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Light and Electron Microscopic Localization of Alpha-1 Adrenergic Receptor Immunoreactivity in the Rat Striatum and Ventral Midbrain

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Abstract

Electrophysiological and pharmacological studies have demonstrated that alpha-1 adrenergic receptor (α1AR) activation facilitates dopamine (DA) transmission in the striatum and ventral midbrain. However, because little is known about the localization of α1ARs in dopaminergic regions, the substrate(s) and mechanism(s) underlying this facilitation of DA signaling are poorly understood. To address this issue, we used light and electron microscopy immunoperoxidase labeling to examine the cellular and ultrastructural distribution of α1ARs in the caudate putamen, nucleus accumbens, ventral tegmental area, and substantia nigra in the rat. Analysis at the light microscopic level revealed α1AR immunoreactivity mainly in neuropil, with occasional staining in cell bodies. At the electron microscopic level, α1AR immunoreactivity was found primarily in presynaptic elements, with scarce postsynaptic labeling. Unmyelinated axons and about 30–50% terminals forming asymmetric synapses contained the majority of presynaptic labeling in the striatum and midbrain, while in the midbrain a subset of terminals forming symmetric synapses also displayed immunoreactivity. Postsynaptic labeling was scarce in both striatal and ventral midbrain regions. On the other hand, only 3–6% of spines displayed α1AR immunoreactivity in the caudate putamen and nucleus accumbens,. These data suggest that the facilitation of dopaminergic transmission by α1ARs in the mesostriatal system is probably achieved primarily by pre-synaptic regulation of glutamate and GABA release.

Keywords

norepinephrine; Parkinson's disease; addiction; dopamine; axon; electron microscopy

Introduction

Norepinephrine (NE) is a monoamine neurotransmitter that is synthesized in distinct brainstem nuclei that project widely throughout the brain. The mesolimbic (ventral tegmental area, VTA; nucleus accumbens, NAc), and nigrostriatal (substantia nigra, SN; caudate putamen, CP) dopamine (DA) systems receive noradrenergic innervation. The locus coeruleus (LC), A1 and A2 noradrenergic nuclei project directly to midbrain DA neurons and to the dorsal and ventral striatum (Glowinski and Iversen, 1966, Lindvall and Bjorklund, 1974, Liprando et al., 2004),

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where in general, NE facilitates DA transmission (reviewed by Weinshenker and Schroeder, 2007). For example, stimulation of the locus coeruleus (LC), the major brainstem noradrenergic nucleus, promotes burst firing of midbrain DA neurons (Grenhoff et al., 1993), while pharmacological or genetic NE depletion diminishes basal and evoked striatal DA release (Russell et al., 1989, Lategan et al., 1990, Schank et al., 2006).

Of the three classes of adrenergic receptors (Gilsbach and Hein, 2008), α1-adrenergic receptor (α1AR) activation likely accounts for the majority of the noradrenergic influence on DA transmission. The increase in DA neuron burst firing induced by LC stimulation can be blocked by systemic administration of the α1AR antagonist prazosin (Grenhoff and Svensson, 1993). Pharmacological or genetic blockade of α1ARs can also attenuate psychostimulant-induced locomotor activity and DA release in the NAc (Darracq et al., 1998, Weinshenker et al., 2002, Auclair et al., 2004). While the importance of α1AR activation in the prefrontal cortex for the regulation of striatal DA release has been well described (Blanc et al., 1994, Darracq et al., 1998), local α1ARs in the mesolimbic and nigrostriatal pathways also contribute to DA transmission and behavior. For instance, the stimulation of DA neuron burst firing by NE in midbrain slices can be mimicked by the α1AR agonist phenylephrine and blocked by the α1AR antagonist prazosin (Grenhoff et al., 1993, Grenhoff and Svensson, 1993). Direct infusion of prazosin into the striatum reduces basal extracellular DA levels (Sommermeyer et al., 1995), and intra-VTA infusion of prazosin attenuates the facilitation of DA release in the NAc caused by intra-VTA infusion of amphetamine (Pan et al., 1996). Finally, direct infusion of NE into the NAc induces locomotor activity in rats, while infusion of the α1AR antagonist into the NAc attenuates exploratory activity in mice (Svensson and Ahlenius, 1982, Stone et al., 2004), and striatal NE loss exacerbates L-DOPA-induced dyskinesias in DA-depleted rats (Fulceri et al., 2007).

Despite these behavioral, pharmacological, and electrophysiological data showing a significant regulatory influence of NE on the dopaminergic system, the interpretation of these functional studies remains speculative because very little information is available on the precise localization of α1ARs within the mesolimbic and nigrostriatal systems. While α1ARs are detectable in the NAc using radioligand binding and in the dorsal striatum by *in situ* hybridization, α1AR mRNA has not been found in accumbal neurons (Young and Kuhar, 1980, Rainbow and Biegon, 1983, Jones et al., 1985, Morrow and Creese, 1986, Day et al., 1997, Domyancic and Morilak, 1997). Furthermore, neither radioligand binding nor *in situ* hybridization have reliably detected α1ARs in midbrain DA neurons (Jones et al., 1985, Palacios et al., 1987, Pieribone et al., 1994), although the α1bAR was detected in the VTA using laser capture microdissection and expression microarray analysis (Greene et al., 2005). Although informative, the functional significance of these localization studies is limited by the lack of data on the exact sites of α1AR protein expression in the mesolimbic and nigrostriatal DA systems. Therefore, in order to further understand the potential substrate that underlies NE-mediated regulation of DA transmission, we used specific antibodies at the electron microscopic level (Nakadate et al., 2006) to characterize the ultrastructural localization of α1ARs in the rat mesolimbic and nigrostriatal DA pathways.

Experimental Procedures

Animals and tissue preparation

Six male Sprague Dawley rats (250 g; 2 months old) were used for this study. All procedures were approved by the animal care and use committee of Emory University and conform to the U.S. National Institutes of Health guidelines. All rats were anesthetized with a cocktail of ketamine (60–100 mg/kg i.p.) and dormitor (0.1 mg/kg). Rats were transcardially perfused with cold oxygenated Ringer's solution, followed by a fixative containing 2% paraformaldehyde and 3.75% acrolein (or 4% paraformaldehyde and 0.1% glutaraldehyde) in phosphate buffer

(0.1 M; pH 7.4). After perfusion, brains were removed from the skull and postfixed in 2% (or 4%) paraformaldehyde for 24hrs, cut into 60 μm sections with a vibrating microtome, and stored in phosphate-buffered saline (PBS) to 4°C until processing for immunocytochemistry. Prior to immunocytochemical processing, a series of sections including the caudate putamen, nucleus accumbens shell and nucleus accumbens core or the ventral tegmental area and substantia nigra were placed in a 1% sodium borohydride solution for 20 minutes, then washed with PBS.

Alpha-1 receptor antiserum

Commercially available polyclonal antibodies to the α1AR (Affinity BioReagents Inc.; Golden, CO, USA) were used at a concentration of 1:1000 as described in the original manuscript that published data on the synthesis and specificity of this antiserum (Nakadate et al., 2006). The antibody was raised against the amino acids 339–349 (FSREKKAAK) of the 3rd intracellular loop of the human α1AR, which is common to all alpha-1 receptor subtypes, but is not found in other GPCRs (Stewart et al., 1994, Schwinn et al., 1995, Strausberg et al., 2002). Western blots in rat brain tissue identified a single band between 70–80 kDa (Nakadate et al., 2006), which was eliminated by preadsorption with preincubation of a synthetic peptide corresponding to the α1AR antibody epitope.

Immunoperoxidase labeling for electron microscopy

After sodium borohydride treatment, sections were placed in cryoprotectant solution for 20 minutes (PB 0.05 M, pH 7.4, 25% sucrose, 10% glycerol), frozen at −80°C for 20 minutes, returned to a decreasing gradient of cryoprotectant solutions, and rinsed in PBS. Sections were then pre-incubated for 1 hour at room temperature (RT) in PBS containing 10% normal goat serum (NGS), 1% bovine serum albumin (BSA), and then followed by incubation in primary antibody solution containing 1% NGS, 1% BSA, and the α1AR antibodies (1:1000) for 48 hours at 4°C. After 3 rinses in PBS, sections were incubated in secondary biotinylated goat anti-rabbit IgGs (1:200 dilution; Vector Laboratories, Burlingame, CA) for 90 minutes. The sections were rinsed again in PBS and then incubated for another 90 minutes with the avidin-biotin peroxidase complex (ABC) at a dilution of 1:100 (Vector Laboratories). Then, sections were washed in PBS and Tris buffer (50 mM; pH 7.6) and transferred to a solution containing 0.025% 3,3'-diaminobenzidine tetrahydrochloride (DAB; Sigma), 1 mM imidazole, and 0.005% hydrogen peroxide in Tris buffer for 10 minutes. Sections were then rinsed in PB (0.1 M, pH 7.4) and treated with 1% OsO_4 for 20 minutes. The tissue was then returned to PB and dehydrated with increasing concentrations of ethanol. With exposure to 70% ethanol, 1% uranyl acetate was added to the solution and the tissue was incubated for 35 minutes to increase the contrast of the tissue at the electron microscope. After dehydration, sections were treated with propylene oxide and embedded in epoxy resin for 12 hours (Durcupan ACM; Fluka, Buschs, Switzerland), mounted onto slides, and placed in a 60°C oven for 48 hours. Samples of the caudate putamen, nucleus accumbens shell and core, ventral tegmental area, and substantia nigra were mounted onto resin blocks, and cut into 60 nm sections with an ultramicrotome (Leica Ultracut T2). The 60nm sections were collected on Pioloform coated copper grids, stained with lead citrate for 5 minutes to enhance tissue contrast, and examined on the Zeiss EM-10C electron microscope. Electron micrographs were taken and saved with a CCD camera (DualView 300W; Gatan, Inc., Pleasanton, CA) controlled by DigitalMicrograph software (version 3.10.1; Gatan, Inc.). Some of the digitally acquired electron micrographs were adjusted only for brightness and/or contrast in either the DigitalMicrograph software or Adobe Photoshop 8.0. Micrographs were then compiled to figures using Adobe Illustrator 11.0.

Data Analysis

Data were collected from a total of 27 blocks of tissue, i.e. 1 block/animal in each brain region from 5–6 rats. About 100 electron micrographs of randomly selected immunoreactive elements were digitized at X25,000 from each rat per brain region, which resulted in a total tissue surface of 19,851 μm^2 for the caudate putamen, 30,332 μm^2 for the nucleus accumbens shell, 29,946 μm^2 for the nucleus accumbens core, 25,502 μm^2 for the VTA and 28,159 μm^2 for the SN. Labeled elements were categorized as dendrites, spines, axons, axon terminals and glia on the basis of ultrastructural features described by Peters and colleagues (Peters et al., 1991). The density of labeled elements was calculated by dividing the number of elements labeled by the total area of tissue examined. Significant differences were assessed by using SigmaStat software for one-way ANOVA and Tukey's posthoc test. The density of labeled elements was compared within brain regions. The percentage of labeled terminals forming asymmetric synapses and the percentage of labeled spines in the nucleus accumbens and caudate putamen were compared between brain regions by determining the proportion of labeled and unlabeled terminals involved in asymmetric synaptic contact or spines in ultrathin sections collected from the surface of striatal and accumbens tissue with complete antibody penetration. One more block was taken in CP, VTA and SNc to examine the general pattern of labeling in immunoreactive neuronal cell bodies.

Results

Light Microscopy

At the light microscopic level, the immunoperoxidase labeling in both striatal and ventral midbrain regions was mainly associated with fine punctate structures. In both regions, neuronal cell body labeling was scarce, except for the cell bodies of a small proportion of large striatal interneurons and putative dopaminergic neurons in the SNc that displayed low to moderate immunoreactivity. In contrast, putative striatal projection neurons and cells in the substantia nigra pars reticulata (SNr) did not display a significant level of immunoreactivity (Fig. 3A). The rich neuropil labeling is consistent with the large number of immunoreactive unmyelinated axons seen at the electron microscope level (see below).

Electron Microscopy: Caudate Putamen and Nucleus Accumbens

In general, α1AR labeling in both the nucleus accumbens (NAc) and caudate putamen (CP) was localized primarily in presynaptic elements including unmyelinated axons and axon terminals. To a much lower extent, labeling was also detected in postsynaptic structures such as dendrites and spines, but these accounted for less than 5% of total labeled neuronal elements. In both regions, sparse labeling was found in glia (Fig. 1B–F, Fig. 2A–C). Labeling in axons was significantly greater than in any other elements identified in the NAc shell ($F_{(4,25)}$ =78.258, $p<0.001$; Fig. 2B), NAc core ($F_{(4,25)}$ =52.958, $p<0.001$; Figs 2B,C) and CP ($F_{(4,20)}$ =76.782, $p<0.001$; Fig. 2A). In the NAc shell, but not the core or the CP, the prevalence of terminal labeling was significantly greater than in spines or glia ($F_{(4,25)}$ =78.258, $p<0.001$; Fig. 2B). In other striatal regions, no significant difference was found between the relative abundance of labeled terminals, dendrites, spines and glia (Figs. 2A–C). The majority of immunoreactive terminals displayed the ultrastructural features of putative glutamatergic boutons. In total, 771 terminals were identified in the CP, 1054 in the NAc shell, and 1152 in the NAc core. In the CP, 456 of identified terminals formed a clear asymmetric synapse and 130 of these (~30%) displayed α1AR immunoreactivity (Fig. 1B,C,Fig. 2). This proportion was significantly higher in the NAc shell (221 labeled of 455 identified asymmetric synapses; $F_{(2,14)}$ =4.823, p=0.025 compared to CP) and core (320 labeled of 618 asymmetric synapses; $F_{(2,14)}$ =4.823, p=0.025 compared to CP; Fig. 2D). In contrast, a similar analysis of postsynaptic labeling in spines revealed that only about 4–7% of total spines in the CP and NAc displayed α1AR immunoreactivity (Fig. 2E). Spine labeling was found in both the neck and head of the labeled

structures (Figs. 1B,D). Dendritic labeling was very rare but when present, it was observed in medium and large shafts. Immunoperoxidase labeling in large cell bodies of putative CP interneurons was discrete and often associated with membrane of the endoplasmic reticulum (Fig. 5A, 5B). There was no evidence of labeling associated with the plasma membrane of immunoreactive cell bodies. Due to the small sample size that could be observed at the electron microscopic level, the number of labeled and unlabeled cell bodies was not quantified.

Electron Microscopy: Substantia Nigra and Ventral Tegmental Area

Like the CP and NAc, α1AR immunoreactivity in the SN and VTA was localized predominantly in presynaptic unmyelinated axons and axon terminals (Fig. 3,Fig. 4), and to a much lower extent in postsynaptic dendrites and glia (Fig. 4). In both structures, the labeling in axons was significantly greater than in any other elements identified ($F_{(3,16)}$ =106.286, $p<0.001$ in VTA; $F_{(3,16)}$ =251.788, $p<0.001$ in SN; Fig. 4). In contrast, no significant difference was found between the density of labeled terminals, dendrites, and glia (Fig. 4). Of the 714 identified terminals in the VTA and 597 terminals in SN, ~30% (210 labeled in the VTA and 184 labeled in the SN) displayed α1AR immunoreactivity. Although the synaptic specialization associated with many of these boutons could not be clearly seen in single sections, a small proportion of terminals could be categorized as forming symmetric axo-dendritic synapses (110 out of 714 terminals in VTA and 22 out of 597 terminals in SN) or asymmetric synapses (82 out of 714 terminals in VTA and 3 out of 597 terminals in SN). Of these terminals with clear symmetric or asymmetric synaptic specialization in the VTA, 30–50% (52 out of 110 symmetric and 26 out of 82 asymmetric) displayed α1AR immunoreactivity. In the SN, ~ 30% (8 out of 22) of identified terminals forming symmetric synapses displayed α1AR immunoreactivity, while none of the 3 terminals with clear asymmetric synapses contained immunoreactivity. Labeled dendrites were rare in both SN and VTA. The pattern of neuronal cell body labeling in these regions was the same as in the striatum, i.e. relatively discrete, patchy and associated predominantly with the endoplasmic reticulum (Fig. 5 C–F), without any significant relationship with the plasma membrane.

Discussion

To provide an anatomical basis for the modulation of DA transmission in the mesostriatal system by α1ARs, we characterized the cellular and ultrastructural localization of the α1AR in the CP, NAc, SN and VTA. At the light microscopic level, the α1AR labeling was mainly associated with punctate neuropil elements in both striatal and midbrain regions. A population of putative interneurons displayed cell body immunoreactivity in the CP and NAc, but medium spiny projection neurons were not significantly labeled. Blocks from SN were taken from the rich neuropil that contained SNr neurons and dendrites of SNc dopaminergic neurons. At the electron microscopic level, α1AR immunoreactivity was mainly located in presynaptic elements (unmyelinated axons and terminals), while sparse labeling was observed in dendrites, spines, neuronal cell bodies and glia. These results suggest that pre-synaptic regulation of excitatory (presumably glutamatergic) and inhibitory (putatively GABAergic) afferents comprises a significant component of the physiological effect of NE on DA transmission in the mesostriatal system.

Direct influence of α1ARs on DA neuron activity

Electrophysiological, pharmacological, and behavioral data indicate that NE signaling via α1ARs increases VTA and SN firing and facilitates DA transmission (Grenhoff and Svensson, 1993, Auclair et al., 2002, Auclair et al., 2004). NE also has direct α1AR-mediated effects on midbrain DA neurons. For instance, activation of α1ARs modulates activity of DA neurons in midbrain slice culture, an effect that was not attenuated by blockade of synaptic transmission with tetrodotoxin (Grenhoff et al., 1995). Another set of slice culture experiments revealed that

activation of α1ARs increases amphetamine-induced DA neuron activity by counteracting the inhibitory effect of mGluR1 activation (Paladini et al., 2001). This effect is also likely due to direct action on DA neurons, both because the noradrenergic drugs were applied iontophoretically, instead of being superfused in the bath, and because the effects persisted in the presence of TTX and in the absence of Ca^{2+}(Grenhoff et al., 1995, Paladini et al., 2001). Interestingly, this group subsequently identified an inhibitory effect of α1AR activation on DA neurons, which is in line with the expression of presynaptic α1ARs in putative inhibitory terminals reported in our study.

However, these results are somewhat difficult to reconcile with our findings that α1ARs are only sparsely expressed on dendrites of presumptive DA neurons in the midbrain. Although we did observe occasional cell body staining by light and electron microscopy, the functional role of α1ARs in these perikarya is unclear because at the electron microscopic level, the immunoreactivity was found to be intracellular, associated predominantly with the endoplasmic reticulum, but not with the plasma membrane. One previous study has demonstrated TH-lableled dendrites directly apposed to NE transporter (NET)-positive axons and terminals in the VTA (Liprando et al., 2004), suggesting that some of the dendritic α1ARs identified in our study could be located on DAergic dendrites in close contact with noradrenergic terminals, but this remains to be investigated in more detail. It is possible that the very small population of dendritic α1ARs on midbrain DA neurons can transduce a signal powerful enough to affect neuronal firing properties when maximally stimulated in slice culture, but may not be the primary site mediating the effects of NE on DA neuron activity *in vivo*. Albeit scarce, α1ARs on striatal dendrites and spines may be functional, as NE can modulate the activity of striatal medium spiny neurons in slices and culture (Nicola and Malenka, 1998, Geldwert et al., 2006).

Indirect influence of α1ARs on DA neuron activity

Although our data do not provide strong anatomical support for a direct effect of postsynaptic α1AR signaling on DA transmission, they rather suggest that the α1AR-mediated facilitation of DA transmission is most likely mediated by presynaptic effects of NE on glutamate and/or GABA signaling. We observed robust α1AR labeling in a significant proportion of putative glutamatergic terminals (i.e. formed asymmetric synapses) in the striatum and VTA, with less in the SN. Because the cerebral cortex is the main source of glutamatergic inputs to both the NAc and CP (Smith et al., 1994, Fremeau et al., 2004, Smith et al., 2004), the majority of α1AR-immunoreactive terminals in the striatum likely originate from corticostriatal axons. However, other sources such as the thalamus, amygdala and hippocampus must also be considered as being potential targets of NE-mediated presynaptic regulation, as well as the recently discovered vGlut2-positive neurons in the VTA (Yamaguchi et al., 2007). Glutamate is also important in the VTA for DA neuron excitability (Tzschentke and Schmidt, 2000, Grillner and Mercuri, 2002). Again, activity of cortical glutamatergic neurons has been implicated in the regulation of midbrain DA neuron firing, but the source of the locally released glutamate is unclear and may involve a relay nucleus (Carr and Sesack, 2000). Based on these results, we propose that NE modulates striatal DA transmission indirectly by stimulating α1ARs on glutamatergic terminals, which facilitates glutamate release and potentiates DA transmission. This interpretation is consistent with the findings that α1AR activation has an excitatory effect on pyramidal neurons in the prefrontal cortex and that local blockade of α1ARs attenuates DA release in the NAc (Sommermeyer et al., 1995, Marek and Aghajanian, 1998). However, double-label EM studies will be required to further characterize the sources and chemical phenotype(s) of the α1AR-containing axons and terminals in the ventral midbrain to confirm our hypothesis. We also observed α1AR expression in a significant proportion of presumptive inhibitory terminals that may be GABAergic (i.e. those which form symmetric

synapses) in the midbrain, which could contribute to the reported, but not extensively studied, inhibitory effect of α1ARs on midbrain DA neurons (Paladini and Williams, 2004).

Because of the sparse noradrenergic innervation of the striatum, our finding of significant α1AR immunoreactivity in this structure raises questions about the source of activation and function of α1ARs in this region. On the other hand, the ventral striatum, particularly the caudomedial nucleus accumbens, is densely innervated by noradrenergic fibers probably originating from the LC and nucleus tractus solitarius, and the tissue levels of NE approach those of DA in this brain region in both humans and rodents (Delfs et al., 1998, Tong et al., 2006b,a). Although tissue levels of NE in the dorsal striatum are very low compared to dopamine (~ 50–100 fold difference), basal extracellular NE levels are higher than expected (~ 20-fold lower than dopamine), suggesting that the noradrenergic terminals in the striatum are quite active (Gobert et al., 2004, Fulceri et al., 2007, Weinshenker and Schroeder, 2007). Most, if not all of the NE in the striatum comes from the LC, since 6-OHDA lesions of the LC severely reduce striatal NE levels (Fulceri et al., 2007).

Axonal localization of α1ARs

Consistent with previous electron localization studies of other G protein-coupled receptors, including DA receptors, metabotropic glutamate receptors, and $GABA_B$ receptors, the majority of pre-synaptic labeling for α1ARs was found in unmyelinated axons in both the striatum and ventral midbrain (Villalba et al., 2006, Dumartin et al., 2007, Mitrano and Smith, 2007). Although the functional significance of such axonal labeling remains unclear for most transmitter systems, it strongly suggests that the stimulation of such receptors relies on extrasynaptic spillover or volume transmission of the activating neurotransmitter. This mechanism of neural communication is consistent with the current view that NE is released by volume transmission and may stimulate non-synaptic receptors located far away from the transmitter release sites (Fuxe, 1991). Based on recent immunogold studies of other GPCRs showing that axonal immunoreactivity for D1, metabotropic glutamate receptor and $GABA_B$ receptor labeling is largely bound to the axonal plasma membrane (Villalba et al., 2006, Dumartin et al., 2007, Mitrano and Smith, 2007), it is reasonable to suggest that the α1ARs axonal immunoreactivity described in our study represents functional pre-synaptic NE receptors that could be activated by extracellular NE to regulate glutamate and GABA release in the striatum and ventral midbrain.

Localization of α1ARs in glia

Although NE is traditionally considered a neuron-to-neuron messenger in the central nervous system, NE has also been shown to promote communication between neurons and glia (Duffy and MacVicar, 1995). The localization, although sparse, of α1AR receptors in astrocytic processes is of interest given the recent evidence that glial cells actively participate in mediating responses to drugs of abuse (Narita et al., 2006). Intracellular Ca^{2+} release in glia can be initiated by glutamate and NE (Duffy and MacVicar, 1995), and α1ARs can regulate intracellular Ca^{2+} in cultures of striatal astrocytes (Delumeau et al., 1991a, Delumeau et al., 1991b, Giaume et al., 1991).

Implications for diseases of dopaminergic dysfunction

NE has been implicated in a number of diseases of dopaminergic dysfunction, such as Parkinson's disease (PD), drug addiction, and attention-deficit and hyperactivity disorder (ADHD). For example, severe LC degeneration and NE loss is observed clinically in PD and experimental models of PD have shown that LC or NE loss exacerbates PD-like motor abnormalities (Mavridis et al., 1991, Marien et al., 2004, Rommelfanger et al., 2004, Rommelfanger et al., 2007). The regulation of SN activity by α1ARs could account for these findings (Grenhoff and Svensson, 1993, Grenhoff et al., 1995). In addition, α1ARs in the

nigrostriatal DA system may be critical for the effects of L-DOPA treatment. For example, the α1AR antagonist prazosin attenuates some beneficial effects of L-DOPA in MPTP-treated Parkinsonian monkeys (Visanji et al., 2006, Fox, 2007). The subthalamic nucleus (STN), which has been a target for therapeutic lesioning and deep brain stimulation in PD (Krack et al., 1998, Limousin et al., 1998), may also be of interest for NE-mediated PD pharmacotherapy. α1AR immunoreactivity has been detected postsynaptically in STN cell bodies, and α1AR activation modulates the firing properties of STN neurons (Arcos et al., 2003, Belujon et al., 2007). A brief survey of STN tissue from rats used in the present study did reveal immunoperoxidase staining in this region (data not shown), but a more detailed examination of the subcellular localization of α1ARs in STN neurons will be required to clarify the substrate by which NE can mediate its functional effects in these cells.

α1ARs have also been implicated in the neural mechanisms that underlie behavioral and neurochemical changes in response to drugs of abuse in rodents (Drouin et al., 2002, Villegier et al., 2003, Auclair et al., 2004). Genetic or pharmacological blockade of α1ARs attenuates the effects of psychostimulants and opiates on DA release in the NAc, locomotor activity, sensitization, reward, the escalation of cocaine seeking produced by extended access to drug or prior cocaine exposure, and cocaine-induced reinstatement of drug seeking following extinction (Zhang and Kosten, 2005, Olson et al., 2006, Weinshenker and Schroeder, 2007, Zhang and Kosten, 2007, Wee et al., 2008). While the importance of prefrontocortical α1ARs for some of these phenomena has been established (Darracq et al., 1998), the prominent presynaptic expression of these receptors in the NAc and VTA suggests that local α1ARs also contribute to drug-induced changes in DA neurochemistry and behavior.

Abbreviations

CP, caudate putamen
NAc, nucleus accumbens
VTA, ventral tegmental area
SN, substantia nigra
LC, locus coeruleus
STN, subthalamic nucleus
TH, tyrosine hydroxylase
alpha1AR, alpha1 adrenergic receptor
ABC, avidin-biotin-complex
DA, dopamine
NE, norepinephrine
mGluR, metabotropic glutamate receptor
GABAR, gamma-amino-butyric acid receptor
PD, Parkinson’s disease
ADHD, Attention Deficit/Hyperactivity Disorder
PBS, phosphate buffered saline

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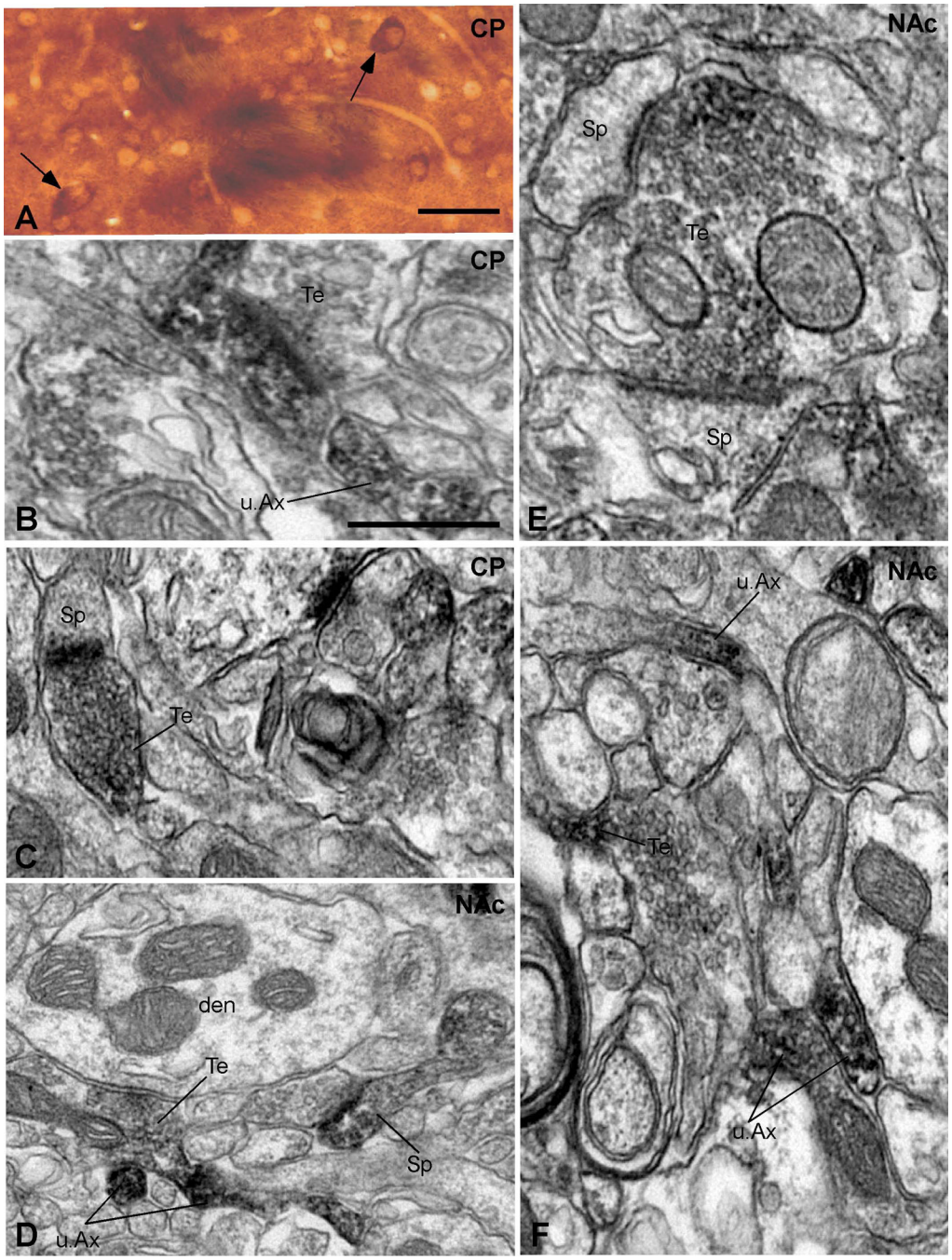

Figure 1. Representative examples of α1AR-immunoreactive elements in the rat caudate putamen and the nucleus accumbens

(A) Example of immunoreactivity seen in large neurons of the caudate putamen (CP). (B) Example of an immunoreactive spine (Sp) and immunoreactive unmyelinated axons (u.Ax) in the rat CP. The labeled spine is contacted by an unlabeled terminal (u.Te) forming an asymmetric synapse. (C) An immunoreactive terminal (Te) forming an asymmetric axo-spinous synapse in the CP. (D) Example of a labeled terminal (Te) that forms a symmetric axo-dendritic synapse, some immunoreactive unmyelinated axons (u.Ax) and a labeled spine (Sp) in the nucleus accumbens (NAc). (E) Example of an immunoreactive terminal (Te) forming

multiple asymmetric axo-spinous synapses in the NAc. (F) Immunoreactive unmyelinated axons (u.Ax) in the NAc. Scale bars: 0.5 μm.

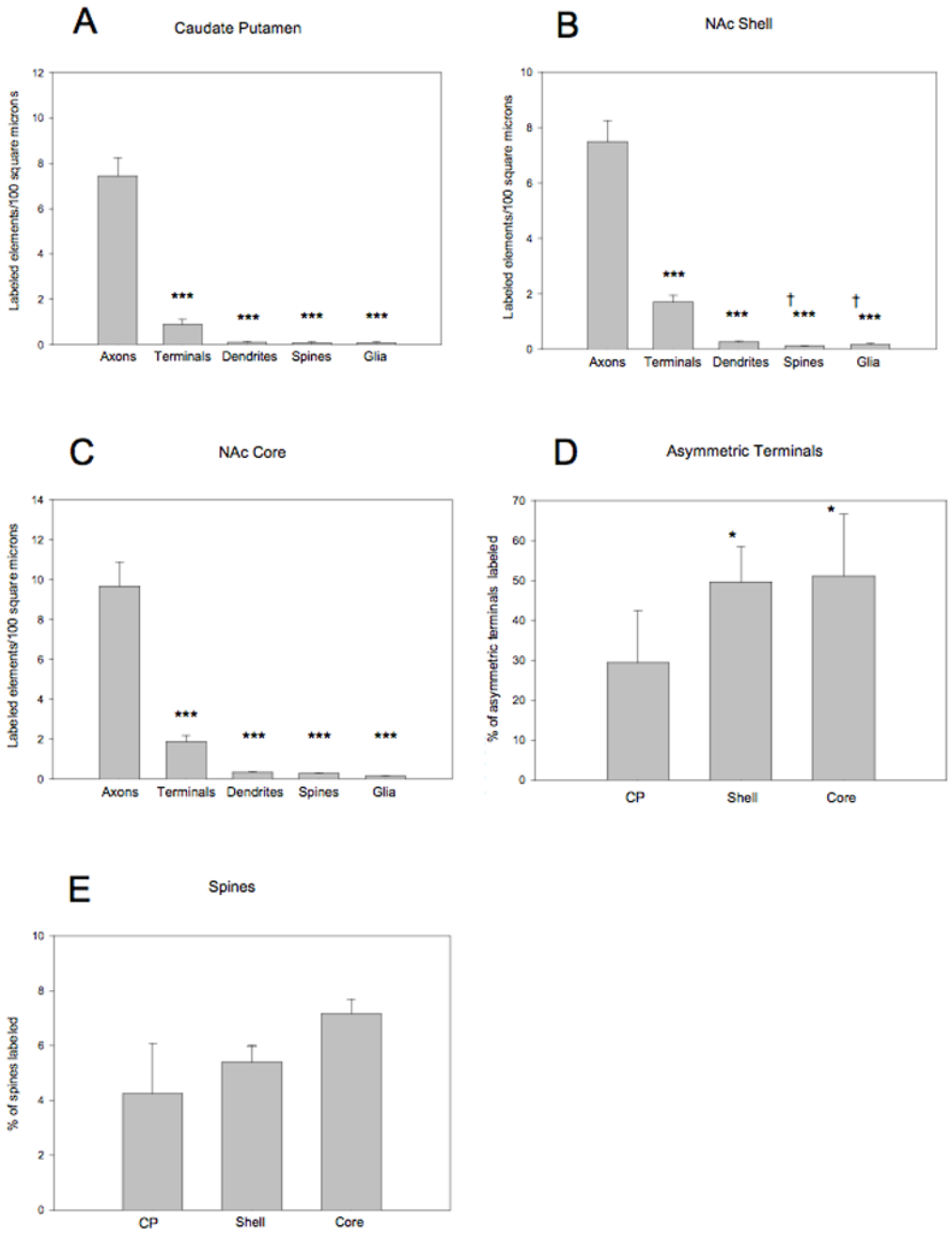

Figure 2. Quantification and Distribution of α1AR labeling in caudate putamen and nucleus accumbens

Histograms showing the density of α1AR-immunoreactive elements in the caudate putamen (CP) (A) and nucleus accumbens (NAc) shell (B) and core (C). ***p<0.001 compared to axons, †p<0.05 compared to terminals. N=5–6 animals per brain region. (D) Shows the percentage of total terminals forming asymmetric synapses that display α1AR immunoreactivity in the CP and NAc. (E) Illustrates the percentage of α1AR-immunoreactive spines in the CP and NAc. *p<0.05 compared to CP. N=94–114 micrographs from 5–6 animals for each brain region.

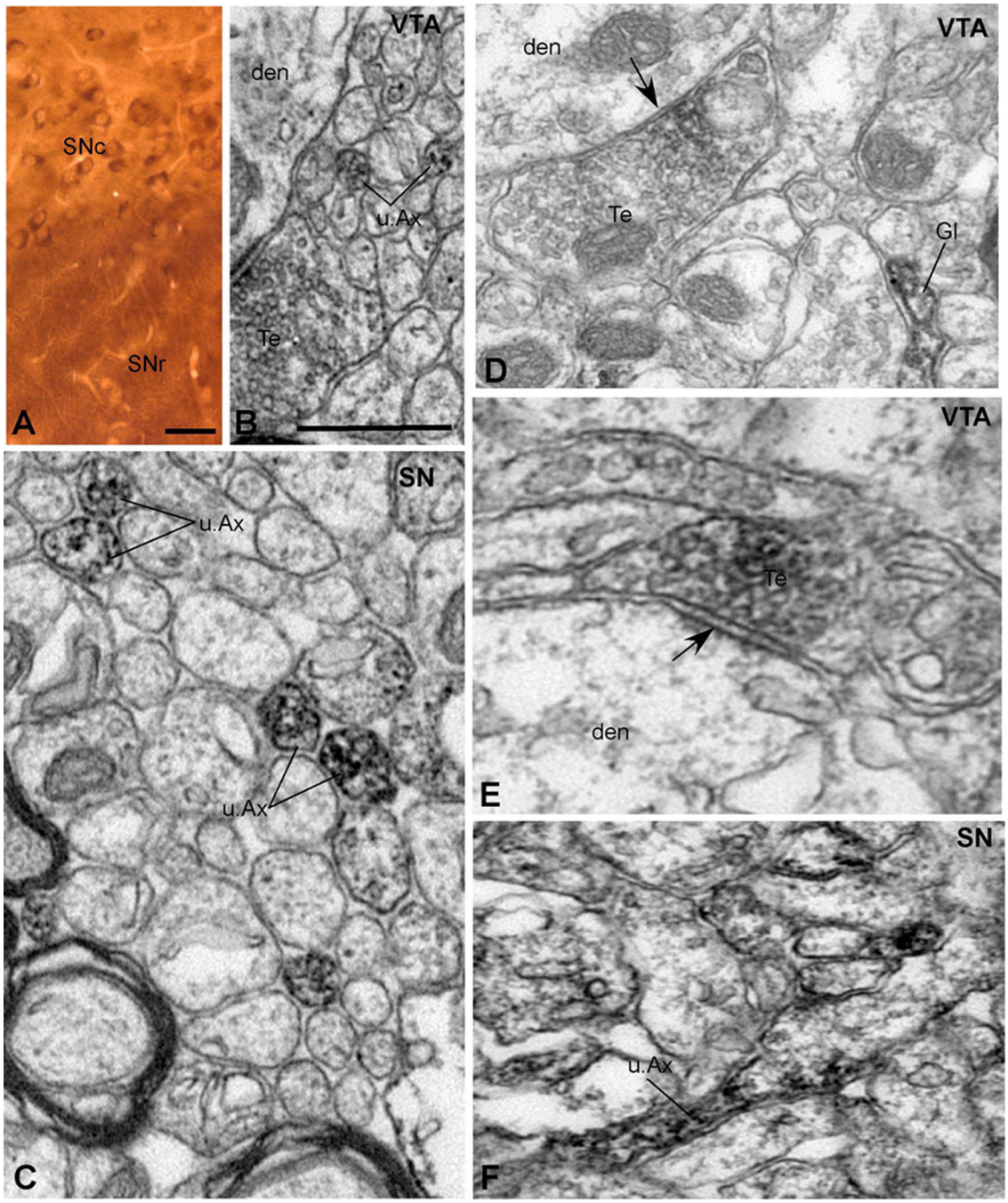

Figure 3. Representative examples of α1AR-immunoreactive elements in the substantia nigra and ventral tegmental area of rat

(A) Examples of immunoreactive neurons in the substantia nigra pars compacta (SNc), but not the substantia nigra pars reticulata (SNr) (B) Examples of labeled unmyelinated axons (u.Ax) and an axon terminal (Te) that forms an asymmetric axo-dendritic synapse in the ventral tegmental area (VTA). (D, E) Labeled boutons (Te) forming symmetric axo-dendritic synapses (arrow) in the VTA. An immunoreactive glial process (Gl) is also shown in (D). (C, F) Examples of immunoreactive unmyelinated axons (u.Ax) in the SN. Scale bars: 0.5 μm.

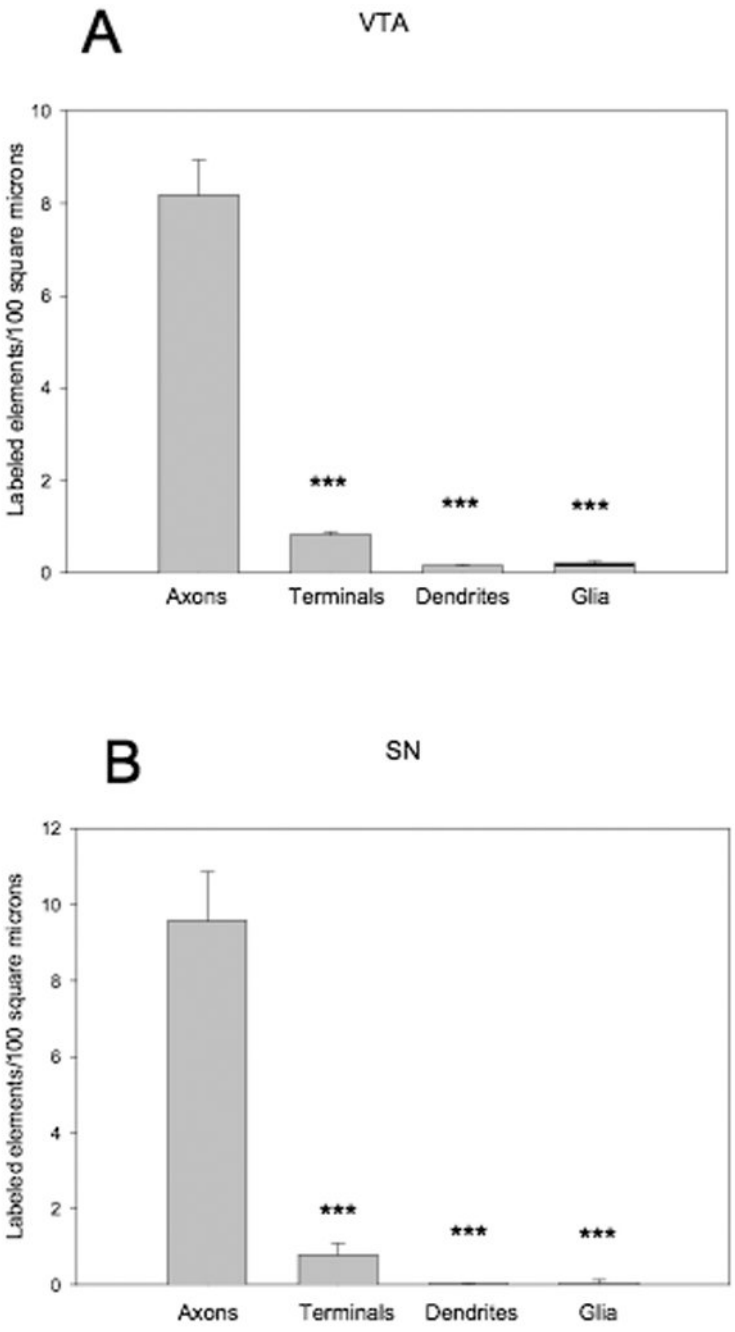

Figure 4. Quantification and distribution of α1AR labeling in ventral tegmental area and substantia nigra

Histograms showing the density of α1AR-immunoreactive elements in the ventral tegmental area (VTA) (A) and substantia nigra (SN) (B). ***$p<0.001$ compared to axons, †$p<0.05$ compared to terminals. N=5–6 animals per brain regions.

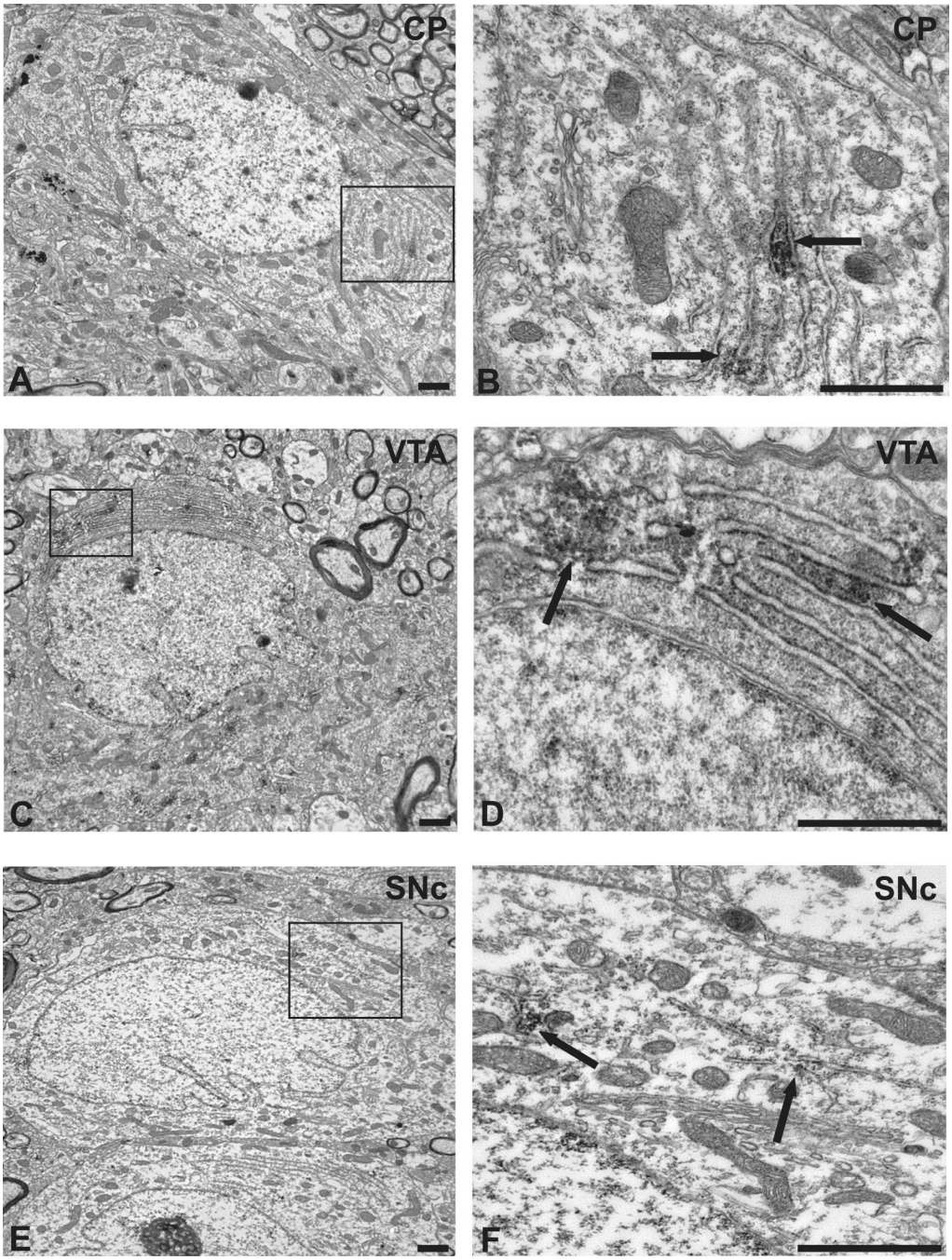

Figure 5. Representative examples of α1AR cell body labeling in the caudate putamen, ventral tegmental area, and substantia nigra

(A) Example of a labeled cell body in thecaudate putamen (CP) (magnification: 5000X). (B) Magnified image (20000X) of the area indicated in the square in (A). Note the labeling indicated by the arrows, adjacent to the endoplasmic reticulum (ER). (C) Example of a labeled cell body in the ventral tegmental area (VTA) (5000X). (D) Magnified image (25000X) of the area indicated in the square in (C) with labeling close to the ER, also indicated by the arrows. (E) Example of labeled cell bodies in the substantia nigra pars compacta (SNc) (5000X). (F) Magnified image (25000X) of area in the square of (E) with labeling close to the ER as shown by the arrows. Scale bars = 1 μm.