

Data Analysis: Analyzing electrophysiological recordings from Parkinsonian rat model

Presentation is made by Olga Y. Salyp and Tim Gervois

19.11.2024

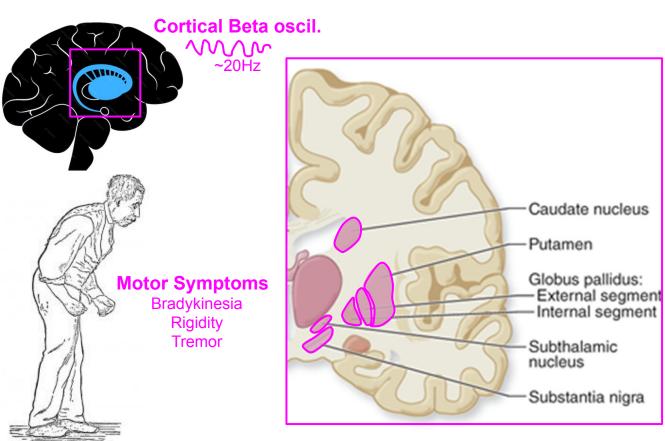
Parkinson's disease: Symptoms and Markers



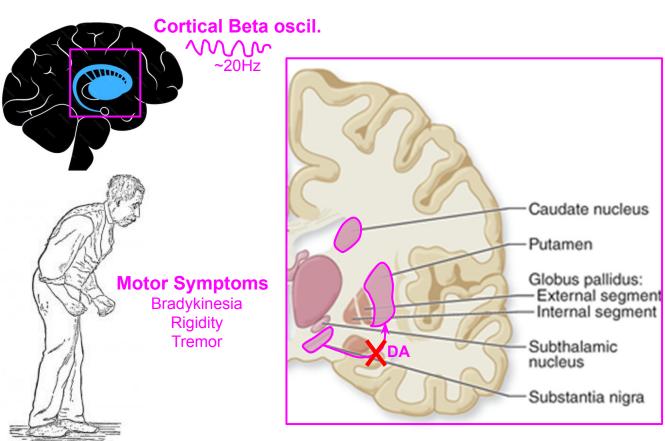
Parkinson's disease: Symptoms and Markers



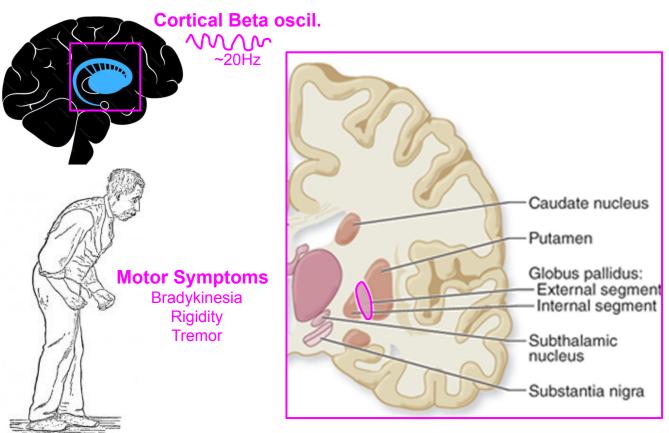
Parkinson's disease: Basal Ganglia Dysfunction



Parkinson's disease: Basal Ganglia Dysfunction

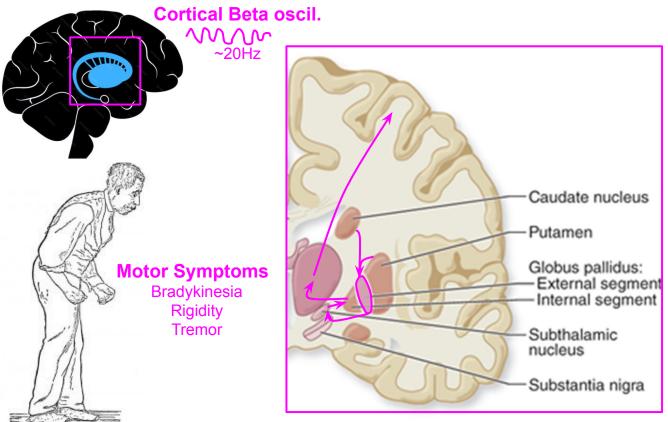


External Globus pallidus: Diverse functions...



External Globus pallidus (GPe)

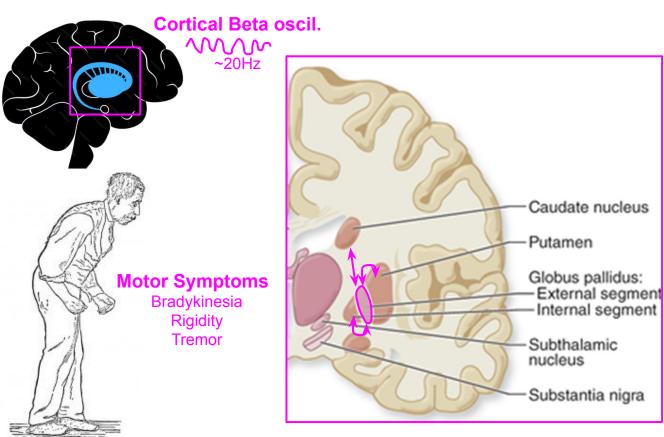
External Globus pallidus: Diverse functions...



External Globus pallidus (GPe)

mediates indirect pathway

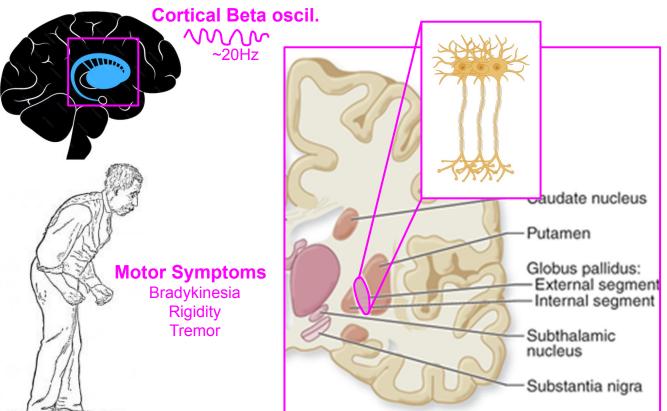
External Globus pallidus: Diverse functions...



External Globus pallidus (GPe)

- mediates indirect pathway
- is a coordinating hub of Basal Ganglia

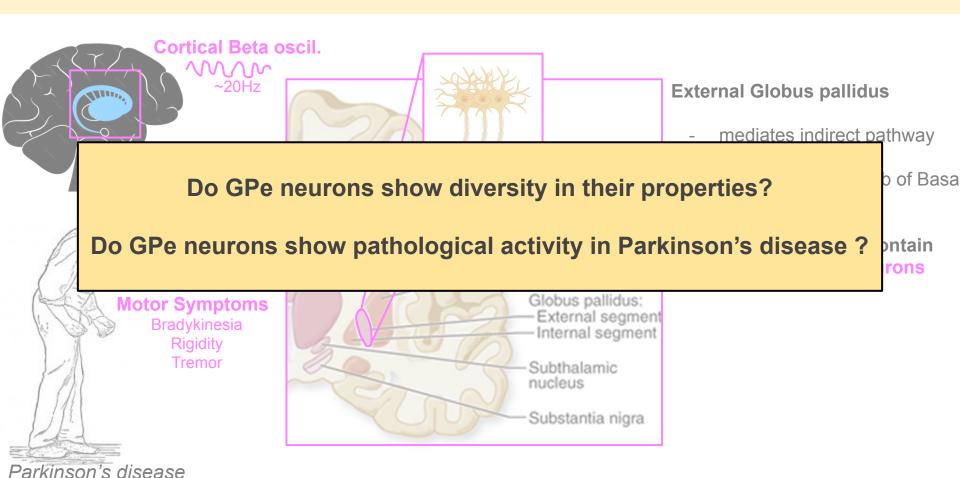
External Globus pallidus: Diverse functions but homogeneous neurons?



External Globus pallidus (GPe)

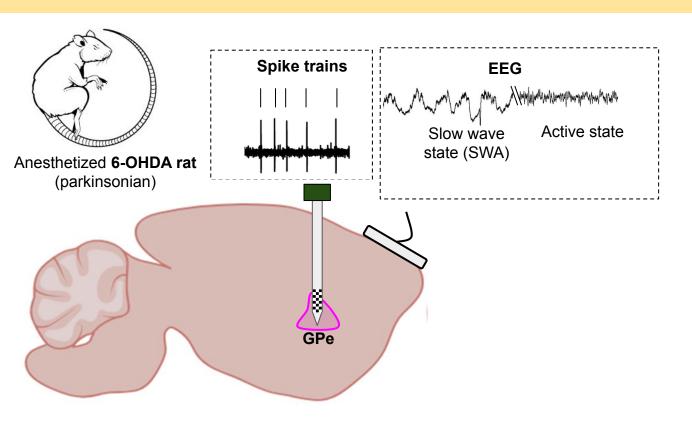
- mediates indirect pathway
- is a coordinating hub of Basal Ganglia
- Is considered to contain homogeneous neurons

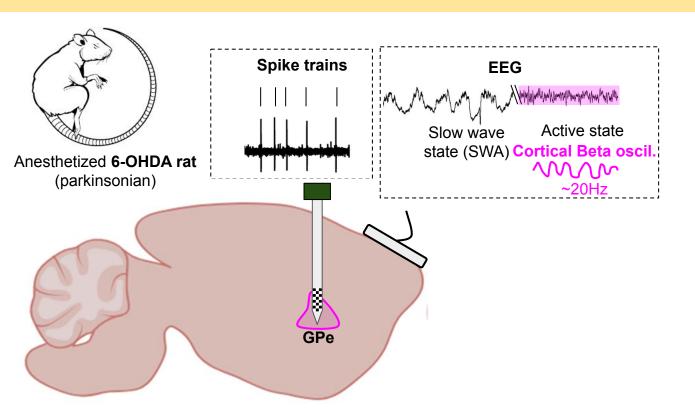
External Globus pallidus: Diverse functions but homogeneous neurons?

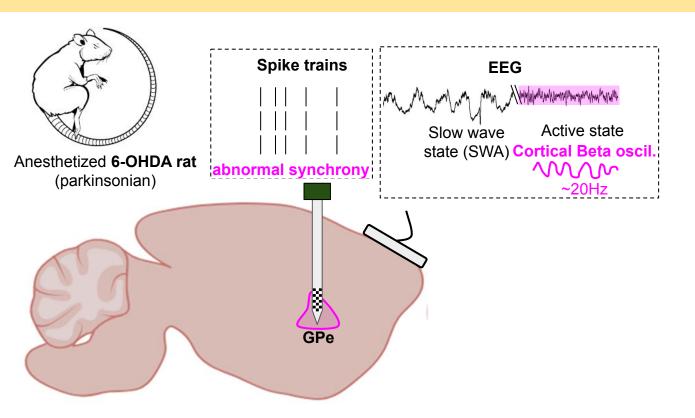


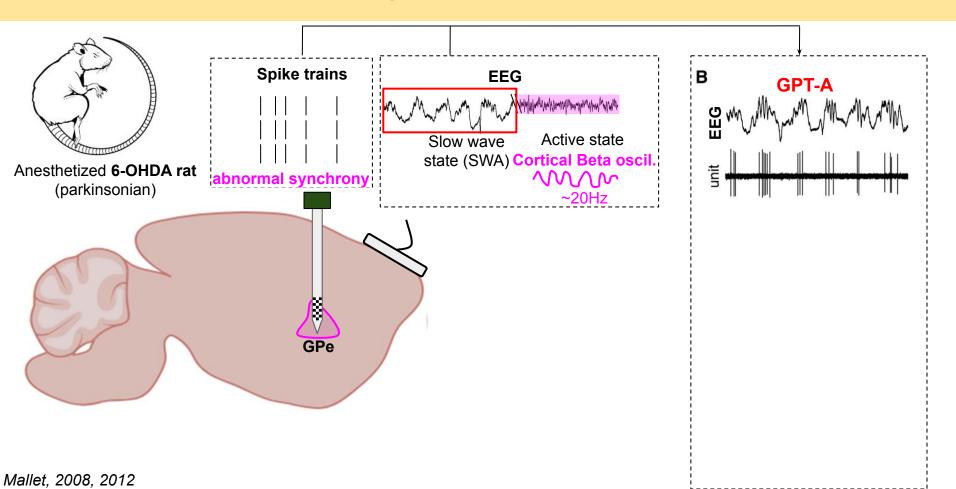


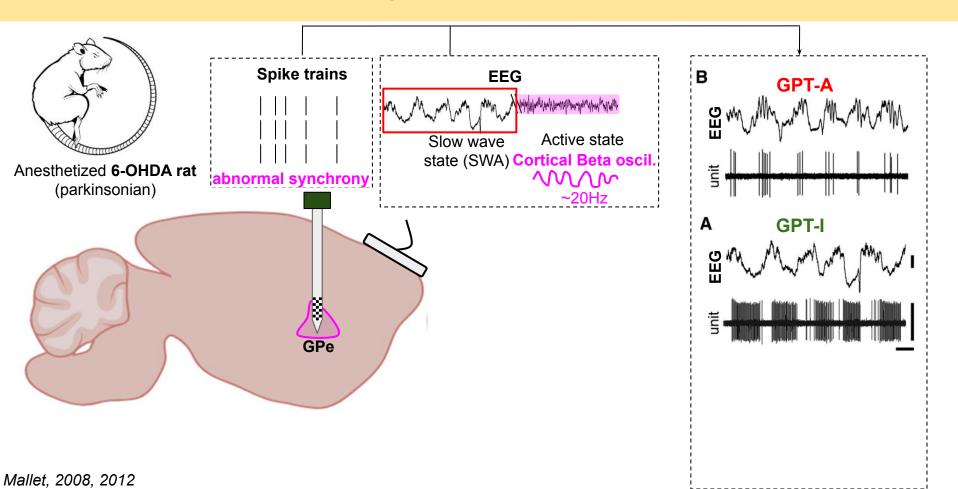
Anesthetized **6-OHDA rat** (parkinsonian)

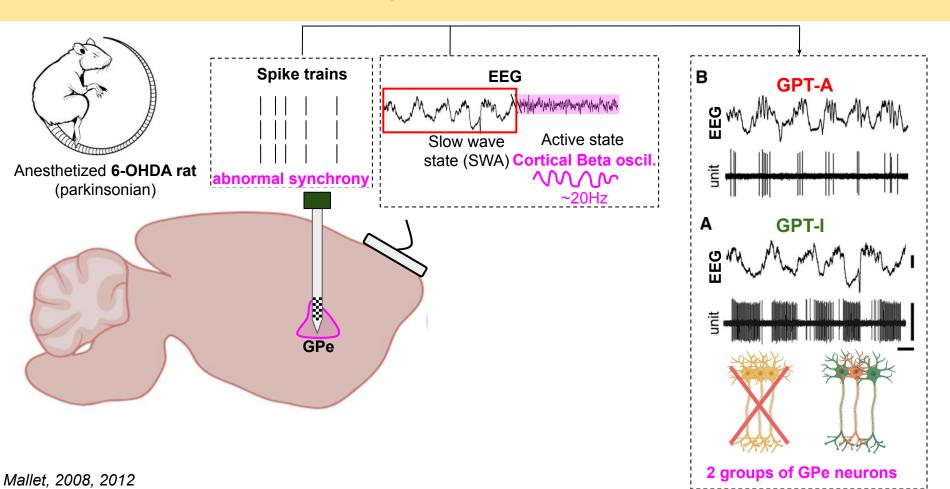


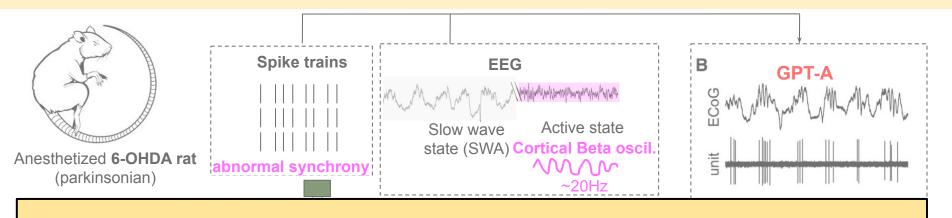










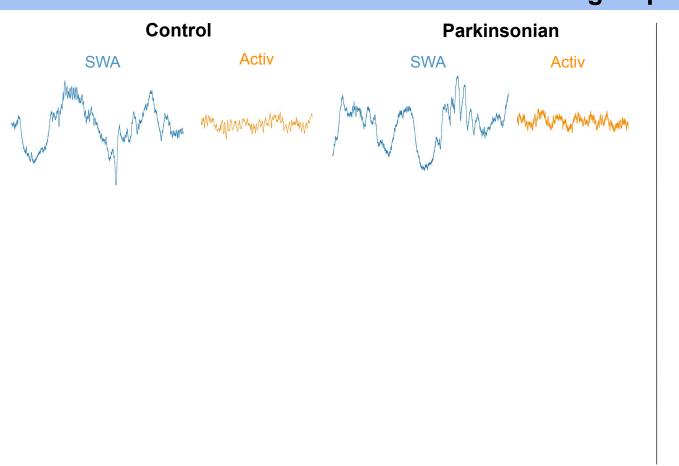


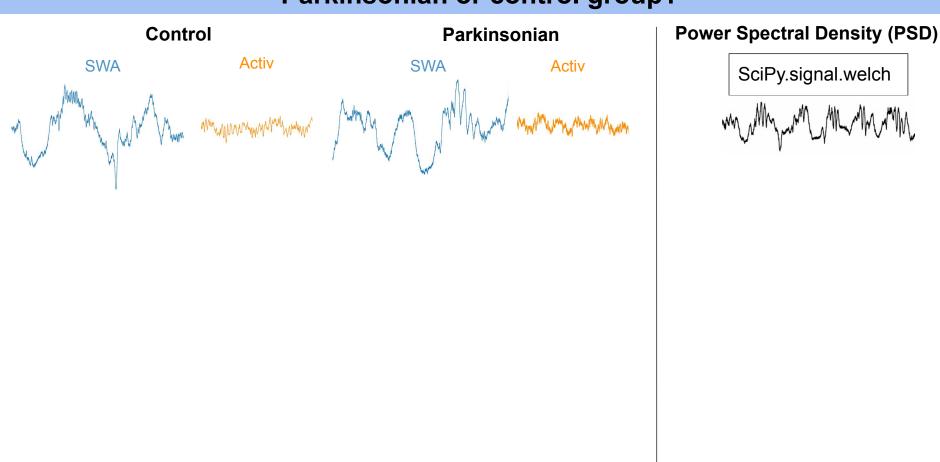
We reanalysed those data and confirmed that:

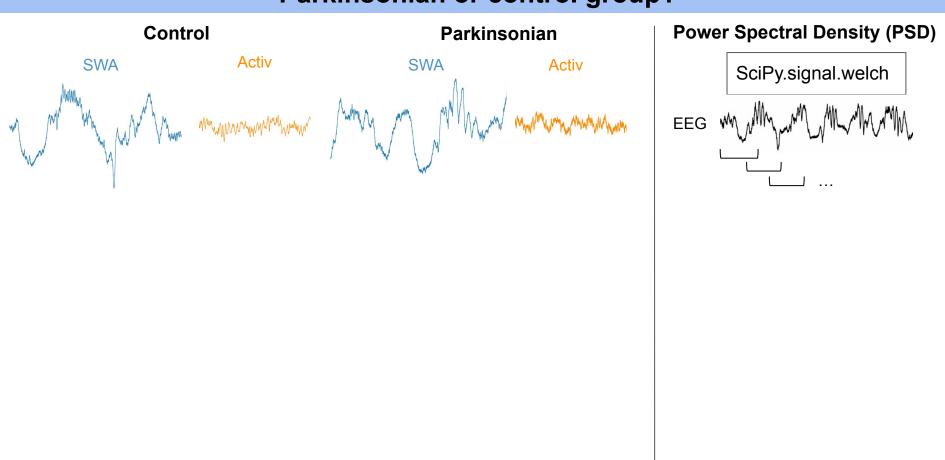
- 1) Exaggerated Beta frequency within active state EEG is a marker of PD
- 2) Their is abnormal synchrony between pairs of GPe neurons and between GPe neurons and active state EEG in parkinsonian rats
- 3) GPe neurons can be classified in two groups based on their tendency to fire at specific phases of the SWA EEG cycle

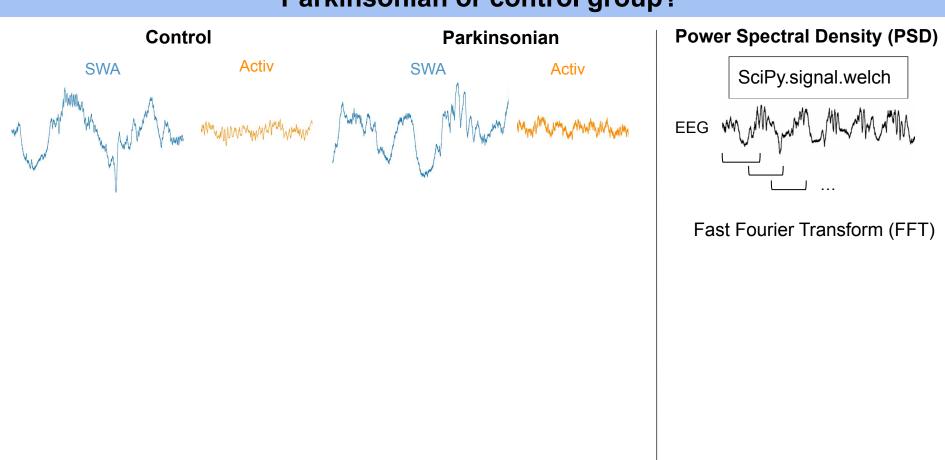
Mailet, 2006, 2012

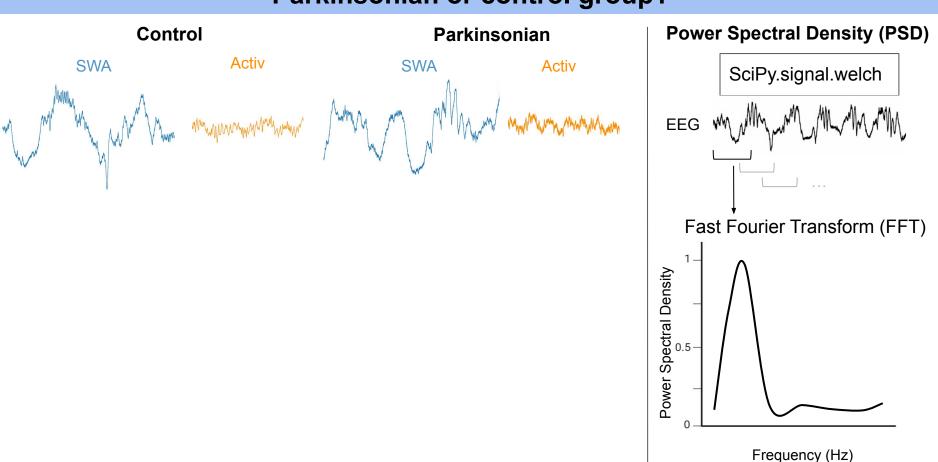
Aim 1: reveal the difference between frequency composition of EEG recordings of parkinsonian and control rats

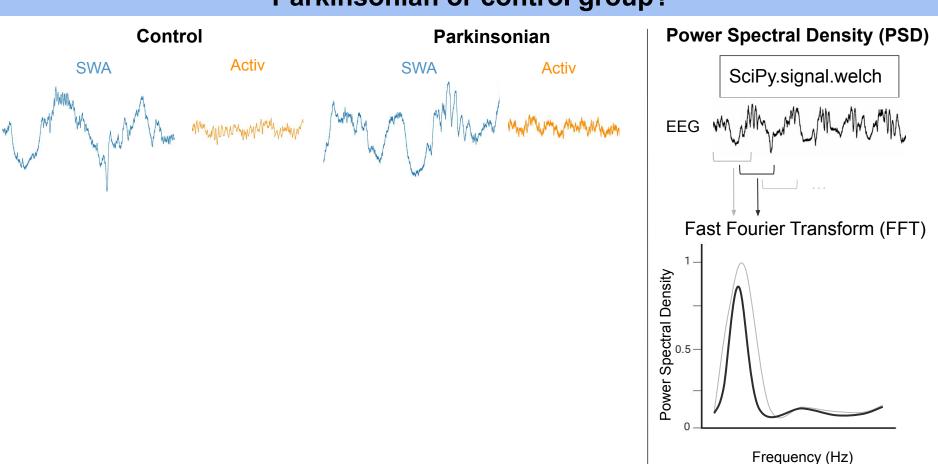


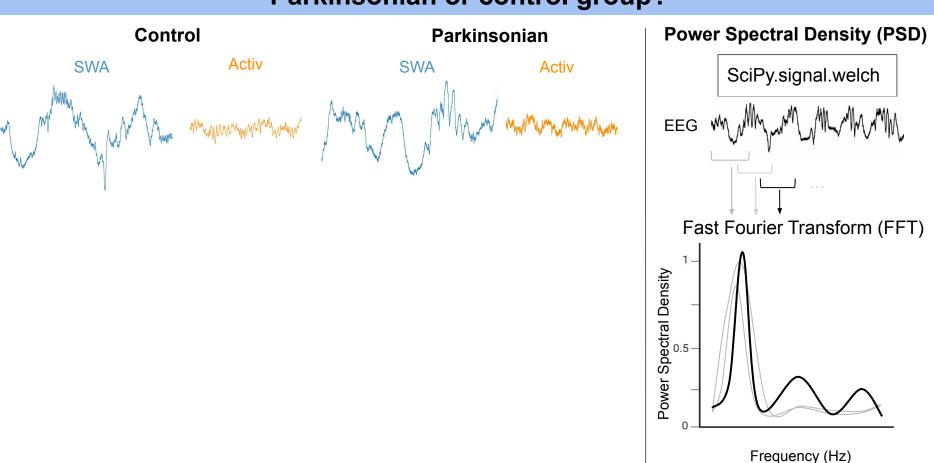


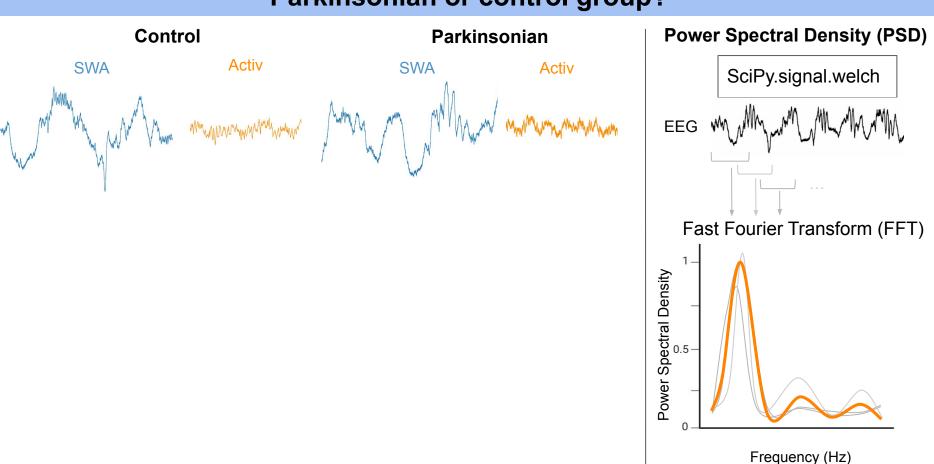


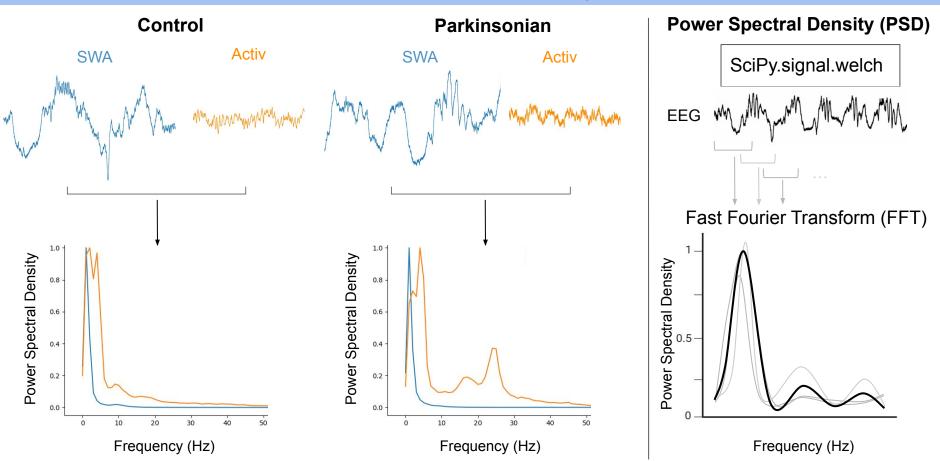


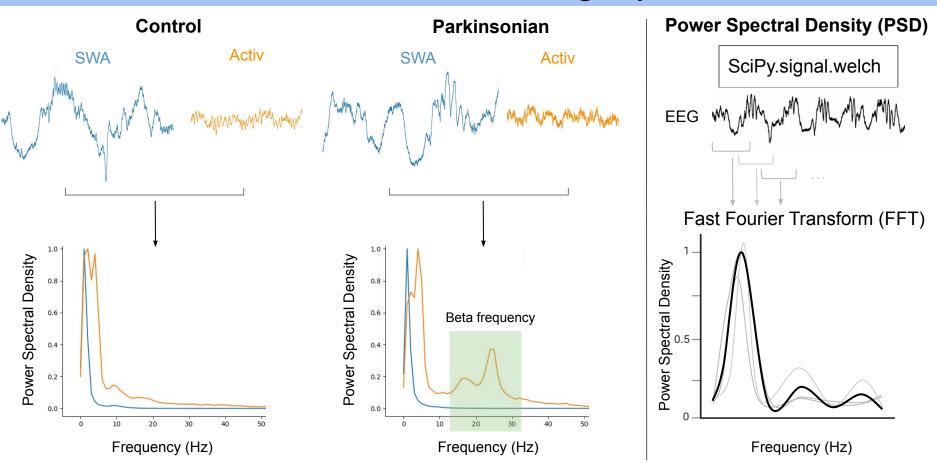


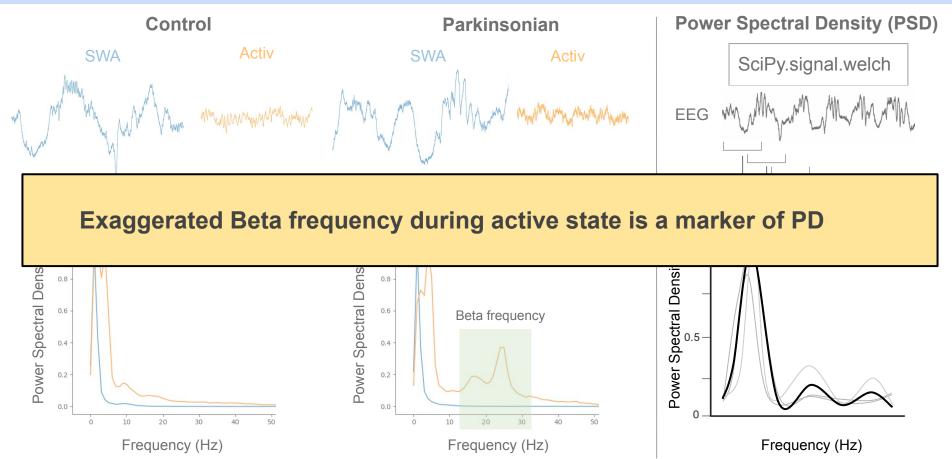




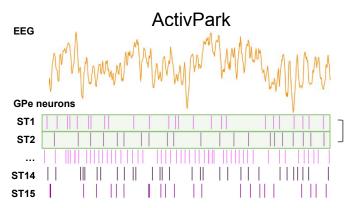


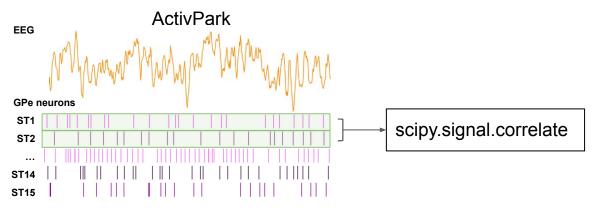


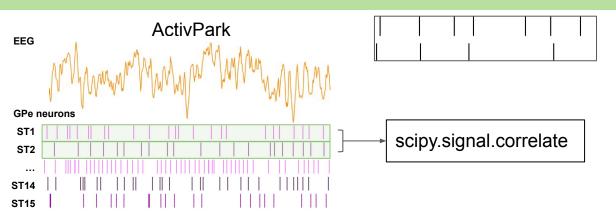


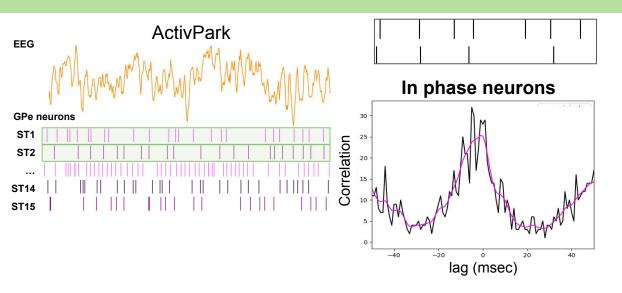


Aim 2: to reveal synchrony between <u>pairs of GPe neurons</u> - and between <u>GPe neurons and EEG</u> in parkinsonian rats during active state

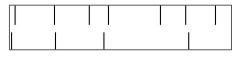


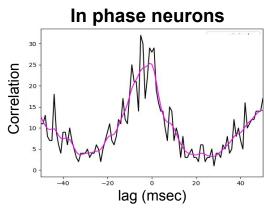


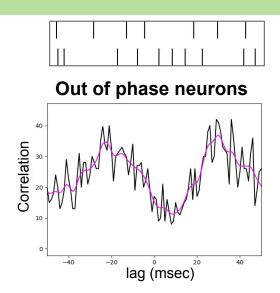


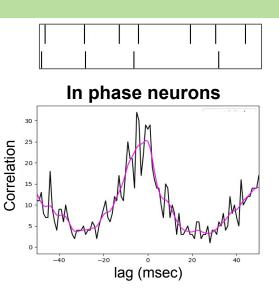


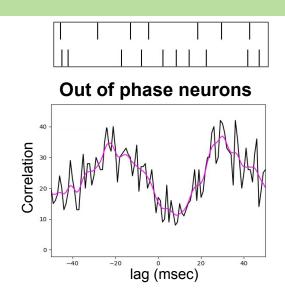


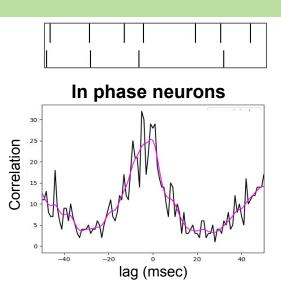


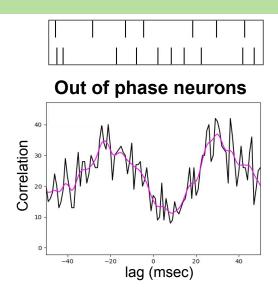


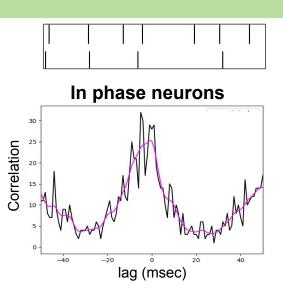




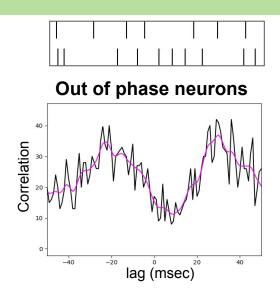


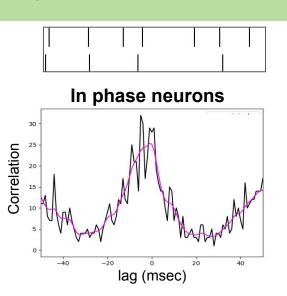




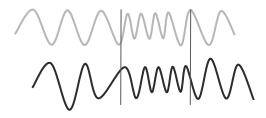


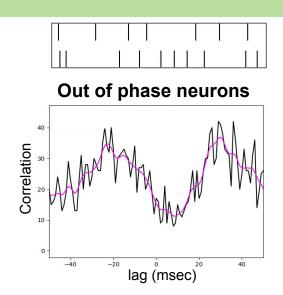
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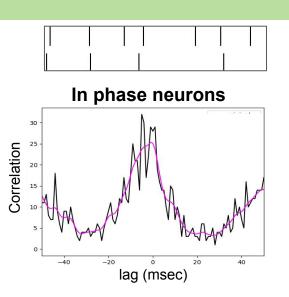




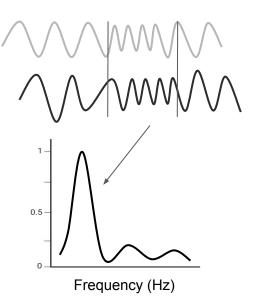
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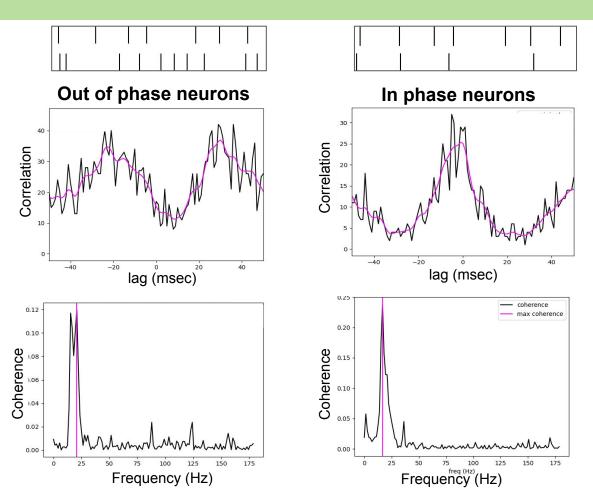




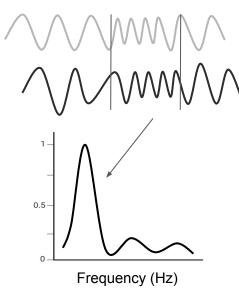


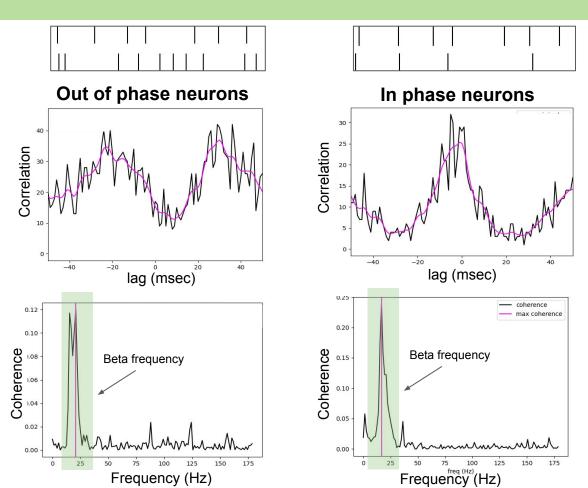
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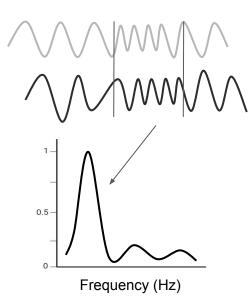


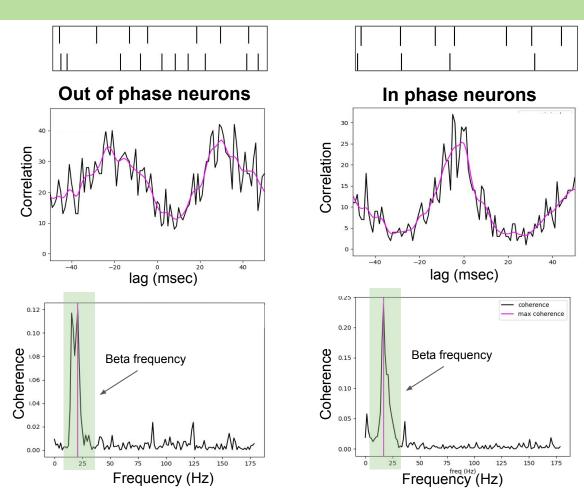
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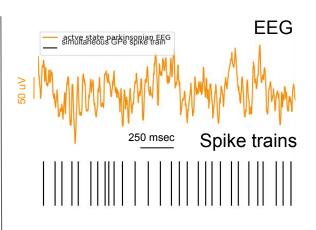


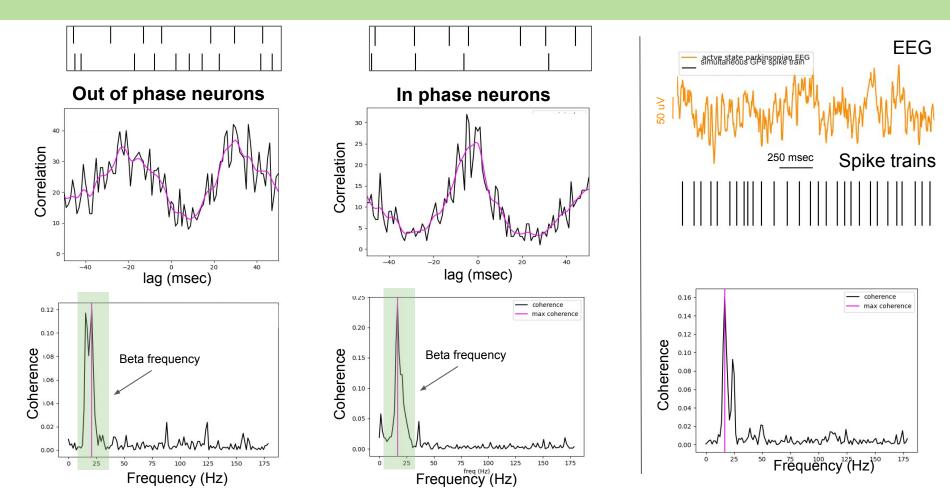


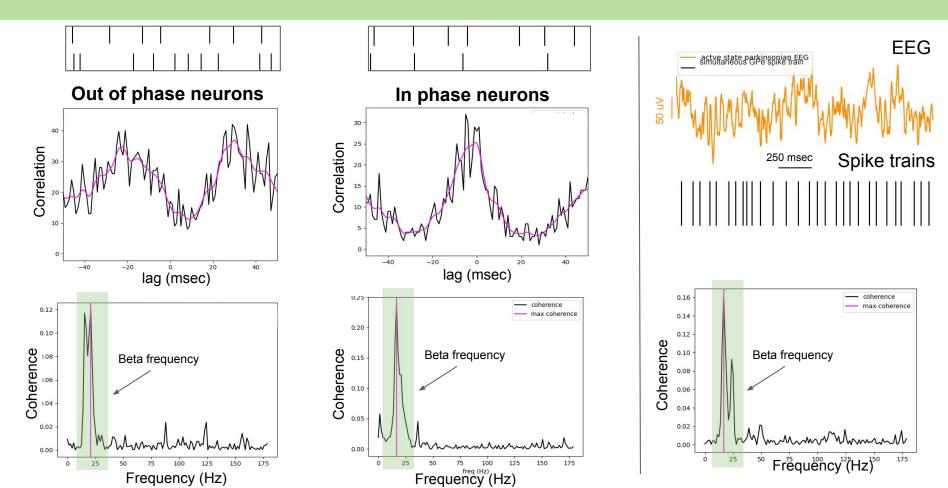
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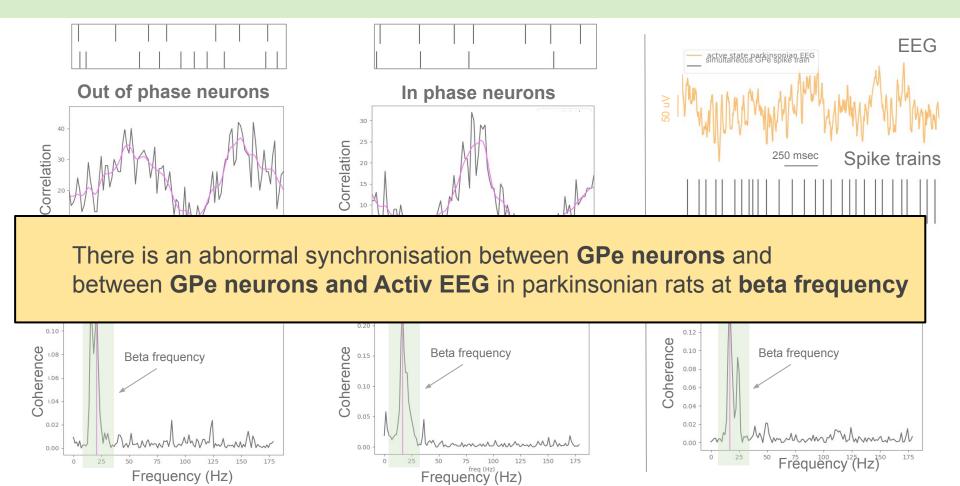


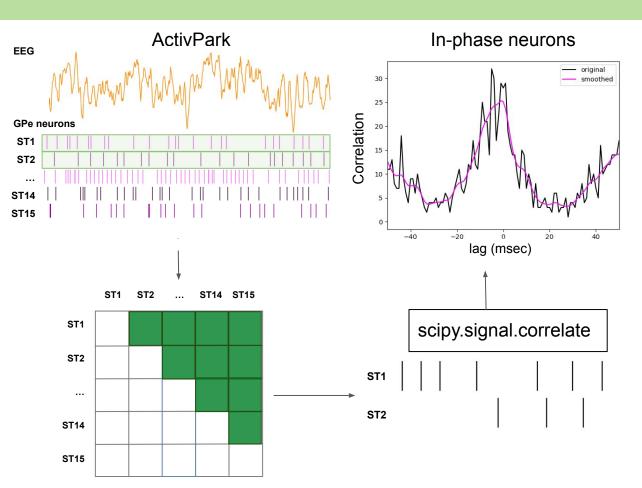


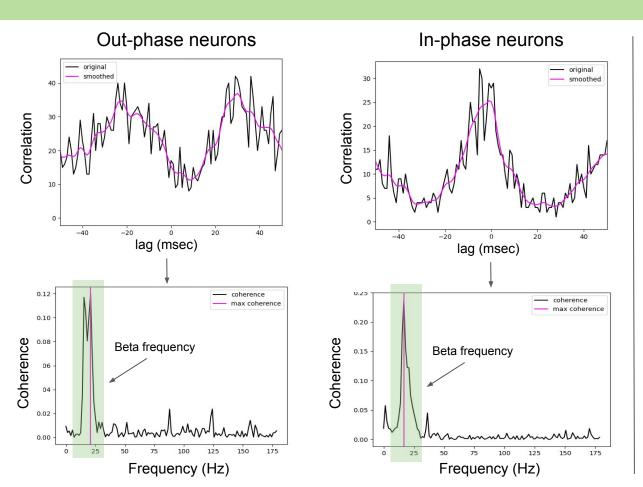




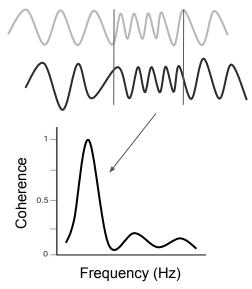




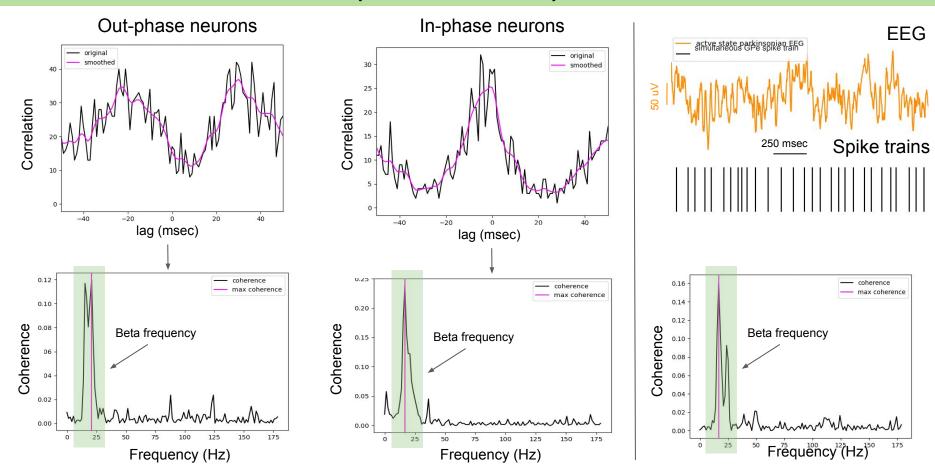


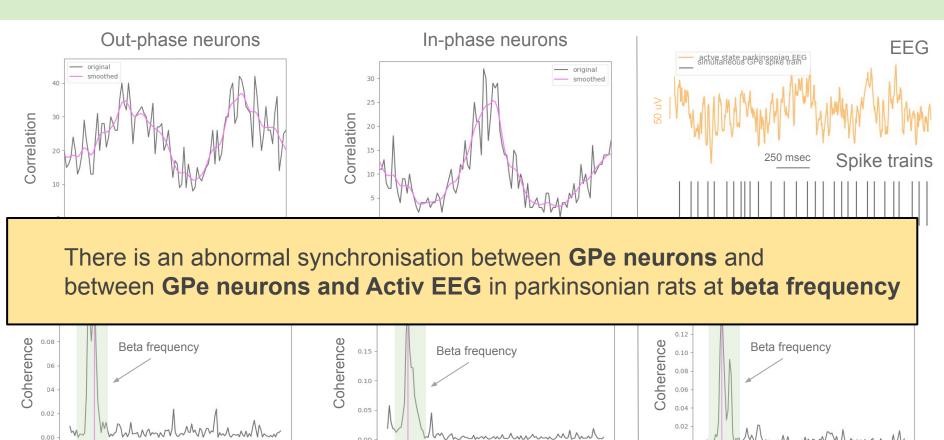


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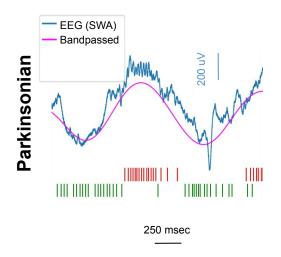
Is there abnormal synchrony <u>between GPe neurons</u> in parkinsonian rats (ACTIVE STATE)?

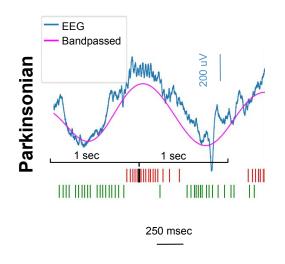




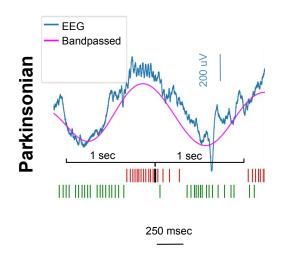
Frequency (Hz)

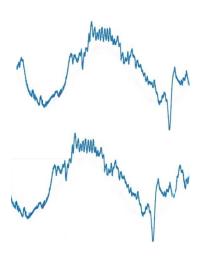
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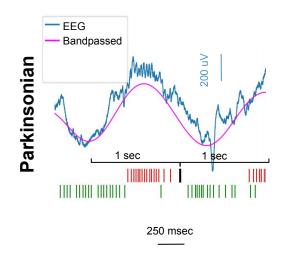


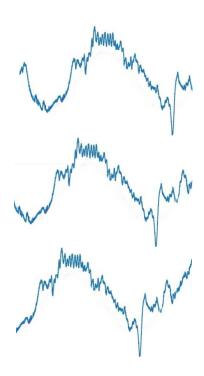


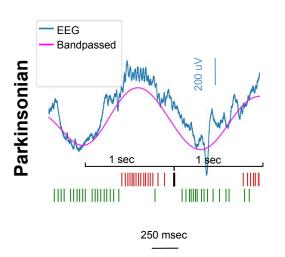


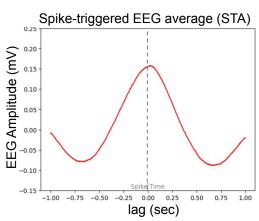


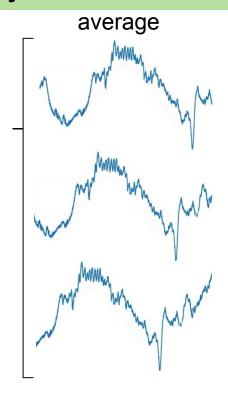


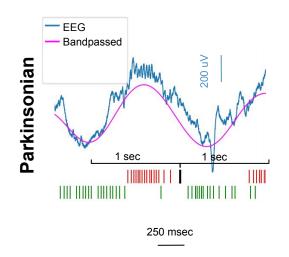


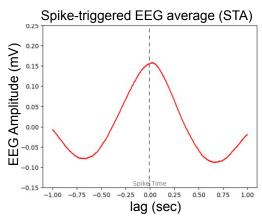


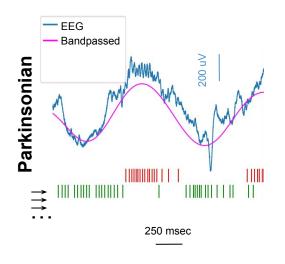


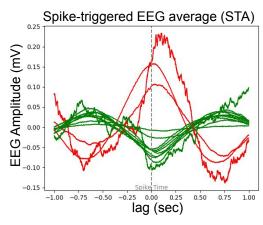


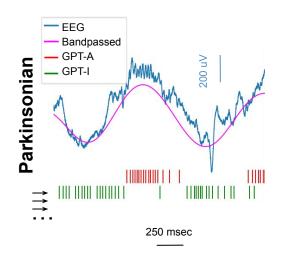


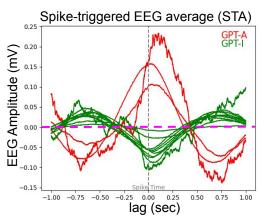


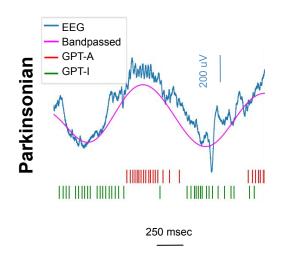


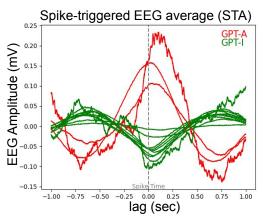


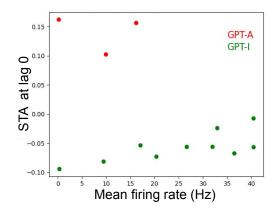


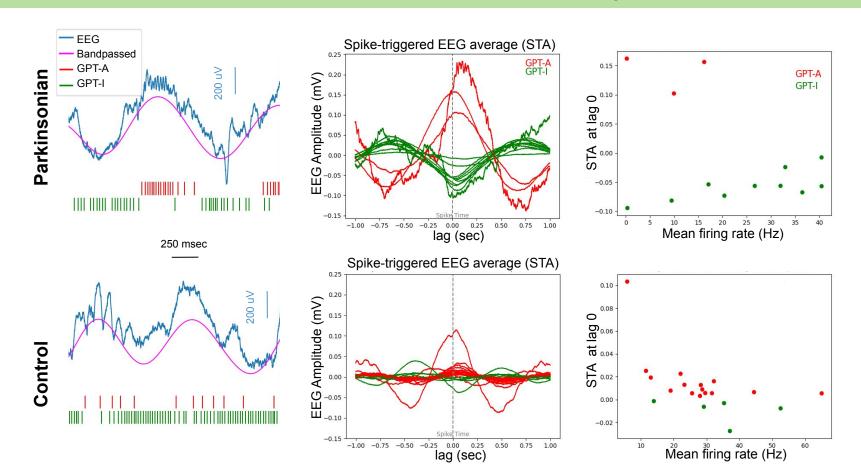


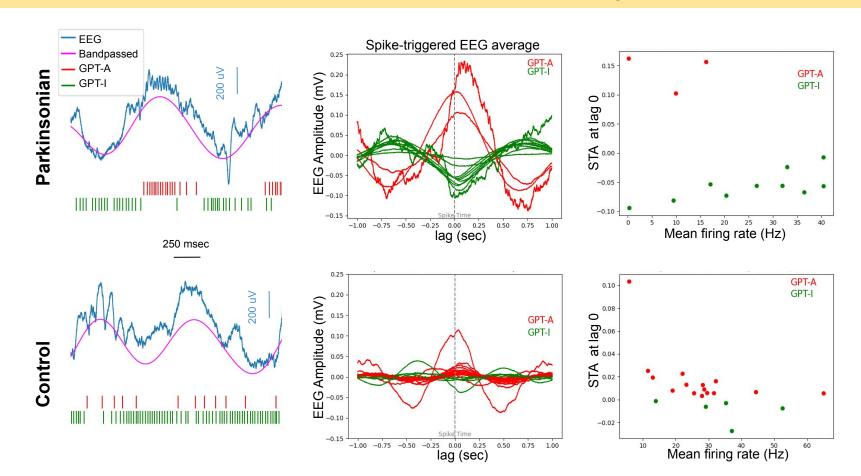










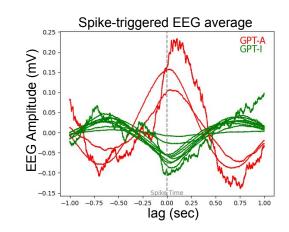


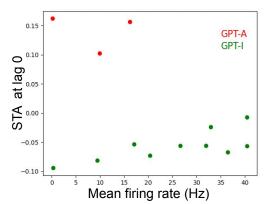
Conclusion

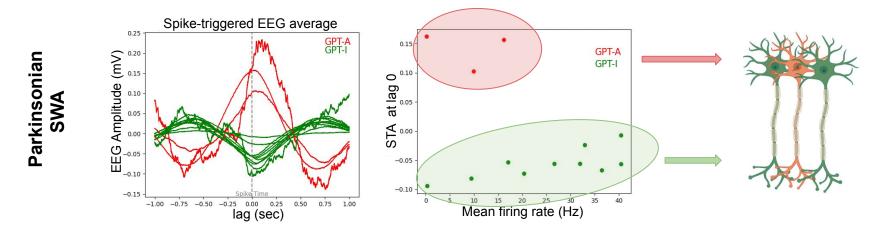
We reanalysed those data and confirmed that:

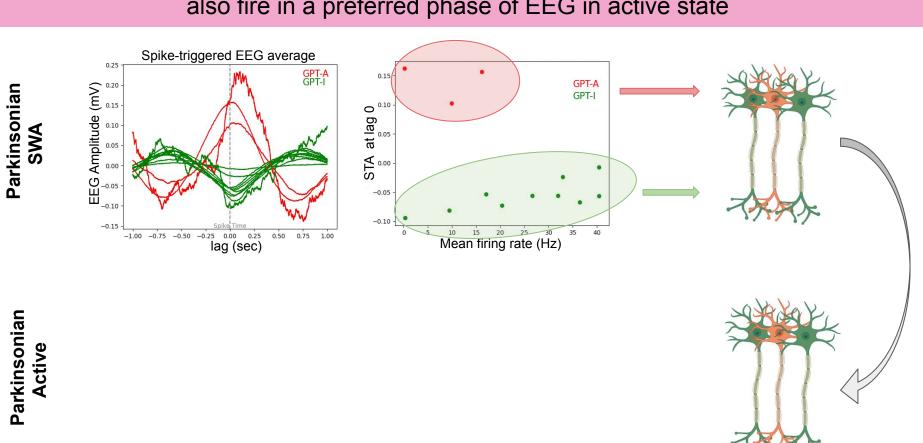
- 1) Exaggerated Beta frequency during active state is a marker of PD
- 2) Their is abnormal synchrony between pairs of GPe neurons and between GPe neurons and EEG in parkinsonian rats
- 3) GPe neurons can be classified in two groups based on their tendency to fire at specific phases of the EEG cycle (in parkinsonian rat)

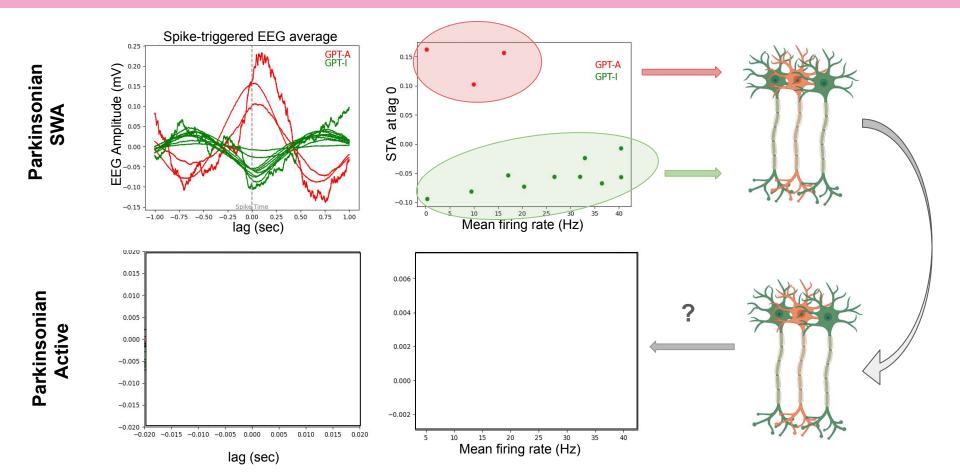


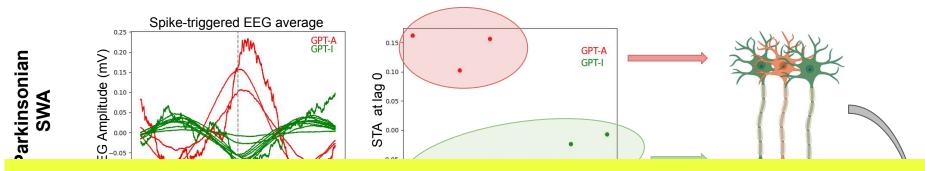






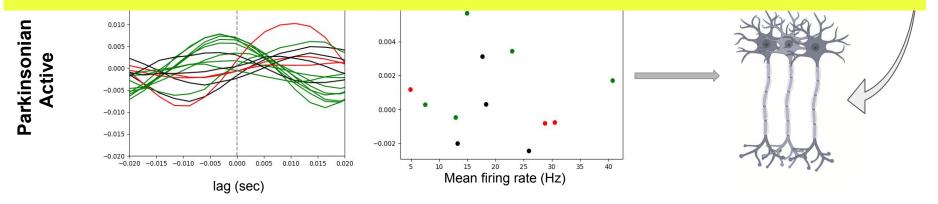


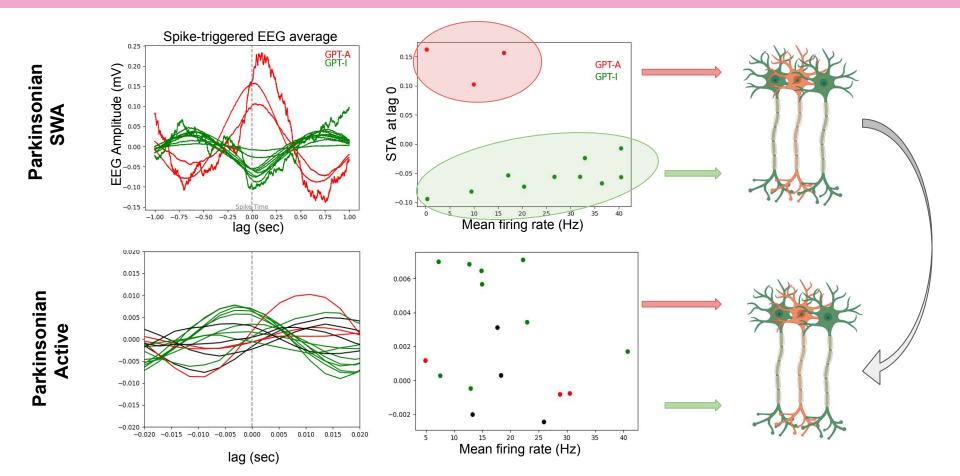




Doing so, we could see here that neurons classified as GPT-A during SWA state tended to fire just **before** the upward peak of EEG in Activ state. Conversely, GPT-I - just after.

In addition, when looking at the mean firing rate, it seems like GPT-A neuron fire at higher frequency during this state, but it is difficult to conclude because of the small sample size. This is actually one of the findings of Mallet and colleagues have done in 2012.

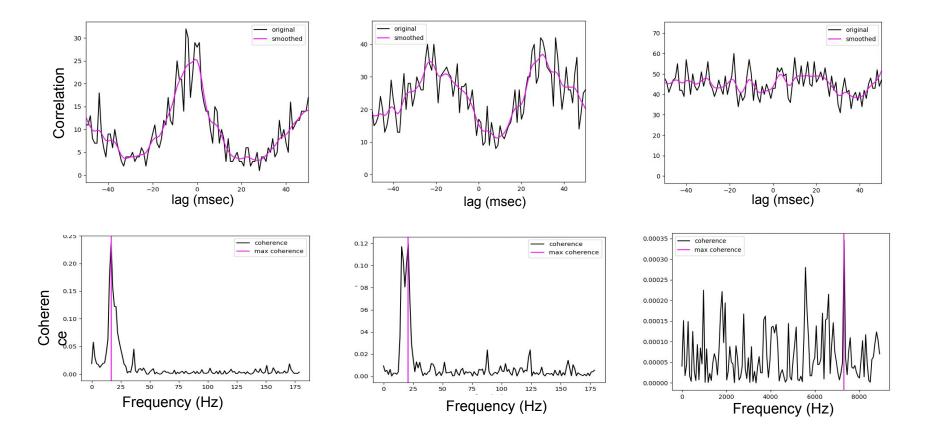




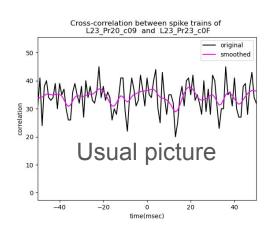
The End

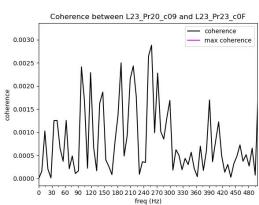
Supplementary

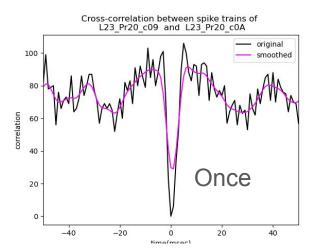
Suppl: Three types of the GPe neurons based on synchrony in parkinsonian rats?

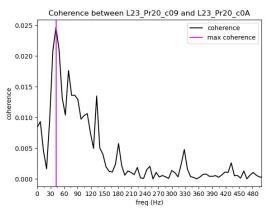


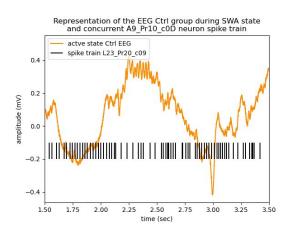
Suppl: beta synchrony is abnormal and there is no coherence observed at beta frequency between GPe neurons and between GPe neurons and EEG during SWA in Prkinsonian rats NOT REALLY TRUE

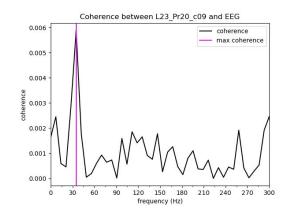




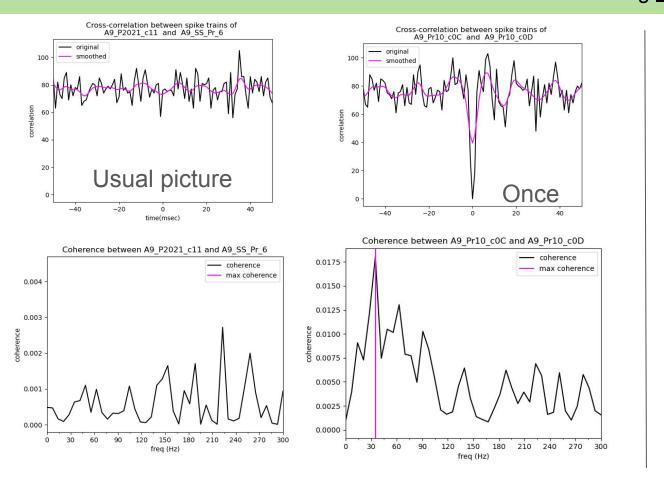




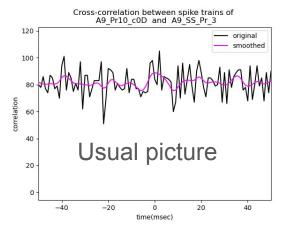


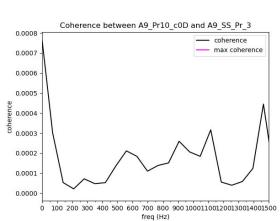


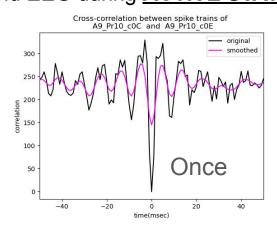
Suppl: beta synchrony is abnormal and there is <u>no coherence observed at beta frequency</u> between GPe neurons and between GPe neurons and EEG during **SWA in control rats**

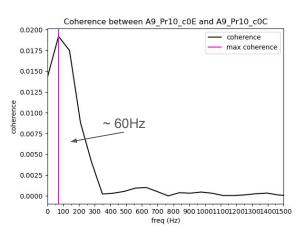


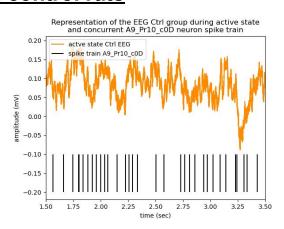
- **Supplished.** Committing that beta synchrony is abnormal,
 - 1. Is there any synchronization
- 2. The is no coherence observed at beta frequency between GPe neurons and between GPe neurons and EEG during **ACTIVE STATE in control rats**

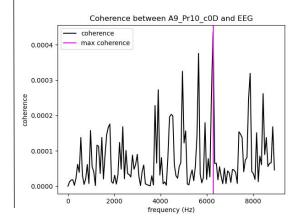






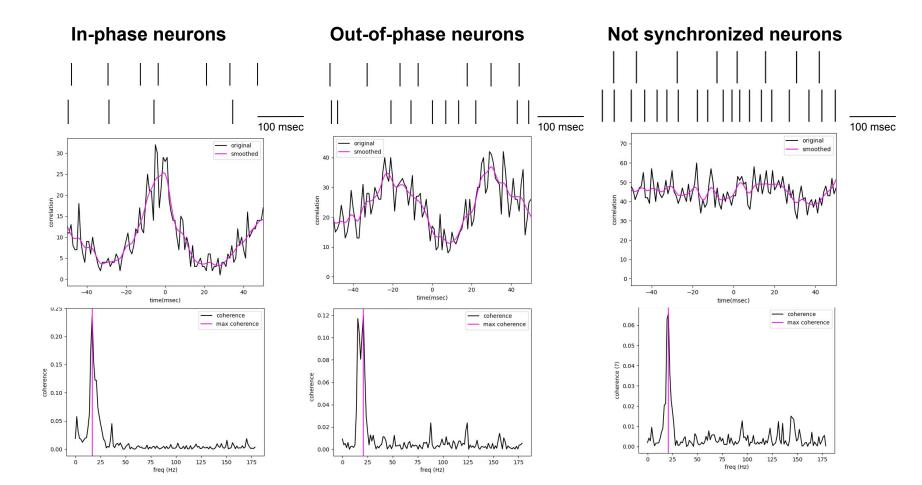




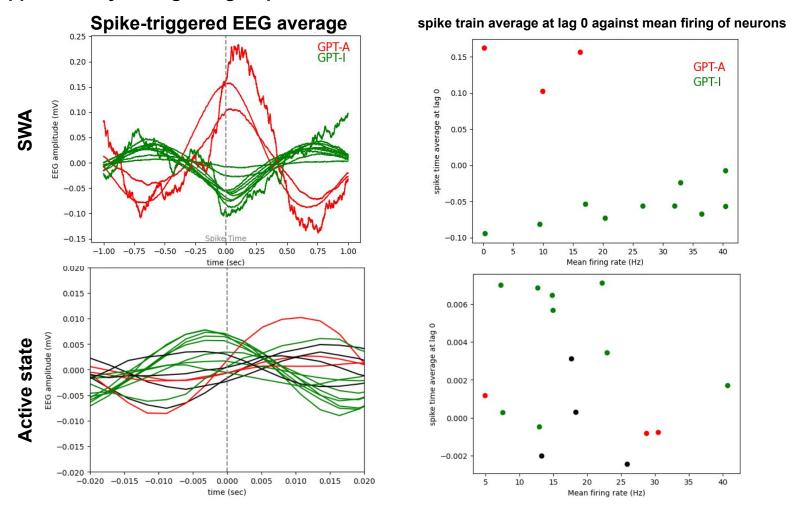


Suppl Figs

Supplementary for Fig2: tree groups of synchrony between spike trases



Supplementary for Fig3: 2 groups of cells of Parkinsonian model in SWA and Active states



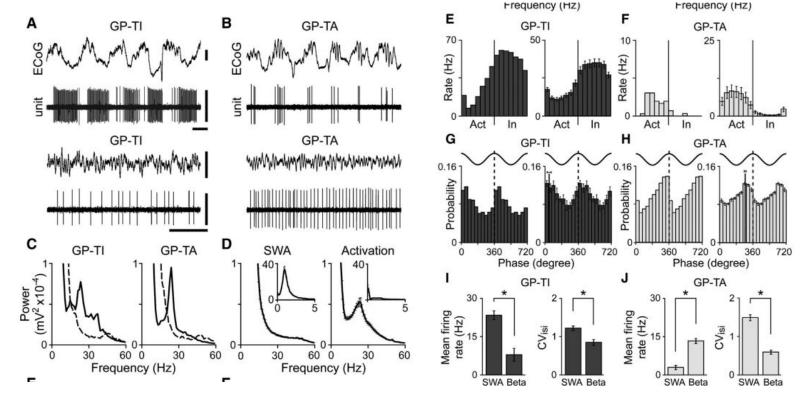


Figure 1. Dichotomous Firing Rates and Patterns of Identified Neurons in External Globus Pallidus

(A and B) Typical single-unit activities of the two types of GPe neuron in 6-OHDA-lesioned Parkinsonian rats. The same GP-TI neuron (A) and GP-TA neuron (B) were recorded during cortical slow-wave activity (SWA, above) and activation (below), as defined in electrocorticograms (ECoG). Note their inversely-related firing patterns and rates. Single neuron data in (E)-(H) are from these same neurons. Vertical scale bars: 250μV (ECoG), 2mV (units).

TA neurons sampled with the relatively high-impedance glass electrodes used here does not match that which we previously reported for recordings made with low-impedance multielectrode arrays (Mallet et al., 2008a). The use of high-impedance electrodes, which were advanced with submicron precision, meant that we were better able to target GPe units with very low firing rates, thus shifting the ratio more in favor of GP-TA

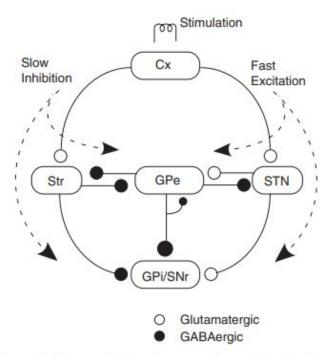
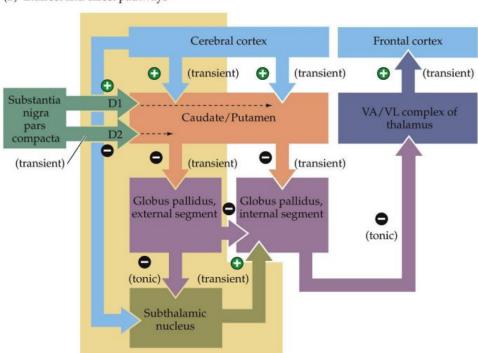
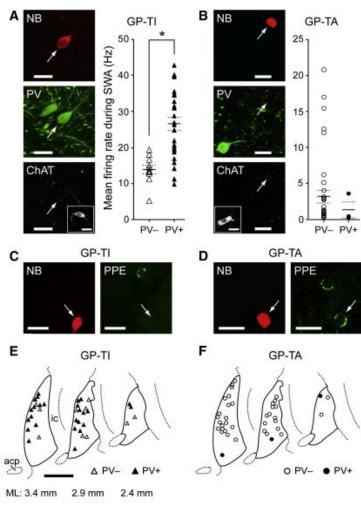


Fig. 1. A diagram of the major connections of the basal ganglia. The external segment of the pallidum (GP_e) receives major inputs from two input nuclei of the basal ganglia, the neostriatum (Str) and the subthalamic nucleus (STN). The internal segment of the pallidum (GP_i) and the substantia nigra pars reticulata (SNr) are the main output nuclei of the basal ganglia. The cortico (Cx)-Str-GP_i/SNr pathway is a slowly conducting inhibitory connection and the Cx-STN-GP_i/SN, pathway is a fast excitatory connection.

(Kita, 2007)

(B) Indirect and direct pathways





(Kita, 2007)

Figure 2. Electrophysiological Dichotomy of Identified Globus Pallidus Neurons Is Mirrored by Their Diverse Molecular Profiles

After recording, single units were juxtacellularly labeled with neurobiotin (NB) and tested for expression of various molecular markers.

- (A) Confocal fluorescence micrographs of a typical GP-TI neuron that expressed parvalbumin (PV) but not choline acetyltransferase (ChAT).
- (B) Typical GP-TA neuron that did not express either PV or ChAT. Most GP-TI neurons expressed PV (PV+), whereas most GP-TA neurons did not (PV-). All identified GPe neurons tested in this study were ChAT- (insets show ChAT+ ventral pallidal neurons in the same focal plane acting as positive controls).
 (A and B) Firing rates of individual PV+ and PV- GPe neurons during slow-
- (A and B) Firing rates of individual PV+ and PV- GPe neurons during slowwave activity. Group means ± SEM are indicated by solid and dashed lines, respectively. *p < 0.001 Mann-Whitney rank sum test.
- (C) GP-TI neurons did not express preproenkephalin (PPE).(D) GP-TA neurons expressed PPE.
- (E and F) Schematics of parasagittal sections of GPe (delineated by solid lines) illustrating the approximate locations of the somata of all recorded and identified PV+ and PV- neurons in this study. ML (mediolateral) numbers denote positions with respect to midline. acp, anterior commissure; ic, internal capsule.

Scale bars: (A–D) 20 μm; (E) 1.0 mm.

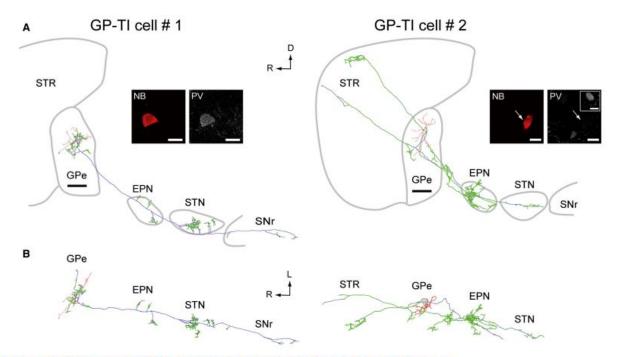


Figure 3. GP-TI Neurons Have the Prototypic Structure of Globus Pallidus Neurons

(A and B) Full reconstructions of two neurobiotin-labeled GP-TI neurons in parasagittal (A, medial view) and horizontal planes (B, dorsal view). Somata and dendrites are drawn in red, axons in blue, and axonal boutons in green. Each neuron was prototypic in its long-range axonal projections descending to the subthalamic nucleus (STN) and other basal ganglia nuclei (EPN, entopeduncular nucleus; SNr, substantia nigra pars reticulata). Each neuron also gave rise to extensive local axon collaterals in external globus pallidus (GPe), and some cells additionally innervated striatum (STR). Confocal fluorescence micrographs illustrate tests for co-localization of the neurobiotin (NB) signal with immunoreactivity for parvalbumin (PV). Inset in (A) shows another PV+ GPe neuron in the same focal plane as Cell #2 acting as a positive control.

(C) Three more identified GP-TI neurons, with only somata, dendrites, local axon collaterals, and proximal extrinsic projections reconstructed. Arrows indicate directions of long-range axonal projections as they exited GPe ("upstream" in black, "downstream" in blue).

Scale bars: 0.5 mm for all reconstructions, and 20 µm for all fluorescence micrographs. R, rostral; D, dorsal; L, lateral.

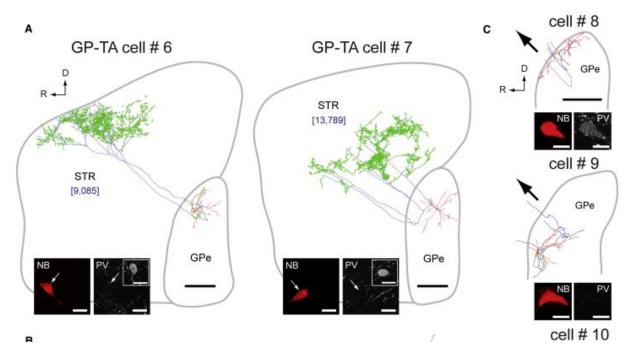
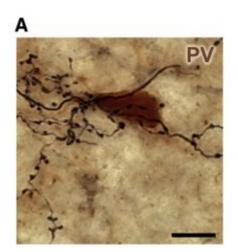


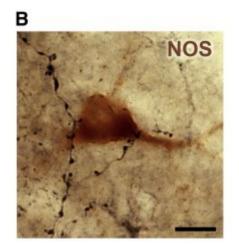
Figure 4. GP-TA Neurons Have Unique Structural Properties and Are a Novel Cell Type in the Globus Pallidus

(A and B) Full reconstructions of two neurobiotin-labeled GP-TA neurons in parasagittal (A, medial view) and horizontal planes (B, dorsal view). Somata and dendrites are drawn in red, axons in blue, and axonal boutons in green. The long-range axonal projections of each neuron provided a massive, dense, and specific innervation of striatum (STR). Numbers of axonal boutons in striatum are given in blue brackets. Confocal fluorescence micrographs illustrate tests for colocalization of the neurobiotin (NB) signal with immunoreactivity for parvalbumin (PV). Insets in (A) show other PV+ GPe neurons in the same focal planes acting as positive controls.

(C) Three more identified GP-TA neurons, with only somata, dendrites, local axon collaterals, and proximal extrinsic projections reconstructed. Black arrows indicate direction of long-range axonal projections as they exited GPe (only "upstream" to striatum).

Scale bars: 0.5 mm for all reconstructions, and 20 µm for all fluorescence micrographs. R, rostral; D, dorsal; L, lateral.





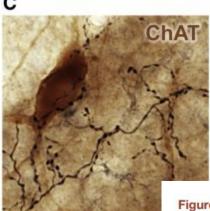


Figure 5. GP-TA Neurons Target Interneurons and Projection Neurons in Striatum

(A–C) Extended-focus light micrographs of GP-TA neuron axons, revealed by a black precipitate, in close apposition to the somata and proximal dendrites of striatal interneurons expressing parvalbumin (PV), nitric oxide synthase (NOS), or choline acetyltransferase (ChAT), as revealed by brown precipitate.

(D–F) Electron micrographs of GP-TA neuron axon terminals (asterisks) making symmetrical synaptic contacts (arrows) with the dendritic shafts of spiny striatal neurons (D and E) or the neck of a dendritic spine (F). The heads of spines in (D and F) formed asymmetrical synapses (double arrowheads) with unidentified axon terminals. Note that spines emanating from the dendrite in (E) were identified in serial sections.

Scale bars: (A-C) 10 μm; (D-F) 1 μm.

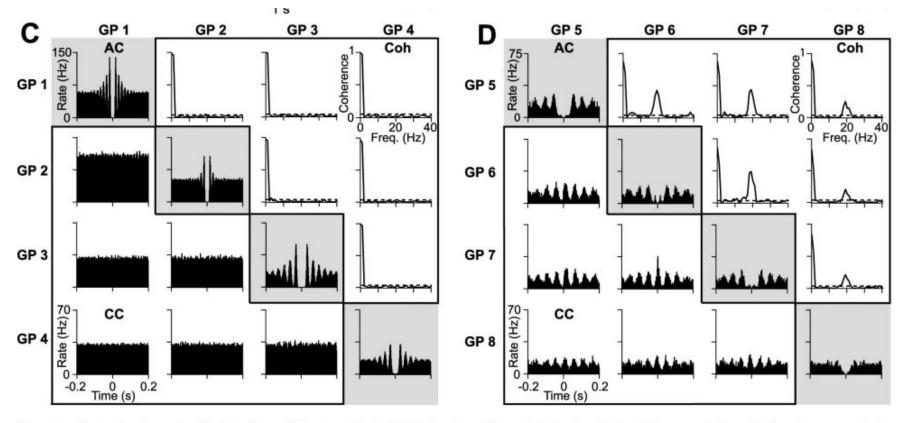
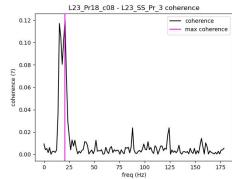


Figure 4. Single-cell and network activity in the globus pallidus of control and 6-OHDA-lesioned rats during cortical activation. **A, B,** Same GP neurons as in Figure 1 during subsequent epochs of activated brain states. Power spectra of field potentials in cortex (ECoG) and globus pallidus (GP-LFPs) show the excessive β oscillations (\sim 20 Hz) in the lesioned rat compared with the control. Calibration: 200 μ V (ECoG), 100 μ V (units). **C, D,** During activated state in control rats (**C**) pairs of GP units are typically not correlated (flat cross-correlograms; CC). Peaks and troughs in auto-correlograms (AC, 2 ms bins) indicate the fast oscillatory nature of single-cell firing. Coherence (Coh) values for pairs were typically below significance (p = 0.05, dashed line). In contrast, in lesioned rats (**D**), pairs of GP neurons tend to synchronize and coherence values peak at β frequencies (\sim 20 Hz).

figures



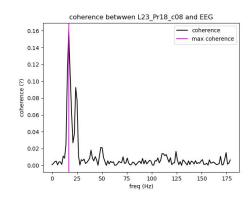
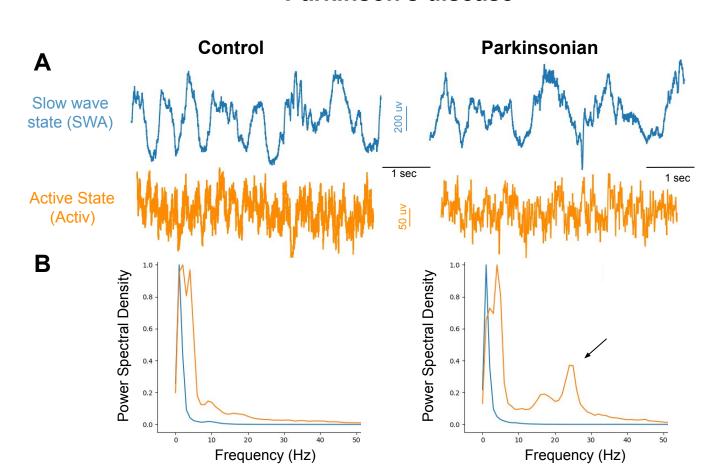


Fig1 : Beta frequency during EEG active state : a marker of Parkinson's disease



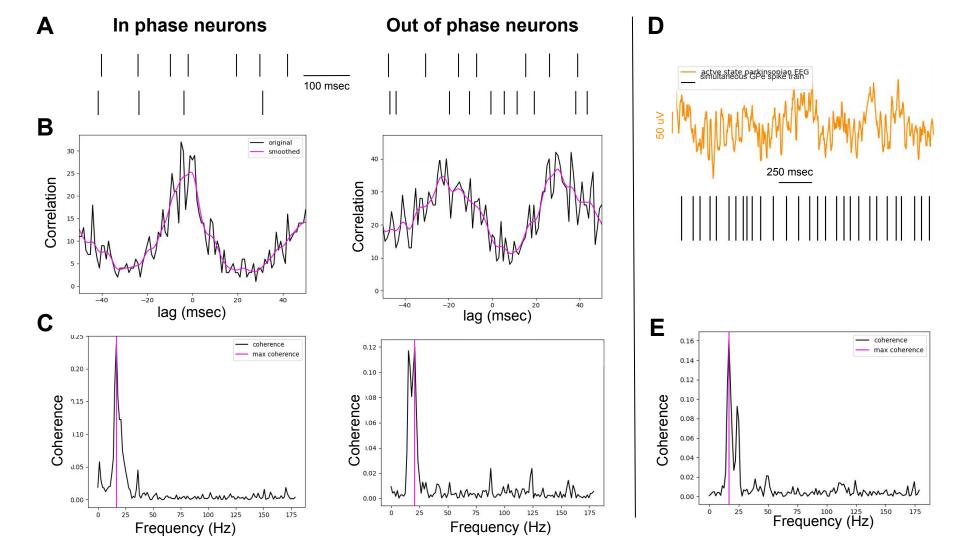
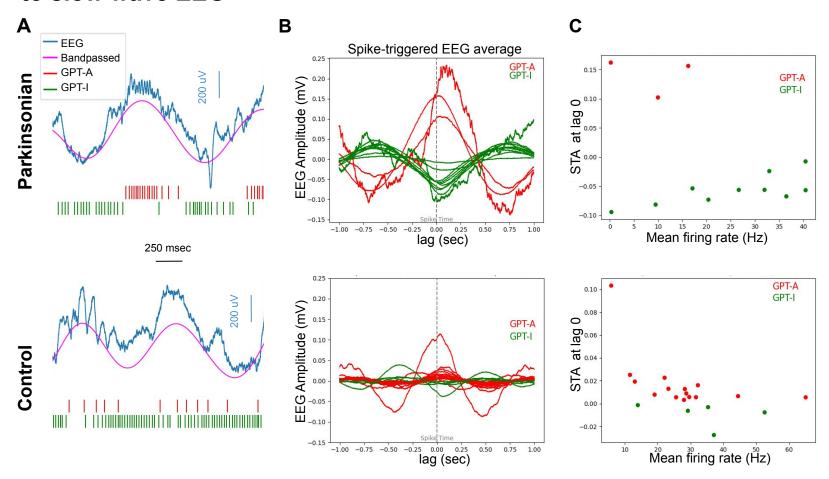


Fig3 : Classification of GPe neurons based on preferred firing phase relative to slow wave EEG



Parkinson's disease

