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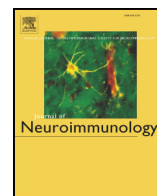
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Persistent phagocytic characteristics of microglia in the substantia nigra of long-term Parkinsonian macaques

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ABSTRACT

Patients with Parkinson's disease show persistent microglial activation in the areas of the brain where the degeneration of dopaminergic neurons takes place. The reason for maintaining this activated state is still unknown, but it is thought that this persistent microglial activation may contribute to the degeneration of dopaminergic neurons. In this study, we report the microanatomical details of microglia and the relationship between microglia and neurons in the substantia nigra *pars compacta* of Parkinsonian monkeys years after insult with MPTP. We observed that microglial cells appear polarized toward dopaminergic neurons in MPTP-treated macaques compared to untreated animals and present clear phagocytic characteristics, such as engulfing gliaptic contacts, an increase in Golgi apparatus protein machinery and ball-and-chain phagocytic buds. These results demonstrate that activated microglia maintain phagocytic characteristics years after neurotoxin insult, and phagocytosis may be a key contributor to the neurodegenerative process.

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1. Introduction

Microglial activation is associated with the release of cytokines, an increase in particular membrane receptors, changes in morphology, and the self-induction of directional motility and polarity (Kreutzberg, 1996; Nimmerjahn et al., 2005; Barcia et al., 2011). The purpose of microglial activation in neurodegenerative diseases remains unclear. In Parkinson's disease (PD) patients, the areas of the brain that contain dopaminergic degeneration show high levels of activated microglia that are maintained for decades (McGeer et al., 1988). The same effect is observed in humans intoxicated with MPTP (Langston et al., 1999) as well as in long-term MPTP-induced Parkinsonian macaques (McGeer et al., 2003; Barcia et al., 2004). However, the specific role of microglia during this persistent stage is still under scrutiny. We and others have recently reported that in an experimental mouse model of PD in which Parkinsonism was induced by MPTP, microglial motility leads to the

formation of complex body-to-body contacts between microglia and dopaminergic neurons that result in the phagocytosis of neuronal cell bodies in the areas of degeneration (Barcia et al., 2012; Depboylu et al., 2012). In mice, interactions between microglia and neurons are transient and precede the elimination of damaged dopaminergic neurons (Barcia et al., 2012).

To study the phagocytic characteristics of microglia in long-term Parkinsonism, we analyzed different parameters of microglial activation in the substantia nigra *pars compacta* (SNpc) in a group of macaques suffering from MPTP-induced Parkinsonism. We observed clear phagocytic features of microglia years after MPTP treatment, such as an increased number of contacts between microglia and dopaminergic neurons and the increase of phagocytic domains, as indicated by the increase of the Golgi apparatus protein machinery and ball-and-chain phagocytic buds. These characteristics are persistent years after MPTP treatment, which suggests that phagocytosis is an important aspect of microglial activation in chronic Parkinsonism that contributes to neurodegeneration.

2. Material and methods

2.1. Parkinsonian monkeys

In this study, we used a brain bank to obtain brain tissue samples from chronic Parkinsonian macaques (*Macaca fascicularis*). We studied

Abbreviations: PD, Parkinson's disease; MPTP, 1-methyl-4-phenyl-1,2,3,6 tetrahydropyridine; SNpc, substantia nigra *pars compacta*; TH, tyrosine hydroxylase; Iba-1, ionized calcium binding adaptor molecule 1; GM130, *cis*-Golgi matrix protein 130; DAPI, 4',6-diamidino-2-phenylindole.

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these macaques throughout the years in our primate unit. All of the studies that were performed in primates before 2006 were carried out in accordance with the protocols from the states members of the European Union (2003/65/CE) and the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Guide, revised 1996). The experiments that were performed in monkeys after 2006 met the recommendations of the Weatherall report, 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 the University of Murcia Approved protocols.

We analyzed samples obtained from young adult macaques from both sexes. Samples from eight animals were used in the present work. Four animals were treated with MPTP and four animals were untreated controls (Table 1). The monkeys were treated weekly with low doses of intravenous MPTP (0.3 mg/kg) according to previous protocols (Herrero et al., 1993; Barcia et al., 2003). Each animal received a different number of doses according to the level of Parkinsonism and their susceptibility to MPTP. None of the animals received L-DOPA or other dopaminergic agonists. Several researchers defined the degree of Parkinsonism in the animals after each dose as well as the progression of the Parkinsonian syndrome. Motor symptoms were assessed using a previously described rating scale ranging from 0 to 25 (Herrero et al., 1993). The degree of disability in the monkeys increased with every new injection and remained stable for months or years. Susceptible MPTP-treated monkeys showed Parkinsonian symptoms and displayed different degrees of bradykinesia and akinesia, and sporadic freezing phenomena. Some of the monkeys presented action tremor, paradoxical kinesia and occasionally vertical and horizontal saccadic ocular movements. Susceptible monkeys also displayed a typical Parkinsonian posture. The animals were euthanized one to 5 years after the last MPTP administration.

2.2. Tissue staining

The monkeys were euthanized by injection with pentobarbital after ketamine anesthesia one, two or five years after the last dose of MPTP. The brain was quickly removed, dissected and fixed for 3 days in 4% paraformaldehyde that was dissolved in 0.1 M phosphate buffer. The fixed mesencephalon was sectioned into 40 μ m thick serial sections (Microm, HM400). Series of sections that were regularly spaced at intervals of 1440 μ m were stained for tyrosine hydroxylase (TH) (sheep polyclonal antibody 1:500; Chemicon, Temecula, CA, USA) to identify dopaminergic neurons, Iba-1 (rabbit polyclonal antibody 1:500; Wako, Chuo-Ku, Osaka, Japan) to identify microglial cells, and cis-Golgi Matrix protein 130 (GM130) (1:500; Abcam, Cambridge, UK) to detect the Golgi apparatus. Sections from all of the animals were stained simultaneously and under the same experimental conditions.

2.2.1. Immunohistochemistry and immunofluorescence

2.2.1.1. DAB detection. Sections of the SNpc (40 μ m) were used to detect specific cells and structures via immunohistochemistry.

Table 1

Motor score and MPTP accumulative dose of used monkeys.

| Monkey | Sex | Motor score | MPTP accumulative dose mg/kg |
|--------|--------|-------------|------------------------------|
| C1 | Male | 0.0 | 0 |
| C2 | Male | 0.0 | 0 |
| C3 | Female | 0.0 | 0 |
| C4 | Male | 0.0 | 0 |
| P1 | Male | 3.5 | 1.8 |
| P2 | Female | 4.2 | 0.9 |
| P3 | Male | 5.0 | 1.8 |
| P4 | Female | 9.2 | 0.9 |

Endogenous peroxidase activity was inhibited with 0.3% H₂O₂, and non-specific Fc binding sites were blocked with 10% horse serum. The sections were incubated for 48 h (room temperature, constant shaking) with the primary antibody (see above) diluted in PBS containing 1% horse serum, 0.5% Triton X-100, and 0.1% sodium azide. The sections were incubated for 4 h in the secondary antibody that was diluted in antibody solution. Binding of the antibody was detected with the avidin–biotin peroxidase ABC kit (Vectastain, Vector Labs). Sections were mounted on gelatin-coated slides and dehydrated in graded ethanol series and xylene before the addition of a coverslip.

2.2.1.2. Immunofluorescence. For immunofluorescence, 40 μ m sections were treated with 0.5% citrate buffer (65 °C, with constant shaking) for 30 min to maximize antibody penetration into the tissue. Non-specific Fc binding sites were blocked with 10% horse serum, and the sections were incubated for 48 h (room temperature, constant shaking) with primary antibody diluted in PBS containing 1% horse serum, 0.5% Triton X-100, and 0.1% sodium azide. The sections were incubated for 4 h with the labeled secondary antibody and were then washed with PBS and incubated with the DAPI solution (1:1000) in 1 \times PBS for 30 min. The sections were washed, mounted, and examined using fluorescence (Zeiss Axioplan 2) or using confocal microscopy (Leica DMIRE2). The secondary antibodies used are as follows: Alexa 488-conjugated, Alexa 594-conjugated or Alexa 647-conjugated (1:1000) (Molecular Probes). For F-actin staining, sections were incubated with Alexa Fluor 488-phalloidin or 647-phalloidin (1:500 in PBS; Molecular Probes) for 2 h at room temperature after immunostaining. After washing, the sections were incubated with a DAPI solution for 30 min. The sections were washed again, mounted and examined by fluorescence (Zeiss, Axioplan 2) or confocal microscopy (DMIRE2, Leica Microsystems, Exton, PA).

2.2.1.3. Quantification and stereological analysis. The nigrostriatal pathway was defined according to the Monkey Brain Atlas (Paxinos et al., 1999). Quantification of DAB or fluorescently labeled cells in the SNpc was performed on coronal sections from each animal. The number of cells was estimated with an optical fractionator probe using a computer assisted image analysis system with a Zeiss Axioplan 2 microscope connected to a digital camera. The region of interest was traced using the 1.25 \times objective. The number of cells was measured in 200 \times 200 μ m dissectors covering the surface of the analyzed region. Labeled cells were counted using the 40 \times objective, and frames were counted throughout the delineated area of the SNpc in each section via the optical fractionator. Two representative sections of the SNpc were used for staining in monkey brains. Representative sections from the monkeys were chosen according to the criteria previously defined by Olzewski and Baxter (1954). The sections contained the ventral area of the SNpc (in primates corresponding to the $\alpha + \beta$ area at the level of the III cranial nerve output), which is known to show the highest degree of dopaminergic loss and glial activation in humans (Damier et al., 1999) and non-human primates (Herrero et al., 1993). The data are expressed as the absolute number of positive cells in each anatomical region analyzed. The results are expressed as the mean \pm SEM.

3. Confocal analysis and 3D rendering

The brain sections were examined using a Leica DMIRE2 confocal microscope with a 63 \times oil objective and Leica Confocal Software (Leica Microsystems). A series of optical sections were analyzed to determine an upper and lower threshold using the Z/Y position for the Spatial Image Series setting. The optical series covered a 20 μ m thick slice of tissue with 0.5 μ m per optical section. The confocal microscope settings were established and maintained by Leica and local technicians for optimal resolution. (For further details, see previous publications (Barcia et al., 2011). Contacts were defined as areas where co-localization of both markers (Iba-1 and TH) occurred between two

cells in at least two 0.5- μm -thick optical sections. A thin area of co-localization can be observed between the two fluorophores. The final confocal images are illustrated as they appear throughout the stack of sections as a simple 0.5- μm -thick layer or as a transparency of all of the layers merged together.

Three-dimensional reconstructions of the stacks of confocal images were rendered in the Imaris software (Bitplane Scientific Software, Zurich, Switzerland). Different surface effects were applied in the 3D rendering to show the structures in the real planes. Videos of the rotations were also generated with Imaris and were edited with iMovie Software (Apple Inc, Cupertino, CA) to obtain the final versions.

3.1. Quantification of activated microglia phenotypes

Sections of the SNpc from monkeys underwent immunofluorescence to visualize microglial cells (Iba-1⁺ cells). The sections were scanned with a confocal microscope as described above, and the terminal microglial tips per microglial cell were quantified along the z-axis. The microglial area and terminal tips were quantified using confocal images of the Iba-1⁺ cells. The scanned confocal planes with a thickness of 0.5 μm that contain Iba-1⁺ cells were processed with the image software Image-J in order to obtain a black and white image. The area of 50 microglial cells (specified by Iba-1 staining) in the SNpc of each animal was measured in the processed images. Similarly, sections were co-stained for Iba-1 and GM130, and the area occupied by GM130 was quantified in Iba-1⁺ cells using the same method described for Iba-1. Two different researchers did all of the quantifications blindly.

3.2. Quantification of intercellular contacts

Intercellular contacts between microglial cells and dopaminergic neurons or fibers were quantified using stereological methods in the confocal stacks of images of the SNpc. The number of contacts per field between cells that are positive for Iba-1 and TH was quantified in 0.5 μm single layers from the 20- μm -thick confocal stacks of images. The contacts between microglial cells and dopaminergic neurons were identified by the convergence of red and green fluorescence, which formed a thin yellow area. The contacts were classified into four categories: **Pr-Pr**, contact between a microglial process and a dopaminergic process; **B-Pr**, contact between a microglial cell body and a dopaminergic process; **Pr-B**, contact between a microglial process and a dopaminergic neuron cell body; and **B-B**, contact between a microglial cell body and a dopaminergic neuron cell body. The number of contacts per DA fiber (**Pr-Pr** and **B-Pr**) was quantified using the total number of remaining fibers, which was quantified using unbiased stereological methods (Gundersen, 1986). The number of **B-B** and **Pr-B** contacts per neuron was quantified for each neuron along the z plane. The length of the contacts between processes and neurons was quantified using Leica Confocal software. All parameters (number of contacts, number of dopaminergic fibers and neurons, number of contacts per neuron and the length of the contact) were quantified in 5 dissectors (sample fields) per single layer of the 20- μm -thick stacks. For each analysis, 8 stacks per brain region per animal were analyzed. The final result for the length of the contact was the average of at least 150 events per animal.

4. Statistical analysis

The data are expressed as the mean \pm SEM. A statistical analysis was performed using a student's t-test or one-way ANOVA test followed by a post hoc analysis (Tukey's test). The null hypothesis was rejected for an α risk equal to 5%.

5. Results

5.1. There is an increase in microglial polarity in chronic Parkinsonian macaques

To understand whether microglial polarization and the formation of engulfing gliapses takes place in chronic Parkinsonian primates, we analyzed the brain sections of macaques with stable Parkinsonism by applying techniques and imaging protocols that were previously used in mice (Barcia et al., 2012). The primates were analyzed years after MPTP treatment. The results indicate that macaques with Parkinsonism show loss of dopaminergic neurons in the SNpc and a persistent increase in microglial activation (Fig. 1A–C), which has been the focus of recent extended studies in our group (Barcia et al., 2011). Although the number of Iba-1⁺ microglial cells did not change in the SNpc of Parkinsonian monkeys compared to control animals, the microglia clearly showed an activated phenotype, which was characterized by an increased cell body size and an increased number of terminal tips (Fig. 1D–E).

Because this increase in terminal tips may reflect the polarization of microglial cells toward damaged neurons, we analyzed the anatomical relationship between microglia and neurons in the SNpc of chronic Parkinsonian monkeys. Strikingly, years after the induction of Parkinsonism, microglial cells remain in a polarized state toward neighboring dopaminergic neurons when compared to those of control animals. A detailed confocal analysis indicated that there were an increased number of contacts between microglia and the remaining dopaminergic neurons in the SNpc years after the last MPTP insult. The analysis of the entire SNpc showed a prominent increase of interactions between microglial filopodia and dopaminergic structures (Fig. 2A and B). However, the contacts between microglial cell bodies and dopaminergic neurons are similar to those of the controls. Importantly, an analysis of particular sub-regions of the SNpc revealed that in Parkinsonian macaques, the number of body-to-body gliapse-like contacts remained higher than controls only in the ventral area of the SNpc, which is an area that is known to undergo the highest loss of dopaminergic neurons in Parkinsonian primates (Fig. 2C–E). In addition, the area of contact between microglia and dopaminergic neurons, including the neurites and neuronal bodies, was also increased in Parkinsonian monkeys (Fig. 2F).

5.2. Microglia show persistent phagocytic characteristics

A confocal analysis of the micro-anatomical characteristics of microglial cells in the SNpc of Parkinsonian macaques revealed that microglia exhibit phagocytic characteristics in the areas of neurodegeneration. One of the key elements, observed previously in the mouse MPTP-induced model, is an increase of Golgi apparatus protein machinery in microglial cells. This increase indicates that the microglia in the degenerating areas contain phagocytic and secretory functionalities. In macaques, we found that there is an increase in Golgi apparatus protein machinery, as indicated by GM130 staining, in Parkinsonian macaques compared to control animals (Fig. 3A–C). This increase observed in the SNpc suggests that microglial cells remain in a persistent phagocytic state in chronic Parkinsonism. Further micro-anatomical analyses indicate that microglial cells contain numerous phagocytic buds in the SNpc of Parkinsonian monkeys (Fig. 3D–F and Supplementary Movies 1 and 2). These structures are rounded enlargements located at the microglial branches and display a ball-and-chain-like structure (Fig. 3D). These structures are reminiscent of the phagocytic events responsible for the elimination of apoptotic newborn cells in the adult mouse hippocampus (Sierra et al., 2010). In addition, a zoom-in confocal analysis indicated that phagocytic buds specifically contain GM130-expressing cisternae (Fig. 3E). Finally, three-dimensional reconstructions revealed the existence of engulfing gliapses in the SNpc of Parkinsonian monkeys

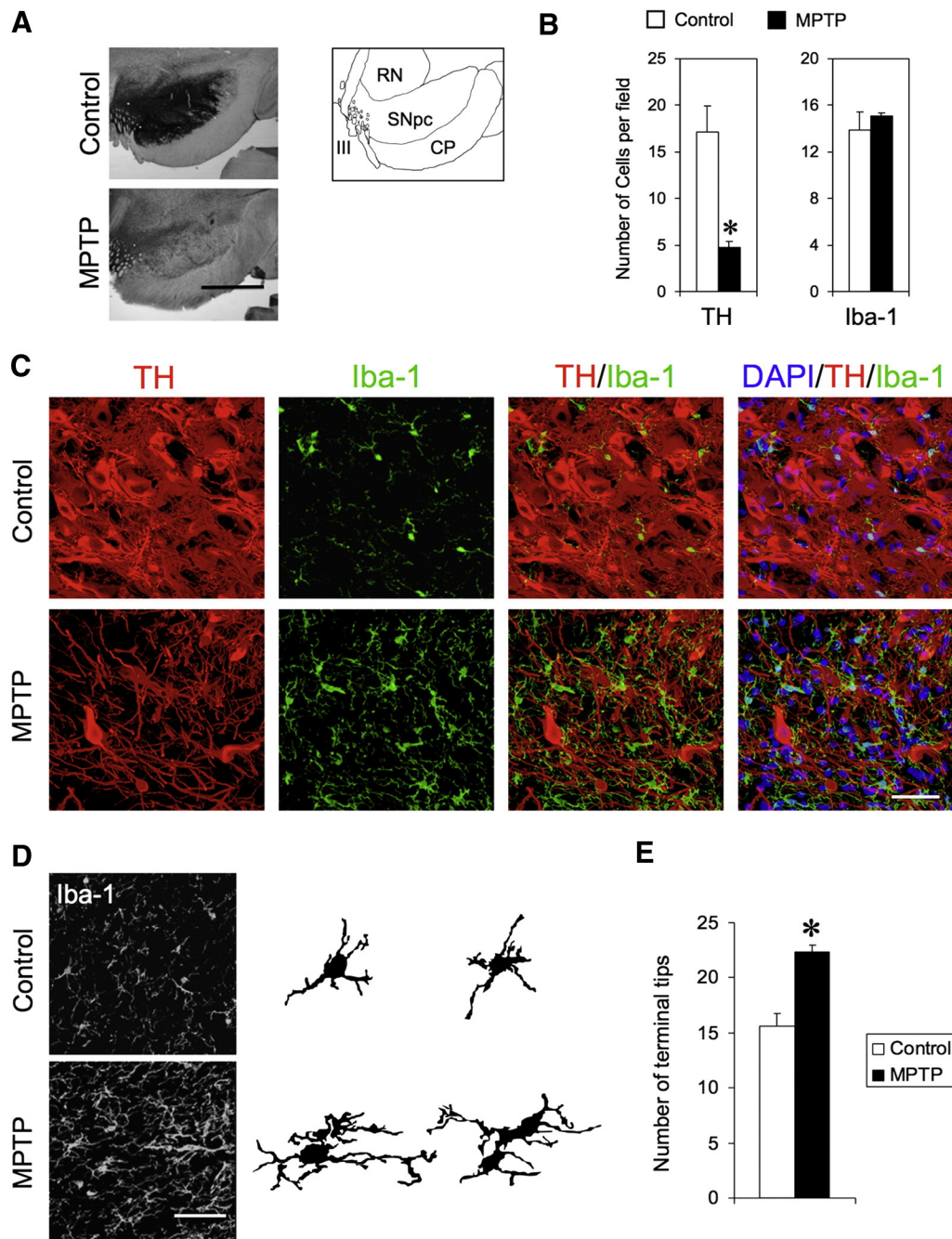


Fig. 1. Microglial cells undergo morphological changes in Parkinsonian monkeys. (A) Chronic MPTP-treated macaques present with a high depletion of dopaminergic neurons in the SNpc. Pictures of TH immunostaining are depicted. The illustration shows the anatomical area of the SNpc. RN; red nucleus, SNpc; substantia nigra *pars compacta*, CP; Cerebral Pedunculus, III; third cranial nerve output. Scale bar: 2 mm. (B) A stereological quantification of TH⁺ neurons indicated a dramatic decrease in dopaminergic neurons in chronic Parkinsonian monkeys compared to control animals. No changes were observed in the number of Iba-1⁺ microglial cells. (C) Microglial cells undergo drastic morphological changes in the SNpc in MPTP-treated monkeys. Confocal images of the SNpc immunostained for TH and Iba-1 reveal that microglial cells increase in size and contain a greater number of terminal tips that contact the dopaminergic neurons in chronic Parkinsonian monkeys. Scale bar: 50 μ m. (D) Microglial cells show an increased shape and size for years after MPTP treatment. Confocal images from the SNpc were analyzed in detail, and representative images of the cells are shown. Scale bar: 40 μ m. (E) The numbers of terminal tips of microglial cells are significantly increased in MPTP treated monkeys. * $p < 0.05$ (student's t-test).

(Fig. 4). These structures were characterized by the engulfment of a pycnotic nucleus by a microglial cell, suggesting an ongoing degenerative process (Fig. 4). Three-dimensional reconstructions revealed that the microglial Golgi apparatus also surrounds the engulfed nucleus, suggesting an active secretory and phagocytic domain.

6. Discussion

In this study, we show that microglial cells maintain phagocytic characteristics in the areas of the brain where the degeneration of

dopaminergic neurons takes place in chronic Parkinsonian monkeys years after MPTP insult. These microglial cells also possess an ongoing polarization of motility towards the degenerating neurons. These results indicate that microglia phagocytize dying dopaminergic cell bodies years after treatment with a neurotoxin, which is consistent with the well-established idea that there is a long term activation of microglial cells in Parkinsonian patients.

Contacts made between microglial and neuronal cell bodies, which are defined as gliapses, are significantly increased only in the ventral SNpc, which is the area with the highest vulnerability to

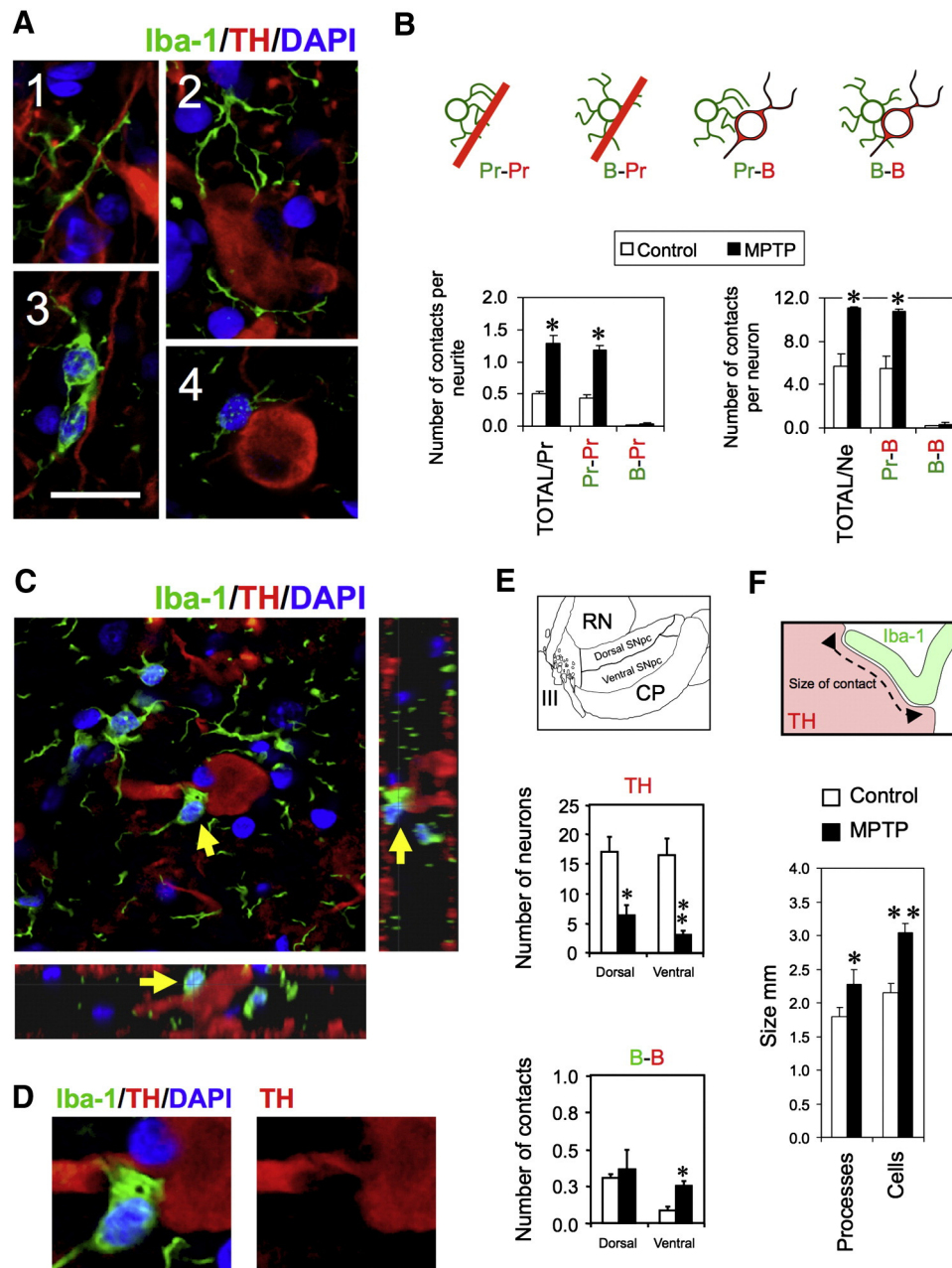


Fig. 2. Microglial cells establish gliapses with dopaminergic neurons in the SNpc of chronic Parkinsonian macaques. (A) Microglial cell processes and cell bodies establish contacts with dopaminergic neurites and cell bodies in monkeys. (1) Confocal image showing a microglial process (green) in contact with a dopaminergic neurite (red). (2) Microglial processes contacting a dopaminergic neuron cell body. (3) A microglial cell body in contact with a dopaminergic neurite. (4) A microglial cell body in contact with the cell body of a dopaminergic neuron. The cell nuclei were counterstained with DAPI (blue). Scale bar in 3: 30 μm . (B) Increase of contacts between microglia and dopaminergic neurons in MPTP monkeys. The types of contact between microglial cells and dopaminergic neurons were classified into four categories: **Pr-Pr**, contact between a microglial process and dopaminergic process; **B-Pr**, contact between a microglial cell body and dopaminergic process; **Pr-B**, contact between a cell body of a dopaminergic neuron and processes of microglial cells; and **B-B**, contact between a microglial cell body and a dopaminergic neuron cell body. A stereological quantification of the detailed confocal examination demonstrates that the number of microglial cell processes in contact with dopaminergic processes [Pr-Pr] and with dopaminergic cell bodies [Pr-B] increases significantly in chronic Parkinsonian macaques. The contacts between microglial cell bodies and dopaminergic structures (B-Pr and B-B) were unchanged. (TOTAL/Pr = [Pr-Pr] + [B-Pr]), TOTAL/Ne = [Pr-B] + [B-B]. * $p < 0.05$. (C) Gliapse-type contact between a microglial cell and a dopaminergic neuron in the ventral SNpc of a Parkinsonian monkey. Lateral z-axis views are also shown. The yellow arrows indicate the contact. (D) Detail of the gliaptic contact. (E) Quantification of the number of body-to-body contacts in the sub-regions of the SNpc. A significant increase in body-to-body contacts was observed only in the ventral area of the SNpc, which is an area where dopaminergic degeneration is high * $p < 0.05$, ** $p < 0.02$. (F) The quantification of the contact surface between microglial processes and dopaminergic neurons, either neurites or dopaminergic cell bodies, increases in chronic Parkinsonian macaques. * $p < 0.05$; ** $p < 0.01$.

MPTP. It is thought that the vulnerability of dopaminergic neurons of this area is due to many factors such as the levels of neuromelanin and calbindin (Yamada et al., 1990; Gibb and Lees, 1991). Thus, the present results indicate that there is an association between neuronal damage and gliapse formation, which suggest that microglial phagocytosis is an important event in Parkinsonian degeneration especially in vulnerable regions.

These microglial characteristics are specific to primates because the results found in macaques are in contrast with previous results obtained in mice (Barcia et al., 2012). In monkeys, the physical contact between microglial cells and neurons persists throughout the course of the disease, while in mice, cell-to-cell contact between microglia and neurons is transient and precedes neuronal elimination (Barcia et al., 2012). In the mouse model of PD, the engulfing process

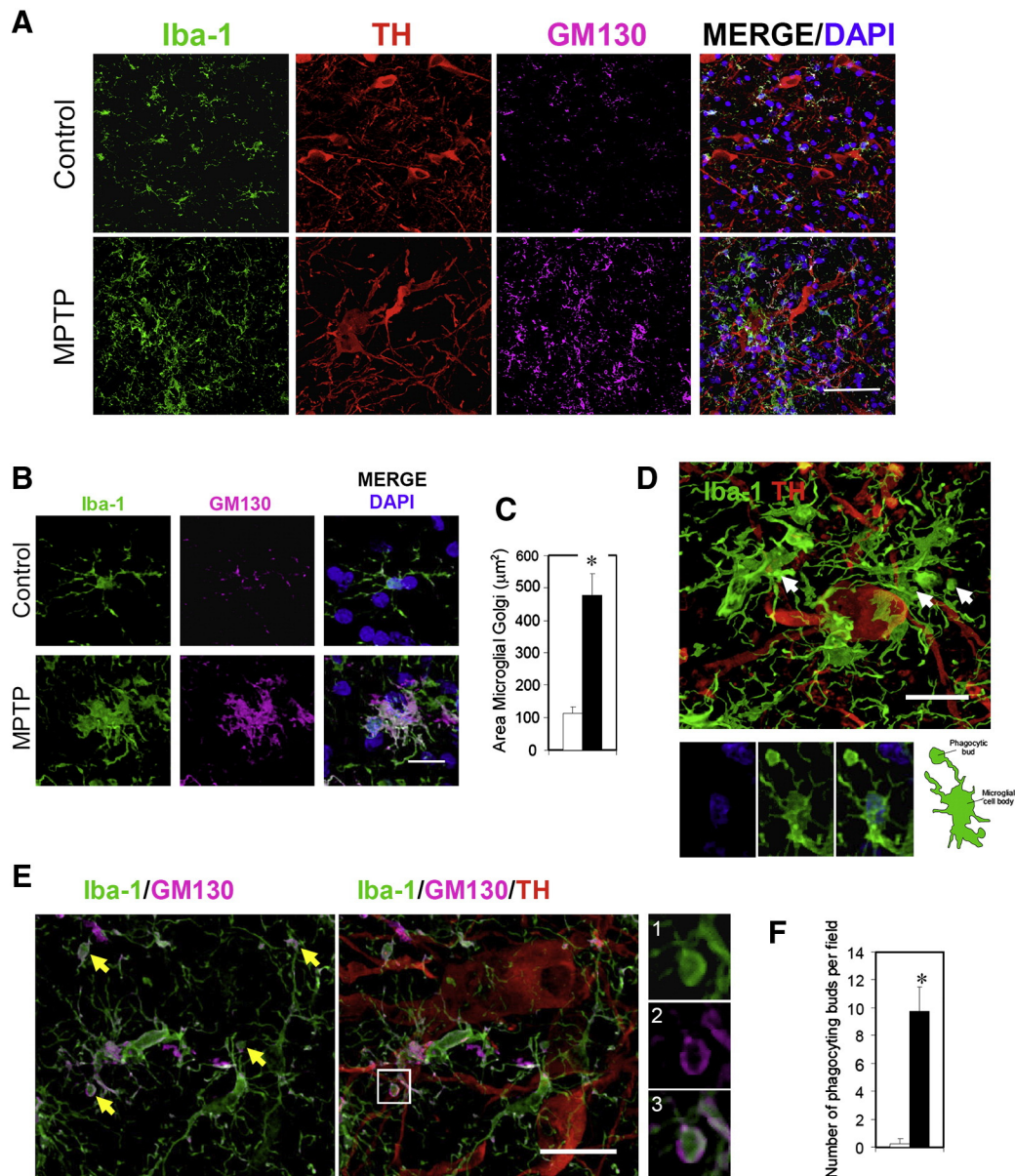


Fig. 3. Microglial cells present phagocytic features in the SNpc of chronic Parkinsonian macaques. (A) Microglial cells in the SNpc of chronic Parkinsonian monkeys show increased expression of GM130 compared to control animals. Scale bar: 50 µm. (B) Detailed confocal analysis in the SNpc indicated that microglial GM130 shows peri-nuclear staining with some cisterns in the microglial processes of control monkeys. However, microglial cells from Parkinsonian macaques present a hypertrophic Golgi in the SNpc. Scale bar: 20 µm. (C) Quantification of the area occupied by GM130 expression. (D) Top panel: three-dimensional transparency of a dopaminergic neuron (red) surrounded by microglial cells (green) in the ventral area of the SNpc of a chronic Parkinsonian monkey. Microglial cells show numerous phagocytic ball-and-chain buds in the areas of dopaminergic degeneration (white arrows). Bottom panel: detail of a ball-and-chain phagocytic bud in a microglial cell from the SNpc of a Parkinsonian monkey. Scale bar: 25 µm. (E) Confocal images of the SNpc of a Parkinsonian macaque show that microglial cells form numerous buds (yellow arrows) that are in close proximity to the remaining dopaminergic neurons. Images 1, 2 and 3 show higher magnification from the white insert depicted in the merged image Iba-1/GM130/TH. Iba-1 ball-and-chain buds (1) contain Golgi cisterns (2), which co-localize in the same structure (3). Scale bar: 25 µm. (F) The quantification of the number of phagocytic buds revealed an increase in microglial cells in the SNpc of Parkinsonian macaques.

and the formation of gliapses lasts until the occurrence of cell death in dopaminergic neurons, at which point cellular contacts and polarizations return to their normal state. In the present study, we show that the phagocytic features of microglial cells are persistent in primates, suggesting that there is ongoing neuronal damage. The reasons for the differences between the two species remain unclear. Different protocols to administer MPTP to non-human primates have been used, and all have resulted in similar levels of microglial activation (Vazquez-Claverie et al., 2009). In contrast, mice downregulate the inflammatory response once the degenerative process resolves (Czlonkowska et al., 1996). These results suggest that dopaminergic neurons in primates are more sensitive than dopaminergic neurons

in rodents. The neurons remaining in the SNpc in primates are likely to be in a damaged state and may release signals that induce phagocytosis mediated by the surrounding microglial cells. Furthermore, the persistent production of cytokines, such as IFN- γ and TNF- α , in primates may also induce microglial activation (Barcia et al., 2011).

Taken together, our results suggest that persistent microglial activation in PD patients may lead to phagocytosis in the areas of the brain where neurodegeneration occurs. This increase in phagocytic activity may exacerbate the degenerative process. Therefore, monitoring microglial activation during the progression of PD and treating patients with anti-inflammatory drugs may prevent neuronal loss that is mediated by phagocytosis.

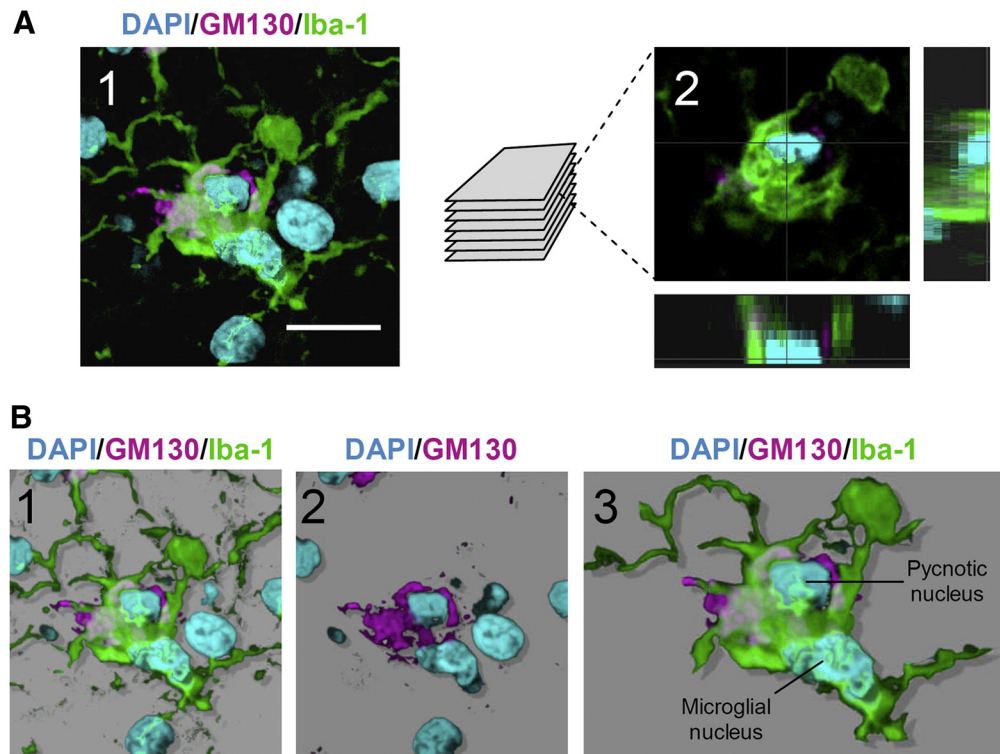


Fig. 4. Engulfing events in the SNpc of Parkinsonian macaques. (A) Phagocytic gliapse in chronic Parkinsonian monkeys. (1) Confocal three-dimensional transparency of a stack of images of a phagocytic gliapse in the ventral SNpc of a Parkinsonian monkey. Iba-1 (green) and GM130 (magenta) are shown together with DAPI counterstaining (cyan). (2) Central 0.5- μm -thick optical section of the stacked images at the level of the pycnotic engulfed nucleus. Scale bar: 15 μm . (B) Three-dimensional rendering of the engulfing gliapse from 1A. (1) A microglial cell is engulfing an extra-nucleus. (2) The pycnotic extra-nucleus is surrounded by several Golgi cisterns. (3) Isolated detail of the phagocytic event with higher magnification.

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.jneuroim.2013.05.001>.

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