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Alpha-1 Adrenergic Receptors are Localized on Presynaptic Elements in the Nucleus Accumbens and Regulate Mesolimbic Dopamine Transmission

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Brainstem noradrenergic neurons innervate the mesocorticolimbic reward pathway both directly and indirectly, with norepinephrine facilitating dopamine (DA) neurotransmission via α1-adrenergic receptors (α1ARs). Although α1AR signaling in the prefrontal cortex (PFC) promotes mesolimbic transmission and drug-induced behaviors, the potential contribution of α1ARs in other parts of the pathway, such as the ventral tegmental area (VTA) and nucleus accumbens (NAc), has not been investigated before. We found that local blockade of α1ARs in the medial NAc shell, but not the VTA, attenuates cocaine- and morphine-induced locomotion. To determine the neuronal substrates that could mediate these effects, we analyzed the cellular, subcellular, and subsynaptic localization of α1ARs and characterized the chemical phenotypes of α1AR-containing elements within the mesocorticolimbic system using single and double immunocytochemical methods at the electron microscopic (EM) level. We found that α1ARs are found mainly extra-synaptically in axons and axon terminals in the NAc and are enriched in glutamatergic and dopaminergic elements. α1ARs are also abundant in glutamatergic terminals in the PFC, and in GABA-positive terminals in the VTA. In line with these observations, microdialysis experiments revealed that local blockade of α1ARs attenuated the increase in extracellular DA in the medial NAc shell following administration of cocaine. These data indicate that local α1ARs control DA transmission in the medial NAc shell and behavioral responses to drugs of abuse.
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INTRODUCTION

Norepinephrine is a catecholamine neurotransmitter involved in arousal, stress, and attention. Noradrenergic dysfunction is believed to contribute to neuropsychiatric disorders ranging from depression and schizophrenia to ADHD and addiction (Berridge and Waterhouse, 2003; Weinshenker and Schroeder, 2007). Norepinephrine (NE) signals via three classes of adrenergic receptors (ARs: α1ARs, α2ARs, and βARs), with the α1-adrenergic receptors (α1ARs) regulating mesocorticolimbic dopamine (DA) transmission and multiple responses to drugs of abuse (Darracq *et al*, 1998; Drouin *et al*, 2002; Weinshenker *et al*, 2002; Zhang and Kosten, 2005; Weinshenker and Schroeder, 2007; Alsene *et al*, 2010). α1ARs are found throughout the mesocorticolimbic system, and the importance of these receptors in the prefrontal cortex (PFC) for DA release and behavioral responses to drugs of abuse has been well characterized. For example, infusion of the α1AR antagonist, prazosin, into the rodent medial PFC attenuates psychostimulant- and opiate-induced locomotion and DA release in the nucleus accumbens (NAc) shell (Darracq *et al*, 1998; Drouin *et al*, 2001; Weinshenker and Schroeder, 2007); however, the functional contribution to mesolimbic transmission and drug responses of local α1ARs in the NAc shell and ventral tegmental area (VTA) has not been studied. Similarly, the ultrastructural localization of these receptors throughout the mesocorticolimbic system remains poorly characterized, which significantly hampers our understanding of the neural mechanisms by which the modulation of α1ARs in this circuit could regulate communication between these structures and the behavioral and neurochemical effects associated with addictive drugs.

To better understand the potential target sites and mechanisms through which α1ARs regulate DA transmission

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and mediate responses to drugs of abuse, we previously investigated the EM localization of α1ARs in the rat striatum and ventral midbrain to determine their subcellular localization and found that α1AR immunoreactivity was mainly expressed presynaptically in the dorsal and ventral striatum, VTA, and substantia nigra, with sparse postsynaptic labeling in dendrites and spines (Rommelfanger *et al*, 2009). These findings revealed that α1ARs in the NAc were enriched in axons and axon terminals, but their specific neurochemical phenotype and functional relevance was not identified.

To expand on these initial findings, we undertook a detailed anatomical analysis of the ultrastructural organization of α1ARs in the NAc, PFC, and VTA using EM immunocytochemical approaches. To understand the functional relevance of these α1ARs, we used a combination of *in vivo* microdialysis with a simple drug-induced behavior (locomotor activity) following local infusion of the α1AR antagonist, terazosin, as a readout for mesolimbic transmission.

MATERIALS AND METHODS

Animal Treatment for Behavioral Experiments

Procedures were approved by the Animal Care and Use Committee of Emory University and the University of Chicago. Male Sprague–Dawley rats were individually housed in a temperature-controlled environment on a 12-hr light/dark cycle with *ad libitum* access to food and water. Experiments were performed during the light cycle between 1000 and 1600 hours.

Cannula Surgery for Intracranial Infusions

Rats were anesthetized with a ketamine (60–100 mg/kg)/xylazine (0.1 mg/kg) cocktail. Bilateral guide cannulae were implanted overlying the medial NAc shell or VTA by 2 mm. The medial shell was chosen due to prominent projections from noradrenergic nuclei to the medial NAc shell (Berridge *et al*, 1997; Delfs *et al*, 1998). Coordinates for the medial NAc shell were AP = +1.3, ML = ±2.5, DV = −7.1, angled at 10°. Coordinates for VTA were AP = −4.8, ML = ± −1.0, DV = −7.0 (Paxinos and Watson, 1998).

Infusion Procedures

Subjects were restrained and artificial cerebrospinal fluid (aCSF) or the selective α1AR antagonist terazosin (3 μg/0.5 μl/side; Sigma-Aldrich, St Louis, MO) (Kyncl, 1986; Stone *et al*, 2001) were administered over a 3 min period using 5-μl Hamilton microsyringes. Doses of terazosin chosen were reported to blunt psychostimulant-induced behaviors, but not impair basal motor behavior (Stone *et al*, 2005; Alsene *et al*, 2010).

Locomotor activity

Drug-naïve subjects ($n = 5$–8 per group) were placed in locomotor activity chambers (San Diego Instruments, San Diego, CA) for 1 h, then infused intracranially with aCSF or terazosin and injected with saline, morphine (5 mg/kg, ip), or cocaine (15 mg/kg, ip). Ambulations (consecutive beam breaks) were recorded. Preliminary experiments showed that cocaine primarily increased locomotion during the first 30 min, whereas morphine increased locomotion during the second hour of the session (data not shown). Thus, we analyzed the effects of terazosin during the first 30 min following cocaine administration and the last 60 min following morphine administration.

Animal Treatment for Immunocytochemistry

Fourteen male, adult Sprague–Dawley rats (200–300 *g*) were anesthetized with a ketamine/medetomidine cocktail, transcardially perfused, and brain tissue was cut and prepared with $NaBH_4$ and cryoprotectant for immunocytochemistry experiments as described previously (Mitrano and Smith, 2007).

Primary Antibodies

Table 1 describes all primary antibodies and peptides and their concentrations used in this study. The specificity of each of these antibodies has been characterized previously

Table 1 Antibodies Used for Immunocytochemistry Experiments

Antigens	Immunogen	Manufacturer data	Dilution used
Alpha-1 adrenergic receptor	Synthetic peptide corresponding to residues K(339)FSREKKA KT(349) of 3rd intracellular loop of human alpha 1AR	Thermo Scientific, Pierce Antibodies, Rabbit Polyclonal, #PA1-047	1 : 1000
vGluT1	Clone N28/9; Produced against fusion protein amino acids 493–560 of C-terminus of rat vGluT1	UC Davis/NIH NeuroMab Facility, Mouse Monoclonal #75-066	1 : 5000
vGluT2	Produced against amino acids 565–582 of rat vGluT2	Millipore Guinea Pig Polyclonal (#AB5907; No longer available)	1 : 5000
TH	Clone LNC1; Tyrosine hydroxylase purified from PC12 cells	Millipore, Mouse Monoclonal, #MAB318	1 : 1000
GABA	γ-aminobutyric acid conjugated to BSA	Sigma, Rabbit Polyclonal, #A2052	1 : 1000
Alpha-1 adrenergic receptor peptide	Synthetic peptide: FSREKKAAKT (for pre-adsorption experiments; 5 mg/ml)	Peptide 2.0 (custom made)	1 : 25

(Dallvechia-Adams *et al*, 2002; Montana *et al*, 2004; Nakadate *et al*, 2006; Masilamoni *et al*, 2010), and pre-adsorption of the α1AR antibody with the synthetic α1AR peptide eliminated all labeling (data not shown).

Single Immunoperoxidase Labeling for α1ARs for Light Microscopy

Sections were pre-incubated for 1 h as described previously (Mitrano and Smith, 2007), followed by primary antibody solutions overnight and then secondary biotinylated goat anti-rabbit IgGs (1 : 200; Vector Laboratories, Burlingame, CA). Next, sections were incubated with the avidin-biotin-peroxidase (ABC) complex (1 : 100; Vector Laboratories), transferred to a 3,3′ diaminobenzidine tetrahydrochloride (DAB) solution (Sigma), mounted on slides and scanned with the Aperio ScanScope (SS5115). Micrographs were taken with ImageScope software (Version 10.2.0.0) and compiled into figures using Adobe Illustrator (version 11.0, Adobe Systems, San Jose, CA).

Single Pre-embedding Immunogold Labeling for α1ARs for EM

Sections were pre-incubated for 30 min in PBS containing 5% dry milk at room temperature (RT) (Mitrano and Smith, 2007). Sections were transferred to primary antibody solutions for the α1AR overnight at RT and treated for 2 h with secondary goat anti-rabbit IgGs conjugated with 1.4 nm gold particles (1 : 100; Nanoprobes, Yaphank, NY), followed by silver intensification of gold particles, osmification, dehydration and embedding procedures described previously (Mitrano and Smith, 2007). Tissue samples of the different brain regions (Figure 1) were chosen from areas corresponding to the following stereotaxic coordinates: NAc core and medial shell (AP coordinates between: +1.7 and +1.2), PFC (prelimbic, layers IV and V; AP: +3.7 and +3.2), and VTA (AP: −5.6 and −5.8; Paxinos and Watson, 1998). They were then cut from the larger sections, mounted onto resin blocks, and cut into 60-nm sections using an ultramicrotome (Leica Ultracut T2). The shell and core of the NAc were differentiated based on previous studies using Calbindin-D_{28k} (Meredith *et al*, 1996; Mitrano and Smith, 2007). The 60-nm sections were collected on Pioloform-coated copper grids and examined on the Zeiss EM-10C.

Analysis of Single Immunogold Labeling

Data were collected from 16 blocks (from four animals) of α1AR-immunostained NAc core, shell, VTA, and PFC tissue as described above. Approximately 40 electron micrographs of randomly selected immunoreactive elements were taken at ×31 500 and saved with a CCD camera (DualView 300W; Gatan, Pleasanton, CA) controlled by DigitalMicrograph software (version 3.10.1, Gatan). The total amount of tissue examined was: 940.39 μm^2 in the NAc core, 869.54 μm^2 in the shell, 966.15 μm^2 in the VTA, and 979.03 μm^2 in the PFC. Labeled elements were categorized as dendrites, spines, unmyelinated axons, axon terminals, and glia as described by Peters *et al* (1991). Spines are usually mushroom-shaped and have a visible postsynaptic density apposed to an axon terminal, identified by the presence of vesicles. Unmyelinated axons are small, regular, circular elements that are relatively smooth in shape, travel straight in the neuropil when seen in longitudinal plane, often contain tubules, and are frequently clustered, forming a bundle. Dendrites display different sizes and shapes, contain mitochondria, microtubules, stacks of endoplasmic reticulum and often receive synaptic contacts. Glial plexesses are usually thin, have an irregular shape, are not found in bundles, and often display a tortuous trajectory across the neuropil.

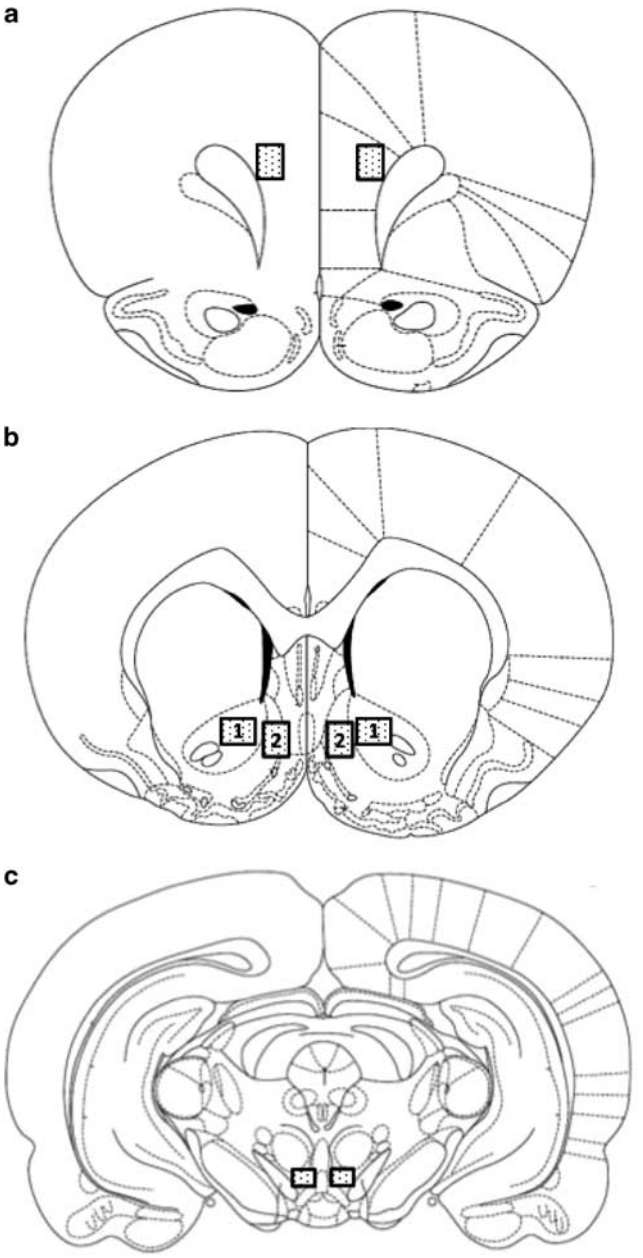

Figure 1 Schematic of brain regions analyzed in immunocytochemical studies. Shown are the (a) PFC (AP: +3.2), (b) NAc (AP: +1.6; 1 = core, 2 = shell), and (c) VTA (AP: −5.8). 1 = core, 2 = shell). Shaded boxes indicate approximate location of tissue samples analyzed. Adapted from Paxinos and Watson (1998).

Gold particles were classified as either intracellular or plasma membrane-bound (PMB; Mitrano and Smith, 2007). PMB gold particles were further classified as perisynaptic, synaptic, or extrasynaptic (Mitrano and Smith, 2007).

Digitally acquired micrographs were adjusted for brightness or contrast using either the DigitalMicrograph or Adobe Photoshop software (version 8.0, Adobe Systems), and then compiled into figures using Adobe Illustrator.

Double Pre-embedding Immunogold for α1ARs and Immunoperoxidase Labeling for Neurotransmitter Markers (TH, vGluT1, and vGluT2) for EM

Sections were treated as described above and transferred to solutions that contained a mixture of the α1AR antibody and one of the neuronal markers (TH, vGluT1, and vGluT2). Silver intensification, ABC, and DAB procedures were performed as described above. Immediately following the DAB reaction, sections were subjected to osmification, dehydration, and resin embedding protocol as described previously (Mitrano and Smith, 2007).

Analysis of Double Pre-embedding Labeling

Data were collected from a total of 48 blocks. Four animals were used for each of the neuronal markers. Approximately 40 electron micrographs of randomly selected α1AR-immunoreactive elements were taken at 31 500 ×. The total amount of tissue examined was: TH: NAc core: 2061.12 μm^2, shell: 2093.33 μm^2, VTA: 1977.39 μm^2, and PFC: 798.68 μm^2; vGluT1: core: 1357.63 μm^2, shell: 1250.64 μm^2, VTA: 1043.44 μm^2, and PFC: 1345.04 μm^2; and vGluT2: core: 998.36 μm^2, shell: 1011.24 μm^2, VTA: 1062.77 μm^2, and PFC: 1062.77 μm^2. Labeled elements were categorized as described above. The number of axon terminals containing immunogold labeling for the α1AR and the percentage that also contained immunoperoxidase labeling for each of the markers (TH, vGluT1 or vGluT2) were determined.

Double Pre-embedding Immunoperoxidase for the α1AR and Post-embedding Immunogold Labeling for GABA for EM

Sections were incubated in primary and secondary antibody solutions for the α1AR, identical to those used for light microscopy, with the exception that incubation in primary antibody lasted for 48 h at 4 °C.

After the DAB reaction, the tissue was rinsed, treated with osmium, and dehydrated. Following dehydration, sections were treated, embedded, and baked as described previously with the exception of placing the 60-nm sections on Pioloform-coated gold grids. Post-embedding procedures have been described previously (Dallvechia-Adams *et al*, 2002), taking note that secondary goat anti-rabbit IgGs were conjugated with 15 nm (1 : 50; BBInternational, Cardiff, UK). The grids were then stained with 1% uranyl acetate (in dH_20), rinsed, and examined at the EM.

Analysis of GABA Post-embedding Labeling

Tissue was examined from four animals, from each of the four regions, totaling 16 blocks. Approximately 40–50 micrographs of α1AR immunoreactive axon terminals were taken at 31 500 ×. Total amounts of tissue examined were: 1190.88 μm^2 in the core, 1165.11 μm^2 in the shell, 1387.47 μm^2 in the VTA, and 1107.85 μm^2 in the PFC. The determination of whether or not a terminal was positive for GABA has been described previously (Dallvechia-Adams *et al*, 2002). The percentage of α1AR-immunoreactive terminals that were GABAergic was calculated with the aid of ImageJ software (NIH).

In Vivo Microdialysis

Bilateral guide cannulae were implanted in the medial NAc shell as described above. Procedures for the day before the experiment were performed as described previously (Vezina *et al*, 2002; Kim *et al*, 2005). The next day, infusion rates increased to 1.5 μl/min, and samples were collected every 20 min for 1 h (baseline), followed by reverse dialysis of terazosin (10 or 100 μM) or vehicle for 3 h. Cocaine (15 mg/kg, ip) was administered 1 h after the start of vehicle/terazosin ($n = 6$–8 per group). Terazosin doses were reported to blunt α1AR agonist-induced neuronal activation and psychostimulant-induced DA overflow, but not impair basal neuronal activation or extracellular DA (Stone *et al*, 2006). After 5–7 days, a second microdialysis experiment was performed in the same animals with the probe inserted contralaterally. Probe placement was verified histologically.

Chromatography

DA and glutamate chromatography was performed as described previously (Kim *et al*, 2005). Extracellular concentrations, corrected for individual probe recoveries, were estimated from peak areas using EZChrom Elite (Agilent Technologies, Santa Clara, CA) and Shimadzu CLASS-VP (Shimadzu Scientific Instruments, Columbia, MD) software, respectively. Probe recoveries were determined *in vitro* and ranged from 6–11%.

Data analysis. Data were analyzed by ANOVA followed by Bonferroni or Tukey's *post hoc* tests using Prism 4.0 for Macintosh.

RESULTS

Intra-NAc, but not intra-VTA Administration of Terazosin Attenuates Drug-induced Locomotion

Locomotor activity was recorded following infusion of aCSF or terazosin (3 μg/0.5 μl/side) into the medial NAc shell or VTA and administration of saline, cocaine (15 mg/kg, i.p.), or morphine (5 mg/kg, ip). For the NAc, two-way ANOVA showed a main effect of treatment ($F(1, 24) = 13.87$, $p < 0.01$) and a pre-treatment x treatment interaction ($F(1, 24) = 3.56$, $p < 0.05$) on cocaine-induced locomotion, and a main effect of treatment ($F[1, 24] = 12.9$, $p < 0.01$) and pre-treatment ($F(1, 24) = 12.9$, $p < 0.01$) for morphine-induced locomotion. Tukey's *post hoc* analysis revealed that intra-NAc terazosin attenuated the locomotor activating effects of both drugs (Figures 2a and b). By contrast, there was only a main effect of treatment in the VTA for cocaine ($F(1, 19) = 30.4$, $p < 0.0001$) and morphine ($F(1, 23) = 31.28$, $p < 0.0001$); Tukey's *post hoc* analysis revealed that intra-VTA infusion of terazosin had no effect on cocaine- or morphine-induced locomotion (Figures 2c and d).

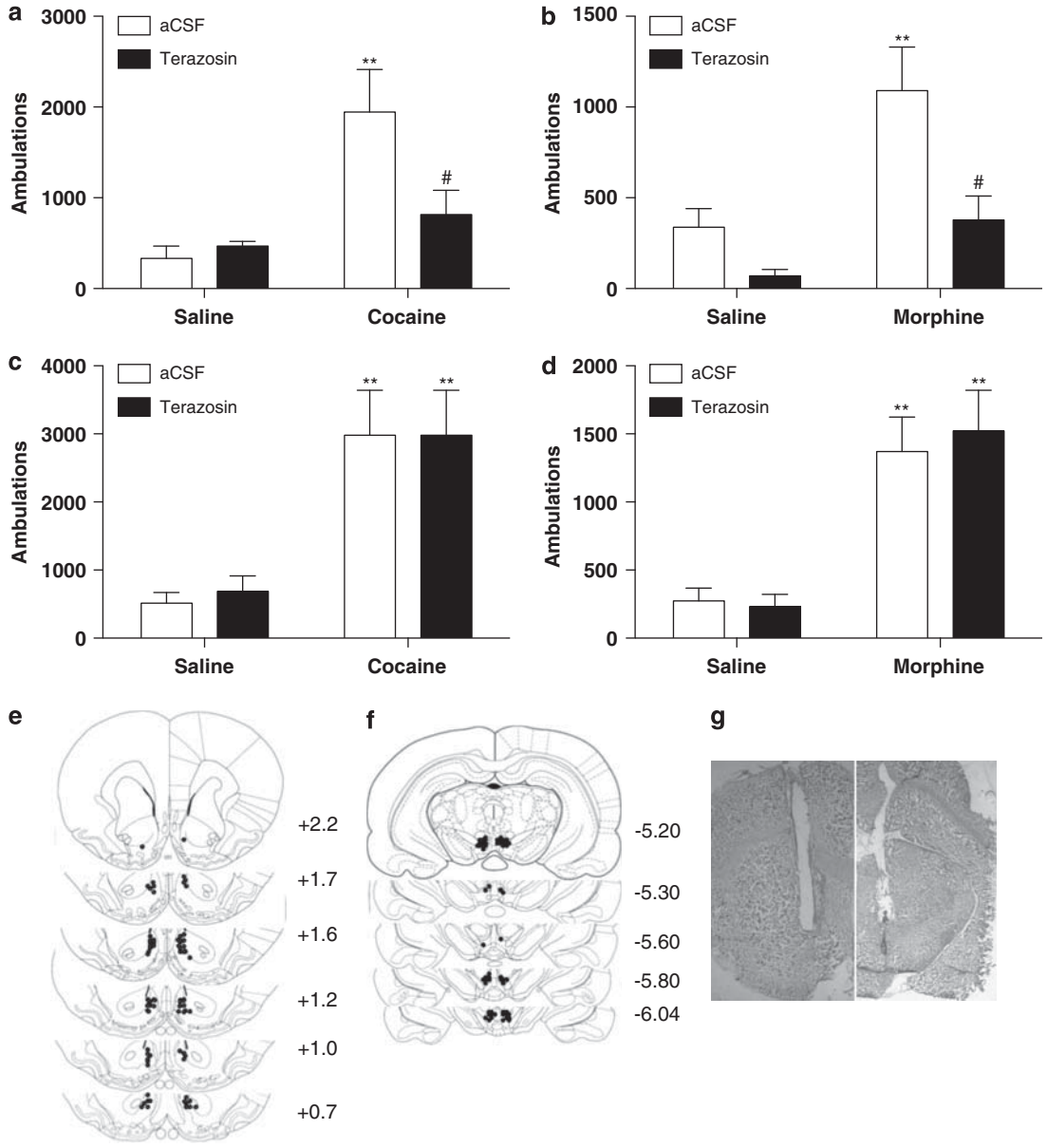

Figure 2 Blockade of α1ARs in the NAc, but not VTA, attenuates morphine- and cocaine-induced locomotion. aCSF or terazosin (3 μg/0.5 μl/side) was infused into the NAc shell (a and b) or the VTA (c and d), followed by systemic administration of saline or cocaine (15 mg/kg, i.p.; a and c), or saline or morphine (5 mg/kg, i.p.; b and d). Shown are the total ambulations (mean ± SEM) during the first 30 min following cocaine administration (a and c), and the total locomotor activity over the second hour following morphine administration (b and d). Also shown are schematic representations of cannula placements for the NAc (e) and VTA (f), and sample photomicrographs of cannula tracts (g) for the NAc (left panel) and VTA (right panel). $n = 5$–8 per group. $^{**}p < 0.01$ compared to aCSF-saline group; $^{\#}p < 0.05$ compared to aCSF-cocaine/aCSF-morphine group.

Immunogold Labeling for α1ARs Reveals Primarily Presynaptic Localization

Using immunoperoxidase labeling of α1ARs within the mesolimbic DA system, we showed previously that α1ARs are localized primarily in axons and axon terminals (Rommelfanger *et al*, 2009). We used immunogold labeling to further assess the subcellular and subsynaptic localization of α1ARs in the NAc, VTA, and PFC. Across all brain regions examined, the majority of immunogold particles were found pre-synaptically (unmyelinated axons and axon terminals; Figure 3a). Consistent with our previous findings using immunoperoxidase immunocytochemistry (Rommelfanger *et al*, 2009), glial expression of the α1ARs was sparse, comprising only 1–5% of the total elements examined in the NAc, VTA, and PFC.

Approximately 60% of the α1ARs in axons were associated with the plasma membrane, whereas 20–40% of α1ARs in other elements were plasma membrane-bound (Figure 3b). A two-way ANOVA showed significant effects of element ($F(3, 48) = 314.2$; $p < 0.001$), brain region ($F(3, 48) = 62.79$; $p < 0.001$), and an element x brain region interaction ($F(9, 48) = 14.71$; $p < 0.001$). *Post hoc* analysis revealed that unmyelinated axons displayed significantly higher percentages of PMB gold particles compared with dendrites, spines, and axon terminals, whereas spines and axon terminals had significantly higher percentages of PMB gold particles compared with dendrites.

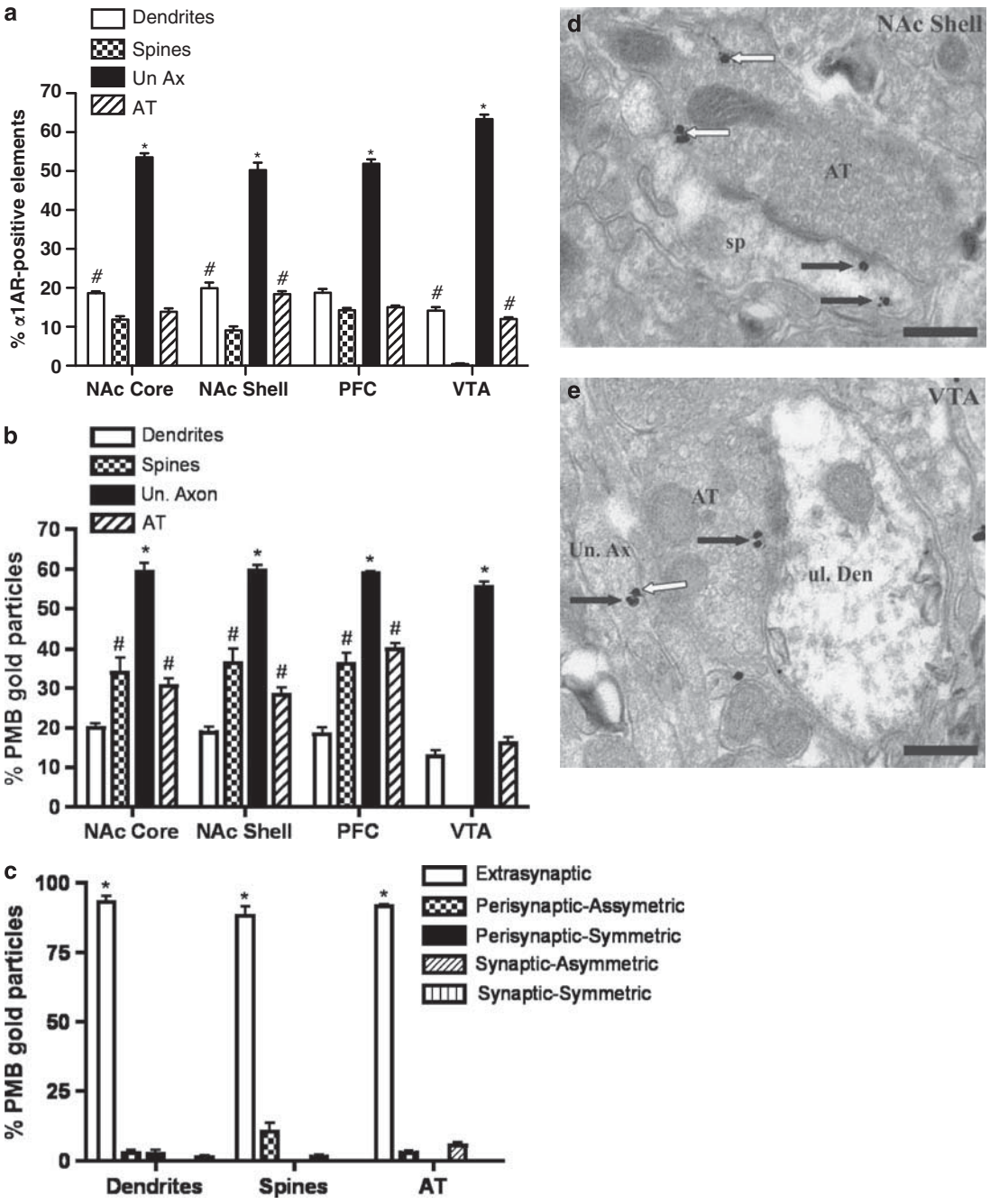

Figure 3 Ultrastructural localization of α1ARs within the mesocorticolimbic system. (a) Percentage of gold particle immunolabeling (mean ± SEM) for the α1AR on the various neuronal elements in the NAc core, NAc shell, PFC, and VTA. $^{*}p<0.001$ compared to all other elements in that brain region, $^{\#}p<0.01$ compared to spines in that brain region. (b) Relative percentage of plasma membrane-bound (PMB) immunogold labeling (mean ± SEM) for the α1AR in various immunoreactive neuronal elements in the NAc core, NAc shell, PFC, and VTA. $^{*}p<0.001$ compared to all other elements in that brain region, $^{\#}p<0.01$ compared to dendrites in that brain region. (c) Subsynaptic distribution of PMB immunogold labeling (mean ± SEM) for α1AR in various immunoreactive neuronal elements in the NAc core. The same overall pattern of labeling was found in the NAc shell, PFC, and VTA (data not shown). $^{*}p<0.001$ compared to all other subsynaptic categories. (d and e) illustrate representative electron micrographs of α1AR immunogold labeling in an axon terminal (AT) in the NAc shell (d) and VTA (e). A labeled spine (sp) and a labeled unmyelinated axon (Un. Ax) are also depicted in (d) and (e), respectively. White arrows indicate extrasynaptic PMB gold particles, and black arrows indicate intracellular gold particles. AT, axon terminal; PMB, plasma membrane-bound; sp, spine; Un. Ax, unmyelinated axon; ul. Den, unlabeled dendrite. Scale bars: 0.25 μm. $n=4$ rats.

The PMB gold particle distribution on dendrites, spines, and axon terminals was further classified by synapse type and proximity to the active zones. α1ARs were almost exclusively extrasynaptic, with <10% of these receptors found peri-synaptically or directly associated with the synaptic active zones of either asymmetric or symmetric specializations. As the data were almost identical for all brain regions examined, only one representative graph of the NAc core is presented (Figure 3c). Two-way ANOVA showed a main effect of location ($F(4,45)=2182$, $p<0.001$), and a location x brain region interaction ($F(8,45)=4.30$, $p<0.01$). *Post hoc* analysis revealed that all neuronal elements had a

significantly higher percentage of extrasynaptic α1AR labeling compared with peri-synaptic or synaptic localization at either asymmetric or symmetric synapses.

Neurotransmitter Phenotype of α1AR-expressing Axon Terminals

To determine the neurotransmitter phenotype of α1AR-expressing axon terminals, we used double immunoperoxidase and immunogold labeling for α1AR and either vGluT1 (glutamatergic terminals originating primarily from the PFC), vGluT2 (glutamatergic terminals originating primarily from the thalamus), tyrosine hydroxylase (TH; catecholaminergic terminals), or GABA (GABA terminals) (Fremeau *et al*, 2001; Varoqui *et al*, 2002; Raju and Smith, 2005). Although TH labels both noradrenergic and dopaminergic elements, α1ARs are not expressed by noradrenergic neurons (eg, Pieribone *et al*, 1994). Thus, any pre-synaptic elements that are positive for both TH and α1ARs are considered dopaminergic. The results of these double labeling experiments are shown in Figure 4. There was some degree of co-localization between α1ARs and all other markers in each brain region analyzed. The total extent of co-localization with all markers in each brain region was close to 100%, indicating that most α1AR-expressing terminals are glutamatergic, dopaminergic, or GABAergic. Two-way ANOVA showed a main effect of neurotransmitter phenotype ($F(3, 48) = 22.42$, $p < 0.0001$) and a neurotransmitter phenotype x brain region interaction ($F(9, 48) = 12.03$, $p < 0.0001$). *Post hoc* analysis revealed a significantly higher degree of α1AR co-localization with vGluT1 compared with all other markers in the NAc core and PFC. By contrast, the degree of α1AR co-localization with GABA was significantly higher compared with all other markers in the VTA. The degree of α1AR co-localization with TH was significantly lower compared with all other markers in the PFC and compared with vGluT1 in the VTA. All markers showed similar degrees of co-localization with α1ARs in the NAc shell. The chemical phenotype of α1AR-expressing unmyelinated axons could not be determined accurately, owing to the low level of immunoreactivity in these elements for the different neurotransmitter markers used.

Intra-NAc Administration of Terazosin Attenuates Local Cocaine-induced DA Overflow

Given the localization of α1ARs to pre-synaptic dopaminergic elements in the NAc and the ability of local α1AR blockade to attenuate cocaine-induced locomotor activity, we tested the effects of intra-NAc terazosin on local basal and cocaine-induced DA overflow by microdialysis. One hour following baseline dialysate collection, vehicle or terazosin (10 or 100 μM) was infused into the medial NAc shell by reverse dialysis, and cocaine (15 mg/kg, ip) was administered systemically 1 h later. Terazosin had no effect on baseline extracellular DA levels (Figure 5a). Although cocaine significantly elevated extracellular DA in the medial NAc shell in each treatment group, both doses of intra-NAc terazosin attenuated the effects of cocaine (Figure 5a). Two-way repeated measures ANOVA showed a significant effect of time ($F(11, 198) = 15.44$, $p < 0.0001$) and a time x treatment interaction ($F(22, 198) = 1.98$, $p < 0.01$). *Post hoc* analysis revealed that both the 10-μM and the 100-μM concentration of terazosin significantly attenuated the effects of cocaine during the first 2 time bins following cocaine administration.

Neither Systemic Administration of Cocaine nor Intra-NAc Administration of Terazosin Alters Extracellular Glutamate Levels

As α1ARs were also enriched on glutamatergic terminals in the NAc, we tested the effects of intra-NAc terazosin on extracellular glutamate levels at baseline and following cocaine administration. There was a main effect of time; glutamate levels decreased over the course of the experiment ($F(11, 198) = 6.52$, $p < 0.0001$). However, neither of the drug treatments significantly altered extracellular glutamate levels in the NAc (Figure 5b).

DISCUSSION

When infused into the medial NAc shell, but not the VTA, the α1AR antagonist, terazosin, blunted cocaine- and morphine-induced locomotion and cocaine-induced increases in local extracellular DA. The enrichment of these receptors on pre-synaptic glutamatergic, dopaminergic, and GABAergic elements suggests that α1ARs facilitate drug-induced behaviors by modulating the release of other neurotransmitters within the mesocorticolimbic system.

The Noradrenergic System Modulates Mesolimbic DA Transmission and Responses to Drugs of Abuse via α1ARs

The locus coeruleus (LC), A1, and A2 brainstem noradrenergic nuclei project to and facilitate mesocorticolimbic DA transmission and behavioral responses to drugs of abuse via activation of α1ARs (eg, Weinshenker and Schroeder, 2007; Smith and Aston-Jones, 2008; Mejias-Aponte *et al*, 2009). For example, genetic or pharmacological blockade of α1ARs impairs psychostimulant- and opiate-induced DA release in the NAc, locomotor activity, conditioned place preference, and re-instatement of drug seeking (eg, Darracq *et al*, 1998; Drouin *et al*, 2001; Drouin *et al*, 2002; Auclair *et al*, 2002; Weinshenker *et al*, 2002; Zhang and Kosten, 2005; Alsene *et al*, 2010). Although α1ARs are found throughout the mesocorticolimbic system that underlies many responses to addictive drugs (eg, Rainbow and Biegon, 1983; Jones *et al*, 1985; Rommelfanger *et al*, 2009), only those α1ARs in the PFC have been studied extensively in this context. Local depletion of NE or infusion of an α1AR antagonist in the prelimbic portion of the PFC attenuates drug-induced locomotion, sensitization, and conditioned place preference (Darracq *et al*, 1998; Drouin *et al*, 2001; Ventura *et al*, 2003, 2005, 2007).

By contrast, very little is known about the contribution of α1ARs in the NAc or VTA to mesocorticolimbic function. Infusion of an α1AR antagonist into the NAc attenuates novelty-induced locomotor activity and amphetamine-induced deficits in pre-pulse inhibition (Stone *et al*, 2004; Alsene *et al*, 2010). We targeted the medial NAc shell for our behavioral and neurochemical experiments because it receives the densest noradrenergic innervation and contains the highest levels of basal and psychostimulant-evoked increases of extracellular NE in the striatum (Berridge *et al*, 1997; Delfs *et al*, 1998; McKittrick and Abercrombie, 2007).

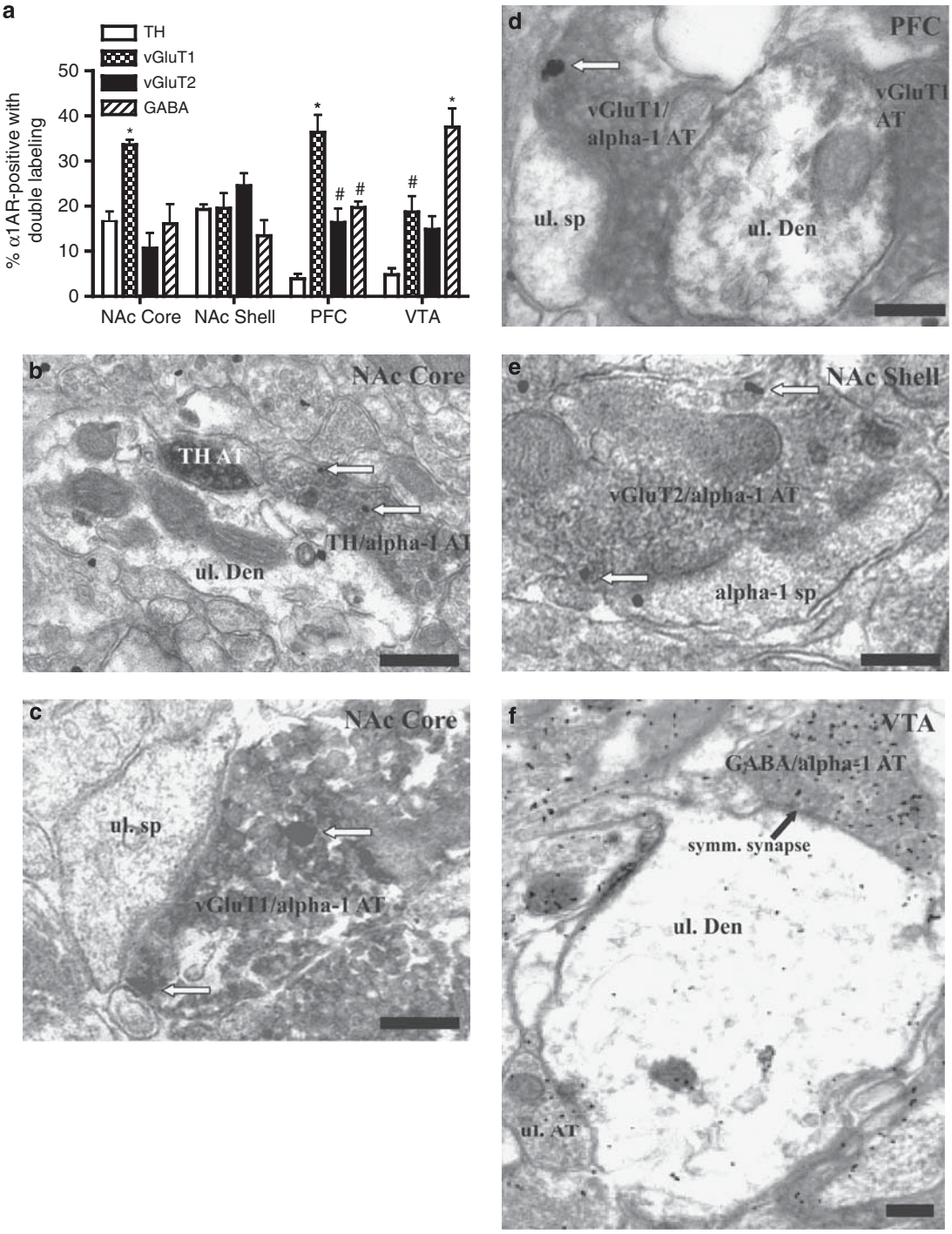

Figure 4 Neurotransmitter phenotype of α1AR-immunoreactive axon terminals. (a) The percentage (mean ± SEM) of α1AR-labeled axon terminals that also contain labeling for TH, vGluT1, vGluT2, or GABA. $^{*}p < 0.001$ compared to all other markers in that brain region, $^{\#}p < 0.05$ compared to TH in that brain region. The micrographs shown in panels (b–f) depict examples of double labeled elements. Panels B-E show immunogold labeling for the α1AR (white arrows) and immunoperoxidase labeling for TH (b), vGluT1 (c and d), and vGluT2 (e) in the indicated brain regions. The micrograph in panel (f) is an example of α1AR immunoperoxidase labeling with postembedding immunogold for GABA. AT, axon terminal; sp, spine; symm. synapse, symmetric synapse; ul. Ax, unmyelinated axon; ul. AT, unlabeled axon terminal; Un. Den, unlabeled dendrite; ul. sp, unlabeled spine. Scale bars: 0.25 μm. $n = 4$ rats.

The data from the present study indicate that activation of α1ARs in the medial NAc shell, but not the VTA, are required for cocaine-induced DA overflow in the medial NAc shell and the full locomotor response to cocaine and morphine. Although we cannot exclude the possibility that the terazosin is diffusing from the NAc and acting on neighboring brain structures that regulate mesolimbic DA transmission, this seems unlikely based on other studies showing behavioral effects of terazosin in the mouse NAc but not in adjacent areas (Stone *et al*, 2006). Although the LC innervates both cortical and subcortical structures, such as the PFC and VTA, the medial NAc shell receives

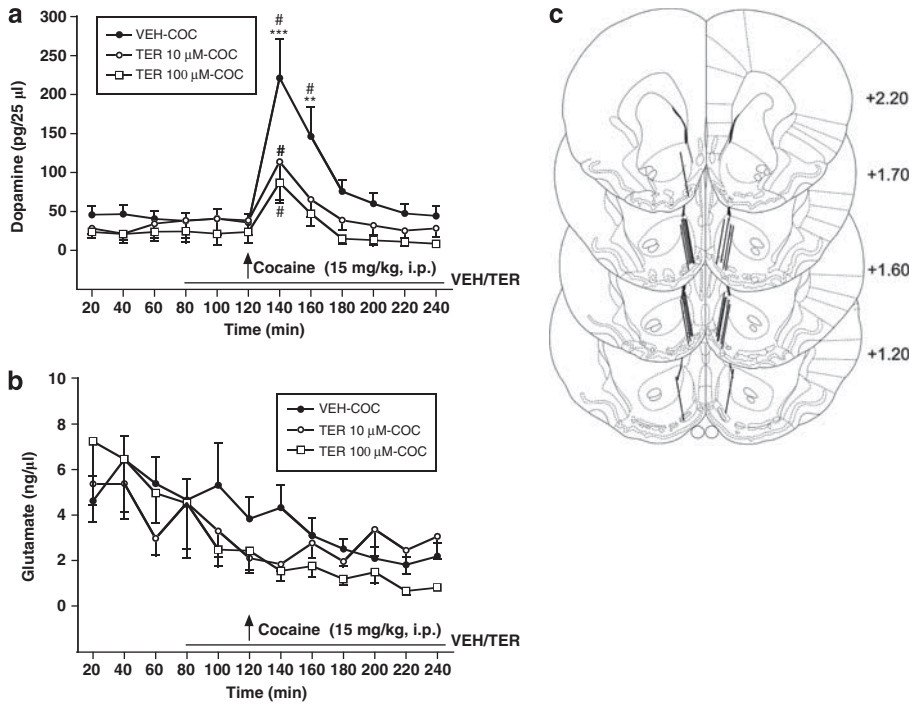

Figure 5 Blockade of α1ARs in the NAc attenuates cocaine-induced DA overflow. Following collection of baseline microdialysis samples, vehicle (VEH) or terazosin (TER; 10 or 100 μM) was infused into the NAc shell by reverse dialysis (lines at abscissae), followed by systemic administration of cocaine (15 mg/kg, i.p.) 1 h later (arrows), and dialysate was collected for an additional 2 h. Shown are mean ± SEM dopamine (a) and glutamate (b) levels, and the probe placements (c) in the NAc. $^{\#}p<0.05$ compared to baseline for that treatment group, $^{***}p<0.001$ compared to either terazosin group, $^{**}p<0.05$ compared to TER 10 μM, $p<0.01$ compared to TER 100 μM. $n=6$–8 per group.

most of its noradrenergic input from the A2 brainstem noradrenergic cell group (Swanson and Hartman, 1975; Morrison *et al*, 1981; Berridge *et al*, 1997; Delfs *et al*, 1998; Liprando *et al*, 2004; Mejias-Aponte *et al*, 2009). These findings are consistent with our earlier data showing that morphine activates A2 noradrenergic neurons, in contrast to its classic inhibitory effect on LC neurons, and noradrenergic projections from A2 are critical for morphine-induced locomotion and conditioned place preference (Olson *et al*, 2006). The role of α1ARs in the NAc core, which receives very little noradrenergic innervation (Berridge *et al*, 1997; Delfs *et al*, 1998) is unclear. The α1ARs in this region could be activated by sparse local NE release or NE released from adjacent areas via volume transmission/diffusion. DA, which has been shown to activate α1ARs under some conditions (eg, Lin *et al*, 2008), could be acting as an α1AR ligand in the NAc core.

Neuroanatomical and Neurochemical Substrates Underlying α1AR-mediated Responses to Drugs of Abuse

It has been established previously that α1ARs in the PFC are critical for DA release in the NAc neurochemical and behavioral responses to drugs of abuse; blockade of α1ARs in the PFC attenuates cocaine- and morphine-induced increases in subcortical DA release and locomotor activity. The data presented here reveal that the NAc itself is another neuroanatomical target through which α1ARs could mediate mesolimbic DA transmission and some of the behavioral effects elicited by psychostimulants and opiates.

α1ARs in the PFC. Activation of α1ARs increases excitatory postsynaptic current frequency in layer V pyramidal cells of the PFC, and this effect is blocked by an AMPA antagonist, indicating that the effects of α1ARs are mediated via glutamate (Marek and Aghajanian, 1999). We found that α1ARs are enriched in vGluT1-positive axons and axon terminals, suggesting that activation of α1ARs on local glutamatergic terminals in the PFC could mediate this excitation. Glutamate transmission in the PFC is dispensable for the acute locomotor effects of psychostimulants and opiates, but is critical for behavioral sensitization to these drugs. Although we observed very little co-localization between α1ARs and TH in the PFC, local α1AR activation does appear to enhance DA release in this brain region (Sommermeyer *et al*, 1995; Pan *et al*, 2004).

α1ARs in the NAc. Our data indicate that in the NAc, α1ARs are enriched presynaptically in axon and axon terminal presynaptic elements, suggesting that α1ARs act primarily by controlling the release of other neurotransmitters, rather than directly affecting the excitability of intrinsic accumbal neurons. Furthermore, α1ARs are co-localized in these presynaptic elements with markers for several neurotransmitters known to have a role in drug-induced behaviors, such as DA and glutamate, being particularly enriched in vGluT1-positive axons and axon terminals originating from the PFC. DA transmission in the NAc is required for locomotor responses to psychostimulants and opiates, and we found that cocaine-induced DA overflow in the medial NAc shell was attenuated by local infusion of terazosin, which is consistent with our finding that α1ARs are found in

TH-positive terminals. Terazosin infusion had no effect on extracellular glutamate levels, although we did find a significant decrease in extracellular glutamate over time in all treatment groups, which has been observed by others (eg, Pierce *et al*, 1996) and could hamper interpretation of our data. However, it is well-established that acute administration of drugs of abuse to naïve subjects does not typically increase glutamate release in the NAc (eg, Pierce *et al*, 1996; Meyer *et al*, 2009). Modulation of glutamate transmission by α1ARs may be critical for more complex addiction-like phenotypes, such as re-instatement of drug seeking, which does require glutamate release from PFC terminals in the NAc (see below for further discussion).

α1ARs in the VTA. Given the profound effect of α1AR blockade on VTA neuron firing, the inability of intra-VTA terazosin to alter cocaine- or morphine-induced locomotor activity was somewhat surprising. Stimulation of the LC in rats increases burst firing of VTA DA neurons, whereas α1AR blockade suppresses burst firing (Grenhoff and Svensson, 1989; Grenhoff *et al*, 1993; Grenhoff and Svensson, 1993). In slices, stimulation of α1ARs depolarizes VTA cells on its own and increases amphetamine-induced DA neuron activity, even when synaptic transmission is blocked (Grenhoff *et al*, 1995; Paladini *et al*, 2001); however, α1AR activation also appears to have direct and indirect inhibitory effects on VTA neurons (Paladini and Williams, 2004). α1AR activation in VTA slices increases the frequencies of inhibitory postsynaptic potentials (IPSPs) in a tetrodotoxin-sensitive manner, suggesting the involvement of interneuron excitation and GABA release onto DA neurons (Grenhoff *et al*, 1995). This inhibitory effect of α1AR activation on VTA firing is consistent with the high prevalence of α1AR co-localization in GABA terminals described in the present study. Thus, α1AR modulation of synaptic transmission in the VTA is clearly complex.

α1AR Subsynaptic Localization Throughout the Mesocorticolimbic System. α1ARs were mainly associated with the extrasynaptic plasma membrane in axons and localized intracellularly in dendrites, spines, and axon terminals. The extrasynaptic pattern of labeling on axons is consistent with what has been reported for other G-protein coupled catecholamine receptors such as DA receptors (Yung *et al*, 1995), and probably reflects the fact that catecholamines signal via 'volume transmission' rather than via classical synaptic transmission (reviewed by Fuxe *et al*, 2010). The high degree of intracellular labeling for the α1AR is also common for G-protein coupled receptors, and may represent receptors traveling to the membrane after synthesis or trafficking to and from the membrane during de-sensitization–re-sensitization (Gainetdinov *et al*, 2004). There is also mounting evidence that internalized receptors have unique signaling capabilities (Calebiro *et al*, 2010).

Conclusion

In this study, we present evidence that local α1ARs on dopaminergic terminals in the medial NAc shell regulate functional mesolimbic neurotransmission, including drug-induced changes in extracellular DA levels and locomotor activity. Because we used a simple, acute behavioral paradigm that is not associated with changes in glutamate, the contribution of α1ARs to glutamate transmission remains unknown. Future research will focus on the role of α1ARs within the mesocorticolimbic circuit that may contribute to some of the more complex behaviors associated with chronic drug administration and withdrawal that require DA and/or glutamate. For example, blockade of α1ARs attenuates psychostimulant- and opiate-induced sensitization (Drouin *et al*, 2001, 2002; Weinshenker *et al*, 2002; Auclair *et al*, 2004; Jiménez-Rivera *et al*, 2006), drug-primed reinstatement of cocaine, and nicotine seeking (Zhang and Kosten, 2005; Forget *et al*, 2010), the ability of cocaine pre-exposure to enhance subsequent cocaine self-administration (Zhang and Kosten, 2007), and the escalation of drug intake in the extended-access model of cocaine and heroin self-administration (Wee *et al*, 2008; Greenwell *et al*, 2009). Although the neuroanatomical and neurochemical substrates underlying these effects of α1AR antagonism have not been identified, our data point to a contribution of alterations in drug-induced DA and/or glutamate release in the PFC and NAc, and potentially the VTA.

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DISCLOSURE

The authors declare no conflict of interest.

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