Corticostriatal Combinatorics: The Implications of Corticostriatal Axonal Arborizations

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Zheng, T. and C. J. Wilson. Corticostriatal combinatorics: the implications of corticostriatal axonal arborizations. J Neurophysiol 87: 1007–1017, 2002; 10.1152/jn.00519.2001. The complete striatal axonal arborizations of 16 juxtacellurally stained cortical pyramidal cells were analyzed. Corticostriatal neurons were located in the medial agranular or anterior cingulate cortex of rats. All axons were of the extended type and formed synaptic contacts in both the striosomal and matrix compartments as determined by counterstaining for the mu-opiate receptor. Six axonal arborizations were from collaterals of brain stem-projecting cells and the other 10 from bilaterally projecting cells with no brain stem projections. The distribution of synaptic boutons along the axons were convolved with the average dendritic tree volume of spiny projection neurons to obtain an axonal innervation volume and innervation density map for each axon. Innervation volumes varied widely, with single axons occupying between 0.4 and 14.2% of the striatum (average = 4%). The total number of boutons formed by individual axons ranged from 25 to 2,900 (average = 879). Within the innervation volume, the density of innervation was extremely sparse but inhomogeneous. The pattern of innervation resembled matrisomes, as defined by bulk labeling and functional mapping experiments, superimposed on a low background innervation. Using this sample as representative of all corticostriatal axons, the total number of corticostriatal neurons was estimated to be 17 million, about 10 times the number of striatal projection neurons.

INTRODUCTION

Because of its large number of neurons, the topographical nature of its cortical input, and the divergence of its cortical inputs, the neostriatum has often been to function as a detector of distributed patterns of cortical inputs (Bar-Gad et al. 2000; Brown 1992; Brown et al. 1998; Graybiel et al. 1994; Wickens 1993). For most authors, this means that specific patterns of activity in the cortex involving cells in many cortical areas trigger spatially localized activation in the neostriatum. This view has been reinforced by the discovery that individual small regions of the cerebral cortex often innervate the neostriatum in a discontinuous fashion, with multiple small projections separated by regions of relatively sparse innervation (e.g., Brown et al. 1998; Gerfen 1989; Malach and Graybiel 1986; Selemon and Goldman-Rakic 1985). Related cortical areas overlap at some but not all of the regions of dense innervation (which are often called matrisomes). This arrangement favors the representation of combinations of cortical inputs by position in the striatum (Graybiel et al. 1994). In this way of thinking, individual neostriatal projection neurons or small localized groups of these cells would fire when excited by combinations of cortical inputs and so would encode specific patterns of cortical activity. If this was true, a large portion of the function of the neostriatum would be implemented simply by the arrangement of synaptic connections within the structure. For this reason, it has been important to understand the rules that govern convergence in the corticostriatal pathway and much has been learned about those rules. One major advance was the recognition that the convergence is mainly along functional, rather than spatial, similarity. For example, the motor and somatosensory cortical representations of an individual body part (i.e., a digit or a portion of a digit) tend to converge in the neostriatum (although they are distant from each other in the cortex), whereas the motor cortical regions corresponding to different digits converge less, even though they are nearby (Brown et al. 1998; Flaherty and Graybiel 1993, 1994). Graybiel et al. (1994) have proposed a specific combinatorial scheme consistent with these observations in which each small region of the cortex is represented multiple times in the neostriatum. In each of these representations, the information converges with input from a different set of other cortical regions.

The results of neurophysiological studies of the striatal projection neurons have been consistent with the notion that these cells respond to combinations of cortical inputs. Striatal spiny projection neurons receive a large number of corticostriatal inputs, each of which is individually weak and are generally not strongly correlated (i.e., they arise from different axons) (Kincaid et al. 1998; Stern et al. 1998; Wilson and Groves 1981). The neostriatal projection neurons have a very negative resting membrane potential and powerful potassium currents active near the resting membrane potential that resist depolarization of the cell by small uncorrelated excitatory synaptic input (Calabresi et al. 1987; Nisenbaum and Wilson 1995; Wilson and Kawaguchi 1996). Thus to be depolarized sufficiently to fire, it is usually necessary for a large number of different cortical neurons to become active at the same time and to sustain their discharge long enough to overcome the time- and voltage-dependent currents that govern the neostriatal projection neuron at rest (Wilson 1992).

Because there are necessarily many more possible combina-
ions of cortical inputs than there are cortical input fibers, a combinatorial encoding of this kind must be very selective (i.e., reject many patterns by failing to respond to them), lack specificity (i.e., respond identically to a large number of input patterns although they are different), or contain at least as many output neurons as there are input fibers. Thus it is useful to compare the number of input fibers, the number of striatal neurons, and the number of different fibers that converge onto single neurons.

A previous study attempted to quantify the number of cortical neurons innervating a small region of the neostriatum corresponding to the volume occupied by the dendritic tree of a single projection neuron (Kincaid et al. 1998). That study concluded that nearby projection neurons have very few cortical inputs in common and that very few of the possible combinations of cortical inputs available in that volume could actually occur on the limited number of cells available in that small region of the neostriatum. Specifically, it was found that the volume occupied by one neostriatal projection neuron in the rat contains about 2,850 projection cells and is innervated by approximately 380,000 cortical neurons. These numbers indicated that the neostriatum could not perform a loss-free combinatorial encoding of the cortical input because the corticostriatal inputs so far outnumber the postsynaptic neurons. It was suggested that the striatum can detect only a relatively small and selected subset of independent cortical combinations. This analysis was based on a study of small parts of corticostriatal axonal arborizations and so may overestimate the degree to which the stratum is cortically innervated. The approach was well suited for those corticostriatal axons with arborizations of the focal type, which are comparable in size to the spiny cell dendritic field. Because the arborizations of the extended-type corticostriatal axons fill a much larger volume than a single neuron, the large number of cortical axons found within a single spiny neuron’s dendritic tree may be mostly identical to those innervating nearby regions. Combinations that are not represented by neurons within the small volume considered in that analysis may well be represented elsewhere. Thus a more complete approach to the problem would center on the volume of a complete axonal arborization rather than that of a postsynaptic neuron’s dendritic tree. In the experiments reported here, entire corticostriatal axonal arborizations were analyzed, yielding an estimate of the total number of combinations of cortical inputs in the neostriatum, the number of striatal neurons within the volume occupied by one afferent axon, and the total number of corticostriatal neurons total for comparison with the number of striatal cells.

**Methods**

Corticostriatal neurons were stained juxtelacally in the medial agranular or anterior cingulate cortex of adult Long-Evans rats weighing 250–350 g at the time of the experiment. The animals were anesthetized with a single intraperitoneal injection of urethan (1.5 g/kg) and received supplemental intramuscular injections of a mixture of ketamine and xylazine (35 mg/kg ketamine, 7 mg/kg xylazine). Anesthetized animals were placed in a stereotaxic device and suspended by a tail clamp to reduce breathing movements. Temperature was maintained at 37 ± 0.5°C using a feedback-controlled heating pad. Stimulating electrodes consisted of pairs of stainless steel insect pins (000 gauge) insulated except for 0.5 mm at the tips. They were placed 0.75–1.0 mm apart in the contralateral striatum (anterior 9.5–10 mm from the interaural line, 2.0–2.5 mm lateral to the midline, and 5.5 mm from the surface of the pia) through small burr holes drilled in the skull. The stimulating electrodes were glass micropipettes with external tip diameters of 1.0–1.5 μm. These electrodes had resistances of 18–26 MΩ when filled with 0.5 M NaCl and 2–4% neurobiotin (Vector Lab, Burlingame, CA) and tested in the brain. After electrodes were placed in the cortex, the hole in the skull was covered with low-melting-point paraffin wax to suppress movements of the brain. Recordings were made using a standard active bridge amplifier (Neurodata IR-283).

Juxtacellular injection was performed in the way described by Pinault (1996) except that passage of current in the juxtacellular configuration did not reliably produce firing in cortical neurons. Instead the characteristic increase in noise described by Pinault as indicating juxtacellular positioning of the electrode was used as the only indicator that the electrode location was suitable for staining the neuron. When possible, cells were selected for staining using antidromic activation from the contralateral neostriatum. In the medial agranular and anterior cingulate cortices, many corticostriatal neurons project bilaterally, and no contralateral cells send axons of passage through the neostriatum en route to other regions (e.g., Wilson 1987). Thus while not a method for identifying all corticostriatal cells, antidromic activation from the contralateral striatum is a sufficient condition for identifying a large group of corticostriatal neurons whose ipsilateral axonal branches could be analyzed morphologically. Antidromic activation was determined by collision with spontaneous action potentials when the stimulus followed a spontaneous action potential by less than the conduction time for the evoked action potential. Because not all corticostriatal axons project bilaterally, a sample of nonidentified cortical cells was stained and their axons inspected for possible branches in the striatum. Those exhibiting such branches were included in the sample. Staining currents were 2–3 nA, 200 ms on/200 ms off for 20–60 min. No more than two injections were made in any animal, and these were separated by at least 0.5 mm, to prevent confusion of axonal branches from different cells.

Animals with injected neurons were maintained anesthetized for 8–12 h after making the last injection. They were then deeply anesthetized with another dose of urethan (1.5 g/kg ip) and perfused intracardially with 4% formaldehyde in 0.15 M phosphate buffer (pH: 7.4). The brains were removed and stored in the same buffer for 8–12 h. Animals were placed in the cortex, the hole in the skull was covered with low-melting-point paraffin wax to suppress movements of the brain. Recordings were made using a standard active bridge amplifier (Neurodata IR-283).

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tions were rinsed, mounted, dehydrated, and coverslipped with Permount.

Stained corticostriatal axons were reconstructed though serial sections using a microscope with a computer-controlled motorized stage and software developed in the laboratory specifically for this purpose. Sections were reconstructed separately and then placed in register and connections established across section boundaries to make a complete reconstruction. Although most of the axons were too fine to allow light microscopic measurement of their diameters, the location of boutons along the axon could be determined. These have previously been shown to correspond to the locations of synapses formed almost exclusively on dendritic spines (Kincaid et al. 1998), and so their distribution within the axonal arborizations were taken as indicative of the distribution of synaptic contacts on spiny neurons. Shrinkage of the sections in the direction normal to the surface of the slide was measured and corrected. There was no measurable shrinkage of the sections in the plane of the slide. Quantitative analyses of the bouton distributions were performed using purpose-designed software or by exporting the bouton locations as coordinate triplets and analyzing them using Mathematica (Wolfram) routines written for this purpose. All purpose-built software used for these analyses are available from the authors on request.

**RESULTS**

Sixteen axons from nine animals were reconstructed completely in animals with satisfactory staining of either the striosomes (with mu-opiate receptor, \( n = 13 \)) or the matrix (with calbindin, \( n = 3 \)). Of these, 5 were located in the medial agranular cortex, and 11 in the anterior cingulate. Of the medial agranular cortical cells, two were brain stem-projecting cells with axon collaterals in the striatum, and three did not project more caudally than the striatum. Of the cingulate cortical neurons, eight projected to the brain stem, whereas three did not. Three of the neurons were identified as bilaterally projecting by antidromic activation from the contralateral neostriatum. All of the axons originated from pyramidal neurons in layer 5. A photomicrograph of one of the anterior cingulate neurons projecting to the brain stem and to the striatum is shown in Fig. 1. In all cases, single neurons, or one darkly stained and one or two very faintly stained cells were found per injection. In seven of nine animals with injected neurons, two cells were stained well enough to reconstruct both axons, but these were always from separate injections and at least 0.5 mm apart. All the axons reconstructed were of the extended type described previously (Cowan and Wilson 1994; Kincaid et al. 1998).

**Extended axonal arborizations end in the striosome and matrix compartments**

We previously described corticostriatal axons from small groups of neurons stained by cortical injections of biotinylated dextran-amine that appeared to be of the extended type and that made synaptic varicosities in both the patch and matrix compartments (Kincaid and Wilson 1996). In the current sample, we were able to examine the distribution of boutons formed by single extended-type corticostriatal axons in tissue sections stained for visualization of the patch and matrix. Both a stain for the patch (mu-opiate receptor) and one for the matrix (calbindin) were employed, but the definition of the compartmental boundaries was much clearer when stained using mu-opiate receptor, so the quantitative analysis was restricted to those 13 extended arborizations collected in seven animals (Fig. 2). Also, the irregular shape of the patch matrix boundaries made it impossible to accurately interpolate the boundaries to adjacent sections, so only boutons that were on the counterstained sections were counted. As approximately 40% of sections containing boutons were counterstained; this allowed about the same proportion of the boutons in the axonal arborizations to be localized to either the patch or matrix compartment. The area of striatum on each section occupied by patch and by matrix compartments was also measured and compared with the proportion of the boutons within each compartment. If boutons in extended axonal arborizations were located preferentially in one compartment or the other, there would be a difference between the two proportions.

All axons made axonal varicosities in both the patch and matrix compartments. The proportion of the area occupied by patches varied among animals 0.09–0.17 of the striatum (mean = 0.11). This result is in good agreement with previous reports (Johnston et al. 1990). The proportion of boutons in the patch varied from 0.02 to 0.18 (mean = 0.09). These results indicated no preference of the axons for the patch or matrix compartment with about 11% of all striatal area occupied by...
opiate receptor-positive patches and about 9% of the boutons located in those patches. There was the possibility of a bias against detection of boutons in the patch compartment because in that region the background texture of the counterstain could interfere with the detection of boutons. This was tested by measuring the proportion of boutons detected in the counterstained versus the uncounterstained sections. On average about 42% of sections containing some portion of the axonal fields were counterstained but only 30% of boutons from the axonal arborizations were observed in those sections. Assuming that the difference was due to undetected boutons in the patch compartment, the average number of boutons in the patches was projected to be underestimated by 110 boutons of 3,054. This assumption could not be verified, but if it were made, the corrected proportion of boutons in the patch compartment was 11%.

**Shape and size of corticostriatal axonal arborizations**

All axons in the sample were reconstructed completely and examined in three dimensions. Extended arborizations in this study were observed arising from neurons in both cortical regions and from cells that had main axonal branches that projected to the brain stem and those that did not (including bilaterally projecting cells). Examples showing the axonal arborizations of two neurons, one projecting to the striatum bilaterally and not to brain stem, and one with unilateral projections to the striatum and to brain stem, are shown in Figs. 3 and 4. In both cases, the axons formed large arborizations with no apparent clustering. Both brain stem projecting and bilaterally projecting neurons also varied widely in the extent of their axonal arborizations, as estimated by the number of boutons in the arborization, and by the apparent volume occupied. The number of boutons formed per cell varied from 25 to 1,729 for brain stem projecting neurons (the axon with 1,729 boutons is shown in Fig. 4) and from 186 to 2,900 for crossed corticostriatal cells (the arborization in Fig. 3 formed 2,582 boutons). The volume of the arborization is much more difficult to characterize. For the purposes intended here, the actual volume of the axon is not interesting but rather the volume of the striatum innervated by it. This depends on the locations of the boutons (but not other parts of the axon) and also the size and shape of the dendritic fields of the postsynaptic neurons. A neuron is within the innervated region of an axon if its dendrites could potentially receive a synapse from a bouton formed by that axon. To calculate that volume, we approximated the dendritic field of the striatal spiny neuron (the main target of corticostriatal connections) as a sphere 400 μm in diameter. This corresponds to the average dendritic field size of striatal spiny neurons in the rat and reflects the fact that nearly all corticostriatal synapses are formed on dendritic spines located on the dendrites of those cells (e.g., Somogyi et al. 1981; Xu et al. 1989). The method used to calculate the volume of the arborization based on the spiny cell dendritic tree is illustrated in Fig. 5. The entire volume of the striatum was divided into volume elements (voxels) 50 μm on a side with values initialized to zero. The three-dimensional locations of all boutons were extracted from each reconstruction and convolved with a 400-μm sphere. To perform the convolution, the spherical volume was centered at the location corresponding to each bouton, and the values of voxels more than 50% contained within the sphere were incremented. The circles in Fig. 5 represent the spherical volumes centered on boutons, and the
Voxel values obtained are interpreted as the number of boutons on the axon that are within range of a striatal spiny neuron whose soma is located within the voxel. The voxel value is therefore an innervation density because it associates the degree to which an axon contributes to the total innervation of the ensemble of cells located within a small distance of each other at a particular location in the striatum. It is unlikely that any one neuron would receive all the synapses formed by an axon within reach of the neuron’s dendritic tree, but if somehow it did, the neuron could not receive more synapses from the axon than the innervation density associated with the voxel containing that neuron’s soma.

The extraction of the spatial distribution of boutons was the first step in this process. As in the case of the entire axonal Arborization, the boutons are distributed over a large region of the striatum and are not organized into obvious clusters. In the horizontal projection shown in Fig. 3, it is apparent that this axon’s Arborization forms a crescent shape, following the contours of the striatum along its rostral and medial boundaries. The volume generated by convolution of the spiny cell’s dendritic field volume with the same axon is shown in horizontal view in Fig. 6A with nonzero voxel values color coded using the color table shown at the right in Fig. 6B (0 voxels are rendered transparent). The volume in Fig. 6A was rendered using a set of isosurfaces with each assigned a color from the color table and with transparency decreasing with increasing innervation density. This reveals the internal structure of the volume, which is a set of approximately tubular regions with innervation density between 10 and 40 (boutons/dendritic field) following axonal branches, embedded in a larger low-density field (innervation density 1–10 boutons/dendritic field). Near branching points for the axon, and in other regions where individual branches of the axon approach each other, hot spots are formed within the volume. These have innervation densities more than 40 boutons/dendritic field. They were occasionally as high as 250 but were usually closer to 100. The distribution of voxel values for each Arborization was approximately exponential, so could be represented by its maximum and mean value, given for each neuron in Table 1. In Fig. 6B, an opaque section through the volume is shown, to illustrate these internal structures.
features of the innervation volume. The hot spots in the arborization did not have discrete boundaries but were peaks in a continuum of innervation density. The overall volume of the arborization was taken to be the volume occupied by all nonzero voxels. The innervation densities shown in Fig. 6 can be converted to average connectivity, by dividing by the number of spiny cells per dendritic field volume (2,850). For the maximum of the hot spots in Fig. 6B, the average connectivity is 0.04 (113/2850).

A summary of the data collected from all axons is shown in Table 1. The total number of boutons from each axon and the volume of each arborization are given there. The volume fraction is the proportion of the total striatal volume [taken to be 32.9 mm$^3$ (Oorschot 1996)] innervated by the axonal arborizations. Like the number of boutons and the volume itself, this number varied widely, from 0.3 to 14% of the striatal volume. On average, the axons in the sample each occupied about 4% of striatal volume. There was no relationship between the cell type (crossed corticostriatal vs. brain stem projecting) or the cortical field of origin (medial agranular vs. anterior cingulate) and the volume occupied in the striatum. Likewise, the proportion of the total corticostriatal innervation contributed by each neuron varied widely, but in all cases was very small compared with the volume occupied. The discrepancy between these two estimates reflects the large degree of convergence in the corticostriatal projection.

Number of corticostriatal neurons

Each axon could be used to derive an estimate of the total number of corticostriatal neurons based on the fraction of the total corticostriatal innervation of the striatum represented by each axon. The total number of corticostriatal boutons was taken as $1.5 \times 10^{13}$. This was calculated as half the asymmetrical synapse density of Ingham et al. (1998) based on the corticostriatal projection times the striatal volume. This number, divided by the number of boutons formed by each axon, was used to calculate the number of axons identical to that one that would be required to generate the entire corticostriatal projection. A similar ratio, but using the average number of boutons for the entire sample of axons, was used to generate an estimate of the number of corticostriatal cells required if the pathway were composed of axons like those in the sample as a
whole. This estimate was 17 million. This should be compared with the estimate of 1.7 million for the total number of striatal spiny neurons.

**DISCUSSION**

*Distributed nature of the corticostriatal projection is a property of single axons*

The earliest experiments on the corticostriatal projection concluded that the cortex axons projected to the striatum in a topographical fashion with nearby regions of the cortex connecting to nearby regions of the neostriatum (e.g., Webster 1961). Since that time, more accurate anatomical studies have revised that view, stressing that small cortical regions innervate very large regions in the neostriatum. However, within those regions the innervation may be very inhomogeneous, so a fine-scale topographical relationship between the cortex and the neostriatum within a cortical area may exist in the form of variations in the locations of high and low density innervation (Malach and Graybiel 1986; Selemon and Goldman-Rakic 1985). This discontinuous topography within the larger pattern has been proposed to be functionally organized based on convergence of sensory and motor cortical representations of the same body part (Brown et al. 1998; Flaherty and Graybiel 1993, 1994; Parthasarathy et al. 1992) and on a similarity between the inhomogeneities innervation and the patterns of glucose utilization during somatosensory stimulation (Brown 1992; Brown and Sharp 1995). These experiments could be interpreted in a variety of ways, depending on how individual corticostriatal axons contribute to the arborizations. For example, each small region of high innervation density within the large innervation field (and each small region of high glucose utilization) could represent the arborizations of individual corticostriatal neurons, and the large innervation seen in bulk labeling studies could be a mosaic of small axonal arboriza-

![Diagram of corticostriatal projections](image1)

**Fig. 5.** The method employed to generate the volume of corticostriatal axonal arborizations. A 400-μm sphere representing the dendritic field of a spiny striatal neuron is centered on each bouton and the value associated with voxels half or more contained within that sphere are incremented. The volume of the arborization is the number of non-0 voxels times the voxel density. The value associated with a voxel gives the number of boutons contained within the dendritic tree of a cell whose soma is located within the voxel.

![Graphical representation of innervation density](image2)

**Fig. 6.** Graphical representation of the innervation density of the axonal arborization showed in Fig. 3 created as shown in Fig. 5. The non-0 (nonwhite) volume shows the volume in which spiny projection neurons could receive input from the axon. The number of boutons within range of neurons located in various regions of the volume are color coded. A: a horizontal projection of the volume color coded for innervation density as shown in the color bar and with isosurfaces drawn at 1, 2, 4, 8, 16, 32, 64, and 112 boutons. Rostral is up and medial is to the left (compare with Fig. 3B). B: an opaque slice cut through the arborization color coded without the use of isosurfaces. Although they are not seen in the reconstructions of the axonal arborization, hot spots in the innervation density are apparent in these views.
tions forming a microtopography within each projection. Alternatively, the entire innervation field of a small region of cortex could arise from the superimposition of a number of large single axonal arborizations that are individually inhomogeneous in the same pattern as the whole. Distinguishing between these two possibilities requires staining of single corticostriatal axons and comparison of their innervation fields with those seen in bulk labeling experiments. This and other studies have shown that the striatal arborizations of single cortical neurons may occupy regions of the neostriatum comparable to those observed after bulk injections of tracers (Cowan and Wilson 1994; Kincaid et al. 1998; Levesque and Parent 1998; Levesque et al. 1996a,b; Wilson 1987). That is, they have shown the region of the striatum innervated by a single neuron in the motor or somatosensory cortex is comparable to that occupied by the entire corticostriatal innervation from a bulk injection of a small region of the cortex. This study has provided a quantitative confirmation of the large and sparse innervations previously reported qualitatively. Individual axonal arborizations occupied as much as 14% of the total volume of the striatum. It is important to stress that in this paper we have specifically studied the axons with extended arborizations, which are expected to have the largest and sparsest innervations. This is because only these axons could have violated the conclusions of the previous paper (Kincaid et al. 1998). A different set of axons, making more focal and discontinuous arborizations, occupy smaller striatal volumes but innervate them very sparsely (Kincaid et al. 1998). We cannot estimate the proportion of neurons making focal versus extended innervations overall. In our experiments the extended arborizations are much more frequently obtained, but this may be due to sampling bias. The focal arborizations always make fewer boutons, and if they are a large proportion of the pathway, our estimate of the number of corticostriatal neurons should be corrected upward.

### Table 1: Axon arborization analysis

<table>
<thead>
<tr>
<th>Cell No.</th>
<th>No. of Boutons</th>
<th>Max. I.D.</th>
<th>Mean I.D.</th>
<th>Volume, mm³</th>
<th>Volume Fraction</th>
<th>Synaptic Fraction</th>
<th>Total Axons, millions</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>J85-1</td>
<td>341</td>
<td>61</td>
<td>12</td>
<td>0.95</td>
<td>0.029</td>
<td>2.28 × 10⁻⁸</td>
<td>44</td>
<td>Medial agranular</td>
</tr>
<tr>
<td>J85-2</td>
<td>735</td>
<td>98</td>
<td>16</td>
<td>1.50</td>
<td>0.046</td>
<td>4.91 × 10⁻⁸</td>
<td>20</td>
<td>Anterior cingulate</td>
</tr>
<tr>
<td>J88</td>
<td>2582</td>
<td>113</td>
<td>22</td>
<td>3.97</td>
<td>0.121</td>
<td>1.72 × 10⁻⁷</td>
<td>6</td>
<td>Anterior cingulate</td>
</tr>
<tr>
<td>J91-1</td>
<td>185</td>
<td>94</td>
<td>17</td>
<td>0.37</td>
<td>0.011</td>
<td>1.24 × 10⁻⁸</td>
<td>81</td>
<td>Medial agranular</td>
</tr>
<tr>
<td>J91-2</td>
<td>756</td>
<td>109</td>
<td>22</td>
<td>1.13</td>
<td>0.034</td>
<td>5.05 × 10⁻⁸</td>
<td>20</td>
<td>Anterior cingulate</td>
</tr>
<tr>
<td>J92-1</td>
<td>277</td>
<td>13</td>
<td>50</td>
<td>0.48</td>
<td>0.015</td>
<td>1.85 × 10⁻⁸</td>
<td>54</td>
<td>Medial agranular</td>
</tr>
<tr>
<td>J92-2</td>
<td>27</td>
<td>13</td>
<td>5</td>
<td>0.17</td>
<td>0.005</td>
<td>1.30 × 10⁻⁹</td>
<td>554</td>
<td>Anterior cingulate</td>
</tr>
<tr>
<td>J94-1</td>
<td>300</td>
<td>90</td>
<td>17</td>
<td>0.59</td>
<td>0.018</td>
<td>2.00 × 10⁻⁸</td>
<td>50</td>
<td>Anterior cingulate</td>
</tr>
<tr>
<td>J94-2</td>
<td>186</td>
<td>66</td>
<td>16</td>
<td>0.40</td>
<td>0.012</td>
<td>1.24 × 10⁻⁸</td>
<td>81</td>
<td>Medial agranular</td>
</tr>
<tr>
<td>J97-1</td>
<td>1155</td>
<td>234</td>
<td>30</td>
<td>1.31</td>
<td>0.040</td>
<td>7.72 × 10⁻⁸</td>
<td>13</td>
<td>Anterior cingulate</td>
</tr>
<tr>
<td>J97-2</td>
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<td>0.04</td>
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</tr>
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</table>

* Maximal innervation density, value of the highest-valued voxel. † Mean innervation density, mean voxel value. ‡ Based on a striatal volume of 32.9 mm³ (Oorschot 1996). § Based on asymmetric synapse density of 0.91/µm² (Ingham et al. 1998) and assuming that corticostriatal axons account for half of all asymmetric synapses.

Corticostriatal projection is heterogeneous

Our experiments have shown that within the extended axonal arborization there are inhomogeneities of innervation density of the right size and distribution to contribute to the functional mapping seen in glucose utilization studies. In addition, quantification of the projections of single neurons revealed a remarkable heterogeneity among the axonal projections of corticostriatal neurons, with some neurons occupying very large regions of the striatum and making thousands of synapses, whereas others occupy much smaller regions and make few. The density of synaptic inputs was relatively constant for axons of these different kinds so that axons with small arborizations innervate small regions but do so at about the same density as seen in the larger volumes innervated by larger arborizations. The reason for such large variations among neurons in the quantity of tissue innervated is not known, but it apparently is not simply a reflection of the different kinds of corticostriatal neurons.

Anatomical studies of the corticostriatal pathway have subdivided the corticostriatal neurons along several dimensions. Some corticostriatal neurons have main axons that descend to the brain stem or to the spinal cord, whereas others may project to a variety of telencephalic regions but have no brain stem projections. These latter are expected to exclusively constitute the crossed pathway (Levesque et al. 1996a; Wilson 1987; Wright et al. 2001), as brain stem-projecting neurons do not project to the contralateral telencephalon in adults. Corticostriatal neurons are also differentiated into two groups on the basis of whether they project to the striosomes or to the matrix. Because injections of tracers in specific cortical regions, or in different lamina within a cortical region, may specifically label axons in the striosomes or the matrix, it was suggested by Gerfen (1989) that different kinds of cortical neurons may project to these two striatal subregions. These observations have been repeated and extended to the arborizations of single corticostriatal axons (Kincaid and Wilson 1996). Finally, studies of single corticostriatal axons have shown that some of...
these arborize in a focal pattern that resembles the shapes and sizes of striosomes (and matrisomes), while others arborize in a much more extended pattern (Cowan and Wilson 1994; Kincaid et al. 1998; Levesque and Parent 1998; Levesque et al. 1996a,b; Wright et al. 1999), apparently ignoring the boundaries between these substructures. In our single-axon studies, focal projections arose primarily from the collaterals of brain stem-projecting neurons, while the extended arborizations arose from axons of cells that did not extend axons caudal to the striatum (Cowan and Wilson 1994; Kincaid et al. 1998). In this larger sample of well-filled extended arborizations, both brain stem-projecting and crossed corticostriatal cells with no descending branch were observed to make large extended arborizations. We conclude that the apparent correlation between target and arborization type observed before was spurious and due only to the restricted sample size. We also conclude that a strong specificity for striosome or matrix compartments is a feature restricted to cells with focal arborizations. Axons forming extended arborizations innervated the two compartments approximately in proportion to their volumes. Despite the dramatic innervation volume differences between the axons making up this sample, they all were extended arborizations according to the criteria described above. It should be noted that the differences observed among these axons may reflect a constant dynamic adjustment of the sizes of arborizations and so not be permanent features.

Individual axons make sparse innervations

On the basis of a more restricted study of individual branches of corticostriatal neurons, we previously concluded that the input to every spiny neuron will be unique, and that sharing of inputs among spiny neurons is minimal (Kincaid et al. 1998). The argument given in that paper was flawed when applied to the extended axonal arborization because it did not take into account the possibility that separate branches of the large arborizations might innervate single spiny cell dendritic trees. The current results allow more general treatment. A typical corticostriatal axon, which innervates 4% of the striatal neuropil, would share this arborization zone with 680,000 other corticostriatal neurons (4% of 17 million). Within that volume, where it would make about 800 synapses would be 68,000 striatal spiny neurons (4% of 1.7 million) (Oorschot 1996), making the average connectivity about 1.2% (or less if there are multiple contacts on single spiny neurons). This estimate is close to that obtained previously (1.4%) (Kincaid et al. 1998). This is because branches of extended corticostriatal axons generally do not approach each other. In the previous study, it was assumed that the maximum innervation density generated in a corticostriatal axonal arborization was about 40 because individual axonal branches make synapses about every 10 μm. In the present study, localized regions of higher innervation density were observed near branch points in the axonal field and regions where axon branches approached each other. Even in the center of these small hot spots in single cortical arborizations, the number of boutons from a single axon within synaptic range of any spiny neuron never exceeded 250. Because there are approximately 2,850 striatal neurons with somata located within the volume of a spiny cell dendritic field, the proportion of neurons innervated by that axon cannot exceed about 9%. In most of the axonal field the average connectivity is much smaller still (less than 1%).

Is the striatum a competitive network?

The striatum has often been compared with a competitive network. The distributed termination of corticostriatal inputs with massive convergence and divergence, the absence of local excitatory interneurons, and the GABAergic, presumably inhibitory synaptic connections among striatal neurons have encouraged this view, which was advanced by the Vogts (Vogt and Vogt 1920) and more recently and quantitatively by Wickens (1993), Plenz and Kitai (2000), and by Bar-Gad et al. (2000). In addition to its relationship to the apparent structural features of the striatum, this view is consistent with the current functional view of the striatum as engaged in action selection (Graybiel 1998; Gurney et al. 2001; Lawrence et al. 2000; Marsden 1984; Wickens 1993). That is, because the pattern of activation in a few neurons of the striatum is supposed to be a more localized and compact representation of a large and distributed pattern of activity in the cortex, the facilitation or inhibition of activity in that compact representation would be a good way to gate the expression of the complex cortical pattern (representing an action, plan or goal). It is also consistent with the cellular neurophysiology of the spiny neostriatal neuron. The striatal neurons receive inputs from large numbers of cortical cells and require the cooperative effort of many cortical inputs before they can become sufficiently excited to fire. Thus the firing of a striatal spiny neuron can be taken as an indication that a large part of the several thousand corticostriatal neurons converging on that one cell are active together. Striatal neurons may then be seen as detecting activity of specific but distributed ensembles of cortical neurons. Each striatal neuron may detect the coordinated activity of a different cortical ensemble, so the activity in the striatum could be seen as a compact representation of cortical activity.

Does the corticostriatal network possess the properties required of such a competitive network? Synaptic plasticity of the required type has been observed at corticostriatal synapses (Centonze et al. 2001; Charpier et al. 1999; Don Santos Villar and Walsh 1999; Partridge et al. 2000; Reynolds and Wickens 2000; Spencer et al. 2000). The use of this kind of synaptic plasticity to train a network, however, requires a considerable overlap of synaptic inputs among striatal neurons. In the standard winner-take-all competitive network (Herz et al. 1991), the neurons in the network all receive the same connections and compete with each other to obtain a monopoly on the responsiveness to a particular input pattern. The cells that lose that competition do not lose their connections to the axons that make up the pattern, but the strengths of those connections are reduced. The continued presence of the connections is essential if the network is to be able to adapt to future changes in input patterns. The structure of the corticostriatal input resembles the end state of a competitive network in which connections that have been weakened were removed, and so each neuron receives a unique set of inputs. Because no two neurons receive more than a very small number of inputs in common, there is no competition for representation of input patterns, and so no need to resolve that competition by mutual inhibition among striatal neurons (Jaeger et al. 1994). If this was true, it would also make the network unable to dynamically adapt to changes.
in the patterns of inputs arriving from the cortex. A deviation from the usual scheme for competitive networks, in which axons grow and regrow branches and make and break synaptic connections dynamically, could restore the essence of a competitive network to the striatal circuitry. Without this, however, we conclude that the sharing of inputs among cells that is critical for learning in a competitive network has not been observed in the corticostriatal projection of adult rats.

**Dimensional reduction in the corticostriatal projection implies loss of information**

If the striatum generates compact representations of distributed patterns of activity in the cortex, then the number of such patterns that can be differentiated in the output depends on the total number of striatal cells and the proportion of striatal cells that participate in the response to each input. To represent all possible corticostriatal input patterns uniquely in the output of the striatum, there must be at least as many striatal neurons as possible corticostriatal input patterns. For example, if corticostriatal cells a and b always fire together, it is unnecessary for the striatum to allocate any neurons to represent patterns which include activity in a but not b or the converse. Our finding that corticostriatal cells outnumber striatal neurons by a factor of 10 suggests that unless the corticostriatal output is overwhelmingly redundant in this way (at least 9 of 10 possible combinations of corticostriatal output patterns never occur because of correlations among the cortical neurons), the striatum cannot simply repackage its input patterns in a more compact form. Actually, the difficulty is probably more severe than this. The compactness of the striatal representation of cortical patterns (in comparison to the cortex) relies on the use of a smaller proportion of striatal neurons in the representation of each pattern than is employed in the cortex. This would make the efficiency of encoding in the striatal output (number of patterns that can be represented by the striatal cells per capita) less than in the cortical output (unless the striatum can transmit information at a faster rate than the cortex, which is unlikely as they generally fire more slowly). Studies of the responses of corticostriatal neurons in behaving monkeys suggest that these cells employ a sparse code of the cortical output, the redundancy of which is even less than is apparent in recordings of cortical neurons overall (Bauswein et al. 1989; Turner and DeLong 2000). For all these reasons, it is likely that the striatum does not simply remove redundancy in the cortical input, but instead large numbers of possible cortical input patterns must be either rejected (not represented in the striatal output) or treated as if they were identical although they are not.

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**REFERENCES**


