

# Region-specific restoration of striatal synaptic plasticity by dopamine grafts in experimental parkinsonism

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Intrastriatal transplantation of dopaminergic neurons can restore striatal dopamine levels and improve parkinsonian deficits, but the mechanisms underlying these effects are poorly understood. Here, we show that transplants of dopamine neurons partially restore activity-dependent synaptic plasticity in the host striatal neurons. We evaluated synaptic plasticity in regions distal or proximal to the transplant (i.e., dorsolateral and ventrolateral striatum) and compared the effects of dopamine- and serotonin-enriched grafts using a rat model of Parkinson disease. Naïve rats showed comparable intrinsic membrane properties in the two subregions but distinct patterns of long-term synaptic plasticity. The ventrolateral striatum showed long-term potentiation using the same protocol that elicited long-term depression in the dorsolateral striatum. The long-term potentiation was linked to higher expression of post-synaptic AMPA and N2B NMDA subunits (GluN2B) and was dependent on the activation of GluN2A and GluN2B subunits and the D1 dopamine receptor. In both regions, the synaptic plasticity was abolished after a severe dopamine depletion and could not be restored by grafted serotonergic neurons. Solely, dopamine-enriched grafts could restore the long-term potentiation and partially restore motor deficits in the rats. The restoration could only be seen close to the graft, in the ventrolateral striatum where the graft-derived reinnervation was denser, compared with the distal dorsolateral region. These data provide proof of concept that dopamine-enriched transplants are able to functionally integrate into the host brain and restore deficits in striatal synaptic plasticity after experimental parkinsonism. The region-specific restoration might impose limitations in symptomatic improvement following neural transplantation.

6-OHDA lesion | DA | 5-HT | LTP | LTD

Nonpharmacological dopamine (DA) replacement approaches to the therapy of Parkinson disease (PD) focus on the transplantation of DA-producing neurons into the striatum. Parkinson disease is indeed viewed as the disease of choice to develop intracerebral transplantation therapies, and promising results have been obtained both in experimental models and in some patients using embryonic DA neurons (1, 2). Embryonic DA neurons are able to innervate the host striatum, release DA, and reverse alterations in neuropeptide expression after a parkinsonian lesion (3). There is a continuous debate about whether these effects are sufficient for transplanted neurons to partially restore clinical symptoms or whether other underlying mechanisms also are required. In particular, a functional integration of the graft into the host microcircuits, with bidirectional synaptic contacts between the host and grafted neurons, may give superior therapeutic benefit than a mere neurochemical restoration. Transplanted DA neurons are able to form synapses with the surrounding striatal medium-sized spiny neurons (MSNs) (4) and receive innervation from the host neurons with bidirectional

synaptic interactions (5–7). It is, however, unknown whether these plastic changes are sufficient to restore the basic functional properties of the host neurons essential for corticostriatal control of movements (8). This study attempts to understand whether neural transplants have the ability to restore activity-dependent synaptic plasticity in the host corticostriatal pathway.

We have herein investigated corticostriatal plasticity after transplantation of DA and 5-HT neurons in host MSNs in an experimental model of PD. Dopamine is critical for inducing long-term striatal plasticity, i.e., long-term potentiation (LTP) and long-term depression (LTD) in MSNs (9). The changes are mediated by the activation of ionotropic glutamate receptors, i.e., alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) and *N*-methyl-D-aspartate (NMDA) receptors, as well as by the activation of DA receptors. Consequently, animal models of severe DA denervation have demonstrated a loss of both forms of corticostriatal plasticity in the dorsolateral (DL) striatum (10, 11). A partial DA denervation, on the other hand, spares LTD in the DL striatum (12). Also clinical studies have revealed

## Significance

This paper identifies long-term synaptic plasticity restoration as an underlying mechanism of progressive motor improvement after neuronal transplantation in a rat Parkinson model. A Parkinson-associated loss of plasticity in the host striatum could be restored by transplanted dopamine neurons with sufficient fiber innervation, suggesting that functional innervation with possible synapse formation is required for the long-term effect of neural transplants. These data support a multisite-grafting procedure to more extensively restore the plasticity in the host parkinsonian brain. Dopamine neuron transplantation could be a future therapy for Parkinson disease and is currently being evaluated in a European Union-sponsored project.

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an impairment of synaptic plasticity in the corticostriatal pathway (13).

Using an experimental model of PD, we here demonstrate that transplanted DA neurons are efficient in restoring corticostriatal plasticity in the densely innervated area close to the graft whereas more sparsely innervated areas remain unaffected. This restoration is in contrast to the effect of transplanted 5-HT neurons that were unable to restore any type of plasticity.

## Results

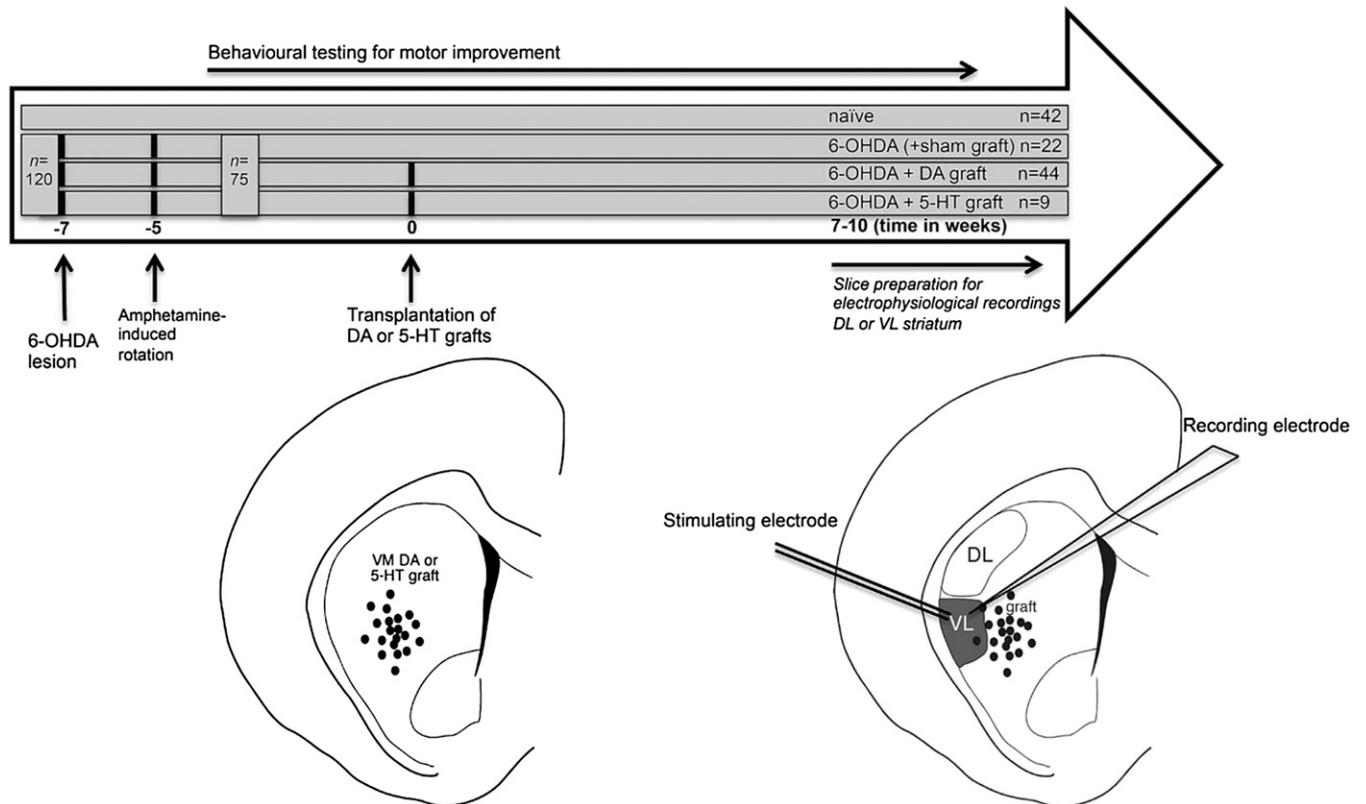
### Distinct Synaptic Plasticity in Dorsolateral Versus Ventrolateral Striatum.

The DL striatum has been extensively studied in previous electrophysiological investigations in the rat 6-hydroxydopamine lesion PD model, playing a role in both axial movements and forelimb use. The ventrolateral (VL) striatum is an area influencing orofacial and forelimb movements (14, 15). Of importance for this study, this region receives rich fiber innervation from grafted neurons being in close proximity to the grafted core with the most traditionally used coordinates for transplantation (16–19). In this study, therefore, LTP was measured in both DL region and the more ventral region of striatum (i.e., VL) that received a denser fiber innervation from the grafted neurons (Fig. 1).

Recordings from MSNs in the DL and VL regions showed similar discharge firing patterns, resting membrane potential (RMP), and membrane resistance (Rm) in physiological condition, i.e., in naïve rats (Fig. 2*A* and *D*) (DL, RMP  $-84.0 \pm 1.2$  mV and Rm  $49.4 \pm 0.8$  M $\Omega$ ; VL, RMP  $-84.6 \pm 1.0$  mV, Rm  $50.5 \pm$

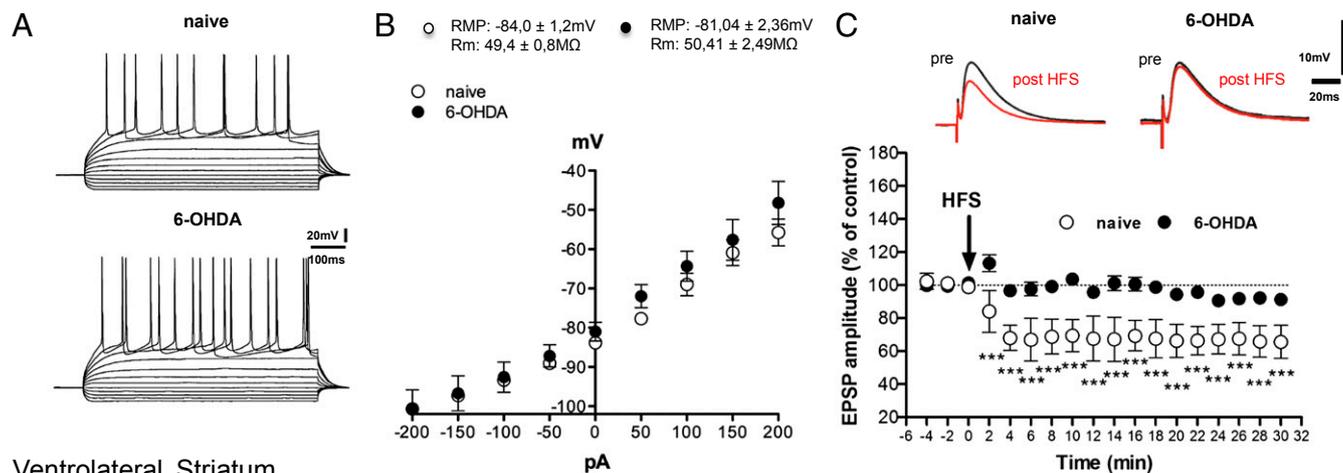
$0.6$  M $\Omega$ ,  $P > 0.05$ ,  $n = 12$  cells from eight rats for VL and  $n = 10$  cells from six rats for DL). Dopamine denervation did not cause any alteration in the intrinsic properties of the neurons in any of the regions, as the firing discharge and the current–voltage relationship showed similar membrane properties compared with naïve condition (Fig. 2*A*, *B*, *D*, and *E*) (DL, 6-OHDA, RMP  $-81.04 \pm 2.36$  mV, Rm  $50.41 \pm 2.49$  M $\Omega$ ; VL, 6-OHDA, RMP  $-81.76 \pm 1.83$  mV, Rm  $50.76 \pm 0.88$  M $\Omega$ ,  $P > 0.05$ ,  $n = 5$  cells from five rats for DL and  $n = 7$  cells from six rats for VL).

To evaluate long-term synaptic plasticity in the two regions, we choose a protocol that induces LTD in the DL striatum, i.e., high-frequency stimulation (HFS) in the presence of physiological concentrations of magnesium (10). This type of plasticity is expressed in neurons both of the direct and indirect pathways (20). Results showed an unexpected regional difference in the synaptic plasticity of naïve animals. As expected, striatal neurons from the DL striatum showed induction of LTD of EPSPs after tetanic stimulation (Fig. 2*C*) (naïve EPSP pre- vs. post-HFS,  $P < 0.05$ ,  $n = 7$  cells from five rats), which was blocked by a severe DA denervation induced by 6-OHDA (Fig. 2*C*) (naïve vs. 6-OHDA,  $P < 0.001$ ,  $n = 9$  cells from five rats). In contrast, in the VL striatum, the same stimulating protocol induced an activity-dependent LTP of the EPSPs in a majority of MSNs (Fig. 2*F*) (naïve EPSP pre- vs. post-HFS,  $P < 0.05$ ,  $n = 7/11$  cells from nine rats). Experiments with retrograde tracer injection, specifically labeling the striatonigral MSNs, showed that both types of responses were present in striatonigral MSNs (Fig. S1) ( $n = 6$  cells from four rats, LTP,  $n = 3$  cells from three rats, no response).

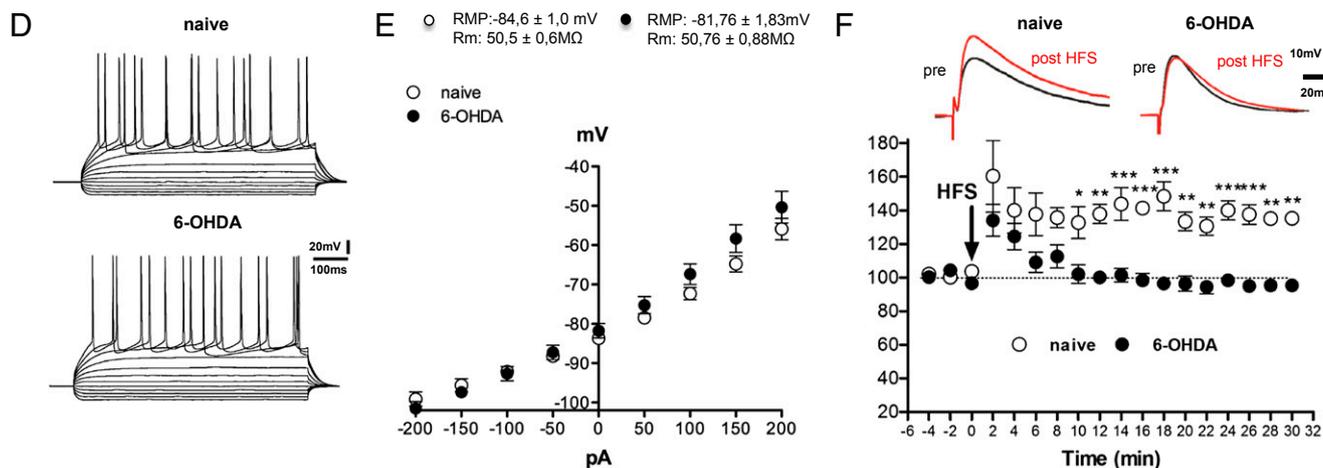


**Fig. 1.** Time line (in weeks) for electrophysiological, behavioral, and transplantation experiments. Four experimental groups were analyzed: (i) naïve, (ii) 6-OHDA, (iii) 6-OHDA+DA graft, and (iv) 6-OHDA+5-HT graft. In the first group, naïve animals were studied. In the remaining three groups, rats were unilaterally lesioned with 6-OHDA to induce a severe DA denervation. After 7 wk, they received transplants rich in either DA or 5-HT neuroblasts. Cell-suspension solution without neuroblasts was injected in 6-OHDA-lesioned rats as sham graft controls. Rats were tested in cylinder tests for assessment of motor improvement by the grafts from week 4 until the end of the experiment. Ten weeks after transplantation, animals were killed for electrophysiological recordings or Western blot analysis. (Lower) Illustrations of the location of the grafts and the region of interest for electrophysiological recordings in the rat striatum. DL, dorsolateral; DA, dopamine; 5-HT, serotonin; SNpr, Substantia Nigra pars reticulata; VL, ventrolateral; VM, ventral mesencephalon.

## Dorsolateral Striatum



## Ventrolateral Striatum



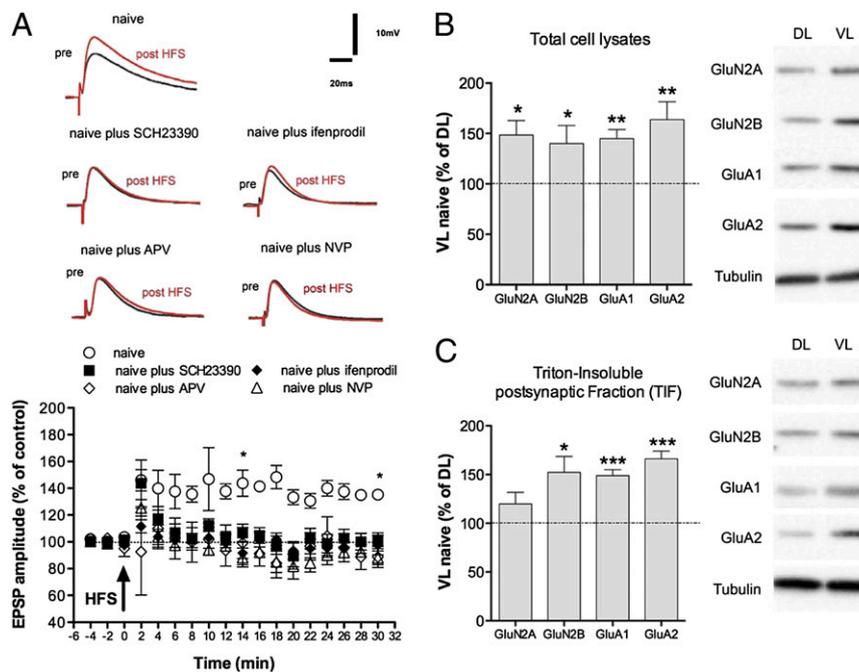
**Fig. 2.** Intrinsic membrane properties and synaptic plasticity in DL versus VL striatum. (A) Illustration of the firing discharge in MSN of naïve and 6-OHDA-lesioned rats recorded in DL striatum. (B) The current–voltage (IV) relationship as evoked by injection of increasing current amplitude did not differ in MSN recorded from DL naïve versus 6-OHDA-lesioned animals. (C) MSN from naïve rats recorded from DL striatum showed induction of LTP after high-frequency stimulation (HFS, arrow) of corticostriatal glutamatergic fibers. The 6-OHDA lesion blocked this induction (naïve vs. 6-OHDA, treatment effect  $F_{(1,238)} = 53.73$ ,  $P < 0.001$ ; time effect  $F_{(17,238)} = 19.04$ ,  $P < 0.001$ ; interaction  $F_{(17,238)} = 10.06$ ,  $P < 0.001$ ;  $***P < 0.001$ ). (Lower) The evoked EPSP tracers pre- and post-HFS in naïve versus 6-OHDA rats. (D) Illustration of the firing discharge in MSN of naïve and 6-OHDA-lesioned rats recorded in VL striatum. (E) The current–voltage (IV) relationship as evoked by injection of increasing current amplitude did not differ in MSN recorded from VL naïve versus 6-OHDA-lesioned animals. (F) HFS was able to induce LTP striatum in a population of cells in VL striatum even in the presence of magnesium in the perfusion buffer. The LTP was completely blocked by the 6-OHDA lesion (naïve vs. 6-OHDA, treatment effect  $F_{(1,216)} = 169.6$ ,  $P < 0.001$ ; time effect  $F_{(17,216)} = 5.10$ ,  $P < 0.001$ ; interaction  $F_{(17,216)} = 2.58$ ,  $P < 0.001$ ;  $*P < 0.05$ ,  $**P < 0.01$ ,  $***P < 0.001$ ). (Lower) The evoked EPSP tracers pre- and post-HFS in VL MSN, naïve vs. 6-OHDA rats. DA, dopamine; DL, dorsolateral; VL, ventrolateral.

Similar to the DL striatum, the induction of LTP in VL striatum was dependent on the endogenous DA innervation as MSNs recorded from 6-OHDA-lesioned animals did not show any potentiation (Fig. 2F) (naïve vs. 6-OHDA from  $n = 6$  rats), treatment effect  $F_{(1,216)} = 169.6$ ,  $P < 0.001$ ;  $n = 7$  neurons for both groups).

**Regional Expression of Glutamate Receptor Subunits in the Striatum and Their Involvement in Plasticity.** In DL striatum, LTP depends on the activation of NMDA and D1 DA receptors (for review, see ref. 21). Long-term potentiation in both hippocampus (22) and nucleus accumbens (NA) (23) is dependent on the GluN2B subunit containing NMDA receptors whereas LTP in DL striatum is purely dependent on GluN2A (24). To examine the involvement of NMDA receptor subunits and D1 DA receptors in the ventrally expressed plasticity, we applied a pharmacological approach.

Despite the presence of magnesium in the buffer, the LTP measured in the VL striatum was dependent on the activity of NMDA receptors as it could be blocked completely by 2-amino-5-phosphonovaleric acid (APV) (Fig. 3A) (naïve vs. naïve plus APV,  $P < 0.05$ ). Similar to the DL striatum, the LTP in this region was also dependent on the D1 DA receptor and the GluN2A NMDA subunit and could be completely blocked by both SCH23390 (D1 receptor antagonist) and NVP-AAM077 (GluN2A subunit-specific antagonist). The ventrally expressed LTP was further blocked by the GluN2B-specific antagonist, ifenprodil (Fig. 3) (naïve vs. all of the applied drugs, treatment effect  $F_{(4,342)} = 76.22$ ,  $P < 0.001$ ; at least  $*P < 0.05$ , APV,  $n = 3$  cells from three rats; ifenprodil,  $n = 5$  cells from four rats; NVP-AAM077,  $n = 4$  cells from three rats; SCH23390,  $n = 5$  cells from four rats).

To further explore the features of LTP observed in the VL striatum, we evaluated the regional protein expression levels of



**Fig. 3.** Pharmacological analysis and protein expression. Pharmacological characterization was performed in naive rats, VL striatum. (A) EPSP tracers pre- and post-HFS for MSNs recorded from naive rats (VL striatum) with or without antagonists. The LTP in VL striatum, which was induced in the presence of magnesium, depended on the DA D1 receptor and GluN2A and GluN2B containing NMDA receptors. All of the applied drugs (applied 10 min pre-HFS) blocked the induction of LTP (naive vs. drugs, treatment effect  $F_{(4,342)} = 76.22$ ,  $P < 0.001$ ; time effect  $F_{(17,342)} = 2.545$ ,  $P < 0.001$ ; interaction  $F_{(68,342)} = 1.277$ ,  $P > 0.05$ ; at least  $*P < 0.05$  for all of the drugs). (B) Western blot analysis of the homogenate from VL striatum, naive rats, showed increased protein levels of glutamate receptor subunits GluN2A, GluN2B, GluA1, and GluA2 compared with DL striatum.  $*P < 0.05$ ,  $**P < 0.01$ . (C) Western blot analysis of the Triton-insoluble postsynaptic fraction (TIF) showed increased localization of GluN2B, GluA1, and GluA2 subunits to the postsynaptic site in VL compared with DL striatum.  $*P < 0.05$ ,  $**P < 0.01$ ,  $***P < 0.001$ . The same amount of protein was loaded per lane in both B and C. Shortenings: DL, dorsolateral; EPSP, excitatory postsynaptic potentials; MSN, medium-sized spiny neurons; VL, ventrolateral.

the NMDA and AMPA receptor subunits in the postsynaptic compartment using Triton-insoluble postsynaptic fraction (TIF) analysis. Western blot of total cell lysate of the DL and VL quadrant showed higher expression levels of the NMDA receptor subunits GluN2A and GluN2B as well as the AMPA receptor subunits GluA1 and GluA2 in the VL striatum compared with the DL striatum (Fig. 3B) ( $*P < 0.05$ ,  $**P < 0.01$ ,  $n = 5$  rats). Western Blot performed in the postsynaptic fraction further revealed higher expression of GluN2B, GluA1, and GluA2 subunits in VL striatum (Fig. 3C) ( $*P < 0.05$ ,  $**P < 0.01$ ,  $***P < 0.001$ ,  $n = 5$  rats). Notably, the expression levels of GluN2A subunit were not different in the postsynaptic compartment in VL striatum ( $P > 0.05$ ).

**Grafted Dopaminergic Neurons Can Restore the Synaptic Plasticity in Region Close to the Graft.** The degree of striatal DA innervation distinctly affects the different forms of synaptic plasticity expressed by MSNs (12). Long-term potentiation in the DL striatum requires a rich DA innervation and is absent in both the fully and partially denervated brain. In contrast, LTD is still present in the same region following a partial DA denervation of the striatum (12). Both types of plasticity can be restored after chronic L-DOPA treatment in animals that do not develop dyskinesia (25, 26). It is so far unknown whether transplanted DA neurons can either alter or restore these two forms of synaptic plasticity after DA denervation. Moreover, the impact of 5-HT neurons, that are often included in the transplants, has never been evaluated in the DA-denervated striatum.

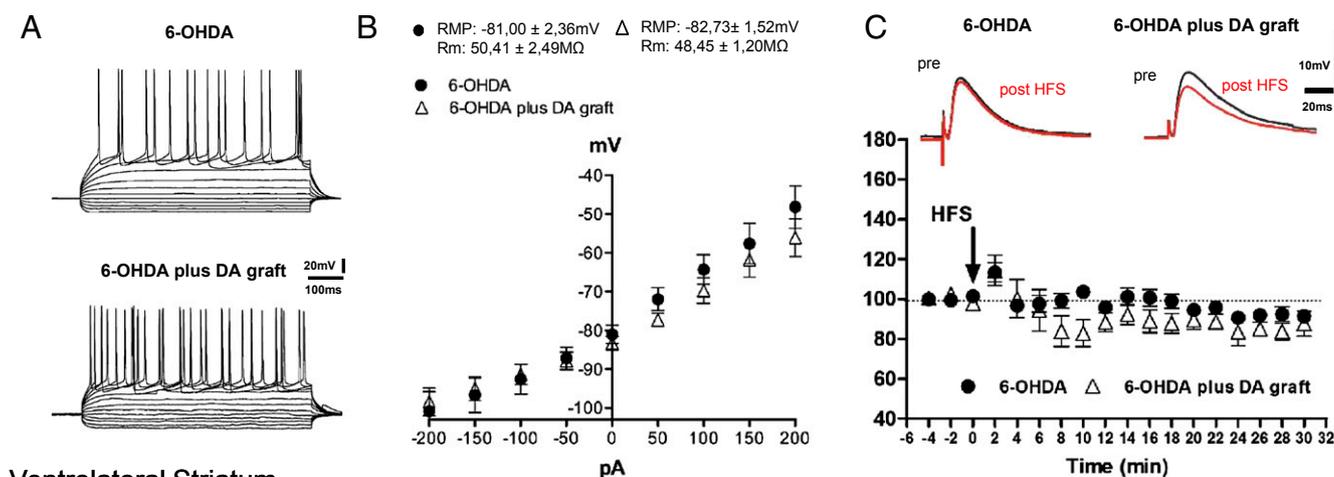
We recorded the synaptic plasticity of host MSNs innervated by transplanted DA and 5-HT precursor neurons. Recordings were performed 7–10 wk posttransplantation, an interval at which the transplant-induced motor improvement is clearly detectable

(27). Medium-sized spiny neurons were selected both in the VL striatum, a region proximal to the grafted core where the grafted neuronal fibers are known to form synapses (5), as well as in the more distal region, i.e., the DL striatum.

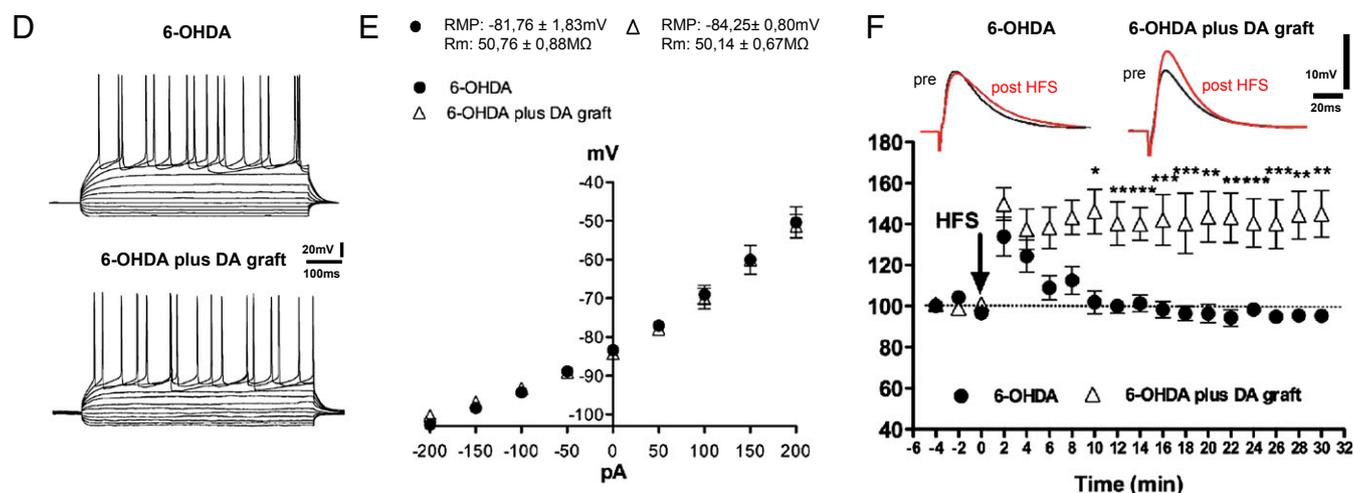
In the DL striatum, the MSNs of 6-OHDA-lesioned DA-grafted rats showed no change in intrinsic membrane properties, resting membrane potential, and membrane resistance compared with the 6-OHDA-lesioned group (Fig. 4A and B) (6-OHDA, RMP  $-81.00 \pm 2.36$  mV, Rm  $50.41 \pm 2.49$  M $\Omega$ ; 6-OHDA plus DA graft, RMP  $-82.73 \pm 1.52$  mV, Rm  $48.45 \pm 1.20$  M $\Omega$ ,  $P > 0.05$ ,  $n = 5$  cells from five rats for 6-OHDA and  $n = 6$  cells from four rats for 6-OHDA plus DA graft). In this region, the grafted DA neurons were not able to restore the deficiency in synaptic plasticity caused by the DA denervation (Fig. 4C) (6-OHDA plus DA graft, EPSP pre- vs. post HFS,  $P > 0.05$ ,  $n = 7$  cells from six rats), and the DA-grafted group did not differ from the 6-OHDA group with sham graft (Fig. 4C) (sham graft vs. 6-OHDA plus DA graft,  $P > 0.05$ ) (Fig. 5).

In the VL striatum, there was no difference in the intrinsic membrane properties of MSNs between the 6-OHDA and DA-grafted rats (Fig. 4D and E) (6-OHDA, RMP  $-81.76 \pm 1.83$  mV, Rm  $50.76 \pm 0.88$  M $\Omega$ ; 6-OHDA plus DA graft, RMP  $-84.25 \pm 0.80$  mV, Rm  $50.14 \pm 0.67$  M $\Omega$ ,  $P > 0.05$ ,  $n = 7$  cells from six rats for 6-OHDA and  $n = 5$  cells from four rats for 6-OHDA plus DA graft). In this region, however, DA-transplanted neurons were able to restore LTP in a number of neurons (Fig. 4F) (6-OHDA  $n = 7/7$  vs. 6-OHDA plus DA-grafted group  $n = 8/19$  cells from 14 rats, treatment effect  $F_{(1,221)} = 11.44$ ,  $P = 0.005$ ;  $*P < 0.05$ ,  $**P < 0.01$ ,  $***P < 0.001$ ) to the same extent as in naive animals (150% increase in EPSP amplitude) (Fig. 5). For proportional expression of LTP in the different groups in DL and VL, see Fig. 5.

## Dorsolateral Striatum



## Ventrolateral Striatum



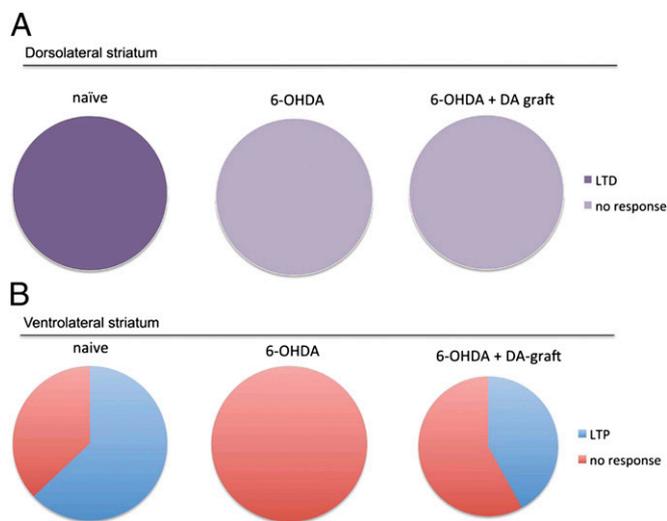
**Fig. 4.** Grafted DA neurons can restore LTP close to the graft. (A) Illustration of the firing discharge in MSN of 6-OHDA-lesioned and 6-OHDA+DA-grafted animals recorded in DL striatum, i.e., distal to the grafted site. (B) The current-voltage (IV) relationship as evoked by injection of increasing current amplitude did not differ in MSN recorded from DL striatum 6-OHDA versus 6-OHDA DA-grafted animals ( $P > 0.05$ ). (C) Grafted DA neurons were not able to restore LTP after 6-OHDA lesion in DL striatum (6-OHDA vs. 6-OHDA+DA graft,  $P > 0.05$ ). Traces for the evoked EPSP pre- and post-HFS in DL striatum are shown for both groups, i.e., 6-OHDA and 6-OHDA+DA-grafted group. (D) Firing discharges evoked by increasing amplitude of current, MSN from rats with 6-OHDA lesion, with or without DA transplants, VL striatum, i.e., close to the grafted site. (E) Current-voltage relationship did not differ between MSN from 6-OHDA and 6-OHDA+DA-grafted VL striatum ( $P > 0.05$ ). (F) In VL striatum, grafted DA neurons were able to restore LTP in a number of cells significantly differing from 6-OHDA group (6-OHDA vs. 6-OHDA+DA-grafted group, treatment effect  $F_{(1,221)} = 11.44$ ,  $P = 0.005$ ; time effect  $F_{(17,221)} = 2.545$ ,  $P < 0.001$ ; interaction  $F_{(17,221)} = 6.815$ ,  $P < 0.001$ ; \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ ). To the right the upper panels represent the traces for the evoked EPSP pre- and post-HFS. DA, dopamine; DL, dorsolateral; EPSP, excitatory postsynaptic potential; VL, ventrolateral.

In contrast to the DA transplants, 5-HT-transplants were not able to restore synaptic plasticity either close or distal to the grafts (Fig. S2B) (6-OHDA vs. 6-OHDA plus 5-HT graft,  $P > 0.05$ ,  $n = 7$  cells from six rats). Immunohistochemical examination of the 5-HT grafts revealed the presence of a rich serotonergic innervation growing from the grafted core into the VL region (Fig. S2C). On the other hand, these grafts did not provide any significant TH-positive reinnervation (Fig. S2D), in agreement with previous studies (28, 29).

**Graft Survival and Symptomatic Effect.** To estimate whether the graft-induced restoration of LTP in the VL striatum correlated with a therapeutic effect in the parkinsonian grafted animals, forepaw akinesia was monitored using the cylinder test from 4 wk before to 10 wk after transplantation (Fig. 1). In line with previous studies (30, 31), the 6-OHDA lesion reduced the use of the

contralateral paw (measured as the number of supporting touches performed against the cylinder's wall) (Fig. 6A and B) (6-OHDA  $n = 4$  rats vs. naïve  $n = 6$  rats, \*\*\* $P < 0.001$ ). Dopamine transplants were able to partially restore this deficiency in all of the grafted rats, an effect that reached significance at 7–9 wk post-transplantation (Fig. 6A and B) (6-OHDA plus DA graft  $n = 8$  rats vs. 6-OHDA, \*\*\* $P < 0.01$ ).

To verify cell survival and fiber growth from the DA transplants in the striatum, TH immunohistochemistry was performed on the recorded sections. Fig. 6C–H shows the results of TH optical density analysis from the DA-grafted, 6-OHDA and naïve groups. The 6-OHDA-neurotoxin injection caused a severe loss of striatal DA fibers down to 10% of control levels (Fig. 6C and D) ( $P < 0.05$  naïve from five rats vs. 6-OHDA from four rats). Dopaminergic neuronal transplantations partially restored TH levels to  $40 \pm 2.89\%$  of controls in the whole striatum (Fig. 6F).



**Fig. 5.** Summary of the synaptic plasticity outcome in the different experimental groups after tetanic stimulation in physiological condition. (A) The proportional synaptic plasticity outcome in the dorsolateral striatum. The dark purple color represents the proportion of MSNs that showed LTD, 100% in naive, 0% of the 6-OHDA, and 0% of 6-OHDA+DA-grafted rats. In the ventrolateral striatum, close to the grafted site (B), the blue color represents the proportion of MSNs that showed LTP, 63% in naive, 0% of the 6-OHDA, and 42% of the 6-OHDA+DA-grafted rats. LTD, long-term depression; LTP, long-term potentiation; MSN, medium-sized spiny neurons.

As expected, the reinnervation was higher in the VL striatum, ( $68 \pm 7.92\%$ ) compared to the DL striatum, ( $45 \pm 4.07\%$ ) (Fig. 6G) (6-OHDA plus DA graft from twenty rats vs. 6-OHDA,  $***P < 0.001$ ; VL striatum vs. DL striatum,  $*P < 0.05$ ).

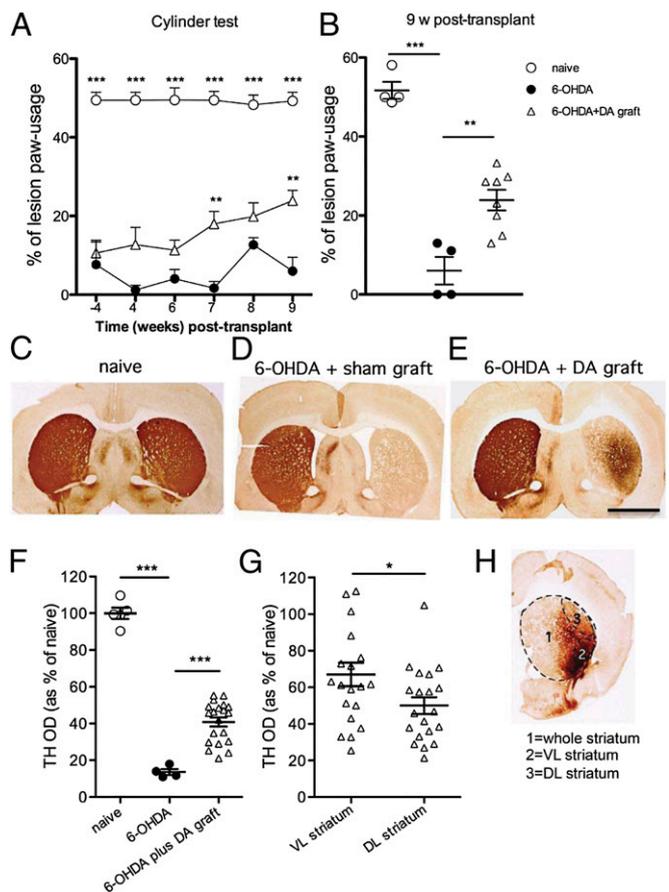
## Discussion

Grafted DA neurons can survive for more than 20 y in the host brain, with possible therapeutic benefits in PD patients (32, 33). Although a small proportion of grafted TH neurons develop Lewy body pathology, this effect does not seem to interfere with the dopaminergic functional restoration produced by the grafts up to 15–20 y posttransplantation (34). With limited treatment options available for patients in the later stages of the disease, DA-cell transplantation could be a very effective approach to PD treatment. Herein, we show that transplanted DA neurons are able to integrate into the host brain microcircuit, restoring corticostriatal synaptic plasticity in a highly reinnervated region. In experimental animal models, PD symptoms are linked to alterations in both LTP and LTD (25, 26, 35). Thus, the ability to restore corticostriatal synaptic plasticity could help determine the therapeutic potential of novel interventions, including cell transplants.

The striatum includes multiple forms of synaptic plasticity that are developmentally regulated and region-dependent (36). Whereas most previous animal studies have focused on either the nucleus accumbens (NA) (23, 37) or the DL striatum (10), this study shows a previous undiscovered region-specific form of synaptic plasticity in the VL striatum. A protocol that induces LTD in the DL striatum led to LTP in a majority of MSNs in the VL region. The induction of LTP under these conditions was unexpected and opened up the possibility for an NMDA receptor-independent mechanism (37). However, our pharmacological experiments demonstrated that the LTP in the VL striatum was NMDA-dependent. Earlier studies have demonstrated that LTP can be facilitated or induced in the adult stage when magnesium is removed from the perfusion solution (8, 38), resulting in a more efficient activation of the NMDA receptors. However, in NA,

this form of synaptic plasticity can also be induced in the presence of magnesium (23). Thus, various conditions—such as presynaptic inputs, the depolarization state of the neurons, the state and activation of glutamate receptors—all together determine the activation and depolarization of the neurons for induction of the NMDA-dependent LTP (9). The fact that both GluN2A and GluN2B receptor subtypes contributed to LTP in the VL striatum in this study suggests a similarity of plasticity features between this striatal region and NA (23).

Several functional compartments could influence the region dependence of synaptic plasticity. The striatum can be divided into several functional compartments based on, e.g., different cortical afferents, the patch and matrix compartments, or the direct or indirect pathway of the MSNs. The differences in synaptic plasticity between DL and VL striatum might be the result of a complex interaction between different fibers innervating the



**Fig. 6.** Striatal innervation and behavioral effect of DA-grafted neurons. (A) Forepaw akinesia (as measured by cylinder test) was induced by 6-OHDA lesion and could be gradually improved in the rats by DA grafts (naive, 6-OHDA, 6-OHDA+DA graft, treatment effect  $F_{(2,75)} = 201.2$ ,  $P < 0.001$ ; time effect  $F_{(5,75)} = 1.891$ ,  $P = 0.106$ ; interaction  $F_{(10,75)} = 1.614$ ,  $P = 0.119$ ;  $**P < 0.01$ ,  $***P < 0.001$ ). (B) At 9 wk posttransplantation, the grafted DA neurons had improved the motor performance significantly from the 6-OHDA group ( $**P < 0.01$ ). (C–E) Photos from TH immunohistochemical labeling illustrating the DA denervation and reinnervation in the striatum from naive (C), 6-OHDA (D), and 6-OHDA+DA-grafted rat (E). (Scale bar: 5mm.) (F) 6-OHDA caused a severe depletion of DA innervation in the striatum as measured by the TH optical density. Transplantation of DA grafts was able to reinnervate the striatum significantly compared to the 6-OHDA group and reached 39% compared with naive rats as measured by TH optical density (6-OHDA vs. 6-OHDA+DA graft,  $***P < 0.001$ ). (G) Dopaminergic fiber innervation was highest in the VL striatum, close to the grafted core, being significantly higher compared with the DL striatum ( $*P < 0.05$ ). (H) A coronal section of the DA-grafted rat striatum with TH-immunopositive staining, illustrating the regions selected for analysis. DL, dorsolateral; VL, ventrolateral.

two striatal compartments (sensorimotor and limbic cortices) as well as distinct postsynaptic signals. There is a commonly accepted view of the dorsolateral (DL or putamen) striatum, receiving terminals from sensorimotor cortex, being responsible for procedural and stimulus-response learning whereas the ventromedial striatum is involved in limbic functions (39). Therefore, it is plausible that, in the VL part, we found an intermediate effect mediated by afferents arising from the sensorimotor cortex and the proximity of the VL area to the more ventromedial zone (NA). Moreover, different striatal striosome and matrix compartments within the striatum also might have relevant implications in the distinct synaptic plasticity patterns found in the two anatomical regions. Nevertheless, the MSNs in DL striatum and NA present uniform general intrinsic and synaptic neurophysiological characteristics (39, 40), and morphological features, electrical membrane properties, and corticostriatal excitatory postsynaptic potentials are similar in MSNs recorded from patch and matrix (41). In line with this study, we assume that, also in our analysis, MSNs in these two distinct morphological compartments (matrix and striosomes) show similar intrinsic and synaptic properties, although they express different molecular markers. Future studies are required to identify the differential expression of LTP and LTD in distinct striatal regions according to the afferent input and matrix and striosome distribution in both physiological and pathological conditions.

The fact that not all of the MSNs recorded from naïve animals in this study showed LTP and the observation that the potentiation was D1 receptor-dependent could also be dependent on the segregation of D1 and D2 DA receptors in two distinct groups of striatal neurons: striatonigral D1R-expressing MSNs and striatopallidal, D2R-expressing MSNs (42). However, our complementing experiments showed that both responses are evident in striatonigral MSNs to similar proportions as in the total number of neurons recorded (i.e., 67% versus 63%), indicating that the induction of LTP is not pathway-specific in the VL striatum. Instead, distinguished state and activation of the glutamate receptors during tetanic stimulation could condition the long-term synaptic response in this region.

In line with the pharmacological experiments, the VL striatum showed higher GluN2B expression levels compared with the DL part. Recent findings suggest that GluN2 subunits have an important role in the induction of LTP within both hippocampus (22) and striatum (36, 43, 44). A modified expression of GluN2B at the corticostriatal synapse could also influence the expression of motor symptoms in PD (45, 46). In addition, the higher postsynaptic expression of GluA1 and GluA2 in the VL striatum suggests a larger AMPA contribution to the postsynaptic events. Higher AMPA receptor expression is expected to enhance the cation influx after afferent stimulation and produce a greater depolarization of the MSN membrane, removing the magnesium blockade from the NMDA receptors and facilitating the induction of LTP. Also, region-specific alterations in NMDA subunit composition found in the postsynaptic density fraction could explain the distinct synaptic plasticity patterns by shifting the probability and degree of LTP induction (47, 48).

**DA Grafted Neurons Can Therapeutically Restore Plasticity in the Rat Striatum.** Dopaminergic neuronal grafts have previously been shown to partially restore spontaneous neuronal firing in striatum in discrete striatal domains up to 2 mm in diameter from the core of the grafted area (49, 50). Embryonic grafts implanted in the striatum of a Huntington disease model are also able to restore synaptic transmission within the grafted core (51). The present study provides evidence of a restoration of long-term striatal synaptic plasticity in striatal neurons after grafting embryonic DA neurons in experimental PD. The graft-induced restoration was present within a distance of 1–1.5 mm from the core of the grafts, a region densely innervated by the transplanted neurons. Results

from animals with partial 6-OHDA lesions have shown that the induction of LTP is critically dependent on a rich DA fiber innervation (12). In this study, also, the more distal region examined, i.e., the DL striatum, was reinnervated by the grafted DA fibers, although to a lesser extent (about 45%) (Fig. 6E). Still, a partial DA innervation would hypothetically preserve the induction of LTD (12). Our result of an absent LTD in the DL striatum points to functional differences between a graft-derived dopaminergic innervation and the residual endogenous DA transmission that persists after a partial lesion (12). Indeed, it has been demonstrated that striatal dopaminergic grafts that reinnervate striatum form aberrant connections with the host MSNs, which are associated with the development of graft-induced dyskinesia (52).

In light of these findings, we cannot exclude that the restoration of synaptic plasticity occurs as a consequence of this altered synaptic rearrangement rather than as a precise reconstruction of the original physiological connections.

**Clinical Implications.** Transplantation of dopaminergic neurons into the striatum can provide long-lasting therapeutic benefit in PD patients. A clinical study has shown a delayed recovery in motor cortical activity occurring first after 18 mo posttransplantation compared with the increase in DA storage capacity that is detected with  $^{18}\text{F}$ -dopa position emission tomography already after 6 mo (53). Also, in animal models of PD, the capacity for DA synthesis and storage in the grafts is detected before a significant improvement in complex sensory motor behavior (1, 54). These data suggest that the function of neural grafts goes beyond that of simple DA delivery and involves more complex mechanisms of functional integration that lead to more substantial clinical recovery. The current experiments suggest that the complex mechanisms most probably involve restoration of synaptic plasticity. Only after some time are synapses and fiber outgrowth from the transplanted neurons formation sufficient to restore the striatal synaptic plasticity deficits associated with the parkinsonian state. Indeed, maturation of the grafts can be a slow process, continuing for many months after initial formation of DA-fiber projections (55), which is also supported by the gradual and protracted recovery of  $^{18}\text{F}$ -dopa uptake in the grafted striatum (56). The clinical outcome after DA cell transplantation thus continues to improve for up to 4 y in patients (57). Taken together, this availability indicates that embryonic DA neurons are able to integrate with the host striatal circuitry, forming anatomically appropriate connections capable of influencing host behavior (32, 56, 58).

Despite these positive clinical findings, there are currently major limitations with the transplantations approach. In the clinical trials conducted so far with DA neurons, the transplants have shown variable effects on both motor improvement and dyskinesia. In some patients, a significant reduction of L-DOPA-induced dyskinesia has been observed whereas, in others, the dyskinesias have been unaffected or worsened (59–63). Moreover, two randomized double-blind placebo-controlled trials demonstrated that fetal nigral transplants with one or four donors per side did not significantly improve the motor features in PD patients but instead induced graft-induced dyskinesia (64, 65). The technique needs refinements before it can be successfully performed in a large series of patients (66).

In this study, the restoration of synaptic plasticity seen after DA neuron transplantation demonstrates that grafted DA neurons are able to restore neuronal network activity in the PD striatum and that this synaptic recovery is accompanied by improved motor function. The restoration in synaptic plasticity was limited to the most richly reinnervated region, possibly explaining the limited efficacy of DA transplants to alleviate clinical symptoms in certain cases (49, 50, 67). The relationship between synaptic restoration and grafted fiber outgrowth would favor

a multisite grafting procedure compared with a single or few-site grafting. Indeed, multisite transplantation of DA ventral mesencephalic cells has shown excellent functional recovery and lower incidence of graft-induced dyskinesia compared with protocols producing unbalanced increases of dopaminergic innervation (so-called “DA hotspots”) (16, 68). This notion is supported by a clinical PET study (69).

The results of the present study also show that 5-HT-grafted neurons do not restore synaptic plasticity in experimental PD. The absence of both LTP and LTD both close and distal to these grafts fits well with the absence of motor recovery seen after this type of transplants (28, 70) and supports the need of a rich DA innervation for restoration of LTP.

The presence of 5-HT-grafted neurons in the striatum that do not form synaptic contacts to the same extent than DA-grafted neurons (71) but still have the capability of releasing excessive amounts of unregulated DA after administration of L-DOPA may support a development of posttransplantation dyskinesia (28). Accordingly, recent PET imaging studies has reported a prominent graft-derived 5-HT innervation (56) and a higher 5-HT/DA ratio in a patient with graft-induced dyskinesia (72).

In conclusion, our data present evidence of region-specific, DA-dependent restoration of synaptic plasticity after neuronal transplantation therapy in experimental PD. The results support the hypothesis that this restoration is an indicator of a successful graft integration underlying motor improvement. Moreover, these results reveal a previously unappreciated difference in plasticity features between the DL and VL striatum, where the latter region appears particularly prone to DA-dependent synaptic plasticity.

## Materials and Methods

**Hydroxydopamine Lesion.** Adult female Sprague–Dawley rats (225 g; Harlan;  $n = 162$ ) were housed under a 12-h light/dark cycle, with ad libitum access to food and water. Animal care and experimental treatments were approved by the Malmö-Lund Ethical Committee on Animal Research and by the Italian Health Ministry. A total of 7.5 and 6  $\mu\text{g}$  of free-base 6-hydroxydopamine (6-OHDA-HCl) were injected into the right ascending DA fiber bundle at two coordinates according to our standard procedure (73, 74). Two weeks post surgery (Fig. 1), an amphetamine-induced rotation test (2.5 mg/kg *D*-amphetamine *i.p.* 90 min recording) was applied to select rats with >90% striatal DA depletion (corresponding to >5 full turns/min ipsilateral to the lesion) (75).

**Embryo Dissection and Transplantation Procedure.** Seven weeks after 6-OHDA lesion, animals (Fig. 1) were transplanted with either DA- or 5-HT-grafts. Time-mated pregnant female Sprague–Dawley rats (Charles River) were anesthetized with lethal dose of  $\text{CO}_2$ , and the embryonic sacs were taken out. Fetal cells were dissected from E14 embryos according to Carlsson et al. (28). A special dissection protocol was used to obtain cell suspension rich in either DA or 5-HT neuroblasts (28). The dissected tissue pieces were incubated in HBSS containing 0.1% trypsin and 0.05% DNase for 30 min at 37 °C and mechanically dissociated to single-cell suspension, concentrated by centrifugation. The total number of cells were counted by trypan blue viability staining. Cells were then resuspended to 130,000 cells per  $\mu\text{L}$  in HBSS plus 0.05% DNase. Sham suspension consisted of HBSS.

Transplantation surgery was performed under Fentanyl-Dormitor anesthesia using a 5- $\mu\text{L}$  Hamilton syringe fitted with a glass capillary (outer diameter 60–80  $\mu\text{m}$ ) using the same coordinated as previously used for behavior and dyskinesia studies in the rat model (16–19). Using these coordinates, the neurons innervate the lateral striatum and improve the motor deficits (16, 18). The 6-OHDA-lesioned rats received injection of 130,000 DA or 5-HT-rich tissue through one needle penetration at two deposits (DV coordinates –5.0 mm and –4.0 mm). One group of animals received only the sham suspension (Fig. 1). The following coordinates were used: AP +0.2 mm and ML –3.5 mm. Tooth bar was set to 0.0 mm. After injection, the syringe was left in place for 2 min before being retracted slowly. Animals received systemic analgesic post surgery.

**Cylinder Behavior.** The cylinder test of forelimb use asymmetry (30) was used to assess the antiakinetin effect of the DA-transplanted neurons (Fig. 6). Each rat was placed in a glass cylinder, and the number of full appositions of the

left and right forepaws to the cylinder wall was counted during a 5-min observation period (26, 31). The number of touches with left forepaw (contralateral to the lesion) was compared with the number of total touches and expressed as a percentage. The cylinder test was performed once a week in a dim-lighted room in the afternoon.

**Retrograde Tracer Injections.** Seven weeks after transplantation, the rats underwent surgery for retrobead injection (Lumafuor). Briefly, rats were anesthetized using Fentanyl Dormitor and put in a stereotaxic frame. The following coordinates were used to target the Substantia Nigra pars reticulata: AP, –5.3; ML, +1.8 and +2.7; DV, 7.5 and 7.1. The retrobeads were dissolved 2 times in milliQ aqua according to the suggestions of the supplier and injected using a 5- $\mu\text{L}$  Hamilton syringe on which a glass capillary was attached. Two deposits of 0.5  $\mu\text{L}$  of solution were injected during 3 min, and the capillary was left in place for 3 min after the injection before being slowly retracted. Animals were given analgesic and antidote directly after the surgery.

**Patch-Clamp Electrophysiology.** Animals were killed by cervical dislocation after halothane anesthesia 7–10 wk posttransplantation, a time sufficient for the transplanted neurons to integrate into the striatum and partially restore behavioral deficits (27) (Fig. 1). Brains were rapidly taken out, and coronal slices were vibratome-cut at 240  $\mu\text{m}$ . Slices were transferred to a recording chamber and submerged in a continuously flowing Krebs solution gassed with 95%  $\text{O}_2$ –5%  $\text{CO}_2$ . The composition of the standard solution was (in mM): 126 NaCl, 2.5  $\text{MgCl}_2$ , 1.2  $\text{NaH}_2\text{PO}_4$ , 2.4  $\text{CaCl}_2$ , 11 Glucose, 25  $\text{NaHCO}_3$ . For synaptic stimulation, bipolar electrodes were placed in the striatum, in close proximity to the recorded neuron, to activate corticostriatal fibers.

Whole-cell recordings were performed using 1.5-mm external diameter borosilicate pipettes. Current clamp electrodes were performed using electrodes (2.8–7 M $\Omega$ ) filled with the following intracellular solution (in mM): 125 potassium gluconate, 15 KCl, 0.04 EGTA, 12 Hepes, 2  $\text{MgCl}_2$ , 4 MgATP, and 0.4  $\text{Na}_2\text{GTP}$ , adjusted to pH 7.3 with KOH.

Medium-sized spiny neurons (MSNs) were identified by somatic size and typical basic membrane properties (input resistance, membrane capacitance, and time constant). Current–voltage relationships were obtained by applying steps of current of  $\Delta 50$  pA in both depolarizing and hyperpolarizing direction (from –200 to 200 pA, 500 ms). Induction of long-term plasticity was performed in current-clamp mode. Patched cells that exhibited the typical intrinsic membrane properties of MSNs (extracted from the firing discharges) were recorded for studies of long-lasting synaptic plasticity. For induction of long-term changes of excitatory postsynaptic potentials (EPSPs), three high frequency stimulation (HFS) trains of 3 s at 100 Hz were delivered at 20-s intervals in the surrounding striatal tissue. Before tetanic stimulation, the intensity was increased to threshold levels. All of the experiments were conducted in the continuous presence of the GABA<sub>A</sub> antagonist picrotoxin (50  $\mu\text{M}$ ). Recordings were made using Multiclamp 700B (Molecular Devices), and signals were acquired at 10 kHz using pClamp10 software and a data acquisition unit (Digidata 1440A; Molecular Devices). Input resistances and injected currents were monitored throughout the experiments. Variations of these parameters by >30% led to the rejection of the experiment. Values are represented as mean  $\pm$  SEM of EPSP peak amplitude. The involvement of specific receptors in the induction of synaptic plasticity in VL striatum was studied during the electrophysiological recordings by *in vitro* bath application of the following antagonists: APV, 30  $\mu\text{M}$  (NMDA antagonist), SCH23390 (3  $\mu\text{M}$ , DA D1 receptor antagonist), ifenprodil (3  $\mu\text{M}$ , GluN2B antagonist), NVP-AAM077 (300  $\mu\text{M}$ , GluN2A antagonist). The drugs were added to the recorded ACSF 10 min before the application of HFS.

**Western Blot Analysis.** For Western Blot (WB) analysis, naïve rats were killed, and coronal sections were cut on a vibratome in oxygenized buffer. In coronal sections, VL and DL striatums were then dissected and directly put on dry ice for WB analysis on either total cell homogenate or Triton-insoluble postsynaptic Fractions (TIFs). The following antibodies were used: polyclonal GluN2A antibody (Sigma-Aldrich); monoclonal GluN2B antibody (NeuroMab); polyclonal GluA1 antibody (Merck Millipore); monoclonal GluA2 antibody (NeuroMab); and monoclonal tubulin antibody (Sigma-Aldrich). Subcellular fractionation of VL or DL striatal tissue was performed as previously described in Gardoni et al. (45) with few modifications. Briefly, striatal tissue was homogenized in ice-cold sucrose 0.32 M containing (in mM): 1 Hepes, 1  $\text{MgCl}_2$ , 1 EDTA, and 1  $\text{NaHCO}_3$ , 0.1 PMSF, pH 7.4. The homogenized tissue was centrifuged at 1000  $\times g$  for 5 min. The resulting supernatant was centrifuged at 13,000  $\times g$  for 15 min to obtain a crude membrane fraction. The pellet was then resuspended in buffer containing 75 mM KCl and 1% Triton X-100 and centrifuged at 100,000  $\times g$  for 1 h. The final pellet, referred to as Triton-Insoluble postsynaptic Fraction (TIF), was

homogenized in a glass-glass potter in 20 mM Hepes and stored at  $-80^{\circ}\text{C}$  until processing. TIF was used instead of the classical postsynaptic density because the amount of the starting material was very limited. All purifications were performed in the presence of a complete set of protease and phosphatase inhibitors (Roche Diagnostics). Protein content of the samples has been quantified by using a Bio-Rad protein assay. After measuring protein concentration, all samples have been standardized at  $1\ \mu\text{g}/\mu\text{L}$  concentration and  $10\ \mu\text{g}$  per sample loaded in each lane. Quantification of Western blotting analysis has been performed by means of computer-assisted imaging (ChemIDoc system and Image laboratory 4.0 software; Bio-Rad) after normalization on tubulin levels.

**Immunohistochemistry.** After the electrophysiological recordings, striatal slices were collected and fixed overnight in 4% paraformaldehyde before being cut into thinner slices and immunohistochemically stained for detection of DA-fiber innervation. Immunohistochemistry was performed as detailed in Westin et al. (2007) (74) using a peroxidase-based detection method and 3–3-diaminobenzidine (Sigma-Aldrich) as the chromogen and tyrosine hydroxylase or serotonin transporter (TH, Pel-Freeze 1:1,000; Millipore 1:800) as primary antisera. Two to four slices, where applicable, were used for quantitative analysis, representing rostro-caudal levels 1.8–0.2 mm anterior to Bregma (76). Optical density measurements of TH immunostaining were obtained using Image J software. Regions of interests included whole, VL, and DL striatum as illustrated in Fig. 4. At levels where graft core was

visible, the level was excluded from delineation. Corpus callosum was taken as the background. All data are expressed as percentage of naïve control in the same experiment of rats.

**Drugs.** Drugs were applied by dissolving them to the desired final concentration in the external solution and by switching the perfusion from control saline to drug-containing saline. APV and SCH23390 were from Sigma-Aldrich. Ifenprodil was from Tocris-Cookson. NVP-AAM077 was kindly provided by Yves Auberson (Novartis, Basel).

**Data Analysis.** Statistical analysis was performed using Graph Prism 4 Software. Group comparisons were analyzed with two-way ANOVA. When a significant interaction was observed, the data were further analyzed with a Bonferroni post hoc test. Data analysis of EPSP amplitude pre- versus post-HFS between the same group of cells was analyzed using a paired *t* test. Data are expressed as mean  $\pm$  SEM. Statistical significance was set at  $P < 0.05$ .

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