

Research report

Overlap and interdigitation of cortical and thalamic afferents to dorsocentral striatum in the rat

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Abstract

Dorsocentral striatum (DCS) is an associative region necessary for directed attention in rats. DCS is defined as the main region in which axons from ipsilateral medial agranular cortex (AGm) terminate within the striatum. In this double-labeling study, we placed a green axonal tracer in area AGm and a red one in an additional brain region. We examined the spatial relationship between terminals from area AGm and other portions of the cortical–basal ganglia–thalamic–cortical network involved in directed attention and its dysfunction, hemispatial neglect, in the rat. These include lateral agranular cortex (AGl), posterior parietal cortex (PPC), ventrolateral orbital cortex (VLO), and secondary visual cortex (Oc2M). One important finding is the presence of a dense focus of labeled axons within DCS after injections in cortical area PPC or Oc2M. In these foci, axons from PPC or Oc2M extensively overlap and interdigitate with axons from cortical area AGm. Additionally, retrograde labeling of striatal neurons, along with double anterograde labeling, suggests that axons from cortical area AGm and AGl cross and possibly make contact with the dendritic processes of single medium spiny neurons. Axons from thalamic nucleus LP were observed to form a dense band dorsal to DCS which is similar to that seen following PPC injections, and a significant number of LP axons were also observed within DCS. Projections from thalamic nucleus VL are present in the dense dorsolateral AGm band that abuts the external capsule, are densest in the dorsolateral striatum, and were not observed in DCS. These results extend previous findings that DCS receives input from diverse cortical areas and thalamic nuclei which are themselves interconnected.

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1. Introduction

Using a rodent model of hemispatial neglect [19,20, 21,33,38–41], our group has determined that the medial agranular cortex (AGm or Fr2 [28]), a multimodal association premotor cortex with diverse cortical connections [30], and posterior parietal cortex (PPC) are components of a cortical–basal ganglia–thalamic–cortical network mediating directed attention in the rat [5]. The dorsocentral striatum (DCS), defined anatomically as the central striatal terminal field of projections originating from cortical area AGm, is critical for normal directed attention. Destruction of DCS results in chronic neglect which does not recover or respond to

Abbreviations: AC, anterior cingulate cortex; AGl, lateral agranular cortex; AGm, medial agranular cortex; cg, cingulum bundle; DCS, dorsocentral portion of the dorsal striatum; Fr1, frontal cortex, area 1; Fr2, frontal cortex, area 2; LD, laterodorsal thalamic nucleus; LP, lateral posterior thalamic nucleus; LO, lateral orbital cortex; lv, lateral ventricle; Oc2M, occipital cortex, area 2, medial part; PPC, posterior parietal cortex; PPCL, posterior parietal cortex (lateral portion); PPCm, posterior parietal cortex (medial portion); VO, ventral orbital cortex; VL, ventrolateral thalamic nucleus; VLO, ventrolateral orbital cortex

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dopamine agonists [29,38–40]. For these reasons, region DCS has become the focus of work on the cortical–basal ganglia–thalamic–cortical network mediating directed attention in the rat.

In previous anterograde and retrograde studies, we demonstrated that the cortical regions AGm, PPC, ventrolateral orbital cortex (VLO), and secondary visual cortex (Oc2M) all send projections to each other and to DCS [4,5,29,33]. Projections from each of these areas form a dense primary projection field in the dorsal striatum and an additional diffuse pattern of labeling in or near DCS. This pattern of labeling in the rat striatum has been described by many authors [1,2,15,25,33,43], and suggests the presence of at least two systems of corticostriatal projections terminating in the rat striatum, termed “discrete” and “diffuse” [43]. The same diffuse region of corticostriatal labeling was also described in the monkey [10,11], and may therefore be an innate property of corticostriatal organization. We have previously published a thorough examination of the band/diffuse pattern of labeling in the rat network for directed attention [33].

Previously, we identified the thalamic nuclei with which the cortical components of the rat network for directed attention are each reciprocally interconnected [29]. Because of the known functional and anatomical relationships between cortical areas and thalamic nuclei in the network [22,29–32], it is important to understand the topography of their striatal projections and the spatial relationship of these projections with respect to DCS. More detailed examination of the relationship between the convergent terminal fields of these key regions will provide a better basis to understand how their inputs interact to influence the activity of neurons in the DCS. Cortical–basal ganglia–thalamic–cortical networks have also been proposed as mediators of directed attention in primates. Similar to the rat network, the primate networks involve regions of prefrontal cortex and posterior parietal cortex, as well as associated regions of the basal ganglia and thalamic nuclei [24,26,36].

Our interest in these specific cortical areas extends to the more general question of whether multiple cortical areas terminate on or near the dendrites of individual striatal medium spiny neurons. This hypothesis has been proposed previously [44] and is supported by both electrophysiological and anatomical data [6,9,27], although it remains untested in a multi-labeling experiment at the light microscopic level. If it exists, this pattern of connectivity would support the hypothesis that the rat striatum, and potentially individual medium spiny neurons, plays a key role in integrating multimodal input from diverse cortical areas, and that the rat DCS is a center for convergence of diverse corticostriatal inputs related to directed attention. Our previous findings support these hypotheses. In a recent report, we found that many cortical areas project to region DCS, but none more consistently than AGm, PPC, and Oc2M [4].

Subsequently, we demonstrated that axons from cortical areas AGm and PPC exhibit both overlap and interdigitation in the rat DCS, using an anterograde tracer placed in individual cortical areas, as well as in two double-labeled cases [33]. The data from our previous papers regarding the spatial arrangement of corticostriatal projections are summarized in Fig. 1.

In the current multiple-labeling study, we directly tested the hypothesis that projections from cortical areas AGI (Fr1 [28]), PPC, and VLO, as well as thalamic nuclei VL and LP, overlap and/or interdigitate with projections from cortical area AGm in the striatal region DCS. Further, we sought to demonstrate whether neurons from anatomically non-adjacent cortical areas form terminals on or near the dendrites of the same individual medium spiny neurons in the rat striatum.

2. Materials and methods

All animal procedures were conducted according to institutional protocols that meet or exceed NIH and Society for Neuroscience guidelines. Animals were anesthetized with ketamine/xylazine (90 mg/kg;10 mg/kg). Upon cessation of tail pinch and eyeblink responses, animals were placed in a small animal stereotaxic device and a hole was drilled in the skull at the selected location. The dura was then incised, exposing the brain surface. All rats received one injection of a 10,000-mw dextran conjugated to a green-fluorescing molecule (100 nl of a 10% AlexaFluor 488 solution [15] in phosphate buffer; Molecular Probes) in area AGm using procedures derived from our previous work [4,30,31,33]. Additionally, each rat received an injection of a red fluorescing molecule conjugated to a 10,000-mw dextran (100 nl of a 10% FluoroRuby [34] or MicroRuby [23] solution in phosphate buffer; Molecular Probes, Inc.) in one of the following regions: cortical area AGI (case DCS 193), PPC (case DCS 178), VLO (case DCS 147), or

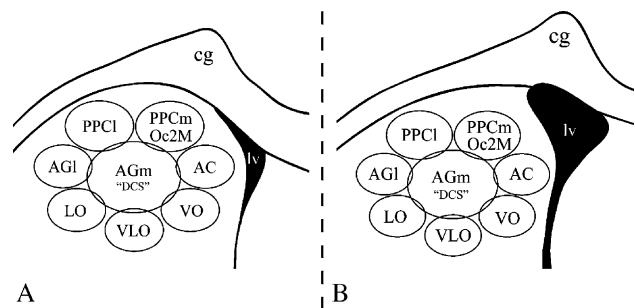


Fig. 1. Schematic representation of corticostriatal terminal fields at levels near: (A) the level of the genu and (B) the level of the septal nuclei. This figure was constructed from previously published data [30]. Corticostriatal terminal fields from many cortical areas project in a topographical way to the dorsal striatum and partially overlap and interdigitate with the terminal field of cortical area AGm. The striatal region containing the terminal field of cortical area AGm in a given brain is referred to as DCS.

thalamic nucleus VL (case DCS 173), or LP (case DCS 191). One rat (case DCS 193) also received a third injection: a 3000-mw dextran conjugated to a biotin molecule (BDA3K [12]; Molecular Probes) was placed in globus pallidus and retrogradely filled the dendritic trees of medium spiny neurons in DCS.

Injections for all cases were placed in the left hemisphere. Intracerebral injections were made through a 27-gauge Hamilton syringe except for injections in orbital cortex, which necessitated the use of iontophoresis. A tracer volume of 100 nl was used for all pressure injections. For iontophoretic injections, glass micropipettes having tip diameters of 20–30 μm were lowered to the proper coordinates and constant current pulses of 4–5 μA were delivered from an iontophoresis unit (Kation Scientific, model BAB-350) in a 5 s on/5 s off pattern for 20–25 min. All thalamic injections were made iontophoretically using the same parameters as cortical iontophoretic injections. After surgical procedures were completed, all skull wounds were filled with gelfoam and antibiotic ointment was placed between the scalp and skull to prevent post-surgical infection. The scalp wound was closed using wound clips. After 10–14 days, rats received an overdose of a barbiturate solution (Beuthanasia-D; 100 mg/kg IP) and were perfused intracardially with 34 °C phosphate-buffered saline followed by 4% phosphate-buffered paraformaldehyde. The brain was extracted and post-fixed in 4% paraformaldehyde fixative for 24 h, then stored in cold 0.4% paraformaldehyde containing 30% sucrose for 2–3 days prior to cutting.

Coronal frozen brain sections were cut at 40 μm on a sliding microtome and collected serially in dilute fixative (0.4% paraformaldehyde). Two series of sections were mounted, dehydrated, and coverslipped using Fluoromount (BDH Chemicals). Fluorescent sections were viewed and photographed on a Biorad MRC-1024 confocal laser scanning microscope using separate red and green filter channels. Final images represent combined red/green image stacks of approximately 20 μm total thickness.

A third series of sections stored in dilute fixative was stained for Nissl substance using cresyl violet and used for cytoarchitectural orientation. Additionally, brains containing microruby or BDA3K injections were processed as previously described [33].

The locations of cortical injection sites were determined in each brain according to the cytoarchitectural criteria of Zilles and Wree [45]. Thalamic nuclei were identified and demarcated according to the criteria and maps of Jones [17] and Paxinos and Watson [28].

Data from single anterograde biotinylated-dextran-amine (BDA10K) injections [2] are previously unreported findings resulting from a reanalysis of 13 rats for which methods were previously reported, as were the methods for calbindin staining on those cases [33]. For the purpose of this report, only rats with injections which affected only one cortical area were analyzed.

3. Results

Injections of anterograde fluorescent tracers in AGm (green) and a second brain region (red) resulted in the simultaneous red and green labeling of terminal fields in the striatum. We attempted to determine whether the striatal terminal fields labeled by each tracer injection overlapped or interdigitated. As noted previously, we viewed the sections on a confocal microscope and imaged ~ 20 μm of each ~ 40 - μm -thick section in ~ 1 - μm intervals. The images from each stack were combined via software into one two-dimensional image. Therefore, axons seen to cross on final images are somewhere between actually touching and passing within 20 μm of one another. Since the two conditions are indistinguishable on our final images, we considered any image in which fibers from different injections were observed crossing as an image depicting overlap. “Interdigitation” was used to describe sections on which very little or no overlap was seen, yet axons from different injection sites (typically large fields of them) were seen adjacent to one another.

Placement of injection sites is described below in anteroposterior (a-p), mediolateral (m-l), and dorsoventral (d-v) dimensions.

3.1. AGm/AGl

In case DCS 193, the AGm injection was centered at a-p +1.0 and affected an area similar to the injection in case 178, extending to all cortical layers without damaging the white matter. The AGl injection was centered at a-p –3.6 and m-l +2.0, and affected all cortical layers, as well as a small amount of the white matter. The AGm labeling in case 193 produced a dorsolateral band and DCS labeling consistent with the previous accounts [33]. A dorsolateral band of labeling was produced in the striatum following the AGl injection, and sparse axonal labeling was observed in DCS, as previously described [33]. The dorsolateral band is distinct from the AGm band, but labeling in DCS overlapped with AGm labeling.

Additionally, case DCS 193 also received an injection of BDA3K in globus pallidus (a-p –0.9), resulting in a robust retrograde labeling of the DCS efferent neurons (medium spiny neurons). With confocal microscopy, both green (AGm) and red (PPC) fine-caliber axons with varicosities were observed to overlap with individual dark-stained medium spiny neuron dendrites in this triple-labeled brain (Figs. 3C, 3D).

3.2. AGm/PPC

Case DCS 178 received fluorescent tracer injections in cortical areas AGm and PPC and both produced a pattern of labeling in the striatum similar to those seen previously after single injections of the anterograde tracer BDA10K [33]. The AGm injection site was centered at

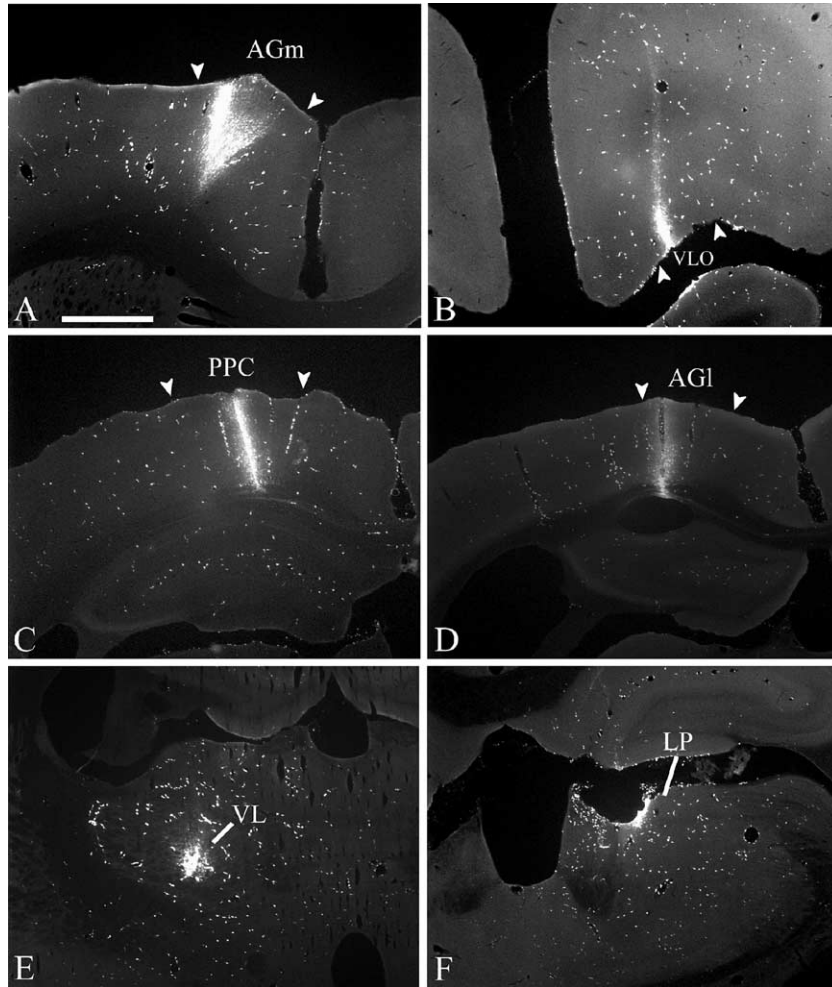


Fig. 2. Representative fluorescent photomicrographs of cortical and thalamic injection sites. 25 \times . Scale bar = 1 mm; applies to A–F. (A) Injection of AlexaFluor 488 in cortical area AGm (case DCS 178). (B) Injection of Fluororuby in cortical area VLO (case DCS 147). (C) Injection of Fluororuby in cortical area PPC (case DCS 178). (D) Injection of Fluororuby in the caudal portion of cortical area AGI (case DCS 193). (E) Injection of Microruby in thalamic nucleus VL (case DCS 173). (F) Injection of Microruby in the medial portion of thalamic nucleus LP (case DCS 191).

a-p +0.7 and affected all cortical layers but did not affect the white matter (Fig. 2A). Terminal fields from area AGm included a dorsolateral band immediately internal to the white matter, as well as a more diffuse area of labeling in DCS, as reported previously [29,33]. The PPC injection site for case 178 was centered at a-p -3.6 (Fig. 2B), and affected all layers of the cortex without extending into the white matter. The injection is centered in PPC and has a narrow mediolateral extent (~ 0.2 mm). The injection is visible over a rostrocaudal distance of approximately 0.3 mm (from spaced serial sections). The primary terminal field of projections from area PPC forms a dense band located dorsal to the AGm projection field in DCS, and it spans the distance between DCS and the dorsolateral AGm band but remains distinct from both fields of AGm axon terminals. The same pattern of termination can be observed along much of the rostrocaudal extent of the striatum in this case.

In case 178, the PPC injection also produced a dense focus of labeling in DCS (Figs. 3A, 3B). The focal PPC

projection in DCS was somewhat distinct from AGm labeling in its center but overlapped extensively with AGm axons at the edges of the focus. Numerous axons from each injection were observed in the focus periphery and crossed each other extensively.

3.3. AGm/VLO

Case DCS 147 had an AGm injection site centered at a-p +0.7, m-l +1.0, which affected all layers of the cortex without impinging on the white matter. The injection spans a rostrocaudal distance of ~ 0.3 mm. Striatal labeling from this injection was typical of that observed following other AGm injections, and was composed of a densely labeled dorsolateral band and a more diffuse area of labeling in DCS.

The VLO injection site for case DCS 147 was centered at a-p +3.2, m-l +2.2, and d-v -4.2 . Terminal fields of axons originating in cortical area VLO were located close to and along the d-v extent of the medial

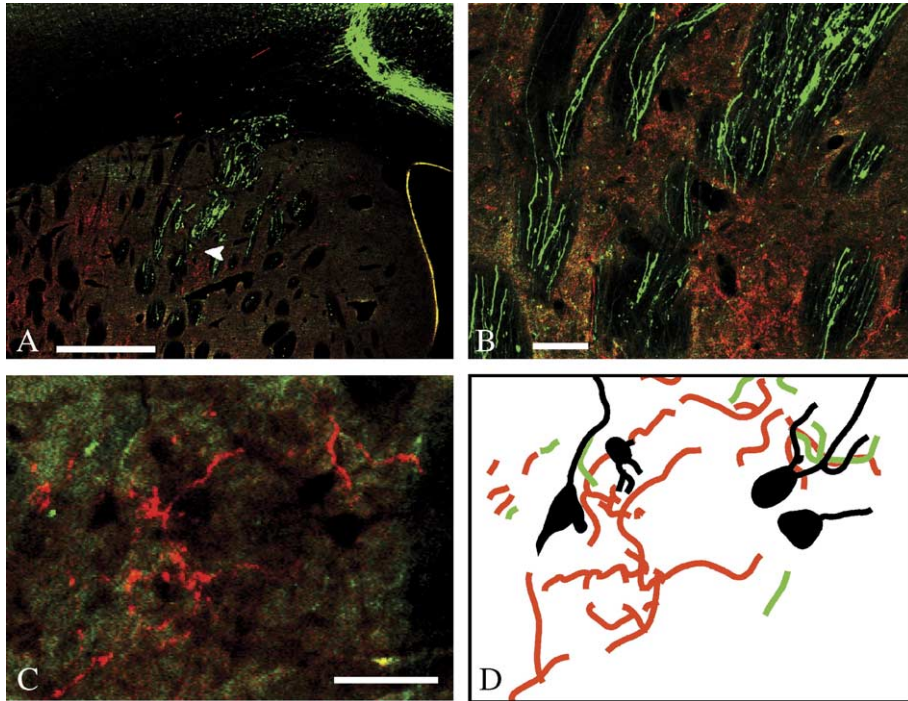


Fig. 3. Striatal labeling following injection of fluorescent anterograde tracers. (A) Labeling following injection in AGm (green) and PPC (red) in case DCS 178. AGm labeling is visible in a green dorsolateral band. Larger-caliber green axons are visible in the white matter fascicles passing through the dorsocentral striatum (DCS). Labeling from the PPC injection also forms a dorsolateral band and terminals are diffusely present in all of DCS. Additionally, a dense focus of labeling is visible in DCS (arrow). 50 \times . Scale bar = 1 mm. (B) Higher-magnification photomicrograph of the dense focus of labeled PPC axons in case DCS 191. Several AGm axons (green) are visible and cross the PPC axons. The two either touch or pass within 20 μ m of one another. Larger-caliber green axons are also visible in the white matter fascicles. 100 \times . Scale bar = 100 μ m. (C) Photomicrograph of axons from cortical areas AGI (green) and PPC (red) in close association with dendritic processes of retrogradely labeled striatal medium spiny neurons (black) in case DCS 193. 200 \times . Scale bar = 100 μ m. (D) Line drawing of axons and cell bodies from C.

wall in this case (Fig. 4A). Some labeling produced by the injection extends to border the AGm labeling in DCS from the medial side and interdigitates at the light microscopic level with some of the axons from area

AGm, forming a region similar to the focal projection described following PPC injections. In contrast to the PPC focal DCS projection, the discrete region of VLO label remained continuous with the primary field of VLO

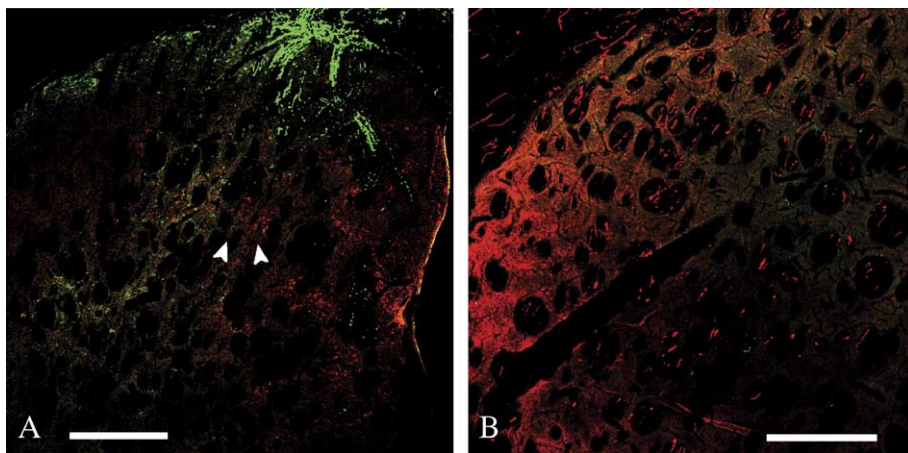


Fig. 4. (A) Striatal labeling in case DCS 147 following injection of fluorescent anterograde tracers in AGm (green) and VLO (red). AGm labeling forms a dense green dorsolateral band and a more diffuse region of labeling in the dorsocentral striatum (DCS). Labeling from the VLO injection is most dense along the medial wall, and terminals are diffusely present in all of DCS. Additionally, a dense focus of labeling is visible in or near DCS (arrow), although no overlap was evident between axons from AGm and VLO in the dense focus. 50 \times . Scale bar = 1 mm. (B) Striatal labeling in case DCS 173 following injection of fluorescent anterograde tracers in cortical area AGm (green) and thalamic nucleus VL (red). Labeling from the VL injection is most dense along the lateral edge of the striatum. No terminals were observed in DCS in this case. Large-caliber fibers can be seen passing through the white matter bundles and probably account for VL labeling observed in a previous retrograde study of DCS. 100 \times . Scale bar = 1 mm.

label along the medial wall. Despite the presence of this seemingly similar focal projection, there was little if any overlap of axons from AGm and VLO evident in this case.

3.4. AGm/VL

Case DCS 173 had an injection centered in rostral AGm (a-p +1.4). The VL injection was centered at a-p -2.5, m-l +1.4, and d-v -6.0, and was limited to nucleus VL (Fig. 2E). The terminals of axons originating in thalamic region VL were located primarily lateral to DCS. Some terminals were observed dorsolateral to DCS, overlapping with the dorsolateral AGm band, but these were less dense. Heavy fascicular labeling was present throughout much of the rostrocaudal extent of the striatum and was especially dense in and around DCS. Despite the rostral AGm injection, the VL injection in this animal produced striatal labeling that was located rostral to the majority of the observed AGm labeling. There was no overlap between AGm and VL terminal field labeling observed in this case (Fig. 4B).

3.5. AGm/LP

Case DCS 191 had an AGm injection centered at a-p +1.2 which affected all cortical layers but did not damage the white matter. It is visible on only one section in each spaced series, and therefore had a narrow a-p extent. The MicroRuby injection site in LP was centered at a-p -3.8, m-l +1.3, and d-v -4.5 (Fig. 2F). The injection site was very focal within medial LP, which resulted in the labeling of a relatively small number of terminal axons in the striatum. However, those that were labeled were very densely filled and easily visualized. The primary terminal field of LP thalamostriatal axons in this case formed a dorsolateral band visually very similar in placement to the one typically seen following injections in cortical area PPC (Figs. 5A–C). Thalamostriatal axons also overlapped diffusely with the dorsolateral AGm band. Within DCS, LP axons extended in long, relatively straight arborizations, which were not observed to this degree or in this conformation following injection in any other cortical area or thalamic nucleus VL (Figs. 5C, 5D). These varicosity-dotted long axons overlapped many AGm axons in both

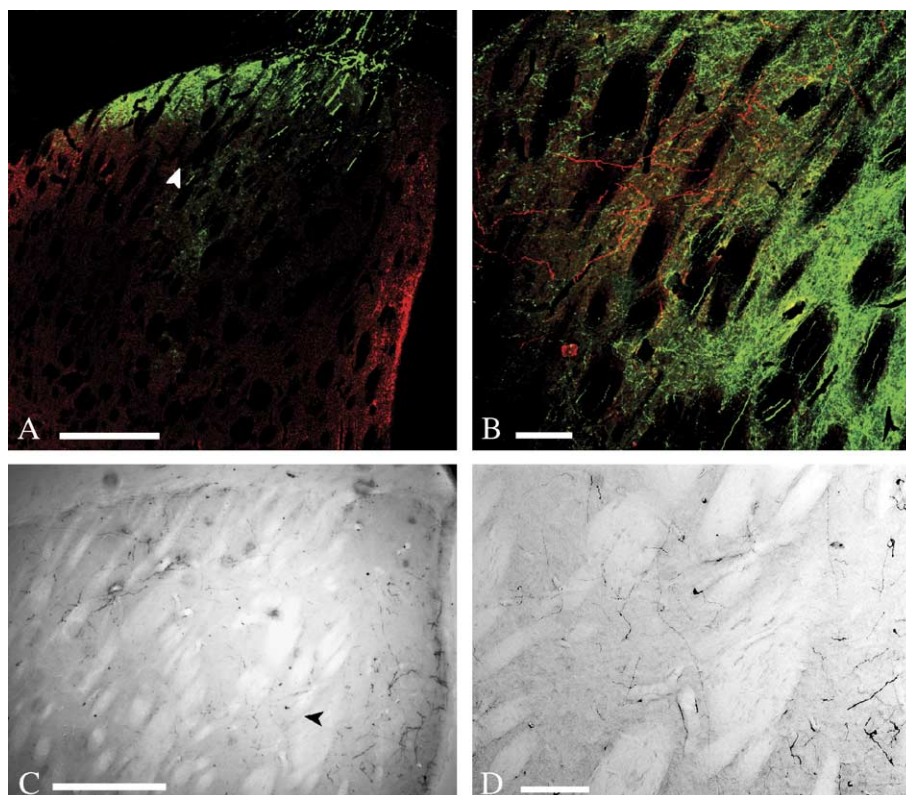


Fig. 5. Striatal labeling following injection of anterograde tracers in case DCS 191. (A) Fluorescent labeling following injection in cortical area AGm (green) and thalamic nucleus LP (red). Axonal labeling from AGm forms a dense green dorsolateral band and a more diffuse region of labeling in the dorsocentral striatum (DCS). Labeling from the LP injection also forms a dorsolateral band (arrow; panel B), in which overlap was observed between AGm and LP axons. 50 \times . Scale bar = 1 mm. (B) Axons from thalamic nucleus LP (red) form a band in the dorsolateral striatum and both overlap and interdigitate with axons from cortical area AGm (green). 100 \times . Scale bar = 100 μ m. (C) Adjacent section to the one depicted in A and B, processed for BDA10K (MicroRuby contains both tetramethylrhodamine and biotin molecules). The dorsolateral band of LP labeling is still evident, and labeling in DCS is more easily visualized. The arrow indicates the location of an especially dense focus of axonal labeling in DCS (depicted in D). 62.5 \times . Scale bar = 1 mm. (D) An especially dense focus of labeled axons following injection of MicroRuby in thalamic nucleus LP. Many axonal varicosities are evident on the labeled axons. 125 \times . Scale bar = 100 μ m.

the dorsolateral AGm band and within DCS. Axons from LP were evident in the dorsolateral band on both the fluorescent sections and those processed for BDA10K labeling, but were only visualized effectively in DCS on BDA10K sections (Fig. 5).

3.6. BDA10K injections in PPC

The brains examined having single injections of BDA10K in PPC (DCS 60, 77, 88, 91, and 114) each exhibited a pattern of striatal labeling which included a dense focus of axonal terminals located at approximately the

level of the anterior commissure (AC), with the exception of DCS 114. Photomicrographs of each PPC focus are presented in Fig. 6. In all brains with foci, the focus was always located ventromedial to the densest portion of the primary labeling field (band) within a given section. Foci were typically ellipsoid in the a-p dimension, having the largest diameter and greatest density in the approximate center of their anterioposterior extent.

As previously noted, the primary terminal bands of PPC shift in position commensurate with a medial or lateral placement of the BDA10K injection site [27]. Case DCS 91 had an injection placed in medial PPC (PPCm) and had the

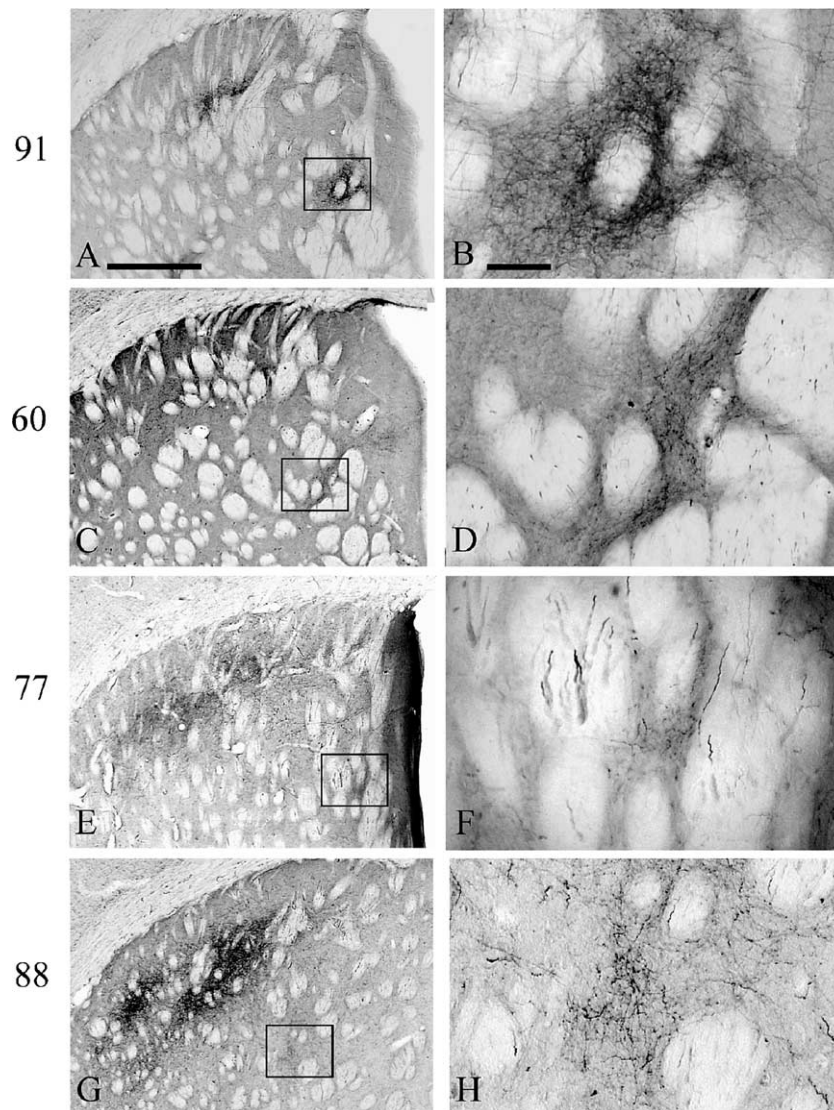


Fig. 6. Labeled axons in the striatum after injection of BDA10K in cortical area PPC. In each case, there was a dense dorsolateral band of labeling, a diffuse projection to DCS, and a dense focus of labeled axons in DCS. Boxes placed in the panels at left represent the approximate field of the corresponding higher magnification panel. (A) Photomicrograph of striatal labeling in case DCS 91. The dense dorsolateral band and dense focus in DCS are clearly visible. $62.5\times$. Scale bar = 1 mm and applies to A, C, E, and G. (B) Higher-magnification view of the dense focus of labeled axons in case DCS 91. $125\times$. Scale bar = $100\ \mu\text{m}$ and applies to B, D, F, and H. (C) Photomicrograph of striatal labeling in case DCS 60. The dense dorsolateral band and dense focus in DCS are clearly visible. (D) Higher-magnification view of the dense focus of labeled axons in case DCS 60. (E) Photomicrograph of striatal labeling in case DCS 77. The dense dorsolateral band and dense focus in DCS are clearly visible. (F) Higher-magnification view of the dense focus of labeled axons in case DCS 77. (G) Photomicrograph of striatal labeling in case DCS 88. The dense dorsolateral band and dense focus in DCS are clearly visible. (H) Higher-magnification view of the dense focus of labeled axons in case DCS 88.

most visibly dense focus of axonal labeling (Figs. 6A, 6B). Cases DCS 60, 77, and 114, also had injection sites confined to PPCm. The primary terminal fields and foci in cases 60 and 77 (Figs. 6C–F) resembled those observed in DCS 91, although foci in both brains appeared less dense overall than those in case 91. Case DCS 114 possessed a primary band of label very similar to those seen in 60 and 77, but lacked an observable focus.

Case DCS 88 received a BDA10K injection in lateral PPC and the primary terminal band was located more dorso-laterally than in the other cases. The focus was also shifted laterally, into the central portion of DCS (Figs. 6G, 6H).

In all PPC cases, labeled foci were in the calbindin-positive matrix with few or no axons visible in nearby patch compartments.

3.7. BDA10K injections in Oc2M

Both Oc2M cases examined (DCS 95 and 101) exhibited a focus of labeled axons ventromedial to the primary terminal field. The observed ellipsoid foci qualitatively resembled those seen in PPC brains and were located at or near the level of the AC, as in PPC cases. All observed foci were in the calbindin-positive matrix with few to no axons visible in nearby patch compartments, as in PPC brains.

3.8. Other BDA10K injections

One brain with a BDA10K injection in VLO (DCS 110), and another with an AGI injection (DCS 100), had robust labeling in the primary terminal fields (bands), but no foci similar to those seen in PPC or Oc2M brains were observed in either case.

4. Discussion

One significant finding in this study is that the cortical areas PPC and Oc2M terminate in DCS diffusely and in a focal manner, overlapping and interdigitating with AGM axons, while other cortical areas and thalamic nuclei do not form dense foci of axonal terminals in DCS (cortical areas VLO and AGI and thalamic nuclei LP and VL). Previously, we reported that cortical areas AGm, AGI, PPC, VLO, and Oc2M were consistently labeled following injections of a retrograde tracer in and around region DCS [4], and all were observed to form terminal fields near or in DCS in the current anterograde study, and in an earlier report [33]. These areas have all been shown previously to be reciprocally interconnected with one another and with several thalamic nuclei [29].

Fluorescent or BDA10K injections in AGI did produce the typical dorsolateral band of primary axonal labeling as well as sparse labeling in DCS, but those brains did not exhibit the focal groups of axons in DCS observed

following PPC or Oc2M injections. This is consistent with previous descriptions of AGI labeling [33]. We demonstrated herein that corticostriatal axons from areas AGm and AGI cross both one another and the dendritic processes of individual retrogradely filled striatal medium spiny neurons at the light microscopic level. This finding is consistent with the hypothesis that individual medium spiny neurons integrate inputs from multiple cortical and thalamic regions, requiring multiple simultaneous input stimuli to depolarize [6,9,27].

Fluorescent tracer injections in AGm and PPC produced a dorsolateral band of labeled PPC axons between DCS and the dorsolateral AGm band, as well as a discrete focus of labeled PPC axons within AGm. These foci were first described in another AGm/PPC double-fluorescent case [33] and appear to be sites of overlap and interdigitation between axons from AGm and PPC. Here, we report the same type of isolated focus of labeled axons in an additional double-fluorescent AGm/PPC case (178). Additionally, we also reexamined brains which received single PPC injections of the anterograde tracer BDA10K. Four of the five PPC BDA10K cases exhibit clear foci which are detached from both the primary terminal field (band) and from terminals scattered diffusely throughout DCS. Therefore, it is probable that these foci are a feature common to the PPC/AGm projection and represent a consistent zone of overlap between axons from the two cortical areas.

In a previous report, we described the results of a AGm/Oc2M double-labeling experiment [33]. We demonstrated that Oc2M projects to the striatum in a pattern consistent with the cortical areas examined herein, forming a dense primary area (band) of terminals and a more diffuse secondary area which overlaps partially with labeling from area AGm in DCS. In the current report, we found that BDA10K-labeled corticostriatal projections from Oc2M form a focal arborization in DCS similar to those seen in PPC brains.

Small groups of labeled axons similar to those seen following PPC and Oc2M injections were reported by Brown et. al following injections of an anterograde tracer into FL and HL [3]. Their presence following injections in both primary somatosensory cortices (FL and HL) and areas involved in secondary and associative tasks (AGm and PPC) suggests that these foci represent a larger pattern of corticostriatal projection.

A visually similar focus of labeling was observed following double labeling of AGm and VLO axons. Although this focus is smaller than the one formed by PPC axons and no overlap was observed, it could also be a zone of overlap on medium spiny neurons located near the interface between AGm and VLO terminal fields. As noted by Wilson [42], the possibility of individual medium spiny neurons integrating information from adjacent yet non-overlapping corticostriatal terminals is a distinct and often overlooked one, due to the size of their dendritic trees. Further, the convergence of axons from non-overlapping

corticostriatal projections has been demonstrated electrophysiologically on single medium spiny neurons in the macaque [27]. This may also be true in the rat striatum but it remains to be demonstrated.

These findings contribute to the strength of the hypothesis that cortical areas AGm, PPC, and Oc2M participate in the function of a cortical–basal ganglia–thalamic–cortical network mediating directed attention in the rat, and that DCS plays a role in the integration of inputs from these non-adjacent, reciprocally interconnected cortical areas.

Incidental uptake by fibers of passage is a concern in any tract-tracing study [4], and therefore great care was taken in the current study to exclude cases with injections in regions that could produce conflicting results. Direct comparison of retrograde and anterograde data also help ensure that observed labeling is real and is not just the result of labeling fibers of passage. Therefore, it is not surprising, that anterograde injections in thalamic nucleus VL, a nucleus primarily associated with motor functions, do not produce labeled axons in region DCS, although VL was heavily labeled following injections of retrograde tracers in and around DCS. Uptake of retrograde tracers by the large number of labeled axons in the fascicles traveling through DCS is the likely explanation for the VL labeling seen in the previous retrograde study [4]. The observed pattern of labeling is consistent with previous anatomical and electrophysiological data localizing the effects of VL stimulation to lateral striatum [13]. However, VL is a large nucleus, and the injection did not completely fill it in this case. It is possible that other portions of VL do project to DCS, although the current data suggest that previously reported retrograde labeling of VL was the result of tracer uptake by fibers of passage [4].

The medial portion of thalamic nucleus LP was found to project densely to a dorsolateral band which overlapped and interdigitated with the primary dorsolateral AGm band, and diffusely to DCS. In a previous retrograde study [4], we reported that LP was labeled only when injections of Fast Blue were placed in DCS itself. Additionally, Ero et al. found LP labeling following injection of a retrograde tracer in the dorsal striatum but not in the ventral striatum [7].

The overlap of corticostriatal terminal fields was noted by Yeterian and Van Hoesen [44]. Many authors have since addressed the topic of overlapping and/or interdigitating corticostriatal terminal fields because of the potent implications. Of particular interest is the overlap in the striatum of efferents from reciprocally interconnected cortical and thalamic areas. In one notable report, a network of reciprocally interconnected cortical and thalamic areas was described in the macaque [25], where interconnected motor-related areas project both to distinct, non-overlapping territories in the basal ganglia, and also project more diffusely to overlapping areas in both the caudate and putamen. Similarly, several groups have demonstrated that forelimb representations from different cortical areas overlap in the macaque putamen [16,37]. This pattern of overlap has also

been described in thalamostriatal and corticostriatal projections from rodent somatosensory cortex as well, where there is more convergence in the striatum from SI areas representing subcomponents of the rodent forelimb than from cortical areas representing noncontiguous body parts [14,15].

In addition to the reports which demonstrated overlap, there are substantial data which suggest that the projections from reciprocally interconnected cortical areas project exclusively to non-overlapping portions of the striatum [35]. One possible explanation for the disparity between our findings and those of Selemon and Goldman-Rakic is that some cortical areas project in an overlapping way and others in a non-overlapping way, despite their reciprocal interconnection. It is possible that functionally associated cortical areas (like AGm and PPC) involved in a cortical–subcortical network that relies on the striatum for some integrating function project to the striatum with some degree of overlap depending on the requirements of the specific network, while other cortical areas are entirely segregated. This pattern of selective overlap and segregation was previously reported in the macaque [8].

In the current study, as in previous reports, we found that both AGm and PPC form their primary terminal fields as densely labeled bands in dorsolateral striatum which do not overlap [29,33], as reported by Brown et al. for rat somatosensory cortex [3]. Also, we demonstrated that areas AGm and PPC project diffusely to region DCS, using a single injection of an anterograde tracer per rat [33]. Herein, we report the existence of a focus of overlap in DCS of projections from AGm and cortical areas PPC and Oc2M. These foci could represent key regions in DCS for multimodal integration of information from AGm with that from PPC and Oc2m. Whether PPC and Oc2M foci overlap with each other in DCS remains an open question and warrants further study. Areas AGm, PPC, and Oc2M all play a critical role in directed attention in the rat. Destruction of DCS, but not dorsolateral striatum, produces robust neglect of contralesional stimuli which does not spontaneously recover and cannot be relieved by the administration of dopamine agonists [38,40]. The location of these foci within DCS is therefore a significant finding. Axons in the dense foci observed in BDA10K brains were located in the calbindin-rich matrix compartment. This is consistent with the descriptions of matrixosomes in the primate basal ganglia [11] and may indicate an anatomical and functional similarity between the rat foci and primate matrixosomes.

It is possible that integration in the diffusely terminating portion of the rat network mediating directed attention is taking place only on specific subtypes of striatal projection cells, which are typically segregated into patch and matrix groups [18]. Diffuse overlap is seen at the edge of the AGm/VLO striatal terminal fields and LP neurons extend relatively long distances in the dorsolateral AGm band as well as in DCS, forming many distinct en passant type axonal varicosities. In a previous paper, we reported that the

majority of labeled axons in DCS were in the matrix compartment following cortical placement of BDA10K [33]. It is likely, therefore, that the majority of the diffuse overlap observed in multiple-labeled cases is occurring in the matrix compartment.

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