Neurobiology of Disease 128 (2019) 40–48

Contents lists available at ScienceDirect

Neurobiology of Disease

journal homepage: www.elsevier.com/locate/ynbdi

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

Laurent Goetz[a,b,*], Brigitte Piallat[a,b], Manik Bhattacharjee[a,b], Hervé Mathieu[a,b,c,d], Olivier David[a,b], Stéphan Chabardès[a,b,e,f]

[a] Grenoble Institute of Neurosciences, INSERM U1216 CEA-UJF-CHUGA, Grenoble, France
[b] Université Grenoble Alpes, Grenoble, France
[c] Unité Mixte de Service IRMaGe, Grenoble Alpes Hospital, F-38000 Grenoble, France
[d] Unité Mixte de Service 3552, CNRS, F-38000 Grenoble, France
[e] Department of Neurosurgery, Centre Hospitalier Universitaire Grenoble Alpes, Grenoble, France
[f] CEA Clinatec-Minatec, Grenoble, France

ARTICLE INFO

Keywords:
Pedunculopontine nucleus
Parkinson disease
Micro electrode recording
Mesencephalic reticular formation
Non-human primate
MPTP

ABSTRACT

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

Here, using micro electrode recording (MER) in 2 primates, we show that dopamine depletion did not alter the mean firing rate of the overall cMRF neurons, particularly the putative non-cholinergic ones, but only a decreased activity of the regular neurons sub-group (though to be the cholinergic PPN neurons). Interestingly, a significant increase in the relative proportion of cMRF neurons with a burst pattern discharge was observed after MPTP intoxication. The present results question the hypothesis of an over-inhibition of the CMRF by the basal ganglia output structures in PD. The decreased activity observed in the regular neurons could explain some non-motor symptoms in PD regarding the strong involvement of the cholinergic neurons on the modulation of the thalamo-cortical system. The increased burst activity under dopamine depletion confirms that this specific spike discharge pattern activity also observed in other basal ganglia nuclei and in different pathologies could play a mojor role in the pathophysiology of the disease and could explain several symptoms of PD including the freezing of gait. The present data will have to be replicated in a larger number of animals and will have to investigate more in details how the modification of the spike discharge of the cMRF neurons in the parkinsonian state could alter functions such as locomotion and attentional state. This will ultimely allow a better comprehension of the pathophysiology of freezing of gait.

1. Introduction

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

In addition to the neurodegenerative process affecting dopaminergic neurons of the *substantia nigra pars compacta* occurring in Parkinson disease (PD), the loss of cholinergic PPN neurons is thought to be involved in the PD pathophysiology (Braak et al., 2003; Coelho and

Abbreviations: cMRF, caudal mesencephalic reticular formation;; DBS, deep brain stimulation;; MER, micro electrode recording;; PD, Parkinson disease;; PPN, pedunculopontine nucleus;; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine

* Corresponding author at: Grenoble Institute of Neurosciences, INSERM U1216 CEA-UJF-CHUGA, Grenoble, France.
E-mail address: laurent.goetz@hotmail.fr (L. Goetz).

https://doi.org/10.1016/j.nbd.2018.08.002
Received 1 April 2018; Received in revised form 24 July 2018; Accepted 3 August 2018
Available online 04 August 2018
0969-9961/

Ferreira, 2012; Galvan and Wichmann, 2008; Hirsch et al., 1987; Jellinger, 1988; Karachi et al., 2010; Pienaar et al., 2013; Rinne et al., 2008; Zweig et al., 1989). Based on experimental data in Parkinsonian animal models, it was suggested that over-activity of the basal ganglia (BG) output structures (internal segment of the *globus pallidus* and *substantia nigra pars reticulata*) could lead to hypoactivity of the PPN and finally could explain akinetic symptoms and rigidity (Aziz and Stein, 2008; Gomez-Gallego et al., 2006; Hamani et al., 2007; Pahapill and Lozano, 2000; Takakusaki et al., 2008). On the contrary, an increased electrophysiological activity of PPN neurons was observed in the 6-hydroxydopamine PD model in rat (Breit et al., 2001), in agreement with a previous metabolic study in rat (Orieux et al., 2000).

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

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

2. Materials and methods

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

2.1. Animals

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

2.2. Surgical procedures and assessment of MER trajectories

Briefly, under ventriculographical X-Ray control, MRI-compatible titanium footed head holder and recording chamber (Crist Instrument Company, Hagerstown, USA) were stereotactically fixed to the skull with titanium screws. The precise assessment of micro electrode trajectories and recording site were performed with methods commonly used in primate electrophysiological studies combined with MRI (pre-, post-operative and *post mortem*) and histological data obtained for each primate (electrolytic lesion and immunohistological staining processed for either a Nissl stain or immunocytochemistry for acetylecholine transferase to localize the cholinergic cMRF neurons).

2.3. MER procedures and data acquisition

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

2.4. MPTP treatment

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

2.5. Signal processing

Spike sorting was performed using a template-matching algorithm (Spike 2, CED Software, Cambridge, United Kingdom). Semi-automatic spike isolation was systematically verified and corrected on the basis of the quality of the spike detction (waveforms overdraw) and by a principal component analysis to discriminate single-unit from other multi-unit activity. Calculation of the neuronal refractory period on interspike interval (ISI) was systematically performed and used as an exclusion criterion when it was found to be < 2 ms (Fee et al., 1996). Firing rate and firing pattern analysis were performed using homemade software developed under Matlab R2009b (The MathWorks Inc., Natick, Massachusetts, USA). A minimum of 500 events in the resulting spike train representing the activity of a single-unit was required to include a neuron in the study.

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

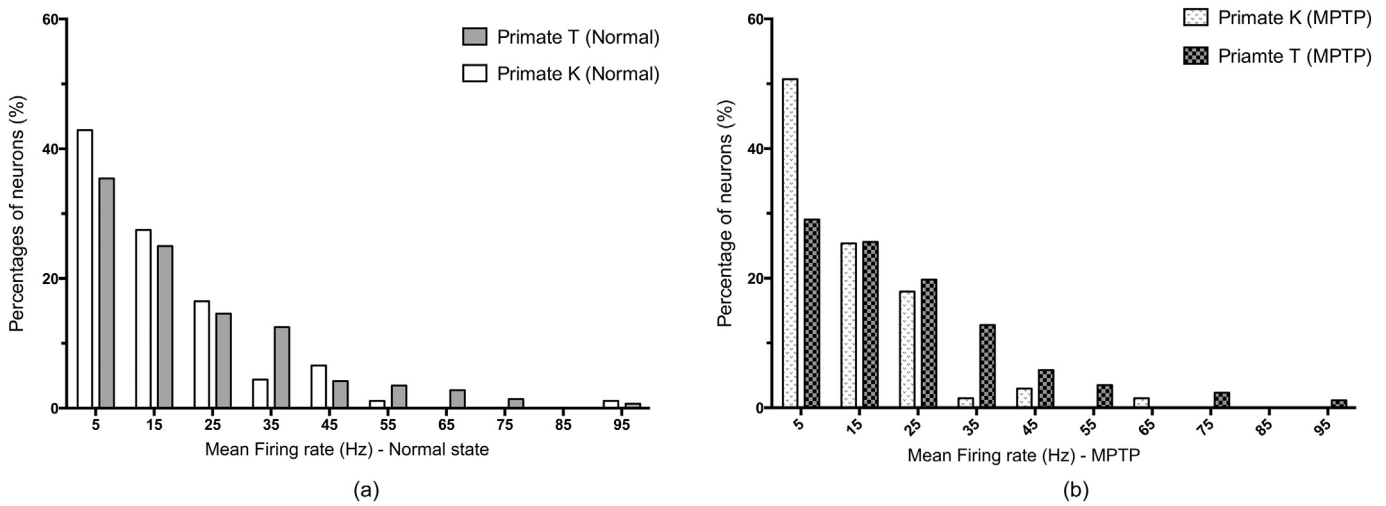

Fig. 1. Base line mean firing rate distribution in primate K and primate T in normal and MPTP states. (a): Normal state. (b) MPTP state. Y axis represents the number of neurons and X axis represents the mean firing rate lustered in bins. The center of the first bin is at 5 Hz and bin width is 10 Hz.

2.6. Firing pattern analysis

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

- Regular activity was characterized by a tonic discharge with a narrow peaked interval interspike distribution and a significant peak on the autocorrelation function usually equal to the mean firing period (*i.e.*, 1/FR).
- Irregular activity was characterized by a wide interval interspike distribution and a flat autocorrelogram.
- Burst activity showed a wide or bimodal interval interspike distribution and a significant single peak on the autocorrelation function. In addition, two different burst detection methods were used to complet the detection: the "Poisson surprise" method of Legéndy and Salcman (1985). Applied to our data, using the Poisson surprise method, bursts were defined as segments including at least 3 spikes with an *S* value of at least 3. Due to the fact that some neurons displayed some long periods of pause that affected the mean FR, thus affecting the Poisson surprise S value, we complemented the burst detection with the interval method that calculates the percentage of spikes in burst derived from the study of Baron et al. (2011). For each neuron, we selected the following final parameters: maximum initial interval signifying burst onset: the median ISI; longest ISI allowed within a burst: median ISI/2; Minimum number of events in a burst: 3. This allowed us to use a burst detection method based on the baseline median rather than the mean FR. Based on these two methods and the complementary ISI and autocorrelogram analysis, the definition of the burst pattern group was statistically significant in the two primates using the two methods (see Fig. 2 C–D).

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

2.7. Statistics

Statistical analyses were performed using SPSS 20 (IBM, Armonk, New York) and Prism (GraphPad Software, Inc., La Jolla, USA). We decided to analyse the data obtained in the two primates in normal state and after MPTP intoxication separately as we could not guarantee that the level of neuronal degeneration was identical between the primates. Thus, we decided to replicate the analysis in the two animals and to evaluate the similarities between the findings. Identification of significant differences of mean firing rate between groups were performed using non-parametric Mann-Whitney *U* test (significance was assumed for $P \leq .05$).

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

3. Results

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

3.1. Firing rate

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

When comparing the firing rate between the 2 states, Normal *versus*

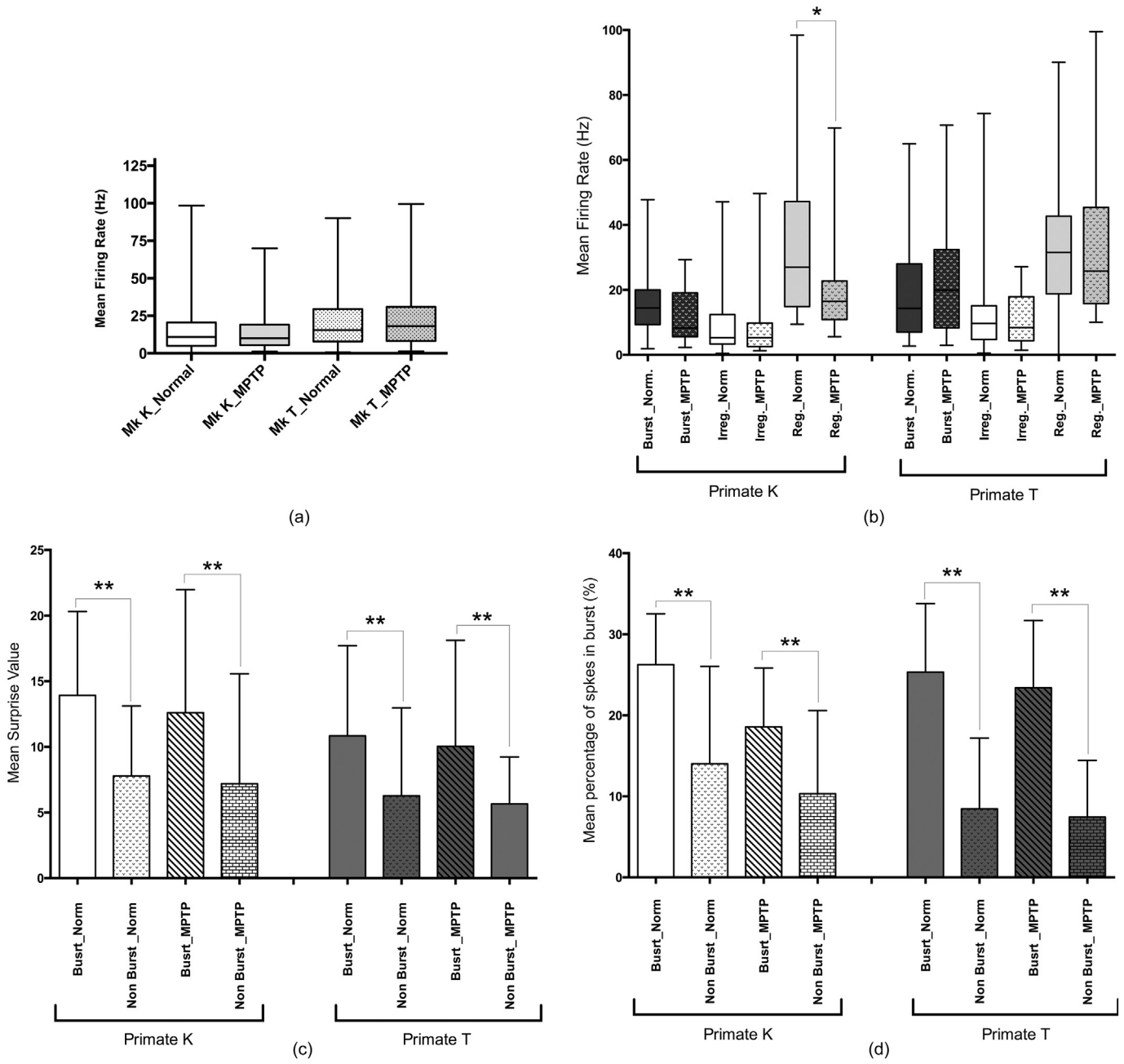

Fig. 2. A: Evolution of the mean firing rate of cMRF neurons after MPTP intoxication. B: Evolution of the mean firing rate of cMRF pattern sub-groups after MPTP intoxication. The box plots summarize the distribution of the firing rate, with the limits of the box representing the 25th and 75th quartiles and the central line showing the median sample value. Thek, whiskers extend to the min and max values. (* Significant $p < .05$ Mann-Whitney U test). C: Burst discharge characteristics: a) Mean Poisson surprise value and b) mean percentage of spikes in burst per pattern group, per primate, in normal and MPTP state according the the methods developped by Legendy and Salcman (Legéndy and Salcman, 1985) and (Baron et al., 2011) respectively (**Significant $p < .01$ - *Significant $p < .05$ - Mann-Whitney U test).

MPTP, in the two primates, the overall mean firing rate were not significantly different between normal and MPTP state (Fig. 2A). In primate K, the mean firing rate between normal and MPTP state changed from 16.0 Hz (SD: 15.7) to 13.8 Hz (SD: 10) (Mann-Whitney U; $p = .63$). In primate T, the mean firing rate between normal and MPTP state changed from 20.5 (SD: 17.7) Hz to 21.8 (SD: 17.8) (Mann-Whitney U; $p = .43$).

By comparing the mean firing rate of the pattern sub-groups between normal and MPTP (Fig. 2B), we did not find significant difference in the mean firing rate of the burst neurons between the 2 states (15.7 Hz (SD: 10.1) and 12.3 Hz (SD: 8.6) in primate K (Mann-Whitney U; $p = .11$) and (18.1 Hz (SD: 13.5) and 22.4 Hz (SD: 15.1) in primate T (Mann-Whitney U; $p = .17$). Similarly, no significant difference was observed in the irregular pattern sub-group in the 2 primates between the 2 states: 9.7 Hz (SD: 10.5) and 9.3 Hz (SD: 11.9) in primate K (Mann-Whitney U; $p = .84$) and 11.37 Hz (SD: 11.4) and 10.6 Hz (SD: 7.9) in primate T (Mann-Whitney U; $p = .87$). On contrary, when considering the regular pattern sub-group, a significant difference was observed in Primate K (Mann-Whitney U; $p = .045$) with a decrease of the mean firing rate from 33.5 Hz (SD: 22.7) in normal state to 21.4 Hz (SD: 16.0) after MPTP intoxication. In Primate T, no significant difference (Mann-Whitney U; $p = .9$) was observed in this pattern sub-group 32.6 Hz (SD: 19.4) in normal state and 33.8 Hz (SD: 22.0) under MPTP condition.

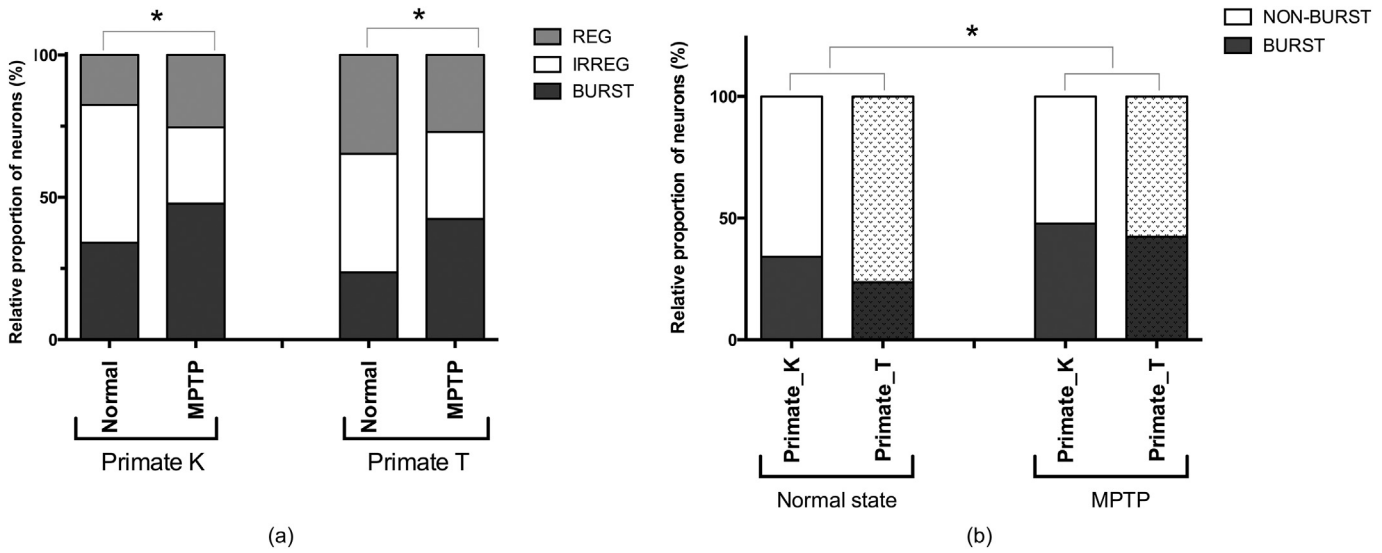

Fig. 3. Spike discharge pattern in normal and MPTP state in the two primates: relative proportion of neurons in the different pattern sub-groups (in percentage). A: Comparison between 3 pattern sub-groups (*Significant $p < .05$ - $\chi 2$-test). B: Comparison between burst *versus* non-burst neurons. Primates are treated as stratum in statistical test (*Significant $p < .05$ - Cochran-Mantel-Haenszel $\chi 2$-test).

3.2. Firing pattern

Significant differences in firing pattern were observed in the two primates between normal and MPTP states ($\chi 2$-test: $p < .05$). More precisely, in primate K, between normal and MPTP states, the percentage of burst neurons increased from 34.1% to 47.8% while the percentage of the irregular ones decrease from 48.4% to 26.9% of the overall number of neurons. Similarly, in primate T, the evolution of the percentages of burst and irregular neurons were from 23.6% to 42.4%, and from 41.7% to 30.6%, respectively. In both primates, the change of percentage of regular neurons was < 10% (Fig. 3A). In addition, by comparing the relative proportions of the burst neurons compared with non-burst neurons (irregular + regular) simultaneously in the two primates (treated in the Cochran-Mantel-Haenszel test as two stratum), we also found significant difference in the percentage of burst *versus* non-burst neurons between normal and MPTP (Cochran-Mantel-Haenszel $\chi 2$-test: $p = .004$) (Fig. 3B). Interestingly, the relative proportion of neurons with a burst oscillatory activity did not change significantly between normal and PD states (Fig. 4A). However, under MPTP condition, those burst neurons displayed an oscillatory frequency around 20 Hz in both primates, which was significantly higher than normal condition in primate K only (Fig. 4B).

4. Discussion

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

4.1. Firing rate in MPTP condition

We did not observe a significant overall change in the mean firing rate of cMRF neurons in the 2 primates, as was first hypothesized. Indeed, convincing datasets obtained in primate by lesion or chemical manipulation suggested that hyperactive GABAergic afferences from GPi/SNr could lead to hypoactivity of the PPN and consequently to the development of akinetic symptoms (Gomez-Gallego et al., 2006; Kojima et al., 1997; Nandi et al., 2002b; Palombo et al., 1990). This led to the well-known hypothesis supported by the “rate model”: in the context of PD, hyperactivity of the basal ganglia output structures leads to inactivation/hypoactivity of the PPN (Hamani et al., 2007; Matsumura, 2005; Mena-Segovia et al., 2004; Pahapill and Lozano, 2000; Stein and Aziz, 2012; Takakusaki et al., 2008). Of interest, DBS of the human PPN was proposed in 2005 as a new therapeutic strategy to treat gait disorders in PD based on those results and hypothesis (Mazzone et al., 2005; Plaha and Gill, 2005). However, some electrophysiological data obtained in a rodent model of PD provided opposite results, in favor of an hyperactivity of the PPN following dopaminergic depletion (Breit et al., 2001; Orieux et al., 2000). Similarly, this latter result was not observed in the present data in primate treated with MPTP.

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

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

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

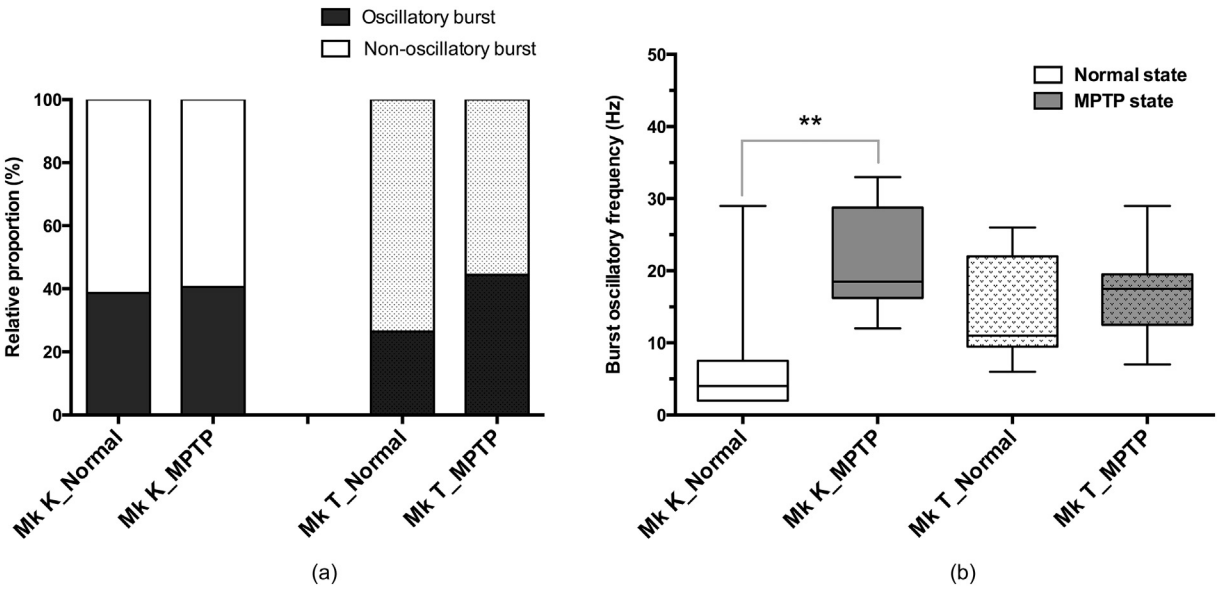

Fig. 4. Relative proportion of oscillatory *versus* non-oscillatory burst neurons in A and mean burst oscillatory frequency in normal and MPTP state in B. The box plots in B summarize the distribution of the neurons coordinates, with the limits of the box representing the 25th and 75th quartiles and the central line showing the median sample value. The whiskers extend to the min and max values. (**Significant $p < .01$ - Mann-Whitney U test).

4.2. Firing pattern in MPTP condition

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

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

4.3. Considerations associated with spike discharge analysis

As mentioned by Israel and Bergman (2008), caution should be observed regarding the large variability of firing rate analysis in the BG studies that led to consider that BG output structures are hyperactive in PD context. Indeed, two studies in primate failed to demonstrate a significant increase in firing rate of the GPi and SNr after MPTP intoxication (Raz et al., 2000; Wichmann et al., 1999). Similarly, in our results, we found a large variability of the mean firing rate of the cMRF neurons in both primates, thus caution should also be observed in the present study when interpreting the evolution of the firing rate after MPTP intoxication. Moreover, as pointed out by several studies, PPN area is composed by a heterogeneous neuronal population (Martinez-Gonzalez et al., 2012; Pienaar et al., 2017; Rye et al., 1987; Wang and Morales, 2009). Thus, the evaluation of the effect of GABAergic projections on the heterogeneous cMRF neuronal population in primate should be considered with caution. Finally, given the constraints related to experimentation on primate, it was not possible to define the neurochemical nature of the recorded neurons. Thus, interpretation of the discharge characteristics was based on studies leaded on other animal models and therefore requires caution.

4.4. Functional implications

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

present data, change in the spiking discharge after MPTP intoxication could lead to disturbance in brain state transitions.

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

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

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

4.5. Implication for DBS

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

5. Conclusion

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

Acknowledgements

We are grateful to Vincente Dicalogero for animal care, Yann Thibaudier for assistance during experiments; Patrick Mouchet in Grenoble for insightful discussions and Prof. Suzanne N. Haber and her team in the Department of Pharmacology and Physiology, University of Rochester Medical Center for the valuable tissue processing and immunohistochemistry.

Funding

This work was supported by grants from Medtronic and by France Parkinson Association. Sponsors had no role in the study design, data collection, data analysis, or data interpretation. The neurophysiology facility and the Grenoble MRI facility IRMaGe were partly funded by the French program Investissement d'Avenir run by the Agence Nationale pour la Recherche (Grant Infrastructure d'avenir en Biologie Santé, ANR-11-INBS-0006.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.nbd.2018.08.002.

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