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Abstract

Recent studies have shown that fibroblast transplantation can modify the activity of basal ganglia networks in models of Parkinson's disease. To determine its effects on parkinsonian motor symptoms, we performed autologous dermal fibroblast transplantation into the internal pallidum (GPi) in two parkinsonian rhesus monkeys with stable levodopa-induced dyskinesias (LIDs). Levodopa responses were assessed every week after transplantation for three months. A reduction of between 58% and 64% in total LIDs on the contralateral side was observed in both animals. No clear LID changes were observed on the ipsilateral side. These effects lasted the entire 3-month period in one monkey, but declined after 6–8 weeks in the other. The antiparkinsonian effects of levodopa did not diminish. The results of this pilot study indicate that fibroblast transplantation into the GPi may have beneficial effects on LIDs and warrant further investigation for potential therapeutic use.

Keywords

globus pallidum; autologous; levodopa; antiparkinsonian; monkeys

INTRODUCTION

In animal models of Parkinson's disease (PD) and patients undergoing stereotactic surgery for deep-brain stimulation or pallidotomy, neuronal recordings indicate that the mechanisms underlying parkinsonian motor symptoms involve increased synchronization of neuronal discharges in the basal ganglia circuitry[1-4]. Using *in vitro* isolated neuronal networks from

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rodents, it has recently been found that fibroblasts reduce the characteristic synchronous electrical activity in culture[5]. It may thus be hypothesized that, similar to the *in vitro* shift from pathological synchronized firing to more physiological desynchronized activity, fibroblasts could normalize the discharge pattern *in vivo*. However, whether *in vivo* fibroblast transplantation desynchronizes neuronal discharges in basal ganglia circuits in a parallel mode to the addition of dopamine[6,7] or direct electrical stimulation[8,9] has yet to be demonstrated. Furthermore, it remains to be established whether the physiological changes induced by fibroblast transplantation translate into specific behavioral effects in PD models.

Transplantation of fibroblasts into the entopeduncular nucleus of hemiparkinsonian rats reduces the contralateral turning in response to apomorphine, but is not accompanied by dopaminergic cell regeneration[5]. This could be interpreted as a reduction of the motor response to dopaminergic stimulation and a potential antidyskinetic effect[10]. In fact, the same study showed that fibroblast transplantation also suppresses locomotor activity and reduces orofacial and limb dyskinesias. Motor abnormalities in PD, including levodopa-induced dyskinesias (LIDs), have been associated with increased synchronization in basal ganglia circuits[11-15]. Therefore, we proposed that the reduction of synchronous discharges *in vitro* after fibroblast transplantation could be responsible for the behavioral effects in transplanted rats, which are equivalent to LID reduction. To further study the potential therapeutic use of fibroblast transplantation, it is necessary to fully characterize the behavioral effects in the parkinsonian setting. Here, we designed a pilot study to examine the motor effects of fibroblast transplantation in the internal segment of the globus pallidus (GPi) in the primate 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) model of PD using two macaques with stable, advanced parkinsonism and LIDs.

MATERIALS AND METHODS

Animals

Three adult male macaques (*Macaca fascicularis* (G, C) and *M. mulatta* (N)) were used for fibroblast transplantation. Animals weighing 6–8 kg were housed and maintained under standard conditions. Studies were conducted in compliance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (1996) and institutional guidelines (IACUC). Monkey G underwent fibroblast transplantation in normal conditions (no MPTP treatment) to assess the safety and survival of transplanted fibroblasts. Monkeys C and N were injected with the neurotoxin MPTP and subsequently given chronic dopaminergic therapy to produce a model of advanced PD.

Production of the Parkinsonian Model with LID

Monkeys C and N received systemic injections of MPTP (0.2–0.8 mg/kg, i.v.) at weekly intervals for one and four months, respectively. To ensure stabilization of moderate to severe parkinsonism (motor disability score >18) for the development of LIDs, motor disability was assessed with a standardized Motor Disability Scale for MPTP-treated primates[16]. After the period of stabilization, both monkeys were treated with oral Sinemet® (100–300 mg/day) until they exhibited LIDs between 2 and 4 months[16,17]. LIDs were choreodystonic,

purposeless movements predominantly in the legs and arms, although some oral dyskinesias also occurred. Thus, the model produced in both monkeys consisted of moderate-to-severe parkinsonism and stable LIDs. Both monkeys were maintained on oral Sinemet® until the studies were completed.

Fibroblast Transplantation into the Internal Pallidum

After the model was produced, the timetable of procedures was: (1) skin biopsy for cell culture; (2) surgery for chamber implant; (3) electrophysiological recordings for basal ganglia mapping; and (4) fibroblast harvest and transplantation into the GPi. In monkey C, the electrophysiological mapping and fibroblast transplantation were done directly without implanting a chamber, because at the time of experiments in this animal the primate physiology equipment compatible with surgery became available. The technique for electrophysiological mapping and the procedure for fibroblast transplantation were identical in all animals.

Skin Biopsy and Autologous Dermal Fibroblast Isolation and Expansion— Under sedation**,** a skin biopsy (1–2 cm^2) was taken from the back of the animal and used for the isolation of dermal fibroblasts and their expansion in cell culture. Briefly, the skin samples were minced and digested in PBS containing collagenase type 1 (15 mg/mL, Worthington Biochemical Corp., Lakewood, NJ). The digested tissue was filtered through a 70-μm cell strainer (BD Biosciences, San Jose, CA). The cell suspension was centrifuged and cells re-suspended in Dulbecco's modified Eagle's medium (DMEM; Hyclone, Thermo Scientific, Waltham, MA) containing gentamycin, penicillin/streptomycin (Hyclone), amphotericin (Hyclone), and bovine calf serum (Hyclone) prior to plating in T25 flasks (Corning Corp., Corning, NY). After 24 h and 5 washes, the medium was replaced. Cells were then expanded in DMEM with antibiotics for 10–12 days, after which expansion was continued in DMEM without antibiotics. Prior to implantation, cells were harvested, centrifuged, and re-suspended in lactated Ringer's solution.

Surgical Procedures—After the skin biopsy, animals underwent surgery for implantation of a recording chamber following stereotaxic coordinates for targeting the basal ganglia[18]. The side of the brain contralateral to the most impaired side with the highest dyskinesia scores was selected for transplantation. A recording chamber was implanted on the skull with dental cement under general anesthesia (1–2% continuous isoflurane administered by inhalation) following standard procedures[19]. The chamber provided access for electrophysiological mapping of the basal ganglia and delineation of the limits of GPi. For mapping purposes, the recording chamber was positioned at an angle in the coronal and sagittal planes to allow electrode trajectories passing through the putamen, the globus pallidus externus, and the GPi (Fig. 1A).

Electrophysiological Recording—Monkeys were seated and restrained in a primate chair for recording sessions using tungsten microelectrodes (FHC, Bowdoin, ME) of 1–2 MΩ impedance at 1 kHz. To examine single-cell activity patterns, amplified and filtered extracellular signals were displayed and stored (data acquisition system, Plexon Instruments, Dallas, TX). Direct waveform examination and frequency analysis on-line using rate

histograms identified patterns typical of basal ganglia regions. After several penetrations, the entire area corresponding to the GPi was mapped[20].

Fibroblast Transplantation—Cultured cells were harvested to a concentration of 25 000/μL and a total of 375 000 cells were grafted into the GPi unilaterally. The fibroblast transplantation was performed at three sites in the posterolateral region of the GPi as delimited by electrophysiological mapping (Fig. 1B–D). The area for fibroblast transplantation into the dorsoposterolateral part of the GPi was selected because this region has been identified as the motor territory in classic studies in monkeys[20]. The target GPi sites were verified on the day of transplantation with neuronal activity recording just before infusion of the cell solution. A Hamilton syringe was used for infusion with the needle inserted through the recording cannula and driven to the same depth as the recording electrode. A total volume of 15 μL (5 μL per site) was infused at a rate of 1 μL/min, and after each infusion, the needle was left in place for 10 min and retracted slowly during the subsequent 10 min.

Behavioral Assessment

The parkinsonian monkeys were assessed for their response to levodopa methyl ester (given subcutaneously (s.c.) along with benserazide) and LIDs in their home cage every week during the four weeks prior to fibroblast transplantation and thereafter for 12 weeks. Monkeys were not assessed during the first week post-surgery to allow full post-operative recovery. For s.c. injections, in order to induce consistent responses in weekly behavioral tests, levodopa methyl ester plus carbidopa was used at preselected doses (the optimal dose to consistently produce clear LIDs was usually 100–150 mg s.c.). The “Motor Disability Scale for MPTP-Treated Monkeys” (see details in[16]) was used for all weekly evaluations. The scale consists of two parts to score motor disability (Part I) and drug-induced adverse reactions including LIDs (Part II). Scoring was carried out before (baseline) and every 20 min after levodopa administration until returning to the baseline score. Monkeys were simultaneously videotaped for deferred blinded scoring. Motor disability scores (MDSs) in the “off” and “on” states (baseline parkinsonian motor disability and its reversal following levodopa administration, respectively) and LID scores on each side of the body (peak-dose and total scores) were analyzed.

Histology

The normal animal was euthanized two weeks after transplantation for histological examination. The brain was blocked and post-fixed in 4% paraformaldehyde in phosphate buffer. Blocks were cryoprotected in 30% sucrose and sectioned at 50 μm on a sliding freezing microtome (Sliding microtome, HM450 Microm, Thermo Scientific, Kalamazoo, MI). Serial sections were collected and stained with cresyl violet or hematoxylin/eosin, or immunostained for the heat-shock protein HSP47, a marker for the implanted dermal fibroblasts. Immunofluorescent labeling was performed as previously described[21]. Briefly, sections were rinsed in PBS, blocked in PBS containing 4% normal donkey serum (NDS) and 1% Triton-X, rinsed, and incubated overnight at 4°C in PBS containing 2% NDS and rabbit anti-HSP47 antibodies (0.2 μg/mL; Abcam, Cambridge, MA). Sections were then rinsed and incubated with Alexa 488 donkey anti-rabbit secondary antibodies in PBS

(1:2000, Life Technologies, Grand Island, NY), rinsed in PBS, mounted on glass slides, and coverslipped using Vectashield hard set mounting medium with DAPI (Vector Laboratories, Burlingame, CA). Some sections were stained for HSP47 using diaminobenzidine as previously described[22] and counterstained with cresyl violet.

RESULTS AND DISCUSSION

Fibroblast Transplantation into the GPi of a Normal Primate Had No Behavioral Effects and Demonstrated Cell Survival

Fibroblasts were successfully transplanted into the GPi of a normal animal and the procedure had no complications. Daily examination for 13 days after transplantation showed that the animal's behavior remained normal with no detectable effects on motor or other central nervous system (CNS) functions. Histological analysis two weeks after transplantation revealed the survival of transplanted fibroblasts in the GPi (Fig. 2). As expected, HSP47 was visible in cells lining blood vessels. With these results, studies in parkinsonian monkeys proceeded using the same methods, but the behavioral assessment period was extended to 12 weeks.

Fibroblast Transplantation into the GPi Reduced LIDs in Primates

Fibroblast transplantation into the GPi of parkinsonian monkeys profoundly reduced choreodystonic LIDs. On average, the peak (at 50 min) improved by 39% and the total dyskinesia score improved by 56% on the side contralateral to the grafted GPi. Also, averaged data showed no substantial changes in peak and total dyskinesia scores on the ipsilateral side. However, analysis of data from individual monkeys revealed different time-spans of the LID changes after fibroblast transplantation.

In the first parkinsonian monkey (monkey N), fibroblasts were transplanted into the right GPi without postoperative motor or other complications. Behavioral assessments of both sides showed that peak and total dyskinesia scores after fibroblast transplantation were reduced by 33% and 35%, respectively (Fig. 3A and C). The effect was more pronounced on the contralateral side reaching 52% and 58% reduction of peak and total dyskinesia scores after transplantation (Fig. 3B and D). Marked improvement of LIDs was noted from the third week and continued for the whole evaluation period.

In the second parkinsonian monkey (monkey C), fibroblasts were transplanted into the left GPi, but the procedure was associated with a small amount of subcortical bleeding and mild weakness in the contralateral limbs that recovered completely after 10 days (the motor behavior was the same as that prior to surgery). After recovery, the levodopa responses on both sides showed that the peak and the total dyskinesia scores after fibroblast transplantation were reduced by 45% and 47% (Fig. 3E and G). Again, a more profound effect was seen on the contralateral side, reaching 63% and 64% decreases in peak and total dyskinesia scores (Fig. 3F and H). The mild, transient weakness of the contralateral limbs caused by the subcortical bleeding could have reduced the global mobility of the contralateral limbs and caused an artificial reduction in dyskinetic movements during the

first week. Thus, in monkey C, the effects started in the second week after transplantation and stabilized thereafter (Fig. 3H).

Fibroblast Transplantation into the GPi Did Not Diminish the Antiparkinsonian Action of Levodopa

Fibroblast transplantation reduced LIDs without compromising the antiparkinsonian efficacy of levodopa. In both parkinsonian monkeys (N and C), the baseline ("off" state) motor disability score (MDS) remained unchanged after transplantation (Fig. 4A and C), and the improvement induced by levodopa was also maintained (MDS at the peak of the levodopa effect in the "on" state; Fig. 4B and D). Slight transient changes in levodopa responses were seen as usual in monkeys with advanced parkinsonism (in monkey N, the "on" state MDS increased from the sixth to eighth week after transplantation, and in monkey C, levodopa responses fluctuated in both higher and lower directions during the 12-week evaluation).

Interpretation of Pilot Results

Autologous dermal fibroblast transplantation into the GPi unilaterally produced a substantial reduction of LIDs on the contralateral side in parkinsonian monkeys. The lack of substantial effects on the ipsilateral side is indicative of specific effects of the transplantation on the contralateral LIDs. In neither of the parkinsonian monkeys did fibroblast transplantation diminish the antiparkinsonian action of levodopa (no clear changes of MDS in the "on" state), and thus, it reduced LIDs directly. The effects on LIDs lasted throughout the whole examination period of 12 weeks in one monkey, but declined after 8 weeks in the other. These pilot data suggest that fibroblast transplantation into the GPi specifically reduces LIDs while maintaining the beneficial effects of levodopa on parkinsonian motor deficits. Therefore, these results suggest that it is necessary to extend these studies to a larger number of animals so as to analyze the efficacy with an appropriate sample size.

Fibroblasts in the GPi were identified in the histological analysis performed two weeks post-transplantation in the normal monkey, demonstrating cell survival. Fibroblasts were delivered to all three monkeys because it was confirmed that following transplantation the material remaining in the needle contained a much lower number of cells than that loaded initially. The long-term survival of transplanted dermal fibroblasts in primates needs to be investigated in future studies with different numbers of fibroblasts. Dermal fibroblast survival has been demonstrated in the CNS more than three months post-transplantation in rodents and rhesus monkeys[5,23]. The finding that the behavioral effects of fibroblast transplantation declined after 8 weeks in one PD monkey suggests that the number of transplanted cells could have been too small for prolonged effects. Taken together, the behavioral and histological results suggest that optimizing the number of grafted cells may produce stronger and more prolonged effects on LIDs.

The ability of unmodified skin-derived fibroblasts to alter the electrophysiological properties of cortical neurons has been reported[5]. In behavioral tests, it was demonstrated that fibroblasts reduce apomorphine-induced rotation and prevent the development of abnormal involuntary movements in rats. Behavioral changes after fibroblast transplantation were then attributed to the desynchronization of GPi neurons due to the fibroblast "current sink"

effect[24]. However, this association needs to be demonstrated, and its underlying molecular mechanisms remain hypothetical. Genetically-modified cells and, more recently, induced pluripotent stem cells that express tyrosine hydroxylase and synthesize dopamine, can induce behavioral recovery in rodent models of PD[25-27]. Also, earlier studies showed that fibroblasts modified to produce brain-derived neurotrophic factor may prevent neurodegeneration in rats[28]. Dopamine or trophic factors cannot be assumed to mediate the effects of the transplanted autologous fibroblasts that are known not to synthesize these molecules. The present pilot results of fibroblast transplantation into the GPi of monkeys showing LID reduction warrant further studies to investigate the underlying mechanisms.

It can be speculated that the benefits of fibroblast transplantation into the GPi could also be associated with a “lesion effect” similar to pallidotomy, which ameliorates LIDs in PD patients[29,30]. Microlesions can be produced with different procedures; one example is deep brain stimulation in patients with PD and dystonia[31]. However, further studies including stereology in histological analysis are necessary to assess the presence of GPi lesion/injury and cell death. Certainly, the present data do not provide definite answers about the effects of fibroblast transplantation. Whether the effects are due to specific actions of the transplanted cells or pallidotomy-like effects needs to be determined in further studies with adequate control experiments.

In conclusion, the results obtained in this pilot study are exploratory, and the data should be interpreted accordingly. Behavioral results suggest beneficial anti-LID effects of dermal fibroblast grafts in the GPi. These behavioral results need to be reproduced in an adequate number of animals and other factors associated with this intervention need to be analyzed to determine the real impact of fibroblasts on the behavioral changes. We also expect that continuing the experiments could also optimize the methodologies and assess the potential of dermal fibroblast transplantation for PD therapy.

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REFERENCES

[1]. Bergman H, Wichmann T, Karmon B, DeLong MR. The primate subthalamic nucleus. II. Neuronal activity in the MPTP model of parkinsonism. J Neurophysiol. 1994; 72:507–520. [PubMed: 7983515]

[2]. Plenz D, Kital ST. A basal ganglia pacemaker formed by the subthalamic nucleus and external globus pallidus. Nature. 1999; 400:677–682. [PubMed: 10458164]

[3]. Rivlin-Etzion M, Marmor O, Heimer G, Raz A, Nini A, Bergman H. Basal ganglia oscillations and pathophysiology of movement disorders. Curr Opin Neurobiol. 2006; 16:629–637. [PubMed: 17084615]

[4]. Soares J, Kliem MA, Betarbet R, Greenamyre JT, Yamamoto B, Wichmann T. Role of external pallidal segment in primate parkinsonism: comparison of the effects of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced parkinsonism and lesions of the external pallidal segment. J Neurosci. 2004; 24:6417–6426. [PubMed: 15269251]

[5]. Finberg JP, Gluzman Z, Reshef M, Loboda Y, Mohsen U, Bressler-Stramer T, et al. Modulation of excessive neuronal activity by fibroblasts: potential use in treatment of Parkinson's disease. Restor Neurol Neurosci. 2010; 28:803–815. [PubMed: 21209495]

[6]. Fahn S. Description of Parkinson's disease as a clinical syndrome. Ann N Y Acad Sci. 2003; 991:1–14.

[7]. Obeso JA, Rodriguez-Oroz MC, Rodriguez M, Lanciego JL, Artieda J, Gonzalo N, et al. Pathophysiology of the basal ganglia in Parkinson's disease. Trends Neurosci. 2000; 23:S8–S19. [PubMed: 11052215]

[8]. Kopell BH, Rezai AR, Chang JW, Vitek JL. Anatomy and physiology of the basal ganglia: implications for deep brain stimulation for Parkinson's disease. Mov Disord. 2006; 21:S238–S246. [PubMed: 16810674]

[9]. Lozano A, Lang A, Galvez-Jimenez N, Miyasaki J, Duff J, Hutchison W, et al. Effect of GPi pallidotomy on motor function in Parkinson's disease. The Lancet. 1995; 346:1383–1387.

[10]. Cenci MA. Dopamine dysregulation of movement control in L-DOPA-induced dyskinesia. Trends Neurosci. 2007; 30:236–243. [PubMed: 17400300]

[11]. Bezard E, Brotchie JM, Gross CE. Pathophysiology of levodopa-induced dyskinesia: potential for new therapies. Nat Rev Neurosci. 2001; 2:577–588. [PubMed: 11484001]

[12]. Heimer G, Bar-Gad I, Goldberg JA, Bergman H. Dopamine replacement therapy reverses abnormal synchronization of pallidal neurons in the 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine primate model of parkinsonism. J Neurosci. 2002; 22:7850–7855. [PubMed: 12223537]

[13]. Krack P, Pollak P, Limousin P, Hoffmann D, Xie J, Benazzouz A, et al. Subthalamic nucleus or internal pallidal stimulation in young onset Parkinson's disease. Brain. 1998; 121:451–457. [PubMed: 9549521]

[14]. Levy R, Hutchison WD, Lozano AM, Dostrovsky JO. Synchronized neuronal discharge in the basal ganglia of parkinsonian patients is limited to oscillatory activity. J Neurosci. 2002; 22:2855–2861. [PubMed: 11923450]

[15]. Wu Y, Levy R, Ashby P, Tasker R, Dostrovsky J. Does stimulation of the GPi control dyskinesia by activating inhibitory axons? Mov Disord. 2001; 16:208–216. [PubMed: 11295772]

[16]. Papa SM, Chase TN. Levodopa-induced dyskinesias improved by a glutamate antagonist in Parkinsonian monkeys. Ann Neurol. 1996; 39:574–578. [PubMed: 8619541]

[17]. Bedard PJ, Di Paolo T, Falardeau P, Boucher R. Chronic treatment with L-DOPA, but not bromocriptine induces dyskinesia in MPTP-parkinsonian monkeys. Correlation with [3H]spiperone binding. Brain Res. 1986; 379:294–299. [PubMed: 3488796]

[18]. Winters, WD., Kado, RT. A stereotaxic brain atlas for Nacaca Nemestrina. 1969.

[19]. Liang L, DeLong MR, Papa SM. Inversion of dopamine responses in striatal medium spiny neurons and involuntary movements. J Neurosci. 2008; 28:7537–7547. [PubMed: 18650331]

[20]. DeLong MR, Crutcher MD, Georgopoulos AP. Primate globus pallidus and subthalamic nucleus: functional organization. J Neurophysiol. 1985; 53:530–543. [PubMed: 3981228]

[21]. Gutekunst CA, Stewart EN, Gross RE. Immunohistochemical Distribution of PlexinA4 in the Adult Rat Central Nervous System. Front Neuroanat. 2010; 4

[22]. Gutekunst CA, Levey AI, Heilman CJ, Whaley WL, Yi H, Nash NR, et al. Identification and localization of huntingtin in brain and human lymphoblastoid cell lines with anti-fusion protein antibodies. Proc Natl Acad Sci U S A. 1995; 92:8710–8714. [PubMed: 7568002]

[23]. Smith DE, Roberts J, Gage FH, Tuszynski MH. Age-associated neuronal atrophy occurs in the primate brain and is reversible by growth factor gene therapy. Proc Natl Acad Sci U S A. 1999; 96:10893–10898. [PubMed: 10485922]

[24]. Fast VG, Darrow BJ, Saffitz JE, Kleber AG. Anisotropic activation spread in heart cell monolayers assessed by high-resolution optical mapping. Role of tissue discontinuities. Circ Res. 1996; 79:115–127. [PubMed: 8925559]

[25]. Bankiewicz KS, Eberling JL, Kohutnicka M, Jagust W, Pivirotto P, Bringas J, et al. Convection-enhanced delivery of AAV vector in Parkinsonian monkeys; in vivo detection of gene expression and restoration of dopaminergic function using pro-drug approach. Exp Neurol. 2000; 164:2–14. [PubMed: 10877910]

[26]. Kaplitt MG, Leone P, Samulski RJ, Xiao X, Pfaff DW, O'Malley KL, et al. Long-term gene expression and phenotypic correction using adeno-associated virus vectors in the mammalian brain. Nat Genet. 1994; 8:148–154. [PubMed: 7842013]

[27]. Wernig M, Zhao J-P, Pruszak J, Hedlund E, Fu D, Soldner F, et al. Neurons derived from reprogrammed fibroblasts functionally integrate into the fetal brain and improve symptoms of rats with Parkinson's disease. Proc Natl Acad Sci U S A. 2008; 105:5856–5861. [PubMed: 18391196]

[28]. Levivier M, Przedborski S, Bencsics C, Kang UJ. Intrastriatal implantation of fibroblasts genetically engineered to produce brain-derived neurotrophic factor prevents degeneration of dopaminergic neurons in a rat model of Parkinson's disease. J Neurosci. 1995; 15:7810–7820. [PubMed: 8613721]

[29]. Jankovic J, Lai E, Ben-Arie L, Krauss JK, Grossman R. Levodopa-induced dyskinesias treated by pallidotomy. J Neurol Sci. 1999; 167:62–67. [PubMed: 10500264]

[30]. Kleiner-Fisman G, Lozano A, Moro E, Poon YY, Lang AE. Long-term effect of unilateral pallidotomy on levodopa-induced dyskinesia. Mov Disord. 2010; 25:1496–1498. [PubMed: 20568091]

[31]. Singh A, Kammermeier S, Mehrkens JH, Botzel K. Movement kinematic after deep brain stimulation associated microlesions. J Neurol Neurosurg Psychiatry. 2012; 83:1022–1026. [PubMed: 22869922]

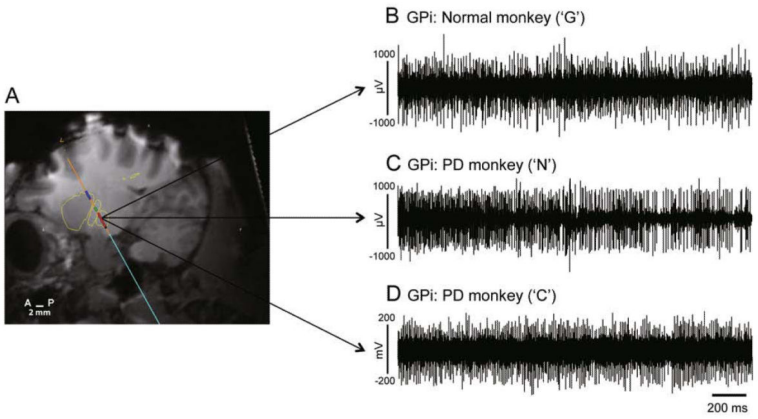

Fig. 1.

Electrophysiological mapping of the GPi. A, Parasagittal MRI image of the monkey brain with delineation of basal ganglia structures and electrode trajectory for mapping. B–D, Single-cell recordings in the GPi of each monkey (B, normal monkey G; C, parkinsonian monkey N; and D, parkinsonian monkey C) demonstrating typical GPi neuronal activity. A, anterior; P, posterior.

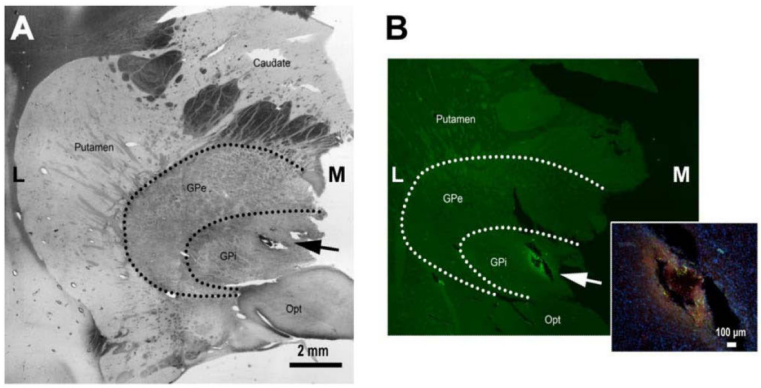

Fig. 2.

Identification of transplanted fibroblasts. Coronal sections through the GPi were stained with cresyl violet (A) or HSP47 antibodies (B). The site of transplantation is stained by cresyl violet in the dorsomedial region of the GPi (arrow in A). Fibroblasts stained for HSP47 were detected by immunofluorescence in the GPi (arrow in B) and can be seen at the edge of the implantation site at higher magnification (insert in B). GPe, external globus pallidus; GPi, internal globus pallidus; Opt, optic tract; L, lateral; M, medial.

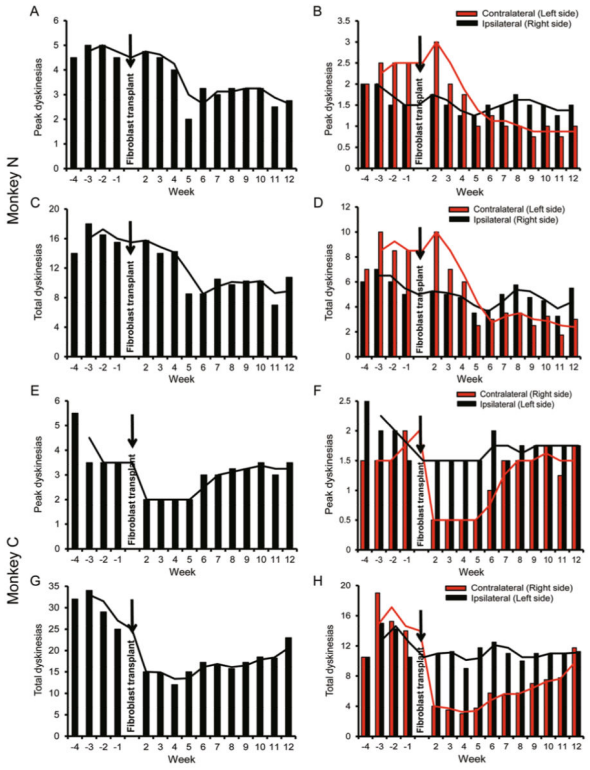

Fig. 3.

Antidyskinetic effects of fibroblast transplantation into the GPi of parkinsonian monkeys. Peak and total LID scores on both sides (A, C, E and G) and on each side (ipsilateral and contralateral to fibroblast transplantation; B, D, F, and H) in monkeys N (A–D) and C (E–H). LID scores on the contralateral side were substantially reduced in both monkeys. LIDs were assessed weekly starting 4 weeks prior to transplantation and subsequently for 12 weeks except for the first week after surgery to allow postoperative recovery.

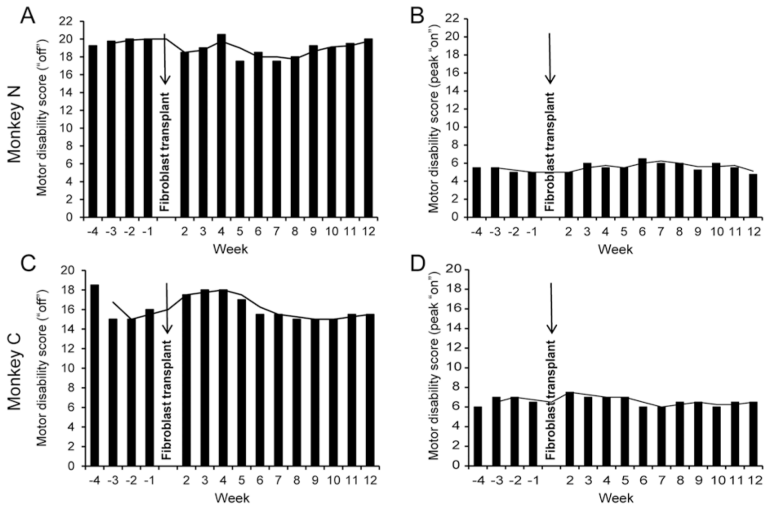

Fig. 4.

Other motor effects of fibroblast transplantation into the GPi of parkinsonian monkeys. Motor disability in "off" and peak "on" periods after injecting levodopa (s.c.) was assessed in monkeys N (A–B) and C (C–D). MDSs in both "off" and peak "on" states did not change after transplantation. Monkeys were assessed weekly starting 4 weeks prior to transplantation and subsequently for 12 weeks with the exception of the first week after surgery to allow postoperative recovery.