Role of the pedunculopontine nucleus in controlling gait and sleep in normal and parkinsonian monkeys

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Abstract

Patients with Parkinson's disease (PD) develop cardinal motor symptoms, including akinesia, rigidity, and tremor, that are alleviated by dopaminergic medication and/or subthalamic deep brain stimulation. Over the time course of the disease, gait and balance disorders worsen and become resistant to pharmacological and surgical treatments. These disorders generate debilitating motor symptoms leading to increased dependency, morbidity, and mortality. PD patients also experience sleep disturbance that raise the question of a common physiological basis. An extensive experimental and clinical body of work has highlighted the crucial role of the pedunculopontine nucleus (PPN) in the control of gait and sleep, and its potential major role in PD. Here, we summarise our investigations in the monkey PPN in the normal and parkinsonian states. We first examined the anatomy and connectivity of the PPN and the cuneiform nucleus which both belong to the mesencephalic locomotor region. Second, we conducted experiments to demonstrate the specific effects of PPN cholinergic lesions on locomotion in the normal and

parkinsonian monkey. Third, we aimed to understand how PPN cholinergic lesions impair sleep in parkinsonian monkeys. Our final goal was to develop a novel model of advanced PD with gait and sleep disorders. We believe that this monkey model, even if it does not attempt to reproduce the exact human disease with all its complexities, represents a good biomedical model to characterise locomotion and sleep in the context of PD.

Keywords

Parkinson's disease
Pedunculopontine nucleus
Gait disorders
Sleep disorders
Monkeys

Abbreviations

CuN	Cuneiform nucleus
DBS	Deep brain stimulation
FOG	Freezing of gait
MLR	Mesencephalic locomotor region
MPTP	1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine
PD	Parkinson's disease
PPN	Pedunculopontine nucleus
REM	Rapid eye movement

Introduction

Most patients with advanced Parkinson's disease (PD) develop gait and balance disorders which worsen with time and become resistant to dopaminergic medication. These gait and postural disorders, the so-called axial symptoms, are characterised by freezing of gait (FOG) and balance deficits. Falls, in particular, have been identified as a major feature in the course of PD (Kempster et al. 2010), because they are the source of serious disability leading to dependency, institutionalisation, and poor quality of life. These resistant gait disorders induce high morbidity mainly related to hip fractures and head injuries, and significantly increased mortality. In this context, the poor alleviation of axial symptoms with

dopaminergic medication and deep brain stimulation (DBS) of the subthalamic nucleus or internal pallidum strongly suggests a dysfunction of non-dopaminergic brain networks. An extensive experimental and clinical body of work has highlighted the crucial role of the pedunculopontine nucleus (PPN), whose cholinergic neurons partially degenerate in advanced PD (Hirsch et al. 1987; Zweig et al. 1989; Rinne et al. 2008; Karachi et al. 2010).

The PPN appears to be a complex gait generator of the brainstem linking cortex, basal ganglia, and the spinal cord. Given its anatomical location and its partial neuronal loss in specific neurodegenerative disorders that impair gait, some clinical teams proposed low-frequency PPN-DBS in PD patients with FOG and falls to activate the remaining PPN neurons to restore locomotor function (Mazzone et al. 2005; Plaha and Gill 2005; Stefani et al. 2007; Ferraye et al. 2010; Moro et al. 2010; Thevathasan et al. 2011; Lau et al. 2015; Welter et al. 2015; Mestre et al. 2016). However, despite an initial great optimism (Mazzone et al. 2005), the overall results of controlled clinical studies using PPN-DBS in PD patients have been disappointing and with unpredictable outcome for the majority of patients (Ferraye et al. 2010; Moro et al. 2010; Thevathasan et al. 2011; Welter et al. 2015). Moreover, a recent double-blind clinical study concluded that stimulation of the PPN area has an initial but not sustained benefit for PD gait disorders (Mestre et al. 2016). Many factors could explain the variability of these clinical outcomes, including patient selection, gait assessment, and target location. These basic questions remain unresolved, but the main interrogation remains the exact anatomy of the PPN and the normal physiology of human gait that is poorly understood at the neuronal network level.

The PPN together with the adjacent cuneiform nucleus (CuN) belongs to the mesencephalic locomotor region (MLR), previously named by experimental physiologists, because its stimulation produced locomotion (see the recent review by (Garcia-Rill et al. 2015). Numerous anatomical and physiological studies have been conducted over many years to analyse the PPN inputs and outputs but mainly in rodents. At the present time, the connections of the PPN and above all of the CuN with the basal ganglia, thalamus, and cortex, as well as their descending output pathways are insufficiently described and required new anatomical investigations in primates. Recent physiological studies show that the PPN is not only

involved in motor control, but also in many other functions as various as sleep, sensory integration, attention, and even emotion. Taken together, these results suggest that the primate PPN has a role that extends beyond simple locomotor motor control. In particular, the PPN appears to integrate information that would be necessary for adapting locomotion to environmental demands (Lau et al. 2015). Several other nuclei of the reticular formation (such as raphe nuclei, locus coeruleus, cuneiform nucleus…) likely share this integrative feature, participating in functions as varied as sleep, attention, emotion, and locomotion. A striking example of the non-motor role of the PPN is its involvement in modulating wakefulness and sleep. In particular, pharmacological and neurophysiological studies in animals have emphasised the importance of brainstem cholinergic neurons in the generation of rapid eye movement (REM) sleep (Steriade et al. 1990; Rye 1997; Datta 2002). These cholinergic neurons are in a position to influence thalamo-cortical activity, sleep–wakefulness states, and muscle tone via its ascending projections to the forebrain and descending projections to the reticulospinal pathway (Pare et al. 1988; Jones 1993). Clinical observations have also reported that PPN-DBS in PD patients can significantly modify sleep quality, with increased daytime alertness and a long-term improvement of night-time sleep (Arnulf et al. 2010; Peppe et al. 2012). The PPN is a component of a group of upper brainstem nuclei that constitute the ascending reticular activating system. As such, the PPN and particularly its cholinergic neurons exert a role in regulating sleep and arousal.

Here, we review the anatomy of the PPN and its role in locomotor and sleep control in non-human primate, describing and discussing our recent experimental studies.

Relevance of non-human primates for studies of locomotion and sleep in normal and parkinsonian states

Primate research is essential to better understand the physiological basis of human neurological disorders which have dramatic repercussions on public health. Moreover, this research is of great assistance in performing pre-clinical therapeutic trials to launch new treatments for patients with the best technical and ethical conditions. Concerning PD, monkeys treated with the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)

constitute the most relevant model available to date, since it can reproduce both dopaminergic neuronal loss and the cardinal parkinsonian symptoms (akinesia, rigidity, and episodes of tremor) that closely resemble those observed in PD patients (Emborg 2007). The MPTP primate model presents some limitations that have already been reviewed (Collier et al. 2003; Emborg 2004). In particular, even though postural deficits are frequently reported, whether they are dopa-sensitive and/or associated with episodes of FOG or falls is unknown.

In our laboratory, we were interested in developing a monkey model which not only displays cardinal parkinsonian symptoms, but also gait and balance disorders which are currently the most debilitating and untreatable symptoms in advanced PD. The physiological mechanisms of gait and posture control have been studied in diverse species, including lamprey, cat, and rodent (Grillner 2003; Clarac 2008; McCrea and Rybak 2008). However, studies on the kinematic and biomechanical properties of gait in macaques have concluded that primate locomotion (including old word primates and humans) is fundamentally different from locomotion in other mammals, and while permanent bipedalism is unique to human (see Vilensky 1983; Courtine et al. 2005; Xiang et al. 2007), it is observed for short durations and over a short distances in non-human primate. It thus seems more appropriate to analyse gait and posture in normal and MPTP-treated macaques than in non-primate species. Since the previous experimental and clinical studies have shown that the PPN is involved in gait control (Pahapill and Lozano 2000), it was of interest to focus on the behavioural effects induced by PPN lesions in normal and parkinsonian macaques to propose a better model of advanced PD.

With regards to the sleep–wake cycle, diurnal habits as well as the nocturnal sleep of monkeys (adult macaques in particular) are much more similar to the sleep architecture in humans than those in rodent or cat. This model could thus allow us to fill a gap between sleep studies in rodent and cat and those in human (Hsieh et al. 2008). MPTP-treated monkeys develop stable sleep disorders with abnormal sleep architecture similar to that seen in PD patients (Barraud et al. 2009). These results demonstrate that monkeys and macaques, in particular, are a good biomedical model to study locomotion and sleep especially in the context of PD.

Anatomical definition and connections of the

PPN in primate

It should be pointed out that only minimal data on the PPN are available for monkeys in comparison to rodent and cat. However, the projections of the PPN are assumed to be similar between these species, the MLR being a highly conserved structure in all vertebrates (Shik et al. 1966; Garcia-Rill et al. 1987; Sirota et al. 2000; Ryczko et al. 2016). Here, the studies available for primates are first discussed together with our recent anatomical data.

The PPN and the adjacent CuN are both located within the reticular formation of the lateral mesencephalon of the upper brainstem. These two structures correspond to open nuclei with unclear boundaries whichever histological stains are used. Both nuclei have a complex cellular organisation with GABAergic and glutamatergic neuronal populations, whereas cholinergic neurons are only present within the PPN itself. This cholinergic cell population has been reported to account for 50% of PPN neurons in human (Manaye et al. 1999). PPN cholinergic neurons are clustered within a dense dorsolateral area (PPN pars compacta) and are scattered more medially (PPN pars dissipata) classically defined as the Ch5 group of cholinergic neurons (Mesulam et al. 1983, 1984, 1989). PPN glutamatergic and GABAergic neurons are observed in the rat (Clements et al. 1991; Wang and Morales 2009; Martinez-Gonzalez et al. 2011) and cat (Jia et al. 2003). In monkeys, GABAergic neurons were not found in the PPN (Lavoie and Parent 1994a), whereas both glutamatergic and GABAergic PPN neurons appear to project to midbrain dopamine neurons (Charara et al. 1996). The possible colocalization of acetylcholine with either glutamate or GABA in PPN neurons is still controversial. About 50% of cholinergic neurons were reported to express GABA (Jia et al. 2003), and about 40% seem to express glutamate in rat and monkey (Clements et al. 1991; Lavoie ~~et al.~~ and Parent 1994a). However, no cholinergic neurons were observed to coexpress GABA or glutamate using a combination of in situ hybridization and immunohistochemistry (Wang ~~et al~~ and Morales~~.~~ 2009). Recent studies reported that GABAergic neurons are more densely concentrated in the rostral PPN, compared to cholinergic and glutamatergic neurons (Mena-Segovia et al. 2009; Martinez-Gonzalez et al. 2011), suggesting that the PPN should be regarded as an heterogeneous structure. The rostral PPN, which contains significantly more GABAergic neurons, would be interconnected with the basal ganglia, whereas the caudal PPN, which contains a larger number of cholinergic and

glutamatergic neurons, would be more related to thalamo-cortical systems and to regions involved in regulation of gait and posture (Martinez-Gonzalez et al. 2011). Whether such a rostro-caudal organisation of neuronal populations within the PPN and the CuN exists in human remains to be determined.

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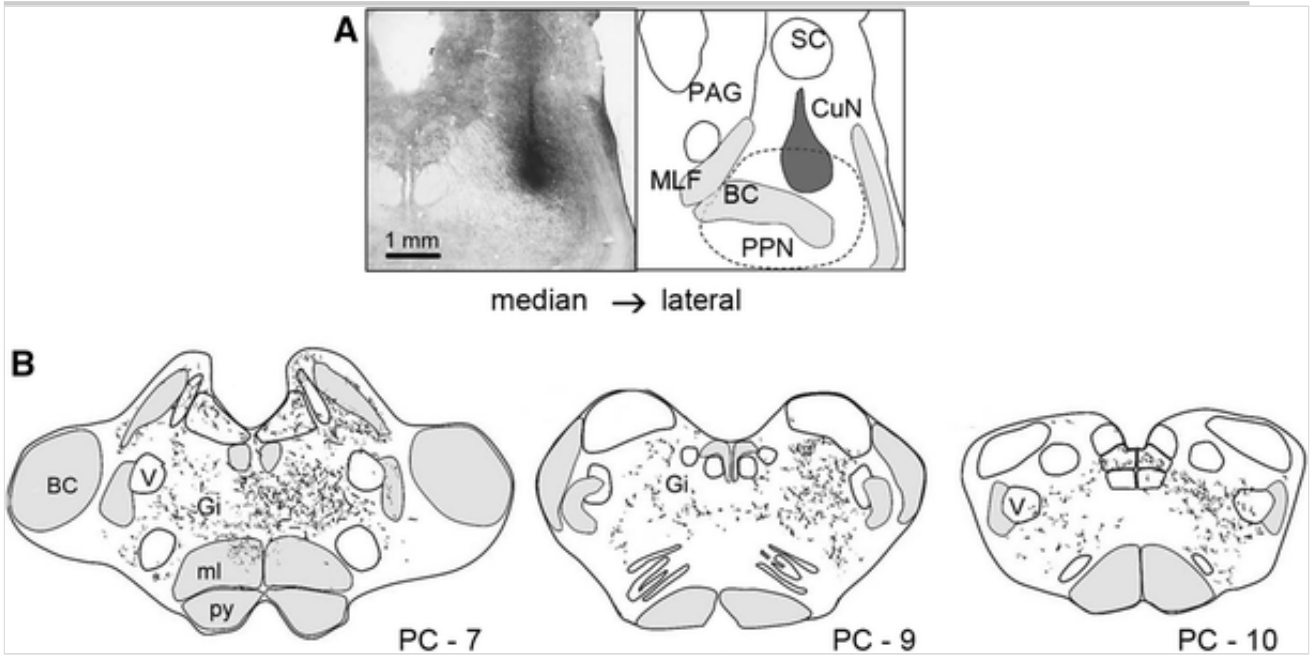
Anatomical and physiological studies have identified many connections to the PPN and CuN. However, due to their open boundaries and their neurochemical heterogeneity, it has been difficult to precisely determine which inputs terminate in the PPN or CuN. Overall, the PPN receives dense cortical afferents from the premotor and motor cortices (Matsumura et al. 2000), suggesting that the PPN integrates motor information. The PPN is also highly interconnected with the basal ganglia (see, for review, Pahapill ~~et al.~~ and Lozano 2000). In particular, it has been demonstrated that the PPN integrates information arising from the three different functional territories of the internal pallidum in monkey (Shink et al. 1997). The PPN also sends profuse cholinergic and non-cholinergic ascending efferent fibers to all thalamic nuclei, although the intralaminar and reticular nuclei are particularly densely innervated in monkey (Steriade et al. 1988; Lavoie et al. 1994b Lavoie and Parent 1994b) and human (Heckers et al. 1992). Moreover, the parafascicular nucleus appears more strongly innervated than the centre median in monkey (Olivier et al. 1970). Projections from the deep cerebellar nuclei have also been demonstrated (Hazrati and Parent 1992).

We conducted a series of tract-tracing experiments in monkey to analyse the input and output connections of the PPN and the CuN, whose connections are poorly understood. Our results show that the descending outputs of both the PPN and the CuN target the pontobulbar reticular formation at the origin of the reticulospinal pathway (Rolland et al. 2011). Figure 1 illustrates the anterograde labelling of fibers obtained after tracer injection into the MLR.

Fig. 1

a Photomicrograph and map of biotin dextran amine injection site into PPN (mainly in its dorsal part) with diffusion within the ventral CuN. **b** Maps of anterogradely labelled PPN terminals on transverse sections of the pontomedullary reticular formation. *BC* brachium conjunctivum, *Gi*

gigantocellular nucleus, *ml* medial lemniscus, *MLF* medial longitudinal fascicle, *PAG* periaqueductal grey matter, *py* pyramidal tract, *SC* superior colliculus, *V* trigeminal nucleus.
Figure adapted from Rolland et al. (2011)

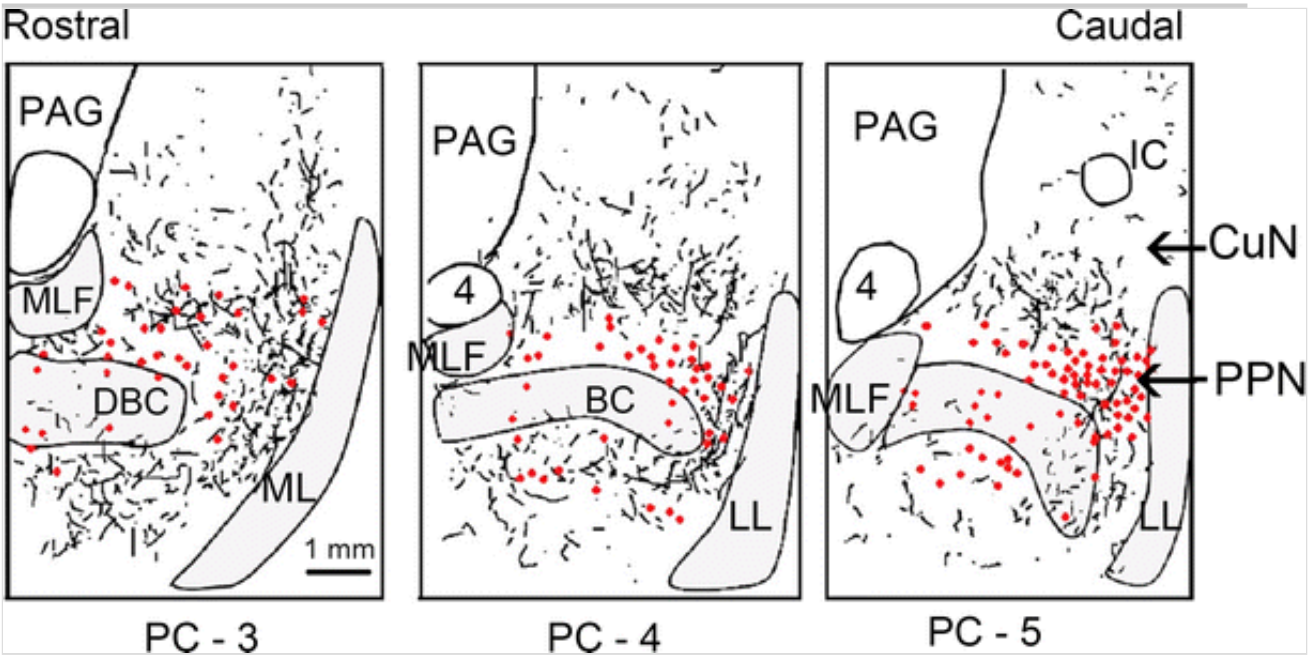


Whereas the internal pallidum principally innervates the PPN, the substantia nigra pars reticulata innervates both the PPN and CuN leading to the conclusion that the internal pallidum and the substantia nigra control different parts of the MLR and can modulate the descending reticulospinal pathway in the primate.

We have also demonstrated that both the PPN and CuN receive dopamine innervation in the macaque (Rolland et al. 2009). The dopaminergic fibers were mainly found in the anterior part of the PPN and tended to avoid cholinergic neurons (Fig. 2). These data suggest that dopamine may have a role in the modulation of MLR neural activity.

Fig. 2

Distribution of dopaminergic fibers (labelled with dopamine transporter) throughout the PPN and CuN in relation to the cell bodies of cholinergic neurons (*red dots*) (labelled with choline transporter) on transverse sections of a macaque. *4* nucleus of the fourth cranial nerve, *BC* brachium conjunctivum, *DBC* decussation of the brachium conjunctivum, *IC* inferior colliculus, *LL* lateral lemniscus, *ml* medial lemniscus, *MLF* medial longitudinal fascicle, *PAG* periaqueductal grey matter.
Figure adapted from Rolland et al. (2009)

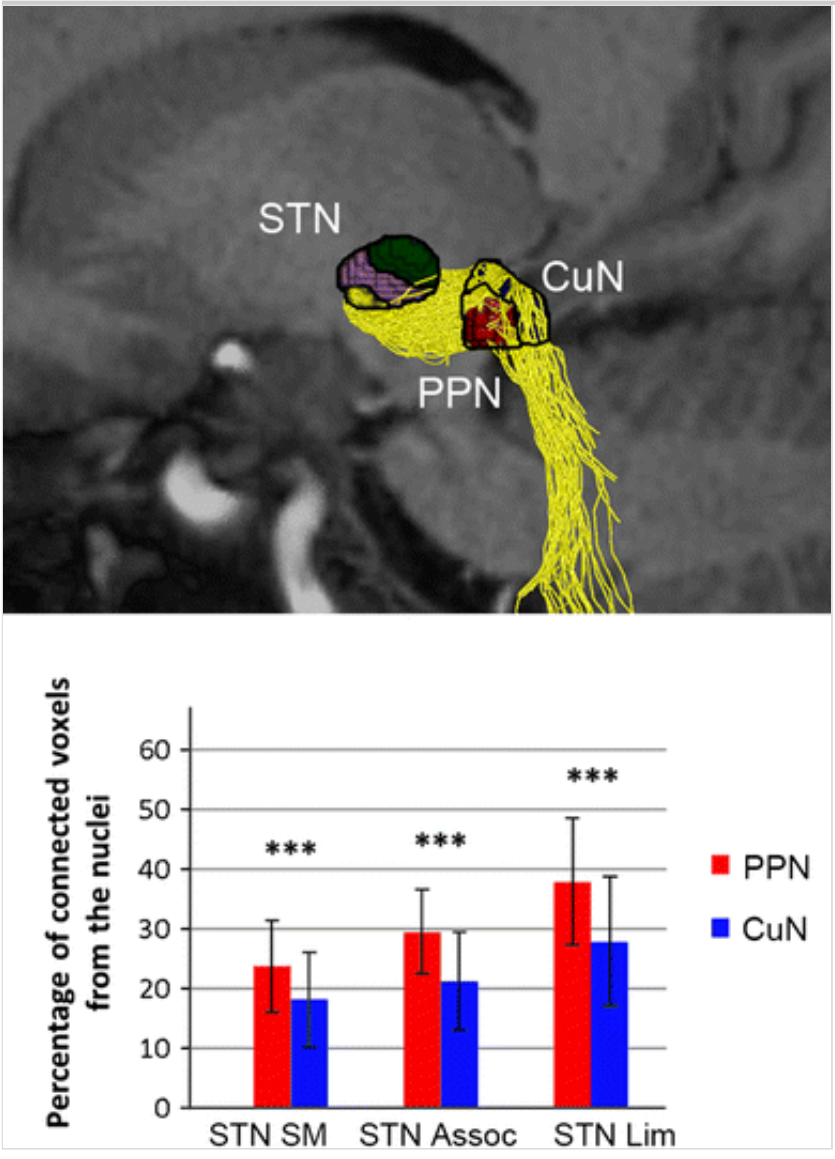


We recently conducted experiments to determine the organisation of both PPN and CuN inputs and outputs in macaque and human with a special focus on the three anatomo-functional sensorimotor, associative and limbic territories of the basal ganglia, and on the thalamus, amygdala, and cortex (Sebille et al. 2016). We found that PPN and CuN connections are generally similar in both species, using tract-tracing methods in monkey, and diffusion-weighted imaging-based methods in healthy human volunteers. The PPN projects to the three anatomo-functional territories of the basal ganglia nuclei, to the centre median-parafascicular complex of the thalamus, and to the central nucleus of the amygdala. The PPN receives from the motor cortical areas, especially in its anterior part, and less strongly from the associative and limbic cortices. The CuN projects to similar cerebral structures as the PPN. However, CuN projections were much stronger to the limbic territories of the basal ganglia and thalamus, and to the basal forebrain (extended amygdala) and the central nucleus of the amygdala. We conclude that the PPN integrates sensorimotor, cognitive, and emotional information, whereas the CuN participates in a more restricted network predominantly integrating emotional information (Karachi et al. 2014; Sebille et al. 2016) (Fig. 3).

Fig. 3

a Sagittal view of fibers (*yellow*) connecting the CuN (which represents the seed) and the limbic part of the subthalamic nucleus (STN) (which represents the target) in a human subject (data provided from the Human

Connectome Project) using diffusion-weighted imaging-based tractography. The sensorimotor, associative, and limbic anatomo-functional territories of the STN are represented in *green*, *pink,* and *yellow*, respectively. In this sagittal view, we have also chosen to illustrate the fiber tract originating from the CuN and descending to the spinal cord to validate the specificity of the reconstructed tracts. Histograms showing percentages of connected voxels from the anatomo-functional territories of the STN to the PPN and CuN, averaged in 30 human subjects. The *error bars* represent standard deviation. Wilcoxon signed-rank test: ***$p < 0.001$.
Figure adapted from Sebille et al. (2016)



Involvement of the PPN in normal locomotor control

The previous experimental studies have shown that a train of electrical stimulation, at 20–60 Hz, within the MLR can produce locomotion in rat,

cat, and monkey decerebrate preparations (Shik et al. 1966; Eidelberg et al. 1981; Garcia-Rill et al. 1987; Garcia-Rill and Skinner 1987). However, a delay is observed between stimulation onset and the induction of the first step, suggesting that the MLR is not a step generator per se and that locomotion is gradually recruited (Garcia-Rill 1991). Notably, increasing current intensity resulted in changes in the frequency of the step cycle, going from a walk to a gallop (Grillner 1981). Moreover, the activity of some PPN neurons was often related to the duration of the stepping episode, while other neurons were shown to be active in relation to left–right alternation (Garcia-Rill and Skinner 1988; Garcia-Rill 1991). The locomotor behaviour obtained following MLR stimulation varied with the location of the stimulating electrode, since stimulation of the CuN in decerebrate cat seemed to elicit locomotor patterns, whereas stimulation of the PPN could change muscle tone (Takakusaki et al. 2003). In the intact cat, stimulation of the PPN area induces complex motor behaviour, the animal exhibiting fast walking and running movements, avoiding collision with obstacles (Mori et al. 1989). In rat, PPN lesions produce motor deficits (MacLaren et al. 2014) if the lesion is restricted to the anterior part of the PPN (Alderson et al. 2008). Moreover, a recent study using an optogenetic approach in mice enabled the identification of distinct locomotor functions of the three neurochemically identified cell types within the MLR showing that PPN glutamatergic neurons, and not cholinergic ones, could provoke locomotion (Roseberry et al. 2016). In summary, while many experimental studies have shown that electrical or pharmacological manipulations of the PPN area can modulate gait on a treadmill in cat and rodent, little is known about the role of these structures in primates.

In monkey, widespread lesion or inactivation of the whole PPN region (by excitotoxic or radiofrequency lesions, local injection of GABAergic agonists, or high-frequency stimulation), induces motor symptoms which have been identified as parkinsonism, since they include akinesia and rigidity (Kojima et al. 1997; MunroDavies et al. 1999; Matsumura and Kojima 2001; Nandi et al. 2002; Pereira et al. 2011). However, dopamine therapy was not tested in these animals, gait and balance were not carefully examined, and it was not known whether these effects are due to degeneration of cholinergic neurons, non-cholinergic neurons, or both.

We thus conducted an initial series of experiments to demonstrate whether

a PPN cholinergic lesion in monkey can induce gait disorders (Karachi et al. 2010). For this study, young macaques (3–5 years old) were trained to walk along a corridor, and various parameters of gait and posture were quantified. Akinesia, rigidity, and tremor were assessed together with the general activity. Stereotaxic injections of diphtheria toxin conjugated with urotensin II specific to cholinergic neurons (Clark et al. 2007) and restricted to the PPN were performed uni and bilaterally. Such injections resulted in a mean reduction of 39% of cholinergic PPN neurons, which is close to the partial PPN neuronal degeneration reported in the PPN of PD patients (Hirsch et al. 1987). Lesioned monkeys developed postural and gait abnormalities. These symptoms were characterised by strong axial rigidity (increase of the back curvature and modification of the tail position), a mild peripheral rigidity (decrease in the knee angle), but without akinesia (normal overall activity) or tremor. If this rigidity looked partially like that obtained following MPTP intoxication, it predominantly involved axial body parts and no cog-wheel rigidity of the limbs was noticed. These postural and gait deficits were not improved by dopaminergic medication, demonstrating that they were unrelated to dopaminergic depletion.

We concluded from these experiments that cholinergic PPN neurons are involved in gait and postural control. However, a small proportion of non-cholinergic PPN neurons was also affected by the toxin and could, therefore, have been involved in the expression of these symptoms.

Involvement of the PPN in parkinsonian locomotor dysfunction

As previously discussed, MPTP intoxication in young macaques induces cardinal parkinsonian symptoms responsive to dopaminergic treatment. However, gait and balance were rarely examined and never described in these animals. Histological analysis and cell counts failed to show any loss of PPN cholinergic neurons (Herrero et al. 1993; Heise et al. 2005). We can conclude, therefore, that this PD model does not produce all the clinical and histological features of advanced PD patients.

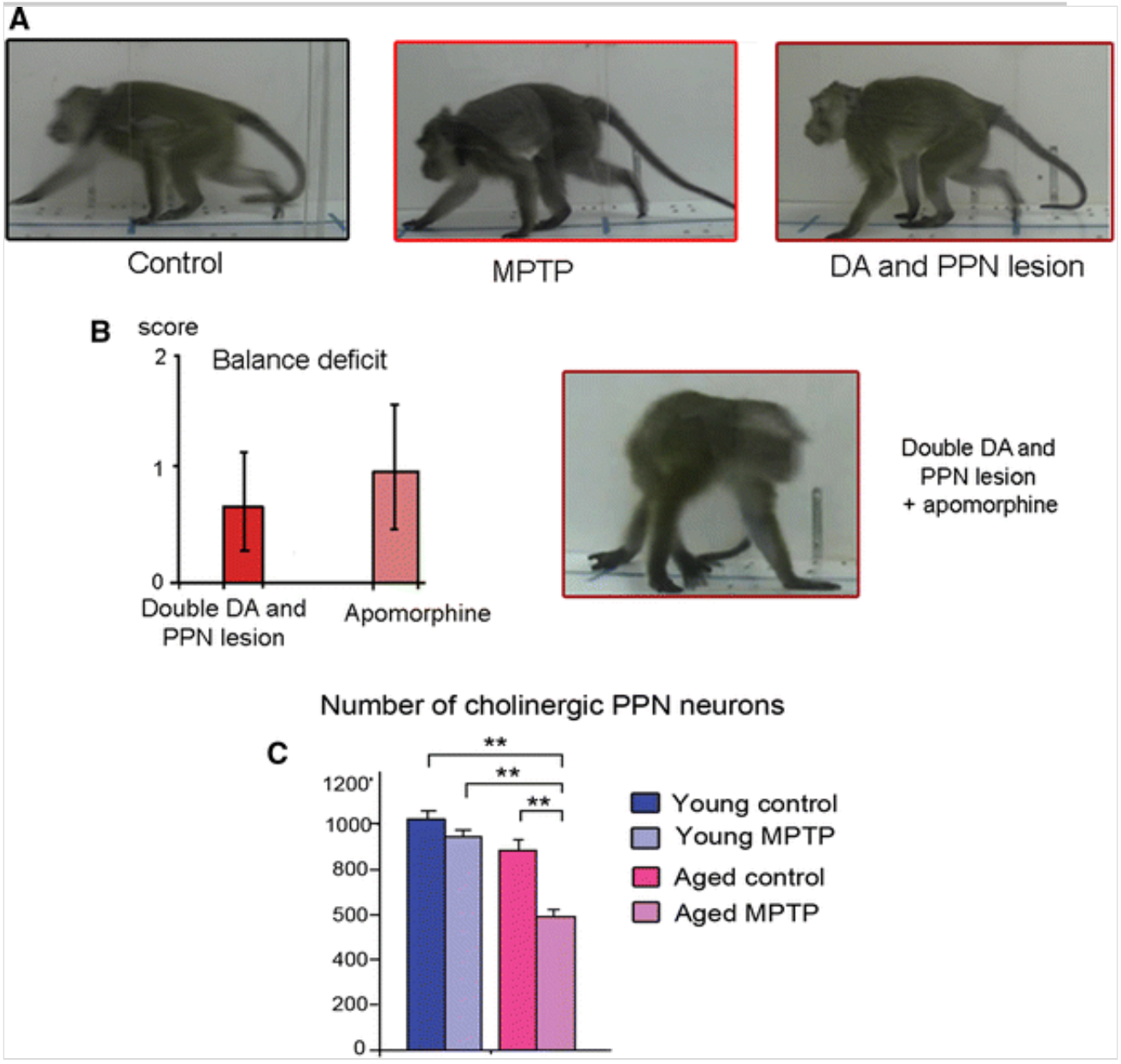
Our aim was thus to develop a new monkey model of advanced PD which displays the cardinal parkinsonian symptoms together with gait and balance disorders with dopaminergic and cholinergic cell loss. Initially, we looked to see whether cholinergic lesions of the PPN in young MPTP-

treated macaques (*Macaca fascicularis*, 3–5 years old) would add gait and balance disorders resistant to dopaminergic drug treatment to the cardinal parkinsonian symptoms (Grabli et al. 2013). Experiments were conducted using similar procedures as those explained above (Karachi et al. 2010). MPTP intoxication was first performed to obtain a mild, stable parkinsonian state. We then performed stereotaxic lesions of the PPN specific to its cholinergic neurons (Karachi et al. 2010). We first observed that adding bilateral partial cholinergic PPN lesions in young MPTP-treated macaque (41% loss of cholinergic PPN neurons) induced a worsening of axial posture (flexed trunk displaced towards the side contralateral to the lesion and erect tail), an increase of knee angle and height of the pelvis, and a weak but persistent balance deficit that induced more falls compared to the parkinsonian state. These parameters did not improve with dopaminergic medication. However, we observed that after PPN lesions, tremor and akinesia improved significantly, with an increase of gait speed but no significant change of step length. Finally, additional MPTP injections gave the clinical association of both a severe DOPA-responsive parkinsonism and a DOPA-unresponsive gait and balance disorders. These complex changes in axial muscle tone induced by PPN lesions may be mediated via its descending input to the reticulospinal tract (Rye et al. 1988; Lai and Siegel 1990; Takakusaki et al. 2003). We also observed that PPN lesions improved tremor and akinesia, with an increase of gait speed but no statistical change of step length compared with parkinsonian state. Such improvement of parkinsonian symptoms could be explained by the reduction of excitatory outputs from the PPN cholinergic neurons to the hyperactive subthalamic nucleus that strongly participates in PD symptom severity.

To reinforce our new monkey model of advanced PD, we performed additional MPTP injections. These dramatically worsened akinesia as well as balance deficits and falls during walking. The symptoms were worsened by dopaminergic treatment, increasing the ipsilateral hypotonia (Fig. 4b), which is consistent with clinical observations of PD patients (Giladi et al. 2001). With these experiments, we were able to obtain robust dopamine-resistant gait and balance disorders associated with the cardinal parkinsonian symptoms in the macaque. This new monkey model of advanced PD will be useful especially for testing new medical or surgical approaches.

Fig. 4

a Photomicrographs of the same animal in control state, after MPTP administration, and then following a PPN lesion (DA and PPN lesion). **b** Development of balance deficit observed after additional doses of MPTP and apomorphine injection. **c** Total number of cholinergic neurons quantified in young and aged MPTP-treated macaques compared with their respective controls ($n = 4$ per group). MPTP did not affect cholinergic neurons in young animals, but induced a 30% loss of cholinergic neurons in aged monkeys. $^{**}p < 0.01$, Mann–Whitney U test.
Figure adapted from Grabli et al. (2013)



In parallel, we also used aged macaques to determine whether balance disorders could be induced using MPTP intoxication only (Karachi et al.

2010; Grabli et al. 2013). We suspected that this could be the case, because aged MPTP-treated macaques have been reported to develop abnormal postures and poor balance (Ovadia et al. 1995). We thus administered MPTP to aged macaques (20–30 years old) of two different species (*Macaca arctoides* and *M. fascicularis*). The macaques developed severe cardinal parkinsonian symptoms that were improved by dopaminergic treatment, but also balance problems during walking with occasional falls that were worsened following dopaminergic medication. Histological examination showed severe dopaminergic neuronal loss in the substantia nigra and a significant partial loss of PPN cholinergic neurons (30% in *M. arctoides* and 22% in *M. fascicularis*) (Fig. 4c). These data strongly suggested a relationship between cholinergic neuronal loss and axial symptoms. MPTP is known to be a selective toxin for the DA neurons. However, the possibility that MPTP finally also kills some PPN cholinergic neurons cannot be ruled out. Indeed, chronically MPTP injections appear to affect non-dopaminergic neurons in the thalamus (Villalba et al. 2014) as well as noradrenergic neurons in the locus coeruleus (Masilamoni et al. 2011). Thus, the cholinergic neuronal loss that we observed in MPTP-treated monkeys is due to both a direct action of MPTP on these neurons as well as the consequences of functional modifications induced by MPTP in basal ganglia activity. However, because PPN cholinergic loss was too low and gait disorders too mild in these aged MPTP-treated monkeys, we considered that these animals could not be used as a model of advanced PD.

Involvement of the PPN in normal and parkinsonian sleep

Many pharmacological and neurophysiological studies have indicated the importance of cholinergic neurons of the PPN and the adjacent dorsolateral tegmental nuclei in the regulation of arousal and REM sleep. Large PPN lesions in cats severely reduce REM sleep with muscle atonia (Webster and Jones 1988; Shouse and Siegel 1992). However, more restricted lesions of the rat PPN have failed to show any alteration in sleep architecture (Deurveilher and Hennevin 2001). It has been shown that pharmacological or electrical stimulation of the PPN in rat or cat is able to elicit REM sleep together with cortical activation and muscle atonia (Jones 1993; Takakusaki et al. 2004). Recently, cholinergic PPN neurons have been shown to discharge maximally during waking and during REM sleep in rat

(Boucetta et al. 2014). Taken together, these experimental data highlight the complex role of the cholinergic PPN neurons in the control of normal sleep, and raise the question of whether this specific cell loss could participate in the emergence of sleep disorders in the context of PD.

Sleep disorders are an important non-motor symptom of PD. They are characterised by excessive daytime sleepiness and nocturnal sleep disorders among which are difficulty in falling and staying asleep with sleep fragmentation (Wailke et al. 2011; Schrempf et al. 2014). Whether these sleep disorders are primarily caused by the PD itself or by the dopaminergic treatment or both is difficult to determine. Moreover, the effects of dopaminergic drugs on sleep are variable, depending on the nature of the pharmacological agent and the doses used. Dopamine agonists have been reported to provoke sleepiness, particularly at high doses, whereas L-dopa seems to have the opposite effect (Bliwise et al. 2012). Cholinergic PPN cell loss may also have a relevant role in the development of sleep disorders in PD, since PPN low-frequency stimulation has been shown to significantly improve sleep efficiency with an increase of REM sleep episodes (Romigi et al. 2008; Alessandro et al. 2010; Arnulf et al. 2010; Peppe et al. 2012). These data suggest that further investigations are necessary to disentangle the roles of dopaminergic cell death, dopaminergic treatment, and PPN degeneration in PD sleep disorders.

The suitability of the macaque model to replicate sleep–wake disturbances in PD patients has been well demonstrated (Barraud et al. 2009). Using a similar macaque model, we performed long-term continuous electro-encephalographic monitoring of vigilance states using an implanted miniaturised telemetry device to examine the sleep–wake parameters. We designed a longitudinal study to record sleep in macaques during different stages: baseline, parkinsonian without and with dopaminergic treatment, and following PPN cholinergic lesions (Belaid et al. 2014). We confirmed that normal macaques slept for 42% of the 24 h, mostly during the night. Sleep includes rapid eye movements (REM), non-REM cycles with light and slow-wave, deep sleep, and waking episodes (Hsieh et al. 2008; Barraud et al. 2009). MPTP-treated macaques displayed a reduction in time spent in REM and slow-wave sleep, and an increased number of nocturnal awakenings. These sleep disorders were similar to those previously reported (Almirall et al. 1999; Barraud et al. 2009; Hyacinthe

et al. 2014) and to those recorded in advanced PD patients (Wailke et al. 2011; Yong et al. 2011). Our MPTP-treated macaques also increased their daytime sleepiness as previously reported (Rye 2010). We concluded that DA lesion alone (severe in our macaques) accounts for the development of sleep disorders, supporting the involvement of the DA system in the regulation of sleep–wake cycle.

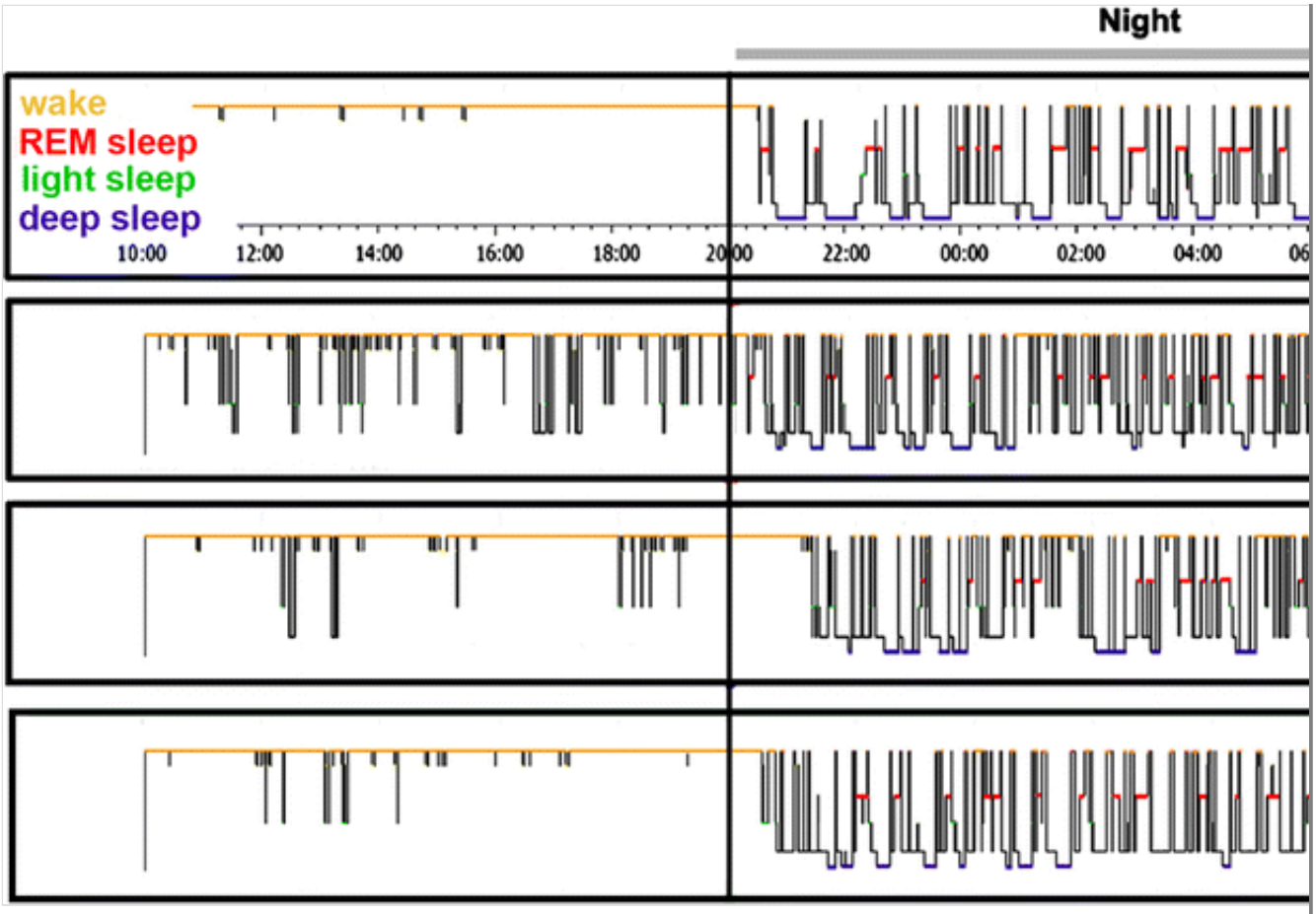
To determine whether dopaminergic treatment can alleviate sleep disorders, we administered L-dopa twice a day for 8–12 days to MPTP-treated macaques. This dopaminergic treatment resulted in a partial but a significant improvement of almost all sleep parameters (reduction of daytime sleepiness and of nocturnal awakenings, sleep pattern benefits). Using this macaque model, it has further been reported that selective D1 receptor agonist administration can improve REM sleep and excessive daytime sleepiness, whereas D2 receptor agonists have no effect (Hyacinthe et al. 2014). These results differ from those reported in PD patients, since it has been shown that dopaminergic treatment has no impact on the altered sleep structure and could even decrease the total sleep period (Wailke et al. 2011). These discrepancies may be explained by the fact that our animals were young and were treated with L-dopa after a 2 week habituation period, whereas L-dopa is administered in middle-aged patients and for years. It is also possible that, compared to MPTP-treated macaques, PD patients have non-dopaminergic lesions which could limit the beneficial effects of the dopaminergic treatment. In conclusion, these experiments demonstrated the efficacy of the dopaminergic treatment in improving sleep disorders in MPTP-treated macaque.

To determine the role of the PPN on sleep disorders, and in particular whether PPN lesions could reduce REM sleep in MPTP-treated macaques, we added a specific cholinergic PPN lesion to the dopaminergic cell loss in the same animal (Belaid et al. 2014). This partial cholinergic PPN lesion induced transient acute sleep disturbances characterised by an increase in sleep fragmentation and a decrease in sleep efficiency and REM sleep. These results confirm the role of cholinergic PPN neurons in REM sleep as proposed by Lu et al. (2006). We also observed the occurrence of high-amplitude slow-wave sleep during daytime, which resembles that described following lesions of the reticular formation in cat (Denoyer et al. 1991) or its pharmacological manipulation in rabbit (Longo 1956). These changes may be explained by the fact that the cholinergic PPN neurons are no

longer able to activate the thalamo-cortical pathway, inducing a suppression of the transition from slow-wave sleep to either arousal or REM sleep (Steriade et al. 1991). However, all the changes we observed were only transient. Surprisingly, 3 weeks after the PPN lesion, monkeys slept slightly better than after MPTP intoxication, with an increase in sleep efficiency and a decrease of nocturnal awakenings (Fig. 5). Several hypotheses could be advanced to explain this benefit. First, compensatory processes could occur and could have masked the acute effects of the PPN lesions. These compensatory processes may involve the remaining PPN cholinergic neurons or other modulatory neurons from systems that are known to be involved in sleep–wake control, such as the adjacent dorsolateral tegmental nucleus, the locus coeruleus, or the raphe nucleus. Second, the cholinergic PPN lesions could be responsible for a reduction of PPN excitatory inputs to the subthalamic nucleus, which is overactive in PD, leading to an improvement of PD motor symptoms and then sleep. This fits well with the reversal of subthalamic hyperactivity reported in a 6-hydroxydopamine rat model after a PPN lesion (Breit et al. 2006). This might suggest that the improvement of sleep quality after a PPN lesion is, at least in part, a consequence of a reduction in night-time akinesia and painfulness.

Fig. 5

Examples of hypnograms of 24 h recordings in the same macaque at baseline (CTL), after MPTP intoxication, after L-dopa administration, and 3 weeks after subsequent PPN lesion.
Figure published in Belaid et al. (2014)



In summary, the combination of dopaminergic and cholinergic PPN lesions allowed us to characterise sleep disorders in a monkey model of advanced PD. We concluded that a partial cholinergic PPN lesion in MPTP-treated macaque is not sufficient to induce consistent additional sleep disorders compared to the parkinsonian state.

Conclusion

From our primate research, we proposed a new monkey model of advanced PD that displays the cardinal parkinsonian symptoms together with gait and balance disorders, and dopaminergic and cholinergic cell loss. However, we are fully aware of several limitations to using a monkey model of PD. In particular, the regular quadrupedal locomotion of macaques cannot be directly compared to the bipedal gait in humans. In that sense, a macaque model of bipedal locomotion has been developed (Goetz et al. 2012) and has been further used as a pre-clinical tool to study gait parameters and their neuronal control after MPTP intoxication (Goetz et al. 2016). However, it should be noted that the bipedal locomotion of macaque, observed over a short distance in its natural environment (Hemmi and Menzel 1995), is obtained under restrained experimental conditions by inducing postural changes that cannot be directly compared

with natural monkey or human gait. Our monkey model that used natural quadrupedal locomotion also cannot be directly compared to bipedal gait in humans. These two approaches are complementary and both are potentially useful in developing monkey models of gait disorders, especially with a pretherapeutic aim.

The pre-clinical studies using monkeys reported here have been made possible by a collaborative interdisciplinary clinical and experimental approach. We believe that this work represents an important step in the validation of the safety and efficacy of new therapeutic procedures prior to their transfer to larger scale clinical trials.

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Compliance with ethical standards

Conflict of interest The authors declare no other conflicts of interest.

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