Neural stem cells restore cognitive function in Parkinsonian macaques

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Abstract

Cognitive deficits as well as disorders of sleep and biological rhythms constitute non-motor symptoms that significantly impact quality of life in Parkinson's disease patients. Few studies have evaluated the impact of cell replacement therapy on such non-motor symptoms. Here we used a multidisciplinary approach to assess the therapeutic potential of bilateral grafts of neural stem cells in a macaque model of Parkinson's disease on both motor and non-motor markers of functional recovery. Grafts led to varying degrees of functional recovery while sham experiments did not. We show unprecedented recovery from cognitive symptoms in addition to a clear clinical motor recuperation. Motor and cognitive recovery but not circadian rhythm recovery correlated with the degree of graft integration into the host environment and with *in-vivo* levels of striatal dopaminergic transporters and function. This study provides empirical evidence that neural stem cells transplantation efficiently restore function at multiple levels in Parkinsonian non-human primates. We demonstrate the promising potential of multiple-sites neural stem cells grafts for Parkinson's disease but furthermore underline the crucial importance of such multidisciplinary approaches for an effective clinical translation.

Main Text

Parkinson's disease (PD) is a neurodegenerative disease affecting up to 10M of the worldwide population with incidences increasing with age, making PD the fastest growing neurological disorder in a globally aging population¹. The clinical manifestation is primarily motor with a typical parkinsonian motor syndrome *i.e.* bradykinesia, rigidity and resting tremor and, further confirmed with clinical diagnostic tools including *e.g.* *in-vivo* imaging of the nigrostriatal dopaminergic (DA) axis denervation². Unfortunately 60-80% of DA cells are already lost when motor symptoms can be clinically diagnosed³. During the so-called premotor period, preceding the clinical threshold, the DA-lesion progresses slowly and is accompanied by the appearance and worsening of non-motor symptoms such as sleep disorders and cognitive impairments⁴. Current palliative therapies mainly correct motor symptoms and often give rise to behavioral deficits⁵. Continued efforts have been made to use non-motor symptoms as premotor markers for the prognosis of PD⁶. Notwithstanding, non-motor symptoms have a significant impact on quality of life; the cognitive abilities of the vast majority of PD patients deteriorate leading to psychiatric disturbances⁷ and with circadian perturbations affecting biological rhythms⁸. However, few studies have addressed the impact of treatment on non-motor symptoms^{5,9}. It appears increasingly important to evaluate the multi-parametric impact of therapeutic treatment on both motor and non-motor aspects of PD^{10,11}. DA lesion is the hallmark of PD and affects multiple frontal lobe functions (*e.g.* performance monitoring or motivational aspects of behavior), motricity via the extrapyramidal system and, circadian regulation of behavior through alterations in the central suprachiasmatic nucleus clock and in multiple downstream central and peripheral structures¹²⁻¹⁴. The multifaceted DA function requires a longitudinal multi-parametric investigation of deficits and of the therapeutic potential of neural stem cells (NSCs) transplantation in a non-human primate (NHP) preclinical model.

We implemented a multidisciplinary approach to assess the therapeutic potential of bilateral grafts of NSCs¹⁵⁻¹⁷ in parkinsonian macaques (nine *macaca fascicularis*, including three histological controls) on both motor and non-motor markers of functional recovery (Extended Data Fig.1, Extended Data Table 1). We used repeat systemic injections of low-doses of MPTP (0.2 mg/kg every 3-4 days) that induce a parkinsonian syndrome in NHP (*macaca mulatta* and *fascicularis*) with a slow and typically dorso-ventral progression of the nigrostriatal denervation, together with the premotor expression of cognitive and circadian *i.e.* non-motor symptoms^{18,19}. We performed *in-vivo* follow-up of the nigrostriatal lesion with Positron Emission Topography (PET) imaging of DA transporters (DAT) prior to and following the induction of stable motor symptoms¹⁹ as well as following grafts of NSCs. Fluoro-DOPA (F-DOPA) imaging was additionally used to assess NSC effect on DA function²⁰.

We derived NSCs from a Rhesus Embryonic Stem Cell line (LYON-ES1) stably expressing the fluorescent marker tau-GFP^{21,22}. We grafted NSCs neural stem cells (Extended Data Figs.2a-c, 3a, Extended Data Table 2) because of their inherent neuroprotective potential through interaction with host-cells²³ and, their higher survival rate as compared to mature DA cell grafts²⁴⁻²⁶. NSCs show both neuronal and astroglial fate *in-vitro* (Extended Data Fig.2d-f), allowing assessment of the influence of the host-environment on the differentiation fate of transplanted cells. Importantly, no post-graft overgrowth was observed (Extended Data Fig.3b-c) and few CD68+ macrophages detected in the vicinity of transplanted sites despite the absence of immunosuppressive treatment (Extended Data Fig.3d-f).

We adapted cumulated MPTP-doses to individual clinical motor scores (Extended Data Table 3) in order to take into account the known variability in MPTP-sensitivity^{12,18,19}. Clinical scores and behavioral measures were divided into quantiles (Q1-5, see Methods for details) in order to compare groups on the basis of the presumed DA-lesion rather than on the time spent in the premotor (MPTP), motor (post-MPTP/pre-Graft) and post-graft periods. During the pre-Graft period, all cases displayed a stable Parkinsonian syndrome with clinical motor scores ranging from mildly to severely symptomatic (Fig.1a, Extended Data Fig.1a). In two out of the five grafted cases, we failed to observe recovery from motor symptoms and accordingly assigned cases to the *recovered* or *non-recovered* group based on clinical motor outcome following NSC grafts (Fig.1a, Extended Data Fig.1a).

During the pre-graft period, non-motor symptoms became manifest in all groups, and worsened during the premotor period *i.e.* before motor scores reached clinical thresholds (Fig.1a-c, Extended Data Fig.1). Alterations of circadian rhythms progressively appeared during the premotor phase (Fig.1c, Extended Data Fig.1c) to reach peak levels during the symptomatic period as described previously¹². This pattern resembles circadian alterations of the sleep-wake cycle observed in PD patients and manifests as increased daytime sleepiness and fragmented sleep structure²⁷. The premotor alteration of rest-wake locomotor rhythms in all cases exhibited reduced Light/Dark (LD) ratios and relative amplitude, increased rhythm variability and fragmentation (Fig.1c, Extended Data Fig.1c). Perturbations of the sleep-wake cycle varied greatly according to the level of motor impairments¹² and thus, were not all stably expressed during the motor period. In all cases cognitive performances gradually deteriorated during the premotor

period (Fig.1b, Extended Data Fig.1b). Increased errors and reduced successes are mainly due to reduced control over motor planning and parallel executive dysfunctions as observed in PD patients^{4,28}. Cognitive performance was dramatically and persistently altered in all cases during the whole motor period following MPTP intoxication offset (Fig.1b, Extended Data Fig.1b). Poor performances could not be imputed to motor impairment as motor-error trials were excluded from all periods. Hence, we can directly relate reduced cognitive performances to the nigrostriatal DA lesion and altered DA innervation of frontal cortex²⁹. Nigrostriatal innervation decreased by more than 80% relative to control measures of striatal DAT levels, with the ventral striatum being the most preserved region (Fig.2a), as previously reported¹⁹.

We delayed NSC grafting by 3 months on average after the last week of MPTP injections (13 ± 6 weeks, see Methods). In order to potentiate graft efficiency on both motor and non-motor symptoms, we performed multiple bilateral transplantations (Extended Data Table 2, 1.5×10^5 to 1×10^6 NSCs per site, see Methods) in the striatal regions involved in cognitive (*i.e.* anterior Caudate - antCdN) and motor functions (*i.e.* posterior Putamen – post-Put.), as well as at the source of nigrostriatal DA innervation *i.e.* *substantia nigra pars compacta* (SNpc). All grafts were correctly placed (Fig.2f-i, Extended Data Fig.4), displaying rather extensive dendritic projections after a post-graft period of 30 ± 8 weeks (Fig.3k, Extended Data Figs.4, 9).

Transplantation of NSCs induced cognitive and circadian recovery in addition to the reduction of the clinical motor score (Fig.1a-c). Following NSC graft, the experimental group started to differ significantly from the *non-recovered* group as of the 2nd quantile concerning clinical score whereas differences were significant after the 3rd quantile concerning non-motor symptoms (Fig.1a-c). Clinical scores dropped below symptomatic thresholds on average 6 weeks after NSC-grafts, achieving maximal motor recovery 5 weeks later (**Fig.1A, Fig.S1A**). Recovery from non-motor deficits was more gradual, with cognitive performances and LD ratio not significantly different from control at an average of 18 weeks following NSC-grafts. Motor and non-motor symptoms persisted in the non-recovered group displaying no sustained improvements following NSC grafts (Fig.1b-c, Extended Data Fig.1b-c). The initial LD ratio recovery after graft was followed by a relapse on the last two quantiles (Fig.1c). Non-parametric circadian rhythm analyses (NPCRA, see Methods) revealed high variability across cases withholding post-graft group comparison (Extended Data Fig.1c).

Markers of DA function and DAT increases following NSC graft correlate with behavioral changes (Fig.2j-k, Extended Data Fig.5a). We show that PET-scan estimation of nigrostriatal innervation through DAT binding was significantly increased bilaterally in the ventral striatum as early as 15 weeks post-graft, suggesting a primarily protective effect of grafts on the remaining pool of nigrostriatal terminals (Fig.1d). F-DOPA scans following grafts confirm a selective effect of NSCs on DA synthesis with maximal motor recovery. Significant increases in DA function and DAT binding are observed in grafted parts of the striatum when compared to the same regions in non-recovered cases (Fig.4a). Notably, we observed no improvement of motor or non-motor symptoms after sham transplantation and functional markers

displayed no difference between recovered vs. non-recovered cases in striatal DAT binding (Extended Data Fig.5b-c, see Methods for details).

Integration of the NSC-graft correlate with clinical, cognitive and functional recovery. Post-mortem immuno-histological examination shows that NSCs integrate into the nigrostriatal network and differentiate into mainly mature neurons and astrocytes (Fig.2b-e). Furthermore, NSCs spontaneously differentiate into aminergic cells thereby potentially restoring a significant proportion of the lost DA pool (Fig.2b). In the recovered group the survival rate varied widely across cases and grafted sites, ranging from 2 -61% (corresponding to the number of surviving tau-GFP⁺ cells per grafted site ranging from 3.1×10^3 to 1.8×10^5 respectively, Extended Data Table 4). Unilateral survival of grafted NSCs did not lead to a systematic difference in recovery across hemispheres (Extended Data Fig.6a-b) suggesting cross-hemispheric compensation³⁰, although we observed an asymmetric time-course of cognitive recovery with unilateral CdN survival (Extended Data Fig.6b, Extended Data Table 4) that could also be detected through a minor unilateral DAT binding decrease (Extended Data Fig. 6c, right). No difference in recovery could be attributed to NSC type grafted (although survival rate did vary, Extended Data Tables 2, 4). Among surviving cells, astrocytic fate accounted for 34-52% (GFAP⁺/tau-GFP⁺, Fig.2b, e) and neuronal fate for 11-18% (MAP2⁺/tau-GFP⁺, Fig.2b, d). Additionally, up to 13% of tau-GFP⁺ cells in SN grafts differentiated into aminergic cells (TH⁺/tau-GFP⁺ cells, Fig.2b-c) which corresponds to an estimated number of 1.5×10^4 cells and hence to a restoration of nearly 18% of the original pool of DA-containing midbrain neurons, as reported for the same NHP species³¹. Computerized reconstruction of tau-GFP⁺ soma and processes showed that grafts are able to extend and project dendrites to distant locations (Extended Data Fig.7). We could further detect local processes (*i.e.* less than 1mm) directed toward surviving TH⁺ cells from the host tissue, that formed synaptic contacts with host neuronal cells (Fig.3a-b, d-f). At some graft sites, aggregates of host-cells (*i.e.* tau-GFP negative) were observed inside the graft-core with numerous DAT⁺ processes (Fig.3g-i), suggesting that differentiated NSCs provide a milieu which orients endogenous cells towards DA fate³², and include a relatively high proportion of GFAP⁺/tau-GFP⁺ cells (Fig.2b, e). Immuno-labeling revealed that non-recovered cases presented no surviving tau-GFP⁺ cells (Extended Data Fig.8). The nigrostriatal DA lesion was most severe in non-recovered cases (Fig.4b), which is considered deleterious for grafted cells and has been shown to decrease the functional impact of the graft³³. Furthermore, immunological rejection of the grafted NSCs cannot be fully excluded as no immunosuppressive treatment was employed. The fact that non-motor symptoms initially improved in the non-recovered group and suddenly worsened at the 4th Quantile (Fig.1b-c, Extended Data Fig.1b-c) suggests a rather chronic graft rejection in these cases.

As an additional control, we compared the time course of behavioral symptoms and functional markers to those following spontaneous recovery. In some cases, spontaneous recovery was initially induced by halting MPTP intoxication as described previously^{18,19}. This allowed better differentiation between features accompanying spontaneous motor recovery from those observed following graft. Spontaneous recovery of clinical motor symptoms had faster dynamics compared to graft-induced recovery (Fig.2I). In

addition there was an absence of cognitive improvements accompanying spontaneous motor recovery, contrasting with the progressive cognitive improvement following grafts (Fig.2l). We previously demonstrated that spontaneous motor recovery could occur despite an average $71.5 \pm 9.7\%$ reduction in striatal DAT relative to control levels and that stable symptoms were observed when the lesion reached $82.3 \pm 7.9\%$ of control¹⁹. We now show graft-induced recovery despite $78.04 \pm 12\%$ average reduction in striatal DAT binding (Fig.4a). These results confirm the specificity of graft effects as compared to spontaneous recovery, especially concerning cognitive recovery (Fig.2l).

In sum, we demonstrate that multisite graft of NSCs allows recovery from cognitive and clinical motor symptoms while the effects on chronobiological rhythms were too variable to be attributed directly to grafts. *In-vivo* imaging confirmed positive effects on nigrostriatal innervation and function (Figs.1d, 2j-k, 3a). Post-mortem histology showed that a substantial proportion of DA fate was assumed by grafted NSCs (Fig.2b) thereby supporting their role in cognitive and motor recovery. Further, the large proportion of glial cells observed in the grafts (Fig.2b) is consistent with the protective effect of grafts on the remaining nigrostriatal innervation after lesion, as evidenced by functional findings on striatal DAT levels (Fig.1d) and as reported after human NSC grafts in MPTP-NHP model³⁴.

Three decades after the first clinical trials of transplanting cells from fetal ventral mesencephalon in PD patients³⁵, cell replacement therapy in PD is still not a proposed treatment. However, this therapeutic approach has recently been shown to be efficient at different levels³⁶⁻³⁸, leading to more recent trials in order to improve grafting procedures, and prepare for stem-cell based transplantation in humans³⁹, while waiting for perspectives from pre-clinical investigations in animal models⁴⁰. Here, we report for the first time efficient recovery from cognitive and clinical motor symptoms following NSC grafts in the NHP model of PD. This study underlines the crucial importance of longitudinal and multifaceted approaches for a better translation to the clinic and appraisal of early-phase clinical trials.

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Methods

In agreement with the 3Rs⁴¹, the rationale for the current study is that each case represents its own control through detailed follow-up of all the periods of the protocol i.e. CTR, MPTP, post-MPTP and Pre- vs. Post-Graft. We published precise and detailed reports for each clinical, behavioral and functional parameter followed as well as for the characterization of NSCs grafted in the present study. These precise and detailed control procedures are described in Extended Data Table 1.

Ethical statements

All procedures were carried out according to the 1986 European Community Council Directives (86/609/EEC) which was the official directive at the time of experiments, the French Commission for animal experimentation, the Department of Veterinary Services (DDSV Lyon, France). Authorization for the present study was delivered by the “Préfet de la Région Rhône Alpes” and the “Directeur départemental de la protection des populations” under Permit Number: #A690290402. All procedures were designed with reference to the recommendations of the Weatherall report, “The use of non-human primates in research”. All monkeys were closely monitored on a regular basis throughout the day, by researchers and animal care staff, in order to ensure that levels of health and welfare were strictly maintained, particularly during the MPTP period. Adaptations to housing and feeding procedures were made in direct response to individual symptoms in the MPTP phase, for example adaptations of water provision to ensure monkeys were able to drink ad libitum. To alleviate physical suffering from motor symptoms progression (such as rigidity) the intoxication procedure was first cautiously stopped when the PMRS-motor score was above ten for two consecutive days following one MPTP injection; further, antalgic and/or anti-inflammatory drugs were delivered to animals experiencing debilitating motor symptoms. Before being sacrificed, animals were first tranquilized with a pre-anesthetic agent (chlorpromazine hydrochloride, Largactil) and anesthesia was induced with Ketamine before deep anesthesia was obtained by means of a large dose of pentobarbital sodium (Vibrac, 100 mg kg⁻¹, i.p.; lethal dose confirmed by complete loss of corneal reflex).

MPTP-intoxication and study design

Six late middle-aged – 11-13 (13-17) years old at protocol onset (end) female macaque monkeys (*Macaca fascicularis*, 4-5kg) were intoxicated with low-dose 6-methyl- 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridin injections (MPTP, 0.2mg.kg⁻¹, i.m). Animals were housed in a room dedicated to MPTP experiments, with free access to water and received food twice a day. The neurotoxin was delivered according to two different regimes: 1) chronically each 3-4 days and 2) acute intoxication (daily injections) as described previously^{19,42}. In cases 1, 3 and 4, the first session of MPTP injections was suspended as soon as the clinical score reached symptomatic threshold (Extended Data Fig.1), allowing investigation of dopaminergic (DA) neurotransmission after spontaneous behavioral recovery^{18,19} and thus providing critical parameters to compare with potential recovery following transplantation. For the last-MPTP intoxication i.e. ending with persistent motor-symptoms in all cases, injections were cautiously stopped when the PMRS-motor score was above ten for two consecutive days following one MPTP injection. For results presentation, animals were grouped according to their clinical state after transplantation (see Fig.1) i.e. 1st group – recovered (n=3, cases 1, 4, 5) and, 2nd group – non-recovered (n=2, cases 3 and 6). Delays between last MPTP injection and i) transplantation were 4-23 weeks (n=6, all cases), ii) sham-grafts were 6-16 weeks (n=2, cases 1 and 6). Data are presented according to the following 5 periods of the protocol: 1- MPTP (during MPTP intoxication period); 2- post-MPTP Recovery (following arrest of first MPTP injections, clinical motor score returned below 5 throughout this period i.e. non-symptomatic); 3- post-MPTP Symptomatic (following arrest of last MPTP injections, clinical motor

score remained stably above 5 during this period i.e. motor-symptomatic); 4- post-Sham (following sham grafts, details in *Surgical procedures*); 5- post-Grafts (following transplantation, details in *Surgical procedures*). Case 4 had a high-score post-MPTP (motor symptoms>15), characterized by tremor at rest and freezing. This motor-symptomatic state persisted during the 4 weeks prior to transplantation (Extended Data Fig.1a). Case 6 had a high-score post-MPTP (motor symptoms>15), characterized by severe rigidity. Musculoskeletal pain is a recognized source of pain syndromes and discomfort in Parkinson's disease patients. We thus delivered regularly ketoprofen (Profenid), from week 5 post-MPTP, in order to relieve suspected apparent musculoskeletal pain. Subsequently the clinical score for this animal progressively improved, due to amelioration on the rigidity scale and ability to manipulate food (from week5 to week10 post-MPTP, Extended Data Fig.1a), but stabilized at week10 and remained largely symptomatic (score >12) despite continuous treatment and disappearance of apparent musculoskeletal pain syndrome. Case 6 was transplanted at week 23 post-MPTP. Case 2 had per operative brain hemorrhage following NSCs transplantation and was thus excluded from comparative analyses pre- vs. post-Graft.

Clinical motor scale – PMRS

Clinical scoring was done independently by three trained experimenters at separate times throughout the day, 3-5 days per week using the Parkinsonian Monkey Rating Scale^{18,19}. The PMRS contains a motor component, employing a previously published scale⁴³ used to define the onset of the motor symptomatic period. The total score for freezing (0–3), resting tremor (0–3, for right and left side), rigidity (0–3), posture (0–2), bradykinesia (0–3), and ability to manipulate food (0–3, for right and left side) was used to frame injections (maximum total score of 23): a score below 5 defined the non-symptomatic state in premotor and recovery periods, and a score above 5 defined symptomatic periods.

Cognitive behavior task – detour task

Cognitive performance was monitored 3-5 days a week using a previously described behavioral test^{18,19}. Briefly, performance on the 'detour task' was evaluated by the percent of successes (retrieval of reward on the first reach, over the total number of trials) and errors (barrier hits, over the total number of responses observed - there could be several responses per trial except in the case of success). Errors due to motor impairments were classified as such and further discarded from the performance evaluation, so as to avoid confounds due to the symptomatic state of the animal. The performance on the 'detour task' depends on the integrity of frontal cortex, the dopaminergic system and DA innervation of frontal cortex⁴⁴ and has been used to assess the impact of lesion of the nigrostriatal axis on cognitive performance as well as potential therapy for cognitive impairments⁴⁵⁻⁴⁷. For inter-individual comparison, percent errors and success were, for each case, first converted in percent change by the average control value and normalized between 0 and 1 by overall maximum and minimum score respectively.

Circadian rhythm follow-up

Animals were maintained under an LD cycle (12h light: 12h dark) of approximately 450–500 lux during the light phase and 0 lux during the dark phase. Locomotor activity was continuously recorded in 1 min intervals throughout the entire duration of the study using passive-infrared motion detectors mounted above each animal's cage. The motion captors were connected to a computerized data acquisition system (Circadian Activity Monitoring, INSERM, France⁴⁸). Data were analyzed using the Clocklab software package (Actimetrics, Evanston, IL, USA).

We previously used Chi-squared periodogram method (Extended Data Table 1) to calculate the period of behavioral rhythm which is defined as the time elapsed for one complete oscillation or cycle (the distance in time between two consecutive peaks or troughs of a recurring rhythm). The stability and the consistency of the rhythm are reflected by the value of the periodogram amplitude which corresponds to the zenith of a periodogram curve. The subjective day and the subjective night are defined as the segment of a circadian cycle during the free-running state that corresponds to, respectively, the light and dark segments during entrainment by a LD cycle.

Non-parametric circadian rhythm analyses (NPCRA) were used to estimate the strength and fragmentation of rest-activity rhythms^{12,18}. These included measurements of inter-daily stability (calculated as the ratio between the variance of the average 24-hour pattern around the mean and the overall variance and gives an indication of the consistency of day to day activity or the strength of coupling to the LD cycle), intra-daily variability (calculated as the ratio of the mean squares of the difference between successive hours and the mean squares around the grand mean and gives an indication of the frequency of transitions between rest and activity periods, corresponding to the fragmentation of the rhythm) and, relative amplitude (ratio between acrophase and nadir of the rhythm, representing the ratio between activity amplitude in light and dark phases). When the rest-activity rhythm is stable, inter-daily stability is high, the intra-daily variability is low and relative amplitude is high.

DA function imaging – [¹¹C]-PE2I and [¹⁸F]-FDOPA

To evaluate the in-vivo DA function, we acquired images from Positron Emission Tomography (PET) scans using (E)-N-(3-iodoprop-2-enyl)-2beta-carbomethoxy-3beta-(4'-methylphenyl)-nortropane labeled with carbon 11 ([¹¹C]-PE2I) at different intervals throughout the protocol as described previously¹⁹ in cases 1,2,3 and 6 and after grafts at different delays (see Extended Data Figs.1, 5). [¹¹C]-PE2I specifically binds with high affinity and selectivity to DA transporters (DAT, K_i = 17nM) and is considered to provide an index of the integrity of the DA pathway that has been used for PD diagnosis, see⁴⁹⁻⁵¹ for a review. Additionally, we used images from PET scans using L-3,4-dihydroxy-6-(¹⁸F)fluorophenylalanine ([¹⁸F]-FDOPA, an analog of the DA precursor L-DOPA) to evaluate the central dopaminergic function of pre-synaptic neurons, i) just before grafts (during stable expression of typical motor parkinsonian symptoms i.e. symptomatic period) and, ii) after grafts at different delays (see Extended Data Fig.1).

We performed [¹¹C]-PE2I and [¹⁸F]-FDOPA PET scans with an ECAT Exact HR+ tomograph (Siemens CTI), in 3D acquisition mode, covering an axial distance of 15.2cm. The transaxial resolution of the

reconstructed images was about 4.1mm full-width and half maximum in the center. We acquired transmission scans with three rotating ^{68}Ge sources to correct emission scans for the attenuation of 511 keV photonrays through tissue and head support. Full procedure for PET-scans acquisition, modeling and ROIs definition for regional assessment of $[^{11}\text{C}]$ -PE2I changes have been published¹⁹. Fluoro-DOPA was labeled with ^{18}F -fluoride (cyclotron-produced isotope, half-life = 109 min). Specific radioactivity was 3.27 ± 1.3 mCi/ μmol . Radiochemical and chemical purity of produced $[^{18}\text{F}]$ -FDOPA (as determined by HPLC) was above 99%. After anesthesia was induced and head secured with a MRI-compatible stereotaxic frame (Kopf, CA, USA) to reduce variability in the measure, a cannula was inserted in the femoral vein. $[^{18}\text{F}]$ -FDOPA was injected as a bolus over a 4 s period followed by a saline flush. Radioactivity was measured in a series of 26 sequential time frames of increasing duration (from 30 sec to 5 min; total time 90 min).

PET modeling and regional assignment of PET changes were done as previously described¹⁹ and applied to F-DOPA measurement⁵². Statistical positive (shown in Fig.1d) and negative (shown in Extended Data Fig.6c, right) difference were determined from 2.5th and 97.5th percentile boundaries of voxel-level distribution statistics of all brain ROIs as described previously¹⁹ and illustrated in Extended Data Fig.6c, left.

Transplanted cells and Surgical procedures

We derived neural stem cells (NSCs) from a rhesus embryonic stem cell line stably expressing tau-GFP (LYON-ESC line)²¹. NSCs were obtained either i) as described in⁵³ where multipotent NSCs were amplified by mild trypsinization and cultured in the presence of EGF and FGF2 (Extended Data Fig.2a, c), or ii) after MS5 induced-neural differentiation followed by early midbrain differentiation, as described in⁵⁴ (Extended Data Fig.2b, c, f). The latter cell type corresponds to early DA midbrain neural precursors. For simplicity and because the two cell types lead to similar recovery when grafted in different hemispheres (Extended Data Fig.6), we used the generic term of NSCs. NSCs express NSC specific markers (Extended Data Figs.2a-c, 3a) and showed *in-vitro* potential to fully differentiate along glial and neuronal pathways (Extended Data Fig.2e) including mature DA neurons (Extended Data Fig.2f). Grafted NSCs retained the capacity to differentiate into glial and neurons *in vivo*, depending on the local environment of the graft (Figs.2b-e, i, 3 and Extended Data Fig.4a).

We performed all transplantations bilaterally using 5 μL Hamilton syringe and small trepanations over target sites. Stereotaxic coordinates were calculated from individuals T1 and T2 structural MRI images. We undertook sham-grafts (trepanation and injection of 5 μL PBS without NSCs) in cases 1 and 6 in order to control for behavioral and clinical effects due to the surgical act in itself. We placed sham-grafts (PBS-only) in posterior caudate and anterior putamen, using the same protocol as for NSCs transplantation. The amount of injected NSCs varied between 3.10^5 - 10^6 cells for SN sites and $1.5.10^5$ - 10^6 for striatum sites (Extended Data Table 2). The syringe was lowered 500 μm further than the target position and then retracted to target; cells were then injected at a rate of 1 μL per min after a 5min delay. After an additional

3 minute delay after injection completion, the syringe was retracted slowly by 2mm; another 2 minute delay was observed before full retraction. We assessed cell viability by trypan blue exclusion before injection and counted the remaining cells after injection. Viability at both time points was found to be between 95 and 100%. Cell mortality due to syringe uptake was estimated at 2%.

Immuno-histological procedures

Deep anesthesia was induced with Ketamine after premedication with chlorpromazine hydrochloride (Largactil) followed by a lethal dose of pentobarbital sodium (Vibrac, 100 mg.kg⁻¹, i.p.; confirmed by complete loss of corneal reflex) before animals were transcordially perfused with saline (0.9% with procaine), 4% paraformaldehyde and 0.05%-glutaraldehyde in phosphate buffer. Cryoprotection was ensured by sucrose gradients (10-30%) perfusion post-fixation. Brains were removed, kept in cryoprotecting liquid overnight and coronal 50µm thick sections cut on a freezing microtome and collected serially. Sections were processed together with equivalent sections from control animals for Tyrosine Hydroxylase (TH; Millipore, #MAB318) and DAT (Millipore, #MAB369) immunochemistry visualized with 3,3-diaminobenzidine, in Ni²⁺+H₂O₂ (0.5-1.0%). All sections were washed, mounted, dried, and dehydrated in increasing gradients of ethanol, cleared in toluene, mounted with mounting medium and cover slipped.

For immunofluorescent staining on free-floating brain slices, sections were washed six to ten times in Tris buffered saline (TBS) and permeabilized in Triton X-100 (0,5%; SIGMA, #T9284). Nonspecific binding was blocked with 10% normal goat serum or donkey serum (Goat: Invitrogen; #16210064; Donkey: SIGMA #D9663) for 45 min at room temperature. Sections were incubated for three days at 4°C, with primary antibodies diluted in Dako antibody diluent (Dako #S3022) supplemented with 0,5% Triton X-100. They were then exposed to secondary antibodies and DAPI (1/10 000; Invitrogen #D1306) for 2 hours at room temperature. Sections were mounted on slides and examined using a confocal microscope (Leica TCS SP5). Sections were regularly (every five sections i.e. every 250µm) processed for GFP immunostaining to detect grafted sites extent.

We determined the length of processes emanating from the graft core by measuring the length of tau-GFP positive projections using the ImageJ software (NeuronJ plugins, <http://imagej.nih.gov/ij/>), presented in Extended Data Fig.5d. We estimated the graft volume through a 3D reconstruction process using contours traced and compiled with the Mercator software (ExploraNova, <http://www.exploranova.com/products/mercator/>), presented in Extended Data Table 3, Fig.2f-h, Extended Data Figs.4, 5a-d. We determined the average cell density by (i) counting all cells present in four random locations within each confocal optical section in several slices at different levels of the graft core to return cell density per mm³ and (2) by scaling those counts to the estimated graft volume in mm³ to return estimated cell number per graft. Finally, we calculated survival rate according to the number of transplanted cells per site (Extended Data Tables 2-4).

Immunostaining and RT-PCR analyses of cultured cells were done according to previously published protocols²¹.

Semi-quantitative immunohistochemistry

Three age and weight matched *Macaca fascicularis* served as controls. We evaluated the extent and amplitude of the nigrostriatal lesion through semi-quantitative immunohistochemistry of striatal structures, as previously described^{19,55}. The mean optical density (O.D.) of TH and DAT in immunopositive regions were computed on at least 5 sections per animal (5-20) from 8bit images, normalized and compared with two-sample t-test separately for each ROI (ttest2, Matlab). In Fig.2a, composite CTR and MPTP images has been enhanced for brightness and contrast, for illustration purpose.

Statistics

We segmented data for MPTP, post-MPTP and post-Graft periods into five equal epochs and grouped variables into each of these epochs. This method of segmentation, described in^{18,19}, thus reveals normalized stages of the progression of processes and allows for comparison of different parameters across an equivalent number of epochs for all subjects, called Quantiles (Fig.1, 27 ± 3 days for the CTR period; duration per quantile for premotor 10 ± 5 days, motor 18 ± 8 days and post-Graft 42 ± 12 days). Results are presented as means $\pm$ standard errors.

Parameters were compared between groups by a treatment-contrast test using one group as the first level and Bonferroni corrected for multiple comparisons and, within groups across periods by a treatment-contrast test using control measures as the first level (estimated standard errors and z-ratio were computed using GLM fit and contrast result was given by two-tailed p-values corresponding to z-ratio based on a Student t-test). Significance was considered at $p < 0.05$. Statistical analyzes were computed using R software (R Foundation for Statistical Computing, Vienna, Austrian <http://www.R-project.org>) and the summary function (Chambers, J. M. and Hastie, T. J. (1992) Statistical Models in S. Wadsworth & Brooks/Cole).

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Declarations

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Authors contributions Data acquisition F.W, K.D, K.F, A.B, V.D, J.V; Data analysis F.W, K.D, K.F, P.M, C.L, J.V; Parkinsonian Monkey Rating Scale K.D, K.F, C.R.E.W, E.P, J.V; Cognitive testing K.D, K.F, E.P, J.V; Circadian analysis K.F, H.M.C, J.V; PET-scan K.D, C.R.E.W, E.P, J.V; PET-scan analysis J.V; Cell culture and NSCs production for graft F.W, A.B, V.D, P.S, C.D; Surgical grafts K.D, E.P, J.V; Histology F.W, K.D, J.V; Experimental design H.M.C, E.P, H.K, P.S, C.D, J.V; Wrote the first draft F.W, H.K, J.V. All authors revised and commented on the manuscript.

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Figures

Figure 1

Motor, non-Motor and Functional Markers of Graft-induced Recovery. **a** Clinical motor score. **b** Cognitive performances, normalized contrast with performance in Control conditions; **c** Chronobiological rhythms of spontaneous locomotor activity, normalized contrast with Light/Dark ratio in Control conditions. **d** NSC grafts increase nigrostriatal innervation. Parametric maps of DAT binding potential with axial (top), sagittal (down-left) and coronal (down-right) views of significant positive difference for the contrast Pre- vs. Post-Graft (color scale from 0.2-1, case1, recovered group). See Extended Data Fig.1 for behavioral markers of all individual cases and Extended Data Fig.6 statistical thresholding done in **d** and negative difference.

Figure 2

Dopaminergic lesion and Differentiation Fate of grafted NSCs. **a** Optical density (O.D.) measures of DA lesion (arbitrary units); **b** Fraction of grafted tau-GFP⁺ NSCs into aminergic (TH⁺, illustrated in **c**, left), neuronal (MAP2⁺, in **b**) and astrocytic phenotypes (GFAP⁺, in **e**); **c** SN (left, case4) and CdN (right, case5); **d** CdN (case5); **e** Put (case5); **f-i** Digital reconstructions of grafts in case5 (**f**, CdN-up, Put-middle, SN-down), case4 (**g**, SN) and case1 (**h**, CdN-up, Put-down left and SN-down right), scale bars 200 μ m; **i**

photomicrographs of sites framed in g, tau-GFP (green) and GFAP (blue). j Images of striatal DAT binding potential (BPND, scale down-right 0-7, case 1 – see Extended Data Fig.5a for all cases). k Functional markers of nigrostriatal innervation (normalized BPND) and DA function (normalized Ki) in recovered (case1, left) vs. no-n-recovered (case6, right) cases during expression of stable symptoms (PD) and following transplantation (10 weeks minimum between Post-Graft measures). Min-max normalization per case. Same color code as in Panel a. l Clinical motor score (left, over weeks) and cognitive performances (right, over quantiles) comparison between spontaneous (cases 1, 3-4) vs. graft-induced (cases 1, 4-5) recovery. Results presented as Mean $\pm$ SEM; scale bars, c left 50 μ m and right 100 μ m, d 200 μ m, e 100 μ m.

Figure 3

Histological evidence of Graft integration and interaction with Host cells. In the substantia nigra, grafted NSCs (a-b) extend dendrites toward surviving host DA cells (arrowheads, scale bars 100 μ m) and, (c) differentiate into aminergic phenotype displaying TH+ dendrites (arrowhead, scale bar 25 μ m). Furthermore, NSCs make synaptic contact with host neuronal cells (d-g arrowheads; scale bars d 100 μ m,

e-f 5µm) through extensive processes outside the graft-core (g scale bar 100µm, see Extended Data Fig.6 for detailed measures). h Host cells within the graft core (center, scale bar 100µm), presenting numerous dopaminergic (DAT+) and sparse serotonergic (SERT+) processes (i-j scale bars, i 25µm, j 5µm). k Some NSCs processes display serotonergic and/or dopaminergic phenotype (arrowheads, scale bar 15µm).

Figure 4

Functional and Histological differences between grafted vs. non-grafted Striatum. a Change in Striatal DAT binding (relative to CTR) after spontaneous clinical motor recovery (case1) and after Graft-induced Recovery. Comparison of grafted and non-grafted Striatal regions between recovered (case1) vs. non-recovered (cases 3, 6). b Comparison of the degree of DA lesion (relative change in DAT and TH O.D. compared to a group of CTR cases) between recovered (cases 1, 4-5) vs. non-recovered (cases 3, 6).

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