Journal of Neurology (2019) 266:2244–2251
https://doi.org/10.1007/s00415-019-09396-9

ORIGINAL COMMUNICATION

Neuronal spiking in the pedunculopontine nucleus in progressive supranuclear palsy and in idiopathic Parkinson's disease

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Received: 28 March 2019 / Revised: 20 May 2019 / Accepted: 22 May 2019 / Published online: 3 June 2019

Abstract

The pedunculopontine nucleus (PPN) is engaged in posture and gait control, and neuronal degeneration in the PPN has been associated with Parkinsonian disorders. Clinical outcomes of deep brain stimulation of the PPN in idiopathic Parkinson's disease (IPD) and progressive supranuclear palsy (PSP) differ, and we investigated whether the PPN is differentially affected in these conditions. We had the rare opportunity to record continuous electrophysiological data intraoperatively in 30 s blocks from single microelectrode contacts implanted in the PPN in six PSP patients and three IPD patients during rest, passive movement, and active movement. Neuronal spikes were sorted according to shape using a wavelet-based clustering approach to enable comparisons between individual neuronal firing rates in the two disease states. The action potential widths showed a bimodal distribution consistent with previous findings, suggesting spikes from noncholinergic (likely glutamatergic) and cholinergic neurons. A higher PPN spiking rate of narrow action potentials was observed in the PSP than in the IPD patients when pooled across all three conditions (Wilcoxon rank sum test: $p = 0.0141$). No correlation was found between firing rate and disease severity or duration. The firing rates were higher during passive movement than rest and active movement in both groups, but the differences between conditions were not significant. PSP and IPD are believed to represent distinct disease processes, and our findings that the neuronal firing rates differ according to disease state support the proposal that pathological processes directly involving the PPN may be more pronounced in PSP than IPD.

Keywords Pedunculopontine nucleus · Progressive supranuclear palsy · Idiopathic Parkinson's disease · Microelectrode recordings · Deep brain stimulation

Introduction

The pedunculopontine nucleus (PPN) is located in the brainstem and is engaged in posture and gait control [1]. Neuronal degeneration of the PPN has been identified in patients with progressive supranuclear palsy (PSP) and idiopathic Parkinson's disease (IPD) [2, 3], and the PPN has become a potential target for deep brain stimulation (DBS) in both conditions [4–8]. Although low-frequency PPN-DBS has been shown to improve posture and gait in IPD [9–11], the clinical outcomes of PPN-DBS in PSP patients differ [12, 13], and it is not clear whether the PPN is differentially affected in these two conditions. Here, we compared the neuronal firing rate in the PPN in patients with PSP with that in patients diagnosed with IPD.

PPN activity has been shown in animal studies to alter in response both to initiation and to modulation of movement [14]. Patient recordings have revealed distinct neuronal populations in which firing differs between rest, passive,

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and active movement and also neuronal populations that respond to movement onset and offset [6]. Differences in action potential width have been associated with the firing of particular neuronal types, with narrow action potentials being in the majority and thought to come from noncholinergic neurons, and wide action potentials being less common and originating from cholinergic neuronal populations [6]. On the basis of these findings, we aimed to identify and compare noncholinergic firing between patients with PSP and IPD. We investigated neuronal firing intraoperatively in the PPN at rest and during passive and active movement. The data were recorded in continuous blocks, to enable a direct comparison between ongoing PPN firing in the different disease states under the different conditions, without the overall changes in firing rate that have already been reported in particular neuronal populations that fire at movement onset and offset.

Methods

Patients

Six patients with a diagnosis of PSP and three patients with IPD were included in this study. Clinical diagnosis of PSP was determined according to National Institute for Neurological Disorders and Stroke (NINDS) clinical criteria [15], and IPD diagnosis was based on the clinical diagnostic criteria for PD from the UK Parkinson's Disease Society Brain Bank [16]. Clinical evaluation was performed using the Unified Parkinson's Disease Rating Scale (UPDRS) [17]. Patient clinical characteristics are provided in Table 1. The study was approved by the Local Ethics Committee of the Otto-von-Guericke University, Magdeburg (protocol no. 38/09), and all participants provided informed, written consent.

Electrode localization

Stereotactic target coordinates were determined pre-operatively based on structural magnetic resonance imaging and confirmed intraoperatively using stereotactic X-rays. The surgical procedure has been reported in detail elsewhere [13]. Electrode localisation is illustrated for patient 1, which is representative of PPN targeting in this cohort (Fig. 1).

Electrophysiological data recordings

We recorded spontaneous neuronal firing activity intraoperatively in continuous 30 s blocks from single microelectrode contacts of a micro–macro electrode (30-µm tip length, axes 800 µm apart, about 1.0 MΩ impedance; Inomed, Medizintechnik GmbH, Emmendingen, Germany), implanted in the PPN in all nine patients, during rest, passive movement, and active movement, with both movement conditions involving bilateral alternating ankle flexion and extension, whereby the left ankle was extended when the right ankle was flexed and vice versa. One to three 30 s blocks were recorded from each PPN for each condition, resulting in 24 blocks from PSP and 9 blocks from IPD patients during rest, 15 blocks from PSP and 5 blocks from IPD patients during passive movement, and 15 blocks from PSP and 5 blocks from IPD patients during active movement. The movement was self-paced by the patients in the active condition and by one of the authors (IG) in the passive condition, with a target rate of 2 s per movement. Recording took place during the electrophysiological mapping procedure used to obtain physiological data for localizing the target for the DBS electrode placement (for further details of the procedure see: [18]). The data analyzed here were recorded from the site determined to be the PPN during mapping, at which DBS was subsequently delivered. All recordings were filtered at 3–5000 Hz, with a sampling rate of 25 kHz, using the ISIS MER System

Table 1 Clinical information

Patient	Diagnosis	Side of recording	Age (years)	Gender	Disease duration (years)	L-Dopa response (%)	Midbrain diameter (mm)	UPDRS III, off/on
1	PSP-RS	L, R	68	M	6	0	13.6	30/30
2	PSP-P	L, R	68	F	5	25	14.5	24/18
3	PSP-RS	L	67	F	7	5.7	14	35/33
4	PSP-RS	L, R	68	F	7	15	11.9	20/17
5	PSP-PAGF	L, R	72	F	6	9	15.1	11/10
6	PSP-PAGF	L, R	71	M	6	0	14.5	28/28
7	IPD	L, R	65	M	6	31.2	18.2	32/22
8	IPD	L, R	66	F	5	29.7	14.6	64/45
9	IPD	L, R	50	F	14	50	18.9	42/21

IPD idiopathic Parkinson's disease, *PSP-P* PSP-parkinsonism, *PSP-PAGF* PSP-pure akinesia with gait freezing, *PSP-RS* PSP-Richardson syndrome, *UPDRS* Unified Parkinson's Disease Rating Scale

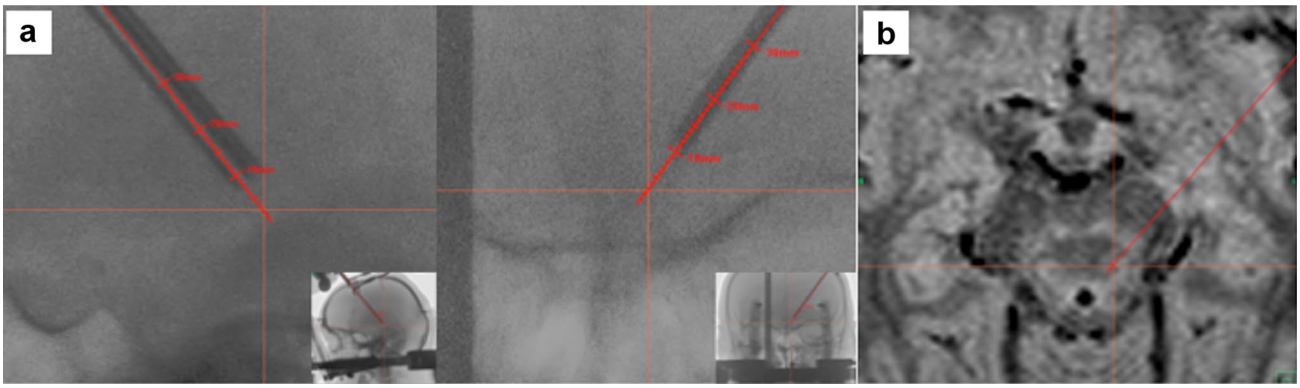

Fig. 1 Illustration of the localisation of the recording site in the left PPN in patient 1. **a** Stereotactic X-ray, lateral and a.p. view: the trajectory of the electrode probe is located 8 mm above the prescribed target (=start of microelectrode recording). Burst activity became visible over a distance from 4 to 0.5 mm above the target point. **b** Preoperative structural MRI, transverse view: projection of the calculated trajectory. The red cross indicates the site from which the microelectrode data were recorded

(Inomed, Medizintechnik GmbH, Emmendingen, Germany), equipped with the ISIS MER software (version 3.2).

Spike clustering

The electrophysiological signals recorded from microelectrodes comprise the firing of multiple neurons. To study the firing rates of particular neurons, it is essential to establish which action potentials are produced by a given neuronal discharging unit. The activity produced by individual neurons can be identified according to the shape of the spikes of electrical activity recorded from them. To define the shape of an action potential, distinctive features are extracted, and these features are then used to cluster spikes with similar shape [19]. We applied a wavelet-based clustering algorithm using the freely available wave_clus toolbox, implemented in Matlab [19]. First, the data were filtered from 300–3000 Hz using a Butterworth filter, and then an amplitude threshold was applied to detect spikes as activity exceeding this threshold. The clustering then proceeded as follows. A set of wavelet coefficients was chosen from each spike, with which to define its shape at different scales and times. These sets of wavelet coefficients were subsequently used as features for clustering using a superparamagnetic clustering algorithm, whose nomenclature is derived from an analogy with magnetic systems. The coefficients selected as features for classification were those that maximally separated the clusters. m selected features were represented in an m-dimensional phase space for clustering. Interactions were simulated between each data point, or feature, and its K nearest neighbours in an iterative process resulting in clusters of spikes with similar shape. The process is described in detail by Quiroga et al. [19]. Two parameters may be set to improve classification of spikes based on visual inspection of the resulting classification: the minimum cluster size and the probability of changing the state, or cluster allocation, of neighbouring data points together, which is referred to as the 'temperature'. At a low temperature, smaller numbers of clusters result, and at a high temperature, spikes are likely to be separated into more clusters. Visual inspection of the shapes of the raw spikes, each plotted together with the cluster to which it has been assigned, allowed evaluation of the appropriateness of the chosen parameters.

To determine the total number of neurons from which spikes were recorded from each PPN, the data from the different recording conditions for each individual patient were concatenated, and clustering was performed again. A particular cluster shape (and hence neuronal discharging unit) could thus be identified across recording conditions.

The PPN comprises different neuronal populations, which may be characterized by the width of the action potentials they produce [6, 20, 21]. We measured the duration of the negative phase of the mean action potential for a given cluster and examined the distribution of spike durations, following the approach taken by Weinberger et al. [6]. Comparisons were then made between individual neuronal firing rates for the neurons thus characterized in the two disease states using a Wilcoxon rank sum test. A non-parametric test was used, as the data were not normally distributed according to the Kolmogorov–Smirnov test. Unpaired T tests were used for the comparisons between firing rates during separate movement conditions, because the small sample sizes did not permit estimation of normality, and simulation has demonstrated the validity of T tests with low sample numbers, which were defined as $N<6$ [22]. Correlation between firing rates and clinical scores was evaluated by calculating Pearson's r.

Finally, we performed an exploratory evaluation of laterality in PPN neuronal firing rates. Where more than one firing rate was available from a PPN on at least one side,

we compared narrow and wide action potential firing rates between left and right PPNs for all patients pooled together and separately according to diagnosis using unpaired *T* tests. The tests were performed both pooling across all three movement conditions and for each movement condition separately. Where firing rates were available for individual patients from both sides in the same movement condition, we also calculated a laterality index (L − R/L + R) and evaluated correlation between laterality and disease duration, baseline UPDRS scores, responsiveness to L-dopa, and midbrain diameter. We compared baseline UPDRS scores between impact on the left and right sides of the body using a paired *T* test and also calculated a laterality index for these scores. We then evaluated correlation between these clinical laterality scores and firing rates as well as a firing rate laterality index. Finally, we examined correlation between the gait freezing sub-score of the UPDRS and firing rate.

Results

Data were recorded from the PPN from six patients with PSP and three patients with IPD. Disease duration did not differ between the groups [PSP: 6.2 years (std 0.8); IPD: 8.3 years (std 4.9); *T* test: $T = 1.13$, $p = 0.30$)]. The mean UPDRS score reflected lower disease severity in the PSP group (24.7, std 8.4) than in the IPD group (46.0, std 16.4) (*T* test: $T = 2.67$, $p = 0.032$).

All but one PSP patient had bilateral recordings, resulting in data from 17 PPN regions. The neuronal spikes were clustered according to their shape, with action potentials with similar shape considered as coming from a single neuronal discharging unit (Fig. 2). Neuronal clusters were identified from 11 PPN sites from PSP patients and 6 PPN sites from IPD patients. Based on the clustering, the recorded spiking was deemed to stem from one to three discharging units in each patient. One IPD patient had clusters identified during sedation (with remifentanil), but no clusters were seen following sedation in the separate recording conditions. No clusters were identified in one patient with PSP, who presented with a distinct microlesion effect post-operatively, with a buccofacial apraxia persisting for several weeks. The number of units from which spikes were recorded is likely to be a function of the precise electrode location and did not differ systematically according to clinical diagnosis or recording condition. Data were recorded from a total of 16 neuronal units in the PSP patients and from 7 neuronal units in the IPD patients.

The neuronal firing rates across both patient groups, and also within the PSP and IPD patient groups separately, showed a bimodal distribution with a cutoff width at around 0.7 ms (Fig. 3). The majority of neurons (67% of the total: 76% in PSP patients; 63% in IPD patients) had narrow action potentials.

Comparing the spiking rates of the PPN neurons with a narrow action potential width, pooled across the rest, passive movement, and active movement conditions, a higher firing frequency was observed in the patients with PSP than in those with IPD (Wilcoxon rank sum test: $p = 0.0141$) (Fig. 4). The rate was higher in the PSP group in each

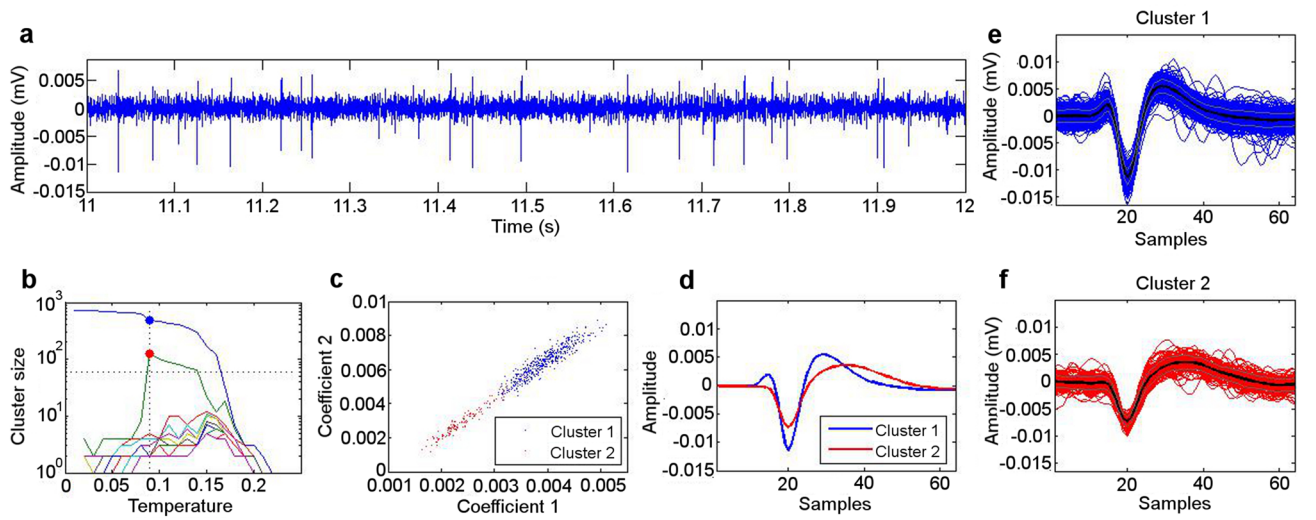

Fig. 2 Illustration of the wavelet-based clustering algorithm used to sort neuronal spikes, applied to electrophysiological data recorded from the PPN of patient 1 during rest. **a** Raw electrophysiological data. **b** Cluster size vs. temperature. The blue and red dots correspond with the selected clusters. **c** Projection of wavelet coefficients for spikes in which clustering is visible. **d** Mean of spikes comprising each cluster, with the peak aligned to data point 20 of the 64 data points taken for each spike. **e** Individual spikes allocated to cluster 1, with the mean in black. **f** As in **e** for cluster 2

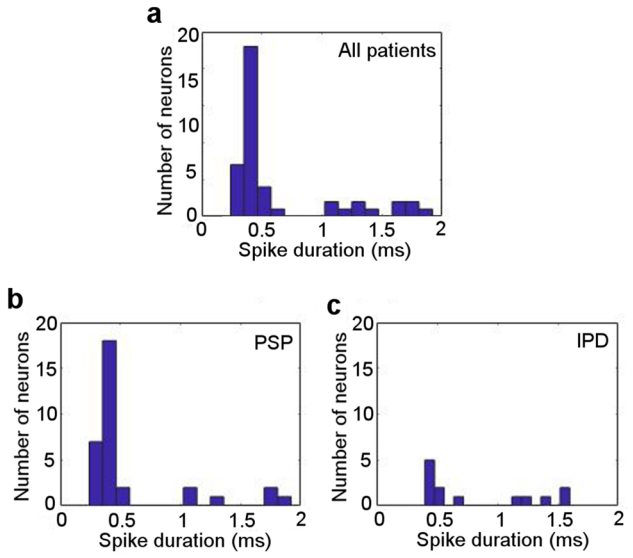

Fig. 3 Bimodal distribution of action potential widths. **a** Including all patients. **b** PSP patients. **c** IPD patients

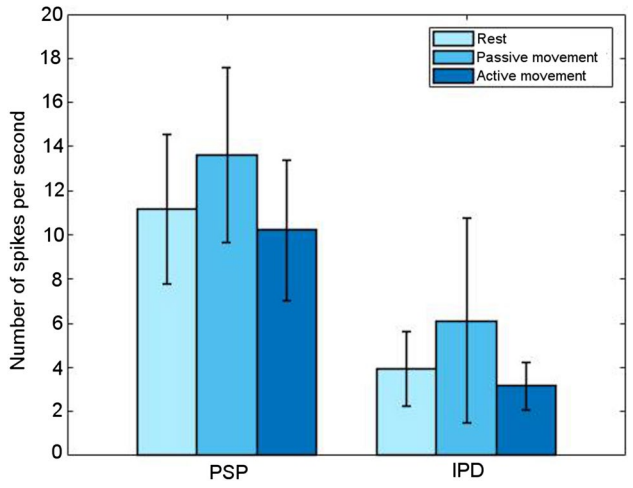

Fig. 4 Mean neuronal spiking rates from neurons firing narrow action potentials (error bars = standard error of the mean)

condition separately, but the inter-group difference did not reach significance. In each patient group, the firing rate was highest during passive movement, with similar firing rates at rest and during active movement. The number of PPN neurons firing with wide action potentials was smaller, with only a single firing rate available for passive and active movement conditions in patients with IPD. In the resting condition, the firing rate with wide action potentials was significantly higher in patients with PSP than in patients with IPD (*T* test: $T=5.12$, $p=0.0144$).

The spiking rate of the neurons producing narrow action potentials was higher than that of neurons yielding wide action potentials, in each of the three conditions and also pooled over conditions, as well as pooling all patients, or taking only the PSP group. The narrow action potential firing rate did not correlate with the UPDRS score on the basis of individual patient mean firing rates across all neuronal discharge units bilaterally ($r=-0.52$, $p=0.29$) and nor did the wide action potential firing rate ($r=-0.30$, $p=0.70$). Moreover, when comparing the UPDRS scores between the PSP and IPD groups only including the patients from whom narrow action potentials were identified, the clinical scores did not differ significantly between the groups (*T* test: $T=-2.34$, $p=0.079$), nor did they differ significantly between the groups including only the patients from whom wide action potentials were recorded ($T=-1.57$, $p=0.26$). No correlation was found between narrow action potential firing rates pooled or in any individual movement condition or wide action potential firing rates pooled or during rest (only measurements from single neuronal discharging units were available for wide action potentials during passive and active movement), and disease duration, baseline UPDRS, responsiveness to L-dopa treatment, or midbrain diameter measured using magnetic resonance imaging [23].

Pooling data from patients with PSP and IPD across all three movement conditions, there was no difference between the narrow ($T=0.36$, $p=0.72$) or wide ($T=0.47$, $p=0.65$) action potential firing rates from left compared with right PPNs. The findings were not altered when examining the firing rates from patients with PSP or IPD separately. The narrow action potential firing rates also did not differ between left and right PPN during the resting condition separately, pooled across patient groups ($T=0.44$, $p=0.67$) or separately for patients with PSP ($T=0.46$, $p=0.66$) and IPD ($T=0.25$, $p=0.83$). The wide action potential firing rates at rest could only be compared pooling across disease states, as none were measured on the right in the PSP group, and no difference was detected ($T=0.49$, $p=0.66$). During passive movement, pooling across disease state, the narrow action potential firing rate was higher from the right than the left PPN ($T=-2.69$, $p=0.036$). Including only patients with PSP, there was a trend in the same direction ($T=-2.33$, $p=0.08$). No wide action potential firing rates could be measured in the IPD group on the right during passive movement, so no comparison could be made. During active movement, the firing rates did not differ between sides, either pooling across patient groups ($T=0.94$, $p=0.37$) or for the PSP patients separately ($T=1.20$, $p=0.26$). No narrow action potentials were detected on the right in the IPD group, so no comparison could be made. Wide action potential firing rates could only be compared by pooling across conditions, as none were recorded on the left in the IPD group, and no difference was seen ($T=-0.52$, $p=0.69$). Laterality indices could be calculated for five patients for narrow action potentials during rest and for one of these patients also during active movement. No significant correlation was

detected between laterality index and either disease duration, baseline UPDRS score, the laterality index of the baseline UPDRS score, responsiveness to L-dopa, or midbrain diameter, whether or not the unit firing during active movement was included. The narrow action potential firing rate during passive movement, which was higher on the right than on the left, also did not correlate with the laterality index of the UPDRS scores or with the UPDRS score for the contralateral side. The UPDRS score was greater on the left (mean 7.67, SD 3.50) than the right (mean 6.5, SD 3.62), including all patients for whom a firing rate laterality index could be calculated, but the difference was not significant ($T = 1.47$, $p = 0.20$). This difference was also not significant when including only the patients with PSP. The gait freezing sub-score of the UPDRS showed no correlation with the laterality of the firing rate during passive movement ($r = 0.42$, $p = 0.34$).

Discussion

Our findings that PPN neuronal firing rates were higher in PSP than IPD patients are consistent with the proposal that pathological processes directly involving the PPN could differ between PSP and IPD. Indeed, PSP and IPD are believed to represent partly distinct disease processes [24]. Despite shared clinical features, PSP tends to involve faster disease progression, and more severe axial motor symptoms, i.e., gait abnormality, compared with IPD after similar disease duration [25], as well as greater PPN neuronal loss, as indicated by histological findings [2, 3] and also suggested by recording from fewer neuronal units in two PSP patients compared with an IPD cohort [6].

The distribution of the numbers of neurons firing according to the width of their action potentials was strikingly similar in shape to that observed by Weinberger et al. [6], with a similar division between neurons firing narrow and wide APs at around 0.7 ms here, compared with 0.65 ms in the study by Weinberger et al. [6]. The distribution found in data recorded from rat PPN was notably more strongly bimodal, with a division at around 1.5 ms [21]. Similarly to Weinberger et al. [6], we observed a lower firing rate in the neurons producing wider action potentials, as well as a lower number of neurons with wide action potentials. These latter neurons are thought to be cholinergic [21, 26].

In addition to cholinergic neuronal populations, glutamatergic neurons have also been identified in the PPN, and the neurons firing narrow action potentials are likely to be glutamatergic. We observed a higher firing rate in PSP compared to IPD. The basal ganglia [substantia nigra (SN), internal globus pallidus, and subthalamic nucleus (STN)] project to the PPN, which sends projections to the SN pars compacta and the STN, suggesting a potential role for the PPN in modulating basal ganglia output [4, 6]. Specifically, direct glutamatergic input from PPN neurons to SN dopaminergic neurons [27–30] and to the STN [31] have been identified, which is deemed to modulate information flow via an indirect pathway, together with cholinergic and GABAergic projections, through the basal ganglia via pallido-subthalamic projections [31]. The higher presumed glutamatergic firing rate in the PPN in PSP patients could reflect altered basal ganglia input to the PPN or direct PPN pathology, given the midbrain atrophy seen in PSP [32], with an impact on movement modulation resulting from the alteration in glutamatergic feedback from the PPN back to the basal ganglia.

The PPN has been proposed to play a role both in movement initiation and modulation [14]. In the current study, we focused on ongoing firing within a particular condition, as opposed to movement onset and offset, identifying a higher firing rate in PPN neurons likely to be glutamatergic, in patients with PSP compared with those with IPD. We tentatively suggest, therefore, that an alteration in glutamatergic firing of PPN neurons has an impact on movement modulation. While the firing rate did not differ significantly between conditions, it is noteworthy that the pattern observed was the same in both patient groups, with the highest firing rate occurring during passive movement and a slightly lower firing rate during active movement than rest.

Increased neuronal firing rates have been observed in the PPN following lesioning of the SN compacta (SNc) to induce a Parkinsonian state in rats [33, 34]. These findings were interpreted as possibly reflecting compensatory mechanisms or an altered balance between excitation and inhibition [34]. SNc pathology is seen both in IPD and PSP [35]. Weinberger et al. [6] did not observe a difference in their findings between patients with PSP and IPD, other than a lower number of neurons in two PSP patients compared to five patients with IPD, which could be consistent with greater PPN degeneration in the PSP group. They do not report mean firing rates or disease duration separately for the two patient groups, but the comparison between the disease duration (mean 12.4 ± 5.0 years) of the patients studied by Weinberger et al. [6] and the PSP cohort presented here reveals that our patients had a much shorter disease process (mean 6.0 ± 0.7 years). The clinical scores in the current cohort did not in fact correlate with the PPN neuronal firing rates, however. We note also that although the current PSP patients were less severely clinically affected than the IPD patients, the PPN firing rates were higher. Therefore, in addition to SNc pathology, the increased neuronal firing rate seen here in the PPN of PSP patients after a relative short disease duration could also reflect local pathology in the PPN.

We observed a higher narrow action potential firing rate in the right than the left PPN, pooling across disease states, during passive movement. It is possible that we

only observed laterality during passive movement, because the higher firing rate seen in this condition resulted in more reliable rate estimations in our small sample. Previous work has shown an association between reduced fibre tracts in the right PPN network and gait freezing [36]. The authors described the loss of fibres as being specific to nodes of an inhibitory network in the right hemisphere. Our finding of a higher firing rate on the right fits with the suggestion that an increased PPN firing rate is pathological. We note, however, that we observed no correlation between the gait freezing sub-score of the UPDRS and laterality of firing rate in our cohort. Moreover, no laterality was observed in an electrophysiological study of the PPN, in which the authors considered the small patient numbers to be a potential factor in the negative finding [37]. As our analysis was exploratory, we did not perform a correction for multiple comparisons. Repetition of our findings in further patients would be required, before conclusions can be drawn.

An important limitation of the current study is indeed the small number of patients. Although we note that the cohort presented is relatively large, given that PPN-DBS is not currently a common treatment for PSP and IPD, our findings should be considered preliminary. In particular, the absence of correlation between firing rates and clinical features could be due to the small sample sizes, especially with reference to the wide action potential firing rates.

Conclusions

In summary, we identified higher neuronal firing rates in extracellular recordings from the PPN of patients with PSP than from patients with IPD. Different reasons for altered PPN neuronal firing have been considered in Parkinsonian disorders, including altered input from the SN, changed modulation of PPN activity through altered neuronal firing in the STN, and local neurodegeneration in the PPN itself. Higher PPN firing rates have been observed in patients with IPD at later disease stages and might, therefore, be an expression of faster disease progression in PSP and higher degeneration in the PPN itself.

Author contributions IG: conceived of and designed the study and recorded the electrophysiological data; JV: surgically implanted the electrodes; IG, JK, and JV: localized the electrodes using imaging; CMSR: designed and performed the electrophysiological data analysis; IG and CMSR interpreted the data analysis, drafted the manuscript, and revised it critically; JK, JV, HH, and HJH: critically revised the manuscript. All the authors approved the final version of the manuscript.

Funding None.

Compliance with ethical standards

Conflicts of interest The authors declare that they have no conflict of interest.

Ethical standards The study was approved by the Local Ethics Committee of the Otto-von-Guericke University, Magdeburg, Germany and was performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. All participants gave their informed written consent prior to their inclusion in the study.

References

1. Mori F, Okada K, Nomura T, Kobayashi Y (2016) The pedunculopontine tegmental nucleus as a motor and cognitive interface between the cerebellum and basal ganglia. Front Neuroanat 10:1–8. https://doi.org/10.3389/fnana.2016.00109
2. Hirsch EC, Graybielt ANNM, Duyckaertst C, Javoy-agid F (1987) Neuronal loss in the pedunculopontine tegmental nucleus in Parkinson disease and in progressive supranuclear palsy. Proc Natl Acad Sci USA 84:5976–5980
3. Jellinger K (1988) The pedunculopontine nucleus in Parkinson's disease, progressive supranuclear palsy and Alzheimer's disease. J Neurol Neurosurg Psychiatry 51:540–543
4. Pahapill PA, Lozano AM (2000) The pedunculopontine nucleus and Parkinson's disease. Brain 123:1767–1783
5. Zrinzo L, Zrinzo L, Hariz M (2007) The peripeduncular nucleus: a novel target for deep brain stimulation? NeuroReport 8:1631–1633
6. Weinberger M, Hamani C, Hutchison WD et al (2008) Pedunculopontine nucleus microelectrode recordings in movement disorder patients. Exp Brain Res 188:165–174. https://doi.org/10.1007/s00221-008-1349-1
7. Pötter-Nerger M, Volkmann J (2013) Deep brain stimulation for gait and postural symptoms in Parkinson's disease. Mov Disord 28:1609–1615. https://doi.org/10.1002/mds.25677
8. Morita H, Hass CJ, Moro E et al (2014) Pedunculopontine nucleus stimulation: where are we now and what needs to be done to move the field forward? Front Neurol. https://doi.org/10.3389/fneur.2014.00243
9. Thevathasan W, Cole MH, Graepel CL et al (2012) A spatiotemporal analysis of gait freezing and the impact of pedunculopontine nucleus stimulation. Brain 135:1446–1454. https://doi.org/10.1093/brain/aws039
10. Mazzone P, Lozano A, Stanzione P et al (2005) Implantation of human pedunculopontine nucleus: a safe and clinically relevant target in Parkinson's disease. NeuroReport 16:1877–1881. https://doi.org/10.1097/01.wnr.0000187629.38010.12
11. Moro E, Hamani C, Poon YY et al (2010) Unilateral pedunculopontine stimulation improves falls in Parkinson's disease. Brain 133:215–224. https://doi.org/10.1093/brain/awp261
12. Scelzo E, Lozano AM, Hamani C et al (2017) Peduncolopontine nucleus stimulation in progressive supranuclear palsy: a randomised trial. J Neurol Neurosurg Psychiatry 88:613–616. https://doi.org/10.1136/jnnp-2016-315192
13. Galazky I, Kaufmann J, Lorenzl S et al (2018) Deep brain stimulation of the pedunculopontine nucleus for treatment of gait and balance disorder in progressive supranuclear palsy: effects of frequency modulations and clinical outcome. Park Relat Disord 50:81–86. https://doi.org/10.1016/j.parkreldis.2018.02.027
14. Matsumura M (2005) The pedunculopontine tegmental nucleus and experimental parkinsonism. J Neurol 252:5–12. https://doi.org/10.1007/s00415-005-4003-x

15. Litvan I, Agid Y, Jankovic J et al (1996) Accuracy of clinical criteria for the diagnosis of progressive supranuclear palsy (Steele–Richardson–Olszewski syndrome). Neurology 46:922–930
16. Hughes A, Daniel S, Kilford L, Lees A (1992) Accuracy of clinical diagnosis of idiopathic Parkinson's disease: a clinico-pathological study of 100 cases. J Neurol Neurosurg Psychiatry 55:181–184
17. Movement Disorder Society Task Force on Rating Scales for Parkinson's Disease (2003) The Unified Parkinson's Disease Rating Scale (UPDRS): status and recommendations. Mov Disord 18(7):738–750
18. Hutchison WD, Allan RJ, Opitz H et al (1998) Neurophysiological identification of the subthalamic nucleus in surgery for Parkinson's disease. Ann Neurol 44:622–628
19. Quiroga RQ, Nadasdy Z, Ben-Shaul Y (2004) Unsupervised spike detection and sorting with wavelets and superparamagnetic clustering. Neural Comput 16:1661–1687
20. Matsumura M, Watanabe K, Ohye C (1997) Single-unit activity in the primate nucleus tegmenti pedunculopontinus related to voluntary arm movement. Neurosci Res 28:155–165
21. Takakusaki K, Shiroyama T, Kitai ST (1997) Two types of cholinergic neurons in the rat tegmental pedunculopontine nucleus: electrophysiological and morphological characterization. Neuroscience 79:1089–1109
22. De Winter JCF (2013) Using the Student's t-test with extremely small sample sizes. Pract Assess Res Eval 10:1-12
23. Warmuth-Metz M, Naumann M, Csoti I, Solymosi L (2001) Measurement of the midbrain diameter on routine magnetic resonance imaging: a simple and accurate method of differentiating between Parkinson disease and progressive supranuclear palsy. Arch Neurol 58:1076–1079
24. Shao N, Yang J, Li J, Shang H-F (2014) Voxelwise meta-analysis of gray matter anomalies in progressive supranuclear palsy and Parkinson's disease using anatomic likelihood estimation. Front Hum Neurosci 8:1–10
25. Egerton T, Williams DR, Iansek R (2012) Comparison of gait in progressive supranuclear palsy, Parkinson's disease and healthy older adults. BMC Neurol 12:1–6. https://doi.org/10.1186/1471-2377-12-116
26. Dormont J, Conde H, Farin D (1998) The role of the pedunculopontine tegmental nucleus in relation to conditioned motor performance in the cat. I. Context-dependent and reinforcement-related single unit activity. Exp Brain Res 121:401–410
27. Scarnati E, Proia A, Di Loreto S, Pacitti C (1987) The reciprocal electrophysiological influence between the nucleus tegmenti pedunculopontinus and the substantia nigra in normal and decorticated rats. Brain Res 423:116–124
28. Futami T, Takakusaki K, Kitai ST (1995) Glutamatergic and cholinergic inputs from the pedunculopontine tegmental nucleus to dopamine neurons in the substantia nigra pars compacta. Neurosci Res 21:331–342
29. Charara ALI, Smith Y, Parent A (1996) Glutamatergic inputs from the pedunculopontine nucleus to midbrain dopaminergic neurons in primates: *Phaseolus vulgaris*-leucoagglutinin anterograde labeling combined with postembedding glutamate and GABA immunohistochemistry. J Comp Neurol 266:254–266
30. Galtieri DJ, Estep CM, Wokosin DL et al (2017) Pedunculopontine glutamatergic neurons control spike patterning in substantia nigra dopaminergic neurons. eLife 6:e30352
31. Bevan M, Bolam JP (1995) Cholinergic, GABAergic, and glutamate-enriched inputs from the mesopontine tegmentum to the subthalamic nucleus in the rat. J Neurosci 15:7105–7120
32. Massey LA, Jäger HR, Paviour DC et al (2013) The midbrain to pons ratio A simple and specific MRI sign of progressive supranuclear palsy. Neurology 80:1856–1861
33. Jeon M, Ha Y, Cho Y et al (2003) Effect of ipsilateral subthalamic nucleus lesioning in a rat parkinsonian model: study of behavior correlated with neuronal activity in the pedunculopontine nucleus. J Neurosurg 99:762–767
34. Breit S, Bouali-Benazzouz R, Benabid A, Benazzouz A (2001) Unilateral lesion of the nigrostriatal pathway induces an increase of neuronal activity of the pedunculopontine nucleus, which is reversed by the lesion of the subthalamic nucleus in the rat. Eur J Neurosci 14:1833–1842
35. Hardman CD, Halliday GM, McRitchie DA et al (1997) Progressive supranuclear palsy affects both the substantia nigra pars compacta and reticulata. Exp Neurol 144:183–192. https://doi.org/10.1006/exnr.1997.6415
36. Fling BW, Cohen RG, Mancini M et al (2013) Asymmetric pedunculopontine network connectivity in parkinsonian patients with freezing of gait. Brain 136:2405–2418. https://doi.org/10.1093/brain/awt172
37. Fraix V, Bastin J, David O et al (2013) Pedunculopontine nucleus area oscillations during stance, stepping and freezing in Parkinson's disease. PLoS ONE. https://doi.org/10.1371/journal.pone.0083919