Report: Analyzing electrophysiological recordings from Parkinsonian rat model

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Abstract

Parkinson's disease is characterized by the alteration of dopaminergic input to striatum leading to altered basal ganglia (BG) activity. Another marker includes excessive beta frequency oscillation in cortical electrocorticogram recordings. Within BG, Globus pallidus external (GPe) neurons are usually considered homogeneous. Nevertheless, in 2012, Mallet and colleagues showed that, in a model of Parkinson's disease, GPe neurons can be classified in two groups based on their tendency to fire at specific phases of the EEG cycle. Here we collected EEG recordings and spike trains of GPe neurons from the Mallet study of 2008. Using those we aim at 1) verifying that exaggerated EEG beta oscillation is a marker of Parkinson's disease, 2) testing for abnormal synchrony between pairs of GPe neurons and between GPe neurons and EEG in parkinsonian animal model, and 3) reproducing Mallet and colleagues classification of GPe neurons (Mallet, 2012).

Introduction

Parkinson's disease (PD) is well known for its motor symptoms including tremor and dyskinesia. Those symptoms are thought to be mediated by alterations of basal ganglia (BG) functioning, through alteration of its dopaminergic input from substantia nigra (Gerfen, 2011). The BG is usually divided in two circuits. First the direct pathway, formed by striatum projection to BG output nuclei (SNr, GPi) is thought to promote movement generation. Second, the indirect pathway formed by striatum projection to external Globus pallidus, which projects to subthalamic nuclei, which projects to BG output nuclei is thought to restrict movement initiation (Albin, 1989). Beside its role in the indirect pathway, the GPe is thought to serve as an integrative hub, coordinating neural activity across BG (Kita H., 2007). In line with this idea, different domains of the GPe are known to mediate distinct functions (Kelly, 2004), with this heterogeneity being further amplified in animal models of PD (6-OHDA-lesioned rats) (Mallet, 2008). Despite this knowledge, GPe neurons are still considered as relatively homogeneous. Nevertheless, a study conducted by Mallet and colleagues in 2012 revealed that GPe neurons can be classified in two groups differing by their structural, molecular and electrophysiological properties (Mallet, 2012). In this paper, authors simultaneously recorded, in anesthetized 6-OHDA-lesioned (later referred as parkinsonian) rats, frontal electrocorticogram (EEG) and single GPe neuron activity. This allowed them to sort GPe neurons in "GPT-A" (20%) neurons, preferentially firing during the up state of slow wave cortical oscillation (SWA, analogous to sleep oscillation) and "GPT-I" neurons (80%), preferentially active during the down state. During their study, the authors also confirmed the occurrence of exacerbated beta frequency oscillations during EEG "active state" (Activ, which is more similar to awake activity). In this report, for training purposes, we gathered some data from this team *(see methods)* and tried to reproduce the authors' findings while including recordings from control animals for comparison. In the first part, through EEG power spectral density analysis, we try to confirm that exaggerated beta frequency oscillation during active state is a marker of PD. In a second part, via cross correlation of neuronal spike trains, we test whether there is different sort of synchrony between neurons (in phase, in phase opposition) which may suggest the existence of different groups. We also test whether GPe neuron firing shows coherence at beta frequency with other GPe neurons and with EEG. Finally, in the third part, we reproduce the sorting of GPT-A and GPT-I neurons based on their synchrony with slow wave EEG activity, confirming the presence of two neuron groups in parkinsonian rats.

Material & methods

Data. The dataset analyzed in this study was obtained from the article of Mallet N. et al., 2008. It comprises : 1) spike train of extracellularly recorded GPe neurons (via silicon probes) and 2) simultaneously-recorded frontal EEG. Those recordings were done in healthy (control) and 6-OHDA (parkinsonian) rats in different anesthesia states : slow-wave and active state.

Data analysis. We used Python (version 3.13.0) within Jupyter Notebook (Jupyter Notebook version 7), utilizing libraries **NumPy** (np) for numerical operations, **Matplotlib** for data visualization, **SciPy** (scipy) for signal processing and **H5py** for uploading and manipulating HDF5 files.
For this analysis we used specific functions from these libraries and certain calculations:

- ***scipy.signal.welch*** *(nperseg = fs_EEG)* was used to compute the Power Spectral Density (PSD) of EEG signals using Welch's method. EEG being acquired with a different sampling frequency in the different animals, and to get a comparable frequency resolution for all, the nperseg value was set to be equal to the EEG sampling frequency (fs_EEG).
- ***scipy.signal.correlate*** (mode = 'full', method = 'auto') was used for the calculation of cross-correlation between spike trains to assess their synchronization. The “Auto” argument allowed using the most efficient computation method based on input signal sizes.
- ***np.convolve*** (mode = "same") / **gaussian.sum**() was used for the smoothing of cross-correlation via gaussian window (length of window is 0.01 sec, sigma=2.5);
- ***scipy.signal.coherence*** (fs = signal sampling frequency) was used to compute the coherence between pairs of spike trains and between spike trains and EEG. This is used to reveal how well the frequency content of both signals matches at different frequencies.
- ***scipy.signal.butter*** (order= 5, btype = 'low' / btype = 'high', output = 'ba') was used to create a band pass filter. A lowpass filter (cutoff=0.5Hz) was first applied to the slow wave EEG and then a highpass (cutoff=1.5Hz). This allowed removing both low-frequency drift and high-frequency noise. Order (=5) was adjusted manually.
- ***np.histogram*** *(nb of bins = len(EEG))* was used to create an **instantaneous firing rate** vector for each neuron. This function determines the number of data points (spike) falling within specific time bins. The number of bins was set equal to the number of EEG samples to allow coherence analysis with EEG.
- ***Spike-triggered average*** (STA) calculation was done by averaging EEG signal surrounding each spike (1 sec before and 1 sec after it) to reveal when neurons are spiking relative to SWA EEG up/down state.

Results

First, we wanted to reveal whether there is a difference between frequency composition of EEG recordings from parkinsonian and control rats (in SWA and active states). We analyzed EEG recordings of each group (fig. 1A) using the *scipy.signal.welch* function to reveal the most prevalent frequencies composing the signal. Our results confirmed the presence of excessive beta oscillation (~20 Hz) during the active state of parkinsonian rats only (fig. 1B). This is a known marker of PD (Mallet N. at 2008).

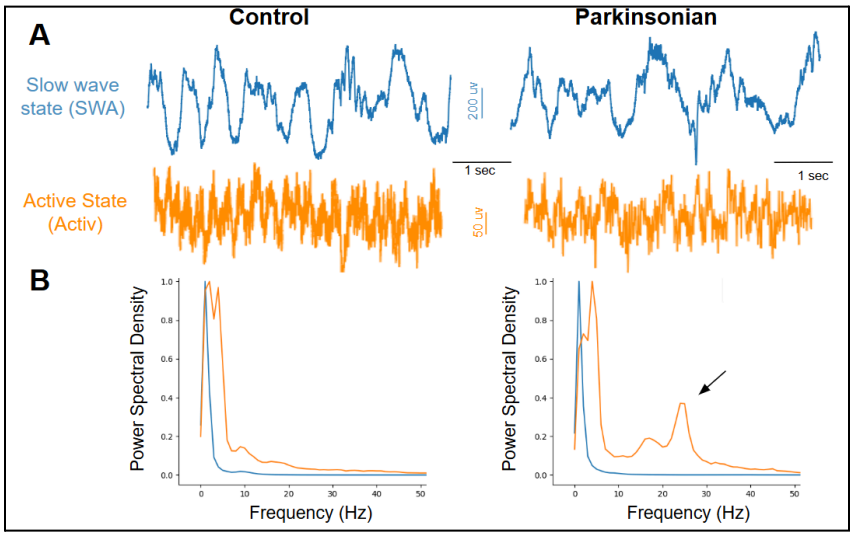

Figure 1| Exaggerated Beta Frequency During EEG Active State is a Marker of Parkinson’s Disease. A, Example traces of EEG during active and SWA state (of parkinsonian and control rats). **B,** Power spectral density analysis of EEG signal represented in (A). Arrow indicates excessive beta frequency (~20 Hz).

Our second question was: Is there any synchrony between GPe neurons and do GPe neurons firing show synchrony with active state EEG in parkinsonian rats at beta frequency? This would indeed indicate abnormal GPe activity. To test for synchronization between pairs of neurons, we performed cross-correlation of their spike trains. We then convolved the obtained correlation with a gaussian window for smoothing (fig. 2A,B). An oscillatory appearance of the cross correlation was considered as a sign of synchrony. The results showed that some recorded neurons are synchronized in phase (upward peak of correlation at lag 0, fig. 2B left), synchronized out-phase (downward peak of correlation at lag 0, fig. 2B right) and that some are not synchronized (data not shown). Coherence analysis revealed that the synchrony between neurons was mostly occurring at beta frequency (fig. 2C). Those neurons also showed coherence with active state EEG at beta frequency (fig. 2D,E), further confirming their abnormal synchronization with cortical beta oscillations (Mallet, 2008).

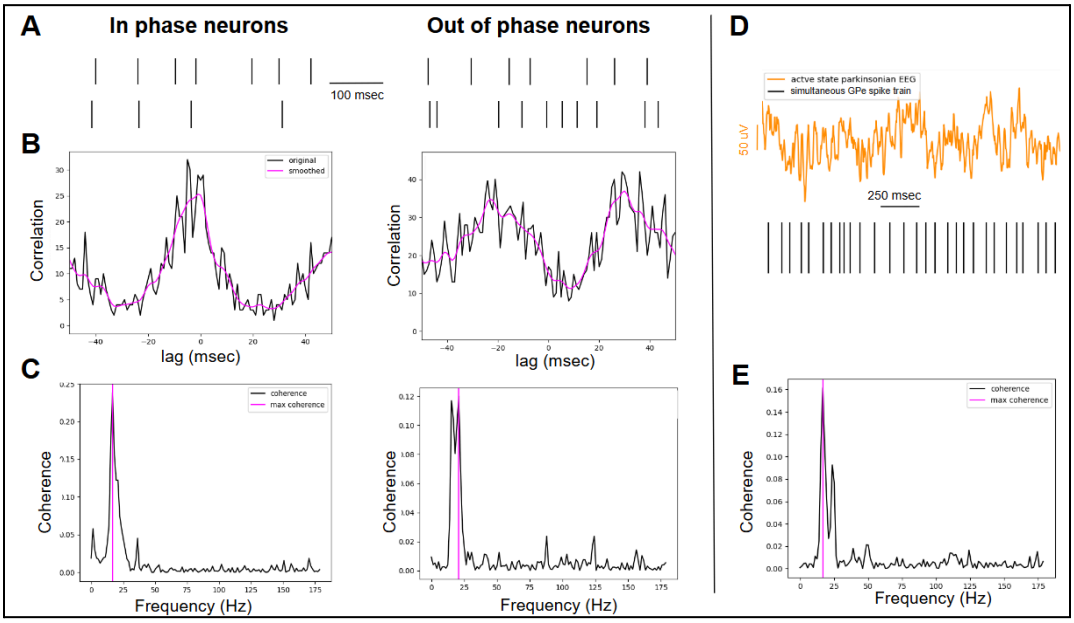

Figure 2| Synchronization Between GPe Neurons Pairs and Between GPe Neuron and EEG in Parkinsonian Animals During the Active State. A, examples of the spike train of neurons synchronized in-phase (left) and out-of-phase (right); **B,** correlation and **C,** coherence between those spike trains; **D,** example of EEG and neuron spike trains in parkinson group during active state; **E,** coherence between this EEG recording and this spike train.

Neurons' spike trains synchronization in phase or phase opposition led to the idea that there might be different neuron groups (though other things can explain this). We thus then attempted to classify neurons based on their tendency to fire at specific phases of the EEG cycle. Indeed, doing so, and using EEG and spike train recording during SWA state, Nicolas Mallet and colleagues were able to distinguish two groups of neurons (Mallet, 2008). Here we aimed at reproducing their findings and testing if this classification works for control rats. First, looking at SWA EEG and simultaneously recorded GPe neurons spike trains (fig 3A), we could already see that some neurons were firing preferentially during the up or down state of EEG slow oscillation. Then, we computed and plotted the spike-triggered averages (STA) of the SWA EEG trace with respect to all spike trains recorded in parkinsonian and control rats. In parkinsonian rats, this revealed two different types of neurons, firing either during up or down state of EEG (fig 3B). Similarly to Mallet and colleagues, here we proposed a classification method of the GPe neurons based on the STA value at lag 0: if the STA value was positive then the neuron was considered as a "GPT-A" neuron (preferentially firing during EEG up-phase), and if negative, GPT-I (preferentially firing during EEG down-phase). Plotting neurons STA values at time 0 against their mean firing frequencies further revealed these two neuron groups as distinct clusters of GPe neurons (fig 3C). Doing the same classification for control rats, the GPT-A and GPT-I neurons could not be well distinguished (fig 3 B,C, *bottom*, see discussion).

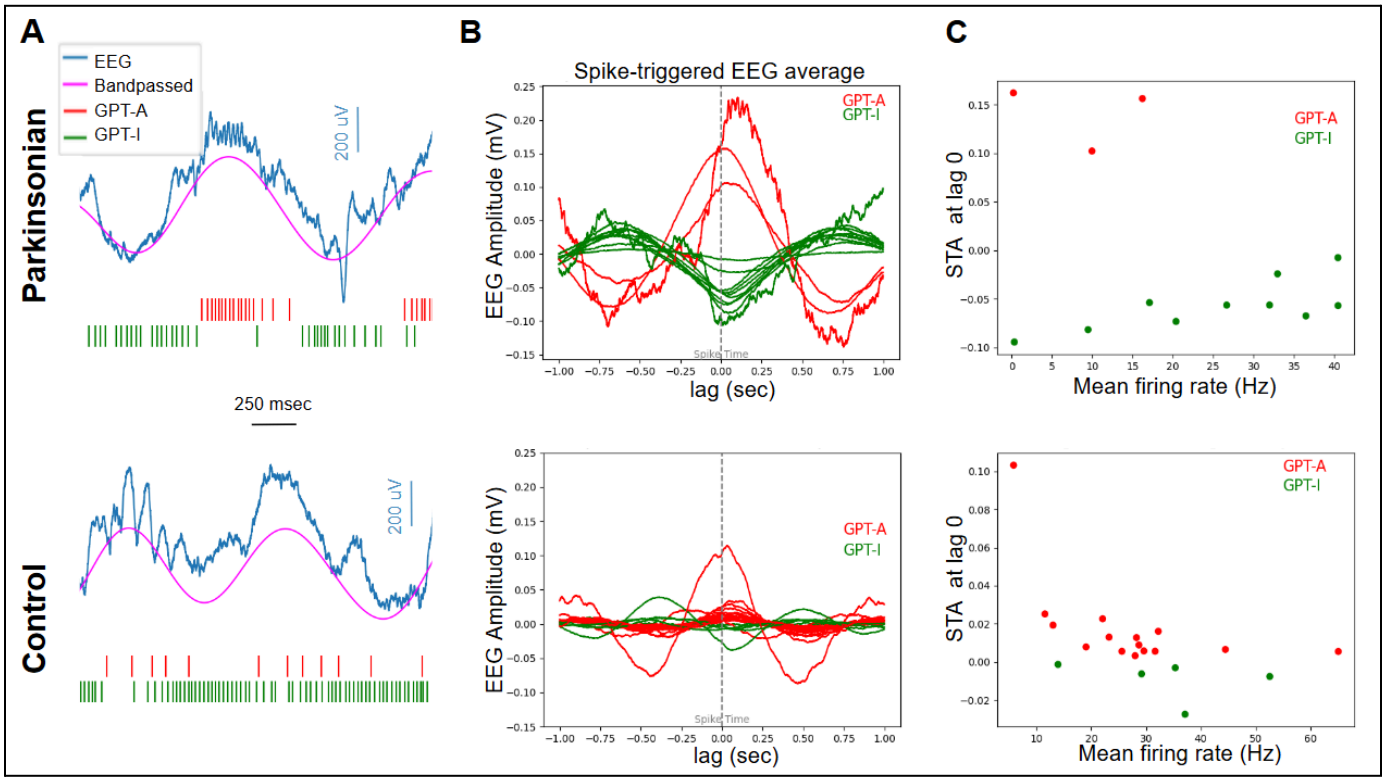

Figure 3| Classification of GPe Neurons by Firing Preference Toward SWA EEG Up or Down States. A, Example of GPT-I and GPT-A neurons spike times relative to original and bandpassed EEG signal. **B,** Spike-triggered average (STA) of SWA EEG for all recorded neurons. If STA al lag 0 was superior to 0, the neuron was classified as GPT-A (red), if not, it was classified as GPT-I (green). **C,** Plotting STA at lag 0 against neuron mean firing allows clear clusterization of the GPT-I and GPT-A neurons for parkinsonian but not control rats.

Discussion

Overall, here we reproduce Mallet team findings (2008, 2012). First, we confirm that an exaggerated beta frequency oscillation during active state is a marker of PD. Then, we highlight a coherence between GPe neurons firing at beta frequency in parkinsonian rats during active state. We also evidence coherence at beta frequency between GPe neurons firing and active state EEG. Finally, we show that GPe neurons' spike timing relative to EEG phase allows their classification in two groups for parkinsonian but not control rats.

The main limitation of this study relies on the fact that our dataset contained only 1 EEG recording and about 15 spike train recordings for each rat group (parkinsonian or control) in each EEG state (SWA or Activ). That amount of data is not enough for analysis and could lead to sampling bias, where the observed results may not fully represent broader patterns within each group. In addition, for figure 2, the synchronization between neurons and between neuron and EEG was also concluded from a single pair of spike trains and a single pair of spike train/ EEG recording. Those observations will need to be reproduced over more recordings to draw stronger conclusions.

Then, the coherence observed at beta frequency between GPe neurons and between GPe neurons and EEG during active state confirmed abnormal activity of GPe (Mallet, 2008). Still, confirming that this synchrony is abnormal with our dataset would require performing the same analysis in control rats, to check that there is no coherence at beta frequency in normal conditions.

Regarding classification of neurons (in GPT-I and GPT-A) in control rats (fig 3 B,C). The spike-triggered SWA EEG average graph (fig 3B, bottom) revealed that those GPe neurons showed less heterogeneity in their preferred firing time relative to EEG phase compared to parkinsonian GPe neurons. Indeed most values of spike triggered average at lag 0 were near to 0, making it hard to distinguish two neuronal groups. Thus, in this case, a classification of neurons in GPT-A and GPT-I only based on this value might not be really relevant. This probably explains why those 2 groups could not be clearly clustered later on according to their firing frequency (fig 3C). Accordingly, GPe neurons are known to display lower heterogeneity in healthy rats compared to parkinsonian ones (Mallet, 2008). This leads to the question : Is duality in the GPe neuron (GPT-A, GPT-I) specific to PD? Again here a larger data set would be needed to answer the question.

Regarding the classification of neurons in GPT-I or GPT-A in parkinsonian rats, it would be interesting to check if those neurons share more heterogeneity than just their preferred firing time relative to SWA EEG phase. Here, though no quantification was done, we showed that GPT-A neurons generally have lower firing frequency than GPT-I ones during SWA EEG (fig 3C). To go further, we could also test for differences in those neurons coefficient of variation of firing (through deeper analysis of our data), in their structure (through juxtacellular labeling), in the neurotransmitter they express (through immunohistochemistry) etc. All those parameters were actually shown to differ in the two neuronal groups by Mallet and colleagues (2012), further confirming the relevance of the classification that they (and we) used in parkinsonian rats.

Finally, because pathological beta oscillation occurs during the active state, it would be interesting to test whether the neurons identified as GPT-A / GPT-I neurons during SWA state also fire in a preferred phase of active state EEG. Preliminary results tend to indicate that they do, but the two groups preferred firing phase is not so clear and needs verification with a bigger sample size (results not shown, see presentation).

Thanks to

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