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Differential role of ventral tegmental area acetylcholine and N-Methyl-D-Aspartate receptors in cocaine-seeking

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Abstract

Exposure to drug-associated cues evokes drug-seeking behavior and is regarded as a major cause of relapse. Cues evoke burst firing of ventral tegmental area (VTA) dopamine (DA) neurons and phasic DA release in the nucleus accumbens (NAc). Cholinergic and glutamatergic input to the VTA is suggested to gate phasic DA activity. However, the role of VTA cholinergic and glutamatergic receptors in regulating phasic dopamine release and cue-induced drug-seeking in cocaine experienced subjects is not known. In male Sprague-Dawley rats, we found that VTA inactivation strongly inhibited, while VTA stimulation promoted, cocaine-seeking behavior during early withdrawal. Blockade of phasic activated D1 receptors in the NAc core also strongly inhibited cue-induced cocaine-seeking - suggesting an important role of phasic DA activity in the VTA to NAc core circuit. Next, we examined the role of VTA acetylcholine receptors (AChRs) and N-methyl-D-aspartate receptors (NMDARs) in regulating both NAc core phasic DA release and cue-induced cocaine-seeking. In cocaine naïve subjects, VTA infusion of the nicotinic acetylcholine receptor (AChR) antagonist mecamylamine, the muscarinic AChR antagonist scopolamine, or the NMDAR antagonist AP-5, led to robust attenuation of phasic DA release in the NAc core. During early cocaine withdrawal, VTA infusion of AP-5 had limited effects on NAc phasic DA release and cue-induced cocaine-seeking while VTA infusion of mecamylamine or scopolamine robustly inhibited both phasic DA release and cocaine-seeking. The results demonstrate that VTA AChRs, but not NMDARs, strongly regulate cue-induced cocaine-seeking and phasic DA release during early cocaine withdrawal.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords

Drug-seeking; NMDA; acetylcholine; phasic dopamine; ventral tegmental area; nucleus accumbens

1. Introduction

Exposure to drug-associated cues is a major precipitant of drug-relapse in humans (Rohsenow et al., 1990; Shaham and Hope, 2005; Shaham et al., 2003). Clinical studies of cue-induced cocaine craving suggest the involvement of dopamine (DA) neurotransmission within the mesolimbic DA system (Berger et al., 1996; Goldstein et al., 2009; Kranzler and Bauer, 1992; Potenza et al., 2012; Robbins et al., 1992; Volkow et al., 2010a; Volkow et al., 2006; Volkow et al., 2010b; Wang et al., 1999). Specifically, dopaminergic activity in the nucleus accumbens (NAc) is necessary and sufficient to drive reward-seeking in the presence of reward-predictive cues (Nicola et al., 2005). However, the underlying mechanisms that regulate DA activity and cue-induced drug-seeking behavior are poorly understood.

Recent findings have demonstrated that conditioned stimuli (CS) evoke time-locked burst firing of the ventral tegmental area (VTA) DA neurons and subsequent phasic DA release in the NAc (Ito et al., 2000; Kalivas and McFarland, 2003; Nicola et al., 2005; Schultz, 2007; See et al., 2001; Weiss et al., 2000). It has been proposed that CS-induced phasic DA release in NAc serves as a signal to modulate on-going behavior, as the NAc is known to integrate cortical and limbic input and to guide motor action (Kalivas and McFarland, 2003). Indeed, VTA-evoked phasic DA release in the NAc is sufficient to promote reward-seeking behavior (Adamantidis et al., 2011). Further, CS-evoked phasic DA signaling in the NAc diminishes and is eventually eliminated during extinction - an effect accompanied by a decline in goal-directed behavior (Owesson-White et al., 2008; Stuber et al., 2005). In contrast, cue-induced reinstatement of reward-seeking is accompanied by the return of CS-evoked DA transients to pre-extinction amplitudes (Owesson-White et al., 2008; Stuber et al., 2005). Together, these observations suggest a crucial role of VTA DA burst firing and subsequent NAc phasic DA release in cue-mediated reward-seeking behavior. Thus, understanding the mechanisms that regulate phasic DA release can provide novel insight of the underlying mechanisms that control CS-induced cocaine-seeking.

CS-evoked DA release and neuronal activity in the NAc depends upon VTA activity (Sombers et al., 2009; Yun et al., 2004). Burst firing of VTA DA neurons and subsequent NAc DA release is modulated by excitatory afferents from the pedunculopontine tegmental nucleus (PPTg) and the laterodorsal tegmental nucleus (LDTg), both implicated in processing CS-related sensory information to DA neurons (Lester et al., 2008; Lodge and Grace, 2006; Zweifel et al., 2009).

LDTg inputs to the VTA are thought to act as an essential gate that allows DA neurons to transition from tonic to burst firing (Kitai et al., 1999; Lodge and Grace, 2006; Maskos, 2008). Thus, LDTg cholinergic and glutamatergic input into the VTA, and therefore VTA AChRs and NMDARs, may play a critical role in regulating both phasic DA release and cue-induced drug-seeking.

While recent studies have begun to elucidate a role for VTA AChRs and NMDARs in modulating phasic DA release in a drug-naïve state (Blaha et al., 1996; Lester et al., 2008; Wickham et al., 2013, in press), the role of these VTA receptors in regulating phasic DA release during cocaine-withdrawal is unknown. Given the demonstrated alterations in VTA

NMDAR levels after cocaine self-administration and withdrawal (Ghasemzadeh et al., 2011; Hemby et al., 2005; Lu et al., 2003), it is likely that NMDAR regulation of phasic DA release is altered during cocaine withdrawal and such alterations could have a profound influence on cue-mediated behavior. While previous studies have also highlighted the ability of AChR mechanisms to mediate the pharmacological and reinforcing effects of cocaine (Fink-Jensen et al., 2003; Ranaldi and Woolverton, 2002; Rasmussen et al., 2000; Zachariou et al., 2001; Zanetti et al., 2006; Zanetti et al., 2007), the role of AChRs in cue-induced cocaine-seeking is not well understood. The aim of our study was to determine the role of the VTA AChRs and NMDARs in regulating NAc core phasic DA release and cue-induced cocaine-seeking behavior in cocaine-withdrawn rats. Our novel findings demonstrate that after cocaine self-administration and protracted abstinence 1) NMDAR regulation of phasic DA release is greatly diminished and 2) both phasic DA release and cue-induced cocaine-seeking are potently regulated by VTA AChRs, but not NMDARs.

2. Materials and Methods

2.1 Animals

Male Sprague Dawley rats (250-270 g; Charles River Laboratories, Wilmington, MA) (2 to 4 per cage prior to surgery and singly housed after surgery) were housed under a 12 hour light/dark cycle with ad libitum access to food and water. All experiments were conducted according to the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals and were approved by the Yale University Institutional Animal Care and Use Committee (IACUC). Throughout the experiments, animals were monitored daily and efforts were made to minimize animal pain, distress or discomfort, as specified by the Yale University IACUC approved protocol.

2.2 Drugs

All drugs were dissolved in sterile saline and delivered in a 0.5 μL volume over 1 min via micro-infusion pump and syringe (25 gauge, Hamilton, Reno, NE, USA). Lidocaine (Lid; 0.82 μg; Sigma Aldrich, St. Louis, MO, USA), mecamylamine (Mec; 3 or 30 μg; Sigma Aldrich, St. Louis, MS, USA), scopolamine (Scop; 3 or 67 μg; Sigma Aldrich, St. Louis, MS, USA) or AP-5 (0.1 or 1 μg; Sigma Aldrich, St. Louis, MS, USA) were locally infused into the VTA. Muscimol (Musc; 0.003 μg; Sigma Aldrich, St. Louis, MO, USA) and baclofen (Bacl; 0.06 μg; Sigma Aldrich, St. Louis, MO, USA) were delivered in a cocktail at 0.25 μL each (0.5 μL total volume of Musc+Bacl cocktail) into the LDTg. SCH23390 (SCH; 1 or 2 μg; Sigma Aldrich, St. Louis, MS, USA) was locally infused into the NAc core. The internal cannula extended 1 mm beyond the cannula to target either the VTA or NAc core or 2 mm to target the LDTg. Drug doses were chosen based on their ability to inactivate discrete brain regions (Lasseter et al., 2011) or to modulate phasic DA release in the NAc core (Sombers et al., 2009; Wickham et al., 2013, in press) and reward-related behavior (Yeomans and Baptista, 1997). The SCH doses were based on the ability of these doses to alter behavioral responding to reward-predictive cues (Calaminus and Hauber, 2007).

2.3 Cocaine self-administration

Rats were anesthetized with ketamine HCl (100 mg/kg, ip, Sigma Aldrich, USA) and xylazine (10 mg/kg, ip, Sigma Aldrich, USA) and implanted with a silastic catheter in the external jugular vein, as described by others (McFarland and Kalivas, 2001). Following catheter implantation, bilateral cannula (Plastics 1, Roanoke, VA, USA) were stereotaxically implanted above the VTA (AP −5.3 mm, ML ±0.5 mm, DV −7.0 mm from dura), LDTg (AP −8.6 mm, ML ±0.8 mm from Bregma, DV −4.6 mm from dura), or the NAc core (AP + 1.2 mm, ML + 1.5 mm, DV −6.5). Cocaine self-administration training started after 7 days of surgical recovery and was preceded by 2-3 days of food restriction to ~90% of free feeding

body levels. Rats were trained under fixed ratio 1 (FR1) schedule of reinforcement during which each active lever depression led to intravenous cocaine infusion (0.18 mg over a 6 s, ~0.5 mg/kg) and conditioned stimulus (CS) cue presentation (tone + stimulus light for 6s) in standard operant chambers, illuminated by a house light (Med Associate, St. Albans, USA). Inactive lever depressions had no programmed consequences. Each rat received 2 h daily training sessions for 8-10 consecutive days. Acquisition of stable self-administration behavior was defined as less than 15% variability in total active lever pressing over 3 consecutive days. Animals that did not acquire stable self-administration behavior were excluded from the study and were not tested for cue-induced cocaine-seeking.

2.4 Cue-induced cocaine-seeking

After cocaine self-administration training, rats underwent 3 days of forced abstinence in their home cages (Fig. 1A). On withdrawal day 3 (WD3) animals were placed in operant chambers for 2 h where active lever depression led to the CS presentation alone with no cocaine delivery. Inactive lever depressions had no programmed consequences. Cue-induced cocaine-seeking tests were performed on WD3 to avoid confounds from a potential extinction burst on WD1. Intra-VTA infusion of Lid, Mec, Scop or AP-5, intra-LDTg infusion of Musc+Bacl, or NAc core infusion of SCH was performed immediately prior to the cue-induced cocaine-seeking (Fig. 1B; Tab.1). In a separate experiment on WD10, the effects of intra-VTA Mec, Scop and AP-5 administration on locomotion were studied in the open field test (described below). To study the role of VTA-dependent phasic DA release in the NAc core in cocaine-seeking during abstinence, we applied phasic-like electrical stimulation to the VTA during the cocaine-seeking test and compared operant responding in cocaine-withdrawn rats vs. saline-withdrawn controls (described in detail in supplementary materials). Briefly, cocaine-withdrawn or saline-withdrawn rats with implanted VTA stimulation electrode were placed in the operant chamber for a 1 h session. During the session, phasic-like electrical stimulation of the VTA (125 - 150 μA, 24 pulses at 60Hz) was applied every 120s. During the session, every active lever press led to CS presentation in the absence of cocaine delivery. Inactive lever depressions had no programmed consequences. In this experiment, Saline-withdrawn rats served as the control group

2.5 Locomotor activity

On WD10, all rats from the cue-induced cocaine-seeking studies were tested for locomotor activity after intra-VTA drug micro-infusion. In addition, rats that were never trained in cocaine self-administration (due to non-patent catheters) or rats that did not meet criteria for cocaine self-administration were also tested in the open field test as additional control subjects that were VTA drug naive. Immediately after intra-VTA Mec, Scop or AP-5 micro-infusion rats were placed in the middle of a brightly lit open field apparatus (black Plexiglass 40 × 40 cm arena with 60cm high opaque Plexiglass walls). Velocity and distance travelled were assessed using video recoring and the Ethovision analysis program (Noldus Inc, USA). There were no statistical differences in locomotor activity (both before and after drug treatment) between rats previously tested in cue-induced cocaine-seeking and rats that were never trained or did not finished training for cocaine self-administration (data not shown), therefore locomotor activity data after intra-VTA drug administration was collapsed and analyzed irrespective of previous cocaine exposure.

2.6 Fast-scan cyclic voltammetry (FSCV)

FSCV recording was performed on WD3 in rats that had previously self-administered cocaine and in cocaine-naïve age-matched controls (Fig. 1A, C). Rats were anesthetized with urethane (1.5 g/kg, ip.) and placed in a stereotaxic frame equipped with a heating pad (Harvard Apparatus, Holliston, MA, USA). A bipolar, stainless-steel stimulating electrode, coupled with a 26 gauge infusion cannula (Plastics One, Roanoke, VA, USA), was placed

into the VTA (AP −5.2 mm, ML +0.5 to −1.5 mm, DV between −7.4 and −8.1 mm), a carbon-fiber microelectrode (described in the supplementary text) was implanted in the NAc core (AP +1.2 mm, ML −1.4 mm, DV from −6.2 to 6.9 mm) and a Ag/Ag Cl reference electrode was placed in contralateral hemisphere (Fig. 1C). A low-pass filtered (2 kHz) triangular waveform (−0.4 to +1.3 V and back to −0.4 V at a rate of 400 V/s, repeated at 100 ms intervals) was applied to the carbon fiber microelectrode. Data was digitized and processed using NI-6711 and NI-6251 DAC/ADC cards (National Instruments, Austin, TX, USA) and Demon Voltammetry and Analysis Software (Wake Forest Baptist Medical Center, Winston-Salem, NC) or Tarheel CV software (ESA, Chelmsford, MA, USA). Background-subtracted cyclic voltammograms (CVs) were obtained by digitally subtracting stable background currents to resolve CVs for dopamine. Electrical pulses delivered to the VTA were computer-generated with a 6711 PCI card (National Instruments, Austin, TX, USA) and were optically-isolated from the electrochemical system (NL 800A, Neurolog, Digitimer Ltd, Hertfordshire, UK). Electrical stimulation consisted of biphasic square wave pulses (300 μA, 24 pulses with a 4ms pulse width, applied at 60Hz). For each 15 sec FSCV collection, electrical stimulation of the VTA was applied 5 sec after the initiation of the FSCV recording session. VTA stimulation and simultaneous FSCV recordings, at the carbon-fiber microelectrode in the NAc, were performed with a 3 min wait time between each session to prevent the depletion of readily releasable stores and to allow sufficient time for releasable stores to return to baseline. Once VTA-stimulated DA was detected in the NAc core, at least 6 baseline VTA stimulation and FSCV recording samples were collected to ensure the stability of stimulated DA release. We considered the evoked DA signal to be stable when peak evoked DA concentrations varied by less than 10% over a 15 min period. Immediately after the last baseline FSCV recording, an internal cannula was inserted into the VTA cannula to infuse drug at the site of VTA electrical stimulation. Drug was micro-infused into the VTA (see Table 2) at a rate of 0.5 μL/min for 1 min and the internal cannula was left in place for an additional 1 min to allow for complete drug absorption. Then, the internal cannula was removed and post-drug VTA-stimulation and FSCV recoding files were collected at 3 min intervals (Fig 1C).

2.7 Histology

At the completion of behavioral experiments, 0.5 μl of Chicago Blue Dye was infused into the LDTg, VTA or NAc core of the anesthetized rat. Rats were then sacrificed and brains were removed from the skull, frozen in chilled isopentane and stored at −80°C. Brains were coronally sectioned into 100-1000 μm thick slices with a 1 mm brain matrix or by hand. Sections were mounted on slides and viewed under a light microscope to verify cannula placements. All data from subjects with misplaced cannula were removed from the analysis (Table S1). Representative cannula placements are shown in Fig. 6.

After the FSCV experiments, rats were sacrificed and brains were removed from the skull and stored in 10% formaldehyde. Brains were coronally sectioned into 40–50 μm slices with a cryostat (Leica Microsystems, Buffalo Grove, IL, USA). The sections were mounted on slides and viewed under a light microscope to verify working electrode placements. Representative cannula placements for micro-infusion experiments are shown in Fig 6A-C. Representative working electrode placements from FSCV experiments are shown in Fig. 6D.

2.8 Statistics

The effects of intra-VTA, intra-LDTg or intra-NAc core drug administration on total lever presses during the 2 h cue-induced cocaine-seeking test were analyzed using two-way ANOVA. If the two-way ANOVA revealed a significant effect, a Newman Keuls post-hoc comparison was performed. For analysis of drug infusion effects on lever pressing or locomotor activity over time, a two-way repeated measures ANOVA was performed. If the

two-way repeated measures ANOVA revealed a significant main effect or a significant interaction, a subsequent Newman Keuls post-hoc analysis of discrete time points was performed. For locomotor activity, time-dependent analysis was measured in 5 min bins over the entire 2 h session. For all tests (active lever responses, inactive lever responses, and locomotor activity), there were no significant differences between treatment groups after the initial 60 minutes of the session, therefore time course data is reported for only first hour.

Data obtained with FSCV in anesthetized rats is presented as the effects of intra-VTA saline or drug infusion on the peak of stimulated phasic dopamine release ([DA_{max}]). We used Demon Voltammetry for data collection and analysis. Saline and drug data is normalized [DA_{max}] reponse in the 15 minute baseline period directly preceding the infusion. We considered the evoked dopamine signal to be stable when the variation over the 15 minute period was less than 10%. Time-dependent intra-VTA drug administration effects on phasic DA release in the NAc core were analyzed using two-way ANOVA with repeated measures with Newman Keuls post hoc comparison. $P<0.05$ was considered statistically significant for all tests.

3. Results

3.1 VTA activity regulates cue-induced cocaine-seeking during abstinence

To study the role VTA activity in cue-induced cocaine-seeking we used local lidocaine micro-infusion to inactivate both cell bodies and projecting/passing fibers in the VTA. Inactivation of the VTA (n=6) attenuated cue-induced cocaine seeking, as shown by selective attenuation of active, but not inactive, lever pressing in Lid treated animals over the 2 h session (Fig. 2A; treatment × lever: $F_{(1, 20)}=9.9$, $p<0.01$, post hoc test $p<0.001$). These effects were not due to previous differences during training, as “to be saline” (n=6) and “to be Lid” (n=6) subjects showed no significant between-group differences during cocaine self-administration training (Fig. S1A). However, closer examination of Lid effects over time revealed that intra-VTA Lid administration significantly attenuated active and inactive lever pressing during the first 20 min of the WD3 test (Fig.S2A, B; treatment × time: $F_{(23, 460)}=2.7$, $p<0.001$, post hoc test $p<0.05$). Thus, to determine the potential causal link between VTA phasic activity (which evokes phasic DA release in the NAc (Fig 4D, (Phillips et al., 2003)) and cocaine-seeking during abstinence, we examined the effects of VTA phasic-like stimulation on cocaine-seeking in withdrawn subjects. The data revealed that phasic VTA stimulation led to a time-locked increase in active lever pressing in cocaine trained subjects (Fig. 2B; $F_{(5, 47)}=2.7$, $p<0.05$, post hoc test; $p<0.05$), but not saline trained rats (Fig. S4H; n.s.). Importantly, the observed increase in responding was selective for the active, but not inactive, lever responding in cocaine rats (Fig. S4I, stim × drug × lever interaction: $F_{(1, 20)}=4.4$, $p<0.05$, post hoc test $p<0.05$). Thus, phasic-like stimulation of the VTA promoted cocaine-seeking in cocaine withdrawn subjects.

The LDTg sends excitatory projections to the VTA that regulate phasic DA signaling in NAc (Lammel et al., 2012). Inactivation of the LDTg with Musc + Bacl (n=6) attenuated both active and inactive lever responding during the 2 h session in cocaine-abstinent rats on WD3 (Fig. 2C; treatment: $F_{(1, 26)}=11.9$, $p<0.01$, post hoc test $p<0.01$; lever: $F_{(1, 26)}=36.4$, $p<0.001$, post hoc test $p<0.001$). Examination of 5 min epochs during the 2 h session revealed that Musc + Bacl significantly attenuated active and inactive responding during first 10 min (Fig. S2C, D; treatment × time: $F_{(23, 621)}=1.9$, $p<0.01$, post hoc test $p<0.001$). Importantly, the observed intra-LDTg Musc + Bacl effects on cue-induced cocaine seeking were not due to pre-existing differences during cocaine self-administration training, since “to be saline” subjects (n=9) and “to be Musc + Bacl” animals showed no significant between-group differences during training (Fig. S1B). However, given that LDTg inactivation led to a non-specific decrease in active and inactive lever pressing during cue-

induced cocaine-seeking, subsequent experiments with pharmacological inhibition of VTA AChRs and NMDARs was used as an alternative method to determine the potential role that LDTg inputs may exert by acting at these receptors in the VTA.

3.2 Intra-NAc core infusion of a D_1R antagonist attenuates cue-induced cocaine-seeking

SCH (1 or 2 μg) administration into the NAc core selectively attenuated cue-induced cocaine-seeking, as reflected by a decrease in active lever presses (Fig. 2G; dose × lever: $F_{(1, 34)}$=4,5, $p<0.05$, post hoc test $p<0.01$) and no significant effect on inactive lever responding. Time-dependent analysis of cue-induced cocaine-seeking revealed that 1 μg SCH attenuated active but not inactive lever responding during the initial 15 min, whereas 2 μg SCH attenuate active lever responding during first 10 min of the session (Fig. S2E, F; dose × time × lever: $F_{(46, 782)}$=5.5, $p<0.05$, post hoc test $p<0.01$). During cocaine self-administration training, there were no significant differences between “to be saline” and “to be SCH” groups (Fig. S1C).

3.3 Blockade of VTA nAChRs, mAChRs or NMDARs attenuates cue-induced cocaine seeking

Intra-VTA administration of Mec attenuated cue-induced cocaine seeking in a dose-dependent manner (Fig. 3A; dose × lever: $F_{(2, 52)}$=7.9, $p<0.001$, post hoc test $p<0.001$), with the highest Mec dose (30 μg/side) effectively abolishing cocaine-seeking ($p<0.001$). Examination of Mec effects over time revealed attenuated operant responding on both active and inactive levers during first 10 min after Mec 30 μg (Fig. S3A, B; dose × time: $F_{(46, 1104)}$=1.6, $p<0.01$, post hoc test $p<0.001$) and 5 min after Mec 3 μg micro-infusion ($p<0.05$). Intra-VTA administration of Scop attenuated both active and inactive lever responding in cocaine-withdrawn rats on WD3 in dose-response manner (Fig. 3B; dose: $F_{(2, 48)}$=4.3, $p<0.05$, post hoc test $p<0.01$; lever: $F_{(1, 48)}$=35.3, $p<0.001$, post hoc test $p<0.001$; dose × lever: n.s.). Closer examination of cue-induced cocaine-seeking revealed that 67 μg Scop micro-infusion attenuated operant responding during first 10 min (Fig. S3C, D; dose × time: $F_{(46, 828)}$=2.4, $p<0.001$, post hoc test $p<0.001$), whereas 3 μg Scop micro-infusion attenuated responding during the first 5 min of the session ($p<0.05$). Intra-VTA administration AP-5 at either dose did not attenuate cue-induced cocaine seeking assessed over 2 h (Fig. 3C; n.s.). However, time-dependent analysis revealed that 1 μg AP-5 attenuated active lever responding during the first 5 min (Fig. S3E,F; dose × lever × time: $F_{(46, 1104)}$=1.4, $p<0.05$, post hoc test $p<0.001$). Importantly, during cocaine self-administration training in all experiments, the number of active lever presses in “to be Sal” subjects did not statistically differ from “to be drug” subjects (Fig. S1D, Mec; Fig. S1E, Scop, Fig. S1F, AP-5). To control for potential non-specific effects associated with VTA drug-infusion, locomotor activity was subsequently measured on WD10 following VTA infusion. The data showed that intra-VTA administration of Mec, Scop or AP-5 had no significant effect on distance traveled (Fig. 4A, B; no effect of drug: $F_{(3.27)}$=1.1, p=0.3, main effect of time: $F_{(23, 621)}$=10.2, $p<0.0001$, drug × time: $F_{(69, 621)}$=0.9, p=0.5), or velocity (Fig. 4C, D; no effect of drug: $F_{(3.27)}$=1.1, p=0.3; main effect of time: $F_{(23, 621)}$=207.4, $p<0.0001$, drug × time: $F_{(69, 621)}$=0.7, p=0.9)) measured during 2h open field test.

3.4 Differential effects of VTA nAChR, mAChR and NMDAR blockade on phasic DA release in the NAc core after cocaine self-administration and withdrawal

Intra-VTA administration of Mec (n=6), Scop (n=5) or AP-5 (n=6) in doses that attenuated cue-induced cocaine-seeking on WD3 (30 μg, 67 μg and 1 μg, respectively) decreased DA_{max} in the NAc core of cocaine-naïve rats (Fig. 5A; drug: $F_{(3.20)}$=13.2, $p<0.001$, post hoc test $p<0.01$). The VTA drugs effects on DA_{max} in the NAc core remained attenuated during entire 120 min (n.s. time and drug × time factors). Intra-VTA administration of Sal (n=7) had no effect on stimulated phasic DA release when compared to pre-infusion phasic DA

release, whereas intra-VTA Mec, Scop or AP-5 decreased DA release in the NAc core 40-50% (Fig. 5B; representative traces).

Interestingly, in cocaine-withdrawn rats, intra VTA AP-5 administration did not alter phasic DA release over a 2 h period when compared to the saline treated control group (Fig. 5C; drug: $F_{(3.663)}$=6.9, p<0.001, post hoc test n.s.). In contrast, intra-VTA Mec or Scop in cocaine withdrawn rats decreased phasic DA release (p<0.01) similar to what was observed in cocaine-naïve rats (Fig 5A, C). We also observed a significant effect of time, as revealed by a main effect of time irrespective of the intra-VTA drug treatment in cocaine-withdrawn rats (time: $F_{(39.663)}$=2.5, p<0.001, post hoc test p<0.05 for 3 vs. 78 min, and 3 vs. 108 min). In order to directly compare intra-VTA drug effects on phasic DA release in cocaine naïve and cocaine-withdrawn rats, we binned data over the first 15 min, where intra-VTA Mec, Scop and AP-5 had the most robust effects on cue-induced cocaine-seeking (Fig. S2). While intra-VTA infusion of Mec, Scop or AP-5 all attenuated phasic DA release similarly in cocaine-naive rats, only Mec and Scop attenuated DA release in cocaine-withdrawn rats (Fig. 5E; drug × cocaine treatment $F_{(3.41)}$=3.1, p<0.05, post hoc test p<0.01). In contrast, intra-VTA AP-5 micro-infusion had no effect on electrically-evoked phasic DA release in NAc core in cocaine-withdrawn rats (n.s.). This lack of an AP-5 effect could not be attributed to pre-existing differences in cocaine self-administration since “to be AP-5” subjects trained similarly to all other groups in cocaine self-administration (Fig 5F).

4. Discussion

Here, our data shows that activity in the VTA to NAc core circuit, via regulation of phasic DA release, plays an important role in cue-induced cocaine-seeking. We also find that cue-induced cocaine-seeking and phasic DA signaling after cocaine self-administration and subsequent abstinence is potently regulated by VTA AChRs, but not NMDARs. Specifically, contingent cocaine exposure followed by early withdrawal decreases the ability of VTA NMDAR manipulation to alter phasic DA release in NAc, revealing a diminished role of NMDARs in regulating phasic DA during early cocaine withdrawal.

Dopaminergic activity in the VTA to NAc circuit is necessary and sufficient to drive cue-induced reward-seeking in rats (Nicola et al., 2005) and CS-evoked burst firing of VTA DA neurons and the subsequent phasic DA release in the NAc is proposed to underlie reward-seeking (Day et al., 2007; Nicola et al., 2005; Phillips et al., 2003). Mechanistically, VTA DA burst firing and NAc phasic DA release are regulated by VTA ACh and glutamate input from the LDTg (Lester et al., 2008; Lodge and Grace, 2006) and recent work suggests a critical role of the LDTg to VTA circuit in reward-related behavior (Lammel et al., 2012). Indeed, selective optogenetic stimulation of LDTg inputs into the VTA is sufficient to induce a conditioned place preference through downstream actions at phasic activated D_1 receptors in the NAc (Lammel et al., 2012). Phasic DA release preferentially activates low affinity D_1R receptors, leading to the activation of the direct pathway of the basal ganglia (Goto and Grace, 2005). Consistent with a role of phasic activated D_1 receptors in the NAc, we found that intra-NAc core infusion of SCH, to block D_1Rs, abolished cue-induced cocaine-seeking. Even though phasic DA release preferentially activates D_1 receptors (Goto and Grace, 2005), our findings do not eliminate the possibility that D_2 receptor mechanism in the NAc may also contribute to cue-induced cocaine-seeking. Thus, future work will further delineate whether the ability of phasic DA activity to promote cocaine-seeking is mediated by NAc core D_1 receptors, D_2 receptors or both. Importantly, despite the evidence for LDTg regulation of phasic DA activity in the VTA and NAc (Lester et al., 2008; Lodge and Grace, 2006), the potential role of LDTg mechanisms in cue-induced cocaine-seeking had not been previously examined. Here, we find that transient inactivation of the LDTg decreases both active and inactive lever responding during cue-induced cocaine-seeking,

revealing a non-specific effect of global LDTg inactivation. However, it is possible that cell-specific projections from the LDTg to VTA (either glutamatergic or cholinergic) may play a more specific role in cue-induced cocaine-seeking behavior during withdrawal.

The majority of the neurons in the LDTg and the majority of the LDTg projections to the VTA are glutamatergic (Lammel et al., 2012), suggesting that LDTg projections could alter DA burst firing through actions at glutamate receptors in the VTA. Importantly, VTA DA burst firing is dependent on NMDAR, and not AMPA receptor, activity (Chergui et al., 1993; Overton and Clark, 1992; Suaud-Chagny et al., 1992). Furthermore, genetic and FSCV studies point to VTA NMDARs as potent modulators of both phasic DA signaling and cue-mediated behavior (Sombers et al., 2009; Zweifel et al., 2011; Zweifel et al., 2009). Previous studies have also demonstrated that both acute cocaine and chronic contingent cocaine-taking induce persistent neuroadaptations in the VTA, as evidenced by an increased AMPA/NMDA ratio, enhanced DA excitability, and altered expression of NMDARs after early and prolonged withdrawal (Chen et al., 2008; Ghasemzadeh et al., 2011; Lu et al., 2003; Luscher and Malenka, 2011; Stuber et al., 2010; Ungless et al., 2001). However, the consequence of cocaine-induced neuroadaptations on VTA NMDAR-dependent phasic DA signaling was not known. Our data revealed that intra-VTA micro-infusion of AP-5, despite having long-lasting effects on phasic DA release in cocaine-naïve rats, had minimal effects on phasic DA release in cocaine-withdrawn rats. We also observed a transient AP-5 effect on cue-induced cocaine-seeking on WD3. We propose that VTA NMDAR-dependent regulation of phasic DA release and cue-induced cocaine-seeking is diminished due to VTA neuroadaptations during early cocaine withdrawal. Consistent with this interpretation, previous studies showed that extended-access cocaine self-administration and subsequent protracted abstinence led to decreased post-synaptic NMDAR NR1 expression in the VTA (Ghasemzadeh et al., 2011), while acute VTA AP-5 infusion did not alter cue-induced reinstatement following 7 day extinction (Mahler et al., 2012). This diminished role of NMDARs does not eliminate the possibility that AMPARs, or mGluRs, may alter NAc phasic DA release during cocaine-withdrawal. Indeed, both AMPAR and mGluR antagonists have been demonstrated to attenuate cue-induced cocaine-seeking after extinction training (Kumaresan et al., 2009; Mahler et al., 2013). Thus, future studies will examine whether these behavioral effects are mediated by AMPAR or mGluR regulation of NAc phasic DA release. In contrast to the lack of an NMDAR effect, our data revealed that VTA AChR regulation of phasic DA and cocaine-seeking is maintained during early withdrawal.

Previous clinical studies showed that mecamylamine decreased, whereas nicotine augmented, cue-induced cocaine craving in addicted individuals (Reid et al., 1999; Reid et al., 1998). However, the critical CNS locus for AChR regulation of cue-induced cocaine craving had not been previously identified. Our data point to VTA nAChRs as key targets that regulate cue-induced cocaine-seeking. Here we show that intra-VTA micro-infusion of the nonselective nAChR antagonist mecamylamine attenuated NAc phasic DA release and abolished cue-induced cocaine-seeking. Importantly, this inhibition of cue-induced cocaine-seeking was observed in the absence of mecamylamine effects on locomotor activity. Consistent with this suggested role of VTA nAChR regulation of cue-induced drug-seeking, previous work has shown that VTA nAChR blockade attenuates the reinforcing properties of ethanol cues (Lof et al., 2007). In addition, increasing VTA cholinergic tone increases cue-induced heroin-seeking in rats (Zhou et al., 2007). We propose that VTA nAChRs regulate behavioral responses to drug-associated CSs through the regulation of downstream phasic DA release. Indeed, our FSCV experiments demonstrated that intra-VTA mecamylamine micro-infusion in both cocaine naïve and cocaine-withdrawn rats potently attenuated NAc core phasic DA release. Mechanistically, AChRs on axon terminals and DA cell bodies in the VTA are well-positioned to modulate DA release in the NAc (Maskos, 2008).

Accordingly, intra-VTA micro-infusion of scopolamine also attenuated phasic DA release in the NAc core of both cocaine naïve and cocaine-withdrawn rats. Our FSCV results are consistent with previous findings showing the involvement of the VTA nAChRs and mAChRs in phasic DA release in cocaine-naïve subjects (Blaha et al., 1996) and extend these findings to the cocaine-withdrawn state. Importantly, scopolamine effects on cue-induced cocaine-seeking and locomotor activity are similar to the effects of mecamylamine, further supporting the hypothesis that VTA cholinergic neurotransmission that regulates phasic DA signaling underlies cue-induced cocaine-seeking.

In conclusion, our results demonstrate a novel functional dissociation between VTA AChRs and NMDARs and their ability to regulate phasic DA release and cue-induced cocaine-seeking during early withdrawal. In addition, contingent cocaine-taking followed by short abstinence induces functional changes in the VTA receptor mechanisms that regulate phasic DA release and these neuroadaptations may play an important role in compulsive cocaine-seeking. We propose that salient, cocaine-paired cues evoke VTA DA neuronal activity and subsequent phasic DA release in the NAc core that drives cocaine-seeking during abstinence. We further propose that VTA ACh activity may serve as a functional gate that allows cues to evoke phasic DA release and drive drug-seeking behavior. Together, our data provide new insight of the cholinergic mechanisms that underlie cue-induced cocaine-seeking behavior.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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- We examined cue-induced cocaine-seeking in rats after 3 days of forced abstinence
- VTA NMDARs and AChRs regulate phasic dopamine release in cocaine naïve rats
- Cocaine withdrawal limits VTA NMDAR regulation of phasic dopamine release
- VTA AChRs regulate cocaine-seeking and phasic dopamine during early withdrawal

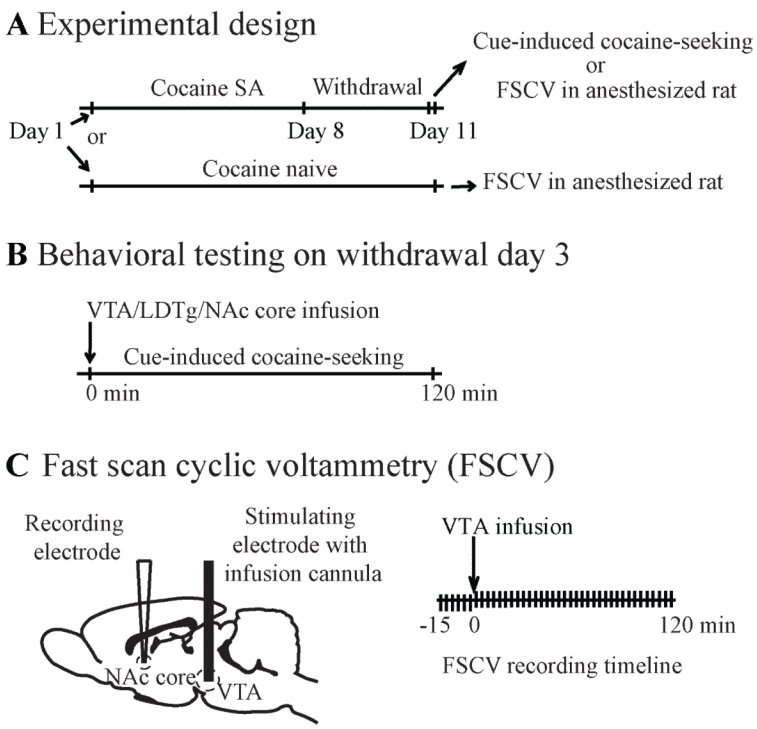

Fig. 1.

Time-line of behavioral and electrochemical experiments. (A) Rats were trained to self-administer cocaine (~0.5mg/kg/inf) for 8 days, after which they underwent forced abstinence for 3 days. (B) On withdrawal day 3 (WD3), rats were tested for cue-induced cocaine-seeking during which active lever depression led to presentation of the cocaine-paired cue alone with no cocaine delivery. Intra-LDTg, VTA or NAc core drug micro-infusions were performed immediately prior to behavioral testing. (C) Fast scan cyclic voltammetry was performed in the NAc core of cocaine-naïve or cocaine-withdrawn, anesthetized rats. NAc phasic dopamine release, evoked by electrical VTA stimulation (300 μA, 60Hz, 24 pulses with a 4 ms pulse width, delivered every 3 min) was measured before (baseline) and after (drug) VTA micro-infusion of saline, mecamylamine, scopolamine or AP-5. The pre-drug baseline period consisted of NAc phasic DA recordings performed over a 15 min period. Sagittal brain images adopted from Paxinos and Watson (2007).

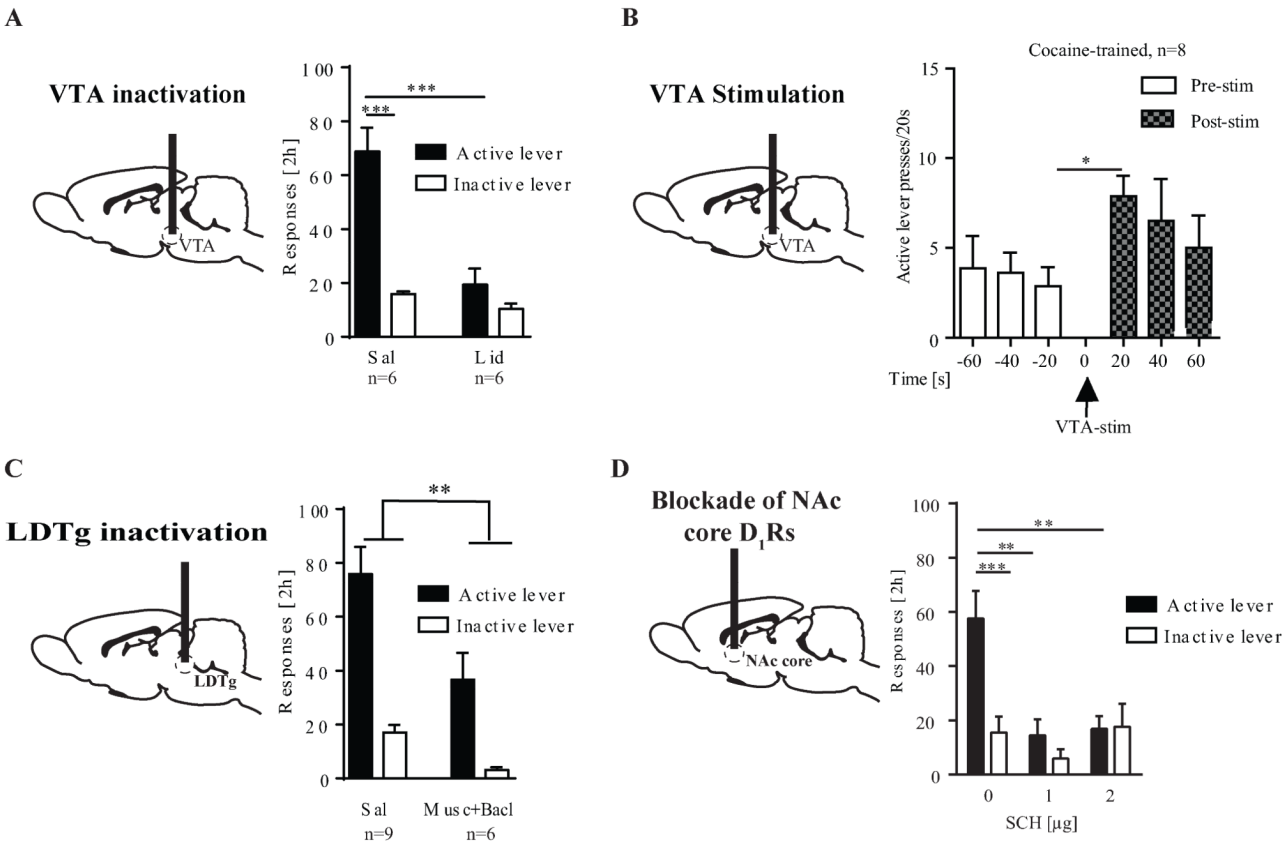

Fig. 2.

The VTA to NAc core circuitry regulates cue-induced cocaine-seeking in cocaine withdrawn rats. (A) Intra-VTA micro-infusion of lidocaine (Lid; 0.82 μg/side) but not saline (Sal) abolished cue-induced cocaine-seeking in abstinent rats (two-way ANOVA: treatment × lever: $p<0.01$). In contrast, (B) phasic stimulation of the VTA (150 μA, 60Hz, 24 pulses electrical stimulation), which leads to phasic DA release in the nucleus accumbens, facilitated cue-induced cocaine-seeking in first 20 s epoch after stimulation in cocaine-trained, abstinent rats (one way ANOVA: $p < 0.05$). (C). Intra-LDTg micro-infusion of a muscimol and baclofen cocktail (0.003 μg/side Musc + 0.06 μg/side Bacl) but not saline (Sal) attenuated cue-induced cocaine-seeking on WD3 (two-way ANOVA: treatment: $p<0.01$). (D) Intra-NAc core micro-infusion of SCH23390 (SCH, 0, 1, 2 μg/side) attenuated cue-induced cocaine-seeking (two-way ANOVA: treatment × lever: $p<0.05$). Data presented as the mean + SEM. *** $p<0.001$; ** $p<0.01$, * $p<0.05$.

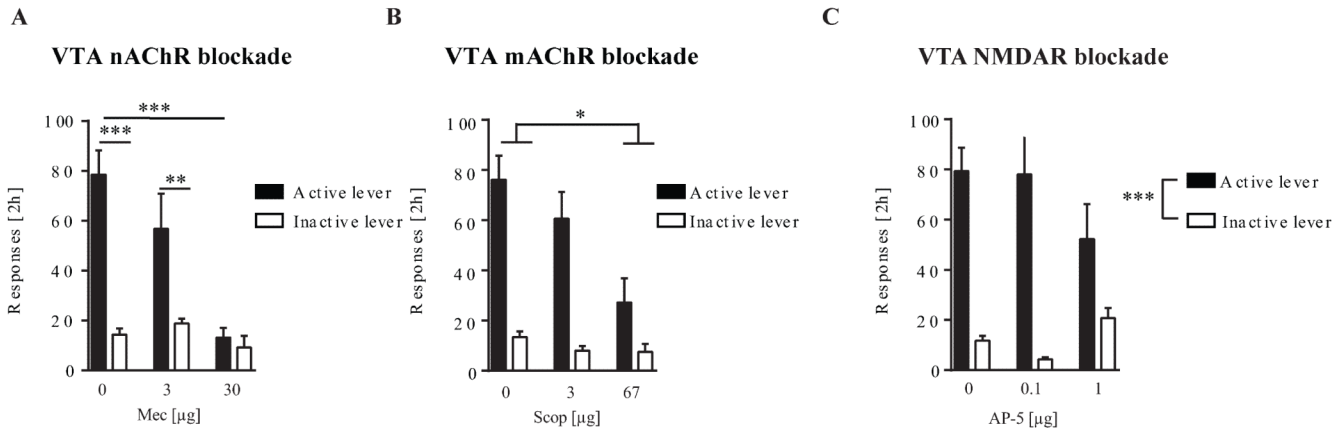

Fig.3.

VTA AChRs, but not NMDARs, are potent modulators of cue-induced cocaine-seeking. (A) Intra-VTA micro-infusion of the nAChR antagonist mecamylamine (Mec; 0, 3, 30 μg/side) abolished cue-induced cocaine-seeking in cocaine-withdrawn rats in a dose-dependent manner (two-way ANOVA: dose × lever: $p<0.001$). (B) Intra-VTA micro-infusion of mAChR antagonist scopolamine (Scop; 0, 3, 30 μg/side) attenuated cue-induced cocaine-seeking in dose-response manner (two-way ANOVA: dose: $p<0.05$; lever: $p<0.001$). (C) Intra-VTA micro-infusion of NMDAR antagonist AP-5 (0, 0.1, 1 μg/side) had no effect on cue-induced cocaine-seeking when measured over 120 min of testing (two-way ANOVA: dose n.s.). Data presented as the mean + SEM. *** $p<0.001$, ** $p<0.01$, * $p<0.05$, Newman Keuls post hoc test.

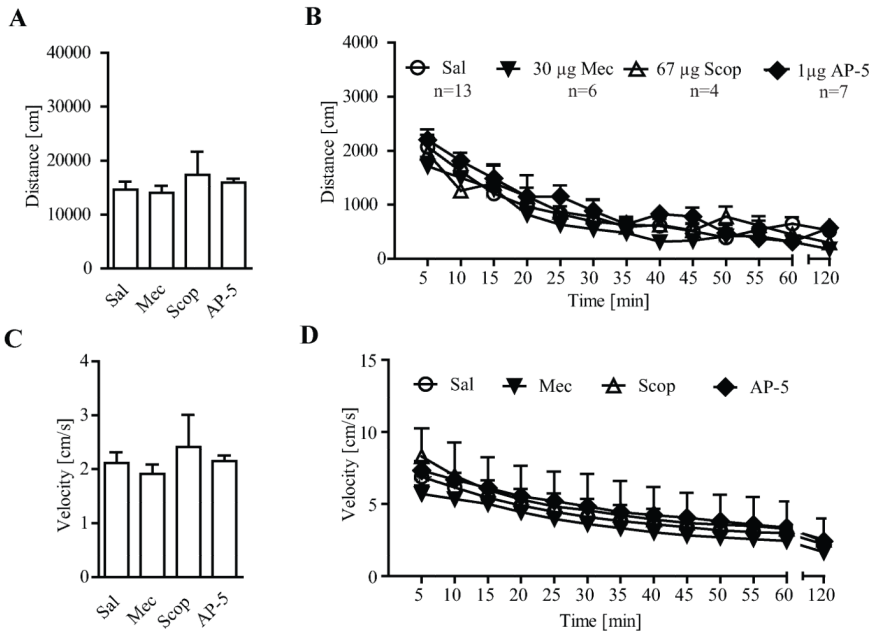

Fig. 4.

Locomotor activity after VTA infusion of nAChR, mAChR or NMDAR antagonists in doses that attenuated cue-induced cocaine-seeking on WD3. Intra-VTA micro-infusion of the nAChR antagonist mecamylamine (Mec; 30 μg/side), mAChR antagonist scopolamine (Scop; 67 μg/side) or NMDAR antagonist AP-5 (1 μg/side) in comparison to saline (Sal) led to no significant differences on locomotor activity as measured by distance traveled (A, B; drug: $F_{(3.27)}$=1.1, p=0.3; time: $F_{(23, 621)}$=10.2, p<0.0001, drug × time: $F_{(69, 621)}$=0.9, p=0.5) or velocity (C, D; drug: $F_{(3.27)}$=1.1, p=0.3; time: $F_{(23, 621)}$=207.4, p<0.0001, drug × time: $F_{(69, 621)}$=0.7, p=0.9) during 2h of the open field test. Data presented as the mean + SEM.

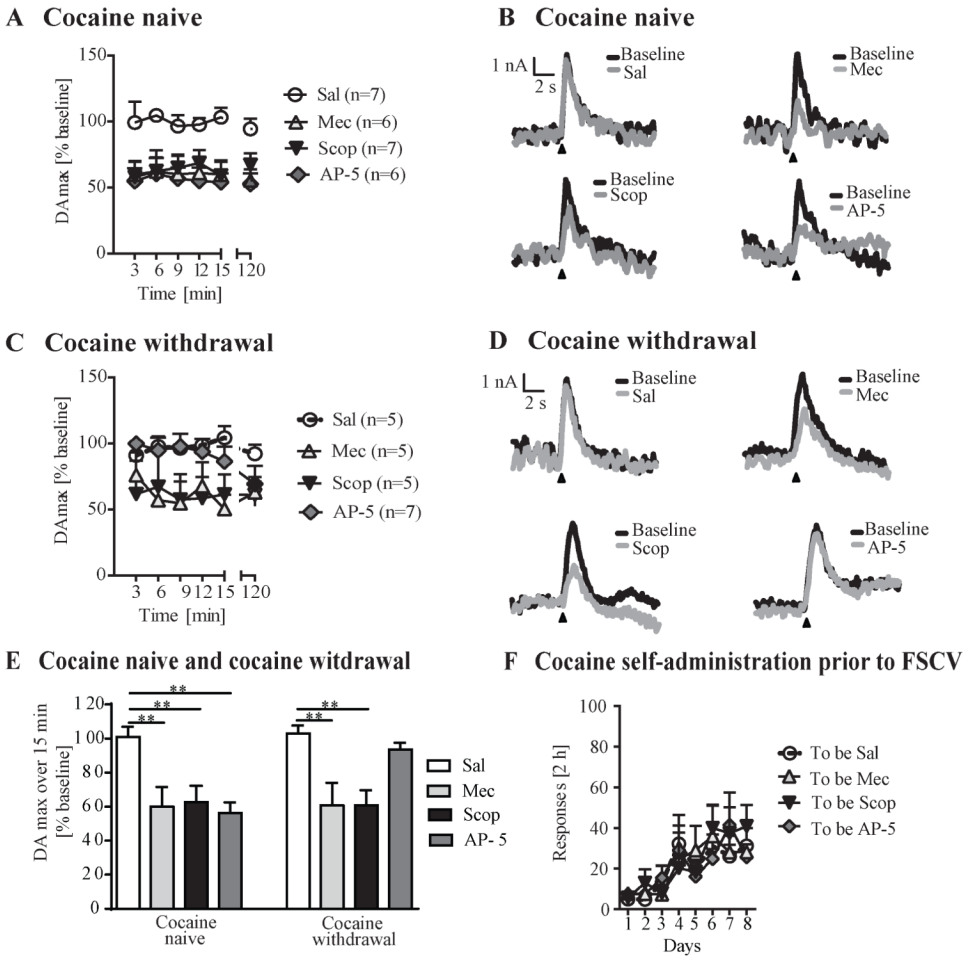

Fig. 5.

VTA AChRs, but not NMDARs, are potent modulators of phasic DA release in the NA core after cocaine self-administration and 3 day withdrawal as measured by fast scan cyclic voltammetry (FSCV). (A) Time course of DA_{max} response in the NAc core after saline (Sal), mecamylamine (Mec, 30 μg), scopolamine (Scop, 67 μg) and AP-5 (1 μg) intra-VTA micro-infusion in cocaine-naïve rats. Two-way ANOVA with repeated measures revealed a significant drug treatment factor ($p<0.001$). Data are presented as the mean + SEM of the peak of stimulated phasic dopamine release (DA_{max}) and are shown for initial 15 min of the FSCV recording. There were no differences between experimental groups during second hour of testing. Saline and drug data was normalized to the baseline evoked dopamine signal obtained during a 15 min period directly preceding the infusion. (B) Representative DA traces vs. time plots during the baseline period (black) and after intra-VTA drug micro-infusion (grey) in cocaine-naïve rats. The triangle indicates the time of VTA stimulation. (C) Time course of DA_{max} response in the NAc core after intra-VTA micro-infusion (Sal; 30 μg Mec; 67 μg Scop; or 1 μg AP-5) in rats following cocaine-self administration training and 3 day withdrawal. Two-way ANOVA with repeated measures revealed a significant effect of drug treatment ($p<0.001$) and post hoc comparison showed significant attenuation of phasic DA release by Mec and Scop ($p<0.01$), but not AP-5 (n.s.). (D) Representative DA vs. time traces during the baseline period (black) and after intra-VTA drug micro-infusions (grey) in cocaine-withdrawn rats. (E) Comparison of VTA receptor regulation of phasic DA release (DA_{max}) in the NAc core in the 15 min period immediately following infusion in cocaine-

naïve versus cocaine-withdrawn rats. (F) Cocaine self-administration in rats subsequently tested in fast scan cyclic voltammetry on WD3 revealed no significant between-group differences self-administration training. During the FSCV test on WD3, rats from “to be sal” group received intra-VTA microinfusion of saline, whereas to be Mec, Scop and AP-5 subjects received mecamylamine (Mec, 30 μg), scopolamine (Scop, 67 μg) and AP-5 (1 μg), respectively. ** $p<0.01$, Newman Keuls post hoc test.

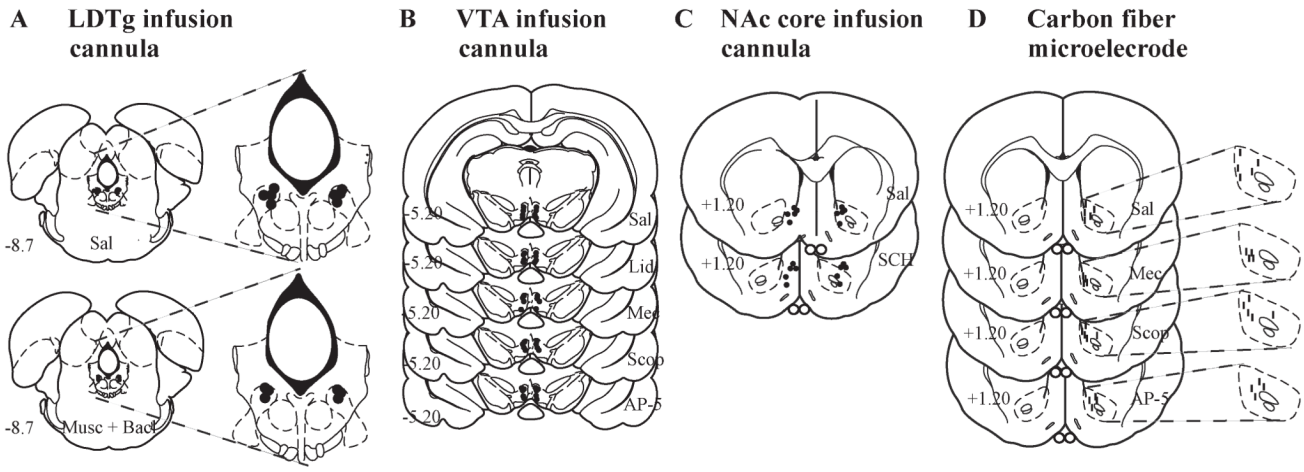

Fig. 6.

Representative (A) LDTg, (B) VTA and (C) NAc core micro-infusion cannula placements as well as (D) NAc core carbon fiber placements (Adopted from (Paxinos and Watson, 2007)). All coordinates for the VTA and NAc core were obtained from the rat brain atlas by Paxinos and Watson, 2007.

Table 1

Behavioral testing. VTA, LDTg, and NAc core drug micro-infusion were performed on withdrawal day 3 (WD3) in rats previously trained in cocaine-self administration. In addition, the effects of phasic stimulation of the VTA were evaluated in cocaine-withdrawn rats (denoted by *). Cue-induced cocaine-seeking during phasic VTA stimulation was performed during WD3-WD20 testing.

Training	Withdrawal day 3 testing	Brain region	Manipulation
Cocaine SA	Cue-induced cocaine-seeking	VTA	Lidocaine (0, 0.82 μg)
			Phasic stimulation*
Cocaine SA	Cue-induced cocaine-seeking	LDTg	Muscimol (0, 0.03 μg) + baclofen (0, 0.3 μg)
Cocaine SA	Cue-induced cocaine-seeking	NAc core	SCH23390: (0, 1 or 2 μg)
Cocaine SA	Cue-induced cocaine-seeking	VTA	Mecamylamine (0, 3, 30 μg)
			Scopolamine (0, 3, 67 μg)
			AP-5 (0, 0.1, 1 μg)

Table 2

Fast scan cyclic voltammetry. VTA drug micro-infusions and phasic DA release in the NAc core after VTA stimulation were performed in both cocaine-naïve rats and in rats that underwent cocaine-self-administration training and subsequent 3 day withdrawal.

Training	Withdrawal day 3 (WD3) testing	Recording site	Infusion site	Manipulation
Cocaine naïve	FSCV in anesthetized rat	NAc core	VTA	Mecamylamine (0, 30 μg)
				Scopolamine (0, 67 μg)
				AP-5 (0, 1 μg)
Cocaine SA	FSCV in anesthetized rat	NAc core	VTA	Mecamylamine (0, 30 μg)
				Scopolamine (0, 67 μg)
				AP-5 (0, 1 μg)