

Muscarinic Receptor Blockade in the Ventral Tegmental Area Attenuates Cocaine Enhancement of Laterodorsal Tegmentum Stimulation-Evoked Accumbens Dopamine Efflux in the Mouse

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KEY WORDS fixed potential amperometry; M5 muscarinic receptor; cocaine; scopolamine; laterodorsal tegmentum; ventral tegmental area

ABSTRACT The reinforcing properties of cocaine have been related to increased extracellular concentrations of dopamine in the nucleus accumbens (NAc). M5 muscarinic acetylcholine receptors (mAChRs) on dopamine cells in the ventral tegmental area (VTA) facilitate mesoaccumbens dopamine transmission and are critically involved in mediating natural and drug reinforcement. We investigated the effects of pharmacological blockade of mAChRs in the VTA on cocaine's ability to enhance electrically evoked NAc dopamine efflux. Using fixed potential amperometry together with carbon fiber recording microelectrodes positioned in the NAc core, we quantified dopamine oxidation currents (dopamine efflux) evoked by brief stimulation (15 monophasic pulses at 50 Hz every 30 s) of the laterodorsal tegmentum (LDT) in urethane (1.5 g/kg, i.p.) anesthetized mice. Compared to predrug baseline responses, cocaine (5 or 10 mg/kg, i.p.) dose-dependently enhanced LDT stimulation-evoked NAc dopamine efflux, whereas the nonsubtype selective mAChR antagonist scopolamine (10 µg/0.5 µl) microinfused into the VTA diminished LDT-evoked NAc dopamine efflux. Preinfusion of scopolamine into the VTA diminished the facilitatory actions of cocaine on LDT stimulation-evoked NAc dopamine efflux, and when infused at the peak effect of cocaine attenuated LDT-evoked dopamine efflux to below predrug baseline levels. These findings suggest that LDT cholinergic inputs to dopamine neurons in the VTA, via activation of mAChRs (probably of the M5 subtype), are involved in modulating the facilitatory effects of cocaine on NAc dopamine neurotransmission. They also suggest that the development of antagonists aimed at selectively disrupting M5 receptor function may be valuable in reducing abuse liability of psychostimulants. **Synapse 64:216–223, 2010.** © 2009 Wiley-Liss, Inc.

INTRODUCTION

The mesoaccumbens dopamine system, comprising neuronal projections from the ventral tegmental area (VTA) to the nucleus accumbens (NAc), is recognized as a neural substrate involved in mediating the reinforcing properties of psychostimulants such as cocaine [for reviews, see Di Chiara (1995) and Koob (1992)]. This is evidenced by dose-dependent “tonic” increases in extracellular dopamine levels during cocaine self-administration in rats (Pettit and Justice, 1989, 1991), whereas 6-hydroxydopamine-induced destruction of either VTA dopamine cells or NAc dopaminergic terminals produces significant decreases in cocaine self-administration (Roberts et al., 1977, 1980;

Roberts and Koob, 1982). Rapid “phasic” changes in extracellular levels of dopamine recorded in the NAc also coincide with cocaine-seeking behaviors and cocaine-related cues (Phillips et al., 2003). Brain-imaging techniques have extended these observations to humans showing that psychostimulant-induced elevations in ventral striatum dopamine activity

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correspond with self-report measures of euphoria (Drevets et al., 2001).

Several lines of evidence indicate that dopamine levels in the NAc are mediated by excitatory cholinergic inputs from the laterodorsal tegmentum (LDT) of the pons (Blaha et al., 1996; Cornwall et al., 1990; Oakman et al., 1995; Woolf, 1991), via direct activation of midbrain muscarinic acetylcholine receptors (mAChRs). Of the five mAChR subtypes, only M5 receptor mRNA has been localized to dopamine neuronal cells in the VTA (Vilario et al., 1990; Weiner et al., 1990). Consequently, the M5 subtype occupies a unique position to modulate behaviors driven by mesoaccumbens dopaminergic activity. Indeed, research suggests that these receptors may have an important role in motivational behaviors (Forster et al., 2001; Yeomans et al., 2000), including a general function in the addictive properties of several classes of drugs of abuse (Basile et al., 2002; Fink-Jensen et al., 2003; Thomsen et al., 2005).

This study investigated the importance of mAChRs in the VTA in modulating mesoaccumbens dopaminergic transmission, particularly during the facilitatory actions of cocaine. Given evidence that scopolamine may diminish dopamine activity via blockade of M5 mAChRs (Forster and Blaha, 2000; Forster et al., 2001), experiments examined the effect this antagonist has upon the ability of cocaine to enhance LDT stimulation-evoked NAc dopamine efflux. Using fixed potential amperometry (FPA) in combination with electrical stimulation of the LDT, this was investigated by monitoring rapid changes in evoked NAc dopamine oxidation current (corresponding with dopamine efflux) in response to microinfusion of scopolamine into the VTA prior to or following systemic administration of cocaine.

MATERIALS AND METHODS

The following experiments were approved by the Institutional Animal Care and Use Committee at the University of Memphis and conducted in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals. Efforts were made to reduce the number of animals used and to minimize animal pain and discomfort.

Animals and surgery

Forty-five male C57BL/6J mice (Jackson Laboratories, Bar Harbor, ME), 8–11 weeks of age and weighing 20–33 g at the time of surgery, were used. Animals were housed five per cage in a temperature controlled environment ($21^{\circ}\text{C} \pm 1^{\circ}\text{C}$) with a 12-h light–dark cycle (lights on at 0600 hrs). Food and water were available ad libitum.

Mice were anesthetized with urethane (1.5 g/kg, i.p.) and mounted in a stereotaxic frame (David Kopf

Instruments, Tujunga, CA) within a mouse head-holder adaptor (Stoelting, Wood Dale, IL), ensuring the skull was flat. Body temperature was maintained at $36^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ with a temperature-regulated heating pad (TC-1000; CWE, NY, NY). In each mouse, a concentric bipolar stimulating electrode (SNE-100; Rhodes Medical, CA) was implanted into the left LDT (coordinates: AP -1.0 mm from lambda, ML $+0.4$ mm from midline, and DV -2.4 mm from dura; Paxinos and Franklin, 2001), and a 31 gauge stainless-steel guide cannula was implanted into the left VTA for drug microinfusions, with the tip of the cannula positioned 2 mm above site (coordinates: AP $+0.9$ mm from lambda, ML $+0.3$ mm from midline, DV -4.0 mm from dura; Paxinos and Franklin, 2001). A stainless-steel auxiliary and Ag/AgCl reference electrode combination was placed in surface contact with contralateral cortical tissue ~ 2.0 mm posterior to bregma. A carbon fiber microelectrode with an active recording surface of $250 \mu\text{m}$ (length) by $10 \mu\text{m}$ (o.d.) (Thornel Type P, Union Carbide, Pittsburgh, PA) was then implanted into the left NAc core (coordinates: AP 1.5 mm from bregma, ML $+1.0$ mm from midline, and DV -4.0 mm from dura; Paxinos and Franklin, 2001). FPA coupled with carbon fiber microelectrodes has been confirmed as a valid technique for real-time monitoring of NAc dopamine oxidation current evoked by brief electrical stimulation of cholinergic nuclei in the pons innervating midbrain dopamine neurons (Forster and Blaha, 2003).

FPA recordings and electrical stimulation of the LDT

All amperometric recordings were made within a Faraday cage to increase the signal to noise ratio (Forster and Blaha, 2003). A fixed potential ($+0.8$ V) was applied to the recording electrode and oxidation current was monitored continuously (10 K samples/s) with an electrometer (ED401 e-corder 401 and EA162 Picostat, eDAQ, CO Springs, CO), filtered at 50 Hz. Approximately 20 min following implantation of the recording electrode, a series of cathodal monophasic current pulses ($800 \mu\text{A}$) was delivered to the stimulating electrode via an optical isolator and programmable pulse generator (Iso-Flex/Master-8; AMPI, Jerusalem, Israel). The stimulation protocol consisted of fifteen 0.5 ms duration pulses at 50 Hz delivered every 30 s over a 1.5-h testing period. Baseline levels of LDT stimulation-evoked dopamine were monitored for 20 min in each mouse prior to drug administration.

VTA drug microinfusions

Drug microinfusions included the nonsubtype selective mAChR antagonist scopolamine hydrobromide ($10 \mu\text{g}/0.5 \mu\text{l}$). To confirm that the observed drug effects were not attributable to nonspecific effects of

the microinfusion procedure, intra-VTA microinfusions of sterile phosphate-buffered saline (PBS, pH ~ 7.4) were also performed. Scopolamine or PBS was backloaded into a fiberglass cannula (80 μm o.d., Polymicro Tech., AZ), connected via PE10 tubing to a 1- μl microsyringe (Scientific Glass Engineering, Austin, TX) mounted on a microinfusion pump (Stoelting, Wood Dale, IL). At the appropriate infusion time, the drug cannula was inserted into the stainless steel guide, extending 2 mm beyond the tip. Scopolamine or PBS was microinfused into the VTA at a rate of 0.25 $\mu\text{l}/\text{min}$ in a volume of 0.5 μl , either 5 min prior to cocaine administration (5 or 10 mg/kg, i.p.) or at its peak effect (15 min) upon LDT stimulation-evoked NAc dopamine efflux. All drugs were prepared immediately before use at doses determined by preliminary studies in this laboratory.

Data collation and statistical analysis

To quantify LDT stimulation-evoked dopamine efflux via the recorded oxidation current, prestimulation current values were normalized to zero current values and data points occurring within the range of 0.25 s pre- and 1.0 s postonset of the stimulation were extracted from the continuous record at 5-min intervals. In each animal, peak changes in dopamine oxidation current evoked by LDT electrical stimulation after drug administration were expressed as mean percent change with respect to predrug baseline evoked response (100%). For each condition, the resulting percentage changes in evoked responses were subsequently averaged across animals. Mean peak LDT-evoked responses following saline or drug administration were statistically compared to preadministration baseline LDT-evoked responses using paired two-tailed *t*-tests; independent *t*-tests were used to statistically compare mean peak LDT-evoked responses between cocaine doses. Mean LDT-evoked responses between control and drug conditions were analyzed with repeated measures analysis of variance (ANOVA) with the between subject factor being drug condition group and the within subject factor being time. If the ANOVA indicated significant interactions, Dunnett's test was used to compare the drug condition groups to the control groups. The alpha level for all analyses was set at 0.05.

Histology

Upon the completion of each experimental session, an iron deposit was made in the LDT stimulation site by passing direct anodic current (100 μA for 10 s) through the stimulating electrode. Mice were then euthanized with a 0.25-ml intracardial injection of urethane (0.345 g/ml). Brains were removed, immersed overnight in 10% buffered formalin containing 0.1% potassium ferricyanide, and then stored in 30%

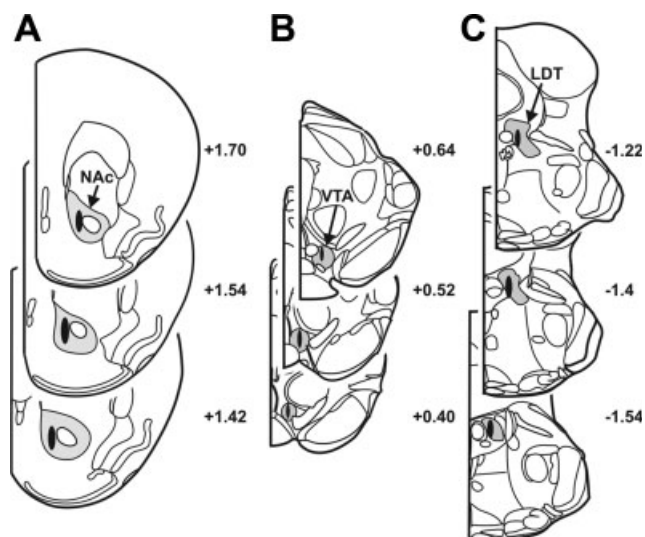


Fig. 1. Representative coronal sections of the mouse brain [adapted from the atlas of Paxinos and Franklin (2001)], with black-shaded areas indicating the placements of (A) amperometric recording electrodes in the core region of the nucleus accumbens (NAc), (B) drug infusion cannulae in the ventral tegmental area (VTA), and (C) stimulating electrodes in the laterodorsal tegmentum (LDT). Numbers correspond to mm from (A) bregma and (B and C) interaural zero.

sucrose/10% formalin solution until sectioning. After fixation, 30- μm coronal sections were sliced in a cryostat at -30°C , with a Prussian blue spot resulting from a redox reaction of the ferricyanide marking the stimulation site. Placements of stimulating electrodes, recording electrodes, and drug infusion cannulae were determined under a light microscope and recorded on representative coronal diagrams (Paxinos and Franklin, 2001).

Chemicals

Urethane, scopolamine hydrobromide, and cocaine hydrochloride were obtained from Sigma-Aldrich Chemical (St Louis, MO). All chemicals, with the exception of urethane (distilled water), were dissolved in sterile PBS (pH ~ 7.4).

RESULTS

Stereotaxic placements of drug infusion cannulae, recording and stimulating electrodes

The placements of the electrochemical recording electrode surfaces ($n = 45$) were confined to the core of the NAc (ranging from 1.42 to 1.70 mm anterior to bregma, 0.80 to 1.20 mm lateral to midline, and 3.90 to 4.20 mm ventral to dura) (Fig. 1A). Infusion cannula tip placements ($n = 45$) were localized within the VTA (ranging from 0.40 to 0.64 mm anterior to interaural zero, 0.20 to 0.40 mm lateral to midline, and 1.30 to 1.70 mm dorsal from interaural zero) (Fig. 1B). The tips of the stimulating electrodes ($n = 45$)

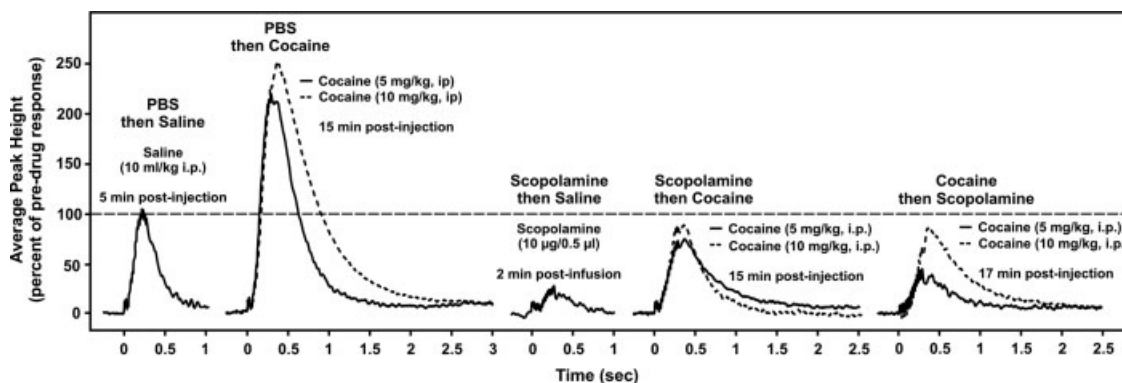


Fig. 2. Amperometric recordings of dopamine oxidation current recorded in the nucleus accumbens, evoked by electrical stimulation of the laterodorsal tegmentum. Profiles illustrate mean peak effects in response to ventral tegmental area microinfusion of phosphate-

buffered saline (PBS) or scopolamine (10 $\mu\text{g}/0.5 \mu\text{l}$) prior to or following intraperitoneal injection of saline (10 ml/kg) or cocaine (5 mg/kg or 10 mg/kg), with respect to predrug baseline responses (100%). Time zero indicates the start of the train of 15 pulses at 50 Hz.

were positioned within the anatomical boundaries of the LDT (ranging from -1.22 to -1.54 mm posterior to interaural zero, 0.40 to 0.80 mm lateral to midline, and 2.20 to 2.50 mm dorsal from interaural zero) (Fig. 1C).

Effects of systemic cocaine and intra-VTA scopolamine on LDT stimulation-evoked dopamine efflux

With respect to preinfusion baseline levels (100%), LDT stimulation-evoked dopamine oxidation current (dopamine efflux) in the NAc was not significantly altered by intra-VTA infusion of PBS administered either 5 min prior to ($n = 3$; $92.8\% \pm 2.2\%$, $P = 0.83$) or 15 min following ($n = 3$; $98.3\% \pm 2.9\%$, $P = 0.62$) systemic injection of saline (10 ml/kg, i.p.; Fig. 2). The temporal pattern of these responses provided LDT-evoked dopamine efflux control values against which to compare the effects of cocaine and scopolamine (Figs. 3A–3D, gray lines). Cocaine (5 or 10 mg/kg, i.p.; $n = 4$ and 3, respectively) significantly increased LDT stimulation-evoked dopamine efflux in the NAc when administered 5 min following intra-VTA infusion of PBS ($215.6\% \pm 16.2\%$, $P < 0.05$ and $241.3\% \pm 13.6\%$, $P < 0.05$, respectively; Fig. 2), with peak effects occurring 10–15-min postinjection (Figs. 3A and 3B). Cocaine (5 or 10 mg/kg, i.p.; $n = 3$ per dose) also significantly increased LDT stimulation-evoked dopamine efflux in the NAc when administered 15 min prior to intra-VTA infusion of PBS ($191.3\% \pm 10.2\%$, $P < 0.05$ and $253.1\% \pm 9.4\%$, $P < 0.05$, respectively; Figs. 3C and 3D). Peak levels of LDT stimulation-evoked dopamine efflux in the NAc were significantly greater following the higher dose of cocaine (10 mg/kg: $247.2\% \pm 19.2\%$) when compared with the lower cocaine dose (5 mg/kg: $205.2\% \pm 28.3\%$) ($P < 0.05$). Intra-VTA infusion of scopolamine (10 $\mu\text{g}/0.5 \mu\text{l}$) significantly decreased LDT stimulation-evoked dopa-

mine efflux in the NAc, when administered 5 min prior to ($n = 4$; $25.2\% \pm 2.8\%$, $P < 0.05$; Fig. 2) or 15 min following ($n = 3$; $30.6\% \pm 4.8\%$, $P < 0.05$) systemic injection of saline, with evoked dopamine efflux returning to levels equivalent to those observed in control animals ~ 25 min postinfusion (Figs. 3A–3D).

Effects of mAChR blockade on cocaine-induced increases in LDT stimulation-evoked dopamine efflux

Intra-VTA infusion of scopolamine (10 $\mu\text{g}/0.5 \mu\text{l}$) 5 min before cocaine administration (5 or 10 mg/kg, i.p.) blocked cocaine-induced elevations in LDT stimulation-evoked dopamine efflux in the NAc (Figs. 2, 3A, and 3B) and attenuated these increases when administered at the peak effect of cocaine (15 min postinjection) (Figs. 2, 3C, and 3D). Figure 4 illustrates the mean differences in the temporal pattern of LDT stimulation-evoked dopamine efflux between drug and control conditions to clearly distinguish the normalized effect of scopolamine infusions either before or after each dose of cocaine administration. In comparison to the normalized effects of cocaine (calculated from the mean difference in dopamine responses to intra-VTA infusion of PBS before systemic administration of cocaine and saline; solid lines, Figs. 4A and 4B), prior administration of scopolamine (calculated from the mean difference in dopamine responses to intra-VTA scopolamine before systemic administration of cocaine and saline; dashed lines, Figs. 4A and 4B) significantly attenuated cocaine-induced dopamine levels in the NAc ($P < 0.05$). In comparison to the normalized effects of cocaine (calculated from the mean difference in dopamine responses to intra-VTA infusion of PBS following systemic administration of cocaine and saline; solid lines, Figs. 4C and 4D), administration of scopolamine during the peak effect of cocaine (calculated from the mean difference in

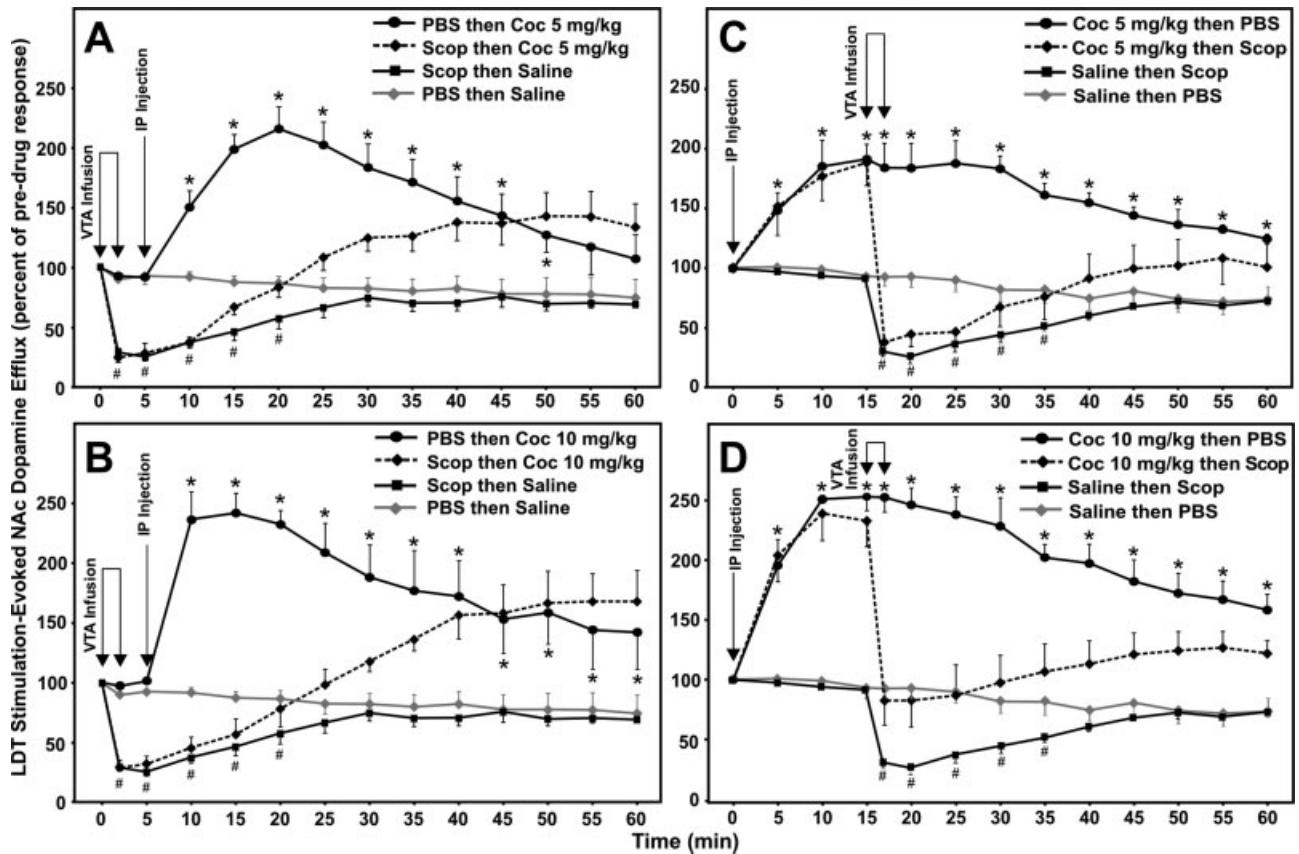


Fig. 3. Amperometric recordings depicting time courses of laterodorsal tegmentum (LDT) stimulation-evoked dopamine oxidation current recorded in the nucleus accumbens (NAc), in response to intraventricular tegmental area (VTA) infusion of scopolamine (10 μ g/0.5 μ l) prior to or following intraperitoneal injection of 5 mg/kg (A

and C) or 10 mg/kg (B and D) cocaine. *Significantly higher dopamine levels following cocaine compared to saline injection. #Significantly lower dopamine levels following scopolamine compared to PBS infusion. Coc, cocaine; PBS, phosphate-buffered saline; Scop, scopolamine; IP, intraperitoneal.

dopamine responses to intra-VTA infusion of scopolamine following systemic administration of cocaine and saline; dashed lines, Figs. 4C and 4D) significantly attenuated elevated dopamine levels ($P < 0.05$).

DISCUSSION

Several lines of evidence indicate that the reinforcing properties of cocaine are primarily mediated by the mesoaccumbens dopamine system (Pettit and Justice, 1989, 1991; Roberts et al., 1977, 1980; Roberts and Koob, 1982). In light of this, the present results demonstrate that the systemic administration of cocaine significantly enhances LDT stimulation-evoked dopamine efflux in the NAc. Thus, cocaine increases both electrically stimulated dopamine release, as measured here using in vivo FPA, as well as basal dopamine levels, measured using in vivo microdialysis (Frank et al., 2008), in the NAc. As anticipated from previous research [see Frank et al. (2008)], in response to cocaine administration peak levels of LDT stimulation-evoked dopamine efflux in the NAc were dose-dependent; however, no differences in the time

to reach peak levels were observed between drug doses. Intra-VTA infusion of the nonselective mAChR antagonist scopolamine significantly attenuates LDT stimulation-evoked dopamine efflux in the NAc. Importantly, preinfusion of scopolamine also diminishes the facilitatory effects of cocaine on LDT-evoked dopamine efflux, and when administered at the peak effect of cocaine attenuates evoked dopamine efflux to below predrug levels.

The inhibitory actions of scopolamine on cocaine facilitated LDT-evoked dopamine efflux described here are supported by the inhibitory effects of atropine, a non-subtype-selective mAChR antagonist, on intravenous cocaine-induced increases in extracellular dopamine in the NAc, measured using in vivo microdialysis (Sziraki et al., 2002). On its own, atropine perfused into the VTA reduces basal dopamine levels in the NAc (Sziraki et al., 2002). These outcomes may reflect two opposing mechanisms whereby scopolamine or atropine act to decrease LDT cholinergic activation of VTA dopaminergic neurons and thus extracellular dopamine levels in the NAc through the blockade of mAChRs (Forster and Blaha, 2000; Forster

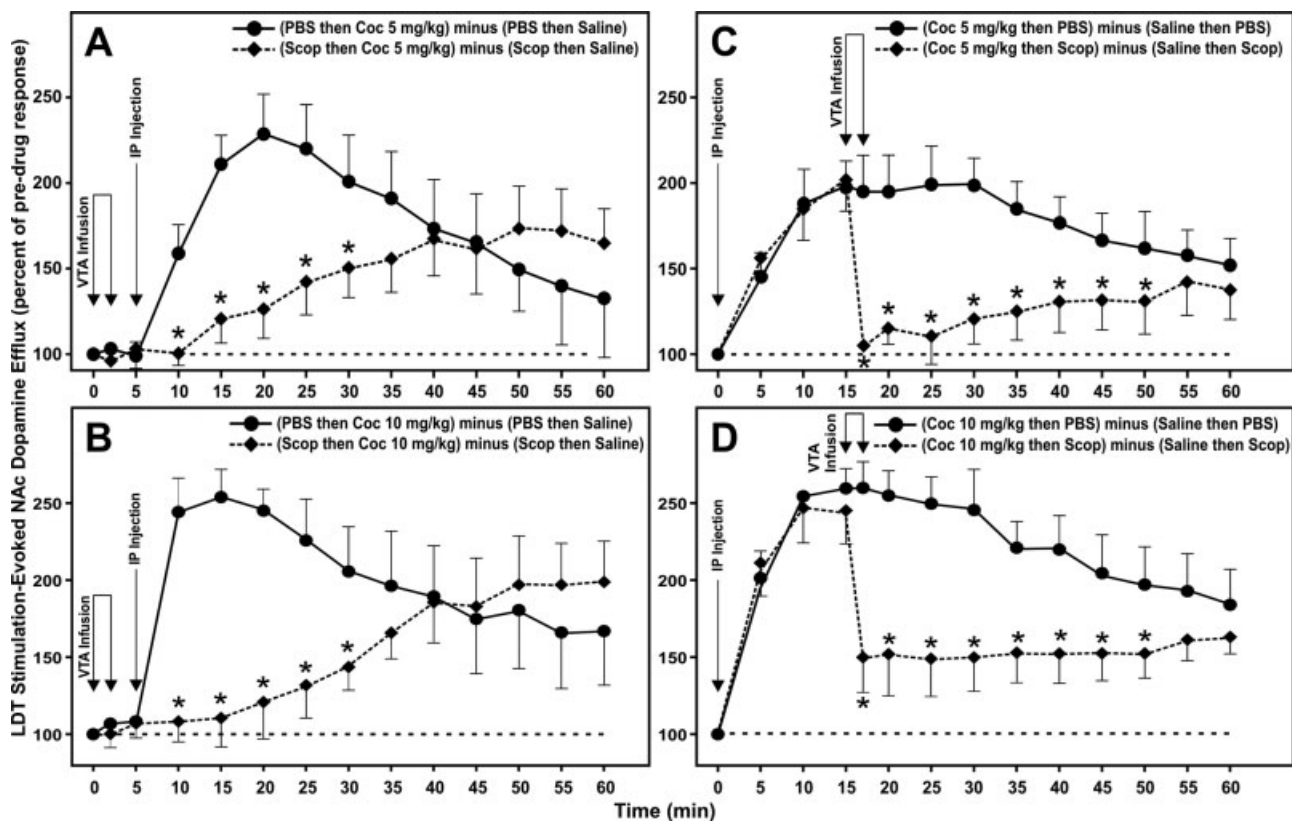


Fig. 4. Mean differences in laterodorsal tegmentum (LDT) stimulation-evoked dopamine oxidation current between drug and control conditions. *Significant differences at a particular time point, depicting the ability of preadministration of scopolamine (10 μ g/0.5 μ l) to attenuate cocaine (**A**: 5 mg/kg and **B**: 10 mg/kg) facilitated

increases in LDT-evoked dopamine efflux and to attenuate these effects when infused at the peak effect of cocaine (**C**: 5 mg/kg and **D**: 10 mg/kg). NAc, nucleus accumbens; VTA, ventral tegmental area; Coc, cocaine; PBS, phosphate-buffered saline; Scop, scopolamine; IP, intraperitoneal.

et al., 2001; Miller and Blaha, 2005; Sziraki et al., 2002), whereas cocaine acts to increase dopamine concentrations in the NAc (Frank et al., 2008; Reith et al., 1997; Sziraki et al., 2002) through blockade of the dopamine uptake transporter [for review, see Koob and Bloom (1988)]. Together, these results suggest that LDT cholinergic inputs to dopamine neurons in the VTA, via activation of mAChRs, are likely involved in modulating the neurochemical effects of psychostimulants.

Potential mAChR subtypes underlying the inhibitory actions of scopolamine on LDT stimulation-evoked dopamine efflux in the NAc

Scopolamine is classified as a broad spectrum mAChR antagonist (Bolden et al., 1992), which therefore precludes identification of specific muscarinic subtype involvement in its antagonistic effects upon LDT stimulation-evoked dopamine efflux. However, the M5 receptor is the only mAChR subtype expressed by dopaminergic neurons in the VTA (Vilaro et al., 1990; Weiner et al., 1990). Furthermore,

in rats and mice sustained increases in NAc dopamine efflux evoked by prolonged stimulation of the LDT (a single bout of 1050 pulse stimulations), measured using *in vivo* chronoamperometry, are diminished by systemic administration or intra-VTA infusion of scopolamine (Forster and Blaha, 2000; Forster et al., 2001) and are abolished in M5 receptor-deficient mice (Forster et al., 2001). These findings imply that the M5 mAChR is integral in LDT cholinergic modulation of dopamine cell activity and forebrain dopamine release. They also suggest that, although M5 selective ligands or toxins are yet to be found (Caulfield and Birdsall, 1998), the inhibitory actions of scopolamine on LDT stimulation-evoked dopamine efflux, including those observed in this study, are M5 dependent.

Although scopolamine shows no significant selectivity for the M3 receptor subtype over the M5 subtype (Bolden et al., 1992), we cannot completely rule out the possibility of scopolamine acting indirectly via blockade of presynaptic M3 mAChRs localized on GABA-containing terminals in the VTA to attenuate LDT stimulation-evoked NAc dopamine efflux (Grillner et al., 2000; Michel et al., 2004). However, previous research has shown that selective blockade

of these receptors via microinfusion of the antagonist *para*-fluoro-hexahydrosiladiphenidol (*p*-F-HHSiD) into the VTA does not alter basal dopamine efflux in the NAc as measured using in vivo chronoamperometry (Miller and Blaha, 2005). Conversely, similar infusions into the substantia nigra enhance dopamine efflux in the striatum suggesting presynaptic M3 mAChRs may serve to counter excessive excitation of nigral dopamine neuronal activity (Miller and Blaha, 2005). These findings align with autoradiographic evidence indicating a preferential localization of M3 mAChRs in the substantia nigra (Frey and Howland, 1992; Zubieta and Frey, 1993).

Significance of M5 mAChRs in mediating psychostimulant-induced behaviors and dopamine transmission

Infusion of 21-mer antisense oligonucleotides into the VTA of awake rats to target M5 mRNA inhibits M5 receptor binding and reduces sensitivity to rewarding hypothalamic stimulation (Yeomans et al., 2000). Given that dopamine neuronal activation in the VTA is integral in brain stimulation reward (Blaha and Phillips, 1990), this suggests that M5 mAChRs may be important in the maintenance of reward-related behaviors that are driven by dopamine activity. M5 mAChRs have also been shown to be directly involved in mediating behaviors associated with the reinforcing and rewarding effects of psychostimulants. For example, cocaine is less potent as a reinforcer in M5 receptor-deficient mice as evidenced by reduced self-administration (Fink-Jensen et al., 2003; Thomsen et al., 2005). In a conditioned place preference procedure, which is considered a standard test for determining the rewarding properties of drugs, M5 receptor-deficient mice also spend significantly less time in a cocaine-paired compartment than wild-type control mice (Fink-Jensen et al., 2003). This is consistent with the finding that morphine-induced dopamine release in the NAc, measured using in vivo microdialysis, and conditioned place preferences are also reduced in M5 receptor-deficient mice (Basile et al., 2002). Moreover, the severity of withdrawal symptoms after chronic administration of either morphine (Basile et al., 2002) or cocaine (Fink-Jensen et al., 2003) is reduced in these mice.

Together, the above findings imply that mAChRs may have a general role in modulating behavioral and neurochemical correlates of both natural and drug reward, in addition to drug withdrawal. Importantly, when microinfused into the VTA of rats, scopolamine decreases the facilitatory effects of intravenous morphine on basal dopamine efflux in the NAc, recorded using in vivo chronoamperometry (Miller et al., 2005). This suggests that when combined with naltrexone and naloxone, the use of scopolamine as

an effective detoxification treatment protocol for heroin addicts (Yang et al., 1999) may in part derive from blockade of M5 mAChRs on dopamine neurons in the VTA. In light of evidence that polymorphisms within gene *CHRM5* that codes for the M5 mAChR have been associated with increased tobacco and cannabis use in humans, this gene may also represent a novel pharmacogenetic target for the treatment of drug addiction (Anney et al., 2007).

CONCLUSIONS

The present findings suggest that LDT cholinergic inputs to VTA dopaminergic neurons, via activation of excitatory mAChRs (likely of the M5 subtype), are involved in modulating the neurochemical effects of psychostimulants. Blockade of these receptors with scopolamine microinfused into the VTA either before or following systemic administration of cocaine, significantly attenuated cocaine-induced elevations in LDT stimulation-evoked dopamine efflux in the NAc. Together with the evidence that the M5 subtype is important in mediating cocaine-associated reinforcement and withdrawal, this suggests that the development of antagonists aimed at selectively disrupting M5 receptor function may be valuable in reducing abuse liability of psychostimulant drugs.

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