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1 **Spike discharge characteristic of the caudal mesencephalic reticular formation and**
2 **pedunculopontine nucleus in MPTP-induced primate model of Parkinson disease**

3
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14
15 **Abstract**

16
17 The pedunculopontine nucleus (PPN) included in the caudal mesencephalic reticular
18 formation (cMRF) plays a key role in the control of locomotion and wake state. Regarding its
19 involvement in the neurodegenerative process observed in Parkinson disease (PD), deep brain
20 stimulation of the PPN was proposed to treat levodopa-resistant gait disorders. However, the
21 precise role of the cMRF in the pathophysiology of PD, particularly in freezing of gait and
22 other non-motor symptoms is still not clear.

23 Here, using micro electrode recording (MER) in 2 primates, we show that dopamine depletion
24 did not alter the mean firing rate of the overall cMRF neurons, particularly the putative non-
25 cholinergic ones, but only a decreased activity of the regular neurons sub-group (though to be
26 the cholinergic PPN neurons). Interestingly, a significant increase in the relative proportion
27 of cMRF neurons with a burst pattern discharge was observed after MPTP intoxication. The
28 present results question the hypothesis of an over-inhibition of the CMRF by the basal ganglia
29 output structures in PD. The decreased activity observed in the regular neurons could explain
30 some non-motor symptoms in PD regarding the strong involvement of the cholinergic neurons
31 on the modulation of the thalamo-cortical system. The increased burst activity under
32 dopamine depletion confirms that this specific spike discharge pattern activity also observed
33 in other basal ganglia nuclei and in different pathologies could play a major role in the
34 pathophysiology of the disease and could explain several symptoms of PD including the

35 freezing of gait. The present data will have to be replicated in a larger number of animals and
36 will have to investigate more in details how the modification of the spike discharge of the
37 cMRF neurons in the parkinsonian state could alter functions such as locomotion and
38 attentional state. This will ultimately allow a better comprehension of the pathophysiology of
39 freezing of gait.

40

41 **Keywords:**

42

43 Pedunculopontine nucleus

44 Parkinson disease

45 Mirco electrode recording

46 Mesencephalic reticular formation

47 Non-human primate

48 MPTP

49

50 **Abbreviations:**

51

52 cMRF: caudal mesencephalic reticular formation ;

53 DBS: deep brain stimulation ;

54 MER: micro electrode recording ;

55 PD: Parkinson disease ;

56 PPN: pedunculopontine nucleus ;

57 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine: MPTP

58

59

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67

68

69

70 **Introduction**

71 Improving therapeutic strategies to treat gait disorders in neurodegenerative diseases requires
72 a better understanding of the pathophysiology at the level of brainstem structures. The caudal
73 mesencephalic reticular formation (cMRF) contains the pedunculopontine nucleus (PPN)
74 (*nucleus tegmentalis pedunculopontinus*) and the cuneiform nucleus thought to be involved in
75 several functions such as the supra-spinal control of locomotion, postural tone, waking state
76 and sleep (Gut and Winn, 2016; Mena-Segovia and Bolam, 2017; Garcia-Rill, 2015).

77 In addition to the neurodegenerative process affecting dopaminergic neurons of the *substantia*
78 *nigra pars compacta* occurring in Parkinson disease (PD), the loss of cholinergic PPN
79 neurons is thought to be involved in the PD pathophysiology (Braak et al., 2003; Coelho and
80 Ferreira, 2012; Galvan and Wichmann, 2008; Hirsch et al., 1987; Jellinger, 1988; Karachi et
81 al., 2010; Pienaar et al., 2013; Rinne et al., 2008; Zweig et al., 1989). Based on experimental
82 data in Parkinsonian animal models, it was suggested that over-activity of the basal ganglia
83 (BG) output structures (internal segment of the *globus pallidus* and *substantia nigra pars*
84 *reticulata*) could lead to hypoactivity of the PPN and finally could explain akinetic symptoms
85 and rigidity (Aziz and Stein, 2008; Gomez-Gallego et al., 2006; Hamani et al., 2007; Pahapill
86 and Lozano, 2000; Takakusaki et al., 2008). On the contrary, an increased
87 electrophysiological activity of PPN neurons was observed in the 6-hydroxydopamine PD
88 model in rat (Breit et al., 2001), in agreement with a previous metabolic study in rat (Orioux
89 et al., 2000).

90 Altogether, these results highlighted a potential role of the PPN neurons in the
91 pathophysiology of the PD. In 2005, deep brain stimulation (DBS) of the PPN was proposed
92 as a new therapeutic strategy to treat levodopa-resistant gait disorders in PD such as freezing
93 of gait (Mazzone et al., 2005; Plaha and Gill, 2005). Despite a growing interest in the
94 neurological community, the precise role of the PPN in the pathophysiology of PD and related
95 gait troubles is still lacking (Mena-Segovia and Bolam, 2017; Pienaar et al., 2017). Regarding
96 the overall considerations on the difficulty to study PPN activity in human beings, we
97 initiated experiments in the behaving primate to study PPN activity during locomotion (Goetz
98 et al., 2016a) and to investigate the role of PPN in the pathophysiology of PD. Regarding the
99 lack of precise anatomical delimitation of the PPN in primate and human, we decided to refer
100 to the cMRF to describe the PPN area, assuming that it encompasses the PPN (cholinergic
101 and non-cholinergic neurons) and the cuneiform nucleus (Goetz et al., 2016a).

102 For several decades, intoxication with the neurotoxin 1-methyl-4-phenyl-1,2,3,6-
103 tetrahydropyridine (MPTP) has proved to be a very robust non-human primate model of PD
104 (Fox and Brotchie, 2010; Morissette and Paolo, 2017; Przedborski et al., 2001; Wichmann et
105 al., 2017). This is the model we considered here, with the goal to evaluate the spike discharge
106 characteristics of the cMRF neurons in the PD context. Using micro electrode
107 recording (MER), we investigated how dopaminergic depletion induced by MPTP
108 intoxication could modify the activity of cMRF neurons by evaluating changes in firing rate
109 and pattern. In particular, we tested the hypothesis of a hypoactivity of the MRF neurons in
110 PD because of basal ganglia dysfunction.

111

112 **Materials and methods**

113 The methods used in the present study were described in detail in our previous study (Goetz
114 et al., 2016a) and followed methods commonly used in primate MER studies of the basal
115 ganglia or PPN area in our lab and by other groups using ventricular landmarks to calculate
116 electrode trajectories (Devergnas et al., 2012; Matsumura et al., 1997; Wichmann et al.,
117 1994).

118

119 *Animals*

120 The same two cynomolgus monkeys (*Macaca fascicularis* - CRP Port Louis, Mauritius) that
121 were used in the previous report (Goetz et al., 2016a) were also used in this present study
122 (Primate K: male, 9 years old, weight: 9 kg ; Primate T : male, 3 years old, weight: 5.5 kg).
123 All experiments were carried out in accordance with the recommendations of the European
124 Community Council Directives of 1986 (86/609/EEC), the National Institutes of Health
125 Guide for the Care and Use of Laboratory Animals. The experimental protocol was approved
126 by the Ethics Committee of Région Rhône-Alpes. Animals were kept with other congeners
127 allowing social behavior, in an air-conditioned room under standard conditions of temperature
128 ($23 \pm 1^\circ\text{C}$), humidity ($65 \pm 4\%$) and light (12 h light/dark cycle). They had access *ad libitum*
129 to food and water and were given fresh fruits and vegetables every day. No food or water
130 restriction was used in the present experiment.

131

132 *Surgical procedures and assessment of MER trajectories*

133 Briefly, under ventriculographical X-Ray control, MRI-compatible titanium footed head
134 holder and recording chamber (Crist Instrument Company, Hagerstown, USA) were

135 stereotactically fixed to the skull with titanium screws. The precise assessment of micro
136 electrode trajectories and recording site were performed with methods commonly used in
137 primate electrophysiological studies combined with MRI (pre-, post-operative and *post*
138 *mortem*) and histological data obtained for each primate (electrolytic lesion and
139 immunohistological staining processed for either a Nissl stain or immunocytochemistry for
140 acetylcholine transferase to localize the cholinergic cMRF neurons).

141

142 *MER procedures and data acquisition*

143 MER were performed using a microdrive set-up (FlexMT-tm and Multi Drive-Tm, Alpha
144 Omega Engineering, Nazareth, Israel). Bevelled mini-tubes were lowered through the dura
145 matter to avoid any damage of the micro electrode when piercing the dura-mater. Two to
146 three tungsten micro electrodes (Impedance: 2-3 MOhm) (FHC Inc, Bowdoin, USA) were
147 lowered independently. The extracellular signal was amplified, analog band-pass filtered
148 (300-6000 Hz) and sampled at 50 kHz. The first cortical neuronal activity defined the null
149 depth of the trajectory. In both primates, the same trajectories were performed in normal and
150 after MPTP intoxication.

151

152 *MPTP treatment*

153 Primates were intoxicated by an intramuscular (IM) injection of MPTP (0.6 mg.kg^{-1} in
154 primate K and 0.5 mg.kg^{-1} in primate T). Primates developed severe Parkinsonian symptoms
155 that necessitated a complete nursing. In primate T, supplementary injection was necessary to
156 induced severe symptoms (0.4 mg.kg^{-1}). L-Dopa therapy (Modopar 50 mg) was provided in
157 the two primates to alleviate the symptoms. Evaluation of the Parkinsonian symptoms was
158 performed every two days, off medication using a modified version of the Imbert scale
159 (Imbert et al., 2000) (See Supplementary Material). Four weeks after the intoxication,
160 primates recovered and symptoms were stable enough to start the electrophysiological
161 protocol. To evaluate dopaminergic degeneration, tyrosine hydroxylase immunostaining was
162 done using a standard immunoperoxidase method, on adjacent coronal serial sections of $50 \mu\text{m}$
163 as previously described (Song and Haber, 2000) (See Supplementary Material).
164 Electrophysiological recordings were always performed off medication.

165

166 *Signal processing*

167 Spike sorting was performed using a template-matching algorithm (Spike 2, CED Software,
168 Cambridge, United Kingdom). Semi-automatic spike isolation was systematically verified and

169 corrected on the basis of the quality of the spike detection (waveforms overdraw) and by a
170 principal component analysis to discriminate single-unit from other multi-unit activity.
171 Calculation of the neuronal refractory period on interspike interval (ISI) was systematically
172 performed and used as an exclusion criterion when it was found to be less than 2 ms (Fee et
173 al., 1996). Firing rate and firing pattern analysis were performed using homemade software
174 developed under Matlab R2009b (The MathWorks Inc., Natick, Massachusetts, USA). A
175 minimum of 500 events in the resulting spike train representing the activity of a single-unit
176 was required to include a neuron in the study.

177 For each neuron, we used the distribution of the inverse of interspike intervals (ISIs) to
178 calculate the mean, standard deviation (SD) and median of the FR.

179

180 *Firing pattern analysis*

181 We determined the firing pattern characteristics by a combined approach using data based on
182 the interval ISI and autocorrelogram analysis further completed with burst detection methods
183 (Abeles, 1982; Wichmann et al., 1994; Baron et al., 2011; Piallat et al., 2011).
184 Autocorrelation histograms of spike trains were calculated for 1s (1 ms bin width) using
185 MATLAB software. Peaks were considered significant if they were found to be above the
186 confidence line set at mean ± 2 SDs. We classified single-unit activities as regular, irregular,
187 or burst as follows:

- 188 • Regular activity was characterized by a tonic discharge with a narrow peaked interval
189 interspike distribution and a significant peak on the autocorrelation function usually
190 equal to the mean firing period (i.e., 1/FR).
- 191 • Irregular activity was characterized by a wide interval interspike distribution and a flat
192 autocorrelogram.
- 193 • Burst activity showed a wide or bimodal interval interspike distribution and a
194 significant single peak on the autocorrelation function. In addition, two different burst
195 detection methods were used to complete the detection: the “Poisson surprise” method
196 of Legendy and Salcman (1985). Applied to our data, using the Poisson surprise
197 method, bursts were defined as segments including at least 3 spikes with an S value of
198 at least 3. Due to the fact that some neurons displayed some long periods of pause that
199 affected the mean FR, thus affecting the Poisson surprise S value, we complemented
200 the burst detection with the interval method that calculates the percentage of spikes in
201 burst derived from the study of Baron et al. (2011). For each neuron, we selected the

202 following final parameters: maximum initial interval signifying burst onset: the
203 median ISI ; longest ISI allowed within a burst: median ISI/2 ; Minimum number of
204 events in a burst: 3. This allowed us to use a burst detection method based on the
205 baseline median rather than the mean FR. Based on these two methods and the
206 complementary ISI and autocorrelogram analysis, the definition of the burst pattern
207 group was statistically significant in the two primates using the two methods (See
208 Figure 2 C-D).

209 For each burst neuron, auto-correlogram analysis was used to assess periodic oscillations
210 defined by the presence of two or more consecutive significant peaks in the autocorrelogram.
211 The main frequency of bursts was defined using the spectral analysis of the transformation of
212 the spike train from discrete series to a continuous function. A power spectrum was computed
213 from the new continuous function (resolution of 0.4 Hz) using a fast Fourier transform within
214 the 0.5–15Hz range (Hanning window, resolution 0.75 Hz). A significant oscillatory activity
215 was considered if the spectrum had one or more peaks above the confidence line
216 (mean + 2 SD).

217

218 *Statistics*

219 Statistical analyses were performed using SPSS 20 (IBM, Armonk, New York) and Prism
220 (GraphPad Software, Inc, La Jolla, USA). We decided to analyse the data obtained in the two
221 primates in normal state and after MPTP intoxication separately as we could not guarantee
222 that the level of neuronal degeneration was identical between the primates. Thus, we decided
223 to replicate the analysis in the two animals and to evaluate the similarities between the
224 findings. Identification of significant differences of mean firing rate between groups were
225 performed using non-parametric Mann-Whitney U-test (significance was assumed for
226 $P \leq 0.05$).

227 Firing rate Chi-2 analysis without correction was performed to test the independence between
228 spiking pattern variables (burst, irregular, regular) and primate status (normal versus MPTP)
229 for each primate. More specifically, to test the association between burst pattern and primate
230 status, we performed the Cochran-Mantel-Haenszel test with 2 stratum (Primates K and T).
231 The null hypothesis was rejected if the P-value of the Mantel-Haenszel test was less than
232 0.05.

233

234

Figure 1 about here

235

236 **Results**

237 We recorded 235 single-unit activities in the cMRF in normal state (91 neurons in Primate K
238 and 144 neurons in primate T). Using the same trajectories, we recorded 152 neuronal
239 activities (single-unit) under MPTP condition (67 neurons in Primate K and 85 neurons in
240 primate T). The electrophysiological mapping of the cMRF extended antero-posteriorly from
241 2 to 6 mm from the anterior border of PC, between 1 and 7 mm laterally from the midline and
242 rostro-caudally from the caudal level of the IC to the pontine nuclei below the superior
243 cerebellar peduncle.

244

245 *Firing rate*

246 Figures 1 A-B show the distributions of the baseline mean firing rate in the two primates in
247 normal and MPTP states. In both primates and in both states, the distributions are wide, with a
248 mean firing rate varying from very low frequencies up to 100 Hz.

249 When comparing the firing rate between the 2 states, Normal *versus* MPTP, in the two
250 primates, the overall mean firing rate were not significantly different between normal and
251 MPTP state (Figure 2A). In primate K, the mean firing rate between normal and MPTP state
252 changed from 16.0 Hz (SD: 15.7) to 13.8 Hz (SD: 10)(Mann-Whitney U; $p= 0.63$). In primate
253 T, the mean firing rate between normal and MPTP state changed from 20.5 (SD: 17.7) Hz to
254 21.8 (SD: 17.8)(Mann-Whitney U; $p= 0.43$).

255 *By comparing the mean firing rate of the pattern sub-groups between normal and MPTP*
256 *(Figure 2B), we did not find significant difference in the mean firing rate of the burst neurons*
257 *between the 2 states (15.7 Hz (SD: 10.1) and 12.3 Hz (SD: 8.6) in primate K (Mann-Whitney*
258 *U; $p= 0.11$) and (18.1 Hz (SD: 13.5) and 22.4 Hz (SD: 15.1) in primate T (Mann-Whitney U;*
259 *$p= 0.17$). Similarly, no significant difference was observed in the irregular pattern sub-group*
260 *in the 2 primates between the 2 states: 9.7 Hz (SD: 10.5) and 9.3 Hz (SD: 11.9) in primate K*
261 *(Mann-Whitney U; $p= 0.84$) and 11.37 Hz (SD: 11.4) and 10.6 Hz (SD: 7.9) in primate T*
262 *(Mann-Whitney U; $p= 0.87$). On contrary, when considering the regular pattern sub-group, a*
263 *significant difference was observed in Primate K (Mann-Whitney U; $p= 0.045$) with a*
264 *decrease of the mean firing rate from 33.5 Hz (SD: 22.7) in normal state to 21.4 Hz (SD:*
265 *16.0) after MPTP intoxication. In Primate T, no significant difference (Mann-Whitney U; $p=$*
266 *0.9) was observed in this pattern sub-group 32.6 Hz (SD: 19.4) in normal state and 33.8 Hz*
267 *(SD: 22.0) under MPTP condition.*

268

269 Figure 2 about here

270 Figure 3 about here

271

272 *Firing pattern*

273 Significant differences in firing pattern were observed in the two primates between normal
274 and MPTP states (χ^2 -test: $p < 0.05$). More precisely, in primate K, between normal and
275 MPTP states, the percentage of burst neurons increased from 34.1% to 47.8% while the
276 percentage of the irregular ones decrease from 48.4 % to 26.9% of the overall number of
277 neurons. Similarly, in primate T, the evolution of the percentages of burst and irregular
278 neurons were from 23.6% to 42.4%, and from 41.7% to 30.6%, respectively. In both primates,
279 the change of percentage of regular neurons was less than 10% (Figure 3A). In addition, by
280 comparing the relative proportions of the burst neurons compared with non-burst neurons
281 (irregular + regular) simultaneously in the two primates (treated in the Cochran-Mantel-
282 Haenszel test as two stratum), we also found significant difference in the percentage of burst
283 versus non-burst neurons between normal and MPTP (Cochran-Mantel-Haenszel χ^2 -test: p
284 = 0.004) (Figure 3B). Interestingly, the relative proportion of neurons with a burst oscillatory
285 activity did not change significantly between normal and PD states (Figure 4A). However,
286 under MPTP condition, those burst neurons displayed an oscillatory frequency around 20 Hz
287 in both primates, which was significantly higher than normal condition in primate K only
288 (Figures 4B).

289 Figure 4 about here

290 **Discussion**

291 We could perform the same mapping of the cMRF, using the same micro electrode
292 trajectories, between normal and Parkinsonian states in two non-human primates to
293 investigate how neuronal activities in the cMRF were affected by dopamine depletion.
294 Recording sessions under MPTP conditions provided two main results. i) **No significant
295 difference in mean firing rate between normal and MPTP conditions of the overall cMRF
296 recorded neurons but a decrease of the mean firing rate observed in the regular neurons
297 pattern sub-group in 1 primate.** ii) A significant increase in the relative proportion of cMRF
298 neurons with a busrt discharge pattern after MPTP intoxication.

299

300 *Firing rate in MPTP condition*

301 We did not observe a significant overall change in the mean firing rate of cMRF neurons in
302 the 2 primates, as was first hypothesized. Indeed, convincing datasets obtained in primate by

303 lesion or chemical manipulation suggested that hyperactive GABAergic afferences from
304 GPi/SNr could lead to hypoactivity of the PPN and consequently to the development of
305 akinetic symptoms (Gomez-Gallego et al., 2006; Kojima et al., 1997; Nandi et al., 2002b;
306 Palombo et al., 1990). This led to the well-known hypothesis supported by the “rate model”:
307 in the context of PD, hyperactivity of the basal ganglia output structures leads to
308 inactivation/hypoactivity of the PPN (Hamani et al., 2007; Matsumura, 2005; Mena-Segovia
309 et al., 2004; Pahapill and Lozano, 2000; Stein and Aziz, 2012; Takakusaki et al., 2008). Of
310 interest, DBS of the human PPN was proposed in 2005 as a new therapeutic strategy to treat
311 gait disorders in PD based on those results and hypothesis (Mazzone et al., 2005; Plaha and
312 Gill, 2005). However, some electrophysiological data obtained in a rodent model of PD
313 provided opposite results, in favor of an hyperactivity of the PPN following dopaminergic
314 depletion (Breit et al., 2001; Orieux et al., 2000). Similarly, this latter result was not observed
315 in the present data in primate treated with MPTP.

316 When considering the pattern sub-groups, our previous observations on the evolution of the
317 mean firing rate between the 2 conditions were confirmed. Indeed, in primate, projections
318 from the BG output structures (GPi and SNr) preferentially target non-cholinergic neurons in
319 the cMRF (M. Parent et al., 1999; Rolland et al., 2011; Shink et al., 1997).

320 In the present study, it was not possible to determine the neurochemical nature of the
321 recording neurons but assuming that neurons with a regular firing pattern could be the
322 putative cholinergic type II neurons (Takakusaki et al., 1996; Leonard and Llinás, 1994;
323 Takakusaki:1996jw; Kang and Kitai, 1990; Gut and Winn, 2016; Mena-Segovia and Bolam,
324 2017) we neither observed a modification of the non-regular neurons (putative non-
325 cholinergic) known to receive projections from the BG output structures projections.

326 However, we observed a significant decrease in firing rate of the regular neurons in one
327 primate while in the other primate, a similar decrease was observed but not significantly. One
328 possible explanation is that only the firing rate of the PPN cholinergic neurons were affected
329 by dopamine depletion. This is surprising because it was observed in primate that DAT-
330 positive fibers tended to avoid PPN cholinergic cell bodies (Rolland et al., 2009). However, a
331 dopaminergic modulation of the cholinergic neurons projections via passive transmission can
332 still be hypothesized as it was demonstrated in several species from vertebrates to mammals
333 including human, that TH+/DAT+ fibers and varicosities can be observed in the vicinity of
334 PPN cholinergic neurons (Ryczko et al., 2016).

335

336 *Firing pattern in MPTP condition*

337 On the other hand, in the two primates, we found a significant increase in the relative number
338 of neurons that discharged in bursts after MPTP intoxication. Furthermore, the proportion of
339 burst neurons with an oscillatory activity remained stable after dopamine depletion but a trend
340 to shift from α to β band frequency was evident. In normal primate, the only studies that
341 investigated the spike discharge of PPN neurons did not perform a detailed analysis of the
342 firing pattern (Kobayashi et al., 2002; Matsumura et al., 1997). But when considering the
343 raster plot provided in the aforementioned studies, it is reasonable to consider that some of the
344 recorded neurons discharged in burst mode. In human, MER during DBS electrode
345 implantation in the PPN area were performed by several groups (Piallat et al., 2009;
346 Shimamoto et al., 2010; Tattersall et al., 2014; Weinberger et al., 2008). They all described a
347 bursting activity in the PPN neurons but the paradigms associated with MER in PD patients
348 makes difficult the comparisons with the present data.

349 The precise mechanisms responsible for the increase in burst pattern and the role of dopamine
350 depletion in this phenomenon remain to be determined. Could an excessive hyperpolarisation
351 of the cMRF neurons by BG output structures lead to an increase bursting rebound is an
352 opened question (Beurrier et al., 1999; Galvan and Wichmann, 2008; Plenz and Kitai, 1999;
353 Wichmann and Soares, 2006). This could explain some effects observed in primate after
354 neurochemical modulation of PPN neurons by GABAergic agent (agonist and antagonist)
355 (Nandi et al., 2002a). This would be in accordance with the rebound spikes observed in PPN
356 neurons (type I & II) after SNr stimulation in rat (Kang and Kitai, 1990). Also, the close
357 interconnection between PPN and STN should be considered in this perspective as an increase
358 bursting activity in the STN has been observed in primate after MPTP intoxication (Bergman
359 et al., 1994).

360 *Considerations associated with spike discharge analysis*

361 As mentioned by Israel and colleagues (Israel and Bergman, 2008), caution should be
362 observed regarding the large variability of firing rate analysis in the BG studies that led to
363 consider that BG output structures are hyperactive in PD context. Indeed, two studies in
364 primate failed to demonstrate a significant increase in firing rate of the GPi and SNr after
365 MPTP intoxication (Raz et al., 2000; Wichmann et al., 1999). [Similarly, in our results, we
366 found a large variability of the mean firing rate of the cMRF neurons in both primates, thus
367 caution should also be observed in the present study when interpreting the evolution of the
368 firing rate after MPTP intoxication.](#) Moreover, as pointed out by several studies, PPN area is
369 composed by a heterogeneous neuronal population (Martinez-Gonzalez et al., 2012; Pienaar
370 et al., 2017; Rye et al., 1987; Wang and Morales, 2009). Thus, the evaluation of the effect of

371 GABAergic projections on the heterogeneous cMRF neuronal population in primate should
372 be considered with caution. Finally, given the constraints related to experimentation on
373 primate, it was not possible to define the neurochemical nature of the recorded neurons. Thus,
374 interpretation of the discharge characteristics was based on studies led on other animal
375 models and therefore requires caution.

376

377 *Functional implications*

378 The decrease activity of the regular neurons (putative PPN cholinergic type II cells) must be
379 confirmed on larger number of cells and animals. This could explain some non-motor troubles
380 including sleep disorders and attentional symptoms observed in the Parkinsonian state
381 (Schapira et al., 2017). Indeed, the PPN cholinergic neurons considered as the cholinergic arm
382 of the reticular ascending system (Garcia-Rill, 2015; Jones, 2005; Shute and Lewis, 1967;
383 Steriade, 1996) are known to project on intralaminar and reticular thalamic nuclei (Lavoie and
384 A. Parent, 1994a), especially on parafascicular thalamic neurons (inhibitory effect) and
385 thalamocortical neurons (excitatory effect) (Ye et al., 2010). Decreased thalamic input by the
386 PPN neurons in the wake state could induce changes in the firing pattern of thalamic neurons,
387 switching spontaneously from a tonic to a bursting discharge mode leading to low-frequency
388 rhythmicity of thalamic neurons (Llinás et al., 2005). Ultimately, a decrease in PPN input
389 observed after MPTP intoxication could cause dysrhythmic state in the thalamo-cortical
390 system, *i.e.* the thalamocortical dysrhythmia (Llinás and Steriade, 2006). In line with the
391 previous arguments, in a previous study in normal behaving primate, we found some neurons
392 in the cMRF mostly with a regular discharge pattern, which modulated their activity during
393 transition from wakefulness to sleep, suggesting an active role in brain state as previously
394 found in other species (Datta and Siwek, 2002; Mena-Segovia et al., 2008; Steriade and
395 McCarley, 2013). Regarding the present data, change in the spiking discharge after MPTP
396 intoxication could lead to disturbance in brain state transitions.

397 Cholinergic and non-cholinergic efferents from the PPN on dopaminergic neurons of the
398 *substantia nigra* has been described in several species including primate and may play a
399 pivotal modulation of the dopaminergic transmission on BG (Dautan et al., 2016). Regarding
400 the location of the synaptic contact between cholinergic axons and dopaminergic SNc neurons
401 (perisomatic and on proximal dendrites) (Lavoie and A. Parent, 1994b), the spike discharge
402 modifications of the cMRF observed in the present study, *i.e.* decrease firing rate of regular
403 neurons and increase bursting activity following MPTP intoxication, could lead to dramatic

404 modification of the dopaminergic innervation of the BG and thus could be a key feature of the
405 pathophysiology of PD.

406 Finally, regarding the potential effect of spike discharge disturbance of the cMRF neurons
407 after MPTP intoxication on both the thalamocortical system and the BG circuitry, the
408 involvement of the cMRF neurons in the pathophysiology of freezing of gait is likely. Indeed,
409 in a previous study, we described the involvement of the same cMRF neurons in the control
410 of locomotion via 2 types of neuronal response, *i.e.* a phasic and/or a tonic discharge mode
411 (Goetz et al., 2016a). We also found that some cMRF neurons were involved in both
412 locomotion and wake state transition (Goetz et al., 2016b), suggesting that the cMRF could be
413 considered as an integrative structure (Lau et al., 2015) for both motor and attentional
414 functions in order to adapt locomotion in the environment via its ascending and descending
415 projections.

416 Using our model of bipedal locomotion (Goetz et al., 2012), we could perform recordings in
417 the cMRF after MPTP intoxication while the bipedal locomotion primate was blocked
418 (impossibility for the primate to initiate the swing phase). Although this cannot be considered
419 as an episode of freezing of gait in primate, in few neurons, we observed abnormal neuronal
420 activities in the cMRF during blocking of the swing phase associated with an abnormal EMG
421 activity of the quadriceps muscle group (see Supplementary Data). These preliminary data
422 will have to be confirmed in further experiments in order to precisely characterize a possible
423 role of spike discharge abnormality of cMRF neurons in the occurrence of freezing of gait.

424

425 *Implication for DBS*

426 While the mechanisms of action of DBS are still not clearly understood at the level of the BG,
427 the investigation of such mechanisms in the reticular formation to treat gait disorders should
428 be regarded very cautiously. If the present data were confirmed on a larger neuronal
429 population and animals, then it is reasonable to consider the hypothesis that DBS could have
430 two effects of the neuronal activity in the cMRF: first stimulation at low frequency (from 10
431 to 30 Hz) could drive the remaining PPN cholinergic neurons to fire with a higher frequency,
432 then restoring a normal PPN-thalamic transmission resulting in the suppression or reduction
433 of thalamocortical dysrhythmia. Second, it has been suggested that the excessive burst activity
434 observed in several nuclei of the BG could play a critical role in the pathophysiology of
435 related symptoms in different pathologies (Galvan and Wichmann, 2008; Lobb, 2014; Piallat
436 et al., 2011; Welter et al., 2011). Thus, we can suggest that a possible mechanism of DBS in
437 the BG and in the cMRF is associated with the reduction of pathological burst activity by

438 acting on the membrane hyperpolarization and the restoration of a physiological discharge
439 activity.

440

441 **Conclusion**

442 Our results did not confirm the hypothesis of an over-inhibition of the PPN by the SNr/GPi
443 complex because we did not observe any significant decrease in the mean firing rate of the
444 overall cMRF neurons, nor on putative non-cholinergic neurons, after MPTP intoxication.
445 However, the decreased activity of the regular neurons (putative PPN cholinergic neurons)
446 could have dramatic consequences on the thalamocortical system and finally could explain
447 some of the non-motor symptoms in PD. In parallel, the increase in bursting activity observed
448 in the 2 primates provides new insights on the key role of the cMRF neurons in the
449 pathophysiology of PD and gait disorders. It is now of special interest in further studies to
450 consider the cMRF as an integrative structure associated with motor function and the reticular
451 activating system regarding its dense ascending and descending projections. This will allow
452 investigating complex symptoms such as freezing of gait or sleep/arousal disturbance.

453

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460

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711

712 **FIGURE LEGENDS**

713

714 **Figure 1:** Base line mean firing rate distribution in primate K and primate T in normal
715 and MPTP states. (a) : Normal state. (b) MPTP state. Y axis represents the number of
716 neurons and X axis represents the mean firing rate lustered in bins. The center of the
717 first bin is at 5 Hz and bin width is 10 Hz.

718

719 **Figure 2:** A: Evolution of the mean firing rate of cMRF neurons after MPTP intoxication.
720 B: Evolution of the mean firing rate of cMRF pattern sub-groups after MPTP

721 intoxication. The box plots summarize the distribution of the firing rate, with the limits
722 of the box representing the 25th and 75th quartiles and the central line showing the
723 median sample value. The whiskers extend to the min and max values. (* Significant $p <$
724 0.05 Mann-Whitney U-test). C: Burst discharge characteristics: a) Mean Poisson surprise
725 value and b) mean percentage of spikes in burst per pattern group, per primate, in
726 normal and MPTP state according to the methods developed by Legendy and Salcman
727 (Legendy and Salcman, 1985) and (Baron et al., 2011) respectively (** Significant $p <$
728 0.01 - * Significant $p < 0.05$ - Mann-Whitney U-test).

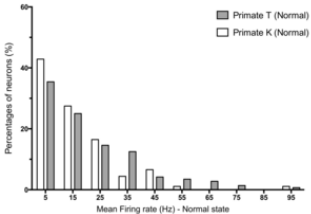
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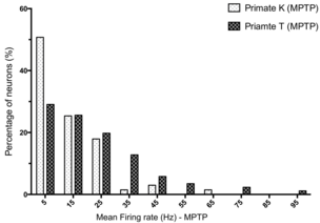
730 **Figure 3:** Spike discharge pattern in normal and MPTP state in the two primates:
731 relative proportion of neurons in the different pattern sub-groups (in percentage). A:
732 Comparison between 3 pattern sub-groups (* Significant $p < 0.05$ - χ^2 -test). B:
733 Comparison between burst *versus* non-burst neurons. Primates are treated as stratum in
734 statistical test (* Significant $p < 0.05$ - Cochran-Mantel-Haenszel χ^2 -test).

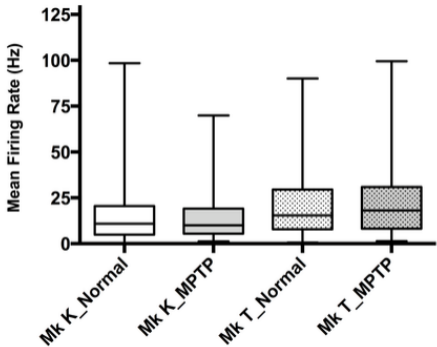
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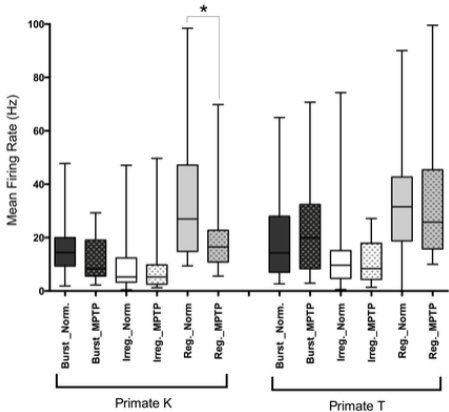
736 **Figure 4:** Relative proportion of oscillatory *versus* non-oscillatory burst neurons in A
737 and mean burst oscillatory frequency in normal and MPTP state in B. The box plots in B
738 summarize the distribution of the neurons coordinates, with the limits of the box
739 representing the 25th and 75th quartiles and the central line showing the median
740 sample value. The whiskers extend to the min and max values. (** Significant $p < 0.01$ -
741 Mann-Whitney U-test).

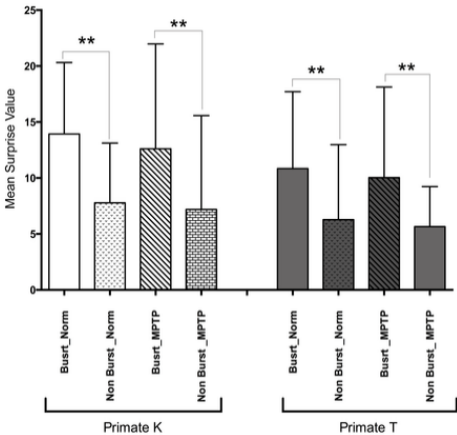
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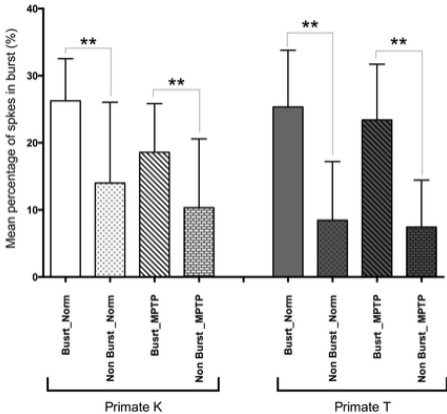


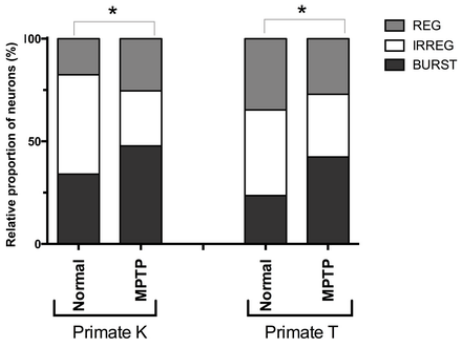


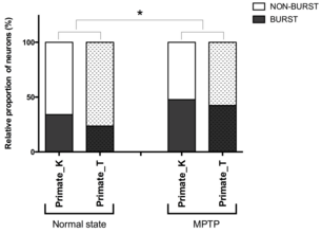












- Oscillatory burst
- Non-oscillatory burst

