Dopamine Depletion Causes Fragmented Clustering of Neurons in the Sensorimotor Striatum: Evidence of Lasting Reorganization of Corticostriatal Input

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ABSTRACT

Firing during sensorimotor exam was used to categorize single neurons in the lateral striatum of awake, unrestrained rats. Five rats received unilateral injection of 6-hydroxydopamine (6-OHDA) into the medial forebrain bundle to deplete striatal dopamine (DA; >98% depletion, postmortem assay). Three months after treatment, rats exhibited exaggerated rotational behavior induced by L-dihydroxyphenylalanine (L-DOPA) and contralateral sensory neglect. Electrode track “depth profiles” on the DA-depleted side showed fragmented clustering of neurons related to sensorimotor activity of single body parts (SBP neurons). Clusters were smaller than normal, and more SBP neurons were observed in isolation, outside of clusters. More body parts were represented per unit volume. No recovery in these measures was observed up to one year post lesion. Overall distributions of neurons related to different body parts were not altered. The fragmentation of SBP clusters after DA depletion indicates that a percentage of striatal SBP neurons switched responsiveness from one body part to one or more different body parts. Because the specific firing that characterizes striatal SBP neurons is mediated by corticostriatal inputs (Liles and Updyke [1985] Brain Res. 339:245–255), the data indicate that DA depletion resulted in a reorganization of corticostriatal connections, perhaps via unmasking or sprouting of connections to adjacent clusters of striatal neurons. After reorganization, sensory activity in a localized body part activates striatal neurons that have switched to that body part. In turn, switched signals sent from basal ganglia to premotor and motor neurons, which likely retain their original connections, would create mismatches in these normally precise topographic connections. Switched signals could partially explain parkinsonian deficits in motor functions involving somatosensory guidance and their intractability to L-DOPA therapy—particularly if the switching involves sprouting. J. Comp. Neurol. 452:24–37, 2002.

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Rats treated unilaterally with 6-OHDA exhibit sensorimotor disintegration contralaterally (Marshall et al., 1971; Ungerstedt, 1971a,b; Ljungberg and Ungerstedt, 1976; Schultz, 1982; Carey, 1988; Cadet et al., 1991). Their failure to orient to contralateral somatosensory stimuli results from a disruption of dopaminergic transmission selectively in the lateral striatum (Fairley and Marshall, 1986), consistent with anatomical and electrophysiological data. The lateral striatum receives patchy, topographic projections from primary somatosensory and motor cortices in rats (Cospito and Kultas-Illins, 1981; McGeorge and Faull, 1989; Ebrahimi, 1992; Brown et al., 1998; Kincaid et al., 1998; Wright et al., 1999; Hoffer and Alloway, 2001) and primates (Kunzle, 1975, 1977, 1978; Goldman and Nauta, 1977; Jones et al., 1977; Yeterian and Van Hoesen, 1978; Goldman-Rakic, 1982; Malach and Graybiel, 1987; DeLong, 1990; Hedreen and Van Hoesen, 1978; Goldman-Rakic, 1987; Ljungberg and Ungerstedt, 1976; Ungerstedt, 1971a,b; Ljungberg, 1971; Ungerstedt, 1971b; Ljungberg and Ungerstedt, 1976; Schultz, 1982; Carey, 1988; Cadet et al., 1991). Their inability to follow contralateral somatosensory stimuli results from damage by 6-OHDA. The effect of the 6-OHDA was tested behaviorally 3 months later by observing the rotational behavior induced by L-DOPA (5 mg/kg plus carbidopa 10 mg/kg, i.p.; Sonsalla et al., 1988). From a set of treated rats, five rats were selected that exhibited the strongest behavioral signs of DA depletion (verified by postmortem DA assay; see below). All five exhibited robust rotational responses to L-DOPA, i.e., bursts of rapid turning. Turning was frequently interrupted by periods of oral stereotypy (e.g., licking), such that overall, the mean number of rotations was 433 turns in 3 hours. These five rats underwent a second surgery to prepare them for single-unit recording.

**MATERIALS AND METHODS**

**Animals**

Data were collected from 16 adult male rats. Sprague-Dawley rats (n = 5) weighing 220–250 g underwent unilateral 6-OHDA treatment, the treated side being termed the 6-OHDA group and the intact side the control group. The control group also included neural data recorded from intact Sprague-Dawley (n = 2) and Long-Evans (n = 5) rats. Other naïve Sprague-Dawley rats (n = 4) underwent no surgery but were used only for DA assay. Animals were housed individually and maintained on a reversed light/dark cycle (On 20:00, Off 08:00) so that experiments were conducted during their active period. Subjects had free access to food (Purina lab chow) and water throughout the course of the study. All procedures were approved by the institutional animal care and use committee and conform to NIH guidelines.

**6-OHDA treatment**

Stereotaxic surgery was performed to destroy the left nigrostriatal dopaminergic projection. Under anesthesia (methohexitol sodium [Brevital] 25 mg/ml; 37 mg/kg, intraperitoneal, i.p.), 4 μl of 6-OHDA (6-OHDA HBr 3 mg/ml) were injected into the left medial forebrain bundle (A-P 4.9; M-L 1.7; D-V 2.4 mm with respect to ear bar zero; Konig and Klippel, 1963). Desmethylimipramine (25 mg/kg, i.p.) was also injected to protect noradrenergic neurons from damage by 6-OHDA. The effect of the 6-OHDA was tested behaviorally 3 months later by observing the rotational behavior induced by L-DOPA (5 mg/kg plus carbidopa 10 mg/kg, i.p.; Sonsalla et al., 1988). From a set of treated rats, five rats were selected that exhibited the strongest behavioral signs of DA depletion (verified by postmortem DA assay; see below). All five exhibited robust rotational responses to L-DOPA, i.e., bursts of rapid turning. Turning was frequently interrupted by periods of oral stereotypy (e.g., licking), such that overall, the mean number of rotations was 433 turns in 3 hours. These five rats underwent a second surgery to prepare them for single-unit recording.

**Chronic implantation and extracellular recording**

At least 3 months after the first surgery to administer 6-OHDA, a miniature microelectrode drive assembly (Josef Biela Engineering, Anaheim CA) for extracellular single-unit recording was installed under sodium pentobarbital anesthesia (50 mg/kg, i.p.; Deadwyler et al., 1980; West and Woodward, 1984; Prokopenko et al., 1997). Bases for attaching microdrives were cemented to the skull overlying the striatum on both sides of each animal treated with 6-OHDA (M-L = 4.0 mm, at bregma, level skull). In intact Sprague-Dawley and Long-Evans rats (300–350 g), one base was implanted on the left side. After 1 week of recovery from installation of the microdrive, recording sessions commenced. The microdrive was equipped for each recording with a tungsten microelectrode (10 MΩm, Heka; Brunswick, ME), which was advanced in 50-μm increments, each increment constituting a recording site during the classification of striatal neuron activity.

Because advancing the electrodes (via manual rotation of the outer cylinder of the microdrive) was accomplished without rotation of electrodes, electrodes could be advanced ventrally from any eccentric position, thereby extending the A-P and M-L ranges by approximately ± 0.8 mm from the A-P and M-L coordinates at which the animal was implanted. Thus several electrode tracks could be
Fourteen body parts, or categories, were examined, which was applied in its entirety to every neuron. Procedures for amplifying, which was coated with silicone. Further details, including procedures for amplifying, filtering, and discriminating extracellularly recorded action potentials of individual neurons, as well as videotape analysis of sensorimotor activity, have been described previously (West et al., 1990; Carelli and West, 1991; West, 1998). Some electrode tracks were not completed, in order to perform lengthy videotaped experiments at a particular recording site.

**Categorization of neural activity**

The procedure used for this study was a sensorimotor exam, which was applied in its entirety to every neuron. Fourteen body parts, or categories, were defined: forelimb, hindlimb, vibrissae, shoulder, and trunk, which were differentiated between the ipsilateral and contralateral side; head, neck, snout, and oral region (lip, chin, or tongue), which were not differentiated between the ipsilateral and contralateral side. At each 50-μm increment in each electrode track, an assessment was made of discharges in relation to each particular category, or BP, by listening through headphones to the audio record (amplified bandpass filter output) of neuronal activity. A hand-held probe (2-mm diameter) was used to deliver cutaneous stimulation (approximately 2 g force). Each body part was tapped with the probe, and the fur or skin also was softly rubbed with light stroking movements (Carelli and West, 1991). Using this approach, each responsive neuron could be assigned unambiguously to a particular category.

The TM was activated to assess relationships between firing and active locomotor movements (West et al., 1990). Passive manipulation was applied to the limbs and neck (the latter by gently manipulating the headstage of the harness). Head-related neurons responded to cutaneous stimulation of the head, other than the face. Firing in relation to the snout was evaluated using only cutaneous stimulation. Testing of the trunk or shoulder consisted of cutaneous stimulation and palpation, either manually or with a blunt wooden probe. Shoulders also underwent passive rotation. Neurons were examined for responsiveness to sensory stimulation of the vibrissae collectively by stroking with the probe in each direction. Firing related to licking was tested using a wooden stick moistened with water or a sweet solution. No attempt was made to determine the precise boundaries of receptive fields. Constructing a complete electrode track profile could involve the delivery of >5,000 individual somatosensory stimuli. Habituation was minimized by the continual shifting of stimulation to different body parts in different sequences. No more than six complete tracks were recorded in each animal.

Neurons related to body parts (BP) were either single body part (SBP) neurons, that fired in relation to only one body part (category), or multiple body part (MBP) neurons, that fired in relation to at least two different body parts. Neurons responding to a broad area within the same body part, or (rarely) to the same body part bilaterally, were considered SBP, not MBP neurons.

Unresponsive (U) neurons and neurons related to general movement (G) constituted separate categories. U neurons were spontaneously active; they exhibited either steady or irregular firing rates that were not affected by any aspect of the sensorimotor exam. G neurons phasically increased firing rate during whole body movement such as locomotion. A relationship to axial musculature was possible but was not tested. The relation of G neurons to whole body movement was not a summation of responsiveness to different body parts (which characterized “multiple body part” neurons; see below). Each recording site that exhibited no spontaneous or evoked neural activity was termed a Space.

**Evaluation of somatotopy, proportionality, and clustering**

To evaluate striatal somatotopy, neurons were divided into four categories: neurons related to 1) head or face (oral region, vibrissae, or snout); 2) neck; 3) forelimb and 4) hindlimb. Each neuron was assigned a depth coordinate, ranging from 0 to 4,000 μm (in 50-μm increments), representing its D-V depth into the striatum. This scheme omitted categories having too few neurons for statistical analysis. MBP neurons were included, because they reliably indicate locations of striatal neurons responding to topographic synaptic inputs. Each MBP neuron was entered a number of times corresponding to the number of categories to which its firing was related.

Only data from complete electrode tracks were used for analyses of proportions among neurons, different combinations of body parts to which MBP neurons were related, number of different body parts represented per unit distance, number of clusters per unit distance, percentage of neurons contained in clusters, D-V depth in striatum, and cluster size. Proportions among neural categories were assessed using the combined data from all tracks in all animals. A Chi square test compared prevalences within categories, followed by Haberman’s post hoc tests (Runyon et al., 1996). For each group, data from the first two complete electrode tracks in each animal were used in certain t-tests or ANOVAs. The observation entered for each animal was the total number of body parts or clusters per length of track, or the percent of neurons contained in clusters, or the D-V depth of each neuron (or Space). Analyses of depth were performed on combined data from the first two tracks after verifying that there were no differences between the first and second tracks (ANOVA with repeated measures). Complete tracks were those that extended ventrally 1) at least 2.9 mm from the dorsal edge of the striatum; or 2) at least 2.0 mm from the dorsal edge, ending with a zone >0.5 mm showing no neurons related to body parts. It was necessary to define an end point for complete tracks because of the increasing likelihood that electrodes exited from the lateral (sensorimotor) striatum as they were advanced ventrally (e.g., into the medial striatum, globus pallidus, or external capsule; see tracks in Fig. 3). The truncation of electrode tracks was equally likely for both control and 6-OHDA groups. The D-V depths of U and G neurons and Spaces, which were not normally distributed (each category failed a normality test; P < 0.0001), were compared between control and 6-OHDA groups, using Wilcoxon matched pairs signed ranks tests.

In the analysis of cluster size, a cluster was defined as neurons at two or more adjacent 50-μm increments related to the same body part. A cluster of two was assigned the value of 50 μm length; a cluster of three was assigned the value of 100 μm length, and so on. The size of each
cluster was entered as an observation into Mann-Whitney ranked sum tests, because cluster size was not normally distributed (each category failed a normality test; \( P < 0.0001; \) e.g., Fig. 4b). In comparisons among control subgroups (i.e., neural data collected from intact Long-Evans and Sprague-Dawley rats, and the intact side of unilaterally DA-depleted rats), for each electrode track, the percentage of clustered SBP neurons or of MBP neurons was entered as an observation into ANOVA.

Entering individual clusters or individual tracks as observations was justified as follows. The normal somatotopic arrangement in the dorsal-ventral dimension is maintained throughout the anterior-posterior and medial-lateral dimensions of the sensorimotor striatum (Carelli and West, 1991; Cho and West, 1997). More tracks were obtained from some animals than others, but sampling within an animal was distributed throughout the A-P and M-L ranges of the microdrive. Therefore, each D-V electrode track profile was considered an independent observation, regardless of the animal from which it was obtained (within the same experimental group). The limitation to six tracks per animal within each group minimized differences in individual rats’ experience with sensorimotor testing. Given this limitation on experience, it was assumed that any other individual differences, although they may exist, are not among the main factors that contribute to variations in the size or prevalence of clusters. This assumption has been supported by hundreds of electrode track profiles (West et al., 1990; Carelli and West, 1991; Mittler et al., 1994; Cho and West, 1997; West, 1998).

**Histology or DA assay**

Electrophysiological depth profiles indicated that all recordings were obtained from neurons intrinsic to the striatum and not from fibers of passage or from cortical neurons. This was confirmed by constructing a depth profile of neuronal activity as each electrode was advanced through the neocortex and into the striatum. Immediately ventral to a region containing large spikes characteristic of neurons in deep cortical layers, a “quiet zone” (200–400-μm distance) was consistently observed in which no neuronal discharges were recorded. Immediately ventral to this, extracellular spikes characteristic of striatal medium spiny neurons were recorded (West, 1998). Histological reconstruction verified that the quiet zone corresponded to white matter of the corpus callosum or external capsule. Hence, it was concluded that the present electrodes did not detect axonal spikes, including those in axon bundles coursing through the striatum. Any track that did not exhibit these characteristics was discontinued.

After the final recording in each animal, histology was performed on intact animals, and DA assay was performed on animals treated with 6-OHDA. In intact animals, under deep pentobarbital anesthesia (150 mg/kg, i.p.) the microdrive was used to lower an electrode (Teflon insulated, stainless steel wire, 200 μm) to a measured depth and produce a small electrolytic lesion that was used for reconstructing the locations of all recorded neurons (details in Carelli and West, 1991; Cho and West, 1997). Animals treated with 6-OHDA and additional naive rats used to provide control data were decapitated (without anesthesia, which would have compromised neuronal chemical assays), the brains were removed, and high-pressure liquid chromatography-electrochemical detection (Sonsalla et al., 1987) was used to measure striatal DA and serotonin (5-HT) concentrations by comparison of peak height ratios of samples with those of standards. Results are reported in micrograms of substance per gram of wet weight of striatal tissue (Sonsalla et al., 1990). Illustrations were constructed with SigmaPlot 5.0, Paint Shop Pro 7.0, Agfa FotoLook 3.0 with scanner (Snapscan 1212), Analyze 1.0 (Unitlab Associates) and Image Pals 2.0.

**RESULTS**

The control group comprised the neurons recorded from seven intact animals and from the intact side of four animals treated with 6-OHDA; the 6-OHDA group comprised the neurons recorded from the 6-OHDA-treated side of five animals. All comparisons between control and 6-OHDA groups refer to this scheme of sorting neurons. A total of 2,449 neurons were recorded at 3,369 recording sites tested in the lateral striatum, 1,647 neurons in the control group and 802 neurons in the 6-OHDA group.

**DA and 5-HT Assays**

All five 6-OHDA-treated rats were selected for study because their rotational behavior indicated substantial unilateral DA depletion (Heikkila et al., 1981). Post mortem, the five rats exhibited 98–100% reduction in striatal DA on the treated side relative to the intact side (Table 1). Thus, depletion of striatal DA by 6-OHDA was nearly complete and highly selective, in that noradrenergic axons were protected by injecting desmethylimipramine. Stratal 5-HT levels showed a small (14%) increase on the DA-depleted side (\( P < 0.05; \) paired t-test; Table 1), in agreement with other studies comparing DA-depleted with intact side in each rat (e.g., Zhou et al., 1991; Gaspar et al., 1993), although 5-HT levels did not differ when comparing between groups (\( P > 0.47; \) unpaired t-test), in agreement with other unpaired studies (Breese et al., 1984; but see Schneider and Rothblat, 1991).

**Behavior**

In DA-depleted animals, postural deviation and spontaneous rotation toward the depleted side was observed during recording, especially in response to sudden stimulation (e.g., tapping on the recording chamber). During somatosensory stimulation contralateral to the lesion, all DA-depleted rats showed clear, consistent, and lasting sensory neglect. In no instance, even up to one year post lesion, did an orienting response occur. That is, no response ever exceeded a score of 0 on a scale of 0 to 4 (0 =

<table>
<thead>
<tr>
<th>Rat</th>
<th>Intact side</th>
<th>6-OHDA side</th>
<th>% Depletion</th>
<th>Intact side</th>
<th>6-OHDA side</th>
</tr>
</thead>
<tbody>
<tr>
<td>D26</td>
<td>10.43</td>
<td>0.18</td>
<td>98.3</td>
<td>0.80</td>
<td>0.85</td>
</tr>
<tr>
<td>D27</td>
<td>12.06</td>
<td>0.07</td>
<td>99.4</td>
<td>0.61</td>
<td>0.95</td>
</tr>
<tr>
<td>D30</td>
<td>11.25</td>
<td>0.09</td>
<td>99.2</td>
<td>1.10</td>
<td>1.20</td>
</tr>
<tr>
<td>D31</td>
<td>11.11</td>
<td>0.12</td>
<td>98.9</td>
<td>0.42</td>
<td>0.58</td>
</tr>
<tr>
<td>D35</td>
<td>9.70</td>
<td>0.00</td>
<td>100.0</td>
<td>0.57</td>
<td>0.77</td>
</tr>
</tbody>
</table>

1DA, dopamine; 5-HT, serotonin; 6-OHDA, 6-hydroxydopamine.
The proportions of functionally different neuronal types were tabulated for complete electrode tracks (N = 51), which contained a total of 2,483 tested recording sites (Table 2). Each site either exhibited neural activity or did not, in which case it was termed a Space. The overall percentage of Spaces, i.e., sites exhibiting no evoked or spontaneous neural activity, was not altered by DA depletion: 19.5% (control) versus 19.6% (6-OHDA). At each site showing neural activity, firing was categorized as unresponsive (U), related to general movement (G), specifically related to sensorimotor activity of a single body part (SBP), or related to somatosensory stimulation of at least two different, or multiple, body parts (MBP). The prevalences within these categories differed as a result of DA depletion (Chi square [4 df] = 91.3; P < 0.0001). Whereas U and SBP categories were not different between control and 6-OHDA groups, the prevalence of G neurons was lower, and the prevalence of MBP neurons was higher, in the 6-OHDA group (P < 0.05, Haberman’s post hoc tests). Among SBP neurons, the prevalences within subcategories differed between control and 6-OHDA groups (Chi square [9 df] = 49.5; P < 0.0001). The prevalence of neurons related only to a forelimb was significantly lower, whereas prevalences of neurons related to the neck and to the oral region were significantly higher in the 6-OHDA group (P < 0.05). The prevalences of neurons related to other body parts either did not change after DA depletion (P > 0.05) or were too low to analyze.

Neuronal firing rates and patterns were not quantified, but it can be stated that no striking differences between control and 6-OHDA groups were observed. Both groups exhibited similar extracellular action potential waveform forms, in nearly all cases negative-positive, 0.5–1.0 msec duration, and 150–250 μV peak-to-peak (noiseband = 50 μV), comparable to previous reports (Crutcher and DeLong, 1984; West, 1998). The neurons reported here are categorized as phasic type II, medium spiny, projection neurons (Kimura, 1990). Control and 6-OHDA groups exhibited generally similar spontaneous firing rates (variations possibly related to level of arousal, muscle tone, etc. were not studied). For neurons responsive to somatosensory stimulation, the form of responses to individual somatosensory stimuli was similar between groups, i.e., increased firing rate from a baseline level near zero (compare Fig. 1 with those in West, 1998). No obvious differences between groups were observed in latency to discharge, number of discharges per stimulus, or duration of response. In both groups, neural responses to cutaneous stimulation were short latency, i.e., began within one or two video frames (33 msec/frame) of stimulus onset (e.g., Fig. 1), before onset of observable behavioral reactions to the stimulus (150–200 msec after stimulus onset).

Neurons related to multiple body parts

Prominent in the 6-OHDA group were certain neurons, the MBP neurons, that responded to somatosensory stimulation of at least two different body parts. Table 2 shows that 53 MBP neurons were observed in the 6-OHDA group, i.e., 8% of 675 total neurons, which was significantly (16-fold) greater than the prevalence observed in the control group (6 MBP neurons of 1,322 total neurons; 0.5%). Perirevent time histograms (PETHs) showed that each MBP neuron responded nearly equally (in discharges per stimulus) to somatosensory stimulation of the different body parts to which it was related (Fig. 1). The combinations of body parts to which individual MBP neurons were related and their proportions are shown in Table 3. In the control group, every MBP neuron showed a relation to only two different body parts. In contrast, in the 6-OHDA group, MBP neurons showed various combinations and included up to five different body parts. The neck or forelimb was most frequently included in the related body parts of MBP neurons in the 6-OHDA group (64 and 60%, respectively). MBP neurons showed no peculiarities in parameters such as action potential waveform or spontaneous firing rate.

Somatotopic organization

For both control and 6-OHDA groups, frequency histograms (Fig. 2) showed that distributions of D-V depth for neurons related to 1) hindlimb, 2) forelimb, 3) neck, and 4) head/face overlapped, but differed from each other in the extent to which they extended ventrally (F[1,3] = 21.0, P < 0.0001, two-way ANOVA followed by Duncan’s multiple range test, P < 0.05). The 6-OHDA group was not different from the control group (F = 1.9), and there were no significant group by body part interactions (F = 1.2).
The mean depth (mm ± SEM) of each distribution was as follows (control vs 6-OHDA, respectively): representation of the hindlimb (0.57 ± 0.04 vs 0.66 ± 0.09) was significantly dorsal to that of forelimb (0.82 ± 0.05 vs 0.81 ± 0.09), which was significantly dorsal to that of neck (0.92 ± 0.08 vs 1.2 ± 0.09), which was significantly dorsal to that of head/face (1.33 ± 0.07 vs 1.38 ± 0.09; further details regarding normal animals in Cho and West, 1997). MBP neurons in the 6-OHDA group were distributed throughout the most dorsal 1.9 mm (Fig. 2).

The D-V depths of U neurons, G neurons, and Spaces did not differ between control and 6-OHDA groups (Wilcoxon matched pairs signed ranks tests, \( P > 0.05 \)), and were distributed throughout the dorsal 3 mm of the lateral striatum (not shown).

![Fig. 1. Responsiveness of MBP neurons to cutaneous stimulation of different body parts. In each column, discharges of one neuron in response to cutaneous tap of each related body part are shown in peri-event time histogram (PETH) and raster above it. The two neurons were recorded from the 6-OHDA side in different animals (D27 and D31). Approximately 50 repetitions in each animal were delivered at 2–3-second intervals. Node (vertical line at time zero) corresponds to approximate onset of stimulus, identified using videotape analysis. Ordinate: neuronal discharges per stimulus per 6-msec bin (Hz). Time base: 1.0 second. D31: Fluctuations in striatal spontaneous activity (pre-stimulus) occur without evidence of changes in overt behavior or changes in the preparation, such as neuronal injury.](image)

**Clustering**

We assessed whether the 6-OHDA group differed with respect to the normal striatal pattern in which clusters of adjacent neurons fire in relation to activity of the same single body part. Clusters (defined as two or more adjacent 50-μm increments exhibiting neurons belonging to the same category) were observed in electrode track profiles of all animals (Fig. 3).

To assess the prevalence of clusters in each group, the number of neurons in each category that were contained in clusters was divided by the total number of neurons in that category for each animal. In the 6-OHDA group, the mean percentage of SBP neurons that were contained in clusters was 62.9%, which was significantly lower than...
the 81.3% observed in the control group (t-test, \( P < 0.05 \); Fig. 4c). The remaining SBP neurons that were not contained in clusters were termed “solitary” SBP neurons. These, by definition, were bordered by sites 50 \( \mu \)m dorsally and ventrally that exhibited neurons belonging to different categories or related to different body parts. Thus the 6-OHDA group exhibited approximately twice as many solitary SBP neurons as the control group: mean 37.1% vs 18.7%, respectively.

The mean number of solitary SBP neurons per 3-mm track (Fig. 4d) or in the number of clusters of G neurons (1.5 \( \pm \) 0.6, control vs 1.5 \( \pm \) 0.6, 6-OHDA), U neurons (4.4 \( \pm \) 0.7, control vs 4.3 \( \pm \) 0.9, 6-OHDA), or Spaces (2.0 \( \pm \) 0.3, control vs 2.2 \( \pm \) 0.9, 6-OHDA), per 3-mm track (t-tests, \( P > 0.05 \)).

The lack of increase in the number of clusters per track indicates that the postlesion reductions in the size of SBP and G clusters were not mediated by changes near the centers of clusters, which might separate them into smaller, more numerous clusters. Instead, the reduced length of these clusters may have been mediated by changes near their borders.

### Borders of SBP and G clusters and of MBP and solitary SBP neurons

We examined the borders of neurons in categories that changed. In the 6-OHDA group, 71% (25/34) of MBP neuron borders with other categories involved SBP neurons (11 SBP clusters and 14 solitary SBP neurons). MBP neurons shared a common body part with 91% (10/11) of the SBP clusters and 71% (10/14) of the solitary SBP neurons that they bordered. For example, every MBP neuron bordering a cluster of forelimb included the forelimb among the body parts to which it responded. These data suggest that, at the borders of clusters, switching of SBP to MBP neurons contributed to the reduction in SBP clustering after DA depletions.

However, consistent with the overall low prevalence (8%) of MBP neurons in the 6-OHDA group, only 18% of all SBP clusters and 17% of all solitary SBP neurons in the 6-OHDA group were bordered by an MBP neuron. The remaining 82% of SBP clusters not bordered by an MBP neuron exhibited a mean length of 88 \( \pm \) 8 \( \mu \)m (nearly identical to the 89 \( \pm \) 8 \( \mu \)m shown for all SBP clusters; Fig. 4a). This was significantly smaller than SBP cluster size in the control group (Mann-Whitney rank sum test, \( P < 0.05 \)). These results suggest that the reduction of SBP clustering involved the switching of some SBP neurons to categories other than the MBP category.

Consistent with the retraction of SBP and G cluster boundaries, these two categories bordered each other less often than expected in the 6-OHDA group, which was opposite the pattern of bordering each other more often than expected in the control group (Fig. 5; Chi square \((5) = 58.1; P < 0.0001 \)). This differential was reversed on the part of MBP neurons and SBP solitary neurons: despite the pattern of bordering SBP and G clusters less often than expected in the control group, SBP solitary and MBP neurons bordered these clusters more often than expected in the 6-OHDA group. Spaces and U neurons showed no differences between control and 6-OHDA groups in bordering SBP and G neuron clusters. The large swings from low to high prevalence at these borders, shown selectively by MBP and SBP solitary neurons, strongly suggests that these are the two categories involved in the changes near the borders of SBP and G clusters that reduced their length.

### Number of different body parts represented per electrode track

In each group, for the first two complete electrode tracks in each animal, the number of different body parts that were represented was divided by the length of each track. The mean number of different body parts represented per unit distance in the 6-OHDA group (5.7 \( \pm \) 0.6 body parts
per 3-mm track) was significantly greater (t test, \( P < 0.05 \)) than in the control group (3.9 ± 0.4). This effect appeared to involve the presence of MBP neurons, because excluding them from a second t-test revealed no significant difference (\( P > 0.05 \)). These data indicate that in the 6-OHDA group, some of the body parts to which MBP (and possibly SBP) neurons were related were not present elsewhere in the vertical track before the lesion.

Different subgroups included in the control group showed similar neural measures

We evaluated the appropriateness of combining, into the control group, three subgroups: neurons recorded from intact Long-Evans and Sprague-Dawley rats, and the intact side of 6-OHDA-treated Sprague-Dawley rats (18, 7, and 10 complete tracks, respectively). The three sub-
groups (L-E, S-D, and intact side of treated S-D, respectively) did not differ from each other in 1) the percentage of neurons that were MBP neurons (0.2, 0, and 1%); 2) the percentage of SBP neurons that were contained in clusters (76 ± 3%, 75 ± 10%, and 89 ± 2%, respectively; one-way ANOVA; F [2, 30] = 2.8; P > 0.05; or 3) cluster size of SBP neurons (125 ± 14, 140 ± 24, and 159 ± 25 μm, respectively) or G neurons (102 ± 16, 100 ± 41, and 125 ± 19 μm; Kruskal-Wallis one-way ANOVAs on ranks; P > 0.05). These results indicate that the control subgroups were similar to each other in all neural measures that differentiated the control from the 6-OHDA group.

**DISCUSSION**

**Partial disintegration of striatal functional organization**

The functional organization of the DA-depleted lateral striatum exhibited fragmentation at the level of clusters of SBP neurons and G neurons. Reduced clustering of SBP neurons was demonstrated both by a decrease in SBP cluster size and by a doubling in the percentage of solitary SBP neurons. G neurons showed reductions in prevalence and cluster size. G neurons fire phasically in relation to general movement (possibly related to axial musculature), and like SBP neurons, are probably innervated by the MI and SI cortex. Thus, the present changes were selective for neurons phasically related to somatic sensorimotor activity. It is assumed that changes were not restricted to the D-V dimension, the only one that could be measured with 50-μm precision. It should be noted that firing rates after DA depletion appeared to be unaffected but were not quantified because that requires further controls for motor variables and behavioral state (e.g., Voloshin et al., 1991, Voloshin et al., 1993; West et al., 1997), although higher striatal firing rates have been observed in DA-depleted animals (Kish et al., 1999; Chen et al., 2001).

**MBP neurons**

A striking result was a 16-fold increase post lesion in the percentage of striatal neurons that fired in relation to sensorimotor activity of multiple body parts, some MBP neurons showing relations to as many as three to five body parts. Together with similar nonselective, nonspecific responses of striatal neurons in cats (Schneider, 1991; Rothblat and Schneider, 1993) and globus pallidus neurons in monkeys (Filion et al., 1988; Tremblay et al., 1989) and cats (Rothblat and Schneider, 1995), the data provide compelling evidence that striatal neurons and their targets in globus pallidus changed responsiveness after DA depletion. The interpretation in those studies that such nonspecific responses represented a population of neurons whose receptive fields had become larger can be evaluated by considering which neurons must have switched category to become MBP neurons.

First, neurons in the SBP and G categories were the only ones that exhibited changes indicative of altered responsiveness. In contrast, U neurons and Spaces were distributed from 0 to 4 mm dorsoventrally. In contrast, MBP neurons were distributed from 0 to 1.9 mm doro-
ventrally, which overlaps with the distributions of most SBP neurons, providing the opportunity for the combinations shown by MBP neurons. Third, the high prevalence of forelimb-responsive MBP neurons (Table 3), coupled to a marked reduction in the prevalence of SBP forelimb neurons after the lesion (Table 2) further suggests that MBP neurons may have been SBP forelimb neurons before the lesion. Every MBP neuron that bordered a SBP forelimb neuron contained the forelimb among its related body parts. Such MBP neurons appear to have belonged to a cluster of SBP forelimb neurons before the lesion. Thus, the present data suggest that MBP neurons were SBP neurons before the lesion, supporting the interpretation of previous studies that some neurons' receptive fields became larger after DA depletion.

Switching of SBP neurons

After DA depletion, the size of SBP and G clusters decreased, more body parts were represented per unit volume, and the prevalence of MBP neurons increased 16-fold. The only parsimonious explanation of these findings is that some striatal SBP and G neurons underwent a change in responsiveness, which, by definition switched the neuron to a different functional category or subcategory, placing it outside the retracted boundary of its former cluster. The most likely categories to which SBP and G neurons switched are those that increased in prevalence at the borders of SBP and G neuron clusters in the 6-OHDA group: solitary SBP neurons and MBP neurons. Switching did not appear to involve loss of responsiveness or activity, because there were no changes in the prevalence per 3-mm electrode track was not significantly different between control and 6-OHDA groups (d). a: Mean length of clusters (measured in 50-μm increments). b: y-axis = % of total clusters of each length, in 50 μm increments. Statistical test was Mann-Whitney rank sum test (a), or t-test (c); asterisks, P < 0.05. Error bars: ± SEM.

Fig. 4. Summary of differences between control and 6-OHDA groups in clustering of neurons related to a single body part (SBP). 6-OHDA group exhibited significantly (a) smaller mean cluster length, (b) smaller clusters of every length, and (c) lower mean percentage of neurons contained in clusters. Note increased percentage of solitary SBP neurons in 6-OHDA group (b). Mean number of clusters
ence of either U neurons or Spaces lacking neural activity. The similar prevalence of Spaces at cluster borders and throughout the striatum further suggests that reduced clustering did not involve spatial shifting of neurons into denser, smaller clusters (which would create Spaces), or alterations in "silent" neurons.

Our findings can be accounted for by two mechanisms: some SBP neurons’ responsiveness switched from one body part 1) to multiple body parts (above); or 2) to a different single body part. The switching of SBP to MBP neurons alone cannot account for the disintegration of clustering of SBP neurons. The majority of SBP clusters were not bordered by MBP neurons and yet exhibited a significant reduction in size. A telling statistic was the increase in the occurrence of solitary SBP neurons. Their overall prevalence doubled in the 6-OHDA group, and they were nearly twice as numerous as MBP neurons. Solitary SBP neurons, along with MBP neurons, showed a significant increase at the borders of SBP and G neuron clusters, the most likely site of switching after DA depletion. Thus, the new evidence provided here is that SBP neurons switched not only to MBP neurons, but more frequently, from a given SBP to a different SBP. Such a switch would be undetectable by single-unit recording without detailed analyses of electrode track profiles.

Possible neural mechanisms
Following DA depletion and the loss of spines on MSNs (McNeill et al., 1988; Ingham et al., 1993, Ingham et al., 1998; Meredith et al., 1995; Nitsch and Riesenberg, 1995; Meshul et al., 1999), changes in striatal responsiveness, most likely to corticostriatal input from MI and SI (Liles and Updyke, 1985; Flaherty and Graybiel, 1993), could involve sprouting of corticostriatal axons (Xu et al., 1989; Jones and Harris, 1995; Napieralsky et al., 1996; Cheng et al., 1998; Ingham et al., 1998; Meshul et al., 1999) to form perforated, asymmetric synapses of substantial efficacy on the shafts of homologous MSNs (Cotman et al., 1981) at the edges of adjacent striatal clusters, causing the newly innervated MSN to respond to stimulation of a different body part. The present changes could also reflect 1) unmasking of existing corticostriatal inputs (Merzenich et al., 1984; Florence and Kaas, 1995; Capozzo et al., 1997); 2) receptor supersensitivity (Bjelke et al., 1994); 3) altered coupling among clusters of spiny striatal neurons (Cepeda et al., 1989; Onn and Grace, 1999); 4) alterations in afferents from intralaminar thalamic nuclei (Schneider and Rothblat, 1996), which could account for decreased specificity of MBP neurons (Matsumoto et al., 2001); or 5) altered activity of striatal interneurons (Girault et al.,...
would ing in the basal ganglia (e.g., a forelimb neuron switching to and motor neurons would change connections. Thus, switches at the striatal level are not accompanied by comparably severe changes in the pallidum (Filion et al., 1988; Tremblay et al., 1995; Sathian et al., 1997). For example, the body parts overlap partially in rats (Fig. 2; cf Cho and West, 1997), monkeys (Liles, 1975; Flaherty and Graybiel, 1993) and humans (Maillard et al., 2000). Overlapping distributions, aside from their normal significance (Brown, 1992; Brown et al., 1998; Alloway et al., 1999), may provide juxtapositions of afferents that enabled the present switching between different body parts following DA depletion. For every "switched" striatal neuron, adjustments to the "new" body part(s) may occur in the pallidum (Filion et al., 1988; Tremblay et al., 1988; Rothblat and Schneider, 1995; Ingham et al., 1997) but VA and VL thalamic neurons of DA-depleted cats did not exhibit loss of specificity, suggesting that switches at the striatal level are not accompanied by comparable switches downstream from the basal ganglia (Voloshin et al., 1991, Voloshin et al., 1993; Schneider and Rothblat, 1996). Further downstream, it is not likely that premotor and motor neurons would change connections. Thus, switching in the basal ganglia (e.g., a forelimb neuron switching to a different part of the forelimb or to the neck or hindlimb) would "switch the wires" and send confounded signals to premotor and motor areas, with negative consequences for behaviors involving basal ganglia function (Marshall et al., 1971; Flowers, 1975; Siegfried and Bures, 1980; Sabol et al., 1985; Fairley and Marshall, 1986; Schneider et al., 1987, Schneider et al., 1992; Rothblat and Schneider, 1993; Miyashita et al., 1995; Sathian et al., 1997). For example, the collective output of the reorganized striatum may falsely signal stimulation of unstimulated or even widespread areas, resulting in failure to orient specifically to a stimulus.

In addition to the fact that DA depletion is known to induce sprouting of corticostriatal terminals, another line of evidence favors sprouting of corticostriatal terminals as the mechanism of the reorganization of connections shown here. Parkinson’s disease patients medicated with L-DOPA were not impaired in movements that were self-initiated or that were made in response to verbal commands, but their performance of somatosensory and proprioceptively guided arm movements was impaired regardless of whether they were currently medicated (Schneider et al., 1986, Schneider et al., 1987). In rats, L-DOPA administration failed to improve 6-OHDA lesion-induced deficits in skilled forelimb usage (Lindner et al., 1997). In parkinsonian patients and animal models, it is important to distinguish between gross motor function and complex sensorimotor behavior (Marsden, 1982; Lidicky et al., 1985; Schneider et al., 1986, Schneider et al., 1987, Schneider et al., 1998; Markham, 1987). The failure of L-DOPA to restore complex sensorimotor behavior could involve the inability of the drug to reverse the effects of reorganized, sprouted corticostriatal connections, underlining the importance of developing preventive strategies against parkinsonism (Brooks, 1993).

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LITERATURE CITED


