Effects of Dopamine Depletion on the Morphology of Medium Spiny Neurons in the Shell and Core of the Rat Nucleus Accumbens

G. E. Meredith,¹ P. Ypma,¹ and D. S. Zahm²

¹Department of Anatomy and Embryology, Faculty of Medicine, Vrije University, Amsterdam, The Netherlands and

²Department of Anatomy and Neurobiology, St. Louis University School of Medicine, St. Louis, Missouri 63104

Nucleus accumbens receives a dense dopaminergic innervation which is important in regulating motivated states of behavior such as goal-directed actions, stimulus–reward associations and reinforcement of addictive substances. The shell and core territories of this nucleus each receive functionally and morphologically distinct dopaminergic inputs and lesions of the ascending pathways totally deprive the core but not the shell of dopaminergic fibers. Medium spiny neurons are the principal targets of dopaminergic terminals. The present study explored whether the loss of dopamine inputs can affect these neurons and whether cells in the shell and core would be equally susceptible to such a loss. Intracellular injection in fixed slices and neuronal reconstruction were used to analyze the dendritic trees of 62 neurons in the shell and core of animals that received a unilateral, chronic 6-hydroxydopamine lesion of the medial forebrain bundle. In the dopamine-depleted core, dendrites are significantly shorter (16% decrease) than in the intact core and in both the dopamine-depleted core and lateral shell, dendrites are less spiny than in respective control regions. Dopamine loss in the medial shell is associated with significantly more tortuous dendrites that are lower in spine density. However, the number of spines is not reduced which may mean that the increase recorded for segment length, although insignificant in tests, could be responsible for the change in spine density. These data suggest that the loss of dopamine can affect accumbal neuronal morphology and, moreover, can affect neuronal structures differentially in the shell and core.

[Key words: 6-OHDA, Lucifer yellow, ventral striatum, dopamine, spine, morphology, core, shell]

The ventral striatum, comprising the nucleus accumbens, ventral parts of the caudate-putamen, striatal parts of the olfactory tubercle and cell bridges interconnecting these structures (Heimer et al., 1985), is densely innervated by dopamine (DA)-contain-

ing fibers (Andn et al., 1966; Beckstead et al., 1979; Voorn et al., 1986). The nucleus accumbens has a morphologically and neurochemically distinct shell which receives DA fibers primarily from mesolimbic neurons in the ventral tegmental area and retrorubral field (Deutch et al., 1988), and a core which is innervated by mesostriatal fibers from the substantia nigra pars compacta as well as by mesolimbic fibers (Nauta and Domesick, 1984; Groenewegen et al., 1991). Dopaminergic axon terminals form predominantly axodendritic terminals in the shell but approximately equivalent numbers of axodendritic and axospinous synapses in the core (Zahm, 1992). In a manner consistent with the morphological data, DA differentially modulates the synaptic responses of shell and core neurons (Pennartz et al., 1992b) and DA receptor antagonism is accompanied by distinct changes in the expression of *c-fos* and Fos-related antigens in the shell and core (Deutch et al., 1992; Dilts et al., 1993). Finally, DA receptor agonists and antagonists alter neuronal dye coupling, differentially in the shell and core (O'Donnell and Grace, 1993).

Neurons in the core project to the dorsolateral part of the ventral pallidum, the subthalamic nucleus and the substantia nigra pars compacta (Groenewegen and Berendse, 1990; Zahm and Heimer, 1990; Heimer et al., 1991; Zahm and Heimer, 1993; Groenewegen et al., 1994; but see Denlau et al., 1994). Cells in the shell project to the neurochemically distinct ventromedial part of the ventral pallidum (Zahm and Heimer, 1990), extended amygdala (Alheid and Heimer, 1988), lateral hypothalamus, and mesencephalic tegmentum (Groenewegen and Russchen, 1984; Heimer et al., 1991; Zahm and Heimer, 1993). Considering the distinct nature of these efferent pathways, activation of shell and core output neurons would be expected to produce different effects, both physiologically and behaviorally. Accordingly, a behavioral dissociation between these regions has recently been shown (Maldonado-Irizarry and Kelley, 1993).

The projection neurons of nucleus accumbens are medium in size and have spiny dendrites. Medium spiny neurons in the shell and particularly in its medial part, have fewer primary dendrites with fewer dendritic segments and lower spine densities than those in the core (Meredith et al., 1992). Insofar as basal and stimulated DA utilization differs between the core and shell (Deutch and Cameron, 1991), DA may contribute to the maintenance of these morphological differences. Dopamine depletion alters neuronal structure in the dorsal striatum (Ingham et al., 1989, 1991) and, presumably, in the ventral striatum as well, although this remains to be demonstrated experimentally. The present report describes experiments to test whether ventral striatal neuronal morphology is altered by DA depletion and whether any changes are expressed differently in the core and shell.

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Correspondence should be addressed to Dr. G. E. Meredith, Department of Anatomy, Royal College of Surgeons in Ireland, 123 St. Stephen's Green, Dublin 2, Ireland.

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Materials and Methods

Thirty-two adult, male Wistar rats (Harlan-Centraal Proefdierbedrijf, Zeist, The Netherlands), weighing 250–325 gm were used in the present study. Sixty minutes after having received an intraperitoneal injection of desmethylinipramine (25 mg/kg in physiological saline, RBI, USA), the animals were deeply anesthetized with a 4:3 mixture of Aescoket (1% ketaminum-HCl) and Rompun (2% xylazine-HCl; both 1 ml/kg, i.m.). Each animal was placed in a stereotaxic frame, the skull was opened and 2.5 μ l of a 6-hydroxydopamine (6-OHDA) solution (4 μ g/ μ l of the bromide in distilled water with 0.04% ascorbic acid) was injected unilaterally in the medial forebrain bundle (MFB) over a 15 min period. The needle of the Hamilton syringe was left in place for an additional 15 min before being removed. Having recovered from anesthesia, the animals were returned to their cages where they were given rat chow and water ad lib. From 9 to 13 d after the lesions were made, rats were injected intraperitoneally with apomorphine (0.25 mg/kg, Sigma) in distilled water. After 30 min, the number and direction of rotations in a large circular bowl were recorded. Animals turning away from the lesion more than 200 times during a 45 min test period are reported to have a striatal DA depletion of more than 90% (Hefti et al., 1980). Rats that met this criterion were kept for further work.

Twenty-one days after the lesions were made, the rats were reanesthetized with an overdose of sodium pentobarbital (60 mg/kg i.p.) and perfused transcardially with Ringer's solution, followed immediately by 500 ml of a solution containing 4% paraformaldehyde, 15% saturated picric acid and 0.05% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) at room temperature during a period of 15 min or less. Following removal of each brain, the forebrain and midbrain were blocked and transverse, 300 μ m thick slices of the rostral forebrain were cut alternately with 50 μ m sections with a Vibratome. In addition, 60 μ m sections were cut transversely through the midbrain. Both forebrain and midbrain sections were subjected to an immunohistochemical protocol with antibodies directed against tyrosine hydroxylase, as described below.

Intracellular injection. The injection procedure is described elsewhere (Meredith and Arbuthnott, 1993). Briefly, the slices were placed for 1–3 min in a 10^{-7} M solution of 4,6 diamidino-2-phenylindole (DAPI; Sigma) in 0.1 M phosphate buffer which served as a counterstain for neuronal nuclei. Numerous neurons containing the fluorescent marker DAPI, were impaled in intact and DA-depleted nucleus accumbens and injected with a 4% solution of Lucifer yellow (LY) made up in distilled water (electrode resistance, 80–250 M Ω). Slices were then placed in a solution of 20% dimethyl sulfoxide in 0.1 M phosphate buffer, pH 7.4, at 4°C and cut transversely at 40 μ m on a freezing microtome the following day.

Immunohistochemistry. The sections cut from the slices were immunoreacted with antibodies directed against LY. The incubation medium consisted of gelatin (0.25%) and TBS-Tx (0.5% Triton X-100 in 0.05 M Tris-buffered saline; 0.9% NaCl); sections were rinsed between incubations in 0.05 M Tris-HCl buffer, pH 7.6. Sections were incubated in rabbit anti-LY (kindly given by Dr. Bastiani, University of Utah, Salt Lake City, UT; Taghert et al., 1982) diluted 1:10,000 to 12,000, overnight at 4°C, followed by swine anti-rabbit IgG, 1:150, for 45 min at room temperature and, thereafter, rabbit peroxidase-antiperoxidase complex (PAP), diluted 1:150, for 45 min at room temperature. The sections were then reacted for approximately 20 min in 0.05% 3,3'-diaminobenzidine (DAB) with 1% ammonium nickel sulphate in 0.05 M Tris-HCL buffer and 0.01% H₂O₂. In order to stain the spines of neurons maximally, sections were reincubated in swine anti-rabbit IgG followed by rabbit PAP and reacted for 10–15 min in the DAB solution without ammonium nickel sulfate for 15 min or until the dendrites and spines of the injected cells appeared blue-black. The sections were mounted onto glass slides in serial order from 0.2% buffered gelatin solution, dehydrated, and coverslipped.

Sections from the forebrain and midbrain were incubated for 30 min in 10% normal goat serum in TBS at room temperature and then in mouse anti-tyrosine hydroxylase (Incstar, USA), 1:2000, at 4°C for 24–48 hr followed by an overnight incubation in goat anti-mouse IgG, 1:50, and thereafter, another 24 hr incubation in monoclonal rat PAP, 1:100 (Sternberger-Meyer, USA). A DAB reaction followed for 10–15 min.

Analysis. Intracellularly injected, LY-immunoreacted neurons were selected for analysis if they fulfilled the following criteria: (1) they were medium spiny neurons and their dendritic processes were filled completely with LY, (2) they could be assigned unambiguously to the shell or core, and (3) during injection, LY did not spread into neighboring

neurons or processes. The territorial assignment of neurons was accomplished by relating the location of injected cells to shell and core borders which were apparent in tyrosine hydroxylase-immunoreacted sections from the intact hemisphere. Since sections from the intact side could not be matched precisely to those from the lesioned hemisphere, care was taken to select neurons well within territorial borders. Cells from the rostral pole were not analyzed.

Approximately 400 cells were injected in both the DA-depleted and intact sides of the nucleus. Seventy-three neurons were reconstructed, and 62 of those were analyzed morphometrically. Using a light microscope equipped with a 100 $\times$ oil-immersion objective and coupled to hardware and a software package dedicated to neuron reconstruction (Neuron Tracing System, Eutectic Electronics Inc., Raleigh, NC), each neuron was digitized in x-, y-, and z-coordinates. Primary dendrites were merged with the cell soma and each dendritic tree was reassembled by matching the truncated ends of dendrites in adjacent sections and merging them. Spines were recorded as points and accurately represented in the reconstruction drawings. Segment length was measured on line by the computer and spine density and dendritic tortuosity were calculated as functions of segment length.

Comparisons of neurons within and between territories were made but because cells near the surfaces of the slices were invariably injected, dendrites that would have extended above the slice surface were truncated during slice preparation. Consequently, this permitted the analysis of some but never all dendrites of any given neuron. Thus, morphometric parameters were evaluated using pooled dendritic segments, a segment being defined as the length between the perikaryon and the first bifurcation, between two branch points or between the most distal bifurcation and the end of the dendrite.

The length (in micrometers), tortuosity (segment length/radial distance between proximal and distal ends of a segment) and the density (spines/ μ m) and number of spines were recorded for each segment. Since dense immunoreaction product frequently accumulated around cell bodies (see, e.g., Figs. 3C, 4A), perikaryal measurements were considered unreliable and were not included in the analysis. Consecutive branch orders 1°, 2°, 3°, etc. were designated, respectively, as the dendritic segments between the soma and the first, the first and the second, the second and the third, etc. bifurcations for each dendritic arbor of a neuron.

Differences between accumbal regions on the control side and between intact and DA-depleted hemispheres were analyzed statistically. Pooled values were examined with a Kolmogorov-Smirnov test to determine if they were distributed normally and normally distributed data were tested with the Student's *t* test for independent measures. Other data were assessed with the Mann-Whitney *U* test. Neuronal differences within each accumbal territory were assessed with a Kruskal-Wallis one-way analysis of variance. Medians and ranges and, where valid, means $\pm$ standard deviations were computed for pooled segments from each territory.

Results

Evaluation of lesions

Necrosis near the midline in the caudal diencephalon was evident in transverse sections through the lesion site and tyrosine hydroxylase-positive fibers appeared to be absent from the ipsilateral MFB. The accumbal core was almost totally depleted of tyrosine hydroxylase immunoreactivity ipsilateral to the lesion (compare sides of Fig. 1A) as was the shell, except for its medial part where some immunoreactive fibers typically remained (Fig. 1B).

General morphology of intracellularly injected neurons

Generally, spiny neurons were injected although a small number of aspiny cells and an occasional glial cell were also filled. Only spiny neurons from middle to caudal levels of nucleus accumbens were selected for analysis (Fig. 2). Filled neurons in the rostral pole were rejected inasmuch as core and shell are not discernible rostrally (Deutch and Cameron, 1991; Zahm and Brog, 1992).

Photomicrographs and digitized reconstructions of filled neurons from DA-depleted and intact sides of the core and shell are

Figure 1. *A*, Photomicrograph of tyrosine hydroxylase immunoreactivity in a transverse section through the brain of a rat that received a unilateral injection of 6-OHDA in the medial forebrain bundle 21 days earlier. Note the near complete absence of tyrosine hydroxylase immunoreactivity on one side. *B*, Higher magnification of a portion of the medial accumbal shell in the DA-depleted hemisphere. Note that remaining tyrosine hydroxylase-immunoreactive fibers and varicosities are visible (*open arrowhead*). The *star* (*B*) marks the location of the anterior commissure. Scale bars: *A*, 500 μ m; *B*, 100 μ m.

illustrated in Figures 3 and 4, respectively. The cell bodies of neurons in the DA-depleted and control sides of the brain appear round to oval in shape (Figs. 3, 4). Each primary dendrite gives rise to a tree consisting of as many as nine branch orders in the core and eight in the shell. Dendrites are aspiny initially and spines appear after 5–20 μ m, typically at the first branch point

(Fig. 3*A–D*). Individual spines vary in length and shape (Fig. 5). Axons were never filled beyond approximately 100 μ m.

The dendrites of neurons in the DA-depleted hemisphere appear to be more tortuous, especially at their distal tips, than their counterparts in the intact side (Fig. 6). Moreover, the density of spines seems to vary between neurons, between dendrites of the

Figure 2. Diagrams showing the position of neurons filled with LY (solid circles) in sections through mid- to caudal levels of the nucleus accumbens. Core, lateral shell (LS) and medial shell (MS) are marked and A shows cells injected in the control side; B shows neurons filled in the DA-depleted hemisphere.

same neuron, and even between different parts of the same dendritic segment (Fig. 5C). These features are particularly pronounced in the DA-depleted hemisphere. Occasionally, dendrites in the DA-depleted side appeared to end in a cone-like process (Fig. 5D).

Quantitative analysis of reconstructed neurons

Intraterritorial comparisons. There is considerable variability among neurons in any one territory. In the intact hemisphere, cells in the medial and lateral shell and in the core differ significantly (Kruskal-Wallis ANOVA) for measures of tortuosity ($p = 0.008$, $p < 0.0001$, $p < 0.0001$, respectively) and spine density ($p = 0.001$, $p = 0.02$, $p < 0.0001$, respectively).

In the DA-depleted hemisphere, core neurons differ in the length ($p = 0.001$) and tortuosity ($p < 0.0001$) of their segments and in the density ($p < 0.0001$) and number of spines ($p = 0.001$). Medial and lateral shell cells differ in spine density ($p < 0.0001$ and $p = 0.003$, respectively) and tortuosity of their segments ($p < 0.0001$, $p = 0.002$, respectively). Such great variability between neurons within each region indicates that comparisons between intact and DA-depleted territories would be difficult to interpret using data for whole cells. Consequently, lesion effects were tested with values derived from pooled dendritic segments from each territory.

Interterritorial comparisons within the intact hemisphere. Comparisons of pooled segments in the intact hemisphere revealed differences between the core, medial shell and lateral shell in all parameters except dendritic segment length (Table 1). Dendritic segments in the entire shell are significantly less tortuous ($p < 0.01$, Mann-Whitney *U*) with fewer spines (density, $p < 0.05$; number, $p < 0.05$) than those in the core. When the shell is divided into medial and lateral subterritories and these are assessed separately, the medial shell segments are significantly less tortuous ($p < 0.01$) and have lower numbers (p

< 0.05) and densities ($p < 0.01$) of spines than those in the core; they also have significantly lower numbers ($p < 0.05$) and densities ($p < 0.01$) of spines than segments in the lateral shell. In addition, segments in the lateral shell are significantly less tortuous ($p < 0.01$) than those in the core but do not differ from the latter in number and density of spines.

Comparisons between intact and DA-depleted hemispheres. The loss of DA in the core is accompanied by decreases in the density and numbers of spines but not by changes in the length and tortuosity of segments (Table 1). However, terminal dendritic segments in the core are significantly shorter with DA depletion ($p = 0.009$, Mann-Whitney *U*) and the segments belonging to the first three branch orders are significantly more tortuous (Fig. 6A) on the DA-depleted side as compared to the intact side ($p = 0.02$, Mann-Whitney *U*).

If the shell is considered in its entirety, the loss of DA is accompanied by a significant reduction in spine density and a significant increase in tortuosity for all segments and for all branch orders, but not by a change in the length of dendritic segments or in the numbers of spines (Table 1). However, when the shell is divided into medial and lateral parts and each is tested separately, a significant reduction in the density and numbers of spines is associated with DA-depletion in the lateral shell (compare Fig. 5C,D), but there is no change in the length and tortuosity of segments (Table 1). In the medial shell, only the tortuosity of segments (Fig. 6B) is significantly different, that is, higher with the lesion (Table 1) but it is noteworthy that even though the difference between segment length in the intact and DA-depleted medial shell is not significant ($p = 0.1$, Mann-Whitney *U*), the median value for length is considerably greater on the DA-depleted side (Table 1).

The ranges of values for dendritic features in the DA-depleted hemisphere are always greater than those for the same features on the intact side (Table 1) which suggests that some segments

Figure 3. Photomicrographs of two LY-immunoreacted core neurons (*A*, *C*) found in the first section of a slice and the full reconstructions of those neurons (*B*, *D*). *A* and *B* are from the intact hemisphere and *C* and *D* from the DA-depleted core. Arrows point to the proximal aspiny segments of these cells. Note that spines begin at or beyond the first branch point. A star marks a filled cell with filamentous dendrites. Scale bars, 25 μ m.

may be more affected than others. When spine density is plotted against segment length for all regions (Fig. 7*A–F*), there is great scatter in density values for segments less than 50 μ m in length. Furthermore, spine densities are normally distributed for segments longer than 50 μ m (not significant, Kolmogorov-Smirnov

test). Statistical analysis revealed that core segments longer than 50 μ m do not differ in length and tortuosity with DA depletion but are significantly reduced in mean spine density ($t = 4.3$, $df = 396$, $p < 0.0001$) and in the numbers of spines ($p < 0.0001$, Mann-Whitney U). Mean spine density for segments longer than

Figure 4. Photomicrographs of LY-immunoreacted medial shell neurons (*A*, *C*) found in the first section of a slice and the full reconstructions of those neurons (*B*, *D*). *A* and *B* are from the intact hemisphere and *C* and *D* from the DA-depleted side. Arrows in *D* point out the tortuous course of some segments in the DA-depleted hemisphere. Scale bars, 25 μ m.

Figure 5. High magnification photomicrographs of LY-immunoreacted dendritic segments in the intact (*A*) and DA-depleted (*B*) medial shell. Arrowheads point out the different shapes and sizes of spines. Segments pictured in *C* and *D* are derived from neurons in the intact and DA-depleted lateral shell, respectively. Arrowheads in *C* point out the asymmetry in spine distribution along the segment, and arrowhead in *D* points to cone-like termination of the dendrite. Scale bar in *A* is valid for *B* and equals 10 μm , and scale bar in *C* is valid for *D* and equals 10 μm .

50 μm is decreased significantly in the DA-depleted side not only in the core and lateral shell ($t = 5.41$, $df = 225$, $p < 0.0001$) but also in the medial shell ($t = 2.51$, $df = 207$, $p < 0.01$). After DA-depletion, segments greater than 50 μm in length have reduced numbers of spines in the lateral ($p < 0.05$, Mann-Whitney *U*) but not the medial shell and segments are significantly more tortuous in the medial shell ($p = 0.001$, Mann-Whitney *U*).

The statistical tests (see above and Table 1) include values for aspiny proximal dendritic segments as well as for some segments that have been artificially truncated in the preparation of the slice. In order to limit the contribution of such presumably invalid measurements to the statistical analysis of length, the data were edited in the following manner. Segments with spine densities outside 2 standard deviations, as calculated for segments greater than 50 μm in length, were removed from each group. Statistical comparisons of these filtered samples revealed that in the core, dendritic segments are 16% shorter on the DA-depleted side (median length = 48) as compared to the intact side (median length = 55) and the decrease is significant ($p = 0.038$, Mann-Whitney *U*). Further in the core, the density ($t = 7.46$, $df = 737$, $p < 0.0001$) and numbers ($p < 0.0001$, Mann-Whitney *U*) of spines are significantly reduced in the DA-depleted side. In the lateral shell, tests with the edited data revealed that segments do not change in length but decrease significantly

in the density ($t = 7.26$, $df = 411$, $p < 0.0001$) and number ($p < 0.0001$, Mann-Whitney *U*) of spines after DA depletion. Edited data in the medial shell reveal that the median length of segments on the DA-depleted side (median length = 59) is 23% greater than that on the intact side (median length = 48); however, this difference is not significant ($p = 0.12$, Mann-Whitney *U*). Nevertheless, spine density decreases significantly ($t = 3.36$, $df = 375$, $p < 0.001$) in the DA-depleted as compared to the intact medial shell, while the number of spines per segment remains unchanged.

Discussion

Dendrites and their spines are the principal targets for synaptic input to striatal neurons (Wilson et al., 1983; Bolam, 1984). Accordingly, any remodeling of these structures in response to deafferentation or neurotransmitter deficits would be expected to compromise the functional integrity of striatal circuits. Indeed, changes in dendritic morphology could contribute, at least in part, to cognitive and motor deficits observed with certain neurodegenerative disorders as well as to the effects, both efficacious and undesirable, of neuroleptic drug treatment. The data presented in this report show that accumbal spiny neurons undergo morphological change following a lesion of the striatal dopaminergic innervation and that neurons in the shell and core are differentially affected by dopamine depletion.

Figure 6. Reconstructions of LY-immunoreacted neurons in the DA-depleted core (A) and medial shell (B). Arrows point to recurved and twisted parts of some dendritic segments. Note that the tortuous segments in (A) in the second or third branch order.

A midbrain lesion with 6-OHDA effectively destroys dopaminergic axons in the MFB. Desmethylinipramine injected shortly before the toxin may reduce noradrenergic depletion (Breese and Traylor, 1971) but probably does not prevent degeneration of all noradrenergic fibers nor spare the serotonergic pathway. Furthermore, various types of fibers may be

damaged at the injection site. Therefore, the morphological changes presented here cannot be linked incontrovertibly to dopamine and may be associated with the loss of other transmitter systems. Nevertheless, since unilateral, nigral lesions with ibotenic acid produce a pattern of striatal tyrosine hydroxylase depletion resembling that with nigral 6-OHDA le-

Table 1. Median values and (range) of the length, tortuosity, spine density and numbers of spines for dendritic segments in the lesioned and intact shell and core

Territory	<i>n</i> ^a	Length (μm)	Tortuosity ratio ^b	Spine density spines/10 μm	Spines per segment
Accumbens, total					
Intact	1103	37 (1–310)	1.48 (1–3.84)	10.3 (0–34.1)	38 (0–359)
Lesioned	1085	33 (1–326)	1.54*** (1–5.35)	8.7*** (0–31.3)	32 (0–414)
Core					
Intact	485	39 (1–258)	1.52 (1–3.84)	11.1 (0–29.7)	50 (0–359)
Lesioned	528	31 (1–254)	1.54 (1–5.35)	9.0*** (0–31.3)	39** (0–414)
Shell, total					
Intact	618	36 (1–310)	1.44 (1–3.14)	9.7 (0–34.1)	34 (0–344)
Lesioned	557	34 (1–326)	1.54** (1–4.3)	8.4*** (0–24.0)	32 (0–262)
Medial shell					
Intact	334	35 (1–310)	1.44 (1–3.14)	8.8 (0–20.9)	28 (0–344)
Lesioned	236	43 (1–326)	1.65*** (1–4.3)	8.4 (0–24.0)	37 (0–262)
Lateral shell					
Intact	284	38 (1–209)	1.44 (1–2.73)	10.8 (0–34.1)	44 (0–292)
Lesioned	321	29 (1–242)	1.46 (1–4.15)	8.6*** (0–20)	25* (0–238)

^a*n*, Number of dendritic segments pooled for analysis.

^bTortuosity is defined as a ratio: segment length/radial distance from start to end of a segment. *, *p* < 0.05; **, *p* < 0.01; ***, *p* < 0.001; differ with respect to control values (Mann-Whitney *U*).

sions (Zahm, 1991), the structural changes could involve the loss of dopamine.

Data analysis: critique and interpretations

The normal structural variability of medium spiny neurons in nucleus accumbens is great (Bayer, 1981; Rueda et al., 1986; Meredith et al., 1992 and present results). For many parameters, differences between DA-depleted and intact hemispheres could be demonstrated with values derived from individual cells (unpublished observations) but since all parameters were measured at the level of the dendritic segment and since the greater sample sizes generated by pooled segments were required to reveal significant differences in certain features that otherwise would have gone unobserved, the dendritic segment was chosen as the optimal unit of comparison (see also, Meredith et al., 1992).

Straightforward quantitative analysis of the present data was confounded by several features of medium spiny neurons and by the methods used in their preparation. Insofar as spines typically appear at or near the first branch point (Wilson et al., 1983), the most proximal segments are aspiny or have very low spine densities. Furthermore, as most filled cells lie near the surface of the slice, their superficial dendrites are often severed (Arts and Groenewegen, 1992). To simply eliminate truncated segments from the analysis is not possible because it is rarely

clear whether segments near the surface terminate naturally or artificially. To reject segments due to their proximity to the slice surface would potentially eliminate a considerable number of valid data points. Consequently, other criteria were applied in an effort to remove probable invalid segments.

As seen in Figure 7, the scatter of spine densities for segments shorter than 50 μm is large and presumably reflects the presence of truncated and aspiny segments. Rapid and dramatic changes (increases) in spine density occur along the proximal 60 μm of a dendrite (Wilson et al., 1983) and frequently, spines are unevenly distributed on segments, especially in the DA-depleted hemisphere (present results). Truncation of a dendrite may therefore produce an abnormally high or low index of segment spine density depending on where the dendrite has been severed. Such peaks and valleys in spine density typically average out for segments longer than 50 μm. Moreover, there is no reason to expect that spine-laden and aspiny segments respond similarly to DA loss. Consequently, segments were accepted only if their spine density fell within 2 SDs of the group mean as calculated for segments longer than 50 μm. By treating the data in this way, the probability of retaining valid segments and rejecting invalid outliers was maximized.

Region-specific structural changes following DA depletion

Morphological changes were evident in the core, where a near total loss of DA was associated with shortened dendrites and spine loss, and in the lateral shell, where DA depletion was associated with fewer spines. However, in the medial shell, where, coincidentally, the loss of tyrosine hydroxylase-immunoreactive fibers was substantial but never complete, higher dendritic tortuosities and lower spine densities were apparent in comparison to controls. Since the significant decline in density was not accompanied by a loss of spines, dendritic lengthening, although insignificant in tests, could account for the decline.

Lesions of the MFB with 6-OHDA ultimately cause considerable loss of DA-containing axon terminals in the entire striatal complex. The loss of these terminals, however, seems to occur more rapidly in some parts of the striatum than in others during the first 2 weeks postlesion (Zahm, 1991). Indeed, DA axons in the shell, and especially its medial part, seem to degenerate less rapidly and less completely (present data) than do those in the core (Zahm, 1991). Furthermore, the integrity of the crossed DA projection (Douglas et al., 1987; Stein et al., 1992) may also be responsible for the residual fibers. Nevertheless, changes in shell and core dendrites presumably reflect the differential distribution of DA inputs (Zahm, 1992) or may be related to extracellular DA levels. Microdialysis experiments have shown that DA depletion must exceed 80% before diminished extracellular DA can be measured (Stachowiak et al., 1987; Robinson and Whislaw, 1988) which suggests that following a partial lesion, increased release and/or diminished uptake by residual terminals compensates for the loss caused by the lesion (Zigmond et al., 1990). Thus, the mitigating influence of remaining, intact DA terminals may be sufficient to preserve spines and bring about increases in dendritic tortuosity in the medial shell but not in the core or lateral shell. However, the available evidence is probably insufficient to conclude that residual DA stimulates dendritic growth since striatal dendrites are reported to atrophy in Parkinsonian patients treated with L-dopa (McNeill et al., 1988) and DA added to cultures causes neurite retraction (Lankford et al., 1988).

Dendritic growth occurs in the striatum of aging mice (Rafols

Figure 7. Scatterplots generated using original pooled data from the intact and DA-depleted medial shell (A, B), core (C, D) and lateral shell (E, F). Segment length is represented on the abscissa and spine density on the ordinate.

et al., 1989; McNeill et al., 1990), in hippocampal neurons of aged humans (Buell and Coleman, 1981; Flood et al., 1985, 1987), and in striatal cells in moderate grade Huntington's disease (Graveland et al., 1985; Ferrante et al., 1991). In each of these instances, however, the effects are transient and dendritic proliferation is followed by degeneration. Deafferented neurons may initially attempt to compensate with dendritic growth but probably independent of the actions of DA. Thus, it is unclear whether the changes observed in the shell reflect the time course, extent of lesion or a response exhibited exclusively by those

neurons. In this regard, there is some evidence that core and medial shell cells comprise distinct populations, the former being purely striatal in nature and the latter having neuronal phenotypes belonging instead to the extended amygdala (Alheid and Heimer, 1988; Heimer and Alheid, 1991).

Potential mechanisms underlying dendritic remodeling

Little is known about the mechanisms involved in neurotransmitter-induced alterations of dendritic structure. Certainly, any major deafferentation can induce dendritic atrophy (Kemp and

Powell, 1971; Lund, 1978; Caceres and Steward, 1983; Deitch and Rubel, 1984). Catecholamines can influence the growth of neurites (Haydon et al., 1984; Lankford et al., 1987, 1988; Davenport et al., 1993), presumably through (de)activation of second messenger systems that open calcium $[Ca^{2+}]$ channels (Doroshenko et al., 1982). Even though a rise in intracellular $[Ca^{2+}]$ has been implicated in neurite outgrowth (Cohan et al., 1987; Mattson and Kater, 1987), substantial increases cause neurite retraction (Mattson, 1988; Choi, 1988) and are toxic to dendrites *in vivo* (Meyer, 1989). Dopamine-depleting lesions of nucleus accumbens are associated with great increases in cAMP (Tassin et al., 1982) and, in the core, cause an upregulation of DA receptors (Jongen-Rêlo et al., 1994) that stimulate adenylyl cyclase activity (Stoof and Keibarian, 1984). Even though $[Ca^{2+}]$ buffers quickly return intracellular $[Ca^{2+}]$ levels to baseline (Guthrie et al., 1991), spines may succumb to excitotoxicity. Dopamine depletion is associated with spine loss in the accumbal core and lateral shell (present study) and in the dorsal striatum (Ingham et al., 1989). Corticostriatal, presumably glutamatergic, terminals contact the heads of spines that often receive DA synapses on their necks (Totterdell and Smith, 1989; Meredith and Wouterlood, 1990; Sesack and Pickel, 1990). Since the DA input appears to modulate the corticostriatal input (Brown and Arbuthnott, 1983; Pennartz et al., 1992a), its removal may produce excitotoxic conditions that result in spine loss. Certainly, small increases of glutamate underlie spine swelling and loss in hippocampal slices (Siman and Card, 1988).

Functional implications of structural changes in shell and core neurons

Recognition of structural change after a lesion requires that proper controls have been employed. For neurons in the intact hemisphere to serve as valid controls, they should not differ morphologically from those in unlesioned animals. The results of interterritorial comparisons made within the intact nucleus accumbens (present study) compare favorably with those published earlier for unlesioned animals (Meredith et al., 1992), in that present measures of dendritic segment length are similar in the accumbal territories, while spine density differs significantly not only between shell and core but also between medial and lateral shell and medial shell and core.

The present data and those of others (Deutch and Cameron, 1991; Pennartz et al., 1992b; O'Donnell and Grace, 1993) suggest that shell and core circuits should respond differently to alterations in DA neurotransmission. Atypical neuroleptic blockade of DA receptors increase Fos expression differentially in the shell and core (Deutch et al., 1992) and mild restraint stress selectively increases DA utilization in the shell (Deutch and Cameron, 1991). Systemically administered cocaine, which enhances extracellular levels of DA in this nucleus, increases the number of Fos-positive neurons in the core as compared to the shell (Graybiel et al., 1993). Thus, the region-specific morphological responses to the depletion of DA and perhaps other monoamines, demonstrated in the present study may reflect specific neurotransmitter-substrate interactions. Further work is clearly needed to establish the precise mechanism(s) involved.

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