Published in final edited form as:

Psychopharmacology (Berl). 2013 September ; 229(1): 73–82. doi:10.1007/s00213-013-3082-0.

Ventral tegmental area $\alpha 6\beta 2$ nicotinic acetylcholine receptors modulate phasic dopamine release in the nucleus accumbens core

Robert Wickham¹, Wojciech Solecki², Liza Rathbun², J. Michael McIntosh⁴, and Nii A. Addy^{1,2,3,*}

¹Interdepartmental Neuroscience Program, University of Utah, Salt Lake City, UT 84112, USA

²Department of Psychiatry, University of Utah, Salt Lake City, UT 84112, USA

³Department of Cellular and Molecular Physiology, University of Utah, Salt Lake City, UT 84112, USA

⁴Yale University School of Medicine, New Haven, CT 06511, Department of Biology, University of Utah, Salt Lake City, UT 84112, USA

Abstract

RATIONALE—Phasic dopamine (DA) signaling underlies reward learning. Cholinergic and glutamatergic inputs into the ventral tegmental area (VTA) are crucial for modulating burst firing activity and subsequent phasic DA release in the nucleus accumbens (NAc), but the specific VTA nicotinic receptor subtypes that regulate phasic DA release have not been identified.

OBJECTIVE—The goal was to determine the role of VTA NMDA receptors (NMDARs) and specific subtypes of nicotinic acetylcholine receptors (nAChRs) in regulating phasic DA release in the NAc core.

METHODS—Fast-scan cyclic voltammetry (FSCV) in anesthetized rats was combined with intra-VTA micro-infusion to evaluate the ability of glutamatergic and cholinergic drugs to modulate stimulated phasic DA release in the NAc core.

RESULTS—VTA NMDAR blockade with AP-5 decreased, while VTA NMDAR activation with NMDA increased NAc peak phasic DA release. Intra-VTA administration of the non-specific nAChR antagonist mecamylamine produced a persistent decrease in phasic DA release. Infusion of the $\alpha 6$ -selective antagonist α -conotoxin MII (α -ctx MII) produced a robust, but transient decrease in phasic DA, whereas infusion of selective doses of either the $\alpha 4\beta 2$ -selective antagonist, dihydro-beta-erythroidine (DH β E) or the $\alpha 7$ antagonist methyllycaconitine (MLA) had no effect. Co-infusion of AP-5 and α -ctx MII produced a similar phasic DA decrease as either drug alone, with no additive effect.

CONCLUSIONS—The results suggest that VTA $\alpha 6\beta 2$ nAChRs, but not $\alpha 4\beta 2$ or $\alpha 7$ nAChRs, regulate phasic DA release in the NAc core and that VTA $\alpha 6\beta 2$ nAChRs and NMDA receptors act at a common site or target to regulate NAc phasic DA signaling.

Keywords

dopamine; nicotinic receptor; NMDA receptor; fast-scan cyclic voltammetry; ventral tegmental area; nucleus accumbens

*Corresponding author: 34 Park Street 3rd Floor Research New Haven, CT 06520 Tel: (203) 737-5646 Fax: (203) 737-2043 nii.addy@yale.edu.

Introduction

Dopamine (DA) cells have two modes of firing: tonic (~3-8 Hz) and phasic, or burst firing (~>14 Hz) (Grace and Bunney, 1984, Schultz et al., 1992, Grace and Bunney, 1995). Burst firing of DA neurons drives phasic DA release in the nucleus accumbens (NAc) as well as in the striatum and other forebrain structures (Wightman et al., 1988, Schultz et al., 1992, Day et al., 2007). Specifically, NAc core phasic DA signaling is important for reward learning and goal-directed behaviors (Day et al., 2007, Day et al., 2010). VTA burst firing and phasic DA release in the NAc core are under the control of both cholinergic and glutamatergic input to the VTA (Lodge and Grace, 2006, Lester et al., 2008). Glutamate modulates burst firing and subsequent phasic DA release through VTA N-methyl-D-aspartate (NMDA) receptors (Overton and Clark, 1992, Lodge and Grace, 2006, Sombers et al., 2009, Zweifel et al., 2009, Parker et al., 2010). Cholinergic input to VTA is also suggested to regulate burst firing as inactivation of the lateral dorsal tegmentum (LDTg), which sends cholinergic and glutamatergic projections to the VTA, ablates VTA burst firing even in the presence of glutamate (Lodge and Grace, 2006). Although cholinergic activity in the VTA and the activity of nicotinic acetylcholine receptors (nAChRs) are important modulators of burst firing and subsequent phasic dopamine release (Kitai et al., 1999, Forster and Blaha, 2000, Forster et al., 2002, Forster and Blaha, 2003, Lodge and Grace, 2006, Mameli-Engvall et al., 2006), the specific VTA cholinergic receptor-subtypes which regulate phasic dopamine release in the NAc have yet to be identified. While a rich literature has begun to elucidate the role of striatal (ST) and NAc nAChRs in modulating phasic DA release (Exley et al., 2008, Exley and Cragg, 2008, Exley et al., 2012), identifying the specific VTA nAChR subtypes that regulate such release will provide the field with novel understanding of the nAChR mechanisms upstream of the ST and NAc that influence phasic DA in these terminal regions. This is particularly important given the proposed role of phasic DA mechanisms in learning and motivation in normal states and in disease states, such as drug addiction (Saddoris et al., 2013).

In the ST and NAc, presynaptic nAChRs on DA terminals act as a frequency-filter to preferentially enhance phasic DA release after high frequency neuronal firing (reviewed in (Exley and Cragg, 2008)). Specifically ACh release from tonically active cholinergic interneurons favors DA release in response to low frequency stimulation but also leads to short-term synaptic depression that inhibits DA release to subsequent burst-like stimulation ((Rice and Cragg, 2004, Zhang and Sulzer, 2004). In contrast, either drug-mediated inhibition or nicotine-induced desensitization of the $\beta 2^*$ nAChRs (where the asterisk indicates other subunits in the pentamer) preferentially decreases dopamine release after low frequency stimulation and removes the short-term depression to maintain (or enhance) dopamine release after burst-like stimulation (Rice and Cragg, 2004, Zhang and Sulzer, 2004, Exley et al., 2008, Exley et al., 2012). In the dorsal striatum, $\alpha 5$ containing nAChRs (specifically $\alpha 4\alpha 5(\text{non-}\alpha 6)\beta 2$ -nAChRs) on DA terminals are critical for regulating ST DA release whereas $\alpha 6$ containing nAChRs (specifically $\alpha 4\alpha 6\beta 2\beta 3$ -nAChRs) regulate phasic DA release in the NAc core (Exley et al., 2008, Exley et al., 2012).

In order to have a circuit level understanding of nAChR modulation of phasic DA release, it is critical that the field identify the role of specific nAChR subtypes in both the VTA and NAc. However, at the level of the VTA, it is not clear how specific nAChR subtypes modulate phasic DA release. Specific subtypes of nAChRs are localized in unique cellular populations within the VTA and depending on their cellular specificity, could differentially modulate phasic DA release. The homomeric $\alpha 7$ nAChRs are located primarily on glutamatergic terminals (Jones and Wonnacott, 2004, Livingstone and Wonnacott, 2009) while the $\beta 2^*$ nAChRs are expressed on both DA cell bodies and on GABA-ergic terminals

making synapses onto the DA cell bodies (McGehee and Role, 1995, Wooltorton et al., 2003, Livingstone and Wonnacott, 2009, Yang et al., 2011). The $\beta 2$ subunit typically assembles with $\alpha 4$ and/or $\alpha 6$ subunits, to form $\alpha 4\beta 2^*$ and $\alpha 6\beta 2^*$ nAChRs that are restricted mostly to GABA-ergic terminals and DA cell bodies (McGehee and Role, 1995, Yang et al., 2011, Brunzell, 2012). Electrophysiological evidence suggests that removal of $\beta 2^*$ nAChRs, and not $\alpha 7$ nAChRs, ablates burst-firing in DA cells (Mameli-Engvall et al., 2006). Thus, unlike in the NAc, one would predict that inhibition of $\beta 2^*$ nAChR on DA cells would decrease phasic DA release. In the present study, we used fast-scan cyclic voltammetry (FSCV), an electrochemical technique with excellent spatial (micron) and temporal (10 Hz) resolution, to determine the VTA nAChR mechanisms that regulate phasic DA release in the NAc core. We found that blockade of $\alpha 6\beta 2^*$, but not $\alpha 4\beta 2^*$ or $\alpha 7$ nAChRs, attenuated dopamine release in the NAc core, thereby identifying subtype-specific VTA nAChRs by which acetylcholine may regulate phasic dopamine release. The data further revealed that a combination of $\alpha 6\beta 2^*$ nAChR and NMDAR antagonists did not have an additive effect on modulating phasic DA release and suggests that $\alpha 6\beta 2^*$ nAChRs and NMDARs act at a common target, likely the DA cell bodies, to regulate phasic DA release.

Materials and Methods

Subjects

Male Sprague Dawley rats (250-350 g) were acquired from Charles River Laboratories. Animals were placed on *ad libitum* food and water and were housed 2 to 3 per cage on a 12 hour light/dark cycle with lights on at 7 am. All experiments were conducted according to the Guide for the Care and Use of Laboratory Animals and were approved by the Yale University Institutional Animal Care and Use Committee.

Drugs—Mecamylamine (0.3, 3, or 30 μ g), AP-5 (1 μ g), MLA (3.437 or 6.875 μ g), NMDA (500 ng), lidocaine (41 ng) (all from Sigma Aldrich, St. Louis, MS), DH β E (1.75 or 17.5 μ g; Santa-Cruz Biotechnology, Santa Cruz, CA), or α -ctx MII (17.1 or 171 ng; synthesized as previously described (McIntosh et al., 2004)), were dissolved into 0.9% saline and infused into the VTA in a 0.5 μ L volume at a rate of 0.5 μ L/min using a Hamilton 25 gauge syringe. After infusion, the internal cannula was left in place for one minute prior to electrochemical recording to allow ample time for drug absorption. The doses for all experiments were calculated based on consensus from published studies using intra-VTA administration of these antagonists (Yeomans and Baptista, 1997, Levin et al., 2002, Brunzell, 2012)

Surgery—Rats were anesthetized with urethane (1.5 mg/kg) and placed in a stereotaxic frame (David Kopf Instruments, Tujunga, CA, USA). All coordinates were obtained from the rat brain atlas (Paxinos and Watson, 2007). Anteroposterior (AP), mediolateral (ML) and dorsoventral (DV) positions were referenced from bregma. The skull surface was exposed, and four small holes were drilled for insertion of the carbon fiber microelectrode, a bipolar stimulator retrofitted with a cannula, a reference wire, and a screw to hold the reference electrode. A bipolar, stainless-steel stimulating electrode (Plastics One, Roanoke, VA, USA) was placed into the VTA/SN (AP -5.2 mm, ML 0.5 - 1.5 mm, DV from 7.4 to 8.1 mm below dura). An Ag/AgCl reference electrode was placed into the contralateral cortex and was held with a screw in contact with the skull to minimize the number of manipulators used. The pia mater was punctured, removed and a carbon-fiber microelectrode was implanted vertically in the NAc core (AP $+1.2$ mm, ML -1.4 mm, DV from 6.2 to 6.9 mm). During FSCV recording, body temperature was maintained at 37°C by a heating pad (Harvard Apparatus, Holliston, MA, USA).

Fast-Scan cyclic voltammetry (FSCV)—T-650 carbon fibers (Thornel, Amoco Corp., Greenville, SC, USA) were aspirated into a glass capillary and pulled with an electrode puller (Narishige International, East Meadow, NY). The carbon fiber protruding from the capillary was then cut under a light microscope to a length of 100 to 200 μm . For FSCV recordings, a triangular waveform (-0.4 to $+1.3$ V and back to -0.4 V versus an Ag/AgCl reference electrode) was applied at 400 V/s and repeated at 100 ms intervals. The triangular waveform was low-pass filtered at 2 kHz. Data were digitized and processed using NI-6711 and NI-6251 PCI cards (National Instruments, Austin, TX, USA) and were analyzed using Demon Voltammetry and Analysis Software (Wake Forest Baptist Medical Center, Winston-Salem, NC). Background-subtracted cyclic voltammograms were obtained by digitally subtracting stable background currents to resolve cyclic voltammograms associated with the electrical stimulation event. To evoke phasic DA release in the NAc core, electrical stimulation was applied to the VTA. Pulses delivered to the stimulating electrode were computer generated with a 6711 PCI card (National Instruments, Austin, TX, USA) and were optically isolated from the electrochemical system using a bi-phasic stimulus isolator (NL 800A, Neurolog, Digitimer Ltd, Hertfordshire, UK). The electrical stimulation train consisted of biphasic square wave pulses (300 μA , 24 pulses, 2 ms each phase, applied at a 60 Hz frequency). Each stimulation train was applied every 3 min to allow time for DA releasable stores to return to their original levels. After the experiment, the carbon fiber electrode was replaced with a tungsten wire electrode and an electrolytic lesion was created by applying 20 μA to the recording site. Animals were then sacrificed and the brains were stored in 10% formaldehyde for at least 72 hr, and coronally sectioned into 40–50 μm thick slices with a cryostat (Leica cm 3050, Meyer Instruments, Houston, TX). The sections were mounted on slides and viewed under a light microscope to verify working electrode placements (Fig. 6).

Experimental design and data analysis—As illustrated in the experimental timeline (Fig. 1A), once stimulated dopamine was detected in the NAc core, 6 VTA-evoked baseline samples were recorded to ensure the stability of stimulated, peak dopamine release (within 10% of average). Next, saline was infused into the VTA and 6 traces were recorded. Subsequently, an additional 6 traces were collected to establish a second stable baseline period. Finally, drug infused into the VTA and 6 traces were recorded. All infusions were made at the rate of 0.5 $\mu\text{L}/\text{min}$ in the volume of 0.5 μL (1 minute total infusion time). After infusion, the internal cannula was left in the guide cannula for an additional minute. Thus, time = 0 corresponds to the first stimulation 1 minute after the end of vehicle or drug infusion.

Data are presented as a comparison of the peak of stimulated phasic dopamine release ($[\text{DA}_{\text{max}}]$) after saline versus drug. We used Demon Voltammetry and Analysis Software (Wake Forest Baptist Medical Center, Winston-Salem, NC) for data collection and analysis. Saline data was normalized to the 15 minute baseline period directly preceding saline infusion, while the drug data is normalized to a 15 minute stable baseline period after saline infusion and immediately prior to drug infusion (Fig. 1). We compared the effects of the drugs versus saline on stimulated phasic dopamine release as well as drug effects over time. Time course analysis was conducted using a repeated-measures two-way ANOVA followed by a Bonferroni post-hoc test (Graph Pad Software, San Diego, CA, USA). We used this time course to detect the epoch which showed the maximal percent change from baseline. Then, one-tailed paired t-tests were used to compare between the maximal drug epoch and the comparable saline epoch in each animal. The baseline for saline is considered to be the average of 6 stimulations, 3 minutes apart, prior to the saline infusion. The baseline for drug is considered to be the average of 6 stimulations, 3 minutes apart, prior to the drug infusion (Fig. 1A). The criterion for a stable baseline period was defined as peak $[\text{DA}]_{\text{max}}$ signals

that were within 10% of the averaged signal. Infusions were at least thirty minutes apart, with saline always being first.

Results

Inactivation of the VTA decreases, but does not completely ablate, stimulated peak DA release in the NAc core

As a positive control, we infused the voltage-gated sodium channel blocker, lidocaine (41 ng) into the VTA. Infusion of lidocaine ($n=9$) decreased, but did not completely abolish, stimulated peak phasic DA release in the NAc core for the entire 15 minute recording period (Fig. 1C: time $\times$ treatment $F_{5,16}=1.00$, $p=0.43$; time $F_{5,16}=0.7$, $p=0.58$; treatment $F_{1,16}=14.12$, $p<0.0001$) compared to saline control. Initial lidocaine infusion reduced stimulated peak phasic DA release by 65% compared to saline control (Fig. 1B: $t=7.98$, $df=8$, $p<0.0001$).

General nAChR blockade in the VTA attenuates stimulated peak phasic dopamine release in the NAc core

In order to assess the role of VTA nAChRs in modulating stimulated peak phasic DA release, the non-selective nAChR antagonist mecamylamine (MEC: 0.3, 3, or 30 μg) was infused into the VTA. MEC infusion of the lowest dose (0.3 μg ; $n=5$) did not significantly alter stimulated peak phasic DA release (Fig. 2A **bottom**: treatment $\times$ time $F_{5,12}=1.41$, $p=0.21$; time $F_{5,12}=0.29$, $p=0.91$; treatment $F_{1,12}=4.7$, $p=0.06$). The effect of 0.3 μg MEC immediately after infusion revealed a strong trend towards reduced stimulated peak phasic DA release (Fig. 2A **top**: $t=1.97$, $df=4$, $p=0.06$). MEC infusion of a moderate dose (3 μg , $n=4$), produced a robust and long lasting decrease in stimulated peak phasic DA release (Fig. 2B **bottom**: treatment $\times$ time $F_{5,12}=0.12$, $p=0.98$; time $F_{5,12}=2.50$, $p=0.05$; treatment $F_{1,12}=13.87$, $p<0.01$). The effect of this 3 μg dose of MEC immediately after infusion significantly reduced stimulated peak phasic DA release (Fig. 2B **bottom**: $t=3.146$, $df=3$, $p<0.05$). A 30 μg MEC dose ($n=7$) also showed similar initial (Fig. 2C **bottom**: $t=3.69$, $df=6$, $p<0.01$) and long-term effects (Fig. 2C **bottom**: treatment $\times$ time $F_{5,12}=0.52$, $p=0.76$; time $F_{5,12}=1.351$, $p=0.26$; treatment $F_{1,12}=15.13$, $p<0.01$) as the 3 μg MEC dose. In addition, the 3 μg MEC dose showed an apparent, greater decrease in phasic DA versus baseline than the 30 μg MEC dose. However, the saline infusion in the “to be” 3 μg MEC group lead to a 24.8% DA decreases while saline infusion in the “to be” 30 μg MEC subjects led to a minimal 3.2% DA decrease. Thus, the apparent enhanced effect of 3 μg MEC versus the DA baseline was likely an artifact of the greater saline-induced decrease in this cohort.

VTA $\alpha 6\beta 2^*$ nAChRs, but not $\alpha 4\beta 2^*$ or $\alpha 7$ nAChRs, regulate stimulated peak phasic DA release in the NAc core

To determine which specific nAChR subtype or subtypes contribute to phasic DA signaling in the NAc core, we infused either the $\alpha 4\beta 2$ antagonist dihydro-beta-erythroidine (DH β E), the $\alpha 6$ -specific antagonist α -conotoxin MII (α -ctx MII), or the $\alpha 7$ antagonist methyllycaconitine (MLA) into the VTA. Infusion of α -ctx MII (17.1 ng; $n=7$) decreased stimulated peak phasic DA release at the first time point (Fig. 3A **top**: treatment $\times$ time $F_{5,12}=0.67$, $p=0.64$; time $F_{5,12}=1.71$, $p=0.14$; treatment $F_{1,12}=9.942$, $p<0.05$), but the signal recovered to baseline levels after 3 minutes (Fig. 3A **bottom**: $t=3.48$, $df=5$, $p<0.01$). A higher α -ctx MII dose (171 ng, $n=7$) produced similar effects at the first time point (Fig. 3B **top**: $t(6)=2.33$, $p<0.05$) and long term (Fig. 3A **bottom**: time $\times$ treatment $F_{5,12}=2.42$, $p<0.05$; time $F_{5,12}=0.62$, $p=0.7$, treatment $F_{1,12}=6.259$, $p<0.05$). However, we also observed a decrease at the 12 minute time point (Bonferonni post-hoc test $p<0.05$).

In contrast to α -ctx MII, infusion of DH β E failed to modulate stimulated peak phasic DA release. Administration of either 1.75 μ g DH β E or 17.5 μ g DH β E failed to modulate stimulated phasic DA release (Fig. 4A, data not shown for 1.75 μ g DH β E). Specifically, the 1.75 μ g DH β E dose (n=4) did not alter phasic DA release during the initial time-point (t=0.47, df= 3, p=0.33, data not shown) or during the entire recording session (time $\times$ treatment $F_{5,8}=0.45$, p=0.81; time $F_{5,8}=0.59$, p=0.71; treatment $F_{1,8}=0.33$, p=0.58, data not shown). A 17.5 μ g dose (n=4) also did not alter stimulated peak phasic DA release initially (Fig. 4A **top**: t=0.80, df=3, p=0.27) or long term (Fig. 4A **bottom**; treatment $\times$ time $F_{5,8}=1.285$, p=0.30; time $F_{5,8}=2.851$, p<0.05; treatment $F_{1,8}=0.1748$, p=0.69).

MLA infusion also had no effect on phasic DA release in the NAc (Fig 4B). Specifically, infusion of 3.437 μ g MLA (n=7) did not significantly decrease stimulated peak phasic DA release (time $\times$ treatment $F_{5,12}=0.60$, p=0.70; time $F_{5,12}=0.76$, p=0.58; treatment $F_{1,12}=0.037$, p<0.58, data not shown) and the maximal change after MLA infusion did not significantly differ from the saline control (t(6)=0.497, p=0.31, data not shown). Similarly, a higher dose of 6.875 μ g MLA (n=5) had no significant effect on peak phasic DA at the initial time-point (Fig. 4B **top**: t=0.40, df=4, p=0.35) and long-term (Fig. 4B **bottom** treatment $\times$ time $F_{5,10}=0.97$, p=0.44; time $F_{5,10}=1.23$, p=0.31, time $F_{1,10}=0.11$, p= 0.74).

VTA NMDARs modulate stimulated phasic peak DA release in the NAc core

The role of VTA NMDARs in modulating stimulated peak phasic DA release was examined by infusing either NMDA (500 ng, n=5) or the NMDAR specific antagonist AP-5 (1 μ g, n=5), into the VTA. NMDA administration robustly elevated stimulated peak phasic DA by 6 minutes (Fig. 5A, time $\times$ treatment $F_{5,8}=1.8$, p=0.15; time $F_{5,8}=1.8$, p=0.14; treatment $F_{1,8}=12.1$, p<0.05) while AP-5 administration into the VTA robustly decreased stimulated peak phasic DA release (Fig. 5B: time $\times$ treatment $F_{5,8}=0.80$, p=0.55; time $F_{5,8}=0.86$, p=0.51; treatment $F_{1,8}=17.1$, p<0.01). The peak effect of NMDA infusion increased $[DA]_{\max}$ to 300% of saline (Fig. 5A **bottom**: t(4)=4.47, p<0.01) while the maximal effect of AP-5 infusion was a 50% reduction of stimulated peak phasic DA release compared to saline control (Fig. 5B **bottom**: (4)=4.03, p<0.01).

VTA α -ctx MII and AP-5 effects on NAc phasic DA release are not additive

To determine whether the effects of NMDAR and $\alpha 6\beta 2^*$ antagonists were additive, we examined the effects of AP-5 infusion alone or in combination with α -ctx MII. In order to directly compare effects between the two treatments, we did not include a saline control, but we collected 6 baseline DA traces followed by 6 drug traces (AP-5 alone or a cocktail of AP-5 plus α -ctx MII, Fig 5C). A between-subjects comparison showed that AP-5 infusion into the VTA without a prior saline infusion (Fig. 5C, n=4) led to a decrease in phasic DA release that did not significantly differ from the AP-5 effect observed in animals who received a saline control infusion prior to AP-5 (Fig. 5B: time $\times$ treatment $F_{5,8}=1.017$, p=0.42; time $F_{5,8}=0.545$, p=0.74; treatment $F_{1,8}=0.187$, p=0.68). A cocktail of AP-5 (1 μ g) plus α -ctx MII (171 ng; n=6) decreased phasic DA release similarly to only AP-5 (n=4) infusion with no significant difference between AP-5 alone versus AP-5 plus α -ctx MII both initially (Fig. 5C **middle**: t(8)=0.337; p=0.745) and over the recording period (Fig. 5C **bottom**: time $\times$ treatment $F_{5,8}=0.578$, p=0.72; time $F_{5,8}=0.558$, p=0.73; treatment $F_{1,8}=0.04$, p=0.844).

Discussion

Here, we demonstrate that both VTA NMDARs and $\alpha 6\beta 2^*$ nAChRs, but not $\alpha 4\beta 2^*$ or $\alpha 7$ nAChRs, regulate NAc core phasic dopamine release. First, we showed that VTA inactivation with lidocaine produced a marked and persistent decrease in stimulated peak

phasic DA release. Second, blockade or activation of VTA NMDARs attenuated and enhanced phasic dopamine release, respectively. The lidocaine and NMDAR results highlight the utility of using pharmacological manipulations to dissect the VTA mechanisms that regulate phasic DA release in the NAc core. Importantly, the magnitude of our observed lidocaine effect was consistent with intra-VTA lidocaine infusions observed in freely-moving animals (Owesson-White et al., 2012). Interestingly, there were instances where limited DA-like signals were observed even in the presence of lidocaine. However, the cyclic voltammograms after lidocaine were less DA-like after lidocaine, suggesting that the remaining signal could partially have resulted from noise that was unmasked by a low evoked signal. Alternatively, the remaining signal after lidocaine infusion could have resulted from non-VTA, glutamatergic input into the NAc that is known to contribute to phasic dopamine release in the NAc (Jones et al., 2010). Indeed, previous work has shown that stimulation of the basolateral amygdala is sufficient to induce dopamine release in the NAc, even in the absence of VTA activity (Howland et al., 2002). Nonetheless, the experimental design provides an assay that allows for the identification of specific VTA receptors that regulate NAc core phasic DA release. The observed attenuation and enhancement of phasic DA with VTA AP-5 and NMDA infusion is consistent with previous work showing that intra-VTA infusion of either AP-5 or NMDA decreased and increased spontaneous DA release in the NAc, respectively, in awake, behaving rats (Sombers et al., 2009).

Although NMDARs are important for driving burst firing in the VTA and phasic dopamine release in the NAc, cholinergic input into the VTA is also suggested to regulate dopamine burst firing (Grace et al., 2007). In a previous study, LDTg inactivation ablated DA cell burst firing, even in the presence of glutamate, suggesting that non-glutamatergic projections from the LDTg are also required for burst firing (Lodge and Grace, 2006).

Electrophysiological data suggest that cholinergic activity is an important regulator of DA burst firing (Kitai et al., 1999) and nAChRs located on terminals and cell bodies within the VTA are well-positioned to modulate both VTA DA burst firing and downstream DA release in the NAc (Maskos, 2008). Both $\alpha 4\beta 2^*$ and $\alpha 6\beta 2^*$ nAChRs in the VTA are found on DA cell bodies and on GABA terminals (Klink et al., 2001, Yang et al., 2011). Indeed, a previous study demonstrated that nicotine-induced increases in VTA DA firing frequencies were blocked by α -ctx MII or by knocking out the $\alpha 6$ or $\alpha 4$ nAChR (Liu et al., 2012). However, this previous work was performed mainly during exposure to nicotine and did not address the role of VTA $\alpha 6$ nAChRs in regulating either DA burst firing or phasic DA release. In the context of this previous study, our α -ctx MII data suggest that the critical $\alpha 6$ nAChRs through which acetylcholine likely regulates phasic DA release are also the critical receptors through which nicotine enhances VTA DA neuronal activity. Here we observed a transient decrease of stimulated phasic DA release by α -ctx MII compared to MEC administration. One possible explanation for the transient α -ctx MII effect is that stimulation of cholinergic terminals in the VTA may elevate acetylcholine to levels which may out-compete α -ctx MII. However, this is unlikely since α -ctx MII has a very slow off-rate and is essentially irreversible during the time-course of the experiment (McIntosh et al., 2004). In addition, it is unlikely that the transient effect is due to low antagonist concentrations since the doses used are pharmacologically selective and behaviorally effective (Cartier et al., 1996, Harvey et al., 1997, Yeomans and Baptista, 1997, Everhart et al., 2004, McIntosh et al., 2004, Lof et al., 2007, Brunzell et al., 2010, Drenan et al., 2010). An alternative possibility is that compensatory mechanisms from other nAChR subtypes may contribute to this transient effect of $\alpha 6$ nAChR antagonism. However, our findings with a higher, non-selective dose of DHBE (35 μ g), which likely inhibits both $\alpha 4\beta 2$ and $\alpha 6\beta 2^*$ nAChRs, produce a similar, transient decrease in phasic DA release as α -ctx MII infusion (data not shown). Similarly, infusion of a high, non-selective dose of MLA (13.75 μ g), which inhibits

both $\alpha 7$ and $\alpha 6\beta 2^*$ nAChRs, also leads to a transient decrease in phasic DA release similar to that of α -ctx MII infusion (data not shown).

These data strongly suggests that $\alpha 4\beta 2^*$ or $\alpha 7$ nAChRs do not play a role in any compensatory process during $\alpha 6\beta 2^*$ nAChR antagonism since phasic DA release is still transient even in the presence of $\alpha 4\beta 2^*$ or $\alpha 7$ inhibition. Instead, it suggests that the transient effect is inherent to the role of $\alpha 6$ nAChRs in regulating VTA-evoked phasic DA release. However, it does not rule out the possibility of non-nAChR compensatory mechanisms that may contribute to the transient effects of α -ctx MII compared to mecamylamine. For instance, high doses of mecamylamine have been shown to inhibit NMDARs (Reynolds and Miller, 1988) and it is possible that the persistent decrease in phasic DA after mecamylamine infusion (Fig. 3) was due to a combination of $\alpha 6\beta 2^*$ and NMDAR inhibition. Mechanistically, our data suggests that $\alpha 6\beta 2^*$ nAChRs and NMDARs both modulate phasic DA release through a common locus, since combining the $\alpha 6^*$ nAChR and NMDA receptor antagonists did not have an additive effect in decreasing stimulated peak phasic DA release (Fig. 5). Specifically, we propose that DA cell bodies in the VTA represent the common locus on which $\alpha 6\beta 2^*$ nAChRs and NMDARs act to regulate downstream phasic DA release in the NAc. The lack of an additive effect further suggests that $\alpha 6\beta 2^*$ nAChRs and NMDARs may act through a common downstream signaling target in VTA DA neurons to regulate phasic DA. It has previously been demonstrated that activation of either VTA NMDARs or AChRs, which is sufficient to induce burst firing, also increases the expression of calcium dependent proteins, such as protein kinase M (PKM) (Liu et al., 2007). Importantly, PKM is capable of inducing DA burst firing (Liu et al., 2007) and is potential common downstream target of both nAChRs and NMDARs that may act as a critical mediator of phasic DA release in the NAc.

An important goal for many nAChR studies is to identify functional roles of specific nAChR subtypes. Previous work using single-cell recordings in the mouse VTA have shown that burst firing, which is thought to be critical for phasic DA release, is attenuated in $\beta 2$ -KO mice but not altered in $\alpha 7$ -KO mice (Mameli-Engvall et al., 2006). Our voltammetry findings are consistent which such a dichotomy and further suggest that the attenuated burst firing in $\beta 2$ -KO mice may have been due specifically to $\alpha 6\beta 2$ and not $\alpha 4\beta 2$ mechanisms. By extension, we propose that a subset of non- $\alpha 4$ containing $\alpha 6\beta 2^*$ receptors are the critical VTA nAChRs that regulate NAc phasic DA release. Consistent with this interpretation, our findings build upon published behavioral data suggesting a functional distinction between $\alpha 6^*$ nAChRs versus $\alpha 4\beta 2$ and $\alpha 7$ nAChRs (Lof et al., 2007) in cue-mediated behavior. Specifically, VTA inhibition of $\alpha 6^*$ nAChRs attenuates cue-induced ethanol-seeking, while $\alpha 4\beta 2^*$ and $\alpha 7$ antagonists have no effect (Lof et al., 2007). Given that the drug-associated cues increase both DA burst firing and phasic DA release in the NAc (Schultz, 1998, Day et al., 2007), VTA $\alpha 6$ nAChR regulation of phasic DA release may be an important underlying mechanism that mediates cue-induced behavior.

Our $\alpha 6$ nAChR findings in the VTA, in combination with previous $\alpha 6$ nAChR mechanisms proposed in the NAc, provide circuit level understanding of the nAChR mechanisms in the mesolimbic DA system that regulate phasic DA release. Given that VTA $\alpha 6$ nAChR inhibition decreases NAc phasic DA (Fig. 3) while NAc $\alpha 6$ nAChR inhibition enhances phasic DA (Exley et al., 2008), our data reveals that $\alpha 6$ nAChRs in the VTA and NAc have opposing effects on NAc phasic DA release. If phasic DA release mechanisms underlie cue-induced behavior, one would predict that nAChR activation in the VTA would increase phasic DA release and cue-mediated behavior while nAChR activation in the NAc would decrease phasic DA and inhibit cue-mediated behavior. Consistent with this proposed mechanism, increasing ACh tone in the VTA has been shown to enhance cue-induced heroin-seeking while increasing ACh tone in the NAc decreases this cue-induced drug-

seeking behavior (Zhou et al., 2007). Thus, at a circuit level, the evidence suggests that cholinergic inputs to the VTA and the NAc modulate cue-mediated behavior in opposite ways. Indeed, cholinergic neurons in the pedunculopontine tegmentum (PPTg) are known to increase their firing rate in response to cues (Pan and Hyland, 2005) which may provide the cholinergic driving force to the VTA which increases burst-firing and subsequent phasic DA release. In contrast, cholinergic neurons in the NAc cease firing, or pause, in response to cues (Morris et al., 2004) - thus reducing cholinergic tone to DA terminals, relieving short-term depression, and enhancing phasic DA release to bursts (Rice and Cragg, 2004, Zhang and Sulzer, 2004, Exley and Cragg, 2008). Our present VTA results, combined with previous studies in the NAc, suggest that $\alpha 6$ receptors are the critical nAChR targets that can facilitate both the ability of VTA cholinergic activation and the NAc cholinergic pause to enhance phasic DA release in the NAc. Thus, by regulating the phasic DA mechanisms that are thought to underlie learned and motivated behavior (Saddoris et al., 2013), $\alpha 6$ nAChRs in the VTA and NAc may powerfully influence both normal and addiction-related behaviors.

In summary, our findings have identified VTA $\alpha 6\beta 2^*$ receptors as critical nAChR subtypes that regulate phasic NAc core DA release. The data further suggests that $\alpha 6\beta 2^*$ nAChRs and NMDARs may act through a similar locus and downstream target to regulate phasic DA release. Given the emerging role of phasic DA mechanisms in reward-related behavior, future voltammetry experiments in awake, behaving subjects will examine the role of VTA $\alpha 6$ nAChRs-regulated phasic DA release in reward-learning and cue-mediated behavior.

Acknowledgments

This work was supported by NIH GM103801 and GM48677 (JMM) and by a NSF Graduate Research Fellowship (RJW).

References

- Brunzell DH. Preclinical evidence that activation of mesolimbic alpha 6 subunit containing nicotinic acetylcholine receptors supports nicotine addiction phenotype. *Nicotine Tob Res.* 2012; 14:1258–1269. [PubMed: 22492084]
- Brunzell DH, Boschen KE, Hendrick ES, Beardsley PM, McIntosh JM. Alpha-conotoxin MII-sensitive nicotinic acetylcholine receptors in the nucleus accumbens shell regulate progressive ratio responding maintained by nicotine. *Neuropsychopharmacology.* 2010; 35:665–673. [PubMed: 19890263]
- Cartier GE, Yoshikami D, Gray WR, Luo S, Olivera BM, McIntosh JM. A new alpha-conotoxin which targets alpha3beta2 nicotinic acetylcholine receptors. *J Biol Chem.* 1996; 271:7522–7528. [PubMed: 8631783]
- Day JJ, Jones JL, Wightman RM, Carelli RM. Phasic nucleus accumbens dopamine release encodes effort- and delay-related costs. *Biol Psychiatry.* 2010; 68:306–309. [PubMed: 20452572]
- Day JJ, Roitman MF, Wightman RM, Carelli RM. Associative learning mediates dynamic shifts in dopamine signaling in the nucleus accumbens. *Nature Neuroscience.* 2007; 10:1020–1028.
- Drenan RM, Grady SR, Steele AD, McKinney S, Patzlaff NE, McIntosh JM, Marks MJ, Miwa JM, Lester HA. Cholinergic modulation of locomotion and striatal dopamine release is mediated by alpha6alpha4* nicotinic acetylcholine receptors. *J Neurosci.* 2010; 30:9877–9889. [PubMed: 20660270]
- Everhart D, Cartier GE, Malhotra A, Gomes AV, McIntosh JM, Luetje CW. Determinants of potency on alpha-conotoxin MII, a peptide antagonist of neuronal nicotinic receptors. *Biochemistry.* 2004; 43:2732–2737. [PubMed: 15005608]
- Exley R, Clements MA, Hartung H, McIntosh JM, Cragg SJ. Alpha6-containing nicotinic acetylcholine receptors dominate the nicotine control of dopamine neurotransmission in nucleus accumbens. *Neuropsychopharmacology.* 2008; 33:2158–2166. [PubMed: 18033235]

- Exley R, Cragg SJ. Presynaptic nicotinic receptors: a dynamic and diverse cholinergic filter of striatal dopamine neurotransmission. *Br J Pharmacol*. 2008; 153(Suppl 1):S283–297. [PubMed: 18037926]
- Exley R, McIntosh JM, Marks MJ, Maskos U, Cragg SJ. Striatal alpha5 nicotinic receptor subunit regulates dopamine transmission in dorsal striatum. *J Neurosci*. 2012; 32:2352–2356. [PubMed: 22396410]
- Forster GL, Blaha CD. Laterodorsal tegmental stimulation elicits dopamine efflux in the rat nucleus accumbens by activation of acetylcholine and glutamate receptors in the ventral tegmental area. *Eur J Neurosci*. 2000; 12:3596–3604. [PubMed: 11029630]
- Forster GL, Blaha CD. Pedunculopontine tegmental stimulation evokes striatal dopamine efflux by activation of acetylcholine and glutamate receptors in the midbrain and pons of the rat. *Eur J Neurosci*. 2003; 17:751–762. [PubMed: 12603265]
- Forster GL, Yeomans JS, Takeuchi J, Blaha CD. M5 muscarinic receptors are required for prolonged accumbal dopamine release after electrical stimulation of the pons in mice. *J Neurosci*. 2002; 22:RC190. [PubMed: 11756520]
- Grace AA, Bunney BS. The control of firing pattern in nigral dopamine neurons: burst firing. *J Neurosci*. 1984; 4:2877–2890. [PubMed: 6150071]
- Grace, AA.; Bunney, BS. Electrophysiological properties of midbrain dopamine neurons. Raven Press {a}; 1995.
- Grace AA, Floresco SB, Goto Y, Lodge DJ. Regulation of firing of dopaminergic neurons and control of goal-directed behaviors. *Trends in neurosciences*. 2007; 30:220–227. [PubMed: 17400299]
- Harvey SC, McIntosh JM, Cartier GE, Maddox FN, Luetje CW. Determinants of specificity for alpha-conotoxin MII on alpha3beta2 neuronal nicotinic receptors. *Mol Pharmacol*. 1997; 51:336–342. [PubMed: 9203640]
- Howland JG, Taepavarapruk P, Phillips AG. Glutamate receptor-dependent modulation of dopamine efflux in the nucleus accumbens by basolateral, but not central, nucleus of the amygdala in rats. *J Neurosci*. 2002; 22:1137–1145. [PubMed: 11826142]
- Jones IW, Wonnacott S. Precise localization of alpha 7 nicotinic acetylcholine receptors on glutamatergic axon terminals in the rat ventral tegmental area. *J Neurosci*. 2004; 24:11244–11252. [PubMed: 15601930]
- Jones JL, Day JJ, Aragona BJ, Wheeler RA, Wightman RM, Carelli RM. Basolateral amygdala modulates terminal dopamine release in the nucleus accumbens and conditioned responding. *Biol Psychiatry*. 2010; 67:737–744. [PubMed: 20044074]
- Kitai ST, Shepard PD, Callaway JC, Scroggs R. Afferent modulation of dopamine neuron firing patterns. *Curr Opin Neurobiol*. 1999; 9:690–697. [PubMed: 10607649]
- Klink R, de Kerchove d'Exaerde A, Zoli M, Changeux JP. Molecular and physiological diversity of nicotinic acetylcholine receptors in the midbrain dopaminergic nuclei. *J Neurosci*. 2001; 21:1452–1463. [PubMed: 11222635]
- Lester DB, Miller AD, Pate TD, Blaha CD. Midbrain acetylcholine and glutamate receptors modulate accumbal dopamine release. *Neuroreport*. 2008; 19:991–995. [PubMed: 18521007]
- Levin ED, Bradley A, Addy N, Sigurani N. Hippocampal alpha 7 and alpha 4 beta 2 nicotinic receptors and working memory. *Neuroscience*. 2002; 109:757–765. [PubMed: 11927157]
- Liu L, Zhao-Shea R, McIntosh JM, Gardner PD, Tapper AR. Nicotine persistently activates ventral tegmental area dopaminergic neurons via nicotinic acetylcholine receptors containing alpha4 and alpha6 subunits. *Mol Pharmacol*. 2012; 81:541–548. [PubMed: 22222765]
- Liu Y, Dore J, Chen X. Calcium influx through L-type channels generates protein kinase M to induce burst firing of dopamine cells in the rat ventral tegmental area. *J Biol Chem*. 2007; 282:8594–8603. [PubMed: 17237234]
- Livingstone PD, Wonnacott S. Nicotinic acetylcholine receptors and the ascending dopamine pathways. *Biochem Pharmacol*. 2009; 78:744–755. [PubMed: 19523928]
- Lodge DJ, Grace AA. The laterodorsal tegmentum is essential for burst firing of ventral tegmental area dopamine neurons. *Proceedings of the National Academy of Sciences of the United States of America*. 2006; 103:5167–5172. [PubMed: 16549786]
- Lof E, Olausson P, deBejczy A, Stomberg R, McIntosh JM, Taylor JR, Soderpalm B. Nicotinic acetylcholine receptors in the ventral tegmental area mediate the dopamine activating and

- reinforcing properties of ethanol cues. *Psychopharmacology (Berl)*. 2007; 195:333–343. [PubMed: 17703283]
- Mameli-Engvall M, Evrard A, Pons S, Maskos U, Svensson TH, Changeux JP, Faure P. Hierarchical control of dopamine neuron-firing patterns by nicotinic receptors. *Neuron*. 2006; 50:911–921. [PubMed: 16772172]
- Maskos U. The cholinergic mesopontine tegmentum is a relatively neglected nicotinic master modulator of the dopaminergic system: relevance to drugs of abuse and pathology. *Br J Pharmacol*. 2008; 153:S438–445. [PubMed: 18223661]
- McGehee DS, Role LW. Physiological diversity of nicotinic acetylcholine-receptors expressed by vertebrate neurons. *Annual Review of Physiology*. 1995; 57:521–546.
- McIntosh JM, Azam L, Staheli S, Dowell C, Lindstrom JM, Kuryatov A, Garrett JE, Marks MJ, Whiteaker P. Analogs of alpha-conotoxin MII are selective for alpha6-containing nicotinic acetylcholine receptors. *Mol Pharmacol*. 2004; 65:944–952. [PubMed: 15044624]
- Morris G, Arkadir D, Nevet A, Vaadia E, Bergman H. Coincident but distinct messages of midbrain dopamine and striatal tonically active neurons. *Neuron*. 2004; 43:133–143. [PubMed: 15233923]
- Overton, P.; Clark, D. *Synapse*. Vol. 10. New York, NY: 1992. Ionophoretically administered drugs acting at the N-methyl-D-aspartate receptor modulate burst firing in A9 dopamine neurons in the rat; p. 131-140.
- Owesson-White CA, Roitman MF, Sombers LA, Belle AM, Keithley RB, Peele JL, Carelli RM, Wightman RM. Sources contributing to the average extracellular concentration of dopamine in the nucleus accumbens. *J Neurochem*. 2012; 121:252–262. [PubMed: 22296263]
- Pan WX, Hyland BI. Pedunculopontine tegmental nucleus controls conditioned responses of midbrain dopamine neurons in behaving rats. *J Neurosci*. 2005; 25:4725–4732. [PubMed: 15888648]
- Parker JG, Zweifel LS, Clark JJ, Evans SB, Phillips PE, Palmiter RD. Absence of NMDA receptors in dopamine neurons attenuates dopamine release but not conditioned approach during Pavlovian conditioning. *Proc Natl Acad Sci U S A*. 2010; 107:13491–13496. [PubMed: 20616081]
- Paxinos, G.; Watson, C. *The Rat Brain in Stereotaxic Coordinates*. Elsevier; London: 2007.
- Reynolds IJ, Miller RJ. [3H]MK801 binding to the N-methyl-D-aspartate receptor reveals drug interactions with the zinc and magnesium binding sites. *J Pharmacol Exp Ther*. 1988; 247:1025–1031. [PubMed: 2849655]
- Rice ME, Cragg SJ. Nicotine amplifies reward-related dopamine signals in striatum. *Nat Neurosci*. 2004; 7:583–584. [PubMed: 15146188]
- Saddoris MP, Sugam JA, Cacciapaglia F, Carelli RM. Rapid dopamine dynamics in the accumbens core and shell: Learning and action. *Front Biosci (Elite Ed)*. 2013; 5:273–288. [PubMed: 23276989]
- Schultz W. Predictive reward signal of dopamine neurons. *J Neurophysiol*. 1998; 80:1–27. [PubMed: 9658025]
- Schultz W, Apicella P, Scarnati E, Ljungberg T. Neuronal-activity in monkey ventral striatum related to the expectation of reward. *J Neurosci*. 1992; 12:4595–4610. [PubMed: 1464759]
- Sombers LA, Beyene M, Carelli RM, Wightman RM. Synaptic overflow of dopamine in the nucleus accumbens arises from neuronal activity in the ventral tegmental area. *J Neurosci*. 2009; 29:1735–1742. [PubMed: 19211880]
- Wightman RM, May LJ, Michael AC. Detection of dopamine dynamics in the brain. *Analytical Chemistry*. 1988; 60:A769.
- Wooltorton JRA, Pidoplichko VI, Broide RS, Dani JA. Differential desensitization and distribution of nicotinic acetylcholine receptor subtypes in midbrain dopamine areas. *J Neurosci*. 2003; 23:3176–3185. [PubMed: 12716925]
- Yang K, Buhlman L, Khan GM, Nichols RA, Jin G, McIntosh JM, Whiteaker P, Lukas RJ, Wu J. Functional nicotinic acetylcholine receptors containing alpha6 subunits are on GABAergic neuronal boutons adherent to ventral tegmental area dopamine neurons. *J Neurosci*. 2011; 31:2537–2548. [PubMed: 21325521]
- Yeomans J, Baptista M. Both nicotinic and muscarinic receptors in ventral tegmental area contribute to brain-stimulation reward. *Pharmacol Biochem Behav*. 1997; 57:915–921. [PubMed: 9259024]

- Zhang H, Sulzer D. Frequency-dependent modulation of dopamine release by nicotine. *Nat Neurosci*. 2004; 7:581–582. [PubMed: 15146187]
- Zhou W, Liu H, Zhang F, Tang S, Zhu H, Lai M, Kalivas PW. Role of acetylcholine transmission in nucleus accumbens and ventral tegmental area in heroin-seeking induced by conditioned cues. *Neuroscience*. 2007; 144:1209–1218. [PubMed: 17184925]
- Zweifel LS, Parker JG, Lobb CJ, Rainwater A, Wall VZ, Fadok JP, Darvas M, Kim MJ, Mizumori SJ, Paladini CA, Phillips PE, Palmiter RD. Disruption of NMDAR-dependent burst firing by dopamine neurons provides selective assessment of phasic dopamine-dependent behavior. *Proc Natl Acad Sci U S A*. 2009; 106:7281–7288. [PubMed: 19342487]

Fig 1.

A. Timeline of experiment. B. Effect of VTA SAL and LID (41 ng) infusion on phasic DA release in NAc core. [DA]_{max} after SAL and LID infusion. *Inset top left:* Representative dopamine traces during the first baseline period and after saline infusion. *Inset top right:* Representative dopamine traces of second baseline period and after LID (41 ng) infusion. Triangle indicates VTA stimulation. C. *Bottom:* Time course of [DA]_{max} response after saline (SAL) and lidocaine (LID) infusion. Data are presented as the mean $\pm$ SEM. For all figures: * p <0.05; ** p <0.01; *** p <0.001; **** p <0.0001.

Fig 2.

VTA MEC infusion attenuates evoked phasic DA in the NAc core. A. *Top:* $[DA]_{max}$ after MEC (0.3 μ g) infusion. *Bottom:* $[DA]_{max}$ time course after SAL and MEC infusion. B. *Top* $[DA]_{max}$ after MEC (3 μ g) infusion. *Bottom:* $[DA]_{max}$ time course after SAL and MEC infusion. C. *Top* $[DA]_{max}$ after MEC (30 μ g) infusion. *Bottom:* $[DA]_{max}$ time course after SAL and MEC (30 μ g) infusion. Data represent the mean $\pm$ SEM.

Fig 3.

VTA α -ctx MII infusion modulates evoked phasic DA in the NAc core. A. *Top* $[DA]_{max}$ after α -ctx MII (17.1 ng) infusion. *Bottom*: $[DA]_{max}$ time course after SAL and α -ctx MII infusion. B. *Top* $[DA]_{max}$ after α -ctx MII (171 ng) infusion. *Bottom*: $[DA]_{max}$ time course after SAL and α -ctx MII infusion. Data represent the mean $\pm$ SEM.

Fig 4. VTA infusion of DHβE or MLA does not modulate evoked phasic DA in the NAc core. A. *Top* [DA]_{max} after DHβE (17.5 μg) infusion. *Bottom*: [DA]_{max} time course after SAL and DHβE infusion. B. *Top* [DA]_{max} after MLA (6.875 μg) infusion. *Bottom*: [DA]_{max} time course after SAL and MLA infusion.

Fig 5.

A. VTA NMDA infusion enhances evoked phasic DA in the NAc core. *Top:* Effect of NMDA (500 ng) infusion on $[DA]_{max}$. *Bottom:* Time course of $[DA]_{max}$ after SAL and NMDA infusion. B. AP-5 infusion attenuates evoked phasic DA in the NAc core. *Top:* $[DA]_{max}$ after AP-5 (1 μg) infusion. *Bottom:* Time course of $[DA]_{max}$ response after SAL and AP-5 infusion. C. VTA co-administration of AP-5 and α-ctx MII infusion does not further decrease phasic DA release compared to AP-5 infusion alone. *Top* $[DA]_{max}$ after AP-5 (1 μg) and a cocktail of AP-5 (1 μg) plus α-ctx MII (171 ng) infusion. *Bottom:* $[DA]_{max}$ time course after AP-5 and AP-5 + α-ctx MII infusion

Fig 6. Representative NAc carbon fiber placements as determined by post-experiment, 20 μ A lesions in 40 – 50 μ m coronal slices that were created at the recording site (Adapted from Paxinos and Watson 2007).