Projections From Primary Somatosensory Cortex to the Neostriatum: The Role of Somatotopic Continuity in Corticostriatal Convergence

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HOOVER, John E., Zachary S. Hoffer, and Kevin D. Alloway. Projections from primary somatosensory cortex to the neostriatum: the role of somatotopic continuity in corticostriatal convergence. J Neurophysiol 89: 1576–1587, 2003. First published November 20, 2002; 10.1152/jn.01009.2002. We characterized the organization of corticostriatal projections from rodent primary somatosensory cortex (SI), testing the hypothesis that projections from SI areas representing subcomponents of the forelimb exhibit greater neostriatal overlap than projections from areas representing separate body parts. The anterograde tracers Fluoro-Ruby (FR), Alexa Fluor (AF), and biotinylated dextran amine (BDA) were injected into physiologically identified regions of rat SI. Injection locations were confirmed by examining the SI barrel fields and limb representations in tangential sections processed for cytochrome oxidase (CO). Experimental animals were divided into two groups: one group received multiple tracer injections in neighboring SI regions that represent separate body parts (whiskers, forepaw, and hindpaw); the other group received injections in SI areas that represent different components of the forelimb (forepaw, antebrachium, and brachium). The distribution of labeled terminals and their varicosities in the neostriatum and in the thalamus were plotted and quantitatively analyzed. For most animals, tracer overlap in the thalamus was either minimal or completely absent. In the neostriatum, projections from the whisker, forelimb, and hindlimb representations terminated in regions that rarely overlap with each other, while those originating from different parts of the forelimb representation were more likely to terminate in overlapping parts of the neostriatum. To the extent that neostriatal activation depends on corticostriatal convergence, the corticostriatal projections in the sensorimotor channel appeared to be organized so that neostriatal neurons may signal when multiple components of the same body part are activated simultaneously.

INTRODUCTION

The neostriatum, the major input nucleus of the basal ganglia, receives widespread projections from the cerebral cortex, including primary somatosensory cortex (SI). Neostriatal processing of sensorimotor information is critically involved in the programming, initiation, and execution of voluntary movements (for review, see Albin et al. 1989, 1995; Alexander et al. 1986; DeLong and Georgopoulos 1981; Wichmann and DeLong 1996). Thus lesions of the neostriatum profoundly affect the sequencing of movements necessary for accurate reaching and grooming behaviors (Aldridge and Berridge 1998; Cromwell and Berridge 1996; Hore and Vilis 1980).

Corticostriatal projections from the SI do not follow a “one-to-one” sensory mapping pattern. Instead, projections from a single cortical site terminate in the dorsolateral neostriatum as multiple dense clusters of axonal arborizations and varicosities spread discontinuously over several millimeters (Alloway et al. 1998, 1999; Brown et al. 1998; Flaherty and Graybiel 1991, 1994; Kunze 1977; Wright et al. 1998). Many of these regions receive overlapping projections from related parts of sensorimotor cortex, including primary motor cortex (MI) and secondary somatosensory cortex (SII) (Alloway et al. 2000; Flaherty and Graybiel 1991, 1993; Hoffer and Alloway 2001; Yeterian and Van Hoesen 1978). Thus a major function of neostriatal modules may be to detect and integrate coincident activity in cortical regions that are co-activated, either synchronously or in close temporal sequence. Indeed, some have hypothesized that neostriatal neurons may have a pattern recognition function in which dopaminergic innervation alters the weighting of corticostriatal synapses to enable neostriatal neurons to recognize corticostriatal input patterns that are behaviorally significant (Houk 1995; Wilson 1995). In this context, such operations in the sensorimotor part of the neostriatum may facilitate motor learning and the coordination of body parts during grooming or object manipulation.

During somesthetic-guided behaviors, the different subcomponents of the forelimb (i.e., paw, distal forelimb, and proximal forelimb) move simultaneously in a cooperative manner. Consequently, somatosensory receptors within each of the forelimb components tend to be stimulated simultaneously during behavioral activities that involve forelimb movements. By comparison, separate body parts such as the head, forelimb, and hindlimb often move independently of each other during a variety of behaviors. Hence, mechanoreceptors and proprioceptors located in these noncontiguous body parts are not always activated simultaneously, and the cortical representations for these body parts are less likely to exhibit synchronous responses than cortical areas that represent subcomponents of the same body part. If the relative organization of corticostriatal projections from different parts of SI is related to their probability of synchronous activation, then the degree of convergence should be greatest for those projections arising from

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areas that represent adjoining subcomponents of the same body part. To test this hypothesis, we injected multiple anterograde tracers into physiologically identified regions of rat SI. The rodent SI cortex is attractive for this type of study because it contains an isomorphic map of the body in which the location of tracer injections can be confirmed with respect to cytochrome oxidase (CO) labeled barrels and barrel-like structures (Land and Simons 1985; Riddle and Purves 1995; Woolsey and Van der Loos 1970). The results of our experiments indicate that SI areas representing the subcomponents of the forelimb exhibit greater neostriatal convergence than equally distant cortical areas that represent separate, noncontiguous body parts such as the forepaw and hindpaw.

**METHODS**

This report is based on data obtained from 20 male Sprague-Dawley rats (330–695 g). All procedures complied with the National Institutes of Health guidelines for the care and use of laboratory animals and were approved by the institutional animal welfare committee.

**Surgery and electrophysiological mapping**

Initially, each rat was placed in a plexiglass induction box and briefly anesthetized with 2.5% isoflurane delivered in a 2:1 mixture of nitrous oxide and oxygen. A prolonged surgical plane of anesthesia was then induced by an intramuscular injection of a mixture of ketamine (20 mg/kg) and xylazine (6 mg/kg). The animal was then orally intubated, positioned in a stereotaxic frame, and artificially ventilated with the 2:1 mixture of nitrous oxide and oxygen. Each animal received atropine sulfate (0.05 mg/kg, im) to reduce bronchial secretions, chloromycetin sodium succinate (50 mg/kg, im) to prevent infection, and dexamethasone (0.5 mg/kg, im) to reduce inflammation. Ophthalmic ointment was applied to prevent corneal drying. Heart rate, end-tidal CO2, and body temperature were monitored continuously. Additional one-quarter doses of the ketamine-xylazine cocktail were administered approximately every 20–30 min or when blink or withdrawal reflexes were evoked.

After exposing the skull, two or three craniotomies were performed over the right hemisphere (see Alloway et al. 1999 for additional details). These were placed at stereotaxic coordinates overlying the whisker, forelimb, or hindlimb representations of SI (Chapin and Lin 1984). A tungsten microelectrode (300–700 kΩ) was inserted through the dura mater that was subsequently used to guide tracer injections into the cortex. Vessels that were encountered on the surface of the dura were preserved. To ensure that the barrel was completely severed, the electrode was lowered into the cortex at an angle perpendicular to the cortical surface. The recording site and the responses evoked by peripheral stimulation were noted on a hand-drawn map of the cerebral blood vessels that was subsequently used to guide tracer injections into specific body part representations. In some cases, electrophysiological recordings were made at many sites to determine the precise boundaries between different somatotopic representations.

**Tracer injections**

A different anterograde tracer was injected into two or three body part representations as listed in Table 1. The tracers were 10% solutions of Fluoro-Ruby (FR), Alexa Fluor (AF), or biotinylated dextran amine (BDA; Molecular Probes, Eugene, OR).

FR and AF were pressure injected through glass micropipettes (tip diameters = 75–125 μm) cemented into 1.0 μl Hamilton syringes. In each case, the pipette was lowered into the cortex at an angle perpendicular to the cortical surface. Injections were typically placed at depths of 1,400, 1,200, and 1,000 μm below the pia. At each depth, 25–50 nl of tracer was injected over the course of 3 min; an additional 3–5 min elapsed before the pipette was raised to the next depth. The volume and rate of tracer delivery was controlled with the aid of a Kopf microinjection unit. In most cases, the pipette was reinserted nearby (50–200 μm) for a second set of injections within the same somatotopic representation.

BDA was injected iontophoretically through glass micropipettes (tip diameters = 45–75 μm) using positive current pulses of 6 μA and a duty cycle of 7 s. Iontophoresis was applied for 9–18 min at

| TABLE 1. Somatotopic location and size of tracer injections in SI |
| --- | --- | --- | --- |
| Across Body Parts | Distance Between Injections (μm) |
| Case | Tracer | Location* | Diameter (μm) | Wh-FP | Wh-HP | HP-FP |
| H5 | BDA | Wh | 694 | 2700 | — | — |
| H6 | FR | FP | 296 | — | — | — |
| H7 | BDA | Wh | 508 | 1709 | — | — |
| H7 | BDA | FP | 1196 | — | — | — |
| H12 | FR | FP | 1333 | 1078 | — | — |
| H12 | FR | HP | 808 | — | — | — |
| H18 | AF | Wh | 556 | 3017 | 2586 | 1552 |
| H19 | BDA | FP | 441 | 978 | — | 1323 |
| H20 | BDA | HP | 833 | — | 2414 | 3161 |
| H21 | BDA | FP | 868 | — | — | 1909 |
| H21 | FR | HP | 348 | — | — | 258 |
| H26 | FR | Wh | 437 | — | 2394 | — |
| H26 | AF | Wh | 474 | — | 2234 | — |
| H29 | FR | HP | 725 | — | 3017 | 2586 |
| H34 | BDA | FP | 333 | — | 1336 |
| H36 | BDA | HP | 900 | — | — | 567 |
| H36 | FR | HP | 405 | — | — | 258 |
| H37 | FR | HP | 474 | — | — | 558 |
| BDA | Wh | 712 | — | — | — |
| BDA | Wh | 574 | — | — | — |

*Wh, whiskers; FP, forepaw; HP, hindpaw; dFL, distal forelimb; pFL, proximal forelimb.

each cortical depth listed above. Injections were generally made along two pipette penetrations within the same body part representation. After completing the injections, the wound margins were sutured and treated with a topical antibiotic, and the rat was returned to its home cage.

**Histology and microscopy**

After a survival period of 7–14 days, each rat was deeply anesthetized with sodium pentobarbital (50 mg/kg, ip). A tungsten electrode coated with India ink was used to make four fiduciary marks at a depth of 4 mm; two of these penetrations were placed at different lateral positions within a coronal plane located 3.0 mm rostral to bregma and the other two were similarly placed in a coronal plane located 4.0 mm caudal to bregma. Then the animal was transcardially perfused using a three-step procedure. The perfusates included heparinized physiological (0.9%) saline, 4% paraformaldehyde in 0.1 M phosphate buffer (PB; 4°C, pH 7.4), and 4% paraformaldehyde with 10% sucrose. Following the perfusion, the brain was removed and postfixed overnight in cold 4% paraformaldehyde with 30% sucrose. In most cases, the cortex was dissected from the injected hemisphere, flattened between glass slides, and postfixed for an additional 24 h. Tangential sections through the cortex were cut at 25- to 75-μm intervals. The subcortical hemisphere was cut coronally at 50-μm intervals; the coronal orientation was confirmed by the fiduciary marks that signaled the start and end of sections that were to be saved for processing. Cortical (tangential) and subcortical (coronal) sections were saved in serial order and then each third section was placed in one of the following three series. For both cortical and subcortical sections, one series was processed for BDA and a second series was left unprocessed for fluorescent microscopy. The third cortical series was processed for CO to reveal the SI barrels. The third subcortical series was stained with thionin to visualize the borders of thalamic nuclei and other brain structures. Anatomic borders were determined using the criteria of Paxinos and Watson (1986).

Sections were processed for BDA labeling as described previously (Alloway et al. 1998; Kincaid and Wilson 1996). Briefly, sections were washed in 0.1 M PB with 0.3% Triton-X100 (pH 7.4) and then incubated in an activated avidin-biotinylated horseradish peroxidase solution (Vector Novocasta Laboratories, Burlingame, CA) for 2 h. After washing in PB, sections were then incubated with 0.05% diaminobenzidine (DAB), 0.005% H2O2, and 0.04% NiCl2 in 0.1 M Tris buffer (pH 7.1) for 11–13 min. Following observation of the reaction product, the sections were rinsed in PB, mounted on gel-coated slides, and dried overnight. The sections were subsequently defatted in xylene and coverslipped with Cytoseal. BDA-labeled terminals and processes were visualized with bright field illumination. Neuronal processes labeled with FR or AF were visualized with epifluorescent illumination in both the BDA-processed and unprocessed sections. Unprocessed sections were mounted on gel-coated slides, dehydrated in alcohol, defatted in xylene, and coverslipped with Cytoseal. FR-labeled terminals and processes were observed with the aid of a TRITC filter set (Chroma Technology, Brattleboro, VT) that permitted excitation from 540–580 nm and emission from 540–660 nm. AF-labeled terminals and processes were observed with the aid of FITC filter set (Olympus, Lake Success, NY) that permitted excitation from 455–490 nm and emission from 535–540 nm.

Serially ordered tangential sections through SI cortex were processed for CO using published techniques (Land and Simons 1985; Wong-Riley 1979). Briefly, sections were washed in PB and incubated at 35°C for 7–8 h in PB with 0.05% DAB, 0.05% cytochrome C (Sigma Laboratories, St. Louis, MO), and 4% sucrose. The sections were then washed in PB again, mounted on gel-coated slides, and dried. Mounted sections were postfixed in neutral formalin for 30 min, dehydrated in a graded series of alcohol, defatted in xylene, and coverslipped with Permount. Tangential sections through SI were then examined to reveal the location of tracer injections with respect to the CO-labeled subfields. Measurements of the size of the injections and the separation between injections were taken from the tangential section through layer V of SI cortex and are listed in Table 1. Injection size was quantified as the average of the minimum and maximum orthogonal diameters; the effective zone of tracer transport was considered to be the dense core of the injection site, where tracer filled the neuropil. The separation between injection sites was measured as the minimum distance between their perimeters (i.e., edge-to-edge).

**Anatomic and quantitative analyses**

Cortical and subcortical sections were inspected with 20× and 40× objectives using epifluorescent (FR and AF) and bright field (BDA) illumination. Initially, we verified that tracer injections were localized to the forelimb, hindlimb, or whisker representations as defined by the pattern of CO labeling. Instances in which tracers were deposited outside the SI representation were excluded from analysis. Cases in which the tracer injections overlapped in the cortex or failed to produce labeling in the neostriatum and thalamus were also excluded. Finally, we also excluded cases in which substantial cortical damage prevented an assessment of the extent of tracer diffusion as well as cases in which the neostriatum was damaged during cortical excision.

Section outlines and the locations of labeled thalamic and neostriatal processes were plotted with the aid of a computerized charting system that uses optical encoders to detect x-y movements of the microscope stage (AccuStage, St. Paul, MN). Using the multiple symbol and color features available in the software, the positions of FR-, AF-, and BDA-labeled cell bodies and terminals were accurately recorded for each section (resolution = 2 μm) and saved to a computer file. Each file was subsequently analyzed with a JAVA-based program that subdivided the section into an array of 35-μm2 bins and determined the number of labeled terminal varicosities in each bin. Bins that contained at least two FR-, AF-, or BDA-labeled terminals were coded with the colors red, green, or blue, respectively. Bins that contained at least two or more beaded varicosities for each of two different tracer combinations (e.g., 2 FR- and 2 BDA-labeled terminals) were coded white. Thus for the animals that received triple tracer injections (Table 1), it was necessary to run the analysis three times so that we could compare each combination of tracers separately. For each animal, we summed the number of colored bins across sections to determine the total number of bins in the thalamus or neostriatum that were occupied by labeled terminals. The total amount of overlap among projections labeled by any pair of tracer injections was determined by the ratio of the white bins to the sum of the relevant colored bins [e.g., FR-BDA total overlap (%) = (white bins)/(white + red + blue bins) × 100].

The absolute amount of tracer overlap in the neostriatum was influenced by bin size and threshold criteria. Simply stated, small bins and high thresholds minimize the amount of overlap, whereas large bins and low thresholds maximize the amount of overlap. We have used 35-μm2 bins and a threshold of two labeled varicosities per bin in our previous reports (Alloway et al. 1999, 2000; Hoffer and Alloway 2001; Leergaard et al. 2000), and we continued to use these criteria to facilitate comparisons with our previous work. In addition, to confirm that significant differences in amount of tracer overlap in our experimental groups did not depend on bin size and threshold criteria, we also used 50- and 75-μm2 bins and 1, 2, 4, or 8 varicosity thresholds in some of our comparisons.

Using the index of overlap described above (i.e., 35-μm2 bins and 2 labeled varicosities of each tracer per bin), we performed a statistical analysis to test whether pairs of tracer injections placed in different SI body part representations revealed less labeled overlap in the neostriatum than pairs of injections placed within contiguous parts of the SI forelimb representation. As indicated by Table 2, cases classified as “across body parts” included instances in which we administered combinations of tracers into the whisker and forepaw (n = 5), whisker and hindpaw (n = 4), or forepaw and hindpaw (n = 8) representa-
equipped with a Kodak 1401-e CCD chip. Although BDA labeling by the SDs were plotted on the distal forelimb (which we administered combinations of tracers into the forepaw and (total overlap separation and 2 factors of somatotopic locations) were plotted onto a appropriate constant that should be added to each observation, the mean and transformation of a zero value is undefined as cases. Cases classified as “within forelimb” included instances in which we administered combinations of tracers into the forepaw and distal forelimb (n = 6), forepaw and proximal forelimb (n = 5), or distal forelimb and proximal forelimb (n = 4) representations. For these comparisons, the distal forelimb representation was defined by RFs on the antebrachium (i.e., wrist to elbow), and the proximal forelimb representation was defined by RFs on the brachium (i.e., elbow to shoulder). A two-way ANOVA for unequal group size was performed using the JMP 3.2 statistics program (SAS Institute, Cary, NC). Cortical separation between injections (greater or less than 1,600 μm) and the relative somatotopic location of the injections (“within forelimb” or “across body parts”) were used as factors or independent variables for this analysis. The dependent variable was total corticostriatal overlap for each pair of tracers. The variances of the groups were not equal; hence, the data were considered heteroscedastic. To express the data in a form that satisfied the condition of equality of variances, a log10 transformation was performed on each observation (i.e., corticostriatal overlap) after adding an appropriate constant. The addition of a constant was necessary because corticostriatal overlap was nonexistent for some pairs of tracer injections, and a log10 transformation of a zero value is undefined. To determine the appropriate constant that should be added to each observation, the mean and SD of each group (4 groups derived from 2 factors of injection separation and 2 factors of somatotopic locations) were plotted onto a Cartesian graph. Thus the group means were plotted on the x axis and the SDs were plotted on the y axis. Ideally, the best linear fit to these data points should intersect the origin because a group with a mean near zero should also have a SD near zero. The best linear fit to our group data (mean vs. SD) intersected the origin only after we added a value of 0.1636 to each observation. Based on this method (Wright 1968), the two-way ANOVA for unequal group size was performed after each observation received the following transformation: log10 (total overlap + 0.1636).

Digital photomicrographs were obtained with a Sensys camera equipped with a Kodak 1401-e CCD chip. Although BDA labeling was unaffected by light exposure, the FR and AF labeling were quenched during the prolonged light exposure that was needed to plot all three tracers in the same section. Hence, digital images of the fluorescent tracers were usually taken from an adjacent unplotted section. The raw digital images, which were acquired using a tagged image file format (TIF), were subsequently imported into Canvas 7.0 (Deneba Systems, Miami, FL) where the gray-scale and color levels were adjusted to produce images that resembled what was seen through the microscope.

**RESULTS**

Corticostriatal projections were quantitatively analyzed in 20 rats that received restricted injections of two or three anterograde tracers (FR, AF, and BDA) into identified regions of SI cortex (Table 1). In 13 animals, the tracers were deposited in different body part representations (whiskers, forelimb, or hindlimb); in 7 animals, the tracers were placed in different subcomponents of the forelimb representation (i.e., forepaw, distal forelimb, proximal forelimb). Injection locations were guided by stereotaxic maps of SI (e.g., Chapin and Lin 1984) and by electrophysiological recordings. Subsequently, we confirmed the location of the tracer injections with respect to the pattern of CO-labeling and measured the spatial extent of each injection in layer V. The edge-to-edge separation between the injection sites of different tracers ranged from 258 to 3,161 μm. As shown by representative examples in Fig. 1, injections “within the forelimb” representation tended to be closer (mean = 1,051 μm) than injections located “across different body part” representations (mean = 1,731 μm). The average diameter of the injections was 623 μm and the range of diameters varied from 236 to 1,336 μm (see Table 1).

Injections of FR, AF, and BDA effectively labeled several cortical and subcortical targets of SI cortex. For example, dense plexuses of labeled axonal arborizations and terminal fields were observed in the primary motor cortex (MI), secondary somatosensory cortex (SII), intralaminar, posterior, and ventrobasal (VB) nuclei of the thalamus, and in the neostriatum. Brain regions that project to SI were also marked by the presence of retrogradely labeled cell bodies [e.g., ventroposterior lateral nucleus (VPL), ventroposterior medial nucleus (VPM), and medial portions of the posterior nucleus (POm)]. In this report, we have focused our analysis on the organization of projections to the neostriatum.

**General pattern of corticostriatal projections**

Labeled corticostriatal axons entered the neostriatum either from the external capsule or from the fiber fascicles that perforate the neostriatum. Consistent with our previous descriptions (Alloway et al. 1998–2000), the labeled fibers were generally quite thin (<0.5 μm in diameter) and contained axonal varicosities of both the en passant and terminaux types. The en passant boutons or “beaded” varicosities were seen at regular intervals along the length of labeled corticostriatal axons and were far more numerous than the terminal boutons. Ultrastructural examination has demonstrated that these structures contain synaptic vesicles, and thus are believed to constitute corticostriatal synapses (Kincaid et al. 1998). We plotted only the locations of the labeled varicosities, not the locations of smooth, labeled fibers. Furthermore, we were careful to distinguish between terminal boutons and the ends of cut axons

**TABLE 2. Total overlap (%) in the neostriatum**

<table>
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<tr>
<th>Case</th>
<th>Wh-FP</th>
<th>Wh-HP</th>
<th>HP-FP</th>
</tr>
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<tbody>
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<td>—</td>
<td>—</td>
</tr>
<tr>
<td>H6</td>
<td>0.29</td>
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<td>H7</td>
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<td>—</td>
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<td>H12</td>
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<td>—</td>
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<table>
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</tr>
<tr>
<td>H25</td>
<td>3.48</td>
<td>0.38</td>
<td>0.48</td>
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</table>

* Wh, whiskers; FP, forepaw; HP, hindpaw; FL, distal forelimb; pFL, proximal forelimb.
by plotting only the axonal swellings or enlargements that suggest the presence of a synaptic ending.

Labeled corticostriatal projections terminated primarily in the dorsolateral neostriatum. Consistent with previous reports, the projections from a single cortical site had a “patchy” distribution, consisting of dense clusters or “puffs” of terminals discontinuously spread over 4–6 mm of the rostrocaudal extent of the neostriatum (e.g., Alloway et al. 1998). In monkeys, these concentrated clusters of corticostriatal terminals have been termed “matrisesomes” (Flaherty and Graybiel 1991, 1993; Parthasarathy and Graybiel 1997). In the rodent neostriatum, heavily labeled clusters of corticostriatal terminals located in close proximity may also form larger aggregates that have been described as strips, bands, laminae, and lamellae (e.g., Alloway et al. 1999; Brown et al. 1998; Wright et al. 1998). In sections posterior to bregma, the lamellar bands were 150–250 μm wide and followed the contour of the external capsule. In the most caudal sections, their positions tended to shift ventrally by several hundred microns. Anterior to bregma, labeled projections were often scattered over a wider mediolateral range but were largely confined to the neostriatum’s dorsolateral quadrant. Patches of labeled terminals were occasionally observed in medial regions of the neostriatum, in a central zone distinctly separate from that described above (see also Alloway et al. 1999). If enough of these patches were present, they sometimes appeared as curved lamellae that resembled those subjacent to the external capsule (e.g., Fig. 2, −0.42 and −0.84 mm).

Topography of corticostriatal projections from different body part representations

The pattern of corticostriatal labeling observed in case H18 is representative of those animals that received tracer injections in different body part representations of SI cortex. In this instance, AF was deposited into whisker barrels D1–3 and into the surrounding septa, BDA was injected into the forepaw representation, and FR was placed in the hindpaw region. For this case, the locations of the tracer injections relative to the SI somatotopic map are depicted in Fig. 1 (left panel).

Tracer injections across different body part representations produced labeling in the neostriatum that was largely nonoverlapping. Corticostriatal projections from the whisker barrel representation formed a curved band along the dorsolateral margin of the neostriatum, just below the external capsule, as shown in Fig. 2 for case H18. Projections from the hindpaw and forepaw representations occupied wide bands of neostriatal neuropil that were located medial to the projections from the whisker representations. Although the projections from the forepaw and hindpaw representations often appeared to terminate in a single large band or lamina, those from the hindlimb representation were always dorsal to those from the forelimb representation, and the total amount of neostriatal overlap was minimal (<1% for case H18 as shown in Table 2).

Corticostriatal projections from the different body part representations also terminated in different rostrocaudal positions as shown in Fig. 3. Whisker projections terminated most caudally, and the densest concentration of these projections extended from about 1.0 mm rostral to 3.0 mm caudal to bregma. Projections from the hindlimb representation terminated most rostrally, the majority terminating in sections between bregma and 3.0 mm more rostrally. Corticostriatal terminals originating from the SI forelimb representation generally had a bimodal distribution in which one group of terminals was concentrated 1–2 mm rostral to bregma and the other group was concentrated 1–2 mm caudal to bregma. The rostrocaudal distribution of labeling for animal H18 is presented in Fig. 3A (top panel). As indicated in that panel, the amount of tracer labeling varied from one section to the next. Furthermore, the
sections selected for illustration in Fig. 2 were those that contained the most labeling for each of the three tracers (indicated by arrowheads in Fig. 3A) and, thus were most likely to contain overlapping projections. Figure 3B is a similar plot that summarizes the data for all animals that received injections across different body part representations of SI. Although there was some variability among animals with regard to the absolute position of the labeled projections relative to bregma, the general scheme was similar to that described above (i.e., whiskers caudal, hindlimb rostral, and forepaw in between).

Topography of corticostriatal projections from the same body part representation

Case H22 is representative of those animals that received double or triple tracer injections within the forelimb region of SI. In this instance, FR was deposited into the digit area of the forepaw representation. The tracer BDA was injected into a region of the forelimb representation where electrophysiological mapping revealed RFs on the antebrachium (i.e., extending from the wrist to the elbow). Finally, AF was placed in a forelimb region where responses were evoked by lightly brushing the hairs of the brachium (i.e., extending from the elbow to the shoulder). The location and spread of these injections is shown in Fig. 1 (right panel).

The pattern of corticostriatal projections seen in H22 and other cases that received all injections in various forelimb representations differed from that observed in the “across body part” cases in two major respects. First, in sections posterior to bregma, where corticostriatal projections are more topographically organized, labeled terminations from all three components of the forelimb representation were usually confined to a single lamellar-shaped region in the neostriatum. Within this region, there was a strong tendency for projections from the proximal limb representation to terminate dorsally, while projections from the forepaw representation terminated more ventrally, and projections from the antebrachium representation terminated between those regions (see Fig. 4, 1.11 and 1.85 mm). Second, the amount of overlap among corticostriatal projections was significantly greater for the “within forelimb” cases than for the “across body part” cases. As shown by Fig. 5, A and A’, some regions of the neostriatal neuropil contained labeled projections from all three subcomponents of the SI...
The precision of the thalamic labeling regions caused labeling to appear in lateral and medial portions of different tracers into the SI hindlimb and forelimb topographic organization. While tracer injections into the SI overlap. Consistent with reports that have described the distribution in every 3rd section as a function of the rostrocaudal distance from bregma. Arrowheads under the abscissa indicate sections that are illustrated for cases H18 and H22 in Figs. 2 and 4, respectively. B: distribution of neostriatal labeling for the 13 animals that received injections into the whisker, forepaw, or hindlimb representations of SI (see Table 1). To construct this summary plot, the total number of labeled bins for each body part was summed across animals and then the percent of total labeling at each rostrocaudal level was plotted relative to the distance from bregma.

A quantitative comparison of the reconstructed sections in Figs. 2 and 4 demonstrates a greater amount of neostriatal overlap for case H22 than for case H18 (see Table 2 for quantitative comparisons of all tracer combinations).

Despite the substantial amount of corticostriatal overlap observed in cases that received tracer injections in the forelimb representation, inspection of the thalamus revealed little tracer overlap was almost always greater when both tracers were placed in the SI forelimb representation. Thus as shown by photomicrographs in Fig. 5B, the thalamic forelimb area was somatotopically organized so that the forepaw was represented most ventrally, the proximal forelimb most dorsally, and the antibrachium or wrist region was located at intermediate sites (see Fig. 4, −2.58 and −2.95 mm).

Quantification of overlap among corticostriatal projections

To estimate the amount of corticostriatal overlap produced by injecting tracers into different somatotopic locations, we used an index of overlap that measures the proportion of the total labeled area in which the number of overlapping labeled varicosities exceeds a specific threshold in a standard unit of area. Although it is possible to measure and illustrate spatial changes in the density of varicosities labeled by a single tracer, such quantitative measures of density are not tractable when multiple tracers are used to determine projection overlap. Indeed, using a color-coded density gradient to illustrate the spatial changes in terminal density and their overlap is simply not feasible when populations of terminals labeled by two or more tracers are superimposed on each other. For these reasons, tracer overlap was quantified initially by using 35-μm² bins and a threshold criterion of at least two labeled varicosities for each injected tracer (see METHODS).

Using the operational definition described above, the amount of total overlap in the neostriatum ranged from 0% to 9.42% (see Table 2), with an overall mean value of 1.518 ± 0.366% (SE). Corticostriatal overlap was higher in the “within forelimb” group (2.769 ± 0.634%) than in the “across body parts” group (0.414 ± 0.126%). Indeed, the highest amount of overlap (9.42%) was observed after a pair of injections into the forepaw and distal forelimb representations (case H24), while injections into separate body part representations often failed to produce any overlap whatsoever (see specific tracer combinations for cases H18, H20, H21, H29, and H34 in Table 2).

The amount of neostriatal overlap varied not only with the somatotopic location of the cortical injections but also with the distance separating the injections. Figure 6 indicates that injections separated by farther distances tended to produce less overlap than those placed closer together. To examine the effects of these two parameters, the “across body part” and “within forelimb” groups were each sorted into two subgroups: those with injections separated by <1,600 μm (“closer separation”) and those with injections separated by more than 1,600 μm (“farther separation”). We then compared the variation in tracer overlap using a two-way ANOVA (see METHODS for details). The ANOVA revealed that both somatotopic location (F = 19.63, P = 0.0001) and cortical separation (F = 4.42, P = 0.0444) had significant effects on corticostriatal overlap. Furthermore, there was no interaction between somatotopic location of injection and cortical separation (F = 0.08, P = 0.7751). Thus the effect of one factor level was not strongly influenced by the level of the other factor. The importance of somatotopic location was emphasized even further by comparing corticostriatal overlap for individual cases in which the injection site separations of the two groups were approximately the same. Thus as shown by Fig. 6, for the nine cases in which the tracer injections were separated by 1,250–2,000 μm, corticostriatal overlap was almost always greater when both tracers were placed in...
the forelimb representation. In summary, these results indicate that the degree of corticostriatal overlap is significantly higher for projections originating from subcomponents of the same body part representation, and this degree of overlap decreases with increasing cortical separation.

To determine whether the effects of somatotopic location on corticostriatal overlap were due to arbitrary technical considerations such as bin size, we repeated the overlap measurements using different bin sizes. Figure 7 indicates that an increase in bin size from 35 to 50 or 75 µm² leads to a noticeable increase in the amount of corticostriatal overlap. However, regardless of the precise bin size that was chosen, mean corticostriatal overlap was always greater for those cases in which the tracer combinations were administered to different subcomponents of the forelimb representation.

We also analyzed corticostriatal overlap using different threshold criteria across a range of bin sizes to determine if the initial values that we chose for these parameters might have biased our results. Because this analysis requires repeated measurements on a large number of sections, we restricted our comparisons to cases H12 and H23 because these cases were highly similar in terms of their injection site separations (1,352 vs. 1,440 µm) and in the number of labeled bins in the neostriatum (3,166 vs. 3,096). These animals received injections of BDA and FR into the forepaw and hindpaw (H12) or into the forepaw and proximal forelimb (H23). As shown by Fig. 8, regardless of the bin size or threshold criteria that were used to calculate tracer overlap for these two cases, the amount of corticostriatal overlap was always greater for case H23 (within forelimb) than for case H12 (across body parts). In fact, if the threshold criteria were more conservative (i.e., 4 varicosities at 35 or 50 µm² or 8 varicosities at 75 µm²), the disparity in the relative proportion of corticostriatal overlap became more apparent.

DISCUSSION

The findings in this study extend our previous report indicating that corticostriatal projections from SI cortex are anisotropically organized (Alloway et al. 1999). Projections from SI areas representing body parts that are functionally and physically separate tend to terminate in parts of the neostriatum that are largely nonoverlapping. By comparison, projections from SI areas representing subcomponents of the forelimb terminate within a single neostriatal lamina and overlap to a much greater degree. Although corticostriatal overlap is related to cortical proximity, somatotopic continuity plays a significant role, and these findings have important implications for understanding...
the relationship between corticostriatal convergence and sensorimotor integration.

**Principles of corticostriatal organization**

There is widespread agreement that corticostriatal projections from sensorimotor cortex exhibit a complex topography of both divergent and convergent connections. Individual body part representations in SI terminate in multiple discontinuous regions of the dorsolateral neostriatum (Alloway et al. 1998, 1999; Brown et al. 1998; Flaherty and Graybiel 1991, 1993; Kunzle 1977; Malach and Graybiel 1986; Wright et al. 1998). Cortical areas representing the same body part or having related sensorimotor functions project to partially overlapping regions of the neostriatum (Alloway et al. 1999, 2000; Flaherty and Graybiel 1991, 1993; Hoffer and Alloway 2001; Inase et al. 1996; Parthasarathy et al. 1992; Takada et al. 1998; Updyke 1993). While these issues are generally agreed on, the extent of corticostriatal convergence remains the subject of some uncertainty. This is an important issue because neostriatal modules may serve a pattern recognition function (Houk 1995). Previous studies have either relied on qualitative statements to describe the degree of overlap or have not addressed the subject at all. This has often been the case because only a single anterograde tracer was injected, projections from two tracers were examined in alternate sections, or the data analysis was limited to contours drawn around labeled regions with the aid of a drawing tube. To overcome these limitations, we injected two or three anterograde tracers that could be visualized in the same tissue section, used a computer-aided plotting system to accurately record the location of labeled terminals, and implemented a computer program to quantify the amount of overlap.
of corticostriatal overlap. Only a few other studies have attempted to examine the issue of corticostriatal convergence in a similar quantitative fashion (Alloway et al. 1999, 2000; Hoffer and Alloway 2001). Our results, taken together with those of other laboratories, suggest that corticostriatal convergence is governed by at least three principles of organization.

The first principle relates to cortical proximity. Cortical areas that are located close to each other are more likely to project to common targets in the neostriatum than those that are more widely separated. Our statistical analysis revealed that the distance separating the tracer injection sites caused significant variation in the amount of corticostriatal overlap that was observed across different animals. This effect was not directly linked to the somatotopic location of the injections, however, as there was no significant interaction between somatotopic representation and cortical proximity, the effect of cortical proximity was not as strong as the effect of somatotopic representation. A similar result was obtained in our previous study that quantified the degree of neostriatal overlap following dual tracer injections into separate columns of the SI barrel field (Alloway et al. 1999).

The effect of cortical proximity may be related to the neurogenetic gradients established in source (cortex) and target (neostriatum) regions during development (e.g., Bayer and Altman 1987; Erzurumlu and Jhaveri 1992).

The second principle relates to somatotopic homology. Corticostriatal projections from separate cortical areas (i.e., Brodmann’s areas) that represent identical body parts are more likely to converge than those from cortical areas that represent different body parts. For example, dual tracer injections into corresponding whisker representations in SI, SII, and MI produce greater overlap in the neostriatum than injections into noncorresponding whisker representations (Alloway et al. 2000; Hoffer and Alloway 2001). In primates, similar results have been reported for corticostriatal projections from corresponding limb representations that occupy different parts of sensorimotor cortex (Flaherty and Graybiel 1991; Inase et al. 1996; Parthasarathy and Graybiel 1992).

The third principle relates to somatotopic continuity. Projections from cortical areas representing subcomponents of the same body part show greater overlap in the neostriatum than projections from cortical areas that are separated by similar distances but represent discontinuous or separate body parts. This principle of organization was revealed by the significant increase in corticostriatal overlap that we observed when different tracers were separately injected into different parts of the SI forelimb representation (case H23 in Fig. 8). By comparison, dual tracer injections into the forepaw and hindpaw representations were often separated by similar cortical distances, but produced substantially less corticostriatal overlap regardless of the bin size or threshold criteria used in our analyses.

We previously reported that corticostriatal overlap is greater following dual injections in the same SI whisker barrel row than when similarly spaced injections are placed in different SI barrel rows (Alloway et al. 1999). This is significant because rats often make repetitive, rostrocaudal sweeping movements with their whisks, and adjacent whisks within a row are often stimulated in close temporal sequence as they contact external objects (Carvell and Simons 1990; Sachdev et al. 2001). The relative timing of stimulation for whisks residing in different rows is more likely to vary, however, as a function of head tilt or changes in the orientation of the objects that are whisked. Thus whisking behavior correlates with our anatomic observation that corticostriatal projections from the same barrel row are most likely to converge. Furthermore, in primates, corticostriatal projections from the thumb and fifth digit representations of area 3b overlap to a greater degree than those from the finger and lip representations (Flaherty and Graybiel 1991). Clearly, fingers on the same hand must work together to grasp objects and manipulate objects, and the fingers are likely to be synchronously stimulated during these activities. Similar arguments should also apply to the subcomponents of the rodent forelimb during nocturnal exploration or other locomotor activities. Thus it is reasonable to expect that cutaneous receptors along the paw, wrist, and elbow are frequently stimulated simultaneously or in a close temporal sequence as the animal brushes against objects in its environment. Taken together, these observations provide strong support for the notion that corticostriatal convergence is related to temporal factors that are inherently associated with somatotopic continuity.

The degree of corticostriatal overlap appears to be strongly influenced by somatotopic continuity, but our results also demonstrate that some neostriatal modules integrate inputs from SI cortical areas that represent separate body parts. Even though we used relatively small bins (i.e., 35 μm²) to quantify corticostriatal overlap, we still detected some corticostriatal overlap when different tracers were placed in the forepaw and hindpaw (H12, H18, H19, H36, and H37) or in the forepaw and whisker...
representations (H5, H6, and H7). If we had used larger bin sizes, the amount of overlap in these cases would have been even greater. In view of these instances of corticostriatal overlap, it is also possible that we would have detected more overlap in the across body part group if our injection sites were larger (e.g., total infiltration of the SI barrel field) or if we had analyzed other combinations of body part representations (e.g., proximal limbs and trunk). Although separate body parts may exhibit a wide range of behavioral expression, many behaviorally significant activities involving the face, forelimb, and hindlimb consist of coordinated body part movements that simultaneously activate certain populations of mechanoreceptors distributed across these body regions. We hypothesize that it is the SI representations for these populations that are most likely to send convergent projections to the neostriatum.

**Functional implications**

The principles governing corticostriatal overlap are important for understanding neostriatal function because several facts suggest that neostriatal activity may depend on convergent corticostriatal inputs. Medium spiny neurons comprise the vast majority (>90%) of cells in the neostriatum and they provide the only source of efferent projections. Consistent with data indicating that medium spiny neurons exhibit long silent periods interrupted by bursts of action potentials (Cowan and Wilson 1994; Wilson et al. 1983), their membrane potentials do not fluctuate randomly, but switch rapidly between two subthreshold levels known as the “down” and “up” states (Kawaguchi et al. 1989; Wilson 1995). In the “down” state, the membrane potential is hyperpolarized to −80 mV; this accounts for the long silent periods. In the “up” state, the membrane is depolarized to −50 mV, and small fluctuations in membrane potential during this state can trigger action potentials. A rectifying potassium current shunts any weak excitatory inputs (Nisenbaum and Wilson 1995; Nisenbaum et al. 1994), and medium spiny neurons probably shift to the “up” state only when they receive many excitatory inputs over a short period of time.

Several observations suggest that corticostriatal projections provide the excitation needed to shift medium spiny neurons into the “up” state. Among the 5,000–30,000 synaptic contacts received by a typical medium spiny neuron, the majority are glutamatergic projections from the cerebral cortex (Fonnum et al. 1981; Hassler et al. 1982; Kemp and Powell 1971; Spencer 1976). The lateral interactions between neostriatal neurons are inhibitory in nature, and their blockade does not cause medium spiny neurons to become tonically active (Nisenbaum and Berger 1992). Finally, corticostriatal neurons have fluctuations in membrane potential that resemble those in medium spiny neurons (Cowan and Wilson 1994). Hence, neostriatal discharges appear to reflect the spatiotemporal integration of their corticostriatal inputs.

The spatial distribution of corticostriatal axons forms an extensive network of axons at regular intervals along their length and most of these axons take a relatively straight course through the neostriatum. This arrangement limits each axon to forming a maximum of 40 synapses within the dendritic volume of a typical medium spiny neuron (Kincaid et al. 1998).

Thus the projections from a focal cortical site may contribute only a small percentage of the synapses on a single neostriatal neuron. To the extent that many corticostriatal neurons must discharge simultaneously to shift medium spiny neurons to the “up” state, activation of a local neostriatal module is likely to be facilitated by co-activation of related cortical areas.

This evidence has led to the hypothesis that medium spiny neurons may perform a pattern recognition function like perceptors (Houk 1995; Rosenblatt 1958). According to this view, neostriatal neurons are trained to respond when related cortical areas are co-activated, either synchronously or in close temporal sequence, thereby coding specific sets of corticostriatal inputs that are behaviorally significant. Our results suggest that corticostriatal overlap—as governed by the principles of cortical proximity, homology, and somatotopic continuity—constitutes an important neural substrate for pattern recognition in the sensorimotor channel. Presumably, the divergence of projections from a single cortical site provides the same information to separate neostriatal modules that perform related computations on different combinations of converging cortical inputs.

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