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 regions. 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. Naive 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 NMDA subunits (GluN2B) and-dependant 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 depletions 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 dopamnergic reinnervation was denser, compared with the distal region. These data support the idea that functional integration of host and grafted neurons 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 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.

6-OHDA lesion | DA | S-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., AMPA and N-methyl-D-aspartate (NMDA) receptors, as well as 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 functional and morphological striatal improvements in DA-stimulated patients with PD.

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 in PD model, playing a role in both axial movements 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 discharges firing patterns, resting membrane potential (RMP), and membrane resistance (Rm) in physiological condition, i.e., in naïve rats (Fig. 2A and D) (DL, RMP −84.0 ± 1.2 mV and Rm 49.4 ± 0.8 MΩ; VL, RMP −84.6 ± 1.0 mV, Rm 50.5 ± 0.6 MΩ, 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. 2A, B, D, and E) (DL, 6-OHDA, RMP −81.04 ± 2.36 mV, Rm 50.41 ± 2.49 MΩ; VL, 6-OHDA, RMP −81.76 ± 1.83 mV, Rm 50.76 ± 0.88 MΩ, 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. 2C) (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. 2C) (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. 2F) (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](https://www.pnas.org/cgi/doi/10.1073/pnas.1311187110 Rylander et al.)

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.
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) (naive vs. 6-OHDA from n = 6 rats), 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 naive versus 6-OHDA rats. DA, dopamine; DL, dorsolateral; VL, ventrolateral.

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) (naive vs. naive 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) (naive 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 NMDA receptor subunits (Fig. 4).
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 compartment further revealed higher expression of GluN2B, GluA1, and GluA2 subunits in the VL striatum compared with the DL 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 and DL 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 ± 2.36 mV, Rm 50.41 ± 2.49 MΩ; 6-OHDA plus DA graft, RMP = –82.73 ± 1.52 mV, Rm 48.45 ± 1.20 MΩ, \( P > 0.05, n = 5 \) 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 ± 1.83 mV, Rm 50.76 ± 0.88 MΩ; 6-OHDA plus DA graft, RMP = –84.25 ± 0.80 mV, Rm 50.14 ± 0.67 MΩ, \( P > 0.05, n = 7 \) 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 \( F_{(1,22)} = 11.44, P = 0.005; \)

\( *P < 0.05, **P < 0.01, ***P < 0.001 \) 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.
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+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 ± 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 ± 7.92%) compared to the DL striatum, (45 ± 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 the striatum and could be gradually improved in the rats by DA grafts (naïve, 6-OHDA, 6-OHDA+DA graft, treatment effect F(2,75) = 1.614, P = 0.106; interaction F(10,75) = 1.891, P = 0.119; ***P < 0.001, **P < 0.01, *P < 0.05). 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 naïve (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 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 naïve rats as measured by TH optical density. TH immunohistochemical labeling was highest in the VL striatum, close to the grafted core, being significantly higher compared with the DL striatum (**P < 0.01). (G) Dopaminergic fiber innervation was highest in the VL striatum, showing a previous undiscovered region-specific synaptic plasticity in PD patients (32, 33). 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 striatum.
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 different input and matrix and striosome distribution in both pathological and pathological conditions. The fact that not all of the MSNs recorded from naive 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, 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 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 18F-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 18F-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 μg of free-base 6-hydroxydopamine (6-OHDA-HCl) was 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 (75) 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 GABAB antagonist, picrotoxin (50 μM). 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 ± SEM of EPSP peak amplitude. The involvement of somatic type NMDA receptors in the induction of synaptic plasticity in VL striatum was studied during the electrophysiological recordings by in vitro in bath application of the following antagonists: APV, 30 μM (NMDA antagonist), SCH23390 (3 μM, DA D1 receptor antagonist), ifenprodil (3 μM, GluN2B antagonist), NVP-AAM77 (300 μM, GluN2A antagonist). The drugs were added to the recorded ACSF 10 min before the application of HFS.

Embryo Dissection and Transplantation Procedure. Seven weeks after 6-OHDA lesion, animals (n = 162) were transplanted with either DA- or 5-HT-grafts. Time-mated pregnant female Sprague–Dawley rats (Charles River) were anesthetized with lethal dose of CO2, 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 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 at 1000 × g for 15 min to obtain a crude cell fraction. The cell suspension was centrifuged at 100,000 × g for 1 h. The supernatant was decanted and the remaining pellet was resuspended in buffer containing 75 mM KCl and 1% Triton X-100 and centrifuged at 13,000 × g for 15 min to obtain a crude membrane fraction. The cell pellet was then resuspended in buffer containing 75 mM KCl and 1% Triton X-100 and centrifuged at 100,000 × g for 1 h. The final pellet, referred to as Triton-Insoluble postsynaptic Fraction (TIF), was

Cylinder Behavior. The cylinder test of forelimb use asymmetry (30) was used to assess the antiakinetic effect of the DA-grafted 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 (P<0.05), compared to the lesioned side (n = 162), was measured and recorded per 10 s and the total number of 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 retrograde injection (Lumaflo). 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 retrobeses were dissolved 2 times in milliQ aqua according to the suggestions of the supplier and injected using a 5-μL Hamilton syringe on which a glass capillary was attached. Two deposits of 0.5-μL 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 antibiotic 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 vibra-tome-cut at 240 μm. Slices were transferred to a recording chamber and submerged in a continuously flowing Krebs solution gassed with 95% O2−5% CO2, at a temperature of 37–38 °C. The solution had the composition (in mM): 126 NaCl, 2.5 MgCl2, 1.2 NaH2PO4, 2.4 CaCl2, 11 Glucose, 25 NaHCO3. 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 electrodos (2.8–7 MΩ) filled with the following intracellular solution (in mM): 125 potassium gluconate, 15 KCl, 0.04 EGTA, 12 Hepes, 2 MgCl2, 4 MgATP, and 0.4 Na3GTP, 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 ±0.5 pA in both depolarizing and hyperpolarizing direction (from −200 to 200 pA, 500 ms). Induction of long-term plasticity was performed using a current-clamp protocol. 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 GABAB antagonist, picrotoxin (50 μM). 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 ± SEM of EPSP peak amplitude. The involvement of somatic type NMDA receptors in the induction of synaptic plasticity in VL striatum was studied during the electrophysiological recordings by in vitro in bath application of the following antagonists: APV, 30 μM (NMDA antagonist), SCH23390 (3 μM, DA D1 receptor antagonist), ifenprodil (3 μM, GluN2B antagonist), NVP-AAM77 (300 μM, 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 (Neuro-Mab); polyclonal GluA1 antibody (Merck Millipore); monoclonal GluA2 antibody (Neuro-Mab); 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 MgCl2, 1 EDTA, and 1 NaHCO3, 0.1 PMSF, pH 7.4. The homogenized tissue was centrifuged at 1000 × g for 5 min. The resulting supernatant was centrifuged at 13,000 × 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 × 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 °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 μg/L concentration and 10 μg 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, representingrostro-caudal levels 1.8–0.2 mm anterior to Bregma (76). Optical density measurements of TH immunostaining were performed 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, representingrostro-caudal levels 1.8–0.2 mm anterior to Bregma (76).

Drugs. Drugs were applied by dissolving them to the desired concentration in Parkinson’s disease. Biochem Soc Trans 38(2):493–497.

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Fig. S1. Striatonigral MSNs show heterogenous response in VL striatum. (A) Current–voltage relationship did not differ between unidentified MSN from naïve rats and retrobead-labeled MSN that project to the Substantia nigra pars reticulata recorded from VL striatum \((P > 0.05)\). (B) Retrobead-labeled MSN showed LTP in a subset of neurons in VL striatum \((n = 6/9)\) and showed no change in EPSP amplitude in another subset \((3/9)\), illustrating the heterogeneity of this neuronal subtype \(\text{retrobead-injected rats, LTP versus no response; response effect } F_{(1,126)} = 13.09, P = 0.008; \text{ time effect } F_{(18,126)} = 7.912, P < 0.001; \text{ interaction } F_{(18,126)} = 3.027, P < 0.001\). EPSP tracers pre- and post-HFS for MSNs recorded from naïve rats (VL striatum) and retrobead-injected neurons are shown above the diagram. EPSP, excitatory postsynaptic potential; LTP, long-term potentiation; MSN, medium-sized spiny neurons; VL, ventrolateral.
Ventrolateral striatum

Fig. S2. Grafted 5-HT neurons fail to restore the plasticity in VL striatum. (A) Illustration of the firing discharge in MSN of 6-OHDA–lesioned and 6-OHDA+ 5-HT–grafted animals recorded in VL striatum. (B) 5-HT–grafted neurons failed to restore LTP in all of the recorded MSN and remained indifferent from the MSN of the 6-OHDA group (6-OHDA vs. 6-OHDA plus 5-HT–grafted group, treatment effect $F_{(1,204)} = 0.692, P = 0.422$; time effect $F_{(17,204)} = 7.211, P < 0.001$; interaction $F_{(17,204)} = 4.485, P < 0.001$. EPSP, excitatory postsynaptic potential; 5-HT, serotonin. (C) Immunohistochemistry of serotonin uptake transporter (SERT) revealed serotonergic cell bodies (see higher magnification) and a rich 5-HT fiber reinnervation into the VL striatum in the 5-HT–grafted rats after an initial 6-OHDA–induced 5-HT denervation. In contrast, there were no dopaminergic cell bodies (higher magnification) or reinnervation of dopaminergic fibers after the 5-HT grafts (D). The DA denervation was similar to 6-OHDA rats. (Scale bar: 1 mm; and 100 μm in higher magnification.)