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1 Serotonergic innervation of the striatum in a non-human primate  
2 model of Parkinson's Disease

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## Abstract

Parkinson's disease (PD) is characterized by dopaminergic neurodegeneration in the *substantia nigra* and dopamine depletion in the striatum. Non-dopaminergic systems are also affected, including the serotonergic system. Enhanced striatal serotonergic innervation is a proposed compensatory mechanism for the dopaminergic deficit. Meanwhile a serotonergic deficit has been suggested as preceding the nigrostriatal dopaminergic pathology in PD.

Our aim was to assess the serotonergic innervation of the striatum in a model of progressive experimental parkinsonism in macaques, from pre-symptomatic to symptomatic stages. The neurotoxin 1-methyl-4-phenyl-1,2,3,6 tetrahydropyridine (MPTP) was administered to adult macaque monkeys using a slow intoxication protocol. The intoxicated animals were classified into asymptomatic, recovered, moderate and severe parkinsonian, based on their motor behavior. The serotonergic innervation was studied by immunohistochemistry against serotonin (5-HT). In the striatum, the density of 5-HT-immunoreactive (5-HT+) axons was estimated with stereology. Images of the striatum in the immunostained sections were taken to compare the distribution patterns of the serotonergic innervation between groups.

These patterns were apparently similar among the groups. Axonal density estimations showed no differences in striatal 5-HT+ innervation between the intoxicated groups and the control group. Accordingly, this study fails to find significant changes in the striatal serotonergic axonal innervation in MPTP-treated monkeys, coinciding with previous biochemical findings in our model. However, it is possible that alterations in the serotonergic system in PD could be independent of axonal density changes. Consequently, the proposed role for striatal serotonin serving as a compensatory mechanism for dopaminergic denervation merits further study.

## Keywords

Parkinson's Disease; serotonin; serotonergic innervation; MPTP monkey; axonal density; stereology.

## 1. Introduction

Parkinson's disease (PD) is mainly characterized by neurodegeneration of the *substantia nigra pars compacta*, leading to striatal dopamine depletion (Ehringer and Hornykiewicz 1960; Hassler 1938). The classic motor features of PD emerge when the striatal level of dopamine has decreased by 60-80% (Bernheimer et al. 1973). Thus, a number of striatal and extra-striatal compensatory mechanisms take place to maintain normal function for a prolonged pre-diagnostic period (Bezard et al. 2003; Blesa et al. 2017). Increased striatal serotonergic activity (Boulet et al. 2008) has been proposed as a possible compensatory mechanism. The serotonergic system has also been implicated in the mechanism behind levodopa action in the striatum (Carta et al. 2007, 2010), and, more recently, it has been suspected of underlying levodopa-induced dyskinesias (Pagano et al. 2018). However, studies on the serotonergic system in PD have provided contradictory results: both depletion and an increase of serotonergic markers in the striatum have been reported (reviewed by Huot et al. 2011).

In PD patients, the striatal serotonergic innervation has been shown to be preserved in a PET (positron emission tomography) neuroimaging study (Politis et al. 2014) and in a *postmortem* investigation (Bédard et al. 2011). Interestingly, a recent *in vivo* neuroimaging study has revealed a widespread decrease in serotonin transporter (SERT) availability prior to detectable dopaminergic changes in premotor stages of genetic PD (A53T SNCA carriers, Wilson et al. 2019). Furthermore, *postmortem* studies in PD patients have reported neuronal loss in some of the raphe nuclei (Mann and Yates 1983; Jellinger 1987; Halliday et al. 1990; Paulus and Jellinger 1991).

In parkinsonian monkeys, enhanced serotonergic innervation has been suspected of compensating for dopaminergic denervation and dopamine loss (Mounayar et al. 2007; Boulet et al. 2008; Ballanger et al. 2016; Gagnon et al. 2016); although this was not fully confirmed in a behavioral-pharmacological study in monkeys (Neumane et al. 2012) or by HPLC (high-performance liquid chromatography) *postmortem* assessment (Blesa et al. 2012). No decrease in the number of serotonergic neurons has been reported in the raphe nuclei of MPTP-intoxicated monkeys (Langston et al. 1984; Gaspar et al. 1993; Gagnon et al. 2016).

Considering the importance of early PD stages and the inconsistencies in the literature on the status of the serotonergic system in both PD and experimental parkinsonism in non-human primates, we undertook the current study to assess the serotonergic innervation of the striatum in an MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) monkey model induced by slow and repeated intoxication (Blesa et al. 2010, 2012), that included both pre-symptomatic and symptomatic subjects with progressive nigrostriatal degeneration. We hypothesized our macaques would present changes in striatal serotonergic innervation either before or during MPTP-induced stable symptomatic parkinsonism.

## 2. Material and methods

### 2.1 Subjects and brain tissue

We analyzed brain tissue from chronic parkinsonian macaques and from control monkeys (*Macaca fascicularis*) stored in our brain bank and used in previous publications (Blesa

et al. 2012). Animals were housed under standard conditions and treated in accordance with European and Spanish guidelines (86/609/EEC and 2003/65/EC European Council Directives; and the Spanish Government). The Bioethics committees of the Universidad de Navarra and the Universidad Autónoma de Madrid approved the experiments.

Animals were treated every two weeks with intravenous doses of MPTP (0.5 mg/kg) according to previous protocols (see Table A.1 for details; Blesa et al. 2010, 2012). Motor status was assessed using a validated motor score (Kurlan scale), range 0–29 points (Kurlan et al. 1991; reviewed by Imbert et al. 2000). Animals received MPTP injections until the motor scale was considered stable (at least one month without changes in the motor state; Table A.1). None of them received L-DOPA or dopaminergic agonists.

Animals that exhibited evident parkinsonian symptoms after MPTP injection and remained affected until sacrifice were considered Parkinsonian (Moderate Parkinsonian: Kurlan scale 1-18, actual scores in our sample: from 9 to 11 before sacrifice; Severe Parkinsonian: Kurlan scale 19-29, actual scores in our sample: from 20 to 22 before sacrifice). Animals with no apparent parkinsonian symptoms after the last MPTP dose or thereafter were considered Asymptomatic. Animals with evident parkinsonian symptoms that disappeared four weeks after their last MPTP dose and were thereafter symptom-free were considered Recovered. The control population was composed of non-MPTP-treated animals. Each group was composed of four animals (n=4; Table A.1). Thus, the subjects and their brains examined in this study represent a scenario mirroring the situation in PD, where dopaminergic neuronal loss can be present without motor impairment until a certain threshold is reached (Blesa et al. 2012).

## 2.2 Histology

Animals were sacrificed at least four weeks after the last MPTP administration. They were anesthetized with sodium pentobarbital and perfused through the ascending aorta with saline, 4% paraformaldehyde in phosphate buffer (PB) and a series of PB sucrose solutions (5-20%). The brains were dissected in the midline; one hemisphere was blocked in the coronal stereotaxic plane (plane 0 corresponding to the interaural plane). The blocks were cut on a freezing microtome into 40 µm-thick coronal sections. Parallel adjacent series of sections were processed to reveal the cyto- and chemo-architecture using cresyl violet staining and acetylcholinesterase histochemistry (Cavada et al. 1995), respectively. These series were used to identify the striatal nuclei and trace their boundaries.

An additional series was processed for immunohistochemical localization of 5-HT. Five to seven sections, regularly spaced 2400 µm apart, were selected from this series in each brain. These sections encompassed a rostrocaudal length of 9.6-14.4 mm. Two to three sections were rostral to the anterior commissure (pre-commissural) and three to four sections were caudal (post-commissural). No striatal tissue was present 2400 µm more rostrally or more caudally than the sections selected except for the caudate tail, which is more caudal and ventral, and was not analyzed. Free-floating immunohistochemistry was performed using a primary monoclonal anti-serotonin rat antibody (MAB352, Chemicon, 1:150), and a secondary polyclonal anti-rat rabbit antibody (BA-4000, Vector Laboratories, 3 µg/ml).

## 2.3 Images of the immunostained striatum

In order to check if MPTP intoxication altered the serotonergic innervation pattern, pseudo-colored images of the immunohistochemical staining for 5-HT from all groups were created.

Mosaics of individual images obtained using a 1.25x objective were generated using a Zeiss Axioskop microscope equipped with a digital camera and processed with StereoInvestigator<sup>®</sup> software (v9, MicroBrightField, USA). Each mosaic served to generate a low power microphotograph that contained the full surface area of the caudate and putamen in each section (Fig. 1A-E and F-J). Pseudo-colored images of immunohistochemical staining against serotonin (5-HT) were generated from the mosaics (Fig. 1A'-E' and F'-J'). Using the program Image J (v1.41, National Institutes of Health, USA), mosaics were transformed into black and white (8 bits) images and the color was inverted. With an LUT filter, the inverted gray values were transformed into a custom color scale, that was used in all images of the study.

## **2.4 Quantitative assessment of the striatal serotonergic innervation**

### **2.4.1 Stereological analysis**

All immunostained sections were used to estimate the total length and length density of axons immunoreactive for 5-HT (5-HT+) in the striatum by means of the optical fractionator method, using 3D hemispherical probes (StereoInvestigator<sup>®</sup> software, MicroBrightField, USA). Section thickness after immunohistochemistry was assessed: it ranged between 11 and 17  $\mu\text{m}$ . Virtual hemispheres (radius=9  $\mu\text{m}$ ) were projected along the Z axis, spaced in a sampling grid of 1000x1000  $\mu\text{m}$  in the caudate and of 1400x1400  $\mu\text{m}$  in the putamen. A guard-zone 2  $\mu\text{m}$  high was used on the top of the section. These parameters were set to reach an error coefficient below 0.10 (Gundersen, m=0) and 0.05 (Gundersen, m=1). The intersections of 5-HT+ axons and the boundaries of the hemispheres were observed with a 100x immersion objective, and were marked and totalled. Total axonal length was calculated as in Mouton et al. (2002; reviewed by West 2018). The volume of each nucleus was calculated by the Cavalieri method (Gundersen and Jensen 1987). Length density was defined as the total axonal length per volume of each striatal nucleus. Caudate and putamen were analyzed separately.

## **2.5 Statistics**

Using SPSS<sup>®</sup> software (v24, IBM Corp., USA), data normality was checked by the Kolmogorov Smirnov test. Differences of axonal length density were analyzed by one-way repeated measure analysis of variance (ANOVA) followed by the Bonferroni post-hoc test. The Pearson correlation coefficient was used to study statistical correlations with already published data from the same experimental subjects (Blesa et al. 2012). The significance threshold was set at  $p < 0.05$ .

All original data were expressed as mean  $\pm$  standard deviation (Table A.2).

## **3. Results**

### **3.1 Distribution of the serotonergic innervation in the striatum**

In controls, the serotonergic innervation showed a dorsoventral gradient and a less pronounced lateromedial gradient (Fig. 1A, 1A', 1F, 1F'), whereby the ventral and medial striatal regions were the most intensely immunostained. In general, immunostaining was more intense in the ventral than in the dorsal striatum, and the caudate was more markedly stained than the putamen.

The distribution pattern of the serotonergic innervation in intoxicated monkeys (Fig. 1B-E, 1B'-E', 1G-J, 1G'-J') matched the one described for controls (Fig. 1A, 1A', 1F, 1F').

### **3.2 Quantitative analysis of serotonergic innervation**

The quantitative analysis of the serotonergic innervation was performed in the caudate and the putamen. The ventral striatum was only represented in one or two of the total sections studied per animal, making it impossible to obtain precise quantitative measures for this structure. We report length density data because we consider this to be the most reliable estimation since it takes into account any variations in striatal volume that would accompany body and brain size changes.

#### **3.2.1 Length density of 5-HT+ axons in the striatum**

No significant differences for length density were found between intoxicated animals and the control animals, in either the caudate or the putamen (Fig. 2, Table A.2).

The total length of 5-HT+ axons, volume of each nucleus and length density of 5-HT+ axons in the caudate and putamen are shown in Table A.2. We found no differences in the volume of either the caudate or the putamen in intoxicated groups compared to the control group.

### **3.3 Correlation between serotonergic and dopaminergic innervation**

Previous studies had associated enhanced striatal serotonergic innervation with dopaminergic denervation in the striatum (Mounayar et al. 2007; Gagnon et al. 2016). Therefore, we analyzed the statistical correlation between the length density of 5-HT+ axons in the striatum and the earlier TH immunoreactivity data in the striatum from the same animals and experimental groups (see Blesa et al. 2012).

5-HT+ axonal length density did not correlate with TH immunoreactivity in either the caudate ( $\rho=-0.011$ , bilateral significance=0.962) or the putamen ( $\rho=-0.165$ ; bilateral significance=0.486).

### **3.4 Correlation between serotonergic innervation and motor status**

Enhanced striatal serotonergic innervation has been related to motor recovery after MPTP intoxication (Mounayar et al. 2007). Consequently, we analyzed the statistical correlation between the length density of 5-HT+ axons in the striatum and the Kurlan scale motor score.

Motor scores did not correlate with 5-HT+ axonal length density in either the caudate ( $\rho=-0.002$ , bilateral significance=0.995) or the putamen ( $\rho=0.117$ ; bilateral significance=0.662).

## 4. Discussion

The present study deals with the status of striatal serotonergic innervation in MPTP-intoxicated monkeys with different stages of parkinsonism. Our stereological analysis did not find any changes in 5-HT+ axonal length density in the caudate or putamen, in either the pre-symptomatic or symptomatic stages of parkinsonism.

### 4.1 Striatal serotonergic innervation in MPTP-intoxicated monkeys

There were no qualitative differences between the distribution patterns for serotonergic innervation in the asymptomatic, recovered, moderate, severe parkinsonian or control groups. All of them showed a dorsoventral gradient, more marked in pre-commissural striatum, and a less evident lateromedial gradient (Fig. 1A-J'), whereby the ventral and medial striatal regions were more densely innervated by serotonin axons. These gradients have been described in non-parkinsonian *Macaca fascicularis* (Azmitia and Gannon 1986).

In the stereological study of 5-HT+ axonal length density in the caudate and putamen, we found no significant differences between MPTP-intoxicated monkeys and controls (Fig. 2). There was no statistical correlation between the estimated density of serotonergic innervation in the caudate and putamen and the TH immunoreactivity in the same nuclei of the same MPTP intoxicated monkeys (Results, section 3.3). Finally, we found no statistical correlation between the estimated density of serotonergic innervation in the caudate and putamen and the motor scale in the MPTP-intoxicated monkeys (Results, section 3.4).

In stable symptomatic MPTP monkeys, Gagnon et al. (2016) demonstrated an increased density of SERT immunoreactive (SERT+) axons in pre-commissural associative territories of the caudate and putamen. In addition, the density of SERT+ axonal varicosities increased in sensorimotor territories of the post-commissural putamen and the commissural caudate (Gagnon et al. 2016). In contrast, Mounayar and colleagues found a decreased 5-HT+ fiber number in the striatum of stable symptomatic MPTP monkeys. Monkeys that recovered motor function after MPTP intoxication had a higher 5-HT+ fiber count in the striatum than did symptomatic MPTP monkeys (Mounayar et al. 2007).

There are several aspects of the above-mentioned studies (Mounayar et al. 2007; Gagnon et al. 2016) that might explain the differences in results. They employed an MPTP intoxication protocol that included more frequent injections than our intoxication method. Our protocol, with doses spaced two weeks apart, may have avoided the accumulation of MPTP, provided for better dose control and allowed the development of plastic



compensatory mechanisms against toxicity (Blesa et al. 2010, 2012). Moreover, the quantitative approaches for the study of serotonergic innervation were different and performed using a highly compartmentalized anatomical framework, in functional territories (Mounayar et al. 2007; Gagnon et al. 2016) and along the anteroposterior axis (Gagnon et al. 2016). This regionalization may allow the detection of subtle localized variations in the markers of interest. We analyzed the caudate and putamen nuclei as whole units with precise quantitative techniques, and concluded that there are no changes in the striatal serotonergic system in MPTP monkeys, whether pre-symptomatic or symptomatic, after a slow, progressive intoxication protocol.

The results of the present work are congruent with previous biochemical findings obtained using the same experimental protocol. HPLC analyses of striatal 5-HT and 5-HIAA showed no concentration changes in MPTP-intoxicated monkeys compared to controls (Blesa et al. 2012), coinciding with the striatal serotonin HPLC results published in other MPTP monkey models (rostral caudate and putamen in Pifl et al. 1991; caudate in Franke et al. 2016).

It is relevant to point out that the PD model used here has several strengths. As previously mentioned, the slow intoxication protocol may permit the emergence of compensatory mechanisms against the effects of dopaminergic denervation and dopaminergic neuronal loss. Also, the experimental groups present a progressive dopaminergic deficit that is closer to the evolutionary stages, both pre-symptomatic and symptomatic, of PD motor alterations than other experimental models (Blesa et al. 2012).

## 4.2 Comparison with human studies

Our results partially resemble those published by Bédard et al. (2011) in *postmortem* brains of PD patients. They demonstrated no significant variation in SERT+ axonal varicosity density as estimated by stereology (Bédard et al. 2011). Other studies in PD patients have revealed a loss of serotonergic neurons (Jellinger 1987; Halliday et al. 1990) and depletion of serotonergic markers in different brain regions (Bernheimer et al. 1961; Fahn et al. 1971; Scatton et al. 1983; Raisman et al. 1986; Wilson et al. 1996). A recent *postmortem* biochemical study (Cheshire et al. 2015) revealed a dramatic loss of 5-HT in the caudate, but not putamen of PD patients. It may be relevant to point out that the samples collected in Cheshire et al. (2015) were mainly from the anterior striatum, where the caudate is much more densely innervated with serotonergic axons (Parent et al. 2011). In general, all *postmortem* studies of serotonergic markers in PD patients face their specific problems added to those of the study of *postmortem* human tissue. For example, patients' pharmacological treatments throughout the disease may alter the studied markers. The L-DOPA metabolism competes with 5-HT metabolism, decreasing the synthesis of 5-HT (Carta et al. 2007). Consequently, biochemical studies may overestimate striatal serotonin depletion in patients treated with L-DOPA. Likewise, inhibitors of serotonin reuptake, a common drug in PD patients, modulate SERT expression (Benmansour et al. 1999).

To the best of our knowledge, only one human *postmortem* study used a stereological approach to estimate the serotonergic innervation of the striatum in PD patients; they did not find significant differences with respect to controls (Bédard et al. 2011). The number of samples was limited (n=2) and several parameters, including axonal length or number of synapses, were not considered, but their results coincide with ours.

In contrast, our results do not reflect the marked decrease of 5-HT and 5-HIAA reported in the caudate of idiopathic PD patients (Cheshire et al. 2015) or the decrease in SERT availability prior to detectable dopaminergic pathology in genetic forms of PD (Wilson et al. 2019). These disparate results could have several explanations, including: (I) differences in the *postmortem* samples analyzed, (II) limitations of studying serotonergic markers in *postmortem* tissue of previously treated PD patients, (III) shortcomings of the progressive MPTP intoxication method that may not completely mimic the non-dopaminergic changes of PD pathology (Porras et al. 2012), and or (IV) differences between macaques and humans in the serotonergic system or in its vulnerability to different conditions.

### 4.3 Serotonergic system and compensatory mechanisms in parkinsonism

Serotonergic neurons can metabolize L-DOPA into dopamine, and then store and release that dopamine (Arai et al. 1994, 1995). Moreover, following L-DOPA administration, striatal serotonin axons may release dopamine in an aberrant manner (Carta et al. 2007, 2010). Thus, serotonergic innervation in the striatum has been proposed as a key factor in levodopa-induced dyskinesias (Pagano et al. 2018). In MPTP parkinsonian monkeys, a serotonergic hyperinnervation in the striatum may help compensate for dopaminergic denervation (Mounayar et al. 2007; Boulet et al. 2008; Gagnon et al. 2016).

Here, we did not find changes in the serotonergic innervation of the striatum in MPTP-intoxicated monkeys. Thus, in our model of progressive experimental parkinsonism, it is difficult to reconcile a paramount role for striatal serotonin (i.e. compensation of early dopaminergic striatal deficit) in the face of the intact axonal innervation. Nonetheless, even though 5-HT axonal length density is preserved in the striatum, the parkinsonian brain may still present serotonergic changes. Other functionally relevant nuclei in the basal ganglia circuitry may undergo serotonergic system changes: increased density of serotonergic axons and serotonergic axonal varicosities in the GPe as well as increased serotonergic axonal density in the GPi have been demonstrated in MPTP-intoxicated monkeys (Gagnon et al. 2018).

Finally, the possibility that serotonergic changes are present in the striatum without 5-HT axonal length density alterations should be considered. Potential changes might include variation in the number and size of the serotonergic axonal varicosities, in the number and size of the established synapses, as well as variations in SERT expression or in 5-HT receptors. Further research is needed to explore these possibilities.

## 5. Conclusions

We have assessed the serotonergic innervation of the striatum in a model of progressive experimental parkinsonism in MPTP-intoxicated macaques. The progressive intoxication protocol is better suited to evaluate compensatory basal ganglia mechanisms than previous models based on acute intoxication. Contrary to expectations, we have found no changes in either the distribution of serotonergic innervation or the density of 5-HT+ axons in the caudate and putamen in the different parkinsonism stages, including pre-symptomatic ones, as compared to controls. These results do not support a compensatory role for the striatal serotonergic system in MPTP-induced parkinsonism.

## 6. Appendices

### 6.2 Appendix A. Supplementary data

Table A.1

| Characteristics and MPTP administration in macaques (modified from Blesa et al. 2012) |                |                |                            |                            |                      |                            |
|---|----------------|----------------|----------------------------|----------------------------|----------------------|----------------------------|
| Group<br>(n=4)  | Age<br>(years) | Weight<br>(kg) | Number<br>of MPTP<br>doses | Total<br>MPTP dose<br>(mg) | Motor scale          |                            |
|   |                |                |                            |                            | After<br>MPTP<br>(1) | Before<br>sacrifice<br>(2) |
| Control   | 5.8 ± 0.7      | 4.9 ± 1.2      | 0                          | 0                          | 0                    | 0                          |
| Asymptomatic  | 4.3 ± 0.2      | 3.6 ± 0.1      | 2 ± 0                      | 3.1 ± 0.2                  | 0                    | 0                          |
| Recovered   | 5.0 ± 0.4      | 5.1 ± 0.9      | 2.5 ± 0.9                  | 6.0 ± 2.4                  | 4.8 ± 1.5            | 0                          |
| Moderate<br>Parkinsonian  | 4.7 ± 0.6      | 3.6 ± 0.5      | 4 ± 0.7                    | 6.0 ± 0.9                  | 19.8 ± 3.5           | 10 ± 0.4                   |
| Severe<br>Parkinsonian  | 6.6 ± 1.5      | 3.6 ± 0.1      | 8.3 ± 2.5                  | 15.1 ± 3.9                 | 22.3 ± 1.1           | 20.8 ± 0.5                 |

Data shown mean ± standard error of the mean (SEM)

(1) Scores one week after the last MPTP injection

(2) Scores one week before sacrifice

Table A.2

| Stereological estimations |  |   |  |  |   |  |
|---------------------------|--|---|--|--|---|--|
| Group                     | Caudate                                  |   |  | Putamen                                  |   |  |
|                           | Total<br>length of<br>5-HT+<br>axons (m) | Nucleus<br>volume<br>(mm <sup>3</sup> ) | Length<br>density of 5-<br>HT+ axons<br>(m/mm <sup>3</sup> ) | Total<br>length of<br>5-HT+<br>axons (m) | Nucleus<br>volume<br>(mm <sup>3</sup> ) | Length<br>density of 5-<br>HT+ axons<br>(m/mm <sup>3</sup> ) |
| Control                   | 1842 ±<br>427                            | 224 ± 26                                | 8.2 ± 1.65   | 2770 ±<br>531                            | 428 ± 102                               | 6.61 ± 1.14  |
| Asymptomatic              | 2485 ±<br>593                            | 251 ± 28                                | 9.79 ± 1.37  | 3729 ±<br>422                            | 504 ± 41                                | 7.45 ± 1.15  |
| Recovered                 | 2157 ±<br>222                            | 263 ± 42                                | 8.26 ± 0.7   | 2857 ±<br>954                            | 457 ± 41                                | 6.19 ± 1.73  |
| Moderate<br>Parkinsonian  | 2804 ±<br>355                            | 284 ± 20                                | 9.93 ± 1.53  | 3356 ±<br>303                            | 463 ± 101                               | 7.48 ± 1.58  |
| Severe<br>Parkinsonian    | 2069 ±<br>390                            | 250 ± 39                                | 8.26 ± 0.37  | 3011 ±<br>432                            | 421 ± 26                                | 7.18 ± 1.17  |

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The sponsors of this research were not involved in the study design, collection, analysis or interpretation of data, in the writing of the manuscript, or in the decision to submit the article for publication.

## Declarations of interest

None.

## Figure captions

Fig. 1. Microphotographs of the immunohistochemical staining against 5-HT in representative cases of the different experimental groups. (A-E) Pre-commissural striatum. (F-J) Post-commissural striatum. The stereotaxic anteroposterior (AP) level is given for each section. Each immunohistochemistry microphotograph is paired with its corresponding pseudo-colored image (A'-J'). A dorsoventral serotonergic innervation gradient can be observed, with the maximum innervation being located in ventral areas of the caudate, putamen and in the ventral striatum. The lateromedial serotonergic innervation gradient is less pronounced. *Abbreviations*: CD, caudate; PT; putamen; VS: ventral striatum.

Fig. 2. Length density of 5-HT + axons in the striatum. The control, asymptomatic, recovered, moderate and severe parkinsonian groups were analyzed. (A) Caudate. No significant differences were found between the groups intoxicated with MPTP and the control group. (B) Putamen. No significant differences were found in the groups intoxicated with MPTP compared to the control group. In each box plot: horizontal lines indicate the median, boxes represent the first and third quartiles and whiskers indicate the minimum and maximum values. *Abbreviations*: CONT, controls; ASYMP, asymptomatic; RECOV, recovered; MOD P, moderate parkinsonian; SEV P, severe parkinsonian.

428   **Abbreviations**  
429  
430   5-HIAA: 5-hydroxyindoleacetic acid  
431   5-HT: 5-hydroxytryptamine, serotonin  
432   5-HT+: immunoreactive against serotonin  
433   GPe: external globus pallidus  
434   GPi: internal globus pallidus  
435   HPLC: high-performance liquid chromatography  
436   L-DOPA: L-3,4-dihydroxyphenylalanine, levodopa  
437   LUT: look-up table  
438   MPTP: 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine  
439   PB: phosphate buffer  
440   PD: Parkinson's Disease  
441   PET: positron emission tomography  
442   SERT: serotonin transporter  
443   SERT+: immunoreactive against serotonin transporter  
444   SNCA: alpha-synuclein gene  
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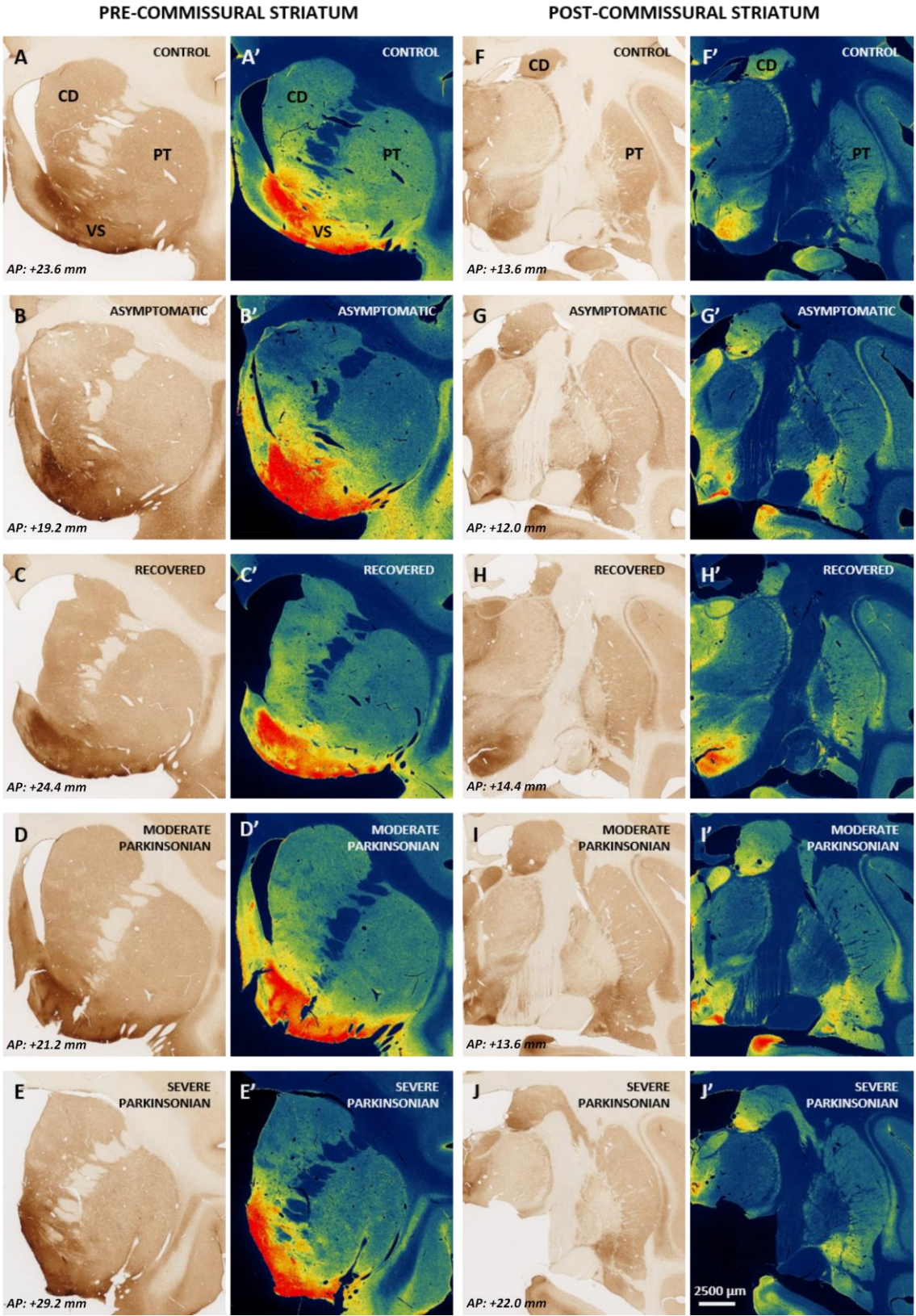
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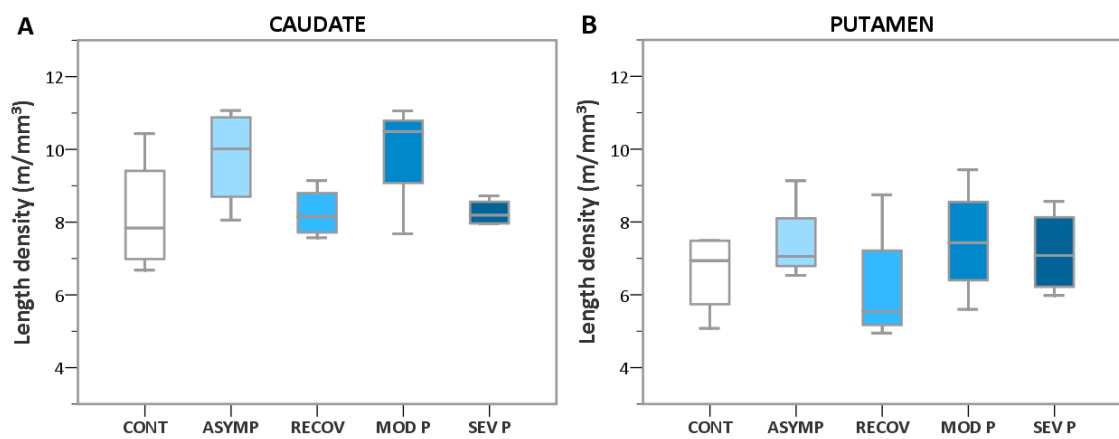
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**Figure 1. Microphotographs of the immunohistochemical staining against 5-HT in representative cases of the different experimental groups.**



624 **Fig. 2. Length density of 5-HT + axons in the striatum.**



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