Evidence of oligodendrogliosis in MPTP-induced Parkinsonism

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Aims: Mice and non-human primates administered with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) represent elective experimental models of Parkinsonism, in which degeneration of the nigro-striatal dopaminergic pathway is associated with prominent neuroinflammation, characterized by activated microglia and astrocytes in both substantia nigra (SN) and striatum. To date, it is unknown whether oligodendrocytes play a role in these events.

Methods: We performed a detailed qualitative and quantitative analysis of oligodendrocyte-associated changes induced by acute and chronic MPTP treatment, in the SN and striatum of mice and macaques, respectively. Oligodendrocytes were immunolabeled by cell-specific markers and analyzed by confocal microscopy.

Results: In both experimental models, MPTP treatment induces an increase in oligodendrocyte cell number and average size, as well as in the total area occupied by this cell type *per* tissue section, accompanied by evident morphological changes. This multifaceted array of changes, herein referred to as oligodendrogliosis, significantly correlate with the reduction in the level of dopaminergic innervation to the striatum.

Conclusions: This event, associated with early damage of the dopaminergic neuron axons **and of the complex striatal circuits of which they are part,** may result in an important, although neglected, aspect in the **onset and progression** of Parkinsonism.

INTRODUCTION

Parkinson's disease (PD) is a neurodegenerative disease **primarily** characterized by the progressive **degeneration** of neurons of the Substantia Nigra *pars compacta* (SN*pc*) and of their fibres projecting to the striatum [1]. **Recently, the idea that axonal damage of the SN*pc* neurons may be a critical step in PD pathology has been put forward [2,3].** Degeneration of the nigro-striatal fibres in human PD is irreversible and the possibility of their partial or complete regeneration is one of the main challenges for researchers in neurosciences. **The most adopted toxin-based model of PD uses 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a selective dopaminergic neurotoxin for humans, primates and mice, which induces clinical symptoms in both humans and monkeys, which mimics idiopathic PD [6,7]. The neurotoxic action of MPTP begins at the level of the dopaminergic terminals in the striatum from where, once its metabolite MPP^+ is captured by these terminals [8], it induces retrograde degeneration of the dopaminergic axons [9] that, if not impeded, leads to a dying-back of the dopaminergic neurons [3,10,11]. In the nervous system, maintenance of healthy axons depends on neurons, as well as on both myelinating and non-myelinating glial cells [12] (oligodendrocytes and Schwann cells). Therefore, severely injuring a neuron, or its axon, may induce axonal degeneration, damage to the periaxonal glial cells and, when present, progressive demyelination [13]. In several neurodegenerative diseases, axon damage and demyelination is followed by microglial and astroglial activation, which, in turn, activate oligodendrocyte precursor cells (OPCs) localized in the vicinity by releasing pro-inflammatory cytokines. In other neurodegenerative scenarios, after migration to the injured area, OPCs differentiate into mature oligodendrocytes (OLs) by increasing the number and complexity of their processes, up-regulating the expression of myelin proteins and forming new membrane wraps around axons [14-16]. Less is known on the behaviour of non-myelinating OLs.**

The involvement of microglial cells and astrocytes in the nigro-striatal pathway of PD

patients and animals with experimental Parkinsonism is a well established phenomenon [5,17,18]. **However, apart from biochemical-based reports on the damage to OLs in the striatum after MPTP toxicity in mice** [reviewed in 20] and the description of α-synuclein-positive inclusions in non-myelinating OLs of the degenerating nigrostriatal pathway of PD patients [19]**,** the correlation between OL and Parkinsonism has been scarcely explored [17]. In the present work, we investigated *in vivo* whether experimental Parkinsonism induced by acute and chronic administration of MPTP in mice and monkeys (*Macaca fascicularis*), respectively, is **associated with OL morphological changes consequent to the primary damage of the distal axon of the dopaminergic neurons and to the secondary alteration of the striatal circuits in which the DA input is integrated.**

MATERIAL AND METHODS

Animals and MPTP treatment

Animals were housed and handled in the facilities of the University of Murcia in accordance with the norms of the states members of the European Union (2003/65/CE), the Guidelines promulgated by the European Convention for the protection of Vertebrate Animals used for Experimental and other scientific purposes of the Council of Europe (no. 123, June 15th, 2006), the European Communities Council Directive 2010/63/ECC, and by the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Guide, revised 1996). The experimental procedures and protocols were approved by the Ethical Committee for Animal Research of the University of Murcia. All efforts were made to minimize animal suffering and to reduce the number of animals used and to utilize alternatives to *in vivo* techniques.

MPTP treated mice

Three-month-old C57BL/6J male mice (Jax®Mice, The Jackson Laboratory, Bar Harbor, Maine USA) received four intraperitoneal injections of MPTP-HCl (Sigma- Aldrich, St. Louis, MO, USA) dissolved in 0.9% NaCl (20 mg/kg) at 2 hours (hrs) intervals according to established protocols [21]. Control animals received saline injections. **Four (TH analysis) to five (confocal quantitative analysis) mice were used for each experimental group.**

*Parkinsonian **macaques***

We used brain sections derived from a colony of chronic parkinsonian macaques (*Macaca fascicularis*), previously established and studied in our Primate Unit. Analyzed samples were obtained from seven young adult animals: four of the seven monkeys had been treated weekly with low intravenous doses of MPTP (0.3 mg/kg) and progressive motor alterations were assessed using a rating scale ranging from 0 to 25, as previously described [5]. MPTP-intoxicated monkeys showing clear impairment of their motor score were classified as parkinsonian; untreated animals

were considered as controls.

Immunohistochemistry and immunofluorescence

Antibodies

Antibodies used were: sheep anti-Tyrosine Hydroxylase antibody (TH) (1:1000, Chemicon, Temecula, CA, USA); mouse anti-Myelin Basic Protein (MBP), diluted 1:30000 (Chemicon, Clone NS-1, Cat. MAB1580), **which detect early and mature oligodendrocytes in mouse brain sections [22], rat anti-2-3-cyclic nucleotide 3-phosphodiesterase (CNPase) (1:200, Abcam Cambridge, UK), and rat anti**-MBP, diluted 1:100 (Chemicon, Cat. MAB395), **which detects mature oligodendrocytes in monkey brain sections.**

Tissue preparation from mice

Mice for each of the following experimental categories were used: control, 72 hours (hrs) and 2 weeks (wks) after the last MPTP injection. Mice were deeply anesthetized with an intraperitoneal injection of ketamine (50 mg/Kg body weight, b.w.) and xylazine (50 mg /Kg b.w.) and **perfused transcardially with a Ringer's oxygenated solutions (pH 7.3), followed by a fixative composed by 4% freshly depolymerized paraformaldehyde in phosphate buffer (PB). Brains were dissected and cryoprotected, for 72 hrs at 4 °C, in 30% sucrose in saline until they sank. Free-floating coronal sections of 25 µm-thick, spanning the entire midbrain and striatum, were cut at a cryostat and stored at -20 °C in a cryoprotectant composed by 0.5 M sucrose diluted in ethylene glycol and 0.2 M PB (pH 7.4) in a 1:1 proportion.**

Tissue preparation from macaques

Two years after the last MPTP administration, monkeys were sacrificed with a lethal injection of pentobarbital after a pre-anaesthesia with an intramuscular injection of ketamine (8 mg/Kg b.w.). Brains were quickly removed, fixed for 3 days in 4% freshly depolymerized paraformaldehyde in 0.1 M PB and cut into 40 µm-thick coronal sections at a sliding microtome (Microm, HM400).

Immunohistochemistry and immunofluorescence

Brain sections were processed for TH immunohistochemistry by using the avidin-biotin complex (ABC) procedure. The primary antibody was diluted in 1% normal horse serum (NHS), 0.5% Triton X-100 and 0.1% NaN_3 in phosphate buffered saline (PBS) (pH 7.4), for 48 hrs at 4°C, under constant shaking. After a rinse in buffer, sections were first incubated with a biotinylated donkey anti-sheep IgG secondary antibody (diluted 1:500, Jackson Immuno Research Laboratories, Inc.), and successively with the ABC, diluted 1:100 (ABC kit; Vectastain Elite, Vector Labs, Burlingame, CA, USA). The peroxidase activity associated with the immune complexes was revealed by incubating the sections in 0.25 mg/ml 3,3'-diaminobenzidine (DAB) (Sigma-Aldrich) and 0.03% H_2O_2 (Sigma-Aldrich) in PBS, pH 7.4. Some of the mouse brain sections immunolabeled for MBP were also processed with the ABC immunohistochemistry, using as the secondary antibody a biotinylated donkey anti-mouse IgG. The following procedures were similar to those described above. Negative controls were obtained by omitting the primary antibodies. Floating sections were mounted on gelatin-coated slides and dehydrated in an ascending series of ethanol alcohol and xylene before being coverslipped.

MBP in mice and macaques was localized by immunofluorescence, using as a secondary antibody a goat anti-mouse IgG and a goat anti-rat IgG, respectively, combined with Alexafluor 594 or Alexafluor 488 (1:1000, Molecular Probes, Carlsbad, CA, USA). Cell nuclei were counterstained with DAPI (1:1000, Molecular Probes, Carlsbad, CA, USA). Sections were mounted on glass slides, coverslipped with the ProLong® Gold Antifade reagent (Molecular Probes) and viewed at a Leica DMIRE2 confocal microscope (Leica Microsystems, Exton, PA, USA). Acquired images were processed with the Leica Confocal Software (Leica Microsystems, Heidelberg 19 GmbH).

Quantitative analysis on immunohistochemical sections

Quantification of immunopositive cells in the SN*pc* was performed on two coronal sections from

each monkey **(+1 mm and +2 mm from the anterior commisure according to macaque brain atlas)** and three coronal sections **(0.86 mm, -046 mm, and -3.08 mm from Bregma according to mouse brain atlas)** from each mouse. Images of the SN*pc* and striatum from both hemispheres were acquired, by using a 20X objective, at a light microscope (Zeiss Axioplan2) connected to a digital camera (AxioCam HRc, Zeiss). TH-immunopositive (TH^+) cells were counted in each photographic field and expressed as the mean number of cells/mm^2 **± the standard error of the mean (SEM). Specifically, each photographic field corresponds to 260 x 370 µm sided rectangles and all cells, including those falling at the border of the photographic rectangle, were counted.** TH immunoreactivity in the striatum was evaluated by densitometric analysis of the immunopositive areas and expressed as optical density (OD, arbitrary gray units), calculated in relation to the background staining of each section by using the ImageJ software (Burger and Burge, Springer Verlag).

Quantitative analysis on confocal microscope images

Brain sections were examined by using immersion oil 63X objective. Each section was scanned in 0.5 µm-thick optical section, the series range being determined by setting the upper and the lower thresholds with the Z/Y Position for Spatial Image Series setting. All quantifications were done blindly. Images were converted into black and white by using the Image-J software and number and area occupied by the immunopositive cells in each photographic field (three image stacks *per* animal taken through the whole thickness of the SN*pc* and striatum) was measured.

Statistical analysis

Statistical analyses were performed by using either the Student's *t* test (when comparing two groups at a time), or the one-way ANOVA test following the post-hoc Dunnet's analysis (when comparing multiple groups against a single reference group). Differences were considered statistically significant for $p \leq 0.05$. Data were expressed as the mean ± the standard error of the mean (SEM). A Pearson coefficient was used to establish the correlation analysis. The

significance of the correlations was determined by using the critical values of the Pearson coefficient table. Differences were considered statistically significant for $p < 0.05$.

RESULTS

*Loss of dopaminergic neurons and axons induced by MPTP treatment in mice is associated with a transient **oligodendrogliosis** in both SNpc and striatum*

We performed detailed confocal and **quantitative analysis on sections of striatum and SN*pc* immunolabeled for MBP,** to evaluate changes in cell number, morphology and area of occupancy in MPTP-treated mice compared to control (Fig. 1). Seventy-two hours after MPTP injection, a significant increase in the MBP^+ cell **number/mm^2; (SN*pc*: control = 205.2 ± 35.4, 72 h MPTP = 537.0 ± 20.9; striatum: control = 425.9 ± 28.6, 72 h MPTP = 714.2 ± 22.2), mean cell size in (SN*pc*: control = 26.1 ± 1.3 µm^2, 72 h MPTP = 76 ± 3.7 µm^2; striatum: control = 21.6 ± 1.7 µm^2, 72 h MPTP = 183.9 ± 60.6 µm^2), and total area occupied by OLs *per* photographic field (which corresponds to 96,200 µm^2) (SN*pc*: control = 997.1 ± 95.5 µm^2, 72 h MPTP = 2920 ± 678.5 µm^2; striatum: control = 535.6 ± 164.2 µm^2, 72 h MPTP = 7650.5 ± 2316.8 µm^2) was observed (Fig. 1A,B and 1D-F, SN*pc*; Fig. 1A',B' and 1D'-F', striatum). To date, this is the first quantitative evaluation, which takes into account this set of parameters in MPTP-treated mice and macaques for evaluating alteration of the normal steady-state condition of OLs.** OLs were characterized by hypertrophic cell bodies, thickened proximal processes and elevated arborization of the distal ones (Fig. 1A,A', control; Fig. 1B,B', 72 hrs MPTP), which accounted for the noticeable enlargement of their cell body size (Fig. 1E,F, SNpc; Fig. 1E',F', striatum). Two weeks after MPTP treatment, confocal (Fig. 1C,C') and **quantitative** (Fig. 1D,F and 1D',F')

analyses showed a significant decrease, with respect to the 72 hrs, in the number of immunopositive cell/mm^2 in both SN*pc* **(72 h MPTP= 537.0 ± 20.9, 2 wks MPTP= 383.1 ± 15.4)** (Fig. 1C and 1D-F) **and striatum (72 h MPTP= 714.2 ± 22.2, 2 wks MPTP= 350.1 ± 17.2)** (Fig. 1C' and 1D'-F'). Except for the number of the MBP$^+$ cells in the SN*pc* and their average area they occupied in the striatum, all these parameters were also significantly lower than, or equal to, those measured in control animals, suggesting a reversion of this process. These results were confirmed by camera lucida reconstructions of anti-oligodendrocyte immunopositive cells, revealed with DAB (Fig. 2A$_{1,2,3}$), performed on sections of control and MPTP-treated mouse striatum. As expected, 72 hrs after MPTP treatment, a significant decrease in both cell number/mm^2 **(control = 228.83 ± 10.39, 72h MPTP = 98.66 ± 5.84)** and intensity of immunolabeling of TH$^+$ neurons and neurites in the SN*pc*, as well as in the intensity of the TH-immunopositivity in the striatum, expressed as OD **(control = 22.06 ± 3.13, 72h MPTP = 9.58 ± 2.89),** were observed (Supplementary Fig. 1). This decrease partially, but significantly, recovered 2 wks after MPTP injection. OL phenotypical changes inversely correlated with the levels of TH immunoreactivity along the dopaminergic pathway, i.e. a higher degree of dopaminergic loss was associated with higher levels of **oligodendrogliosis** (Fig. 2B).

*Persistent **oligodendrogliosis characterizes** the striatum of parkinsonian macaques two years after MPTP treatment*

Since in mice the most prominent oligodendrogliosis was observed in the striatum, a confocal and **quantitative** analysis was carried out on sections of macaque striatum immunolabeled for MBP (Fig. 2). Chronic Parkinsonian macaques showed a striking and significant increase in the number of MBP$^+$ cells in both caudate **(Fig. 2B,B', confocal images; Fig. 2C, cell count: ctrl = 43.02 ± 3.6 and MPTP = 94 ± 10.3 MBP$^+$ cells per mm^2)** and putamen **(Fig. 2G,G', confocal images; Fig.**

2H, cell count, ctrl = 224.07 ± 15.9 and MPTP 540.12 ± 41.01 MBP^+ cells per mm^2) with respect to control. This increase was also appreciated by using DAB as the revelation system in place of fluorescence (Fig. 2A1,2 caudate; Fig. 2F1,2 putamen). OLs in macaque striatum also showed an **apparent increase** in their cell body size, which, however, we were technically unable to measure as cell processes were so intermingled that the software could not identify individual cells. After MPTP treatment, the number of MBP^+ cells in both caudate and putamen inversely correlated with the number of surviving dopaminergic neurons in the SN*pc* (Figs. 2D,I) and with the densitometric values corresponding to the amount of TH^+ fibers projecting into both areas of the striatum (Fig. 2E,J). Chronic macaque MPTP treatment elicited a marked decrease in TH^+ cells and neurites in the SN*pc*, as well as in the dopaminergic projections to the striatum (Supplementary Fig. 2). However, differently from mice, these parameters were persistent 2 years after the last MPTP injection.

DISCUSSION

Our results demonstrate that loss of dopaminergic neurons and of their axons projecting to the striatum, **which is consequent upon MPTP-induced Parkinsonism in mice and macaques, is accompanied by a prominent oligodendrogliosis** along the nigro-sriatal pathway.

In mice, acute MPTP administration triggers a **significant OL response** within 72 hrs. This event is exacerbated in the striatum, possibly connected with the fact that MPP^+, the toxic product of MPTP metabolism in glial cells [23], is captured by the dopaminergic axon terminals and retrogradely transported to the cell body, affecting neuron activity and viability [9]. This mechanism of retrograde degeneration is not peculiar to MPTP, as it is also observed in other pathological conditions induced by administration of different neurotoxins [24,25]. The idea that, at least in some of the common neurodegenerative pathologies, deregulation of axonal activity could be primary with respect to cell body damage and neuron death is interesting, if considering that in both human PD and animal model of Parkinsonism, axons are severely affected [26,27]. In fact, it has been hypothesized that axonal degeneration, probably involving OLs, may indeed precede protein aggregation in nerve fibres (Lewy neurites) and neuronal apoptosis, becoming one of the initial causes of neuronal loss [28]. In our experimental model, oligodendrogliosis may reflect different degrees of axonal damage consequent upon MPTP administration**,** as previously shown by ultrastructural studies [29]. **The anti-oligodendrocyte antibody we used to recognize OLs labels both early and mature OLs. This means that myelinating and non-myelinating cells can be equally immunolabeled. Based on our correlation studies, showing higher levels of oligodendrogliosis when the MPTP-induced loss of the dopaminergic innervation to the striatum is more prominent, we suggest that the first OL response is in part a consequence of the direct lesion of the dopaminergic neuron axons, which are none or scarcely myelinated in different species, including humans [30-33]. On the other hand, we envision that striatal spiny neurons, deprived of the dopaminergic input, may undergo a series of changes characteristic**

of the neuronal response to denervation. These imply also the remodeling of their own axons, as well alteration of other inputs impinging on their cell body and coming, for instance, from the cortical layers (represented by highly myelinated axons). This hypothesis is supported by numerous studies demonstrating how the striatum responds to fluctuation in dopaminergic signaling and how diseases that alter this signaling change striatal functions [reviewed in 34]. An intense and bidirectional cross-talk between glia and axons has been demonstrated both during development [34,35] and in adulthood [34]. Specifically, while glia maintain axolemmal organization, axonal diameter and neuron health, axons maintain glial differentiation and, when present, myelin integrity [34], by means of axonal contact, diameter, electrical activity and different types of molecular signaling [reviewed in 14]. In the peripheral nervous system, altering this signaling by axonal damage initiates an active Schwann cell re-programming [36]. A similar mechanism can, therefore, be advocated in the central nervous system, where damage to axonal transport and neuron physiology, characteristic of dying-back pathologies (here mimicked by MPTP administration), influences OL activity and myelinating properties. On the other hand, one should consider that cytokines released by both microglia and astrocytes, involved in a parallel inflammatory process [5,17,manuscript in preparation], may also contribute to exacerbating an OL response.

A question arising from our results is whether the features we described are due to effective OL morphological changes, or are just a representation of a relocalization of the MBP protein within the cells. It has been reported that mature non-myelinating OLs, identified as MBP^+ cells, are characterized by a highly ramified morphology, which is reduced when the formation of new wraps around nearby axons, to form myelinated internodes, occur [37,38]. These data strongly support the hypothesis that, in our experimental paradigm, axonal degeneration induced by MPTP triggers the shift to an MBP^+-highly ramified-non-

myelinating OL phenotype, which will be reverted with the restoration of peri-axonal wraps. To further investigate this hypothesis, we performed a double immunostaining for MBP and 2-3-cyclic nucleotide 3-phosphodiesterase (CNPase), a protein expressed in myelin-forming cells throughout their lineage, in the striatum of control mice. Interestingly, in contrast to what is reported in the mouse cortex, where CNPase is localized in both cell bodies and processes and MBP is mostly present in cell processes [39] in the mouse striatum MBP immunostaining overlapped that of CNPase (Supplementary Fig. 3). This result does not allow addressing the idea of a putative change in the distribution of MBP after MPTP treatment. However, we cannot exclude the possibility that, beside OL morphological changes, a general increase in MBP immunoreactivity after the MPTP treatment could also occur.

Two weeks after acute MPTP administration in mice, the partial recovery in TH immunoreactivity and the reduction in the inflammatory response [manuscript in preparation] in both SN*pc* and striatum was concomitant to the reduction of oligodendrogliosis, suggesting that re-establishment of some dopaminergic connectivity reflects a more general restoration of striatal circuitry. This result opens up an important avenue of investigation concerning OL behaviour, i.e. their possible involvement in the remodeling and guidance of distal axons of those dopaminergic neurons that survived MPTP treatment, as well as of other neuron axons projecting to the striatum and affected by the disruption of the nigro-striatal circuit. Recurrent OL activation is a peculiar feature of demyelinating diseases, a potentiality that is, however, decreased during aging [40]. As idiopathic PD is a pathology characteristic of the elderly, a decrease in axon plasticity and re-myelination capabilities with age may indeed further exacerbate progressive neuronal death. **To corroborate this hypothesis, future studies on aged mice could give precious information on the development of Parkinsonism by combining both the pathological aspect and the**

physiological aging.

The results obtained in the macaque model of chronic MPTP administration differ, but do not contradict, those described in mice. In chronic parkinsonian macaques, both number and size of OLs in the striatum were still significantly higher than in control animals two years after neurotoxin administration, the oligodendrogliosis being more prominent in macaques suffering more severe dopaminergic depletion. **Oligodendrogliosis** was maintained throughout the years and no apparent recovery of the dopaminergic system was observed**. This discrepancy in the time of occurrence of oligondendrogliosis between mice and macaca, could be partly due to species differences. Moreover, differently from MPTP acutely-treated mice**, in **chronically-induced** Parkinsonism, the presence of a cascade of glia-mediated inflammatory signals, perpetuating themselves, has been well established [5]**. These could sustain a persistent oligodendrogliosis involving both myelinating and non-myelinating OLs, both expressing MBP [41].** This prolonged inflammatory response may also promote a glial scar, creating a hostile environment in which OL activity could be ineffective compared with acute lesions. **As discussed for mice, oligodendrogliosis may not be solely associated with degeneration of dopaminergic neuron axons, as spiny neurons and neuronal populations projecting to the striatum, may undergo progressive degeneration, triggering and perpetuating oligondendrogliosis.**

In conclusion, this study underlines that OLs may play an important role in Parkinsonism, although the function and mechanisms in experimental models and the human disease need to be further investigated. Detailed *post mortem* studies of the nigro-striatal pathway of PD patients will be crucial for understanding type and extent of axonal modifications occurring during the development of the disease, as well as for developing specific therapeutic strategies aimed at preventing, or minimizing, axonal degeneration in Parkinsonism.

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FIGURE LEGENDS

Figure 1. Transient OL activation in both SNpc and striatum of MPTP treated mice. (A-C, A'-C') Confocal analysis of oligodendrocyte immunolabeling (red) in the SN*pc* and striatum of control (A,A') and MPTP-treated mice (B,B', 72 hrs; C,C', 2 wks). Seventy-two hours after MPTP injection (B,B'), a prominent OL activation is observed compared with control animals (A,A'). Oligodendrogliosis drastically reduces after 2 wks (C,C'). Cell nuclei are counterstained in blue with DAPI. (D-F; D'-F') Quantitative analysis shows that OL cell number (D, SNpc and D', striatum) and average cell body size (E, SNpc and E' striatum) significantly increase, respect to control mice, 72 hrs after MPTP treatment. A similar increase is observed in the total area occupied *per* section by the immunopositive cells (F, SNpc, F', striatum). All these values fall 2 wks after MPTP treatment. n= 4-5 animals/group. Histograms represent the mean ± SEM; *$p < 0.05$, calculated by one-way ANOVA followed by the post hoc Duncan's test. Analyzed areas are encircled in red on the brain section drawings at the left hand side.

Figure 2. Correlation analysis between oligodendrogliosis and TH immunolabeling in the SN*pc* and striatum of MPTP-treated mice. (A) MBP immunolabeling with the avidin-biotin-DAB method clearly shows the changes, compared with the control (1), occurring in OL morphology 72 hrs (2) and 2 wks (3) after MPTP treatment, also exemplified in the camera lucida drawings. (B) Increase in number (1, 2 and 1', 2'), mean (3, 4 and 3', 4') and total area (in μm^2) (5, 6 and 5', 6') occupied *per* section by the oligodendrocyte-immunopositive cells inversely correlate to the levels of TH immunostaining in both SN*pc* and striatum. These correlations refer to the number of TH^+ cells in the SN*pc* (1, 3, 5 and 1', 3' 5') and the optical density of the TH^+ fibers in the striatum (2, 4, 6 and 2', 4', 6'). The Pearson correlation coefficient R_2 and the corresponding p values are indicated in each correlation graph. n = 15-20.

Figure 3. Persistent OL activation in the striatum of parkinsonian monkeys.

Caudate (A) and putamen (F) in the monkey brain are put into evidence by the red boxes in the drawings at the left hand side. On the right of each drawing, details of MBP immunolabeling in the striatum of control and MPTP-treated monkeys, revealed by the avidin-biotin-DAB method, are shown (1, 2). The toxic treatment induces an increase in the area occupied by immunopositive cell processes in both caudate (A) and putamen (F). The corresponding confocal analysis is shown in (B,B') for the caudate and in (G,G') for the putamen. A striking increase, with respect to control (B,G), in MBP immunolabeling (red) is still observed two years after MPTP treatment (B',G'). Cell nuclei are counterstained in blue with DAPI. (C,H) Quantitative analysis conducted on the confocal images reveals a significant increase in the number of OLs in both the caudate (C) and putamen (H) of parkinsonian monkeys compared with control animals. (D,E; I,J) The increase in MBP^+ cells inversely correlate with the number of TH^+ neurons counted in the SN*pc* (D, caudate; I, putamen) and with the optical density (O.D.) values of the TH immunolabeling of the dopaminergic axons projecting to caudate (E) and putamen (J). Control group: n = 3 monkeys; parkinsonian group: n = 5 monkeys. The histograms in C and H represent the mean ± SEM; ***$p < 0.001$, calculated by the Student's *t* test. The Pearson correlation coefficient, R_2, is indicated in each of the correlation graphs (D,E,I,J).

SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure 1. TH depletion and partial recovery in both SN*pc* and striatum following acute MPTP treatment in mice. TH-DAB immunostaining in the SN*pc* (A-C) and striatum (D-F) of control (A and D) and MPTP-injected mice (B,C,E,F). (A,D) In control animals, TH immunolabeling is intense in both cell bodies and axons of the dopaminergic neurons. (B,E) Seventy-two hours after MPTP treatment, a drastic decrease in the intensity and number of immunopositive neurons and neurites in the SNpc (B), as well as in the intensity of TH-immunolabeling in the whole striatum, is observed, especially in the dorso-lateral area. (C,F) Two weeks after MPTP injection, a partial recovery of the immunolabeling is observed in both areas (C, SN*pc*; F, striatum). (G,H) Quantitative analysis confirms that changes observed 72 hrs and 2 wks after MPTP injection, with respect to the control (ctrl), in both the number of TH+ cells/mm2 in the SN*pc* (G) and the optical density of the TH^+ fibers in the striatum (H) are statistically significant. A partial, but significant, recovery is observed 2 wks after the MPTP treatment in both SN*pc* and striatum. n = 4-5 animals/time point; bars represent the mean ± SEM; *$p < 0.05$, calculated by one way ANOVA and Duncan test.

Supplementary Figure 2. Persistent TH depletion induced by MPTP chronic treatment in monkeys. (A,B) TH-DAB immunolabeling in the SN*pc* (A1,2) and striatum (B1,2), both caudate (Cd) and putamen (Put), of control (A1 and B1) and parkinsonian monkeys (A2 and B2), shows a marked decrease in TH^+ cells and neurites in the SN*pc* (area circled in red in the left hand drawing) and dopaminergic projections to the striatum (area circled in red in the left hand drawing) in parkinsonian macaques 2 years after the last MPTP injection, compared to control. (C) Quantification of the number of TH^+ cells in the SN*pc* and measurement of the optical densities (O.D.) of the TH^+ immunopositive fibers in the striatum show that these decreases are statistically

significant. Control group: n = 3 monkeys; Parkinsonian group: n = 5 monkeys. Histograms represent the mean ± SEM; *p < 0.05, **p < 0.01 calculated by Student's *t* test.

Supplementary Figure 3. CNPase and MBP co-labeling in the mouse nigro-striatal pathway. Top row of confocal images show a mouse striatum immuno-stained for CNPase (red) and MBP (green). An intense immunostaining for both proteins decorates cell bodies (high magnifications are shown in the top row inserts **together with the lateral view along the z axis**) and fibers coming from the cortex and crossing the striatum. Fibers of the nigro-striatal pathway also co-label for CNPase and MBP (bottom row magnifications). DAPI staining (blue) was used to label nuclei. **Scale bars: 50 μm in the top row, 5 μm in the insert, and 10 μm in the bottom row.**

Figure 1

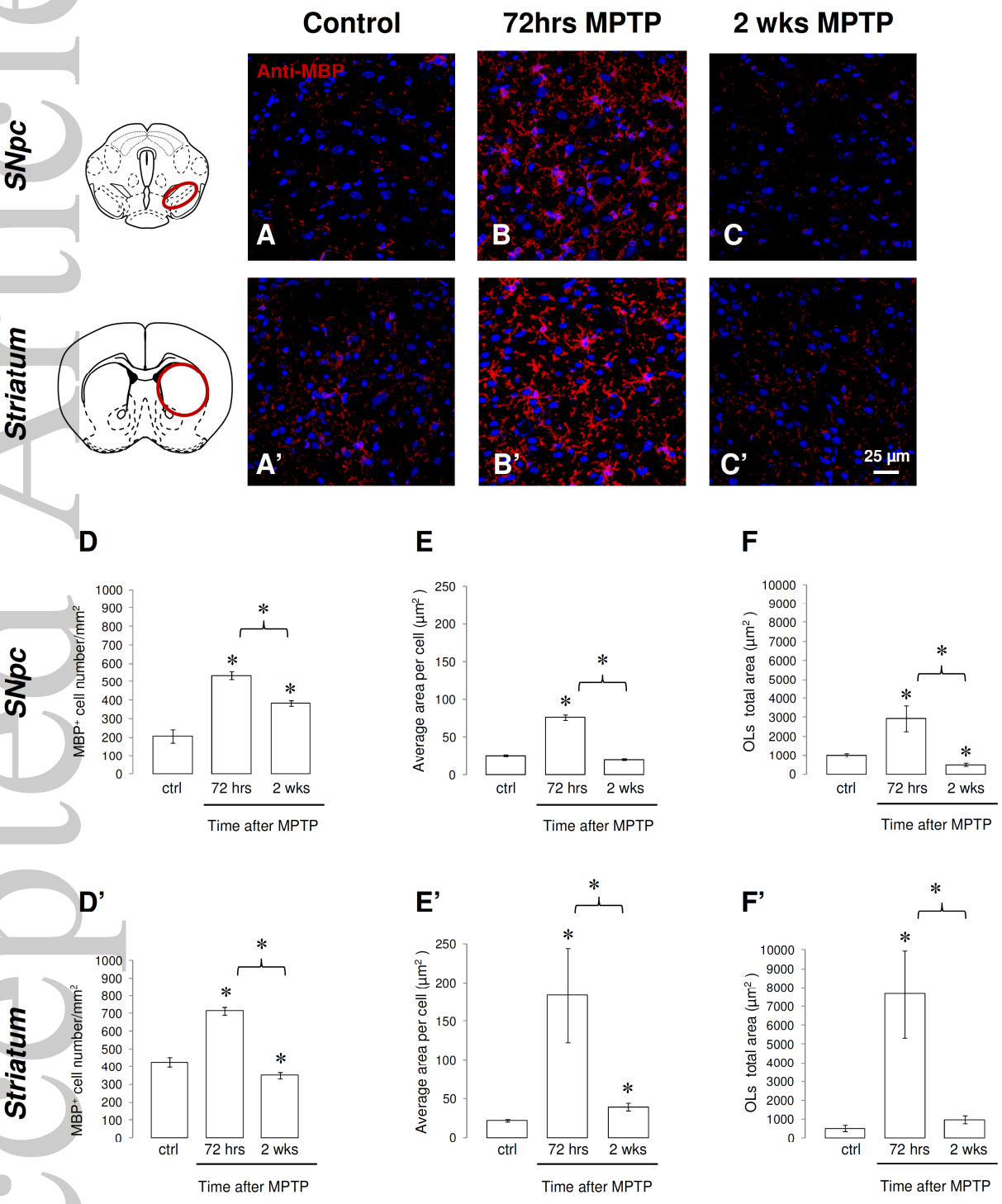

Figure 2

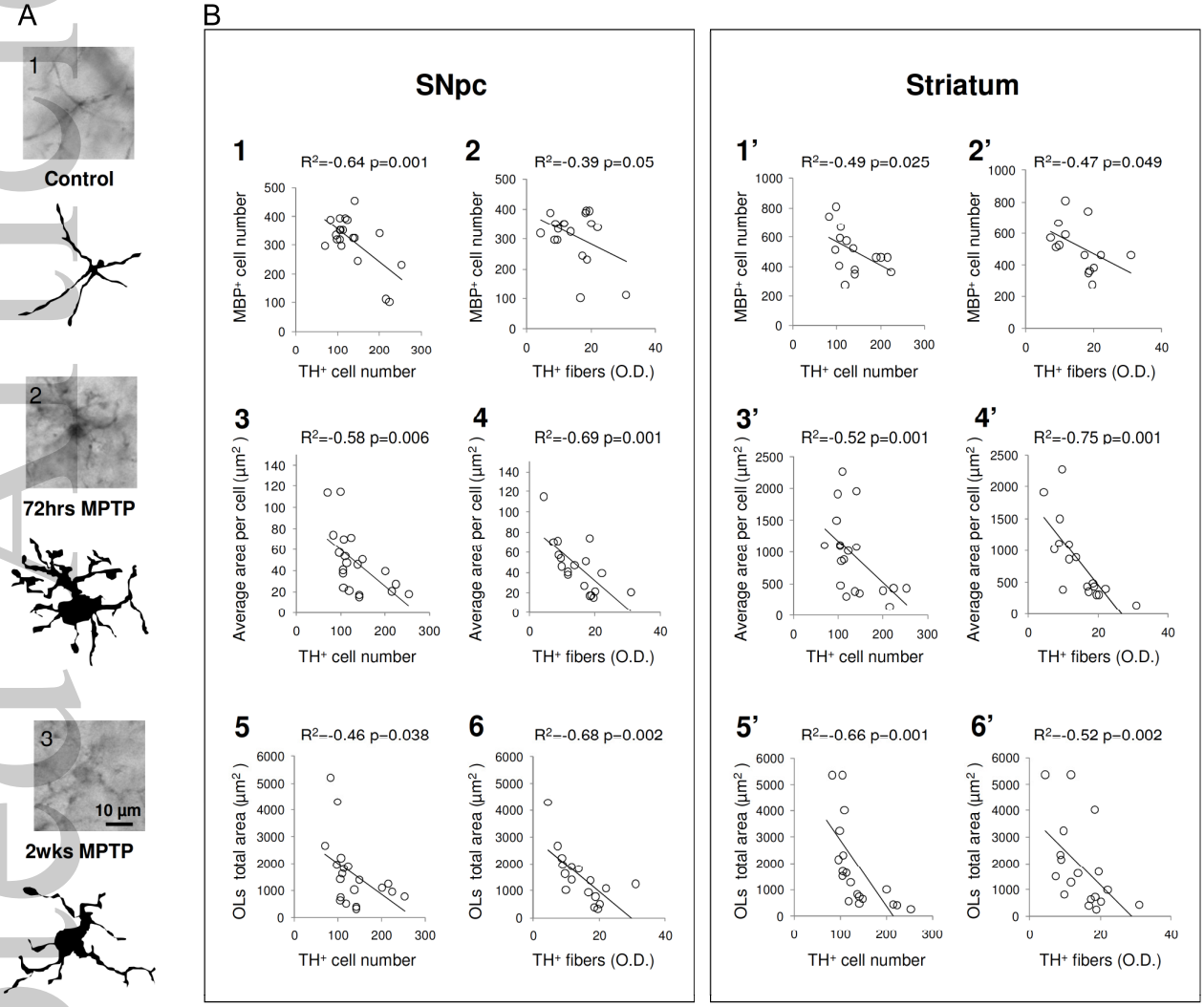

Figure 3