NLX (5 μ M; ratio = 0.84 ± 0.17).

CB1 receptor effects on miniature IPSCs in NAc neurons

The results described above demonstrate that, in contrast to its small effect on EPSCs, WIN 55,212-2 robustly inhibited evIPSCs in NAc MSNs. Moreover the paired-pulse experiments demonstrate a likely presynaptic mechanism for this inhibition. To more fully explore the mechanism of the cannabinoid effects on GABA_A-mediated synaptic transmission and to further examine the possible role that postsynaptic actions may play, we studied the effects of WIN 55,212-2 on TTX-insensitive mIPSCs. These experiments were conducted in MSNs voltage clamped at -80 to -90 mV, using whole cell electrodes containing CsCl and in the presence of glutamate receptor antagonists (40 μ M APV and 10 μ M DNQX) in the ACSF. Under these conditions, small spontaneous inward currents were observed that were completely blocked by addition of BMI (20 μ M; Fig. 10*B*). In contrast to other classes of neurons routinely studied in our laboratory (e.g., hippocampal

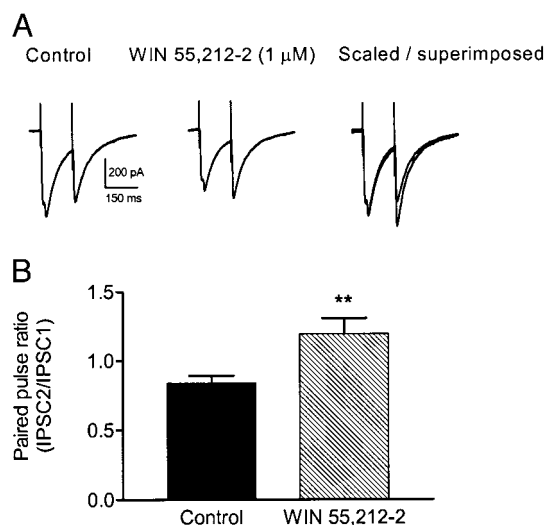


FIG. 9. WIN 55,212-2 reversed the paired-pulse inhibition of evIPSCs in NAc MSNs. *A*: averaged traces (10 sweeps) from a single experiment obtained during the control period and during the application of WIN 55,212-2. For comparison, the traces obtained under each condition have been scaled to the 1st evIPSC (IPSC1). Note that the inhibition of IPSC2 relative to IPSC1 was changed to facilitation. *B*: mean effects of WIN 55,212-2 on paired-pulse inhibition of evIPSCs in MSNs ($n = 10$, ** $P < 0.01$, Student's paired t -test).

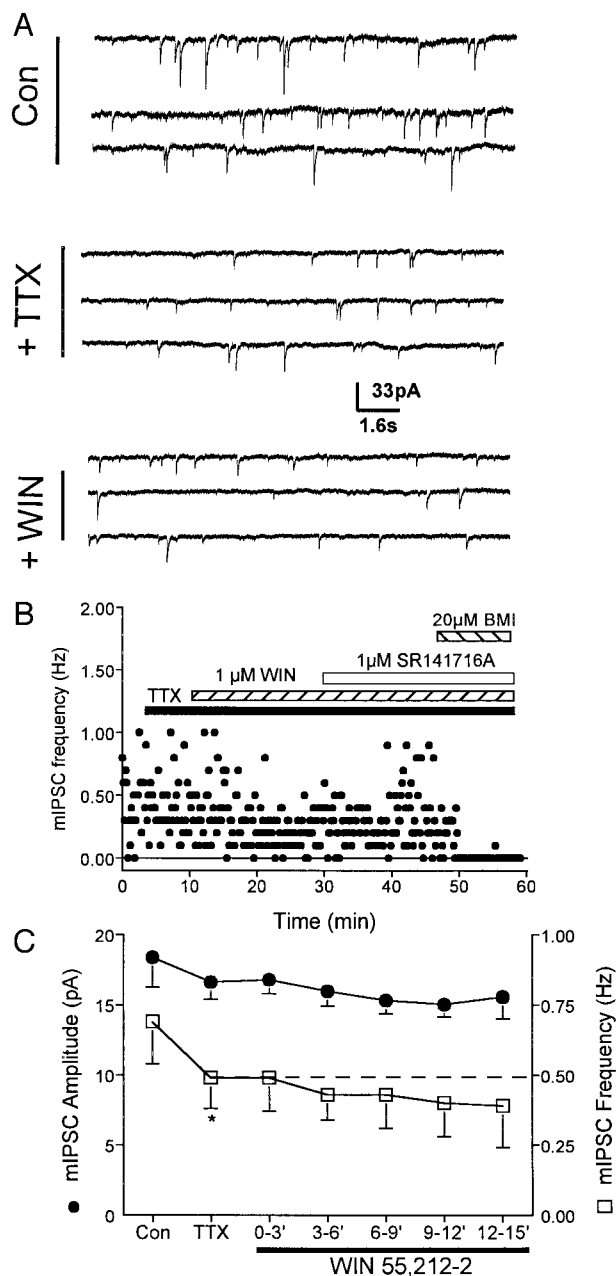


FIG. 10. WIN 55,212-2 effects on mIPSCs in NAc MSNs. A: raw traces from 3-min epochs obtained during control period, during TTX (0.5 μ M) application, and 9–12 min after beginning the WIN 55,212-2 (1 μ M) application. B: time course of mIPSC frequency during superfusion with WIN 55,212-2, SR141716A (1 μ M) and bicuculline methiodide (BMI, 20 μ M) in a single MSN. Note the small effect of WIN 55,212-2 on mIPSC frequency and its reversal with SR141716A. Also note the reduction in mIPSC frequency by BMI. Analysis of this cell using cumulative amplitude and frequency distributions (not shown) indicated that only the frequency was significantly reduced by WIN 55,212-2 ($P < 0.01$, K-S test). C: mean (\pm SE, $n = 6$) time course of the effect of WIN 55,212-2 (1 μ M). The average mIPSC frequency and amplitude were calculated for each 3-min epoch of data and are plotted consecutively (abscissa). --- (C), the control baseline frequency in TTX. The only significant group effect was the small decrease in frequency caused when TTX was applied (* $P < 0.05$, repeated-measures ANOVA, Newman-Keuls post hoc test).

pyramidal neurons) (Hoffman and Lupica 2000), the frequency of the spontaneously occurring currents in MSNs in the absence of TTX was very low (0.7 ± 0.2 Hz, $n = 6$). Because of this, the application of TTX (0.5 μ M) caused only a small

change in the frequency of these events (0.5 ± 0.1 Hz, Fig. 10, A and C). Despite the low frequency of occurrence, the CB1 agonist WIN 55,212-2 (1 μ M) significantly reduced the fre-

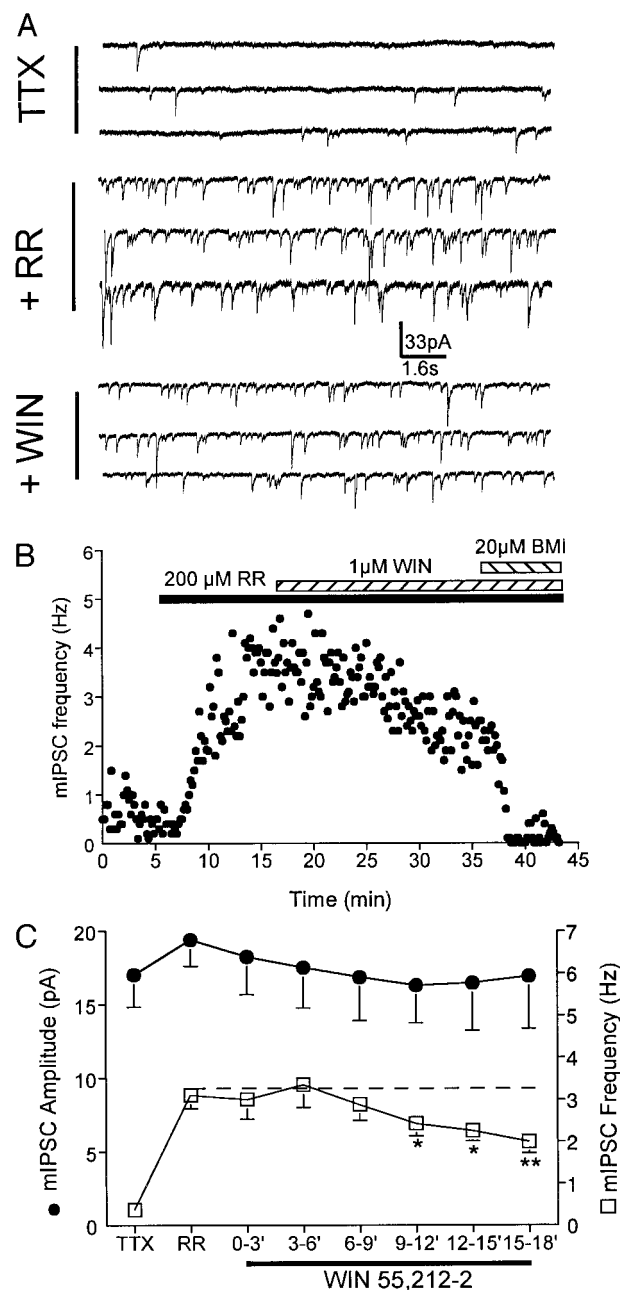


FIG. 11. Effects of WIN 55,212-2 on mIPSCs whose frequency was increased by addition of the polyvalent cation ruthenium red (200 μ M, RR). A: raw traces obtained from 3-min epochs collected during the control period (in TTX), from the 6th to 9th min of ruthenium red application, and during the 12th to 15th min of WIN 55,212-2 (1 μ M) application. B: time course of a single experiment in which ruthenium red, WIN 55,212-2 and BMI were applied. Note the large increase in mIPSC frequency with ruthenium red, the inhibition of mIPSC frequency by WIN 55,212-2, and the elimination of mIPSCs by BMI. C: mean \pm SE time course of the effect of WIN 55,212-2 (1 μ M) during ruthenium red superfusion ($n = 4$). The mean mIPSC frequency and amplitude were calculated for each consecutive 3-min epoch of data. Statistical analysis (repeated-measures ANOVA, Newman-Keuls post hoc test), revealed that WIN 55,212-2 significantly (* $P < 0.05$) reduced mIPSC frequency at between 12 and 18 min of WIN 55,212-2 application by 20–30%. In addition, the cumulative frequency distributions in all 4 cells (not shown) were significantly different from control at these time points ($P < 0.01$, K-S test). --- (C), the control baseline frequency in ruthenium red.

quency of mIPSCs in two of six cells ($P < 0.01$, K-S test; Fig. 10B), and these effects were reversed by the CB1 antagonist SR 141617A (1 μ M; Fig. 10B). However, when the effects of the CB1 agonist on all cells were averaged, this effect was not statistically significant (repeated measures ANOVA, $P > 0.05$). Similarly, there was no significant effect of the agonist on mIPSC amplitude (Fig. 10C).

Because we were concerned that the low frequency of mIPSCs in these MSNs might affect the reliability of our analysis, we attempted to increase their number with the bath application of the polyvalent cation ruthenium red. Ruthenium red has been shown to increase the frequency of GABAergic mIPSCs, in the presence of TTX, through a direct extracellular interaction with secretory mechanisms (Sciancalepore et al. 1998; Trudeau et al. 1996). In addition, ruthenium red has been shown to block voltage-dependent calcium channels, eliminating them as a possible site of action (Cibulsky and Sather 1999). In these experiments, mIPSCs were first monitored in the presence of TTX. Ruthenium red (200 μ M) was then applied for ≥ 10 min prior to application of WIN 55,212-2. Ruthenium red produced a large eightfold increase in the frequency of mIPSCs (mIPSC frequency: TTX = 0.37 ± 0.1 Hz; ruthenium red = 3.1 ± 0.3 Hz, $n = 4$) that was maximal within 6 min of beginning its application (Fig. 11B). The average amplitude of the mIPSCs was also increased by ruthenium red, although not to a statistically significant level (mIPSC amplitude: TTX = 17.0 ± 2.1 pA, ruthenium red = 19.4 ± 1.8 pA, ANOVA, $P > 0.05$). The time constant for the decay of the averaged mIPSCs was unaffected by ruthenium red (decay time constants: TTX = 51.2 ± 2.9 ms, ruthenium red = 53.4 ± 3.1 ms), and these mIPSCs were completely eliminated by BMI (20 μ M, $n = 4$, Fig. 11B), confirming that they were mediated by the activation of GABA_A receptors. WIN 55,212-2 (1 μ M) caused no change in the average amplitudes of these mIPSCs (Fig. 11C) nor did it change the time constant for the mean mIPSC decay (50 ± 2.8 ms). However, WIN 55,212-2 caused a small (20–25%) but significant reduction in the average frequency of mIPSCs 12–18 min after beginning its application (Fig. 11, A and C; $n = 4$; repeated-measures ANOVA $P < 0.01$; $P < 0.01$ Kolmogorov-Smirnov test).

DISCUSSION

The present study reports several novel findings regarding the actions of cannabinoids and opioids on the physiology of MSNs located in the shell region of the NAc. First, the cannabinoid agonist WIN 55,212-2 did not affect postsynaptic passive membrane properties of MSNs in vitro. Second, glutamatergic synaptic inputs to NAc neurons were only marginally inhibited by WIN 55,212-2 but robustly inhibited by the μ -opioid agonist DAMGO. Third, GABAergic synaptic responses were consistently inhibited by WIN 55,212-2 in all cells, inhibited by the μ/δ opioid agonist DALA in some cells, and completely insensitive to the μ -opioid receptor agonist DAMGO. Together, these data demonstrate the differential modulation of excitatory and inhibitory synaptic transmission by cannabinoids in the NAc and distinguishes these actions from those of opioids.

A common action of many drugs of abuse, including cannabinoids, is to increase the levels of dopamine in the NAc (Chen et al. 1990; Di Chiara and Imperato 1988). Since dopa-

mine directly inhibits both excitatory and inhibitory amino acid-mediated synaptic transmission in the NAc (Harvey and Lacey 1996, 1997; Nicola and Malenka 1997; Pennartz et al. 1992), it is likely that this mechanism plays an important role in regulating network activity, and hence reward, in this structure. Therefore since activation of cannabinoid receptors in the VTA (French 1997) increases dopamine levels in the NAc (Chen et al. 1990), it is also possible that this mechanism contributes to the rewarding properties of marijuana. On the other hand, several drugs of abuse, including opioids (Chieng and Williams 1998; Martin et al. 1997), cocaine (Nicola et al. 1996), amphetamine (Nicola and Malenka 1997; Nicola et al. 1996), and PCP (Carlezon and Wise 1996) have been shown to reduce amino acid-mediated synaptic transmission by direct actions in the NAc. Moreover all of these drugs are self-administered by animals into the NAc (Carlezon and Wise 1996; McBride et al. 1999), again suggesting that their rewarding properties are related, at least in part, to their direct actions on NAc synapses. In contrast to these more commonly studied drugs of abuse, direct effects of cannabinoids within the NAc have not been demonstrated until now. However, the moderately high level of CB1 receptor expression in this brain region (Herkenham et al. 1991; Tsou et al. 1998) indicates that these receptors might mediate the effects of cannabinoids in the NAc.

Cannabinoid agonists have been shown to modulate postsynaptic K^+ channels in hippocampal pyramidal neurons (Deadwyler et al. 1995; Schweitzer 2000) and in cellular expression systems (Mackie et al. 1995). However, our data suggest that inwardly rectifying K^+ channels were not modulated in MSNs by WIN 55,212-2 because membrane potential, holding current, input resistance, and the conductance of MSNs were unchanged. This is consistent with results in periaqueductal gray (PAG) neurons (Vaughan et al. 2000) and hippocampal interneurons (unpublished data). However, because we did not attempt to isolate specific voltage-dependent postsynaptic conductances, we cannot exclude possible effects of cannabinoids on these ion channels (Deadwyler et al. 1995; Schweitzer 2000).

Cannabinoid and opioid modulation of glutamatergic transmission

Cannabinoids have been shown to inhibit glutamate release in the hippocampus (Misner and Sullivan 1999; Shen et al. 1996), cerebellum (Takahashi and Linden 2000), and substantia nigra (Szabo et al. 2000). Therefore we examined the effects of WIN 55,212-2 on pharmacologically isolated glutamatergic EPSCs in MSNs (Martin et al. 1997; Pennartz et al. 1991, 1992). These experiments demonstrated that, in contrast to the robust cannabinoid-mediated inhibition of glutamatergic transmission observed in other brain regions, evEPSCs were only modestly inhibited by the cannabinoid agonist WIN 55,212-2 in the NAc, and these effects were only partially reversed by the CB1 antagonist SR141716A. However, the μ -opioid agonist DAMGO reduced these responses to a larger degree (Martin et al. 1997). These data therefore suggest that excitatory inputs to MSNs were more strongly inhibited by μ -opioids than by cannabinoids in MSNs.

To determine whether the cannabinoid- and opioid-mediated inhibition of EPSCs reflected actions at pre- or postsynaptic sites, we examined the effects of WIN 55,212-2 and DAMGO

on the paired-pulse facilitation of evEPSCs (Harvey and Lacey 1996). We found that neither WIN 55,212-2 nor DAMGO modulated evEPSC paired-pulse ratios despite the pronounced facilitation of these responses by dopamine and baclofen (also see Harvey and Lacey 1996). This suggested that, in contrast to dopamine and baclofen, the inhibitory effects of DAMGO or WIN 55,212-2 on evEPSCs were not presynaptic. To more fully explore this possibility, we used a more powerful analysis of TTX-resistant mEPSCs. These quantal release events are a very sensitive measure of the locus of a drug's effect because changes in mEPSC amplitudes are associated with a postsynaptic site of drug action, whereas changes in mEPSC frequency are likely due to interaction with a presynaptic site (Cohen et al. 1992; Lupica 1995; Thompson et al. 1993). In the present study, WIN 55,212-2 decreased mEPSC amplitude, without altering mEPSC frequency, whereas DAMGO reduced mEPSC frequency but did not affect mEPSC amplitude. Thus these data suggested that WIN 55,212-2 acted postsynaptically, whereas DAMGO acted presynaptically to inhibit glutamatergic transmission. Given these results, it is unclear why paired-pulse facilitation was unaffected by the opioid. However, because NMDA receptor function is known to be augmented by both DAMGO (Martin et al. 1997) and repetitive stimulation (Pennartz et al. 1991) in MSNs, it is possible that this postsynaptic change obscured DAMGO's presynaptic actions, which were clearly seen in the mEPSC experiments. This idea is supported by data demonstrating that almost no NMDA component contributes to mEPSCs (Nicola and Malenka 1997; present study). However, despite this disparity, both the mEPSC and the paired evEPSC experiments suggest that the small effect of WIN 55,212-2 on glutamatergic transmission likely occurred through a postsynaptic interaction, whereas the effects of DAMGO were likely presynaptic on mEPSCs, and both pre- and postsynaptic on the paired evEPSC responses (Martin et al. 1997). The mechanism of this apparent postsynaptic effect of WIN 55,212-2 will require further investigation.

Cannabinoid and opioid modulation of inhibitory transmission

In direct contrast to its modest effects on evEPSCs, the cannabinoid agonist more strongly inhibited GABA_A-mediated evIPSCs in NAc MSNs. The effect of WIN 55,212-2 was concentration-dependent ($EC_{50} = 123$ nM) and completely antagonized by the selective CB1 antagonist SR141716A (Rinaldi-Carmona et al. 1994). Furthermore the estimated potency of WIN 55,212-2 was similar to that reported for evIPSCs in hippocampal pyramidal neurons (138 nM) (Hoffman and Lupica 2000). The antagonist also had no effect alone on evIPSCs, suggesting that endogenous cannabinoids do not modulate synaptic transmission in NAc brain slices under the present conditions (Hoffman and Lupica 2000; Katona et al. 1999).

In comparison with the consistent effect of the cannabinoid on GABA release onto MSNs, the effects of the opioid agonists were much more variable. The selective μ -opioid receptor agonist DAMGO had no effect on the evIPSCs, and the non-selective μ/δ -opioid agonist DALA inhibited evIPSCs in only a subset of the MSNs examined (~50%). Since DALA does not have significant activity at κ -opioid receptors (Goldstein and Naidu 1989) and its effects were reversed by NLX, we conclude that these actions were on a subset of inhibitory

terminals that express δ -opioid receptors. A previous study has shown inhibition of evoked synaptic potentials in NAc MSNs by μ -, δ -, and κ -opioid receptors (Yuan et al. 1992). However, it was unclear whether these depolarizing responses were composed of EPSPs, IPSPs, or both. Furthermore in that study, only a small number of the neurons demonstrated clear hyperpolarizing IPSPs that were inhibited by selective μ - or δ -opioid agonists. In contrast, the present data support the more recent observations that δ -opioid receptors are more prominently represented on GABAergic terminals in the NAc than are μ -opioid receptors, which are largely associated with postsynaptic GABAergic cellular profiles (Svingos et al. 1997, 1998). Our data also suggest that δ -opioid receptors may only be found on a subset of the inhibitory terminals making contact with MSNs. Thus unlike the effect of CB1 receptor activation, which inhibited GABA release onto all MSNs, δ -opioid receptors seemed to more selectively regulate GABA release onto only a subset of these neurons.

Unlike its effect on glutamatergic transmission in the present study the cannabinoid agonist, WIN 55,212-2, likely reduced GABAergic transmission in the NAc through, exclusively, a presynaptic action. This was supported by data showing a switch from paired-pulse depression to facilitation of evIPSCs during CB1 receptor (and opioid receptor) activation and a complete absence of WIN 55,212-2 effects on mIPSC amplitudes or decay kinetics. In these experiments, and in a previous study (Nicola and Malenka 1997), the frequency of mIPSCs was found to be very low. This was somewhat surprising because the fast spiking GABAergic interneurons that are thought to provide the major inhibitory input to MSNs in the striatum (and probably the NAc; see following text) are spontaneously active in vivo and in slice preparations (Kawaguchi et al. 1995; Koós and Tepper 1999). This may be explained by the fact that these previous experiments were conducted at higher temperatures (35°C), whereas those in the present study, and in the study by Nicola and Malenka (1997), were conducted at room temperature, which tends to decrease the number of spontaneously active cells. In an attempt to mitigate possible deleterious effects of a small sample size on our analysis of mIPSCs, we also examined these events in the presence of ruthenium red, which enhances mIPSC frequency by a direct interaction with the transmitter release machinery (Sciancalepore et al. 1998; Trudeau et al. 1996). This experiment also demonstrated that WIN 55,212-2 had no effect on mIPSC amplitude or kinetics and only a small effect on mIPSC frequency. Taken together, these results strongly suggest a presynaptic site of action for the cannabinoid in the NAc; an effect that is also found in other brain areas (Chan et al. 1998; Hoffman and Lupica 2000; Szabo et al. 1998; Vaughan et al. 1999, 2000).

Functional implications for the modulation of NAc output by cannabinoids

Prior descriptions of striatal/NAc physiology have attributed GABAergic inhibition of MSNs to synaptic interactions among these cells through an extensive network of axon collaterals (Park et al. 1980; Wilson and Groves 1980). However, more recent data appear to refute this concept, suggesting instead that the major source of inhibitory GABAergic input to MSNs is derived from a much smaller population of striatal interneurons (Jaeger et al. 1994; Kawaguchi et al. 1995; Koós and

Tepper 1999). In particular, one class of these intrinsic interneurons has been shown to express mRNAs for glutamic acid decarboxylase (GAD67) and the CB1 receptor (Hohmann and Herkenham 2000). Assuming that this relationship is found in the ventral striatum/NAc, it is likely that the presynaptic inhibition of GABA release by WIN 55,212-2 seen in the present study was due to activation of CB1 receptors located on the terminals of these interneurons. Furthermore because these interneurons receive substantial cortical glutamatergic input (Bennett and Bolam 1994; Pennartz and Kitai 1991), they represent a critical link in mediating cortico-accumbens feed-forward inhibition of medium spiny projection neuron output. Therefore one of the roles of CB1 receptors in the NAc might be to dampen this feedforward inhibition, thereby disinhibiting MSNs, making them more responsive to excitatory cortical input and increasing NAc output to the ventral pallidum and VTA. In contrast, our data demonstrating a more circumscribed modulation of GABA release onto MSNs by opioid receptors suggest that this system may have a more limited role in the disinhibition of these NAc projection neurons that may allow certain groups of cells to function as discrete NAc output pathways (Groenewegen et al. 1999; Pennartz et al. 1994).

The role of the NAc in drug reward, abuse, and addiction has been the subject of intensive investigation for many decades. While it is clear that the rewarding properties of these drugs are associated with changes in NAc activity, there is still considerable debate as to the specific mechanisms involved. Increases in NAc dopamine levels are a useful neurochemical index of drug reward but do not fully account for the complex processing of fast synaptic activity by this neuromodulator in the NAc. Moreover because both glutamatergic and GABAergic inputs to MSNs are directly inhibited by dopamine, as well as by drugs of abuse, it is likely that these effects contribute to the rewarding properties of these drugs. Thus a demonstration of direct actions at NAc synapses provides a useful context in which to consider the rewarding properties of a drug, either apart from, or in addition to, its effects on NAc dopamine levels. Indeed it is unlikely that the effects of WIN 55,212-2 in the present study were due to an increase in dopamine levels because a recent voltammetric study demonstrated that this agonist did not increase dopamine levels in NAc slices (Szabo et al. 1999). Furthermore we did not see the robust inhibition of glutamatergic EPSCs that would be expected with elevated dopamine levels (Harvey and Lacey 1996). However, because cannabinoids increase dopamine levels in the NAc in vivo (Chen et al. 1990), it is likely that when systemically administered they would indirectly inhibit glutamatergic synapses through this mechanism (Harvey and Lacey 1996). Despite the complexity of these direct and indirect actions of drugs of abuse on amino acid-mediated synaptic processes in the NAc, it is likely that the modulation of the population activity of MSNs through these synaptic mechanisms will contribute to the rewarding and addictive properties of these drugs in the intact organism.

The authors thank Dr. Roy A. Wise for helpful comments on the manuscript. This work was supported by the National Institute on Drug Abuse.

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