

Behavioral improvement in MPTP-treated nonhuman primates in the HALLWAY task after transfer of TH cDNA to host astrocytes

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Parkinson's disease is a neurodegenerative disease, resulting from deterioration of the substantia nigra which in turn leads to a decrease of dopamine levels in the striatum. Clinically the syndrome is characterized by motor alterations that are treated by the oral administration of levodopa. However, this treatment typically loses efficacy over time and therefore new treatments that procure a steady long term supplement of dopamine are needed. Here we tested the expression of a tyrosine hydroxylase (TH) transgene in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) treated primates own astrocytes. The transgene, whose expression of TH cDNA was controlled by a glial fibrillary acidic protein (GFAP) promoter, was injected into MPTP treated primate's brains using liposomes as a delivery system. Monkeys were tested before and after MPTP administration, and after gene therapy treatment on the HALLWAY behavioral task. Results showed both transgene expression and significant behavioral improvements in the hallway task after the TH cDNA transfer. The behavioral recovery observed in the primates whose astrocytes expressed rat TH, is a first step that warrant further studies using primate's astrocytes as a good cell lineage to express therapeutic molecules.

Key words: parkinsonism, MPTP primate model, astrocytes, gene therapy, quantitative evaluation

INTRODUCTION

Parkinson's disease (PD) is a neurodegenerative and progressive pathology characterized by an imbalance in the basal ganglia circuit resulting from a large decrease in dopamine (DA) levels in the striatum. This decrease is caused by neuronal death in the substantia nigra (pars compacta). Clinically the syndrome is known as Parkinsonism, which is characterized by motor alterations such as rest tremor, rigidity to passive movements, difficulty in initiating movements, as well as slowness in voluntary movements (akinesia and bradikine-

sia). Other symptoms include postural alterations, loss of facial expression, gait and balance alterations. PD etiology is unknown, however, it has been reported that both genetic and environmental factors are involved (Fuente-Fernandez and Calne 2002, Le and Appel 2004). One of the most important models that reproduces the histological injuries, as well as behavioral, biochemical and cognitive alterations characteristic of idiopathic PD in both humans (Langston et al. 1983, Burns et al. 1985) and nonhuman primates (Burns et al. 1984, Langston et al. 1984, Di Paolo et al. 1986, Elsworth et al. 1989, Fernández-Ruiz et al. 1995) is the administration of the mitochondrial complex I inhibitor 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). This model has been used to evaluate different antiparkinsonian therapies including phar-

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macological, surgical procedures, transplants and gene therapy (Elsworth et al. 1998, Fernández-Ruiz et al. 1999, Luquin et al. 1999, Muramatsu et al. 2002, Hadj Tahar et al. 2004, Eslamboli et al. 2005, Yokoyama et al. 2011). Diverse pharmacological treatments exist to improve the symptoms of PD. Currently; administration of levodopa is the most used, with extraordinary results at the beginning of the treatment. Nevertheless, between 75 and 80% of the patients with PD that have received high doses of levodopa over the course of 5 to 10 years, develop motor complications such as dyskinesias in combination with rigidity and tremor, short On-Off phenomena and drug-intolerance (Obeso et al. 1989, Golbe 1991, Schrag et al. 1998, Ahlskog and Muentner 2001, Van Gerpen et al. 2006).

One of the more promising therapeutic strategies currently being developed is gene therapy, which can be classified as symptomatic, protective and restorative. Among those, the most used is the symptomatic strategy, in which cells are implanted or genetically modified *in situ*, to make them able to synthesize and release levodopa or DA (Freed et al. 2001, Segovia 2002). Although neural death progresses during PD, neurons have been the target cells of choice for this strategy (During et al. 1994, Bankiewicz et al. 2000, Leone et al. 2000, Shen et al. 2000). However some rodent studies have shown that it is possible to modify glial cells, which are a versatile platform that has the biochemical machinery necessary, except for TH, to produce and release DA into the medium (Juorio et al. 1993, Tsai and Lee 1996, Markiewicz and Lukomska 2006). Studies using glia as a platform for releasing DA have found significant behavioral improvements in rodent models (Segovia et al. 1998a, Trejo et al. 1999b). An important step towards implementing this therapy in humans is to evaluate its effect in primates by modifying their host astrocytes in order for them to express TH which then could lead to behavioral improvement.

In this work we report the behavioral impairment observed in the HALLWAY task after MPTP treatment in vervet monkeys (Campos-Romo et al. 2009). We also report the subsequent significant expression of TH and behavioral improvement following the injection of a transgene into the monkey's striatum. The TH cDNA of the injected transgene was designed to be under the control of the human glial fibrillary acidic protein (GFAP) promoter (Segovia et al. 1998b).

METHODS

Animals

Experiments were carried out in 2 male (4.7 and 5.1 kg) and one female (2.5 kg) vervet monkeys (*Cercopithecus aethiops*). Animals were kept under a controlled environment in individual cages, with light/dark cycles of 12:12 hours, temperature of $24 \pm 1^\circ\text{C}$ and $50 \pm 10\%$ relative humidity, with free access to water, fruits, vegetables and food pellets (High Protein Monkey Chow of Lab Chows, Purina®). The animals were maintained in conditions according to the Mexican official norm (NOM-062-ZOO-1999). We followed the engineering specifications for the well-taken care of production and use of the laboratory animals as well as the regulations for animal care as prescribed by the Faculty of Veterinary Medicine, National University of Mexico.

Behavioral task

Monkeys performance in the HALLWAY behavioral task was videotaped with 4 cameras positioned at different angles (Campos-Romo et al. 2009). In this task the primates walk through a hallway made with a steel frame and transparent acrylic walls, whose dimensions were: 230 cm length, 100 cm high and 100 cm wide. At the end of the hallway there were 8 holes placed at 4 shelves, with one hole to the right and one to the left on each shelf. Each monkey was required to reach to two rewards that were initially placed on the first shelf, one on each side. Once the monkey had taken them, it had to return to the beginning of the hallway. When that happened, more rewards were placed on the second shelf; this process was repeated for the third and fourth shelves; the whole process was repeated twice. This behavioral evaluation was done during 10 days in each of the following consecutive conditions: before and after MPTP treatment, during the acute administration of Levodopa/Carbidopa (L/C), the MPTP baseline after 15 days of L/C withdrawing (to ensure that there is not residual levodopa available in the system), and finally one month after the gene therapy procedure (Fig. 1). Using Adobe Premiere 6.0® software, the four different angle videos were synchronized, and then placed together in a composition fitting the screen using After Effects 5.5® software. The final projects were saved in Quick Time® format. The

data obtained from the videos consisted in frame by frame quantifications of displacement times across the hallway, reaching times towards the rewards, and ingestion times.

It is important to note that each monkey had 10 evaluations during each condition, so we could evaluate the effect of the different treatments in a subject by subject basis.

MPTP treatment

A room with all the necessary security and safety measures for the animals and the researchers was prepared and secured. All the personnel security protocols were followed (Przedborski et al. 2001). Parkinsonism was produced following standard treatment (Taylor et al. 1997) with MPTP hydrochloride (Sigma, St Louis, USA) dissolved in saline solution. After finishing the behavioral evaluation, the monkeys were administered an accumulated dose ranging from 2.0 to 2.5 mg/kg divided into 4 to 5 intramuscular doses of 0.5 mg/kg in a period of 4 to 5 days until they presented a stable extra-pyramidal syndrome (Taylor et al. 1997). After the MPTP treatment, monkeys were closely monitored; they were provided with sufficient water and food to maintain their corporal weight and general well-being. Due to the gradual administration of the MPTP drug, none of the animal required to be

hand-fed. One month after finishing the treatment, monkeys were reincorporated to the behavioral assessment.

Levodopa treatment

Treatment with levodopa in PD patients shows extraordinary results during the initial years, so we wanted to compare the effect of gene therapy with that of the levodopa treatment (Campos-Romo et al. 2009). Once the behavioral evaluation period after MPTP intoxication was finished, Levodopa/Carbidopa (Sinemet® Merck Sharp & Dohme de México S.A de C.V.) (15/1.5 mg/kg) was given orally daily. After one week of LC treatment, we began with behavioral evaluations 30 minutes after the LC administration during 10 days. At this dose we did not observe dyskinesia in any of the subjects for the duration of the experiment.

Magnetic resonance

T1 and T2 magnetic resonances images (MRI) sequences were taken from each monkey’s head. The animals were anaesthetized with Zoletil 50® (Tiletamine/Zolazepam) (Laboratorios Virbac S.A.) injection at dose of 4 mg/kg. These studies were done while monkeys had their head held in a MRI compatible stereot-

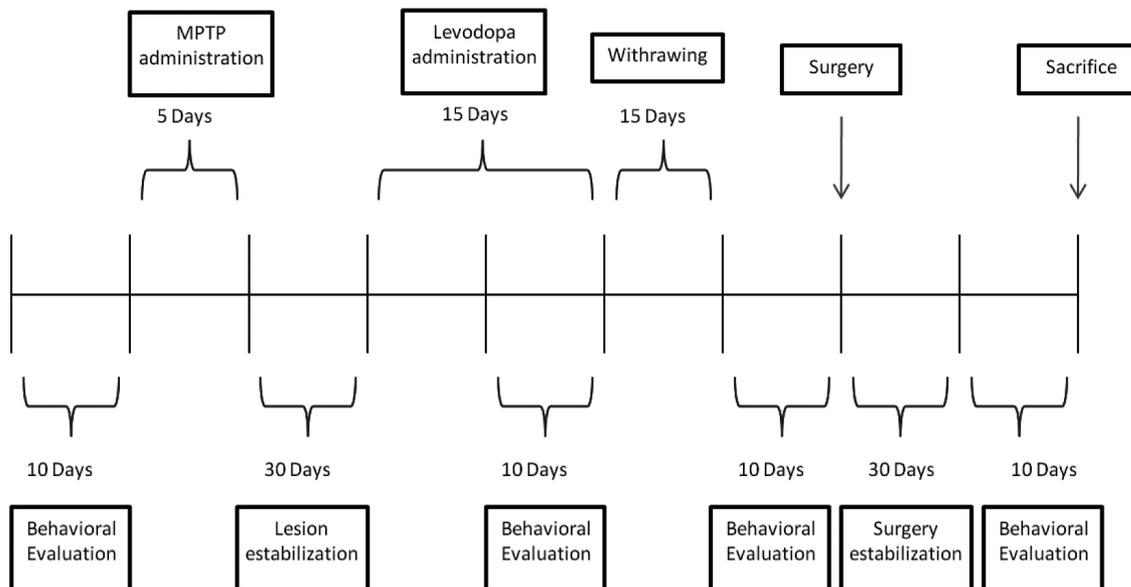


Fig. 1. Schematic chronogram of the experiments

actic frame that was used during both the MRI imaging and the surgery. Using each monkey's MRI we localized the individual coordinates (antero-posterior), (dorso-ventral and mediolateral) for the multiple targets within the putamen. Three sets of coordinates were obtained for each putamen, so a total of 9 injections were performed in each monkey.

Gene transfer procedure

Before the transfer procedure we evaluated the monkeys without any treatment in order to have a new baseline to compare to the gene therapy effect. After the last evaluation without any treatment, gene transfer was accomplished by intracerebrally transferring a TH full-length cDNA. We used a rat TH cDNA driven by a human glial-specific promoter (*gfa2*) (Segovia et al. 1998b). As a control, we transferred the pGfa2-LacZ plasmid, in which the expression of the lac-Z reporter gene is also under the transcriptional control of the *gfa2* promoter (Segovia et al. 1998b). After each monkey was anaesthetized *via* an intramuscular (IM) injection of Zoletil 50® (Tiletamine/Zolazepam) (Laboratorios Virbac S.A.) 4 mg/kg, they were intubated in order to maintain anesthesia with inhaled Dorin® (isoflurane) (Halocarbon Laboratories). Then, standard stereotactic procedures were followed to make the cranial perforations for the injections.

Liposomes for intracerebral injections were prepared following the manufacturer's instructions (Invitrogen), and as previously described (Segovia et al. 1998a) by mixing 6 µg of the plasmid (pGfa2-TH or pGfa2-LacZ) with 15 µl of Lipofectamine 2000 (Invitrogen) followed by an incubation of 10–15 min at room temperature before injecting them into the putamen. Each subject had 9 doses per hemisphere (3 tracts with 3 different depths each), for a total of 54 µg of plasmid per hemisphere.

Each DNA-liposome complex dose was injected through a Hamilton microsyringe at a 3 µl per min rate. The needle was left in each place during 5 min after finishing each injection.

Monkey 1 received pGfa2-TH injections in each hemisphere in the following coordinates in order to cover the whole putamen: 10 mm lateral from the anatomic medial line, 2, 5 and 8 mm superior from the external ear conducts and 24, 26 and 28 mm anterior from the external ear conducts.

Monkey 2 received pGfa2-TH injections in each hemisphere in the following coordinates in order to

cover the whole putamen: 13 mm lateral from the anatomic medial line, 7, 9 and 11 mm superior from the external ear conducts and 10, 12 and 14 mm anterior from the external ear conducts.

Monkey 3 received pGfa2-cLac injections in each hemisphere in the following coordinates in order to cover the whole putamen: 11mm lateral from the anatomic medial line, 10, 13 and 16 mm superior from the external ear conducts and 19, 20 and 21 mm anterior from the external ear conducts.

After the gene therapy procedure, monkeys were closely monitored; they were provided with sufficient water and food to maintain their corporal weight and general well-being. One month after the surgery, monkeys were reincorporated to the behavioral assessment.

Brain tissue analysis

One month and ten days after surgery, and after all the behavioral evaluations were done, each monkey received a pentobarbital overdose. The brains were extracted and cut; the slices of the putamen were separated in two groups of samples. The samples were frozen in dry ice to perform RT-PCR assays using oligonucleotides that exclusively recognize either a rat TH sequence, (Segovia et al. 1998b), and lacZ (Brenner et al. 1994, Domínguez-Monzón et al. 2009) or β-actin (Benítez et al. 2007). The frozen samples were also used for western blot analysis. Briefly, the expression of TH protein was determined as previously described (Segovia et al. 1998b), using a polyclonal antibody (Cell Signaling, 1:1000), and β-galactosidase determined using a monoclonal antibody (Promega, 1:100), blots were incubated overnight at 4°C, washed and then incubated with goat antirabbit (Jackson ImmunoResearch, 1:5000) or goat antimouse (Zymed, 1:2000) peroxidase-coupled secondary antibodies, followed by enhanced chemiluminescence to reveal the proteins (Perkin Elmer). As a positive control, blots were stripped and re-probed with an anti-β-actin antibody (García-Tovar et al. 2001). Images from films were digitally acquired with a BioDoc-It Imaging System (UVP).

Statistical analysis

Intra-individual comparisons of the behavioral results were made using a two-way ANOVA for repeated measures (RM-ANOVA) followed by *post-hoc* Bonferroni test for multiple comparisons ($P < 0.05$).

Greenhouse-Geisser corrections to adjust degrees of freedom were performed in instances where the null hypotheses of sphericity test were rejected. Analyses were made for each subject set of data. Data analysis included those obtained prospectively from the baseline condition, acquired after the MPTP administration, during treatment with L/C, and without any treatment and after the gene therapy transfection.

RESULTS

Behavioral evaluation

RM ANOVA for Monkey's 1 data showed that there were significant differences in displacement time for crossing the hallway under different conditions (Greenhouse-Geisser correction $F=30.299$, $df=2.246$, $P<0.0001$). Bonferroni *post-hoc* analysis showed that there were time differences between all conditions ($P<0.0001$), except between baseline condition and L/C ($P=1.000$) and between MPTP condition and No L/C ($P=1.000$). Reaching time toward rewards also showed significant differences between conditions (Greenhouse-Geisser correction $F=57.261$, $df=2.211$, $P<0.0001$). Bonferroni *post-hoc* analysis indicated that there were time differences between all conditions ($P>0.001$) except between baseline and L/C ($P=0.116$)

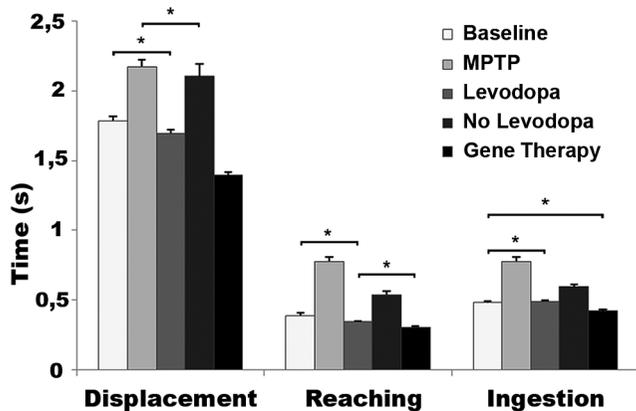


Fig. 2. Monkey 1 (pGfa2-TH) behavioral data. Average amount of time in seconds (s), during displacement, reaching and ingestion at baseline (Baseline), after the administration of MPTP (MPTP), 30 minutes after the treatment with L/C (Levodopa), without any treatment (No Levodopa) and after gene therapy treatment (Gene Therapy). Bars represent the standard error of the mean. Please note that due to the large number of significant differences * indicates no significant differences. All other comparisons are ($P<0.0001$) (ANOVA for repeated measures with Bonferroni *post-hoc* test).

and between L/C and after the gene therapy condition ($P=0.085$). For Ingestion time also showed significant differences (Greenhouse-Geisser correction $F=47.438$, $df=2.293$, $P<0.0001$). Bonferroni *post-hoc* analysis showed that there were time differences between all conditions ($P<0.002$) except between baseline and L/C ($P=1.000$) and between baseline and the after gene therapy condition ($P=0.122$) (Fig. 2).

RM ANOVA for Monkey's 2 data showed a significant displacement time difference for crossing the hallway (Greenhouse-Geisser correction $F=57.267$, $df=1.953$, $P<0.0001$). Bonferroni *post-hoc* analysis showed time differences between all conditions ($P<0.0001$), except between the No Levodopa/Carbidopa and the after gene therapy condition ($P=1.000$). For Reaching time toward rewards also had significant differences between conditions (Greenhouse-Geisser correction $F=42.618$, $df=1.088$, $P<0.0001$). Bonferroni *post-hoc* analysis showed that the MPTP and No Levodopa conditions were different ($P<0.001$) from the other groups. There were no other differences ($P=1.000$). For Ingestion time had significant differences as well (Greenhouse-Geisser correction $F=59.954$, $df=2.204$, $P<0.0001$). Bonferroni *post-hoc* analysis showed significant differences between all conditions, except between Baseline and L/C ($P=1.000$) as well as between baseline and the after

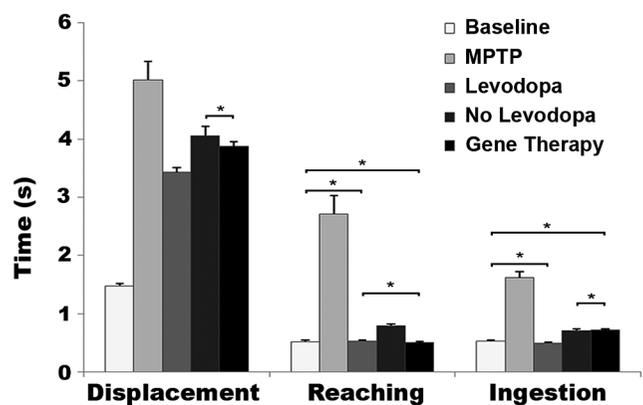


Fig. 3. Monkey 2 (pGfa2-TH) behavioral data. Average amount of time in seconds (s), during displacement, reaching and ingestion at baseline (Baseline), after the administration of MPTP (MPTP), 30 minutes after the treatment with L/C (Levodopa), without any treatment (No Levodopa) and after gene therapy treatment (Gene Therapy). Bars represent the standard error of the mean. Please note that due to the large number of significant differences * indicates no significant differences. All other comparisons are ($P<0.0001$) (ANOVA for repeated measures with Bonferroni *post-hoc* test).

gene therapy condition ($P=0.174$) and L/C and after gene therapy ($P=1.000$) (Fig. 3).

RM ANOVA for Monkey's 3 data showed again significant time differences between conditions for crossing the hallway (Greenhouse-Geisser correction $F=166.719$, $df=3.434$, $P<0.0001$). Bonferroni *post-hoc* time analysis showed that all conditions were different from each other ($P<0.0001$) except between baseline and MPTP ($P=1.000$) for reaching toward rewards time showed significant differences (Greenhouse-Geisser correction $F=60.451$, $df=2.911$, $P<0.0001$). Bonferroni *post-hoc* time analysis showed that all conditions were different between them ($P<0.0001$) except L/C and gene therapy condition ($P=1.000$), and for ingesting, RM ANOVA showed significant differences (Greenhouse-Geisser correction $F=56.151$, $df=2.469$, $P<0.0001$) Bonferroni *post-hoc* analysis showed significant time differences between all conditions ($P>0.03$) except between baseline and L/C ($P=1.000$) and between no L/C and gene therapy ($P=1.000$) (Fig. 4).

Vector expression

Transgene expression was determined through RT-PCR assays using oligonucleotides that specifically recognize TH rat sequences. Figure 5 shows TH transgene expression in the putamen of the two monkeys that received the gfa2-TH plasmid, and the

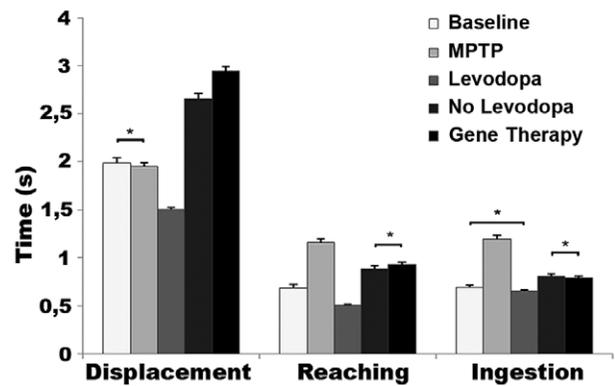


Fig. 4. Monkey 3 (pGfa2-LacZ) behavioral data. Average amount of time in seconds (s), during displacement, reaching and ingestion at baseline (Baseline), after the administration of MPTP (MPTP), 30 minutes after the treatment with L/C (Levodopa), without any treatment (No Levodopa) and after gene therapy treatment (Gene Therapy). Bars represent the standard error of the mean. Please note that due to the large number of significant differences, * indicates no significant differences. All other comparisons are ($P<0.0001$) (ANOVA for repeated measures with Bonferroni *post-hoc* test).

lack of TH expression in the putamen of the monkey that received the pGfa2-LacZ plasmid. The RT-PCR product is of the expected size (258 bp), which is the same size as that obtained from the amplification of the pGfa2-TH control plasmid (Segovia et al. 1998b). Restriction analysis of the amplicons with Apol

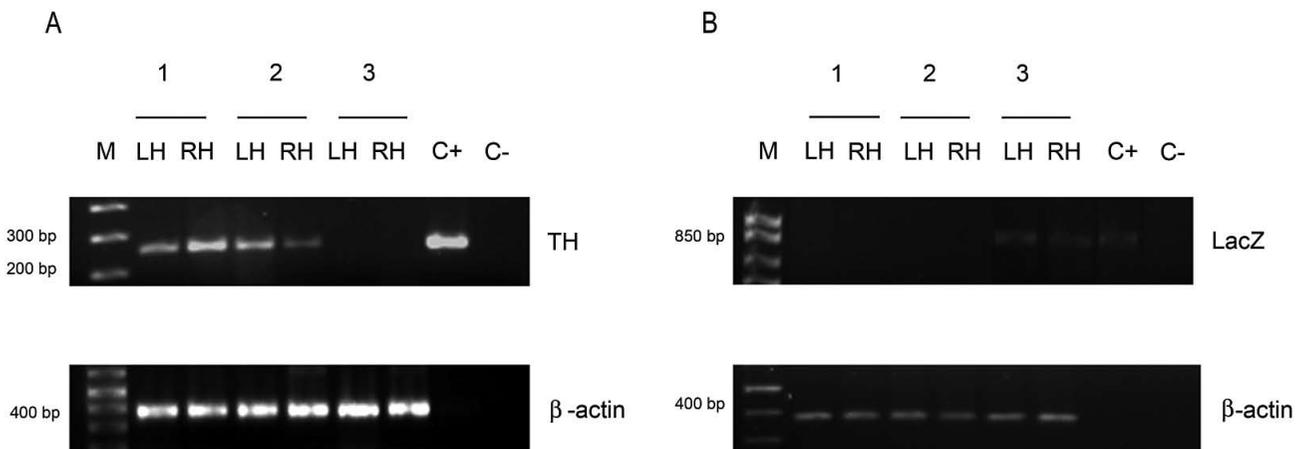


Fig. 5. Transgenic TH and lacZ mRNA detection by RT-PCR. (A) Detection of TH mRNA; 1 and 2 correspond to monkeys receiving the pGfa2-TH transgene, and 3 to the monkey receiving pGfa2-LacZ; LH and RH indicate samples obtained from the left and right putamen, respectively, from each subject, (C+) is the PCR product from the plasmid, and (C-) is a control reaction without cDNA; lower panel shows the detection of β -actin mRNA as a positive control. (B) Detection of lacZ mRNA; 1 and 2 correspond to monkeys receiving the pGfa2-TH transgene, and 3 to the monkey receiving pGfa2-LacZ; LH and RH indicate samples obtained from the left and right putamen, respectively, from each subject, (C+) is the PCR product from the plasmid, and (C-) is a control reaction without cDNA; lower panel shows the detection of β -actin mRNA as a positive control.

resulted in two fragments of 98 and 160 bp, which correspond to the rat sequence. The control reporter lacZ gene was only expressed in the monkey receiving the pGfa2-LacZ plasmid, whereas there was no expression of the control vector in the monkeys that received the TH vector (Fig. 5).

To ensure the appropriate expression of the transgenic proteins, western blot analyses were performed. Figure 6 shows a strong expression of TH in the MPTP animals that received the intracerebral gene transfer of the TH cDNA, as well as a faint TH immunoreactivity signal in the monkey treated with the control gfa2-lacZ vector. Figure 6 also shows the expression of β -galactosidase, which was only detected in the monkey that received the gfa2-lacZ vector.

The pattern of expression of both vectors across the three monkeys suggests that the behavioral recovery observed after the gfa2-TH transfection is associated with the expression of TH in the lesioned striatum, since the transfer of a control transgene gfa2-LacZ, and the surgical procedure did not induce behavioral recovery.

DISCUSSION

The present study tested whether the transfection of the pGfa2-TH plasmid into the astrocytes of the puta-

men of MPTP treated monkeys could lead to behavioral improvements. The results show a significant behavioral improvement only in the monkeys that received the pGfa2-TH plasmid, and not in a control monkey that underwent the same procedures but received a pGfa2-LacZ plasmid. These results suggest that the host astrocytes incorporated the plasmid and were able to make significant behavioral improvements as measured in the HALLWAY task (Campos-Romo et al. 2009).

The initial behavioral analysis using the HALLWAY task showed a clear behavioral deficit after the MPTP administration. The improvement observed after L/C administration support the notion that the monkeys had a significant DA decrease produced by the MPTP neurotoxicity. The behavioral impairment followed after the L/C withdrawing not only supports the notion of the monkeys' impairments, but also confirms that the deficits remained present after several weeks (Taylor et al. 1997).

An important issue to be addressed is the plasmid expression from the host astrocytes. Since the plasmid was injected directly into the host brain, the selection of the appropriate promoter was an important factor. Different strong heterologous promoters have been used for experimental gene therapy of par-

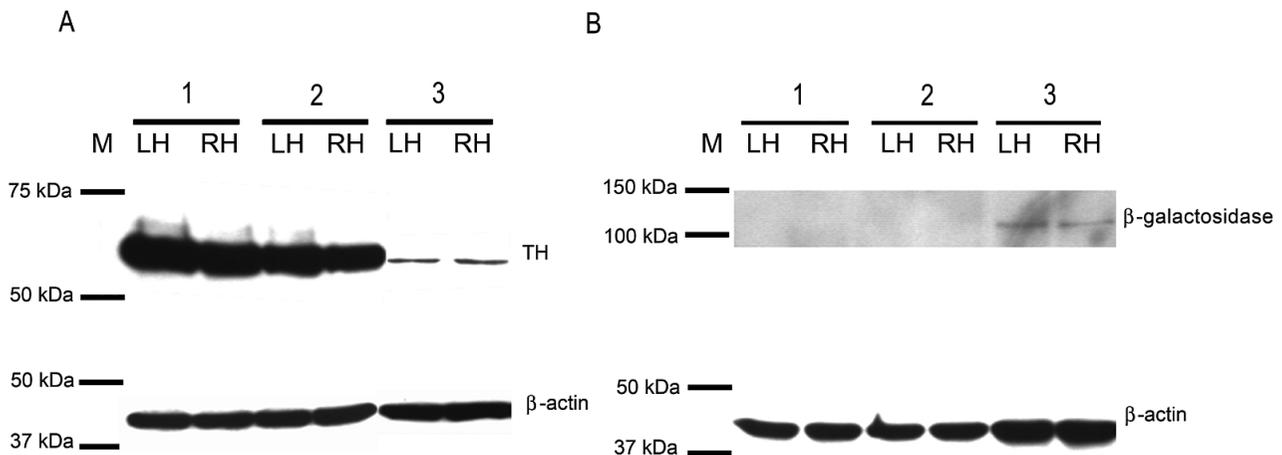


Fig. 6. Expression of transgenic proteins determined by Western blot analysis. (A) Detection of TH protein; 1 and 2 correspond to monkeys receiving the pGfa2-TH transgene, and 3 to the monkey receiving pGfa2-LacZ; LH and RH indicate samples obtained from the left and right putamen, respectively, from each subject; lower panel shows the detection of β -actin as a positive control. (B) Detection of β -galactosidase; 1 and 2 correspond to monkeys receiving the pGfa2-TH transgene, and 3 to the monkey receiving pGfa2-LacZ; LH and RH indicate samples obtained from the left and right putamen, respectively, from each subject; lower panel shows the detection of β -actin as a positive control.

kinsonism, for example, the cytomegalovirus promoter, as well as the chicken β -actin promoter, have been employed in primate parkinsonian models (Ahlskog and Muenter 2001, Eberling et al. 2009). Here we used the GFAP promoter to restrict transgene expression exclusively to astrocytes (Trejo et al. 1999a,b). This promoter has the advantage of being regulated, as it increases its activity in response to gliosis (Brenner et al. 1994, Galou et al. 1994, Johnson et al. 1995) which usually follows after injuries to the brain, including PD (De la Monte et al. 1989, Forno et al. 1992).

The RT-PCR analysis revealed the presence of foreign mRNA into the host putaminal tissue. Both monkeys treated with pGfa2-TH showed expression of transgenic rat TH. Previous reports have shown expression of the pGfa2-TH transgene after intracerebral transfer in 6-OHDA-lesioned rats (Segovia et al. 1998a). Since the oligonucleotides used for the assay only recognize the rat sequence (Segovia et al. 1998b) we can conclude that the detected mRNA is from the injected plasmid, and not from the monkey's endogenous TH.

A second issue to be considered is the functionality of the transfected genes. Western blot analyses showed high levels of TH only in those animals that received the pGfa2-TH transgene; these were also the same animals that showed behavioral improvement.

The monkey that was transfected with the pGfa2-LacZ plasmid did not show rat TH, as determined by the RT-PCR analysis. In addition, the western blot analysis showed a weak TH signal that could be residual from DAergic neurons spared after the MPTP administration. Elsworth and coauthors (2000) found that monkeys with mild to moderate parkinsonism severity, had between 10 to 65% of neurons remaining (Elsworth et al. 2000). The remnant TH-positive neurons, however, were not enough to prevent behavioral impairments (Elsworth et al. 2000). Also, it is clear that the surgery and the transfer of pGfa2-LacZ were not able to produce behavioral improvements (Taylor et al. 1995). As the promoter restricts the expression to host astrocytes, we can infer that the presence of TH and rat TH mRNA must be from the host astrocytes and not from other cell type.

These results suggest that transfection of putaminal astrocytes with the gfa2-TH transgene leads to improved behavior, as we expected this improve-

ment was not as good as the levodopa treatment, probably due to the transfer method that is not as efficient as other methods such as viral vectors. Other approaches have produced behavioral improvements by altering the neurotransmitters and thus the physiological unbalance of the dysfunctional circuits in the Basal Ganglia (BG). For example, the transfer of glutamic acid decarboxylase (GAD) into the STN of MPTP treated primates produced behavioral improvements (Emborg et al. 2007). Also, transfection of human Aromatic L-amino acid decarboxylase (hAADC) into primates previously treated with MPTP lowered l-Dopa requirements and reduced l-Dopa-induced side effects (Bankiewicz et al. 2006).

Unlike other methods, the present approach use astrocytes as the platform of choice. As previously mentioned astrocytes are capable of synthesizing DA from Levodopa, and releasing it to the culture medium (Juorio et al. 1993). Furthermore, the pGfa2-TH vector directly transferred into the striata of 6-OHDA-lesioned rats produces behavioral recovery associated with the transgene expression (Segovia et al. 1998a). Also, the transcriptional control of the gfa2 promoter has been successfully shown in both C6 cells transfected with the gfa2-TH transgene and in primary astrocytes transduced with a retroviral vector expressing TH (Trejo et al. 1999b, Cortez et al. 2000). Another important characteristic shown by genetically modified glial cells, apart from their ability to produce and release DA, is that it has been shown that they can respond to physiological stimuli. For example, they can increase the amount of DA released when depolarized with KCl or even when they are stimulated with glutamic acid (Guerrero-Cázares et al. 2007).

The present results, together with previous reports (Segovia et al. 1998a, Trejo et al. 1999a) show that the expression of TH under the transcriptional control of the gfa2 promoter in cells of glial origin is capable of inducing behavioral recovery in both, murine and primate experimental models of Parkinson's disease. Although these results are promising, long term studies and more efficient transfer methods as well as testing in other PD models (Glud et al. 2011), would be needed before to start exploring the possibility to test its capacity as a potential therapy or adjuvant to treat Parkinson's disease in humans.

CONCLUSION

We report the effects of genetically modifying the own host astrocytes to allow them to produce TH in order to synthesize and release DA into the medium. Our results show that the genetically altered system is able to express TH in the striatum which in turn leads to a significant improvement in the motor performance of primates. These results warrant further studies using astrocytes as a good cell lineage to express therapeutic molecules.

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