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Cortical Regulation of Dopamine Depletion-Induced Dendritic Spine Loss in Striatal Medium Spiny Neurons

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Abstract

The proximate cause of Parkinson's Disease is striatal dopamine depletion. Although no overt toxicity to striatal neurons has been reported in Parkinson's Disease, one of the consequences of striatal dopamine loss is a decrease in the number of dendritic spines on striatal medium spiny neurons (MSNs). Dendrites of these neurons receive cortical glutamatergic inputs onto the dendritic spine head and dopaminergic inputs from the substantia nigra onto the spine neck. This synaptic arrangement suggests that dopamine gates corticostriatal glutamatergic drive onto spines. Using triple organotypic slice cultures comprised of ventral mesencephalon, striatum, and cortex, we examined the role of the cortex in dopamine depletion-induced dendritic spine loss in MSNs. The striatal dopamine innervation was lesioned by treatment of the cultures with the dopaminergic neurotoxin MPP^+ or by removing the mesencephalon. Both MPP^+ and mesencephalic ablation decreased MSN dendritic spine density. Analysis of spine morphology revealed that thin spines were preferentially lost after dopamine depletion. Removal of the cortex completely prevented dopamine depletion-induced spine loss. These data indicate that the dendritic remodeling of MSNs seen in parkinsonism occurs secondary to increases in corticostriatal glutamatergic drive, and suggest that modulation of cortical activity may be a useful therapeutic strategy in Parkinson's Disease.

Keywords

cortex; dendrite; glutamate; MPP^+; Parkinson's Disease; striatum

Parkinson's disease (PD) is a slowly progressing disorder marked by bradykinesia, rigidity, resting tremor, and postural instability (Fahn, 2003, Savitt et al., 2006). The proximate cause of PD is the loss of the striatal dopamine innervation, which originates in the substantia nigra (Fahn, 2003, Savitt et al., 2006). Nigrostriatal dopamine axons synapse onto striatal medium spiny neurons (MSNs), which comprise ~90% of all striatal neurons. These MSNs have radially projecting dendrites that are densely studded with spines (Wilson and Groves, 1980). Postmortem studies of PD have revealed a marked decrease in MSN spine density and dendritic length (McNeill et al., 1988, Stephens et al., 2005, Zaja-Milatovic et al., 2005). Similar morphological changes in MSNs are seen in animal models of parkinsonism (Ingham et al., 1989, Arbuthnott et al., 2000, Day et al., 2006). These changes in dendritic structure are enduring and do not appear to be reversed by levodopa (Stephens et al., 2005, Zaja-Milatovic et al., 2005).

Dendritic spine loss after striatal dopamine depletion has been suggested to depend upon an increase in glutamatergic drive onto MSNs (Deutch, 2006). MSNs receive glutamatergic inputs

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from the cortex onto spine heads, while dopamine axons synapse predominantly onto the necks of spines (Bouyer et al., 1984, Freund et al., 1984). This synaptic triad suggests that striatal dopamine depletion will culminate in an impaired ability of MSNs to gate glutamatergic drive (Surmeier and Kitai, 1997, Meshul et al., 1999, Cepeda et al., 2001). This hypothesis is supported by postmortem studies of PD and examination of animals with striatal dopamine depletion, which have revealed an increased density of perforated synapses (a putative structural marker of increased excitatory transmission) on MSNs (Anglade et al., 1996, Meshul et al., 1999). Moreover, the loss of dopamine removes a tonic presynaptic D2-mediated inhibition of glutamate release from corticostriatal axons (Bamford et al., 2004). Dopamine depletion also appears to increase the excitability of MSNs by diminishing the capacity of these neurons to modulate intracellular calcium levels (Day et al., 2006). Finally, low doses of N-methyl-D-aspartate (NMDA) antagonists potentiate the therapeutic effects of dopaminergic agonists in animal models of parkinsonism (Greenamyre and O'Brien, 1991, Starr, 1995, Blandini et al., 1996).

Despite compelling data concerning an increase in cortically-derived excitatory drive onto striatal MSNs in the dopamine-denervated striatum, it is not clear to what degree this process is critical for the loss of dendritic spines in the dopamine-depleted striatum. A large body of data argues that glutamate is a major determinant of spine development and maintenance (Halpain et al., 1998, Passafaro et al., 2003, Segal et al., 2003). If the loss of spines on MSNs in the dopamine-denervated striatum occurs secondary to increased glutamate release and the decreased ability of MSNs to gate glutamatergic transmission, removal of the cortex should prevent the structural changes in MSN dendrites. We therefore examined the effects of cortical ablation on dopamine denervation-induced dendritic remodeling of striatal MSNs in organotypic slice cultures.

Experimental Procedures

Slice cultures

Triple (ventral mesencephalon–striatum–cortex) organotypic slice co-cultures were prepared from brains of P0 and P1 Sprague-Dawley rats (Harlan; Indianapolis, IN) according to the method of Stoppini et al. (1991). Briefly, 300 μm-thick coronal sections were collected into ice-cold sucrose solution (200 mM sucrose, 1.0 mM KCl, 1.2 mM Na_2HPO_4, 22 mM $NaHCO_3$, 6 mM $MgCl_2$, 0.5 mM $CaCl_2$, 10 mM glucose, and 0.4 mM ascorbate). Dissected pieces of the ventral mesencephalon (VM), striatum, and cortex were plated 1–2 mm apart on Millicell-CM culture inserts (0.4 μm, Millipore). These inserts were kept in 6-well plates with Basal Medium Eagle containing 25% Earle's Balanced Salt Solution (25%), equine serum (Hyclone; Logan, UT; 25% first 3 days, 10% afterwards), 36 mM glucose, 25 mM Hepes-buffer, 250 μM glutamax, and 10 ng/ml GDNF (for the first 8 days *in vitro*) (Peprotech, Rocky Hill, NJ). Starting on day 8 *in vitro*, 10 μM 5-fluoro-2′-deoxyuridine was added to the cultures to inhibit astrocytic overgrowth. Cultures were maintained in an incubator at 37°C and 5% CO_2 and the medium was changed three times weekly.

14 days after the cultures were established (14 DIV) the striatum was fully innervated by both midbrain dopaminergic and cortical glutamatergic axons and the MSNs had a mature morphology. At this time point (14–16 DIV), 15 μM 1-methyl-4-phenylpyridinium (MPP^+) was added to the culture medium for 24 hours, or the ventral mesencephalon was dissected and removed from the cultures, in order to lesion the striatal dopamine innervation. Two weeks after the treatment (28 DIV) the cultures were harvested for analyses. All reagents for tissue culture were obtained from Invitrogen (Grand Island, NY). All other chemicals were purchased from Sigma Chemical Co (St. Louis, MO) unless otherwise noted.

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Diolistic labeling combined with TH-immunohistochemistry

Organotypic cultures were fixed with 1.5% paraformaldehyde in 0.1 M phosphate buffer for 20 min at room temperature, washed in phosphate buffered saline, and then diolistically labeled with the carbocyanine dye CM-DiI (Molecular Probes), as described (Gan et al., 2000). CM-DiI-coated 1.3 μm tungsten particles were ejected with a gene gun (BioRad; Hercules, CA) that was modified with a homemade barrel (O'Brien et al., 2001). After labeling the cultures were stored in 0.04% paraformaldehyde in phosphate buffered saline for two nights at room temperature in the dark. The tissue was then postfixed in 4% paraformaldehyde in 0.1 M phosphate buffer for three hours at room temperature, washed, and incubated with mouse anti-TH IgG (1:250; ImmunoStar, Hudson, WI) in Tris buffered saline (TBS) containing 4% normal serum but no detergent for two nights at 4°C in the dark. This was followed by incubation in Alexa 488-conjugated donkey anti-mouse IgG (1:100; Molecular Probes, Eugene, OR) for two nights at 4°C. The tissue was mounted in ProLong (Molecular Probes). Diolistic labeling under the condition described here resulted on the average in 5 ideally diI-labeled MSNs per culture.

Determination of spine densities and morphologies

Z-stacks of MSN dendritic segments at 0.5μm intervals were acquired with a 63×1.4 NA objective (2.5 zoom factor) using a LSM Meta confocal laser scanning microscopy system (Carl Zeiss). In our initial experiment (Fig. 3) MSN spines were counted on proximal (within a 70 μm radius of the soma) and distal (beyond 70 μm from the soma) dendrite segments and on different dendritic branches (primary, secondary, etc.). In this experiment we analyzed on the average 18 dendritic segments/neuron, 5.3 cells/culture and 3.7 cultures/experimental treatment. In the subsequent experiment in which branch order was not considered (Fig. 4) we analyzed on the average 10 dendritic segments/neuron. We also analyzed spine morphologies, with spines being classified as stubby-, thin-, mushroom-, or filopodia-like, following the criteria of Peters and Kaiserman-Abramof (1970). Spine length was measured by tracing the distance between the most distal tip of the spine and the base of the spine. Spine densities and morphologies were determined by a person unaware of the experimental manipulations of the cultures.

Immunohistochemistry

Cultures were fixed in 4% paraformaldehyde in 0.1 M phosphate buffer, washed, and incubated in 50 mM TBS containing 4% horse serum and 0.2% Triton X-100 (TBS^+) for one hour. Cultures were then incubated overnight in mouse anti-tyrosine hydroxylase (TH; 1:2000; ImmunoStar, Inc.) or rabbit-anti-VGluT1(1:8000; Mab-Technologies, Stone Mountain, GA) in TBS^+. The next day cultures were incubated in Alexa 488-conjugated secondary antibodies (Molecular Probes) in TBS^+, washed, and mounted.

To visualize dopamine axons, the cultures were fixed in 0.1M cacodylate containing 5% glutaraldehyde and 1% $Na_2S_2O_5$ (pH 4), washed in 50 mM TBS containing 1% $Na_2S_2O_5$ and 0.5% Triton X-100 (pH 7.2), and incubated in a rabbit-anti-dopamine antibody (1:2000, Chemicon, Temecula, CA) in the same buffer. The next day the cultures were washed and incubated in donkey-anti-rabbit IgG (Jackson ImmunoResearch, West Grove, PA), after which they were washed and incubated in rabbit-peroxidase-antiperoxidase complex (1:800, Jackson ImmunoResearch). The signal was developed with a nickel/cobalt-intensified diaminobenzidine product.

Anterograde labeling

Biocytin crystals (Molecular Probes) were gently placed onto the cortex and the cultures returned to the incubator for18 hours. The cultures were then fixed in 4% paraformaldehyde,

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washed, and incubated with Cy3-conjugated streptavidin (1:1000; Jackson ImmunoResearch), after which they were dried, cleared, and mounted.

Retrograde labeling

30–50 nl undiluted fluorescent latex microspheres (Lumafluor Corp., Naples, FL) were pressure injected into the VM and the cultures returned to the incubator. After 48 hours the cultures were fixed in 4% paraformaldehyde, washed, and mounted in ProLong.

HPLC analysis of homovanillic acid in culture medium

The extent of dopamine depletion in the cultures was determined in part by measurements of the levels of the dopamine metabolite homovanillic acid (HVA) in the medium. 500 μl of culture medium was removed at the time of media change and added to 125 μl of a 1M perchloric acid solution containing 0.2 g/L $Na_2S_2O_5$ and 0.05 g/L Na_2-EDTA. The samples were centrifuged at 15,000 rpm for 5 min, and injected on a C18 reverse phase column (Alltech, Deerfield, IL), and HVA measured using an ESA 501A Coulochem detector (Chelmsford, MA). HVA levels were expressed as pmols/ml medium.

Propidium iodide staining

To determine if MPP^+ had any toxic effects on striatal and cortical cells, cultures were incubated for two hours in culture medium containing 2.5 μg/ml propidium iodide (Molecular Probes) at 2 days or 14 days after MPP^+ treatment. The cultures were then washed with culture medium and imaged immediately.

Statistical analyses

Differences in spine densities and lengths (on a per cell basis) were assessed by ANOVA, followed by a Bonferroni t-test when indicated. Differences in the relative proportions of different morphological types of spines were analyzed using a Kruskal-Wallis non-parametric ANOVA, followed by a Dunn test.

Results

Characterization of organotypic cortico-striatal-mesencephalic cultures

By 14 days *in vitro* (14 DIV), the striatum was densely innervated by TH-immunoreactive (-ir) axons. The TH fibers emanated from dopamine neurons in the ventral mesencephalon (VM) and ramified extensively throughout the entire striatum. At the striatal-cortical interface a dense plexus of dopaminergic axons was present. Most of these axons turned laterally and remained within the striatum, with relatively few fine-caliber dopaminergic axons innervating the cortex (Fig. 1*A*).

Immunohistochemical studies using an anti-dopamine antibody yielded similar results (see Fig. 1*B*). Anterogradely-labeled cortical axons densely innervated striatal portion of the cultures, with a smaller contingent of cortically-derived axons continuing to innervate the VM (Fig. 1*C*). A dense VGluT1-ir axon plexus was observed throughout the striatum (Fig. 1*D*). Injection of fluorescent latex microspheres into the VM resulted in the retrograde labeling of many medium-sized striatal cells, consistent with MSNs in the cultures projecting to the VM (suppl. Fig. 1*E*).

The development of the dendritic trees of striatal MSNs was analyzed by diolistically-labeling MSNs. At 7 DIV MSNs had a truncated dendritic arbor; in addition, spines were infrequently seen on the dendrites, and many of those that were present were long without a head and thus resembled filopodia (suppl. Fig. 1*A*,*C*). By 14 DIV the dendritic trees of MSNs were

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substantially larger and more complex, spine density had increased, and the spines had assumed mature morphologies (Peters and Kaiserman-Abramof, 1970) (suppl. Fig. 1*B,D*). The morphology of MSNs at 14 DIV was indistinguishable from that observed at later times.

Thus, by 14 DIV the innervations of the striatum from the cortex and VM were present and the morphological features of MSNs in cultures appeared comparable to those observed in adult striatum *in vivo*.

Effects of striatal dopamine denervation on dendritic spines

At 14 DIV striatal dopamine was depleted by either treating the cultures with 15 μM MPP^+ for 24 hours, or by carefully dissecting and removing the VM. Two days after MPP^+ treatment or VM ablation the levels of the dopamine metabolite HVA were sharply reduced ($F_{(2,32)}$ = 12.87, $p \leq 0.001$, Table 1), and did not recover over the duration of the experiment. VM ablation or MPP^+ exposure also caused an almost total loss of striatal TH-ir axons (Fig. 2). We did not see any evidence of striatal reinnervation by TH-fibers from surviving VM cells over the course of the experiment (data not shown). Concentrations of MPP^+ lower than 15 μM did not reliably disrupt the striatal dopamine innervation. We did not observe any toxicity of 15 μM MPP^+ to striatal cells, as reflected by lack of accumulation of propidium iodide by cells in the striatum at 2 and 14 days after neurotoxin exposure (data not shown). At 2 days after MPP^+ treatment we did see a small number of propidium-labeled cells in the central part of the cortical explants; at 14 DIV relabeling of these same cultures revealed no propidium iodide-positive cortical cells (data not shown).

Fourteen to 16 days after dopamine depletion the striatum was diolistically labeled and MSNs identified by their characteristic medium-sized soma and multiple, branched spiny dendrites (Table 1, Fig. 2). Striatal dopamine depletion had no effect on soma size ($F_{2,54}$ = 1.19, NS), but markedly decreased dendritic spine density ($F_{(2,54)}$ = 9.36, $p \leq 0.001$) (Table 1, Fig. 3A); MPP^+ and VM ablation decreased spine density to comparable degrees (Table 1, Fig. 3*A*). The loss of spines was of similar magnitude on proximal and distal dendrites ($F_{(2,104)}$ = 0.13, NS; Fig. 3*B*), and was independent of dendritic branch order ($F_{(8,955)}$ = 1.27, NS; Fig. 3*C*).

The effect of dopamine depletion on spine morphology was also analyzed. In control cultures more stubby than thin spines were present (Fig. 4*B*), with few mushroom spines seen. Filopodia-like extensions (> 4.5 μm in length) were almost never seen, with only 7 out of a total of 5308 analyzed spines being classified as filopodia. Dopamine depletion resulted in a change in the percentage of thin and stubby spines in the total spine pool, with the relative proportions of thin and stubby spines being decreased and increased, respectively (H_{thin} = 32.14, $p \leq 0.001$; H_{stubby} = 31.36, $p \leq 0.001$; see Fig. 4*B*). The average length of stubby and thin spines of MSNs in control cultures were 0.73 ± 0.02 μm and 1.40 ± 0.04 μm respectively; dopamine depletion did not affect the length of either type of spine (suppl. Fig. 2).

Dopamine denervation induced spine loss depends on cortical afferents

In contrast to MSNs in cortex-containing cultures, the spine density of MSNs in cultures that were decorticated at the time of MPP^+ treatment was comparable to control values (Fig. 4*A*). No change in the relative numbers of thin and stubby spines occurred in response to dopamine depletion (Fig. 4*B*). Thus, removal of the cortex rendered MSN spines insensitive to dopamine depletion.

Removal of the cortex only did not affect MPP^+-induced dopamine depletion as judged by inspection of striatal TH-ir. Ablation of the cortex on its own also had no effect on MSN dendritic spine density (Fig. 4*A*) or the percentages of thin and stubby spines when compared to control cultures with a cortex (Fig. 4*B*); however, we did observe a small but significant

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increase in the length of stubby spines when compared to the stubby spines of cortex containing cultures ($F_{4,95} = 9.74$, $p \leq 0.001$; suppl. Fig. 2).

Discussion

Characterization of organotypic cultures and striatal MSNs

The organotypic slice cultures largely recapitulated the *in vivo* connections between the substantia nigra, striatum, and cortex. Dopaminergic neurons were present in the VM, and by 14 DIV had innervated the striatum and to a limited degree the cortex. Anterograde labeling of cortical neurons revealed a dense striatal projection, with a sparse innervation of the VM. These observations are consistent with previous reports (Plenz and Kitai, 1996, Just et al., 1999, Franke et al., 2003, Snyder-Keller, 2004). We also observed an extensive innervation of the striatum by VGluT1-ir axons. Because VGluT1is mainly expressed by cortical but not subcortical neurons (Kaneko and Fujiyama, 2002), the presence of VGluT1-ir axons points to a dense glutamatergic corticostriatal projection. Finally, when fluorescent latex microspheres were injected into the VM, we observed many retrograde-labeled striatal neurons, consistent with the elaboration of MSN axons and their appropriate targeting of the substantia nigra.

The temporal pattern of MSN dendrite development *in vitro* is similar to that reported *in vivo* (Tepper and Trent, 1993, Sharpe and Tepper, 1998). There was a rapid maturation in the dendritic tree between 7 and 14 DIV, and no substantial change thereafter. The soma size and dendritic spine densities of MSNs in the cultures were comparable to those reported in mature MSNs *in vivo* and the dendrites of MSNs had mature spines and lacked filopodia. The only significant difference we observed between MSNs in the organotypic cultures and those in adult rats (Wilson and Groves, 1980) was that the proximal dendrites of MSNs *in vitro* were densely spiny, as noted by Plentz and Aertsen (1996).

Effects of dopamine denervation on MSN morphology

Both MPP^+ treatment and removal of the dopamine cell-containing VM markedly decreased both the density of striatal dopaminergic axons and levels of the dopamine metabolite HVA in the culture media. The decrease in the striatal dopamine innervation was evident within two days after MPP^+ exposure or midbrain removal, and over the ensuing weeks there was no evidence of reinnervation of the striatum. We did not observe non-specific toxicity to cortical or striatal neurons, as reflected by PI uptake, in cultures treated with 15 μM MPP^+. This is consistent with previous reports that concentrations of MPP^+ up to 20 μM are not toxic to non-dopaminergic cells in organotypic slice cultures of the substantia nigra (Kress and Reynolds, 2005).

Striatal dopamine depletion induced by MPP^+ and VM ablation decreased MSN spine density by 34% and 32%, respectively. These changes are similar to those observed in the striatum of PD patients and animals with striatal dopamine depletion (Ingham et al., 1989, Ingham et al., 1993, Stephens et al., 2005, Zaja-Milatovic et al., 2005). Consistent with postmortem studies of PD (Stephens et al., 2005), the magnitude of spine loss in organotypic cultures was the same in the proximal and distal dendrites of MSNs.

We analyzed how striatal dopamine depletion affects spines of different morphologies and found that thin spines were preferentially lost after dopamine depletion. Dendritic spines appear to be independent compartments, with the morphology of the spine determining function. Activation of presynaptic glutamatergic axons in the striatum causes long-lasting increases in intracellular calcium in MSNs that are restricted to the spine head, with little calcium invasion of the dendritic shaft (Carter and Sabatini, 2004). The neck of thin spines restricts diffusion of calcium, suggesting that calcium surges last longer in spines with longer thinner necks, thus

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rendering these spines more susceptible to glutamate toxicity (Segal, 1995). The observation that dopamine depletion does not cause any change in the length of thin or stubby spines, together with the overall decrease in spine density, suggests that the preferential loss of thin spines is not due to a gradual transformation of thin into stubby spines, but due to spines that fully collapse. This conclusion is supported by an ultrastructural study, which suggests that dopamine-depletion results in the retraction of the spine apparatus into the dendritic shaft of striatal MSNs (Nitsch and Riesenberg, 1995).

Our data were obtained from randomly selected MSNs. MSNs comprise neurons projecting primarily to either the substantia nigra (direct pathway neurons) or the globus pallidus (indirect pathway neurons) (Kawaguchi et al., 1990, Wu et al., 2000). These two types of MSNs express D_1 and D_2 receptors, respectively, and are differentially regulated by dopamine (Gerfen et al., 1990, Zeng et al., 1995, Gerfen, 2000). Recent data indicate that dopamine depletion causes spine changes only in D_2 receptor expressing MSNs, with decreases in spine density of ~50% in these MSNs (Day et al., 2006). This is roughly double the decrease in spine density reported in randomly-selected (thus including direct and indirect pathway) MSNs *in vivo* (Ingham et al., 1989), and in our cultures. It therefore appears likely that MSNs of both, the direct and indirect pathways, are present in our organotypic cultures and, that as *in vivo*, the spines of only one type of MSNs are affected by dopamine depletion. It has recently been shown that the majority of MSNs in the rat striatum project to both targets, the substantia nigra and the globus pallidus, but with quite different terminal arbor sizes (Kawaguchi et al., 1990, Wu et al., 2000). This is consistent with the suggestion that our cultures, with only one projection target present (the substantia nigra), nonetheless contain both types of MSNs, direct and indirect pathway cells.

Role of cortex in dopamine depletion-induced MSN spine changes

No loss of MSN spines or change in the relative distribution of thin and stubby spines was observed in cultures in which the cortex had been removed at the time of dopamine depletion. These observations support the hypothesis that corticostriatal glutamatergic inputs play a critical role in dopamine depletion-induced MSN spine loss (Deutch, 2006). Activation of ionotropic and metabotropic glutamate receptors, glutamate-induced calcium release from internal stores and the voltage-dependent L-type calcium channel have all been shown to affect spines (Halpain et al., 1998, Korkotian and Segal, 1998, Korkotian and Segal, 1999b, Vanderklish and Edelman, 2002, Carter and Sabatini, 2004, Day et al., 2006). Which of these molecular pathways plays the most prominent role in the dopamine-depletion induced changes in densities and morphologies of MSN spines will have to await future studies.

Decortication of the cultures on its own resulted in a trend towards increased spine density and a statistically significant increase in the length of stubby MSN spines. Our findings are thus similar to *in vivo* studies where prolonged blockade of excitatory transmission led to increased spine density (Rocha and Sur, 1995) and observations made by Kirov and Harris, (1999) who found that blocking excitatory transmission in acute slices from adult rats results in an increase in spine density in CA1 pyramidal neurons (Kirov and Harris, 1999, Kirov et al., 2004).

However, early reports suggested that focal cortical ablation *in vivo* causes loss of MSN spines and a decrease in synapse density (Kemp and Powell, 1971, Chen and Hillman, 1990, Cheng et al., 1997). There are two potential explanations for the discrepancy between our observations made in organotypic cultures and these *in vivo* studies. First, surgical ablation of the cortex *in vivo* can result in indirect damage (such as that caused by edema or by the involvement of the corpus callosum) thereby causing dystrophic changes in MSNs; indeed, different methods of cortical ablation have different effects on striatal neurons (Napieralski et al., 1996). Second, the use of organotypic cultures allowed us to remove the glutamatergic innervation of the striatum in its entirety, while the *in vivo* studies left in place the glutamatergic projections from

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the contralateral cortex and thalamus. These remaining glutamatergic axons can compensate by sprouting, leading to increased striatal glutamatergic activity (Chen and Hillman, 1990, Napieralski et al., 1996, Meshul et al., 2000), which could potentially cause excitotoxic spine loss (Halpain et al., 1998, Korkotian and Segal, 1999a).

Conclusion

Dystrophic changes in dendrites are present in a variety of brain disorders including PD (Fiala et al., 2002, Blanpied and Ehlers, 2004). Because dopamine receptors are localized to the dendritic spines of MSNs, the loss of spines on these neurons may contribute to the decreased responsiveness to levodopa treatment seen late in the course of PD (Marsden and Parkes, 1977, Rinne et al., 1981, Clissold et al., 2006, Deutch, 2006). We observed that dopamine depletion-induced MSN spine loss critically depends on corticostriatal projections. The involvement of an extended corticostriatal system in determining spine loss suggests that modifying either cortical drive onto MSNs or the response of MSNs to corticostriatal neurons may lead to useful therapeutics for late-stage parkinsonism.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

DIV
: days in vitro

GDNF
: glial-derived neurotrophic factor

MPP^+
: 1-methyl-4-phenylpyridinium

MSN
: medium spiny neuron

NMDA
: N-methyl-D-aspartate

PD
: Parkinson’s disease

TBS
: Tris-buffered saline

VM
: ventral mesencephalon

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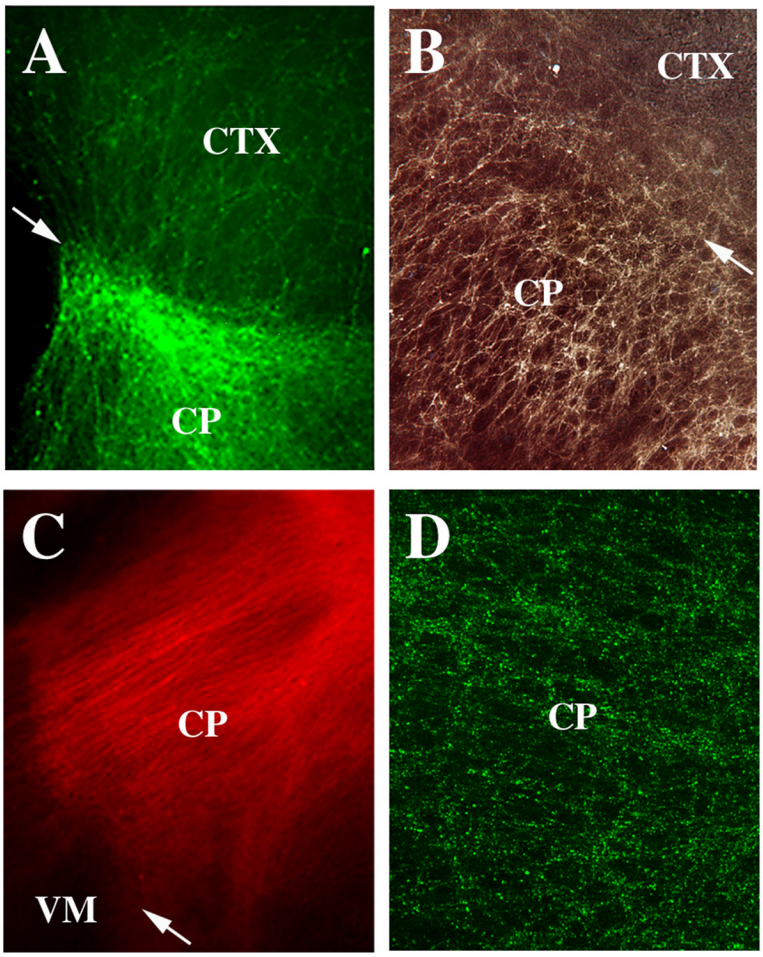

Figure 1. Mesencephalic and cortical innervation of striatum in organotypic triple cultures

A. TH-immunoreactive axons are abundant in the striatum, but turn at the interface between the striatum and cortex, and rarely enter the cortical tissue. **B**. The striatum is also rich in dopamine-immunoreactive axons. The image of the DAB labeled dopaminergic axons has been inverted. **C**. Cortical deposition of biocytin reveals an abundant striatal innervation from the cortex, but cortical axons appear to largely avoid the VM. **D**. Staining of the cultures with an anti-VGluT1 antibody reveals a dense plexus of glutamatergic axons in the striatum. Arrows indicate borders between tissues. Abbreviations: VM, ventral mesencephalon; CP, striatum; CTX, cortex.

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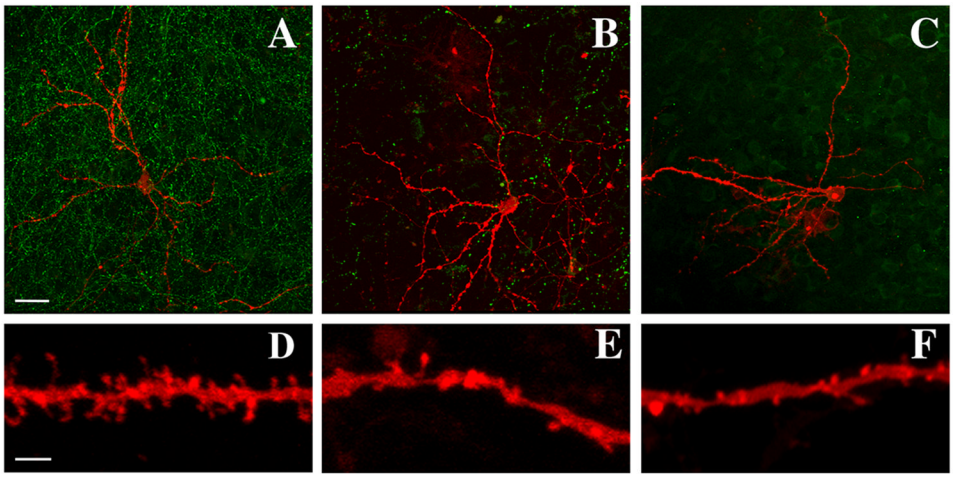

Figure 2. Striatal TH-innervation is depleted after MPP^+exposure or removal of the VM

Two weeks after MPP^+ treatment or removal of the VM, the cultures were diolistically labeled with CM-DiI (red) and incubated with anti-TH antibodies (green). The images show the projections of confocal z-stacks displaying MSN neurons (red) and TH-positive fibers (green). **A**. In control cultures MSNs are surrounded by a dense network of TH-positive fibers. **B,C**. Exposure to 15 μM MPP^+ for 24 hours (B) or removal of the VM (C) results in a near complete loss of TH-immunoreactive fibers in the striatum. **D–F.** Dendritic segments from a control MSN (**D**), a neuron exposed to 15 μM MPP^+ for 24 hours (**E**), and a neuron in a VM-ablated culture (**F**). The scale bar in A–C is 25 μm and in D–F is 2.5 μm.

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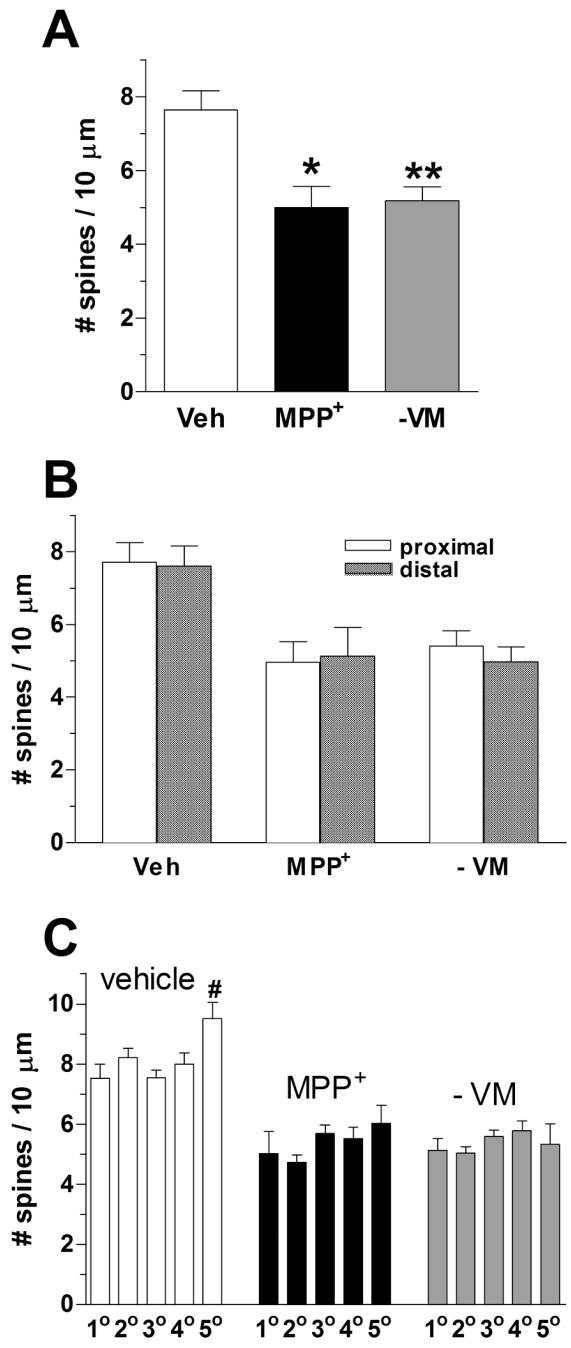

Figure 3. Dopamine-depletion results in decreased spine density along the whole dendritic arbor of MSNs

A. Striatal dopamine denervation by MPP^+ or VM ablation decreased spine density relative to vehicle-treated cultures (Veh). **B**. The extent of dopamine depletion-induced spine loss was the same in proximal (within 70 μm of the soma) and distal (>70 μm from the soma) dendritic segments. **C**. Spine loss was also independent of branch order (primary = 1°, secondary = 2°, tertiary = 3°, quarternary = 4° and higher = 5°).

$^{\#}p \leq 0.05$, $*p \leq 0.01$, $**p \leq 0.001$

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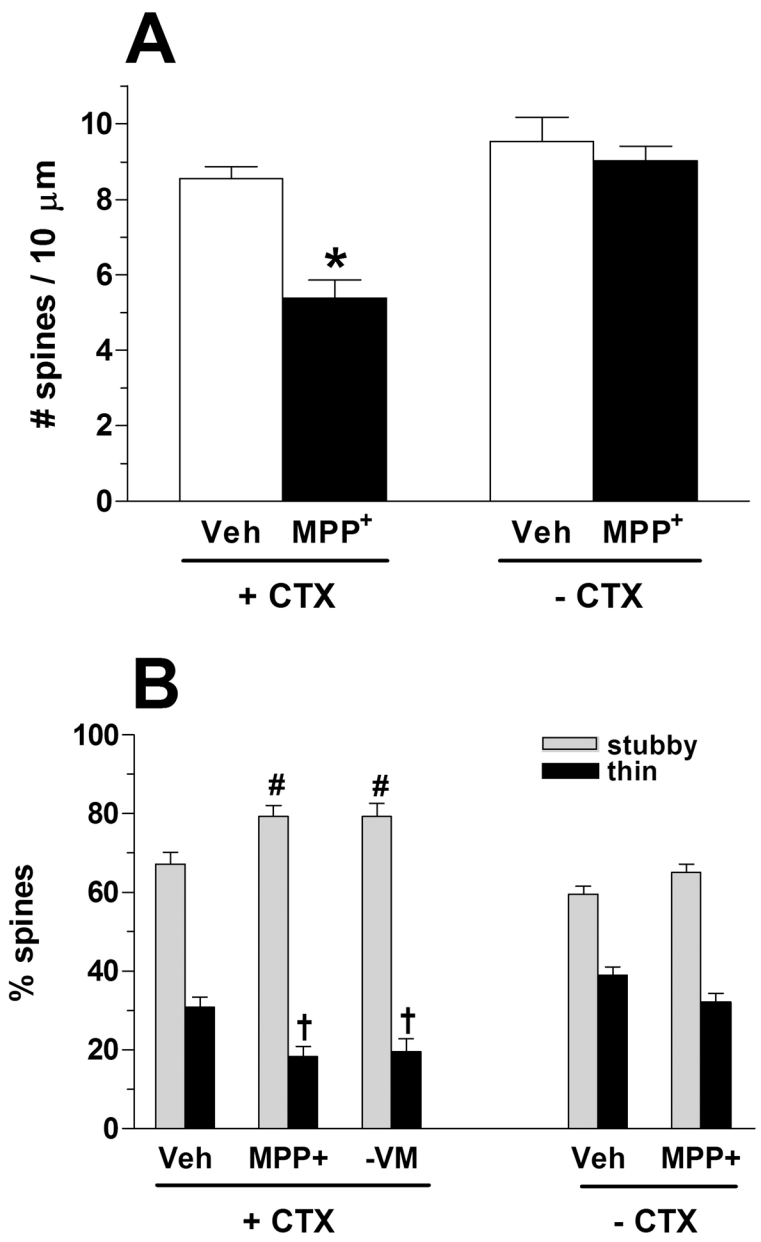

Figure 4. Decortication prevents dopamine-depletion induced changes in spines

Cultures with or without cortex were depleted of dopamine at 14 DIV and MSN spines analyzed 14–16 days later. **A**. MPP^+ significantly decreased MSN dendritic spine density in cultures containing a cortex but had no effect in cultures without a cortex. **B.** In cultures with an intact cortex, MPP^+ treatment or removal of the VM significantly decreased the percentage of thin spines and caused a corresponding relative increase in the percentage of stubby spines when compared to control cultures. In the absence of the cortex MPP^+ did not elicit any change in the relative proportions of thin and stubby spines.

$^{\#}p_{stubby} \leq 0.05$; $^{\dagger}p_{thin} \leq 0.05$; $^{*}p \leq 0.01$

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Table 1

Dopamine depletion affects MSN spine density, but not soma diameter

Soma diameter was unchanged but overall dendritic spine density was decreased in the dopamine-denervated striatum. HVA levels in the medium at 48 hours after initiation of dopamine depletion were significantly decreased in both the MPP^+-treated and VM-ablated cultures.

	Veh	MPP^+	− VM
HVA (pmols/ml)	204.8 ± 25.7	64.4 ± 18.6**	9.7 ± 3.2*
Soma diameter (μm)	14.2 ± 0.6	13.6 ± 0.4*	12.9 ± 0.6
Spine density (spines/10 μm)	7.6 ± 0.5	5.0 ± 0.6*	5.2 ± 0.4**

* $p \leq 0.01$,

** $p \leq 0.001$