Basal-ganglia 'Projections' to the Prefrontal Cortex of the Primate

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We used retrograde transneuronal transport of the McIntyre-B strain of herpes simplex virus type 1 to examine the extent and organization of basal-ganglia–thalamocortical projections to five regions of prefrontal cortex in the cebus monkey (*Cebus apella*): medial and lateral area 9 (9m and 9l), dorsal and ventral area 46 (46d and 46v) and lateral area 12 (12l). All of these prefrontal areas were found to be targets of basal-ganglia output that originated in the internal segment of the globus pallidus (GPi) and/or the pars reticulata of the substantia nigra (SNpr). Approximately one-third of the total volume of these nuclei was directed toward prefrontal cortex, a volume comparable to that directed at the cortical motor areas. The origins of the outputs to different prefrontal areas were topographically organized. Different portions of SNpr (the rostral and caudal thirds) projected to areas 9m and 12l. Similarly, different output nuclei (GPi and SNpr) projected to adjacent portions of the same cytoarchitectonic field (46d and 46v). Furthermore, the outputs to prefrontal areas were segregated from those to motor areas of cortex. Thus, basal-ganglia outputs to prefrontal cortex are both extensive and topographically organized, forming a rich anatomical substrate for basal-ganglia influences on the cognitive operations of the frontal lobe.

Introduction

Classically, the output of the basal ganglia is thought to influence primarily motor areas of the cerebral cortex (Kemp and Powell, 1971). The movement disorders which are so characteristic of basal-ganglia dysfunction are thought to be due, in part, to abnormal signals in basal-ganglia–thalamocortical pathways to these cortical areas. On the other hand, it has become increasingly clear that damage to the basal ganglia has non-motor consequences as well (Alexander *et al*., 1986; Rogers, 1992; Cummings, 1993; Bhatia and Marsden, 1994). The neural substrates that mediate this aspect of basal-ganglia dysfunction have not been as clearly delineated.

It has been proposed (Alexander *et al*., 1986) that the basal ganglia participate in five parallel segregated circuits with the cerebral cortex. Two of these circuits involved loops with skeletomotor and oculomotor areas of cortex: the primary motor cortex (M1) and the frontal eye field (FEF). The remaining three circuits were thought to target non-motor areas in the frontal lobe: dorsolateral prefrontal cortex, lateral orbitofrontal cortex and anterior cingulate cortex. Each of these cortical regions is thought to be involved in various aspects of cognitive behavior, including working memory, planning, attention and rule-based learning (Goldman-Rakic, 1987; Funahashi *et al*., 1989, 1993, 1997; Petrides, 1995; Fuster, 1997). Thus, Alexander *et al*. (Alexander *et al*., 1986) suggested that some of the non-motor symptoms of basal-ganglia dysfunction are due to alterations in basal-ganglia output to these frontal areas.

The disynaptic nature of basal-ganglia–thalamocortical connections has made it difficult to define the specific cortical areas that are the target of basal-ganglia output and to evaluate the proposal of Alexander *et al*. (Alexander *et al*., 1986), but see Ilinsky *et al*. (Ilinsky *et al*., 1985). We developed the use of herpes simplex virus type 1 (HSV1) as a retrograde transneuronal tracer in the central nervous system of primates to overcome this problem (Zemanick *et al*., 1991; Strick and Card, 1992; Hoover and Strick, 1993, 1999; Lynch *et al*., 1994; Middleton and Strick, 1994). In a preliminary study (Middleton and Strick, 1994), we used this method to examine the dorsolateral prefrontal circuit and demonstrated that area 46 is the target of a distinct basal-ganglia–thalamocortical pathway from the internal segment of the globus pallidus (GPi). Observations from experiments with conventional tracers suggest that the output of the dorsolateral prefrontal circuit is not limited to area 46 (Goldman-Rakic and Porrino, 1985; Ilinisky *et al*., 1985; Barbas *et al*., 1991; Dermon and Barbas, 1994; Middleton and Strick, 2001). Therefore, in the present study, we examined the extent and organization of basal-ganglia output to five regions of prefrontal cortex: medial and lateral area 9 (areas 9m and 9l), dorsal and ventral area 46 (areas 46d and 46v) and lateral area 12 (area 12l).

Our major finding is that all of the prefrontal areas we examined are targets of basal-ganglia output. The neurons that project to each area are largely separate from those that project to the other prefrontal areas. Furthermore, the outputs to prefrontal areas as a whole are largely segregated from those to the motor areas of the cerebral cortex. Thus, the dorsolateral prefrontal circuit and the basal-ganglia outputs to prefrontal cortex are more extensive and topographically organized than previously suspected. This system forms a rich anatomical substrate for the basal ganglia to influence the cognitive operations of the frontal lobe.

Short reports of some of the results reported in this manuscript have appeared elsewhere (Middleton and Strick, 1994, 1997, 2000).

Materials and Methods

The animals and procedures that are the subject of this report are the same as those included in our recent publication on cerebellar projections to prefrontal cortex (Middleton and Strick, 2001). A complete description of the experimental methods is provided in that publication. Here we will briefly review the essential features of our experimental procedures.

The procedures adopted for this study and the care provided experimental animals conformed to the regulations detailed in the *National Institutes of Health Guide for the Care and Use of Laboratory Animals*. All protocols were reviewed and approved by the relevant institutional animal care and use committees. The biosafety precautions taken during these experiments conformed to or exceeded the BSL-2 regulations detailed in *Biosafety in Microbiological and Biomedical Laboratories* (Health and Human Services Publication 93-8395). A detailed description of the procedures for handling virus and virus-infected animals is presented elsewhere (Strick and Card, 1992; Hoover and Strick, 1999).

Injection Sites

Injections of HSV1 were made at multiple sites in areas 9, 46 and 12 of 13 hemispheres in 9 cebus monkeys (*Cebus apella*, see Table 1). These cytoarchitectonic fields have a relatively fixed relationship to various surface landmarks such as the principal and arcuate sulci, and the medial wall of the hemisphere. Therefore, the location of each injection site was guided by these features. Our injection sites were placed to avoid spread to either the FEF or the supplementary eye field (SEF) Tian and Lynch (1995, 1996).

Tracers

To determine the origin of thalamic input to the cortical regions under analysis, we injected one or two fluorescent tracers [fast blue (FB), diamidino yellow (DY), rhodamine dextran (RD), or nuclear yellow (NY)] into different sites within areas 9, 46 and 12 [for a detailed description of these injection sites see Table 1 in Middleton and Strick (Middleton and Strick, 2001)]. Multiple small injections of tracer were made in each cortical area (10–35 injections, 0.1–0.25 µl/site to a total volume of 1.7–3.5 µl/area). To determine the origin of basal-ganglia input to prefrontal cortex, we used the McIntyre-B strain of HSV1 as a trans-synaptic tracer (Middleton and Strick, 2001). This strain travels transneuronally in the retrograde direction in the central nervous system of primates (Zemanick *et al*., 1991; Strick and Card, 1992; Hoover and Strick, 1993, 1999; Strick *et al*., 1993; Lynch *et al*., 1994; Middleton and Strick, 1994). Three different preparations of this virus were used. In four hemispheres, we injected McIntyre-B obtained from Dr David I. Bernstein, Gamble Institute of Medical Research, Cincinnati, OH; for method of preparation, see McLean *et al*. (McLean *et al*., 1989). In nine hemispheres, we injected a preparation of McIntyre-B that had been passaged in African green monkey kidney (Vero) cells by Dr Richard D. Dix, Jones Eye Institute, Little Rock, AR or by Dr Jennifer H. LaVail, University of California San Francisco, San Francisco, CA; for method of preparation, see LaVail *et al*. (LaVail *et al*., 1997). No substantial differences were observed in the overall patterns of labeling produced by these different preparations. Multiple small injections of virus were made into areas 9, 46 and 12 (17–59 injections, 0.05–0.25 µl/site to a total volume of 2.1–5.0 µl/area).

Survival Period

After 5 days, each animal was deeply anesthetized (ketamine hydrochloride, 25 mg/kg, i.m.; pentobarbital sodium, 36–40 mg/kg, i.p.) and transcardially perfused using a three-step procedure (Rosene *et al.*, 1986). The perfusates included 0.1 M phosphate buffered saline (PBS), 4% (w/v) paraformaldehyde in PBS and 4% paraformaldehyde in PBS with 10% (v/v) glycerine. Following the perfusion, the brain was photographed, stereotaxically blocked, removed from the cranium and stored in buffered 4% paraformaldehyde with 20% glycerine (4°C) for 4–7 days.

Histology

Blocks of neural tissue were frozen (Rosene *et al*., 1986) and serially sectioned in the coronal plane at a thickness of 50 µm. Every tenth section was counterstained with cresyl violet (Nissl stain) for cytoarchitectonic analysis. To identify neurons labeled by virus transport, we processed free-floating tissue sections according to the avidin–biotin–peroxidase method (ABC; Vectastain, Vector Laboratories Inc., Burlingame, CA) using a commercially available antibody to HSV1 (Dako Corp., Carpinteria, CA; 1:2000 dilution). At least every other section from these animals was reacted. Sections were mounted onto gelatin-coated glass slides, air dried and then coverslipped with either Artmount or DPX.

Analytic Procedures

Tissue sections reacted for HSV1 were examined under bright-field, dark-field and polarized illumination. At least every fourth section was plotted through the injection site and at least every other section was plotted through the output nuclei of the basal ganglia. Data from all experiments were plotted using a PC-based charting system (MD2; Minnesota Datametrics Inc., St Paul, MN). This system uses optical encoders to sense *x*–*y* movements of the microscope stage and stores the coordinates of charted structures (e.g. section outlines, injection site zones and labeled neurons). Digital images of selected structures were 'captured' from the microscope using a video camera coupled to a high-resolution video processing board in a PC. Software written in the laboratory enabled us to generate high-resolution composites from multiple images.

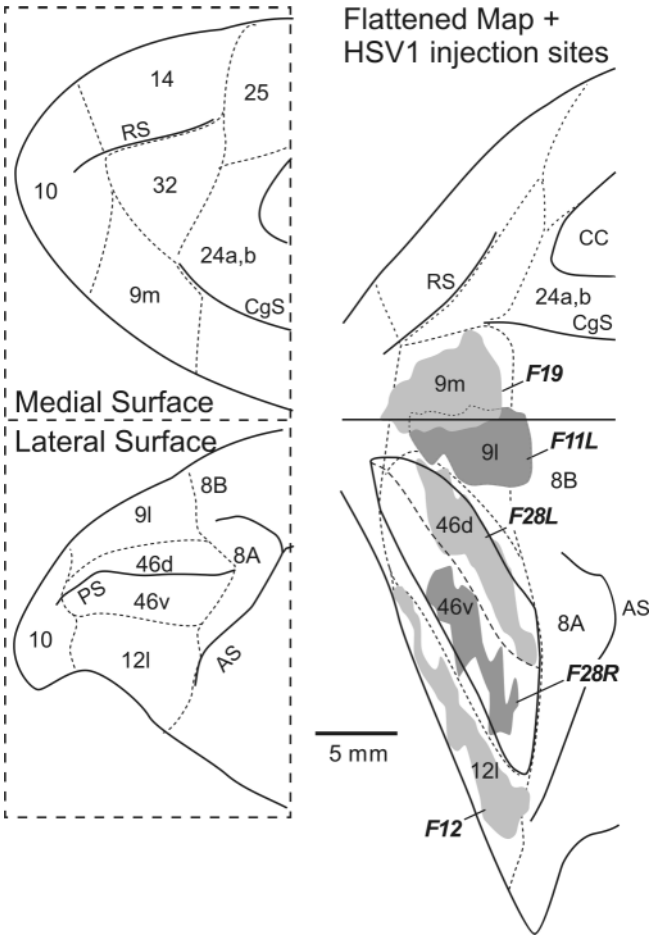

Figure 1. Cortical injection sites. A lateral view of the prefrontal cortex of the cebus monkey (left) indicating approximate cytoarchitectonic borders (dashed line) and the unfolded view (right) indicating the locating of representative HSV1 injection sites (shading). Cortical areas are numbered according to Walker (Walker, 1940). AS, arcuate sulcus; CC, corpus callosum; CgS, cingulate sulcus; PS, principal sulcus; RS, rostral sulcus.

Reconstruction of Injection Sites

Three concentric zones of labeling surrounded each virus injection site. Zone I contained the needle track and the highest density of viral staining and pathology. In some instances, the tissue in this zone disintegrated during tissue processing. Zone II contained a dense accumulation of infected neurons and glia, as well as a high degree of background staining. Zone III contained large numbers of labeled neurons, but little or no back- ground staining. We included both zones I and II in our reconstructions of injection sites [Fig. 1; see also Figs 1 and 2 of Middleton and Strick (Middleton and Strick, 2001)].

Once we defined the borders of each injection site, we displayed this on a flattened map of the frontal lobe (Fig. 1). To generate this map, the plots of individual sections were aligned on the junction of the medial wall of the hemisphere with the lateral surface (i.e. the midline of the hemisphere). Then, the medial wall and the lateral surface of the hemisphere, as well as the dorsal and ventral banks of the principal sulcus, were unfolded. Cytoarchitectonic borders were added to this map using published criteria (Walker, 1940; Barbas and Pandya, 1989).

Distribution and Density of Basal Ganglia Labeling

The numbers of labeled neurons and their locations in the basal ganglia were recorded using the computer-based charting system described

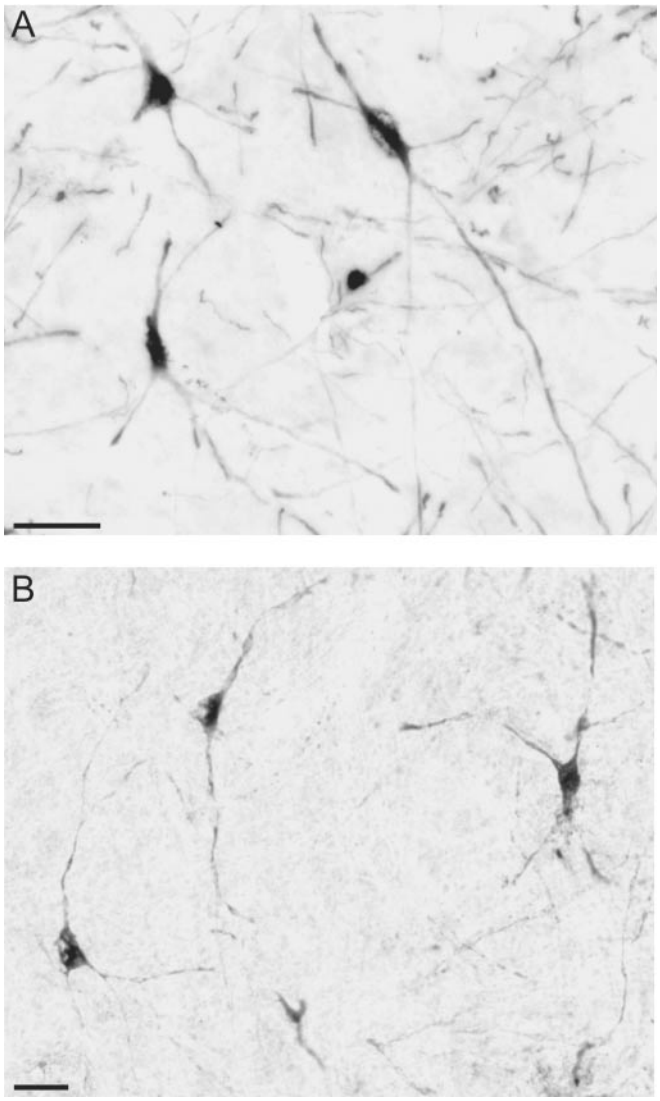

Figure 2. HSV1 labeled cells in GPi and SNpr. Representative cells in the SNpr (top) and GPi (bottom) labeled following injections of area 12l (top) or area 9m (bottom). Scale bar = 40 μm.

above. This information was used to estimate the relative density of neurons in GPi and SNpr that were labeled by virus transport from each cortical injection. The proportion of GPi and SNpr containing labeled neurons was then calculated using Cavalieri's estimator (Rosen and Harry, 1990) as previously described (Middleton and Strick, 2001).

Results

We successfully injected the McIntyre-B strain of HSV1 into five subregions of prefrontal cortex, including the dorsal and ventral parts of area 46 (46d and 46v), the medial and lateral parts of area 9 (9m and 9l) and the lateral part of area 12 (12l) (Fig. 1). The present results focus on the patterns of transneuronal labeling found in the output nuclei of the basal ganglia after these injections. Our prior report presented a detailed descrip- tion of the injection sites, the patterns of thalamic labeling and the patterns of cerebellar labeling seen in these animals (Middleton and Strick 2001).

Retrograde transneuronal transport of HSV1 from each subregion of prefrontal cortex consistently labeled second-order neurons in the output nuclei of the basal ganglia (GPi and/or SNpr). The numbers of labeled neurons, the ratio of GPi to SNpr

Table 1
Distribution of basal ganglia labeling following prefrontal HSV1 injections

Area	Case	GPi			SNpr		GPi:SNpr
		No. of cells	% inner	% ipsi	No. of cells	%ipsi	
9m	F19	911	18	76	721	90	1.26
	F25 R	499	25	–	134	–	3.72
	Mean	705	20	–	427.5	–	1.65
91	F11 L	1273	49	–	2084	–	0.62
	F21	222	58	83	382	92	0.58
	Mean	747.5	50	–	1233	–	0.61
46	F1	326	62	97	65	92	5.02
	F6	140	41	91	149	91	0.94
	F11	690	64	–	515	–	1.38
	Mean	385.3	61	95	243	92	1.59
46d	F24 L	16	31	–	0	–	~
	F28 L	52	37	–	7	–	7.43
	Mean	34	35	–	3.5	–	9.71
46v	F28 R	4	75	–	36	–	0.11
	F24 R	0	–	–	7	–	0.0
	Mean	2	–	–	21.5	–	0.09
121	F12	8	100	100	248	96	0.03
	F27 L	6	83	–	370	–	0.02
	Mean	7	93	–	309	–	0.02

The numbers and distribution of labeled cells in the two major output nuclei of the primate basal ganglia (GPi and SNpr) are indicated after HSV1 injections of each prefrontal area. '% inner' refers to the inner portion of GPi; '% ipsi' is the percentage of cells labeled in the same hemisphere as the injection site (in animals with only single hemisphere injections).

labeled neurons and the topographic distribution of labeled neurons within the output nuclei varied with the injection site (see below). The vast majority of the GPi and SNpr neurons that were labeled through retrograde transneuronal transport had darkly stained cell somas that were spindle-shaped (Fig. 2). In many instances, well-stained dendrites radiated from each pole of the cell body, giving the labeled neurons a bipolar appearance. These morphological features are typical of GPi and SNpr neurons that project to the thalamus (Beckstead and Frankfurter, 1982; Parent and Bellefeuille, 1982; Zemanick *et al.*, 1991; Hoover and Strick, 1993; 1999; Middleton and Strick, 1994, 1996).

Inputs to Different Prefrontal

We have at least two cases for each of the five regions examined (Table 1). In general, virus injections into the same region in different animals produced comparable results. The only qualification to this general statement is that smaller injections of virus resulted in fewer numbers of labeled neurons. Our figures will illustrate data from one representative animal for each area examined. The quantitative descriptions will include data from all cases.

Area 9m

Injections of HSV1 into area 9m (n = 2) labeled an average of >700 neurons in GPi and 400 neurons in SNpr (Table 1). Over 75% of the labeled neurons in GPi and >90% of the labeled neurons in SNpr were found ipsilateral to the injection site. Neurons labeled following area 9m injections were found entirely within the rostral pole of GPi and were most concentrated within the rostral third of SNpr (Figs 3–6). These labeled neurons were located dorsomedially within GPi and ventrolaterally in SNpr (Figs 3–6). Twenty percent of the labeled neurons in GPi were found in its inner portion. Labeled neurons after area 9m injections occupied ~8% of the volume of GPi and 16% of the volume of SNpr. On single sections, the proportions

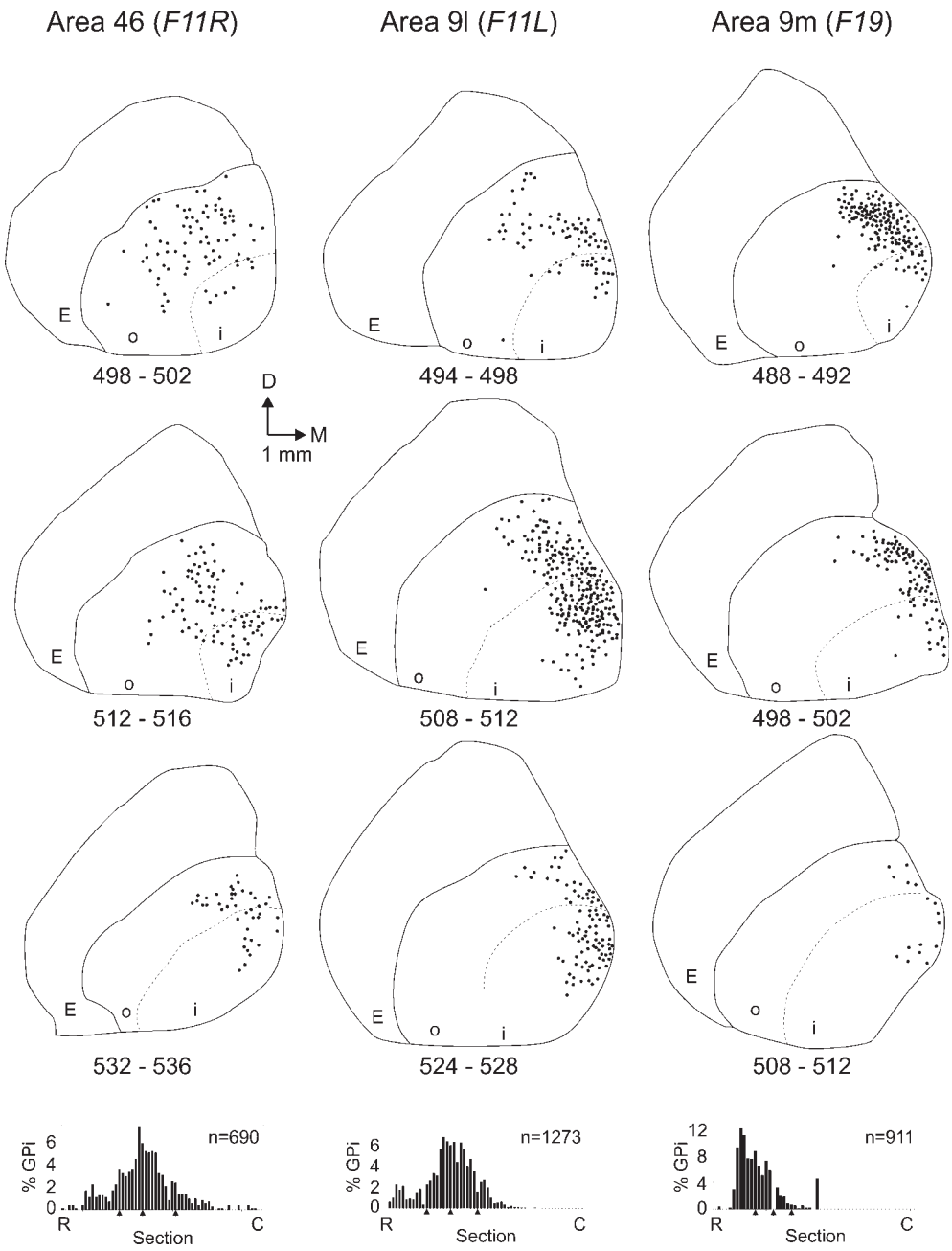

Figure 3. Distribution of HSV1 labeled cells in GPi following HSV1 injections. The location of cells labeled following HSV1 injections of areas 46, 9l and 9m is shown on representative cross-sections (top), with the rostrocaudal distribution of all labeled cells indicated below each series. Each section shows the labeled cells present on three adjacent sections (at the locations shown by the arrows on the plots). C, caudal; D, dorsal; E, external globus pallidus; i, inner GPi; M, medial; o, outer GPi; R, rostral.

of GPi and SNpr that contained labeled neurons were as high as 23 and 35% of the total cross-sectional area of these nuclei.

Area 9l

Injections of HSV1 into area 9l (n = 2) labeled an average of >700 neurons in GPi and 1200 neurons in SNpr (Table 1). Over 83% of the labeled neurons in GPi and >92% of the labeled neurons in SNpr were found ipsilateral to the injection site. Neurons labeled following area 9l injections were most concentrated within the rostral half of GPi and within the rostral half of SNpr. These labeled neurons were located dorsomedially in GPi and laterally in SNpr (Figs 3–6). Fifty percent of the labeled neurons in GPi were found in its inner portion. Labeled neurons after area 9l injections occupied ~10% of the volume of GPi and 20% of the volume of SNpr. On single sections, the proportions of GPi and SNpr that contained labeled neurons were as high as 38 and 36% of these nuclei.

Area 46

Large injections of HSV1 into area 46 (n = 3) labeled an average of >360 neurons in GPi and 240 neurons in SNpr (Table 1). Over 95% of the labeled neurons in GPi and >92% of the labeled neurons in SNpr were found ipsilateral to the injection site. Neurons labeled following area 46 injections were most concentrated in the middle third of GPi and in a caudal segment of SNpr. These labeled neurons were located dorsally within

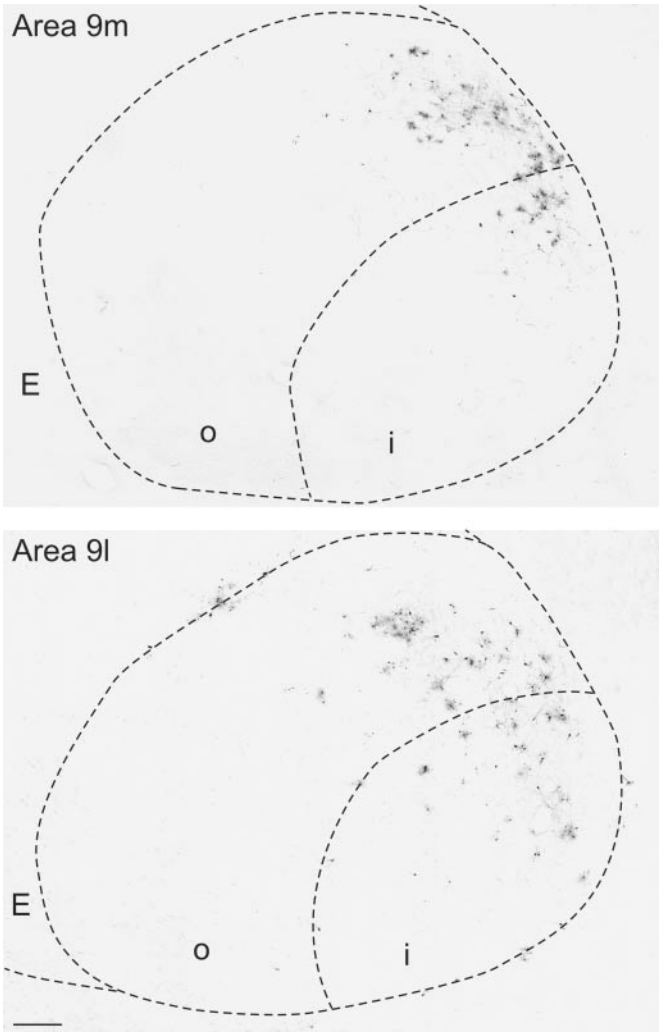

Figure 4. Discrete regions of GPi labeled after injections of different prefrontal area subdivisions. Even after injections of different subregions of the same cytoarchitectonic region (areas 9m and 9l), the location of labeled cells in GPi is differentially localized. Images are taken at the same rostrocaudal level of the nucleus. Abbreviations as in Figure 3. Scale bar = 500 μm.

GPi and dorsolaterally in SNpr (Figs 3–6). Sixty percent of the labeled neurons in GPi were found in its inner portion. Labeled neurons after area 46 injections occupied ~9% of the volume of GPi and 7% of the volume of SNpr. On single sections, the proportions of GPi and SNpr that contained labeled neurons were as high as 29 and 22% of these nuclei.

Although large injections of virus into area 46 resulted in labeled neurons in both GPi and SNpr, more restricted injections of virus demonstrated the presence of a clear topography in the distribution of GPi and SNpr projections to this region of cortex. Labeled neurons were found mainly in GPi after virus injections into area 46d, whereas labeled neurons were found mainly in SNpr after virus injections into area 46v. These observations are consistent with our data on the organization of thalamic inputs to area 46d and 46v (Middleton and Strick, 2001). We found that VApc, a target of pallidal efferents, is a major source of input to area 46d. In contrast, VAmc and MDmf, targets of nigral efferents, are the major source of input to area 46v.

Area 12l

Virus injections into area 12l labeled very few neurons in GPi, <2 cells in any given section (Table 1). Those that were labeled were located ipsilateral to the injection site, in the inner portion of GPi (>90%). In contrast, the same injections labeled an average of >300 neurons in the SNpr. Approximately 95% of these neurons were located ipsilateral to the injection site. Within the SNpr, neurons labeled by area 12l injections were located predominantly in the caudal half of the nucleus, where they were densely clustered in a dorsomedial region (Figs 5 and 6). Neurons labeled following injections into area 12l occupied ~7% of the volume of SNpr. On single sections, this proportion was as high as 14% of the nucleus.

General Pattern of Basal-ganglia Projections to Prefrontal Cortex

GPi

Overall, there were several trends in the pattern of GPi labeling produced by retrograde transneuronal transport of HSV1 from different areas of prefrontal cortex. First, dorsomedial areas of prefrontal cortex (areas 9m, 9l and 46d) receive more GPi input than ventral and lateral areas (areas 46v and 12l; Table 1, Figs 3–6). Second, the origin of projections from GPi to different cortical areas is topographically organized. For example, GPi neurons labeled by virus injections into area 9m were located medial, dorsal and rostral to those labeled after injections into area 46d (Figs 3–6). GPi neurons labeled after injections into area 9l were located between the area 9m and 46d populations. Regardless of the area injected, there was a tendency for the location of labeled neurons in GPi to be more ventral and lateral in rostral regions of the nucleus and to shift dorsally and medially at more caudal levels (Fig. 3). Finally, the proportion of labeled neurons in the inner portion of GPi increased as the injection sites moved from dorsomedial to ventrolateral regions of prefrontal cortex. Similarly, the proportion of labeled neurons located ipsilateral to the injection site increased as the injection sites moved from dorsomedial to ventrolateral regions (Table 1).

SNpr

The pattern of nigral projections to prefrontal cortex also could be characterized by a number of trends. First, areas 9m and 9l receive more nigral input than the other areas. However, the ratio of nigral to pallidal input is greater for ventrolateral regions of prefrontal cortex (areas 12l and 46v) than for dorsomedial regions (areas 9m, 9l and 46d; Table 1). Second, the origin of projections from SNpr to different cortical areas is topographically organized. For example, dorsomedial regions of prefrontal cortex (areas 9m and 9l) receive input from SNpr regions that are rostral to those that project to ventrolateral regions of prefrontal cortex (areas 12l and 46v; Figs 5 and 6). Within the rostral portion of SNpr, neurons that project to area 9m are located ventral to those that project to area 9l (Figs 5 and 6). Less distinct shifts in the origin of projections to areas 12l and 46v were present in the caudal portion of SNpr (Figs 5 and 6).

Discussion

The basal ganglia traditionally have been considered to be motor structures. The movement abnormalities that accompany many basal-ganglia disorders provide ample support for this view. Our results, however, clearly support an expanded concept of basal-ganglia function that extends well beyond the realm of motor control (Ilinsky *et al*., 1985; Alexander *et al*., 1986; Cummings, 1993). Retrograde labeling of neurons following cortical injections of virus has provided us with a means to assess the portion of basal-ganglia output that is devoted to prefrontal function. We

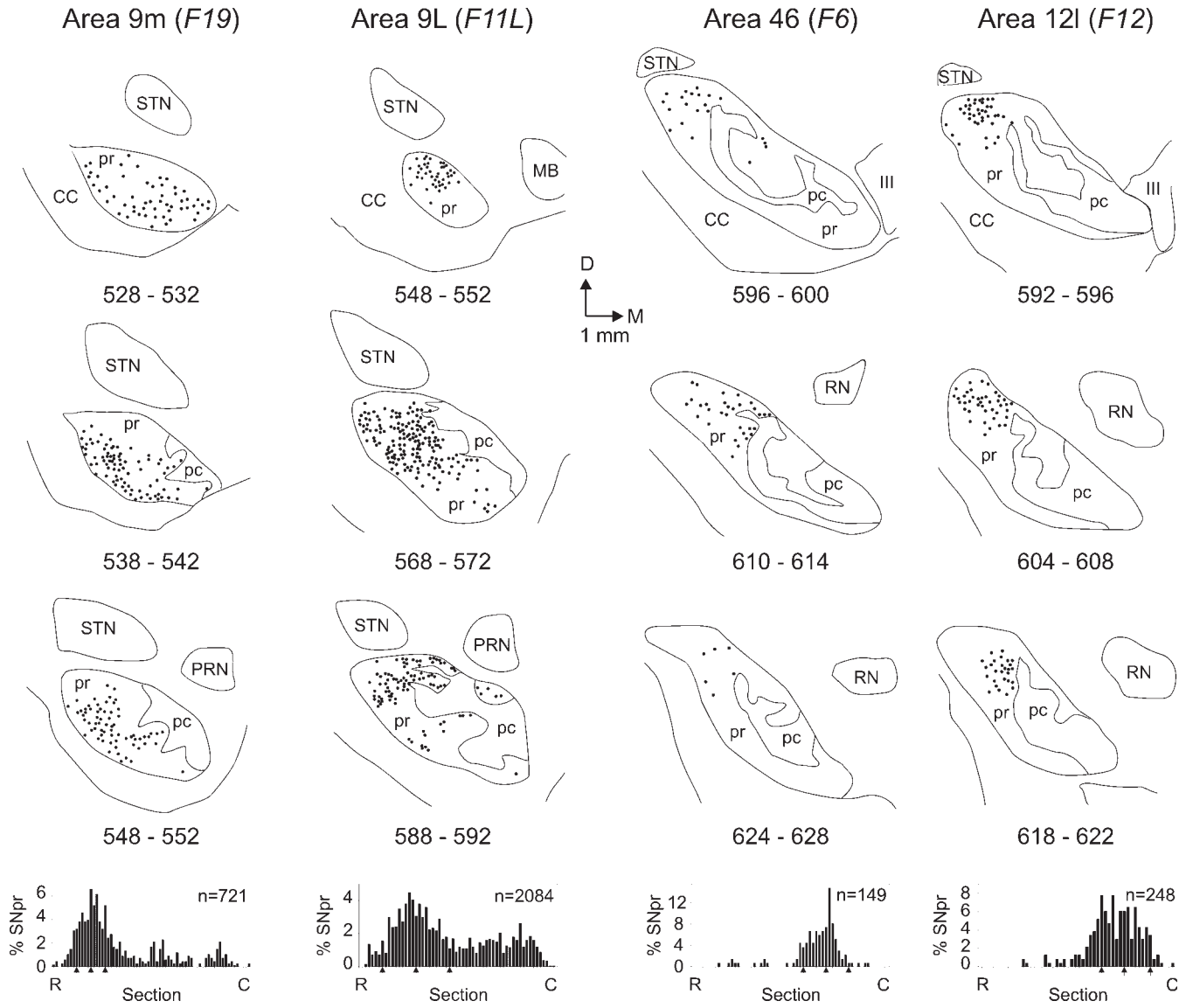

Figure 5. Distribution of HSV1 labeled neurons in SNpr after prefrontal injections. Conventions as in Figure 3. III, third cranial nerve; CC, crus cerebri; MB, mamillary body; pc, pars compacta of substantia nigra; pr, pars reticulata of substantia nigra; PRN, pre-reubral nucleus; RN, red nucleus; STN, subthalamic nucleus.

found that ~27% of the volume of GPi and 45% of SNpr projected to areas of prefrontal cortex. The volume of SNpr is ~60% of GPi. Thus, about one-third of the total output from the basal ganglia is directed at the prefrontal areas we examined. Our current estimate is that a comparable volume of basal-ganglia output is directed at the cortical motor areas (Hoover and Strick, 1993, 1999; Strick *et al.*, 1993; Lynch *et al.*, 1994; Dum and Strick, 1999; Akkal *et al.*, 2001). We have not yet examined the basal-ganglia projections to all of the cortical motor areas or to all portions of the prefrontal cortex. Thus, these numbers will be revised as more data become available. However, the fact remains that a substantial portion of the output from the basal ganglia is directed at prefrontal cortex. This suggests that a considerable component of basal-ganglia function is devoted to cognition.

Our findings further indicate that basal-ganglia output is not restricted to a single area of prefrontal cortex, but innervates multiple areas. Each of the prefrontal regions we examined received input from one or both output nuclei of the basal ganglia. Areas 12l and 46v received the vast majority of their input from SNpr, whereas area 46d received its input largely from GPi. Areas 9m and 9l received their input from both GPi and SNpr. This creates the anatomical substrate for the basal ganglia to have a broad influence over the diverse operations performed by prefrontal cortex. In this respect, basal-ganglia output differs from cerebellar projections to prefrontal cortex, which appear to be more focused on specific subfields of areas 46 and 9, i.e. areas 46d, 9l and 9m (Middleton and Strick, 2001).

Retrograde transneuronal transport of HSV1 provided us with the unique opportunity to examine the topographic organization of GPi and SNpr projections to prefrontal cortex. We found that the projection to an individual cortical area originates from a largely distinct cluster of neurons in the output nuclei. We have termed such clusters 'output channels' (Hoover and Strick, 1993). Our evidence suggests that the output channels to different prefrontal areas are topographically organized. For example, entirely different portions of SNpr (the rostral and caudal thirds) project to areas 9m and 12l. Similarly, entirely

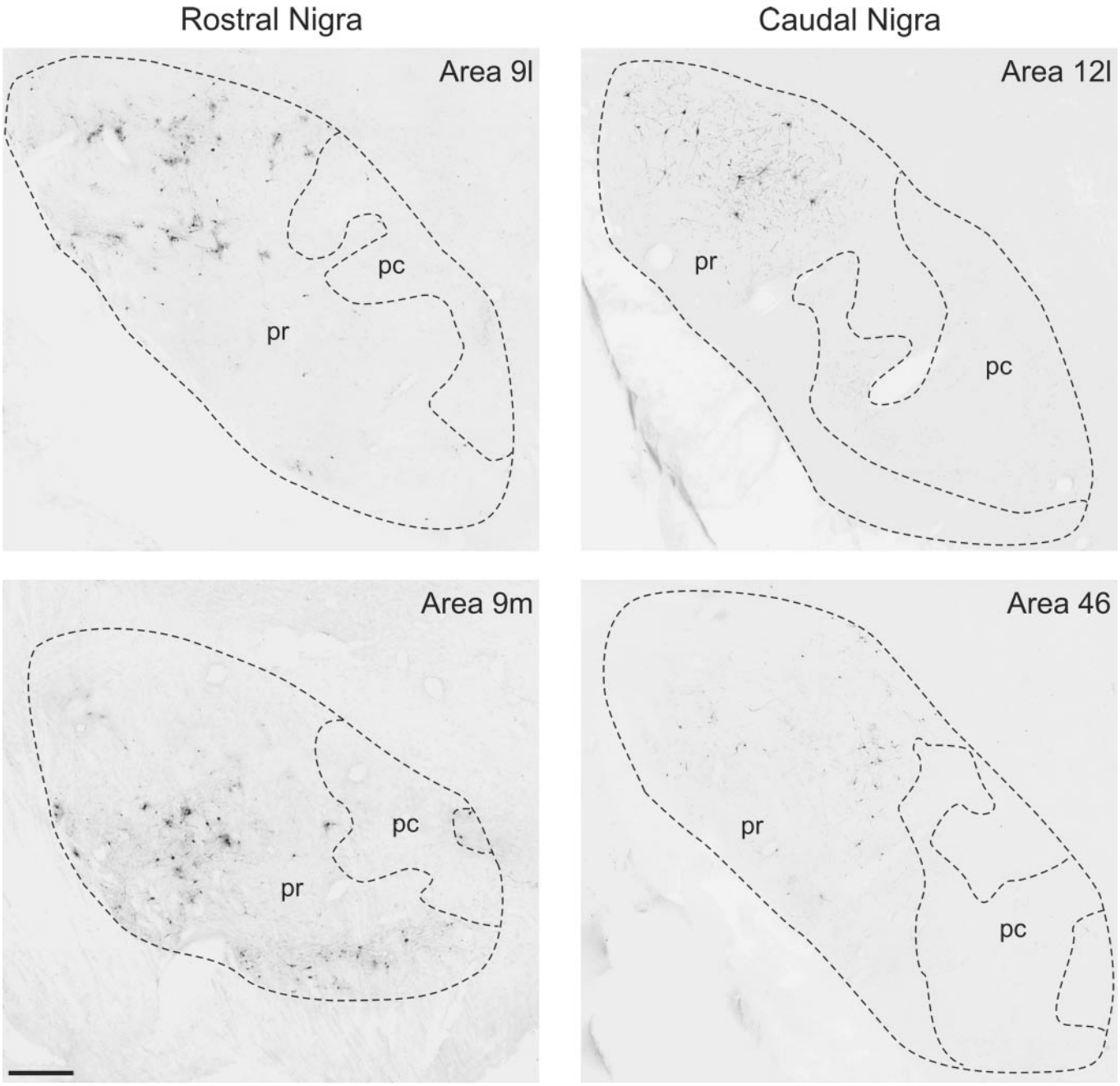

Figure 6. Discrete regions of SNpr labeled after injections of different prefrontal subdivisions. In both the rostral SNpr (left) and caudal SNpr (right), the location of labeling following injections of different prefrontal areas is different. Both pairs of images through the rostral and caudal levels of the nucleus are matched for rostrocaudal location. The dashed lines indicate the borders of the SNpr and SNpc as determined by comparison with adjacent Nissl-stained sections. Abbreviations as in Figure 5. Scale bar = 500 μm.

different output nuclei (GPi and SNpr) project to adjacent portions of the same cytoarchitectonic field (areas 46d and 46v). Thus, there are clear examples where the output channels to nearby prefrontal areas display no overlap.

Within GPi, we observed a progressive medial to lateral shift in the location of neurons that projected to adjacent prefrontal areas (areas 9m, 9l and 46d). We estimate that the overlap in the output channels to these areas is in the range of 10–15%. However, this comparison has obvious limitations because it is made between data from different animals. In addition, the 'overlap' may represent regions where neurons at the borders of two different output channels are simply intermingled. The actual extent of overlap between adjacent channels remains to be determined using techniques capable of revealing multiple channels in a single animal. At this point, although the potential for some overlap exists, our results indicate that the output channels to different prefrontal areas display a marked degree of spatial organization.

Origin of basal-ganglia output to other cortical areas

An important issue about basal-ganglia loops with the cerebral cortex is the extent of segregation between motor and non-motor circuits. There is a growing body of data that can be used to evaluate this issue. Using methods identical to those of

the present study, we have defined the origin of basal-ganglia projections to the face, arm and leg representations of the primary motor cortex, the arm representation of several premotor areas, the saccade region of the frontal eye field and area TE in inferotemporal cortex (Zemanick *et al*., 1991; Strick and Card, 1992; Hoover and Strick, 1993, 1999; Lynch *et al*., 1994; Middleton and Strick, 1994, 1996; Dum and Strick, 1999; Akkal *et al*., 2001). We found that all of these areas are the target of basal-ganglia output. This output appears to originate from regions of GPi and/or SNpr that are separate from those that project to prefrontal areas of cortex. For example, within GPi, the neurons in output channels that project disynaptically to motor areas of the cortex are generally located caudal to those in output channels that project to the prefrontal areas. At levels of GPi where both types of output channels are found, those that target motor areas are generally found ventral and lateral to those that target prefrontal cortex. Similar separations are found between the output channels in SNpr that project disynaptically to prefrontal areas and those that project to the face area of M1, the saccade region of the frontal eye field and area TE (Lynch *et al*., 1994, Hoover and Strick, 1999; Middleton and Strick, 2001). The caution expressed above about data comparisons between animals also applies here. However, the existing data suggest that there is little overlap between the basal-ganglia output to prefrontal cortex and that to the other cortical areas that have been examined.

The anatomical segregation seen in the outputs of these circuits is supported by the distribution of neurons with different response properties in the output nuclei of the basal ganglia. Specifically, the results of recording studies in GPi and SNpr show that neurons with activity related to motor aspects of behavior are located in regions of these output nuclei that are separate from those containing neurons with activity related to non-motor aspects of task performance (DeLong, 1971; DeLong *et al*., 1983; Hikosaka and Wurtz, 1983; Anderson and Horak, 1985; Schultz, 1986; Mink and Thach, 1991; Hikosaka *et al*., 1993; Handel and Glimcher 2000). For example, earlier studies (Hikosaka and Wurtz, 1983; Hikosaka *et al*., 1993) recorded the activity of neurons in the SNpr of monkeys trained to perform an oculomotor spatial-delayed response task. The authors found a group of SNpr neurons with activity that was modulated during the delay period of the task. The location of many of these neurons appears to correspond to output channels in SNpr that project to prefrontal cortex. Indeed, the response properties of these SNpr neurons appear to be very similar to those of neurons in area 46, a potential target of this nigral channel (Funahashi *et al*., 1989, 1993, 1997). In contrast, the location of many purely saccade-related neurons appears to correspond to an output channel that projects to the saccade region of the FEF (Lynch *et al*., 1994). Thus, neurons with cognitive or motor properties appear to be differentially located within SNpr channels that innervate prefrontal or motor areas of cortex.

A number of functional imaging studies provide further support for the differential involvement of specific output channels in cognitive components of task performance (Owen *et al*., 1996, 1998; Jueptner, 1997 a,b). For example, Jueptner and colleagues (Jueptner *et al*., 1997a,b) found that activation in rostrodorsal portions of the globus pallidus was enhanced during the learning of new movement sequences compared to the activation seen during the performance of highly practiced sequences. The same task comparisons also demonstrated enhanced activations in portions of areas 9 and 46, dorsolateral portions of the caudate and the ventral anterior nucleus of the thalamus. Thus, many of the brain regions thought to participate in basal-ganglia loops with areas 9 and 46 were specifically activated during a sequence-learning task in humans.

Perhaps the best evidence for the differential involvement of specific output channels in cognitive or motor components of behavior comes from numerous recent studies of the consequences of pallidal lesions (Bakay *et al*. 1992) that have been made in Parkinson patients to ameliorate some of their motor symptoms. In one of these studies, the cognitive and motor effects of pallidotomy were found to depend significantly on the location of the lesion (Lombardi *et al*., 2000). Lesions located in the most anteromedial region of GPi, the likely origin of output channels to prefrontal cortex, produced the greatest degree of cognitive impairment. In contrast, lesions in the intermediate region of GPi, the likely origin of output channels to motor areas of cortex, led to maximal effects on motor performance, but produced little effect on cognition. These results indicate that the cognitive effects of pallidotomy can be dissociated from its motor effects. Furthermore, they provide a convincing demonstration of the functional segregation of motor and cognitive output channels in the human GPi.

It should be clear from our results that the output of the basal ganglia has the potential to have a substantial influence on processing in prefrontal cortex. This implies that basal-ganglia dysfunction, in addition to producing disorders of movement, should also provoke cognitive disorders. Although a complete review of the literature on this topic is beyond the scope of this report, it is important to note that patients with classical basal-ganglia disorders such as Huntington's and Parkinson's diseases can display marked cognitive and neuropsychiatric symptoms. Furthermore, these symptoms can appear early in the course of these disorders when the pathology is largely confined to the basal ganglia. For example, in a recent study of patients with Huntington's disease, 98% of the patients exhibited neuropsychiatric symptoms (Paulsen *et al*., 2001). The cognitive decline associated with this disease, including impairments in visuospatial memory and executive functions, often precedes the onset of motor symptoms (Butters *et al*., 1978; Heindel *et al*., 1989; Jacobs *et al*., 1995; Lawrence *et al*., 1996). The diversity and range of frontal-lobe-like dysfunction seen in early and mid-stage Parkinson's and Huntington's patients are not surprising given the broad extent of the prefrontal cortex that is a target of basal-ganglia output.

Finally, an interesting pattern has emerged from our analysis; namely, all those cortical areas that are the target of basal-ganglia output also project to the input stage of basal-ganglia processing (e.g., caudate-putamen). Furthermore, the parallel and segregated nature of output channels in the basal ganglia appears to a large extent be mirrored in the pattern of cortical inputs to this system. For example, efferents from prefrontal cortex mainly terminate in regions of the caudate that innervate pallidal and nigral output channels to prefrontal cortex, whereas efferents from the cortical motor areas terminate mainly in regions of the putamen that innervate pallidal output channels to the motor areas (Alexander *et al*., 1986; Hedreen and DeLong, 1991; Yoshida *et al*., 1993; Francois *et al*., 1994). Thus, the basal ganglia appears to participate in multiple closed loops with a large number of cortical areas in the frontal lobe. Furthermore, the involvement of the basal ganglia in this type of circuit extends beyond the frontal lobe to include at least one area of inferotemporal cortex involved in higher-order visual processing (Middleton and Strick, 1996).

Not all basal-ganglia operations fit this processing scheme. As noted above, there is evidence for overlap in the output channels to different cortical areas. Likewise, there is evidence for overlap

in the striatal territories that receive inputs from different cortical areas (Parthasarathy *et al*., 1992; Takada *et al*., 1998). Some cortical areas that project to the striatum do not appear to be the target of basal-ganglia output (e.g. primary somatic sensory cortex). In addition, some systems intrinsic to the basal ganglia appear to have a more global influence on basal-ganglia processing. None the less, the number and diversity of the output channels we have identified suggest that closed loop circuits with the cerebral cortex are a fundamental unit of basal-ganglia architecture.

Notes

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