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Prior MDMA administration aggravates MPTP-induced Parkinsonism in macaque monkeys

Mathilde Millot, Yosuke Saga, Sandra Duperrier, Elise Météreau, Maude Beaudoin-Gobert, Véronique Sgambato*

Univ Lyon, CNRS UMR 5229, Institut des Sciences Cognitives Marc Jeannerod, 67 Boulevard Pinel, Bron F-69675, France



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ABSTRACT

The aim of this study was to investigate the causal role of an early serotonin injury on parkinsonian-like motor symptomatology. Monkeys were pretreated with 3,4-methylenedioxy-N-methamphetamine (MDMA, or “ecstasy”), known to lesion serotonergic fibers, before being administered 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). We combined behavioural assessment, PET imaging, and immunohistochemistry. Strikingly, prior MDMA administration aggravated MPTP-induced Parkinsonism and associated dopaminergic injury. Monkeys with early MDMA lesions developed parkinsonian deficits more rapidly and more severely. Interestingly, not all symptoms were impacted. Bradykinesia, rigidity and freezing were not affected by early MDMA lesions, whereas spontaneous activities, tremor and abnormal posture were significantly aggravated. Finally, as expected, MDMA induced a decrease of the serotonergic transporter availability. More surprisingly, we found that MDMA evoked also a decreased availability of the dopaminergic transporter to a lesser extent. Altogether, these results show that MDMA administration in non-human primates not only damage serotonergic terminals, but also injure dopaminergic neurons and enhance MPTP neurotoxic action, a completely new result in primates.

1. Introduction

It is widely known that Parkinson's disease (PD) is characterized by the degeneration of dopaminergic (DA) neurons in the substantia nigra pars compacta resulting in motor symptoms (Rodríguez-Oroz et al., 2009). However, the neurodegenerative process affects other neurotransmission systems such as the serotonergic (5-HT) system in raphe nuclei (Pagano et al., 2017; Hirsch et al., 2003; Jellinger, 1999). Correlations have been found between the alteration of the 5-HT system and the expression of tremor or dyskinesia (Pagano et al., 2018). This 5-HT dysfunction within the brain also plays a role in non-motor manifestations such as depression, fatigue or rapid-eyes movement sleep disorders (Pagano et al., 2017). Of note, alterations of the 5-HT system can be detected in de novo PD patients and be linked to the expression of psychiatric symptoms (Melse et al., 2014; Maillet et al., 2016).

In accordance with Braak's hypothesis, the pathological process which involves alpha-synuclein-immunopositive Lewy neurites and Lewy bodies would affect the raphe before reaching the substantia nigra (Braak et al., 2003). Whether this early 5-HT alteration has an impact on the severity of specific symptoms remains to be investigated. To do

so, we first damaged the 5-HT system, using 3,4-methylenedioxy-N-methamphetamine (MDMA) before lesioning the DA neurons with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in macaques, the neurotoxicity of these toxins being well known (Morissette and Di Paolo, 2018; Ricaurte et al., 2000). We used behavioural assessment, PET imaging and immunohistochemistry to evaluate the impact of this early MDMA-driven lesioning on the severity of MPTP-induced Parkinsonism in non-human primates (NHPs).

2. Materials and methods

2.1. Ethical statement

All studies were in accordance with the recommendations of the European Communities Council Directive of 2010 (2010/63/UE) and the French National Committee (2013/113). There were also approved by the local ethical committee CELYNE C2EA.

* Corresponding author.

E-mail address: veronique.sgambato@inserm.fr (V. Sgambato).

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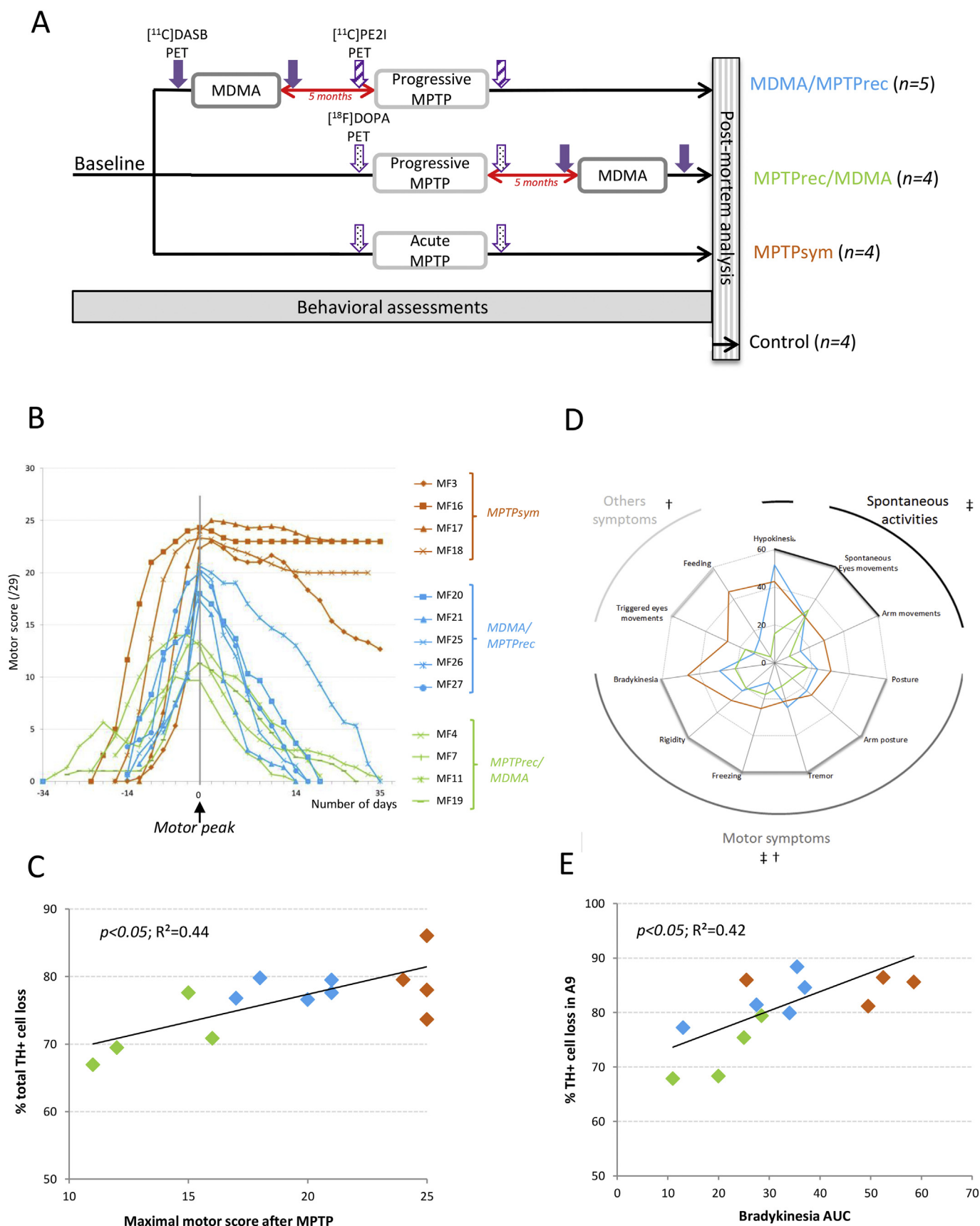


Fig. 1. Study design and Characteristics of MPTP-induced Parkinsonism between groups. (A) Flowchart illustrates experimental design, treatment and group assignments. (B) Evolution of motor score for each monkey during and after MPTP intoxication. Timelines were aligned at Day 0 corresponding to the motor peak (maximal motor score) for each monkey. (C) Positive correlation found between the severity of the score and the percentage of total TH+ cell loss (in A8, A9 and A10). (D) Radar diagram performed with areas under the curve (AUCs) after gathering symptoms into 3 groups. ‡ MDMA/MPTPrec versus MPTPrec/MDMA monkeys; † MDMA/MPTPrec versus MPTPsym monkeys. (E) Correlation between bradykinesia AUC and percentage of TH+ cell loss in A9.

2.2. Animals

We used male macaque *fascicularis* monkeys, weighing between 5 and 9 kg, aged between 3 and 6 years, and kept under standard conditions with 12 h light cycles, 23 °C and 50% humidity. Taking into consideration the three R's for animal experimentation, we also utilized some data obtained from a previous study (Beaudoin-Gobert et al., 2015).

2.3. Experimental design and statistical analysis

Fig. 1A describes the experimental design. Behavioural assessments were carried out longitudinally on fourteen monkeys. The 5 monkeys treated by MDMA then MPTP were MF20, MF21, MF25, MF26 and MF27. The 4 monkeys treated by MPTP then MDMA were MF4, MF7, MF11 and MF19. The 4 monkeys only treated with MPTP were MF3, MF16, MF17 and MF18. Finally, control monkeys used for post-mortem analyzes were MF0, MI69, MF14 and MF15 for TH counts and MF0, MF14, MF15 and MF24 for TPH2 counts. PET scans (arrows) were performed before and after MDMA or MPTP. Post-mortem analysis were undertaken at the end of the protocols. All statistical analysis was performed using R software. Severity of motor symptoms was compared between groups using the non-parametric Kruskal-Wallis test. Analysis at the motor peak represented the variation at the motor peak and 3 days before and after. For the area under the curve (AUC) analysis, we arbitrarily included scoring data until day 23 for all items of the scale. For PET imaging, the statistical differences were assessed using the non-parametric Wilcoxon signed-rank test allowing intra-group comparisons of the different states. For immunohistochemical data, we used a one-way ANOVA [Group]. Linear regressions were used to analyze relationships between behavioural and immunohistochemical data.

2.4. 5-HT and DA lesions

MDMA injections were performed twice daily for four consecutive days (Fig. 1A). All macaque monkeys received the same dose, 5 mg/kg per injection subcutaneously, according to a previous study (Ricaurte et al., 2000). In the case of monkeys treated with MPTP first, MDMA has been administered five months after the motor peak (see Beaudoin-Gobert et al., 2015).

MPTP injections (0.3–0.5 mg/kg i.m.; see Table 1 for details) were performed under light anesthesia (ketamine 0.5 mg/kg, atropine

0.05 mg/kg) on consecutive days (acute protocol; one injection per day) or every 4 to 5 days (progressive protocol) (Fig. 1A). The progressive administration of MPTP was continued until the monkeys reached a motor peak allowing them to recover from their motor symptoms. The progressive MPTP protocol is used to mimic a moderate stage of the disease and triggers a moderate DA lesion associated to transient motor symptoms. Monkeys are therefore able to recover from their motor symptoms (they are called recovered, MPTPrec). It is important to note that the progressive administration of MPTP does not lead to a loss of 5-HT fibers (Beaudoin-Gobert et al., 2015; Mounayar et al., 2007). By contrast, the acute MPTP protocol mimics a more advanced stage of the disease and induces a severe DA lesion (and also affects 5-HT terminals (Mounayar et al., 2007)) associated to stable motor symptoms (they are called symptomatic, MPTPsym). All monkeys pretreated with MDMA received the progressive MPTP protocol five months after (because the potential impact of MDMA on behavioural disorders was investigated during this period; not published yet) (Fig. 1A). However, one MDMA/MPTPrec monkey was excluded from the study as it remained symptomatic. All other MDMA/MPTPrec monkeys were compared to either MPTPsym monkeys (n = 4; monkeys which remained symptomatic and did not receive MDMA) or to MPTPrec monkeys (n = 4; called MPTPrec/MDMA as they had received MDMA after their motor recovery) (Fig. 1A). This MPTPrec/MDMA group has been used in a previous study (Beaudoin-Gobert et al., 2015).

2.5. Parkinsonism

The severity of parkinsonian symptoms was assessed daily in the morning using the rating scale proposed by Schneider and Kovelowski (1990) (Schneider and Kovelowski, 1990), which includes several items rated with a maximal total score of 29; the higher the score, the more symptomatic the monkey. The presence of symptoms was evaluated both in the primate chair and the homecage (with also video recordings). Muscular rigidity and eyes movements were assessed only in the primate chair. Rigidity was assessed by joint manipulation. Items were clustered into three categories: motor symptoms, spontaneous activities and other symptoms. The motor symptoms were: rigidity (assessed by manipulation), action tremor (arms and legs), freezing, bradykinesia, global posture and arm/ft posture (flexed or dystonic). The spontaneous activities were: arm and eye movements and home-cage activity (hypokinesia). Spontaneous eyes movements were rated by counting saccades during 3 min. Triggered eyes movements were tested in chair.

Table 1
Characteristics of intoxications and immunohistochemical data for each monkey.

	Protocol	Number MDMA injections	Total MDMA dose (mg/kg)	Number MPTP injections	Total MPTP dose (mg/kg)	Maximal motor score	Time (days)			TH+ cell loss (%)				TPH2 cell loss (%)		
							To peak	To recover	To stabilize	A8	A9	A10	Total	DRN	MRN	
MPTPsym	Acute															
MF3		N/A	N/A	5	2.2	25	22	-	56	74	86	73	78	-	-	
MF16		N/A	N/A	3	1.2	24	11	-	16	89	86	83	86	47	31.6	
MF17		N/A	N/A	3	1.2	25	6	-	28	78	81	62	74	15.8	-24	
MF18		N/A	N/A	3	1.2	24	14	-	28	83	86	70	80	46.5	17.9	
MPTPrec/MDMA	Progressive															
MF4		8	40	4	1.6	16	26	62	-	72	75	65	71	8.7	-3	
MF7		8	40	3	1.2	11	35	35	-	71	68	61	67	10.6	-6	
MF11		8	40	4	1.6	12	34	38	-	81	68	58	70	2.6	45.8	
MF19		8	40	3	1.2	15	15	38	-	72	79	80	77	8.7	31.7	
MDMA/MPTPrec	Progressive															
MF20		8	40	4	1.5	19	24	25	-	89	81	68	80	6	13.7	
MF21		8	40	3	1.2	19	18	18	-	86	77	66	77	3	9.7	
MF25		8	40	5	1.6	21	25	21	-	79	80	70	77	0	23.8	
MF26		8	40	2	0.8	21	11	34	-	81	88	68	80	8	19.2	
MF27		8	40	3	1.2	21	18	18	-	84	85	63	78	-4	4.9	

Abbreviations: DRN, dorsal raphe nucleus; MRN, median raphe nucleus.

The home-cage activity was measured twice daily (early morning and mid-afternoon) using Phenorack (Viewpoint). Other symptoms were: triggered eyes movements and feeding. Vocalization was not assessed due to its absence in the control state.

2.6. PET imaging

2.6.1. Acquisition

PET (positron emission tomography) and MRI (magnetic resonance imaging) acquisitions were performed at the imaging center (CERMEP) under anesthesia (atropine 0.05 mg/kg intramuscularly followed 15 min later by zoletil 15 mg/kg intramuscularly).

Anatomical MRI acquisition consisted of a 3D T1-weighted sequence using a 1.5-T Magnetom scanner (Siemens). The anatomical volume covered the whole brain with 176 planes of 0.6 mm cubic voxels. PET imaging was performed using either a Siemens CTI Exact HR+ or a Siemens Biograph mCT/S64 scanner (due to a replacement of the scanner during the study; see Supplementary Table 2). The HR+ tomograph had a nominal in-plane resolution of 4.1 mm full-width at half-maximum. Tissular and head support 511 keV gamma attenuation was obtained by a 10-min transmission scan of 68Ge rotating rod sources before emission data acquisition. HR+ emission images were reconstructed with all corrections by a 3D filtered back projection algorithm (Hamming filter; cut-off frequency, 0.5 cycles/pixel) and a zoom factor of three. Reconstructed volumes were 63 slices (2.42-mm thickness, 128×128 matrices of 0.32×0.32 mm² voxels), pixels in 63 2.42-mm spaced planes. The Biograph mCT had a spatial transverse resolution of 4.4 mm. Attenuation was obtained using a 1-min low-dose CT scan acquired before emission. Biograph mCT/S64 emission images were reconstructed using the Siemens ultraHD PET algorithm with 12 iterations, eight subsets and a zoom factor of 21. Reconstructed volumes were 109 slices (2.027-mm thickness, 256×256 matrices of 0.398×0.398 mm² voxels). For both PET scans, dynamic acquisition started with the intravenous injection of radiotracers synthesized in the cyclotron unit at CERMEP. To assess the lesions of 5-HT and DA terminals, the following different tracers were used: [¹¹C]DASB (11C-*N,N*-dimethyl-2-(2-amino-4-cyanophenylthio) benzylamine) for 5-HT transporter binding (Nørgaard et al., 2019), [¹¹C]PE2I (11C-*N*-(3-iodoprop-2E-enyl)-2beta-carbomethoxy-3beta-(4-methylphenyl)nor-tropane) for DA transporter binding (Chalon et al., 2019) and [¹⁸F]DOPA (18-fluoro-L-DOPA) for studying amino acid decarboxylase activity (Vallabhajosula, 2009). Indeed, most monkeys from the MPTP/MDMA group in this study have been scanned with the [¹⁸F]DOPA ligand while all monkeys from the MDMA/MPTP group have been scanned with [¹¹C]PE2I.

2.6.2. Regions of interest and kinetic modelling

Individual PET images were registered to their corresponding individual anatomical MRI, which was registered to the *M. fascicularis* MRI template. Transformations from native PET to individual MRI and individual MRI to template were then concatenated to provide direct (and inverse) affine transformations from PET native spaces to the template space. PET were analyzed by tracer kinetic modelling at a voxel-based level. The parameters computed were the non-displaceable binding potential (BP_{ND}) of [¹¹C]DASB and [¹¹C]PE2I using a simplified reference tissue model. For [¹⁸F]DOPA, the uptake rate (K_i, 10⁻³/min) was calculated using frames recording between 30 and 90 min for the linearization and the Patlak graphical analysis. The cerebellum (excluding the vermis) was considered as the reference area for the two models. Region of interest analysis was conducted using the MAXPROB method to define region delineation as previously (Beaudoin-Gobert et al., 2015).

2.7. Immunohistochemistry

2.7.1. Tissue preparation

Monkeys were anesthetized (ketamine at 1 mg/kg followed by a lethal dose of pentobarbital), and perfused transcardially with 400 ml of saline (0.9% at 37 °C) before 5 l of 4% paraformaldehyde (in 0.1 M phosphate-buffered saline (PBS), pH 7.4 at 4 °C) followed by 1 l of PBS with 5% sucrose. The brains were removed from the skull, rinsed in PBS complemented with 10% sucrose for 1 day and 20% sucrose for further one day. Then, frozen brains were cut into 50-μm thick sections coronally on a freezing microtome.

2.7.2. Immunostaining

Free-floating sections were rinsed in Tris-buffered saline (TBS; 0.25 M Tris and 0.25 M NaCl, pH 7.5), incubated for 5 min in TBS containing 3% H₂O₂ and 10% methanol, and then rinsed three times for 10 min each in TBS. After 15-min incubation in 0.2% Triton X-100 in TBS, the sections were rinsed three times in TBS. These were incubated for three days at 4 °C with the following primary antibodies: anti-tyrosine hydroxylase (TH) 1/5000 mouse monoclonal from Euromedex (catalogue number 22941) and anti-tryptophan hydroxylase 2 (TPH2) 1/800 sheep polyclonal from Millipore (catalogue number AB1541). After three rinses in TBS, the sections were then incubated for two hours at room temperature with the corresponding secondary biotinylated antibody (1/500 from Abcys) in TBS. After being washed, the sections were incubated for 90 min at room temperature in avidin-biotin-peroxidase complex solution (final dilution, 1/50; Abcys). The sections were then rinsed twice in TBS and twice in Tris buffer (0.25 M Tris, pH 7.5) for 10 min each, placed in a solution of Tris buffer containing 0.1% 3,3'-diaminobenzidine (DAB; 50 mg/100 ml), and developed by H₂O₂ addition (0.02%). The specificity of the immunostaining was assessed by omission of the primary antibody from the protocol. After processing, the tissue sections were mounted onto gelatin-coated slides and dehydrated through graded alcohol to xylene for light microscopic examination using a computerized image analyzer (Mercator, ExploraNova).

2.7.3. Soma quantification

Soma quantification was performed as in Beaudoin-Gobert et al., 2015. For each animal, TH+ cells were counted on nine regularly spaced sections encompassing the A8 (peri- and retrorubral area), A9 (substantia nigra pars compacta) and A10 (ventral tegmental area) regions. TPH2+ cells were counted throughout five regularly spaced sections covering the antero-posterior extent of the raphe. All counts were estimated after correction by the Abercrombie method. Percentages of TH and TPH2 cell loss were estimated using the mean of TH and TPH2 positive neurons from control male cynomolgus monkeys ($n = 4$).

3. Results

3.1. Impact of early MDMA administration on MPTP-induced Parkinsonism

A double lesion was induced by sequential use of MDMA and MPTP (Fig. 1A). It is important to remind that monkeys called MPTP/MDMA (represented with green lines and symbols) did receive progressive MPTP and were assessed for Parkinsonism before MDMA treatment (MDMA was applied to this experimental group once the monkeys did recover from their motor symptoms; that's why they are called MPTP/MDMA). As expected, all monkeys receiving MPTP developed motor symptoms (Fig. 1B, D and Fig. 2). However, striking differences were observed between groups, especially when comparing monkeys which received progressive MPTP (all monkeys except the ones indicated by orange lines and symbols on Figs. 1 and 2 (i.e. MPTPsym) which received an acute MPTP protocol, see methods). Indeed, the monkeys which had a prior MDMA lesion (MDMA/MPTP)

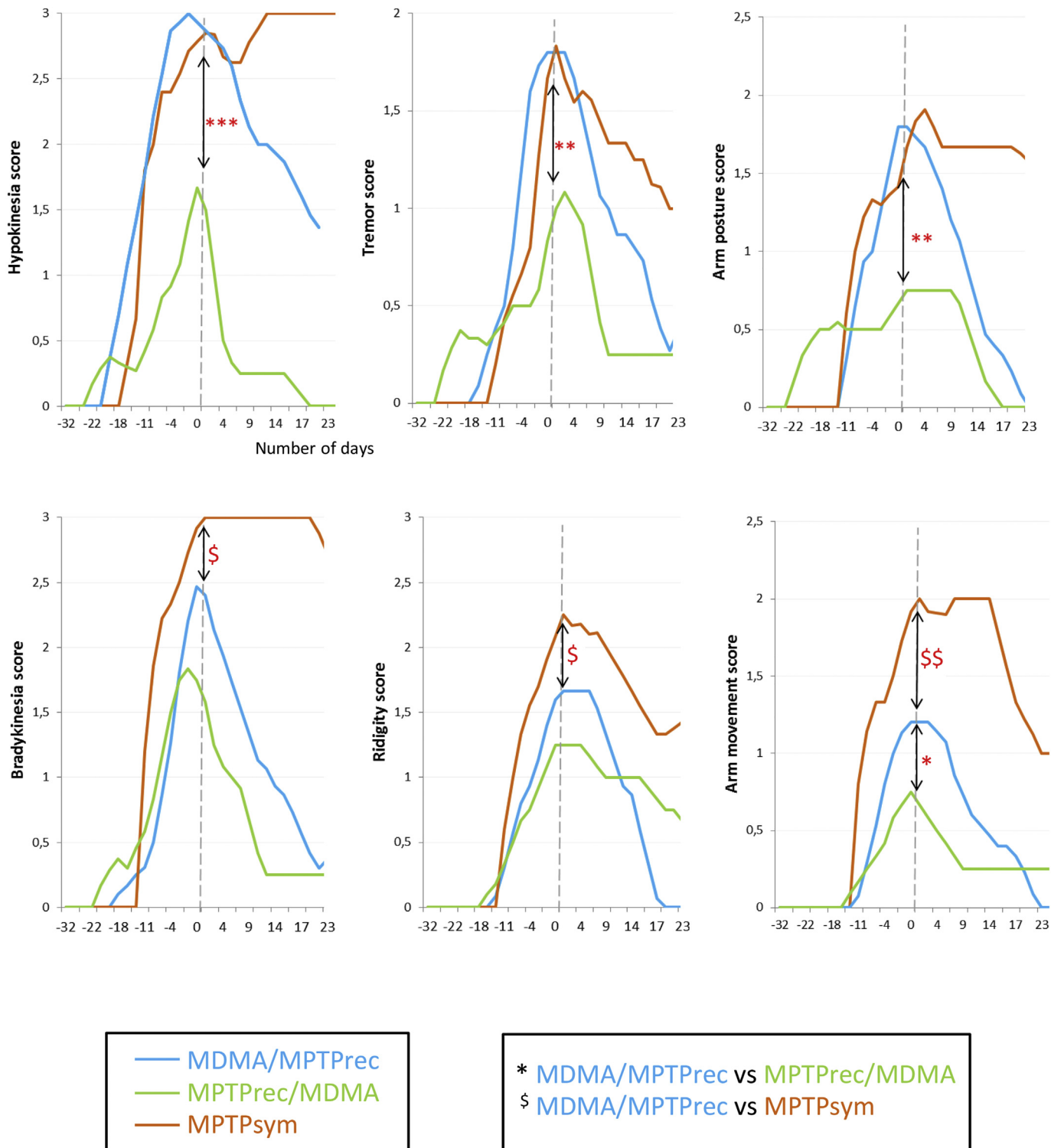


Fig. 2. Severity of individual symptoms between groups. Score at peak reached by MDMA/MPTPrec monkeys was compared to the score at peak obtained by monkeys from the two others groups. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ versus MPTPrec/MDMA. \$ $p < 0.05$; \$\$ $p < 0.01$ versus MPTSym. MPTSym, MDMA/MPTPrec, MPTPrec/MDMA groups are represented in orange, blue and green respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

systematically reached a higher motor score than the MPTPrec/MDMA did (mean maximal motor score 13.5 ± 2) in less time (mean time to reach the peak after the start of MPTP administration: 34 and 18 for MPTPrec/MDMA and MDMA/MPTPrec, respectively). When compared to MPTSym monkeys (mean maximal motor score 24.5 ± 0.5), MDMA/MPTPrec monkeys exhibited Parkinsonism very rapidly even

though they had a lower global motor score (mean maximal motor score 20.2 ± 1) and recovered (Fig. 1B). No significant correlation could be established between the total MPTP dose or the number of injections and the progression of the motor symptoms. However, as expected from post-mortem quantification, we found a positive correlation ($R^2 = 0.42, p = 0.016$) between the total loss of TH-positive cells

(the tyrosine hydroxylase marks DA neurons) and the severity of maximal motor score (Fig. 1C), indicating that the stronger the DA lesion, the more symptomatic the monkey. Of interest, monkeys with an early MDMA lesion exhibited higher maximal motor scores after MPTP, which was associated with a higher percentage of TH-positive cell loss compare to MPTP/MDMA monkeys. On the contrary, the percentage of TPH2-positive cell loss (the tryptophan hydroxylase 2 marks 5-HT neurons) in the raphe was not different between MDMA/MPTP and MPTP/MDMA groups (see supplementary data), indicating that the severity of Parkinsonism developed by MDMA/MPTP monkeys is not due to the loss of 5-HT soma.

To evaluate both severity and duration of motor symptoms, we used an area under the curve (AUC) analysis (Fig. 1D). MDMA/MPTP monkeys had more severe and persistent deficits of spontaneous activities ($p = 0.004$) and motor symptoms ($p = 0.017$) compared to MPTP/MDMA monkeys and, as expected, their motor ($p = 0.04$) and other symptoms ($p = 0.001$) were less severe than MPTP/MDMA monkeys. Interestingly, we observed no difference in spontaneous activities between those two groups ($p = 0.51$). Moreover, among the motor symptoms, we found a positive correlation between the percentage of TH+ cell loss in A9 and bradykinesia AUC ($p = 0.011$; $R^2 = 0.37$) (Fig. 1E). No other significant correlation could be established.

We then looked at each individual symptom at the motor peak (Fig. 2). As already mentioned, monkeys called MPTP/MDMA were assessed for Parkinsonism before MDMA intoxication (as they had recovered from their motor symptoms before MDMA administration). Although MDMA/MPTP (represented in blue lines) and MPTP/MDMA (indicated by orange lines) monkeys received different MPTP protocols (progressive versus acute, respectively; Table 1), MDMA/MPTP had an equivalent severity score to MPTP/MDMA monkeys for hypokinesia (home-cage activity), tremor and arm posture scores. When compared to MPTP/MDMA monkeys, MDMA/MPTP monkeys reached a higher score for hypokinesia ($p < 0.001$), tremor ($p < 0.01$) and arm posture ($p < 0.01$). Only the arm movement score was significantly different between the MPTP/MDMA ($p < 0.05$) and MPTP/MDMA ($p < 0.01$) groups. Compared to symptomatic monkeys, MDMA/MPTP monkeys had significant smaller bradykinesia ($p < 0.05$) and rigidity ($p < 0.05$) scores.

3.2. MDMA, prior to MPTP, affects both 5-HT and DA PET imaging

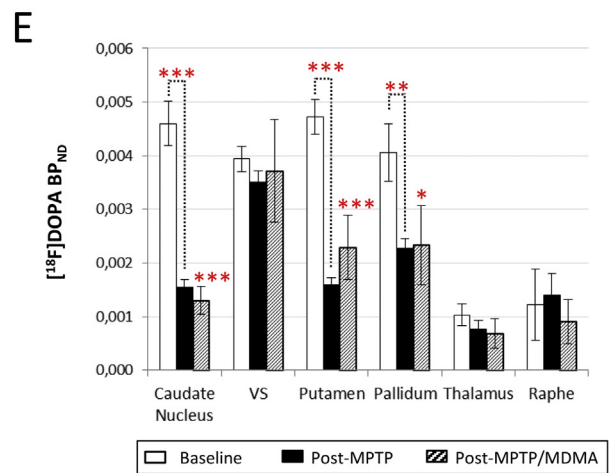
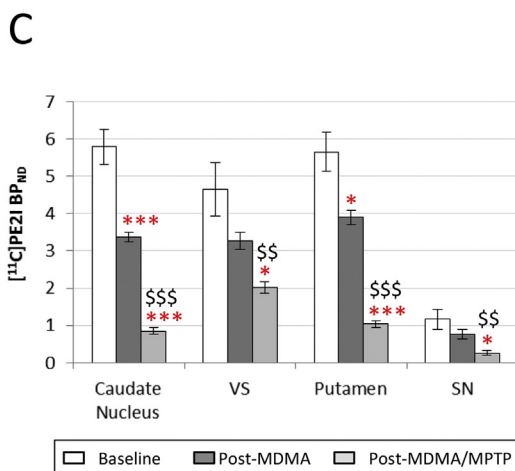
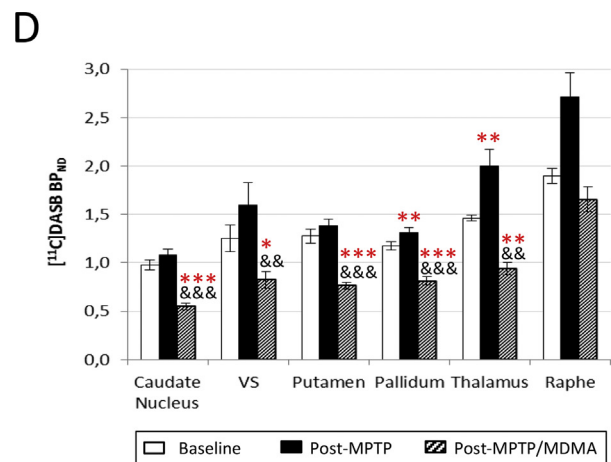
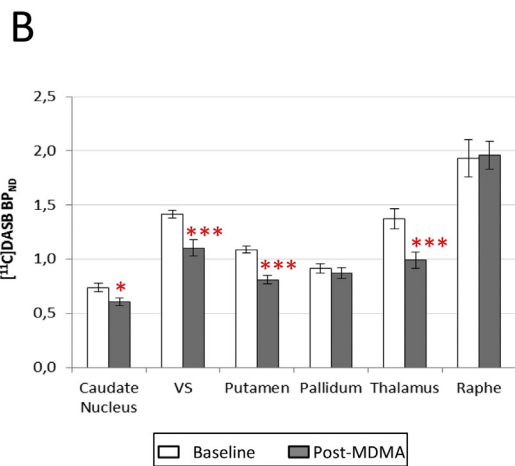
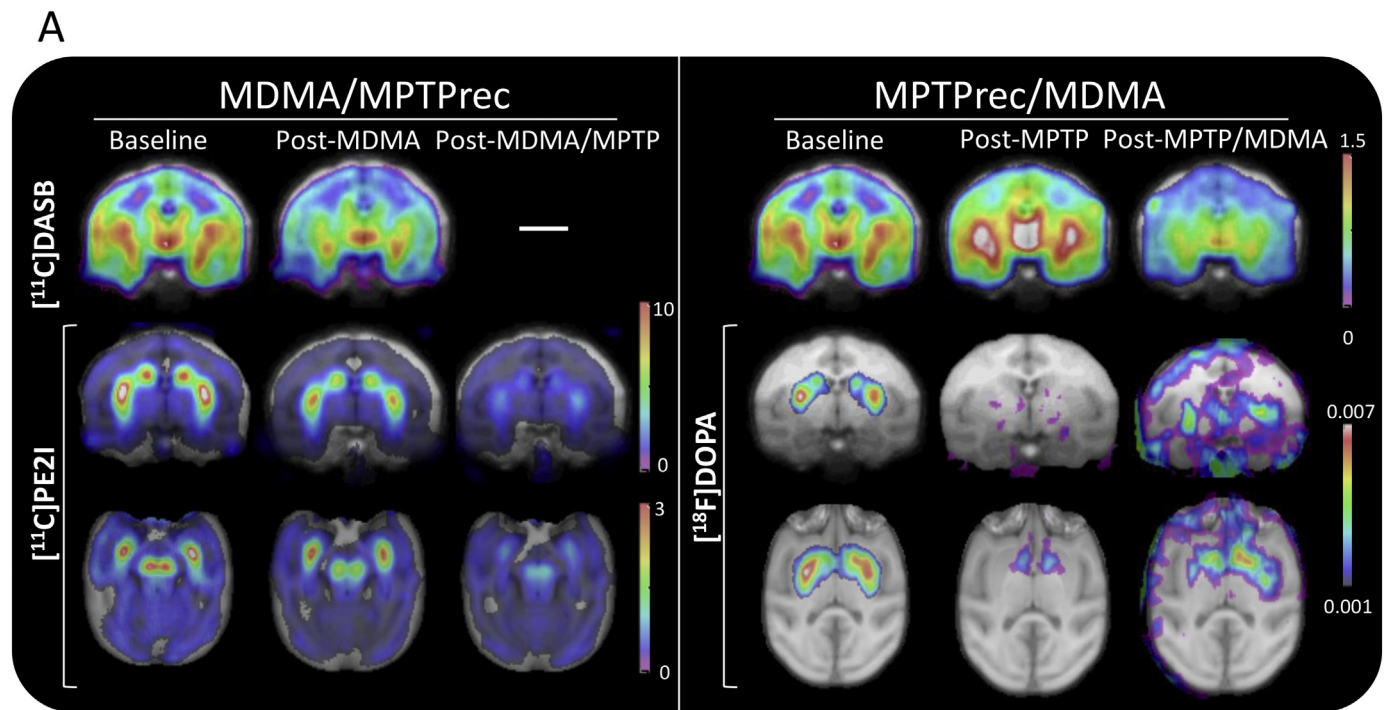
We used 5-HT and DA ligands, which allowed us to analyze 5-HT and DA functional changes (Fig. 1A and Fig. 3). For MDMA/MPTP monkeys (Fig. 3A, left part and Fig. 3B), [^{11}C]DASB BP_{ND} was significantly reduced after MDMA in the striatum and the thalamus. Unfortunately, SERT availability was not measured after the MPTP state for these monkeys because the [^{11}C]DASB ligand was only used initially to assess the injury performed on the serotonergic system by MDMA. Surprisingly, after MDMA, [^{11}C]PE2I BP_{ND} (Fig. 3A, left part and Fig. 3C) was significantly diminished in the caudate nucleus and the putamen (Fig. 3C) and also had the tendency to diminish in the ventral striatum and the substantia nigra. As expected, [^{11}C]PE2I BP_{ND} then collapsed after MPTP administration (Fig. 3, panels A and C). For MPTP/MDMA monkeys (Fig. 3A, right part), [^{11}C]DASB BP_{ND} was not reduced after MPTP but significantly increased in the pallidum and the thalamus (Fig. 3D). After MDMA, [^{11}C]DASB BP_{ND} was significantly decreased in the striatal, pallidal and thalamic regions. [^{18}F]DOPA uptake (Fig. 3A, right part and Fig. 3E) was strongly diminished after MPTP in the caudate nucleus, the putamen and the pallidum, and not further altered after MDMA (Fig. 3E). Of note, similar results were obtained with [^{11}C]PE2I BP_{ND} on another subgroup of MPTP/MDMA monkeys (Beaudoin-Gobert et al., 2015).

4. Discussion

The findings reported here raise several important points. First,

prior MDMA worsened MPTP-induced Parkinsonism. After MDMA alone, monkeys displayed no motor deficits while they exhibited a slight brain DA loss (detected by PET imaging with [^{11}C]PE2I). Of note, once those monkeys received progressive MPTP, they exhibited a severity of Parkinsonism (and associated DA cell loss) which was intermediate between the moderately- and severely-MPTP lesioned animals. Of note, the decrease of [^{11}C]PE2I binding within the posterior putamen positively correlated with the DA loss, the latter being positively linked to the parkinsonian score. These results indicate that repeated MDMA administration in NHPs may injure dopaminergic neurons and enhance MPTP neurotoxic action, as already reported in MPTP-treated mice (Costa et al., 2013). Whether this is the MDMA-evoked 5-HT or DA injury which worsens DA cell loss in response to subsequent MPTP needs further extensive investigation. However, the fact that MDMA, when administered after MPTP, does not kill the remaining mesencephalic DA neurons (Beaudoin-Gobert et al., 2015 and supplementary data) is an argument against the possibility that the MDMA-evoked 5-HT injury could directly worsen DA degeneration. Moreover, the fact that, raphe serotonergic somas resist to both MDMA and progressive MPTP administrations (raphe somas were affected by acute MPTP administration; see supplementary Fig. 1) strongly suggest that MDMA impacts 5-HT terminals and not 5-HT somas or another cell type (i.e. dopaminergic). Instead, there is probably a synergistic effect of the MDMA-evoked partial DA injury with the neurotoxicity of MPTP towards DA neurons which boots DA degeneration. Therefore MDMA, like other amphetamine analogs, favors DA vulnerability. While neurotoxicity towards DA neurons is well accepted in mice (Moratalla et al., 2017), it has been questioned for a long time in other species. A recent study has clearly demonstrated that MDMA also has a neurotoxic effect on DA neurons in rats (Cadoni et al., 2017), but there was no evidence that MDMA could damage DA nerve terminals in humans (Vegting et al., 2016) and NHPs (Beaudoin-Gobert et al., 2015) until now. Although we cannot exclude the possibility of DAT internalization, this study is the first to show a reduced DAT availability as well as a reduced number of mesencephalic DA neurons in MDMA-pretreated MPTP monkeys. The mechanisms that form the basis of the increased vulnerability to MPTP of MDMA-pretreated monkeys are probably attributable to the ability of MDMA to increase the formation of reactive oxygen species and cause oxidative stress, which may render neurons more vulnerable to subsequent MPTP (Moratalla et al., 2017). Illicit stimulant use has recently been associated with abnormal substantia nigra morphology and increased risk of PD (Todd et al., 2013; Rumpf et al., 2017). A link between ecstasy use and PD has been flagged several years ago (Kish, 2003; Jerome et al., 2004) with the reporting of three cases of juvenile Parkinsonism in ecstasy users (Mintzer et al., 1999; O'Suilleabhain and Giller, 2003; Kuniyoshi and Jankovic, 2003).

The second important finding reported here is that while bradykinesia, rigidity and freezing were not affected by prior MDMA administration, tremor, arm posture and spontaneous activities (hypokinesia, arm movement) were significantly impacted. These data indicate that MDMA pretreatment has an impact on particular deficits, which are also detected in PD patients. Bradykinesia, rigidity and freezing are often related to DA system deficits and associated with akinetic-rigid patients (Jellinger, 1999; Kim et al., 2018). The fact that prior MDMA administration potentiated action tremor severity is in line with studies showing that the 5-HT system modulates tremor in parkinsonian rodents (Vanover et al., 2008; Kolasiewicz et al., 2012). This is also in agreement with the existing link between 5-HT dysfunction and tremor in PD patients, although studies are not always concordant regarding the form of tremor involved (rest or re-emergent, postural and kinetic tremors) (Jankovic, 2018). More severe raphe dysfunction correlates with more severe tremor scores in early PD (Qamhawi et al., 2015; Pasquini et al., 2018). Tremor-dominant PD patients also have less severe DA deficits compared to akinetic-rigid patients. Prior MDMA administration enhanced dystonic posture. MDMA/MPTP monkeys had marked dystonic posture of the hand or foot. Of note, dystonia can be



(caption on next page)

Fig. 3. Consequences of MDMA and MPTP intoxications on PET imaging. (A) PET images (in colour) on coronal and horizontal plans in baseline, post-MDMA, post-MDMA/MPTP, post-MPTP and post-MPTP/MDMA according to experimental groups. PET images are superimposed on the *M. fascicularis* MRI brain template (in grey). [¹¹C]DASB PET coronal images were performed in MDMA/MPTPrec (*n* = 7) and MPTPrec/MDMA (*n* = 5) groups. [¹¹C]PE2I was used for MDMA/MPTPrec group (*n* = 5) and [¹⁸F]DOPA for MPTPrec/MDMA group (*n* = 5). Colours represent the level of BP_{ND} or Ki uptake using the cerebellum as reference region (red indicates high BP_{ND} or Ki uptake whereas blue indicates low BP_{ND} or Ki uptake on scales). (B–C) Histograms representing the BP_{ND} of [¹¹C]DASB (B) and [¹¹C]PE2I (C) in baseline, post-MDMA and post-MDMA/MPTPrec for the MDMA/MPTPrec group. **p* < 0.05; ***p* < 0.01; ****p* < 0.001 versus baseline; [§]*p* < 0.05; ^{§§}*p* < .01; ^{§§§}*p* < .001 versus post-MDMA. (D–E) Histograms representing the BP_{ND} of [¹¹C]DASB (D) and [¹⁸F]DOPA (E) in baseline, post-MPTPrec and post-MPTPrec/MDMA for the MPTPrec/MDMA group. * *p* < 0.05, ***p* < 0.01, ****p* < 0.001 versus baseline; [§]*p* < 0.05, ^{§§}*p* < 0.01 versus post-MPTP. Abbreviations: VS, ventral striatum; SN, substantia nigra; MRI, magnetic resonance imaging. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

the presenting symptom of untreated PD, especially common in patients with young-onset PD and responds variably to DA treatments (Ashour et al., 2005). A 5-HT component might therefore have also been involved. Finally, prior MDMA administration affected global spontaneous activities. Indeed MDMA/MPTPrec monkeys displayed a greater lack of activity compared to severely MPTP-lesioned monkeys while they were less bradykinetic and rigid. This could reflect a lack of motivation or a higher level of anxiety-like behaviour. We have also recently shown that apathy, depression and anxiety are linked to 5-HT deficits in de novo PD patients (Maillet et al., 2016). It remains to assess whether severity of action tremor, abnormal posture or spontaneous activities, exhibited by our MDMA-pretreated monkeys, can be related to a specific 5-HT damage within projection regions.

This study has several limitations. First, MDMA does not affect raphe cells, as already shown in other non-human primate studies (Hatzidimitriou et al., 1999; Beaudoin-Gobert et al., 2015). And this result contrasts with the loss of serotonergic somas seen in the dorsal raphe of PD patients (Paulus and Jellinger, 1991). Second, the direct impact of MDMA on DA cell bodies and axons has not been performed at the post-mortem level due to the lack of an experimental group only treated with MDMA. Finally, it would be particularly interesting to investigate the impact of MDMA on the expression of psychiatric-like symptoms.

Despite its limitations, this NHP study provides an important conceptual advance by showing that MDMA damages the 5-HT system and, to a lesser extent, the DA system and worsens parkinsonian deficits induced by subsequent MPTP administration, especially action tremor, abnormal posture and spontaneous activities. The use of a specific 5-HT toxin, such as the 5,7-dihydroxytryptamine (Caillé et al., 2003; Man et al., 2010), combined to MPTP in NHPs, would be extremely useful to address the impact of a selective 5-HT lesion on both DA degeneration and PD symptomatology.

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Declaration of Competing Interest

The authors declare no competing financial interests.

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