

Prefrontal Cortical Projections to the Midbrain in Primates: Evidence for a Sparse Connection

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Frontal cortical efferent fibers are thought to have important regulatory influence on cortico-basal ganglia (BG) circuits. The cortico-midbrain (substantia nigra/ventral tegmental area, SN/VTA) pathway has received particular attention in psychiatric diseases, most notably schizophrenia. Work in rodents demonstrates that the prefrontal cortico (PFC)-midbrain pathway plays a central role in regulating the firing pattern of dopamine (DA) neurons. These findings have led to some important hypotheses concerning PFC/BG interaction in schizophrenia. Descending PFC projections to the SN/VTA have been primarily documented in the rodent. The aim of this study was to determine the degree and organization of PFC afferents to these areas in the Macaque monkey. Anterograde tracer injections were made into discrete orbital, cingulate, and dorsolateral prefrontal areas. Projections were charted to the SN and VTA. Overall, there were very few fibers in the ventral midbrain following injections confined to specific areas of the PFC. To determine the relationship of the descending fibers to the midbrain DA neurons, sections were double stained for the tracer molecules and for tyrosine hydroxylase. In all cases, the prefrontal projections and the TH-positive cells did not appear to be in close juxtaposition. The results show a very limited projection from the PFC to the midbrain DA neurons in primates, terminating both within the SN proper as well as in the VTA. They arise from a broad region of the PFC, including the DLPF, cingulate, and orbital cortices. However, despite the relative lack of cortical input to the midbrain cells, these neurons are rich in glutamate receptors in primates. Thus, while, based on these anatomical studies, direct cortical control of DA neurons remains debatable in primates; the cortex may directly impact other sources of glutamatergic control.

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INTRODUCTION

Understanding how the midbrain dopamine (DA) neurons are regulated is critical for gaining insight into the reward system and into the abnormalities seen in diseases such as drug addiction and schizophrenia. Based on rodent studies, cortical control of the midbrain is one important mechanism by which glutamatergic input either directly or indirectly can drive or modulate DA cells (Maurice *et al*, 1999; Sesack and Carr, 2002). It has been demonstrated with microdialysis that release of DA in the medial striatum can be induced by the infusing the GABA-A receptor antagonist, bicuculline, into the prefrontal cortex (PFC). This effect can be blocked by

the application of excitatory amino-acid receptor antagonists in the ventral tegmental area (VTA) (Karreman and Moghaddam, 1996). More specifically, application of the selective AMPA/kainate antagonist, LY293558, to the VTA reduces DA release in the PFC, but increases DA levels in the nucleus accumbens (NAc) (Takahata and Moghaddam, 2000). These results support dual AMPA-receptor mediated regulation of DA in the VTA; inhibitory regulation of DA neurons projecting to the NAc and excitatory regulation of DA neurons projecting to the PFC. Anatomical studies show that afferents from the PFC innervate the VTA GABAergic cells that project to the NAc (Christie *et al*, 1985; Sesack *et al*, 1989a,b; Hurley *et al*, 1991; Sesack and Pickel, 1992a,b) and the VTA DA cells that project back to the PFC (Carr and Sesack, 2000a,b). In this way, blockade of excitatory stimulation from the PFC to the VTA can produce two opposite results: (1) an increase in DA in the NAc via a reduction in inhibitory GABA drive to the DA neuron that project to the NAc; and (2) a decrease in DA in the PFC via a reduction in excitatory drive to DA neuron that project back to the PFC (Sesack and Carr, 2002).

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This cortico-DA interaction supports the 'revised' DA hypothesis of schizophrenia (Weinberger, 1987; Davis *et al*, 1995). The 'classical' DA hypothesis proposed that there is a hyperactivity of DA transmission, which is responsible for the positive symptoms (Carlsson and Lindqvist, 1963; Seeman and Lee, 1975; Creese *et al*, 1976). Imaging studies in patients with schizophrenia show increased subcortical DA release in response to an amphetamine challenge and increased baseline subcortical DA levels (Laruelle *et al*, 1996; Breier *et al*, 1997; Abi-Dargham *et al*, 1998, 2000), lending additional support to the hypothesis of increased subcortical DA in schizophrenia. However, recent studies implicate a deficit in DA transmission at D₁ receptors in the dorsolateral prefrontal cortex (DLPFC) in the cognitive impairments present in patients with schizophrenia (Goldman-Rakic and Selemon, 1997; Goldman-Rakic, 1999; Goldman-Rakic *et al*, 2000). Impairment of higher cognitive functions, such as working memory, is one of the most enduring symptoms of schizophrenia and a strong predictor of poor clinical outcome (Green, 1996). Furthermore, the negative and cognitive symptoms of the illness are generally resistant to treatment by antipsychotic drugs (Keefe and Gerfen, 1999). Functional brain imaging studies suggesting that these symptoms are associated with a dysfunction of the DLPFC (Manoach *et al*, 1999, 2000; Callicott *et al*, 2000) are supported by non-human primates experiments that demonstrate a deficit in DA transmission in the DLPFC and a lack of D1 receptor stimulation induces cognitive impairments similar to those observed in patients (Goldman-Rakic and Selemon, 1997; Goldman-Rakic, 1999; Goldman-Rakic *et al*, 2000). Thus, the current view of DA and schizophrenia proposes that schizophrenia might be associated with a dopaminergic imbalance, involving an excess of subcortical DA function and a deficit in cortical DA function: DA projections from the midbrain (VTA and substantia nigra (SN)) to subcortical structures might be hyperactive, while mesocortical DA projections to the PFC might be hypoactive.

A general model, based on glutamate modulation of the midbrain DA neurons, for schizophrenia has been proposed by Carlsson *et al* (1999). This model provides the anatomical framework for the putative neurochemical abnormalities involved in the pathophysiology of schizophrenia including: a deficiency of glutamate transmission, a deficiency of cortical DA transmission and an excess of subcortical DA transmission. However, although the data describe above support cortical glutamatergic control of DA midbrain cells, a direct pathway has been difficult to demonstrate definitively in primates. Previous studies using either fiber degeneration techniques or tritiated amino-acid injections were unable to clearly demonstrate that fibers actually terminated in the ventral midbrain (Leichnetz and Astruc, 1976; Kunzle, 1978). Therefore, using modern anterograde tracers that demonstrate axon terminals, we studied projections from the PFC to the ventral midbrain in the Macaque monkey to determine the extent to which the PFC projects directly to the midbrain. The density of labeled fibers in these midbrain regions was compared to the density of cortical fibers from the same injection sites terminating in the striatum.

MATERIALS AND METHODS

To examine the organization of prefrontal cortical-midbrain connections, we injected anterograde tracers into the orbital, cingulate, and dorsolateral prefrontal areas. In each case, the distribution and pattern of terminal fibers were charted in both the striatum and midbrain. To determine the relationship of the descending fibers to the midbrain DA neurons, sections were double stained for the tracer molecules and for tyrosine hydroxylase.

Surgery and Tissue Preparation

In total, 27 adult macaque monkeys (*Macaca Nemistrina*) were used for these experiments. The University Committee on Animal Resources approved all procedures. Before surgery, monkeys were tranquilized by intramuscular injection of ketamine (10 mg/kg). Anesthesia was maintained by intravenous injection of pentobarbital (initial dose 20 mg/kg i.v. and maintained as needed). Temperature, heart rate, and respiration were monitored throughout the surgery. Monkeys were placed in a Kopf stereotaxic, a midline scalp incision was made, and the muscle and fascia were displaced laterally to expose the skull. A craniotomy (~2–3 cm²) was made over the region of interest and small dural incisions only at recording or injection sites. Serial electrode penetrations were performed to determine the dorsal-ventral coordinates for the orbital and cingulate cortical injection sites (see Haber *et al*, 1993). Dorsal injection sites were determined visually. Accurate placement of tracer injections was achieved by careful alignment of the injection cannulae with the electrode. The dorsolateral injection sites were determined by visual inspection of frontal cortical gyri, indicating general frontal cortical areas.

Monkeys received an injection of one of the following anterograde tracers, Lucifer Yellow, Fluororuby, or Fluorescein conjugated to dextran amine (LY, FR, or FS; 40–50 nL, 10% in 0.1 M phosphate buffer (PB), pH 7.4; Molecular Probes, Eugene, OR, USA), or *Phaseolus vulgaris*-leucoagglutinin (PHA-L; 50 nL, 2.5%; Vector laboratories). Tracers were pressure-injected over 10 min using a 0.5 µL Hamilton syringe. Following each injection, the syringe remained *in situ* for 20–30 min. At 12 to 14 days post-op, monkeys were again deeply anesthetized and perfused with saline followed by a 4% paraformaldehyde/1.5% sucrose solution in 0.1 M PB, pH 7.4. Brains were postfixed overnight and cryoprotected in increasing gradients of sucrose (10, 20, and 30%). Serial sections of 50 µm were cut on a freezing microtome into 0.1 M PB or cryoprotectant solution.

Immunocytochemistry

Immunocytochemistry was performed on free-floating sections to visualize LY, FR, FS, and PHA-L tracers. Prior to incubation in primary antisera, tissue was treated with 10% methanol and 3% hydrogen peroxide in 0.1 M PB to inhibit endogenous peroxidase activity, and rinsed 1–2 h in PB with 0.3% Triton X-100 (TX, USA from Sigma). Sections were preincubated in 10% normal goat serum (NGS) and 0.3% TX in PB for 30 min. Tissue was placed in the primary antisera-anti-LY (1:2000 dilution, Molecular Probes, Eu-

gene, OR, USA), anti-FS (1:1000; Molecular Probes, Eugene, OR, USA), anti-FR (1:1000; Molecular Probes, Eugene, OR, USA), or anti-PHA-L (1:500; EY Laboratories, Inc.) in 10% NGS and 0.3% TX in PB for four nights at 4°C. Following extensive rinsing, the tissue was incubated in the avidin-biotin complex solution (Vectastain ABC kit, Vector Labs). Immunoreactivity was visualized using standard DAB procedures. Staining was intensified by incubating the tissue for 5 to 15 min in a solution of 0.05% 3,3'-diaminobenzidine tetra-hydrochloride, 0.025% cobalt chloride, 0.02% nickel ammonium sulfate, and 0.01% H₂O₂ to yield a black reaction product. Sections were mounted onto gel-coated slides, dehydrated, defatted in xylenes, and coverslipped with Permount. Sections that were double labeled were first processed for the tracer and then incubated with antisera to TH (1:20 000 Eugene Tech, Inc.) (ADD fluorescence section).

Analysis

Cortical injections with contamination or weak labeling were eliminated from the analysis. Fiber distributions in the midbrain were charted using a Nikon research microscope with brightfield/darkfield illumination, equipped with an imaging system, and Neurolucida software by MicroBright-Field. Individual charts were imported into Adobe Illustrator to create composite images. Cortical experiments employed multiple tracers molecules (FS, FR, LY, and PHA-L) that have intrinsic differences in uptake and transport properties. Injections of different tracer molecules into the same site demonstrated similar projection patterns and relative densities between the striatum and midbrain. Therefore, the comparisons between distribution densities between cortico-striatal and cortico-midbrain projections are critical for illustrating the relative influence, based on fiber density. We determined the boundaries of the SN and VTA using counterstained or adjacent, Nissl-stained coronal sections (Figure 1).

RESULTS

In total, 29 anterograde tracer injections were placed into discrete regions of PFC. The injection sites encompassed three main regions: orbital, cingulate and DLPFC cortex. There were nine cases of orbital prefrontal injections, areas, 14, 11, 13, and 12 (orbital prefrontal cortex); seven cases of cingulate injections that targeted areas 24, 32, and 25; and 13 cases with injection sites in the DLPFC that included areas 9 and 46. The injection sites selected for analysis and illustration were all confined to the orbital, DLPFC, or cingulate cortices. We selected six cases, two from each general region, cingulate (areas 24 and 25; cases 36 and 83 respectively) orbital (areas 13 and 12, cases 133 and 115 respectively), dorsolateral (areas 9 and 46, cases 106 and 121, respectively) to illustrate the projections throughout the midbrain. A representative section was also illustrated for three additional cases in each general region (cingulate area 32, case 124; OPFC 14, case 47; DLPFC area 46, case 131). When more than one case was available for a particular injection site, the case containing the largest projection was selected for illustration. Given the sparse

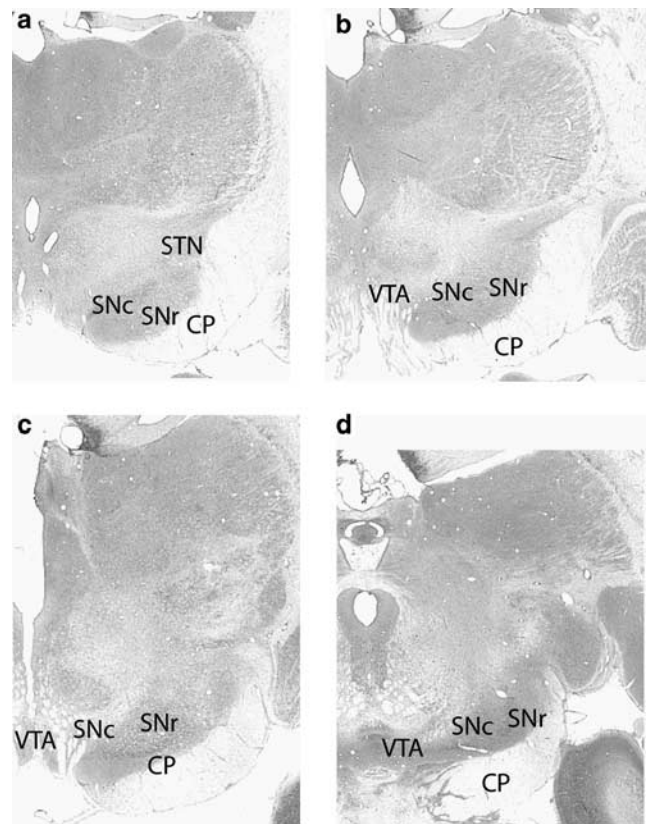


Figure 1 Photomicrographs of Nissl stained coronal sections through the brainstem of the macaque monkey to illustrate the regions in which descending cortical fibers were charted. CP, cerebral peduncle; SNc, substantia nigra pars compacta; SNr, substantia nigra pars reticulata; STN, subthalamic nucleus; VTA, ventral tegmental area.

nature of the projection fibers in all cases, each fiber in each section was illustrated. Terminal fibers were determined by the presence of fine arborizations and terminal boutons. The terminal fields also contained scattered larger fibers, lacking evidence of termination. These fibers were assumed to be fibers of passage and were not illustrated. However, if there was doubt about whether a fiber was terminating or passing, we chose to illustrate it, given the relatively few fibers present in the projection fields. Therefore, if an error exists, it is on the side of over representation.

In general, as indicated by the illustrations, overall there are very few fibers in the ventral midbrain following small injections confined to specific areas of PFC. In contrast, these injections produced very dense labeling in the striatum, as illustrated for comparison, and in the thalamus (not illustrated). Projections to the midbrain remained ipsilateral to the injection site and demonstrated some topography. The ventral cingulate and orbital cortical areas projected primarily to the VTA and the medial SN. In contrast, the dorsolateral prefrontal and dorsal cingulate cortices projected primarily to the SN.

Cingulate Projections (Figure 2)

The injection sites in the cingulate PFC are represented by cases 36, 124, and 83. Case 36 was an injection of LY in area

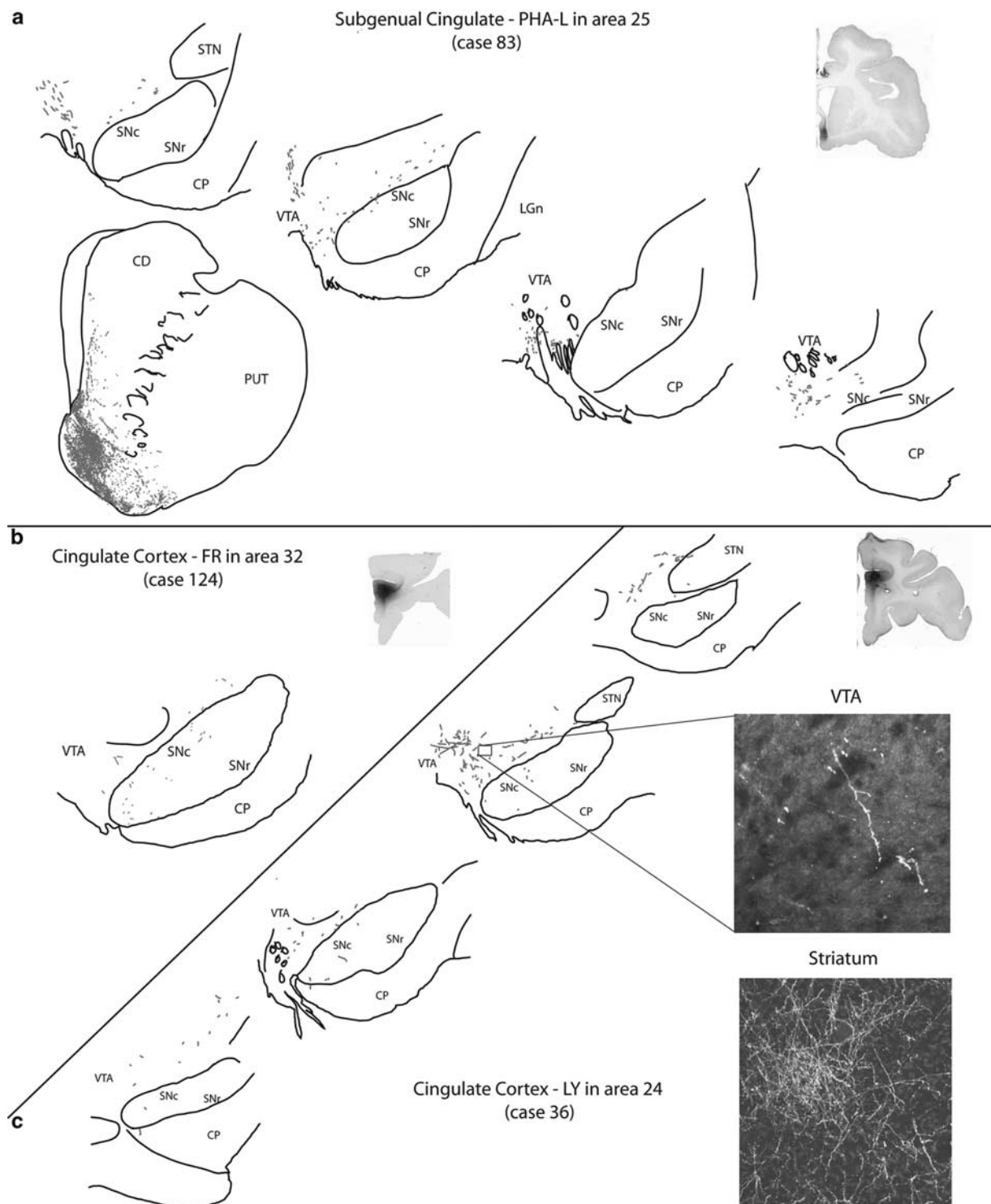


Figure 2 Camera lucida drawings of transverse sections through the midbrain of macaque monkeys illustrating the distribution of anterograde labeled fibers from cingulate cortical regions. Injection sites, shown in the inset photomicrographs, were localized to areas 25, 32, and 24 (a, b, and c, respectively). For comparison, anterograde labeled fibers in a section through the basal forebrain at the level of the striatum were illustrated for the injection site in area 25 (a). In (c), photomicrographs of cortical terminal field in the striatum and in the midbrain following are shown. CD, caudate; CP, cerebral peduncle; PUT, putamen; SNc, substantia nigra pars compacta; SNr, substantia nigra pars reticulata; STN, subthalamic nucleus; VTA, ventral tegmental area.

24, case 124 an injection of FR in area 32, and case 83 an injection of PHAL in area 25. The number of projection fibers from these injection sites was somewhat greater than

that seen with injections into the orbital PFC (see below and Figure 3). However, relative to the dense projections seen in the striatum from these same injections, the total projection

remains very sparse. Fibers projected largely to the VTA and retrorubral area. Following an injection into area 25 individual fibers were noted in the VTA throughout the midbrain. There were no fibers in the region of the SN. In contrast, following injections into areas 24 and 32, there were fibers both in the VTA and in the medial and dorsal SN. All injections resulted in very dense, topographically organized terminal fields in both the striatum and thalamus.

Orbital Prefrontal Projections (Figure 3)

Case 115 was an injection of LY into area Opro (orbital preisocortex), case 133 represents an injection of FR into area 11, and case 47 was an injection of LY into area medial 14. There were isolated labeled fibers at all levels of the midbrain in each case. Furthermore, descending fibers continued caudally and were noted in the other brainstem regions, including the central grey. In contrast to the sparse labeling in the midbrain, the striatum had very dense topographically organized terminals (Figure 3). Following the injection into Opro, labeled fibers were found primarily in the SN, with few labeled fibers in the VTA. At rostral levels, labeled fibers were located in the medial and dorsal SN; however, unlike the other cases, at caudal levels, they were also observed more ventrally. Following an injection of FR into area 11, the labeled fibers were located primarily within the SN proper, in the medial and dorsal region. However, compared to case 115, there were more labeled fibers in the VTA, particularly at rostral levels. Case 47, an injection into area 14 was the most medial case of the orbital prefrontal cortices. This injection resulted in the greatest number of labeled fibers in the VTA, extending dorsal to the SN in the region of the retrorubral cell group. In this case, there were relatively few labeled terminals in the SN, pars compacta. Overall, projections from orbital prefrontal areas yielded minimal projection fibers in the midbrain and were distributed somewhat topographically. The injection sites in the ventral-medial orbital cortex producing labeled fibers primarily in the VTA. Injection sites placed more laterally gave rise to fibers distributed within the SN proper.

Dorsolateral Prefrontal Projections (Figure 4)

Projections from the dorsolateral PFC were assessed by injections into areas 9 and 46. Representative cases from these injection sites are, case 106 for area 9, case 121 primarily area 46, with some inclusion of area 9 and case 131 for area 46. Injections into areas 9 and 46 resulted in labeled fibers seen primarily in the medial and more dorsal aspect of the SN, with fewer fibers in the VTA. This was similar to our observations from the other cases with injections in this region (data not shown). Case 121, which was a relatively large injection site compared to the other injections, resulted projection fibers localized to the medial and dorsal aspect of the SN. Labeled fibers were concentrated in the central part, with fewer labeled fibers in the most rostral and caudal regions. Despite the relatively large injection site, the density of labeled fibers in the midbrain remained low and, as with the other cases, each fiber is illustrated.

Relationship with TH-Positive Cells

Double staining for TH and tracer was performed to examine the relationship between the cortical projection fibers and TH-positive cell bodies and processes. Examination of all cases yielded similar results. Positively stained projections from prefrontal areas could be visualized interspersed with TH-positive cells and processes in both the substantia nigra pars compacta and pars reticulata. However, in all cases, the prefrontal projections and the TH-positive cells did not appear to be in close juxtaposition. There were no sections in which a prefrontal projection fiber and a TH-positive fiber were visualized in the same z-axis plane (Figure 5).

DISCUSSION

The results show a very limited projection from the PFC to the midbrain DA neurons in primates, terminating both within the SN proper as well as in the VTA. They arise from a broad region of the PFC, including the DLPF, cingulate, and orbital cortices. It is difficult to compare the density of projections from different cortical areas to the midbrain, given the scarcity of this projection. This is particularly apparent when compared to the density of the other descending projections of the prefrontal region, particularly to the striatum and thalamus.

Although no other studies have specifically examined projections from the PFC to the midbrain DA neurons in primates, several authors have reported on PFC projections to this area in the context of studying projections to other brain regions. A set of two publications by the same group which described the prefrontal projections to the hypothalamus and the periaqueductal gray in the macaque monkey show results similar to the current study, with a limited number of labeled axons in the mesencephalon resulting from injections in the PFC (An *et al*, 1998; Öngür *et al*, 1998). Consistent with the findings from anterograde studies, retrograde tracer injections into the SN pars compacta and the VTA result in some labeling in medial cingulate cortex (see Figure 5 in Öngür *et al*, 1998). Freedman *et al* (2000) studied the subcortical projections from area 25 and, although they did not comment specifically on termination of fibers in the SN and VTA, they noted fibers passing dorsal to the SN pars compacta giving rise to collaterals with few varicosities that pass through the VTA (see Figure 4c). In a similar study, efferent projections from areas 25, 24, 32, 10, and 14 showed some projections to the VTA from areas 25, 24, and 32, with the strongest projection from area 25 (Chiba *et al*, 2001).

The findings in rodents differ somewhat from that is seen in the primate. In rodents, a region of the cortex, analogous to the primate PFC, that represents a multimodal association area, separate from the motor and premotor areas, is defined based upon thalamocortical and cortico-cortical connectivity (Reep *et al*, 1984; Van Eden *et al*, 1992; Groenewegen *et al*, 1997). The rodent PFC is subdivided into a ventromedial area, comprising the infralimbic, prelimbic and anterior cingulate areas, and a dorsocentral region comprised largely by the medial agranular cortex (McGeorge and Faull, 1989; Berendse

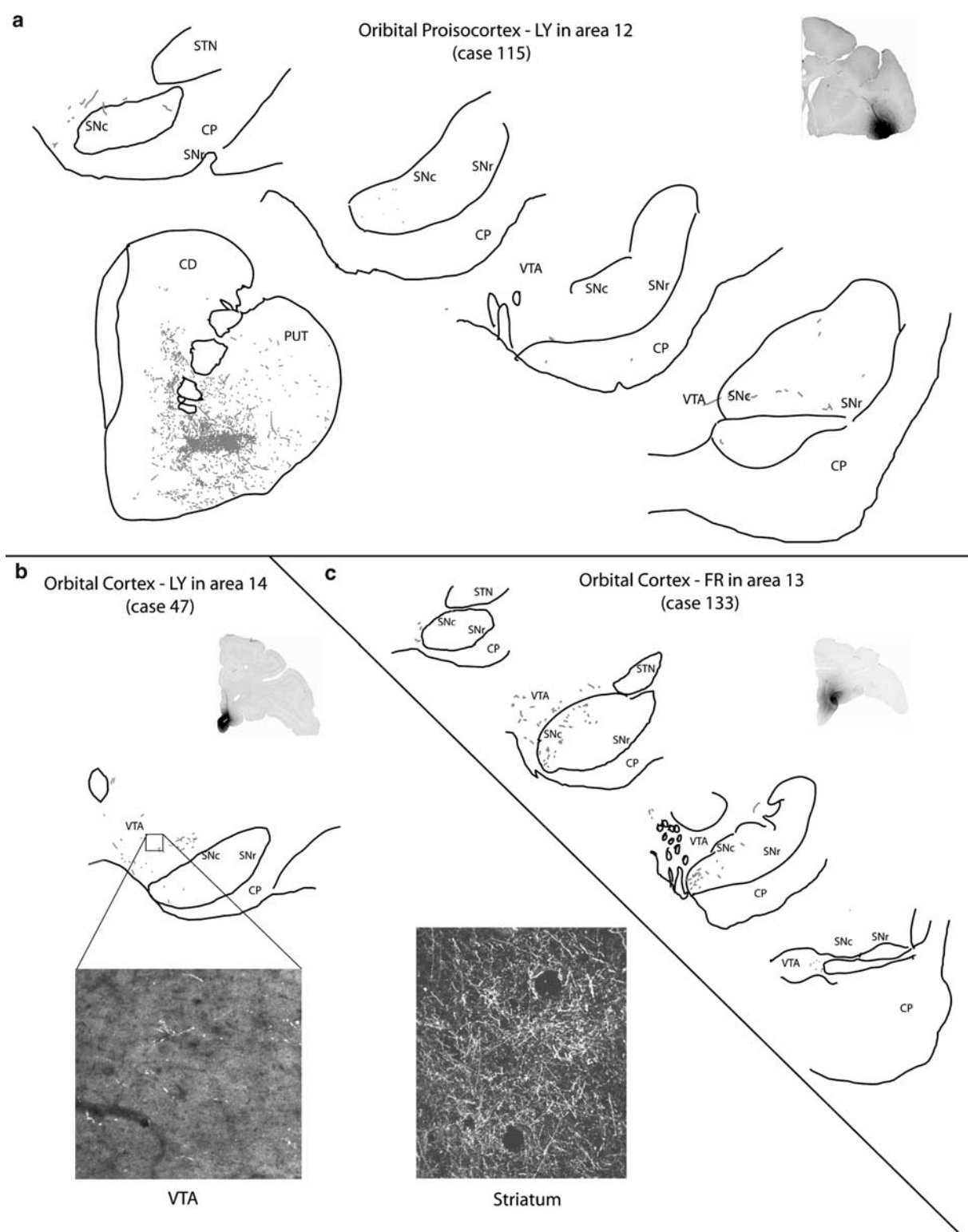


Figure 3 Camera lucida drawings of transverse sections through the midbrain of macaque monkeys illustrating the distribution of anterograde labeled fibers from the orbital frontal cortical regions. Injection sites, shown in the inset photomicrographs, were localized to areas 12, 14, and 13 (a, b, and c, respectively). For comparison, anterograde labeled fibers in a section through the basal forebrain at the level of the striatum were illustrated for the injection site in area 12 (a). In (b), photomicrographs of cortical terminal field in the striatum and in the midbrain following are shown. CD, caudate; CP, cerebral peduncle; PUT, putamen; SNc, substantia nigra pars compacta; SNr, substantia nigra pars reticulata; STN, subthalamic nucleus; VTA, ventral tegmental area.

et al, 1992). In contrast to the weak projections observed in the primate, there is a relatively dense projection from the prelimbic and infralimbic areas to the midbrain (Sesack

et al, 1989a,b; Hurley *et al*, 1991; Takagishi and Chiba, 1991). The discrepancy in the degree of projections from the PFC to the midbrain between the rat and the primate is

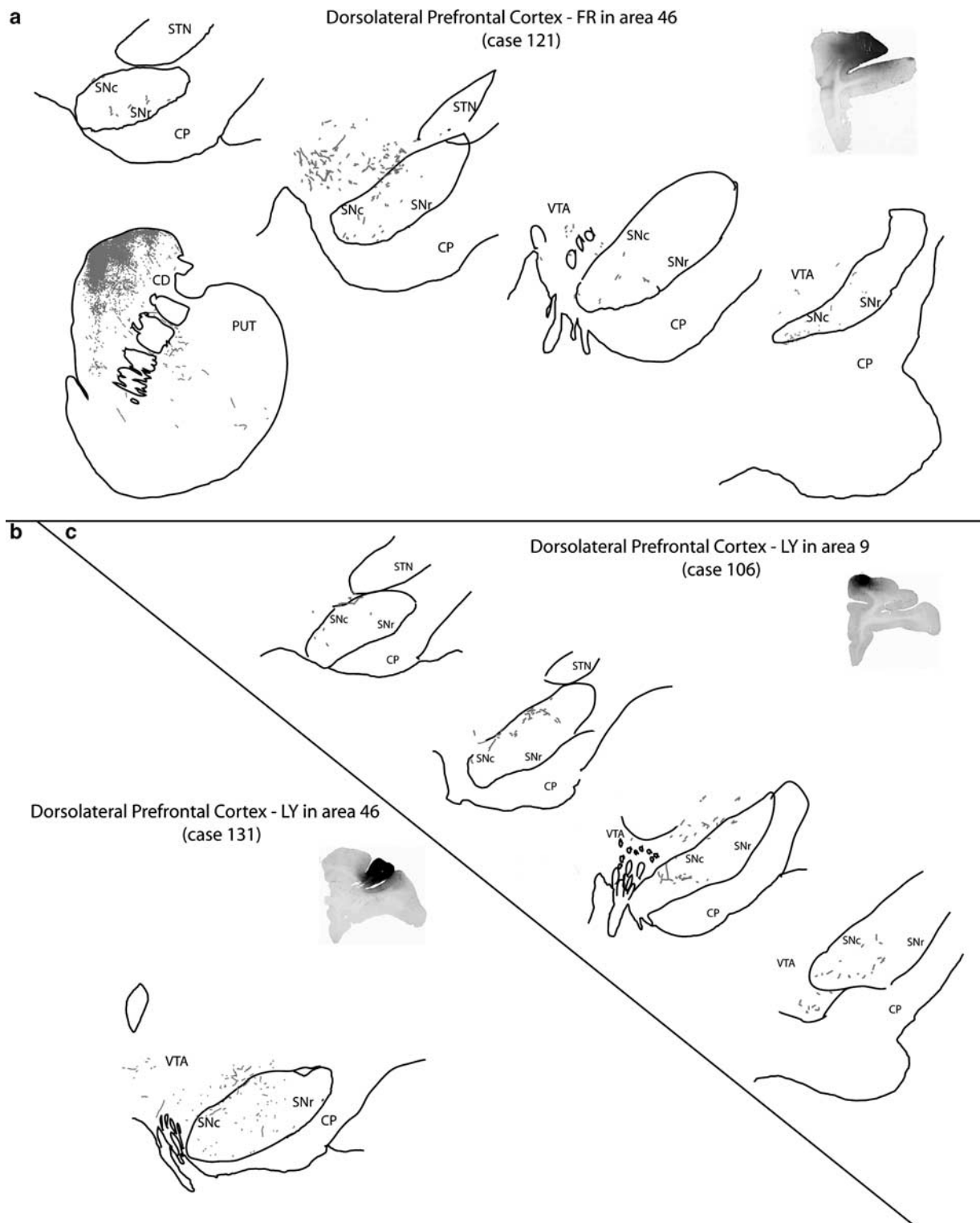


Figure 4 Camera lucida drawings of transverse sections through the midbrain of macaque monkeys illustrating the distribution of anterograde labeled fibers from the dorsolateral prefrontal cortex. Injection sites, shown in the inset photomicrographs, were localized to areas 46 and 9 (b and c, respectively) with a large injection in area 46 with some encroachment into area 9 (a). For comparison, anterograde labeled fibers in a section through the basal forebrain at the level of the striatum were illustrated for the injection site in area 46/9 shown in (a). CD, caudate; CP, cerebral peduncle; PUT, putamen; SNc, substantia nigra pars compacta; SNr, substantia nigra pars reticulata; STN, subthalamic nucleus; VTA, ventral tegmental area.

interesting given that in both species projections to other regions, such as the striatum and thalamus are dense. With the large cortical expansion in primates compared to

rodents, it is possible that larger injection sites within PFC are needed to appreciate the extent of the projection to the midbrain.

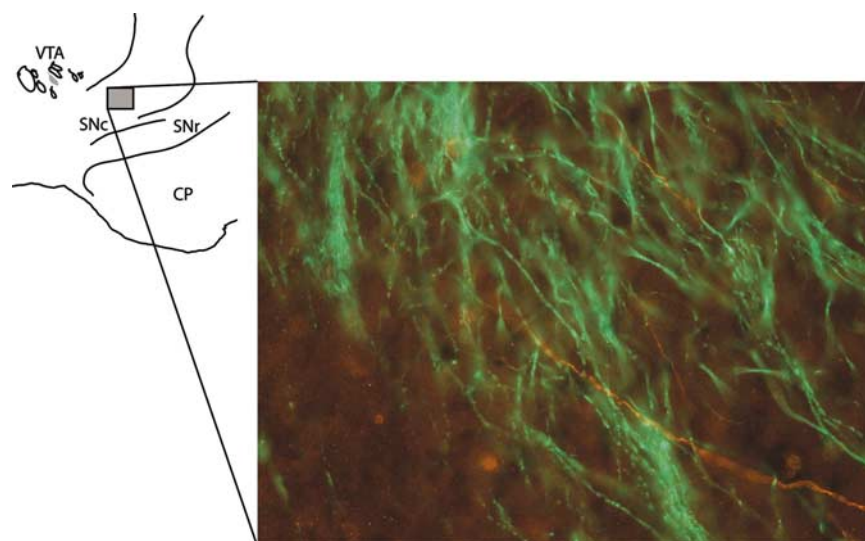


Figure 5 A schematic diagram of the ventral midbrain with a fluorescent photomicrograph from an individual case in which the anterograde tracer LY was placed in the prefrontal cortex. The section is double-stained for TH (green) and LY (red). CP, cerebral peduncle; SNc, substantia nigra pars compacta; SNr, substantia nigra pars reticulata; VTA, ventral tegmental area.

The importance of direct control of DA function by glutamatergic cells in the PFC has been shown in rodents (Afifi *et al*, 1974; Bunney and Aghajanian, 1976; Carter, 1980; Kornhuber, 1984; Sesack *et al*, 1989a,b; Hurley *et al*, 1991; Sesack and Pickel, 1992a,b). The specificity of synaptic connections between the PFC and the VTA/SN may indicate that a large projection may not be necessary for the PFC to exert significant control over the relevant midbrain cells (Carr and Sesack, 2000a,b). While our findings do not show an extensive anatomical basis for direct PFC control of the VTA or SN DA cells, it is possible that a relatively small projection can indeed have a large affect. However, it is important to note that we were unable to visualize any projection fibers in close apposition with TH-positive cells or processes. Taken together, the results raise the issue of possible alternative pathways for PFC glutamatergic control of DA.

Despite the relative lack of cortical input to the midbrain cells, these neurons are rich in glutamate receptors in primates (Paquet *et al*, 1997). Thus, while, based on these anatomical studies, direct cortical control of DA neurons remains debatable in primates; the cortex may directly impact other sources of glutamatergic control. The main input to the DA cells (either directly or indirectly) is from the striatum. Other sources of regulation of DA are derived from the globus pallidus (GP), the pedunculopontine n. (PPN), and the subthalamic n. (STN) (Carpenter *et al*, 1981a,b; Parent, 1986; Smith *et al*, 1990; Rosales *et al*, 1994; Charara *et al*, 1996; Haber, 2003). Of these inputs, both the striatal and pallidal afferent fibers to the SN are GABAergic. In contrast, the STN and the PPN are known to be glutamatergic (for a review see Parent, 1986; Smith *et al*, 1998; for a review see Haber, 2003). In addition, recent evidence indicates that input to the SN from the bed n. of the stria terminalis (Fudge and Haber, 2001) is also glutamatergic (Georges and Aston-Jones, 2002; Dong and Swanson, 2004).

Among the alternative pathways for excitatory control of the DA neurons, the prefrontal cortical influence via the STN on basal ganglia output structures is of particular

recent interest. The frontal cortex provides a main and topographically organized, monosynaptic, glutamatergic input to the STN (Monakow *et al*, 1978; Carpenter *et al*, 1981a,b; Nambu *et al*, 1996; Nambu *et al*, 1997). The cortico-STN projection, which has been a focus of study for motor and premotor cortico-basal ganglia system, is termed the 'hyperdirect' basal ganglia pathway in that impulses reach the basal ganglia output nuclei faster via this route than through the cortico-striatal pathway (Kolomiets *et al*, 2003). Given that the STN impacts directly on DA cells (Rosales *et al*, 1994; Meissner *et al*, 2003), this route (PFC to the STN to the midbrain cells) may represent an alternative in the primate to the direct PFC-VTA/SN pathway observed in other species. In fact, PFC stimulation results in both excitatory and inhibitory responses in the primate SN pars reticulata, which are mediated via the STN and striatum, respectively (Kitano *et al*, 1998). The PFC-STN connection in primates (Frankle *et al*, 2003), along with the direct STN connection to the SN, is likely to have an impact not only on the SN pars reticulata cells, but also on the DA neurons (Rosales *et al*, 1994).

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