Doctoral Thesis

Correlated activity of pallidal neurons in awake monkeys in health and disease

Wongmassang, Woranan

Doctor of Philosophy
Completion in August 2019

Department of Physiological Sciences,
School of Life Science,
SOKENDAI

Contents

General introduction 4

Abbreviations 7

Part I 8

Abstract 9

Introduction 10

Methods 12

Results 18

Discussion 21

Figures and legends 23

Part II 35

Abstract 36

Introduction 38

Methods 40

Results 43

Discussion 46

Figures and legends 48

Part III 54

Abstract 55

Introduction 56

Methods 57

Results.....59

Discussion 61

Figures and legends 64

Conclusion.....68

References 70

General introduction

The basal ganglia (BG) play an essential role in controlling voluntary movement and posture. The BG receive inputs from the cerebral cortex and project back to the original cortex through the thalamus. The output nuclei of the BG are the internal segment of globus pallidus (GPi) and the substantia nigra pars reticulata (SNr). According to the basic circuits of the BG, the motor cortical inputs are transmitted to the output nuclei of the circuit via the three pathways: that is *hyperdirect, direct*, and *indirect pathways* (Nambu 2011) (Fig.1). In the *hyperdirect* pathway, the subthalamic nucleus (STN) relays direct inputs from the cerebral cortex to the GPi/SNr. The *direct* pathway originates from the striatum (Str) and projects to the GPi/SNr. The *indirect* pathway originates from the Str projects to the GPi/SNr via the external segment of globus pallidus (GPe) and STN. Thus, the GPe can be considered as a connecting nucleus within the indirect pathway, while the GPi is the output nucleus of the BG.

Recent studies have emphasized the importance of synchronized neuronal activity in information processing in the brain circuits, such as the cerebral cortex and cerebellum (Person & Raman 2012; Rosenbaum et al. 2014; Salinas & Sejnowski 2001; Singer 1993). On the other hand, previous studies in the BG have reported that the lack of GPe/GPi neuronal correlated activity in monkeys (Nini et al. 1995; Raz et al. 2000). In these studies, the correlated neuronal activity in the BG of monkeys at rest was examined, but the activity during movements has not been studied yet. The GPe/GPi

neuronal activity of monkeys showed evident neuronal discharge changes with limb movements (DeLong 1971; Hamada et al. 1990; Mushiake & Strick 1995). In addition to the discharge rate changes, correlated activity may also convey movement-related information (de la Rocha et al. 2007). In Part I, to answer this question, I simultaneously recorded multiple neurons in the GPe/GPi of monkeys during hand reaching movement by using the multi-channel electrodes and analyzed the cross-correlation of spike trains.

Alternation of activity through BG circuit causes the movement disorders such as Parkinson's disease (PD) and dystonia (Blandini et al. 2000; Brown 2007; DeLong & Wichmann 2007; Miguelez et al. 2012). The electrophysiological studies of PD model monkeys have shown that oscillatory and synchronized activity is observed in the GPe and GPi (Bergman et al. 1998; Nini et al. 1995). The non-synchronized independent activity of GP neurons in normal state became the oscillatory and synchronized activity in PD state. The emergence of GP oscillation could be due to the changes in the intrinsic properties and/or the altered network connection of the BG circuit. However, their study was conducted only in monkeys during resting state, because the monkeys exhibited severe PD symptoms and could not perform behavioral tasks. The correlation of GPe/GPi neuronal activity of PD monkeys during movements has not been studied yet. It is possible that movement-related oscillatory and synchronized activity may disturb execution of neuronal voluntary movements. In Part II, to address this issue, I generated a monkey model of mild PD using 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a neurotoxin selective to dopaminergic neurons. Then, the activity of multiple neurons in the GPe/GPi

was simultaneously recorded during hand reaching movement, and the cross-correlation of spike trains was analyzed.

To clarify pathophysiology of PD, it is necessary to investigate relationship between abnormal neuronal activity and PD symptoms. Dopamine replacement therapy using the dopamine precursor, L-dihydroxyphenylalanine or L-dopa, is the main standard treatment for PD patients (Hornykiewicz 2010). Next, I would like to examine causal relationship between oscillatory/synchronized activity in the GPe/GPi and PD symptoms by combining dopamine replacement therapy, which alleviated PD symptoms, and electrophysiological recording of GPe/GPi neurons. In Part III, I administrated L-dopa into a severe PD monkeys induced by injection of MPTP, and examined the oscillatory firing and cross correlated activity of GPe/GPi neurons before and after L-dopa treatment.

Abbreviations

BG,	Basal ganglia
CSD,	Cross spectral density
Cx,	Cortex
FR,	Firing rate
GABA,	Gamma-aminobutyric acid
GPe,	The external segment of globus pallidus
GPi,	The internal segment of globus pallidus
Hz,	Hertz
L-dopa,	L-dihydroxyphenylalanine
LED,	Light-emitting diode
M1,	Primary motor cortex
MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
ms,	milli-second
PD,	Parkinson's disease
PEEK,	polyether ether ketone
PETH,	Peri-event time histrogram
PSD,	Power spectral density
SMA,	Supplementary motor area
SNc,	Substantia nigra pars compacta
SNr,	Substantia nigra pars reticulata
STN,	Subthalmic nucleus
Str,	Striatum
Th,	Thanlamus

Part I

Correlation of GP neuronal activity

during task performance in normal monkeys

Abstract

The basal ganglia (BG) play a crucial role in control of voluntary movements. There are many reports on movement-related activity in the internal (GPi) and external (GPe) segments of the globus pallidus: the former sends BG outputs to the thalamo-cortical and brainstem motor systems, and the latter projects to many areas of the BG and may control the whole BG activity. Thus, the modifications of GPe/GPi neuronal activity affect the thalamic activity and control of voluntary movements. On the other hand, task-related correlated activity among neurons has yet to be examined in the BG. In the present study, I simultaneously recorded multiple neurons in the GPe/GPi during a performance of a simple hand reaching task from two female Japanese monkeys (*Macaca fuscata*) by using the multi-channel electrodes and analyzed their spike correlation. Following results have been obtained. (1) GPe/GPi neurons responded to the cortical stimulation through chronically implanted electrodes in the forelimb regions of the motor cortex, and their response was mainly composed of early excitation, inhibition and late excitation. (2) GPe/GPi neurons changed their activities in relation to reaching movements during task performance. (3) However, only a limited number of neurons showed significant correlations during reaching movements. These results suggest that GPe/GPi neurons in normal monkeys encode movement-related information as the population firing rate but not as the correlated activity.

Introduction

The globus pallidus (GP) is a part of basal ganglia (BG) circuit, which is essential for controlling voluntary movement and posture. In primates, the GP is divided by the medial medullary lamina into the internal (GPi) and external (GPe) segments. Previous experiments have revealed that these two segments show different firing patterns and may have different functions. In normal primates at rest, GPe neurons fire spontaneously at high frequencies with pauses, whereas GPi neurons fire continuously without any pauses (DeLong 1971). According to the basic circuits of the BG (Fig.1), the motor cortical inputs are transmitted to the output nuclei of the BG, the GPi and substantia nigra pars reticulata (SNr), via three pathways: That is hyperdirect, direct, and indirect pathways (Nambu 2011). In the hyperdirect pathway, the subthalamic nucleus (STN) relays direct inputs from the cerebral cortex to the GPi/SNr. The direct pathway originates from the striatal neurons containing γ-aminobutyric acid (GABA) and substance P, which mono-synaptically project to the GPi/SNr. The indirect pathway originates from striatal neurons containing GABA and enkephalin, which polysynaptically project to the GPi/SNr via the GPe and STN. Thus, the GPe can be considered as a connecting nucleus within the indirect pathway, and affects GPi activity through the STN. Thus, the modifications of GPe/GPi neuronal activity affect thalamic and cortical activity and contribute to controlling voluntary movement.

The GPe/GPi neurons of monkeys showed evident phasic discharge change with limb movements (DeLong 1971). Other studies also showed close relation of the GPe/GPi discharge changes to active hand movements in monkeys (Hamada et al. 1990; Mushiake & Strick 1995). In addition to the discharge rate changes, correlated activity is proposed to convey neuronal information or neuronal code in other brain areas (de la Rocha et al. 2007). The previous study has revealed that GPe/GPi neurons do not show correlated activity at rest (Raz et al. 2000). However, the correlation of GPe/GPi neuronal activity during movements has not been studied. To address this question, I simultaneously recorded activity of multiple neurons in the GPe/GPi during a hand reaching task and analyzed the cross-correlation of spike trains.

Methods

1. Animal preparation

Two female Japanese macaque monkeys (monkey H and L) weighting 5-6 kg, were used for this study. The experimental protocols were approved by the Institutional Animal Care and Use Committees of National Institutes of Natural Sciences, and all experiments were conducted according to the guidelines of the National Institutes of Health Guide for Care and Use of Laboratory Animals. Monkeys were trained daily to sit in the monkey chair quietly during the experimental recording described below. They were given *ad libitum* access to food and water in the home cage.

2. Hand reaching task

Prior to the experiments, each monkey was trained to sit quietly in a monkey chair and to perform a hand reaching task (Fig.3). Monkey used a hand contralateral side to the recorded brain hemisphere. An infrared optical imaging touch panel (ARTS-015, O'HARA, Tokyo) was placed in front of the monkey, and two light-emitting diodes (LEDs) were arranged horizontally and separated by 16 cm on the touch panel. Each trial was initiated after the monkey placed its hand onto the home position at least for 300 ms. When the left or right LED was randomly lit for 500 ms, the monkey was required to release its hand from the home position and reach out to the target area indicated by the LED. The timings of hand release and touch were detected by infrared photoelectric sensors (PS-46, PS-52C, FU-A100,

Keyence, Osaka), and the position of touch was detected by the touch panel. If the monkey touched the target area within 6000 ms and hold at least for 40 ms, the trial was considered successful and a small amount of juice was dispensed as reward with a delay period of 200 ms. The LED target on the same side as the reaching hand was named as ipsilateral target LED, whereas the opposite side of LED target was named as contralateral target LED.

3. Surgical procedures

After learning the hand reaching task, the monkeys received surgical operation to fix their heads painlessly in a stereotaxic frame that was attached to a monkey chair. After general anesthesia with ketamine hydrochloride (10 mg/kg body weight, i.m.) and xylazine hydrochloride (1-2 mg/kg, i.m.), propofol was continuously injected intravenously during surgery using target-controlled infusion (TCI) pump (TE-371, Terumo; 6-9 μg/mL target blood concentration) with fentanyl administrations (2-5 μg/kg, i.m.). Each monkey was positioned in a stereotaxic apparatus and the skull was widely exposed. Small screws made of polyether ether ketone (PEEK) were attached to the skull as anchors. The exposed skull and screws were completely covered with transparent acrylic resin, two PEEK pipes were mounted in parallel over the frontal and occipital areas for head fixation. A titanium pin was fixed on the skull at 50 mm dorsal to the interaural midpoint a stereotaxic reference point. All surgical procedures were performed under aseptic conditions, and arterial oxygen saturation and heart rate were continuously monitored. Antibiotics and analgesics (ketoprofen) were injected (i.m.) after surgery.

4. Implantation of stimulating electrodes in the cerebral cortex

A few days after the head-fixation surgery, each monkey was positioned in a stereotaxic apparatus with its head restrained using the PEEK pipes. Under anesthesia with ketamine hydrochloride (5 mg/kg body weight, i.m.) and xylazine hydrochloride (1 mg/kg, i.m.), the skull over the primary motor cortex (M1) and the supplementary motor area (SMA) contralateral to the hand for the task performance was removed. According to the electrophysiological mapping, two pairs of bipolar stimulating electrodes made of 200-μm-diameter Teflon-coated stainless steel wires (inter-tip distance, 2 mm) were chronically implanted into the forelimb region of the M1, and one pair into the forelimb region of the SMA (for detail, see Nambu et al. 2000). Exposed areas were covered with transparent acrylic resin with the exception of M1 area (10-15 mm in diameter) for accessing to the GPe and GPi. A rectangular plastic chamber that covered the exposed brain area was fixed onto the skull with acrylic resin. (Fig.2).

5. Multi-channel recordings of GPe/GPi activity

GPe and GPi neuronal activity was record 2 or 3 days per week, 4-7 days after implantation of the stimulating electrodes, for several months. During the experimental sessions, the body weight, activity of daily living and food intake of the monkeys were routinely monitored. The multi-channel recording electrodes (Plextrode U-Probe; Plexon Inc), consisting of 16 contacts (275 ± 50 kΩ) in linear formation (the inter-contact spacing was 150 μm) were used (Fig.4). Total length of recording areas was 2.25 mm. The electrode was inserted obliquely (40 degrees from the vertical in the

frontal plane) through a guide tube (OD 570 μm, ID 450 μm) into the GPe and GPi contralateral to the hand for the task performance using a hydraulic microdrive (MO-971-S, Narishige Scientific Instrument, Tokyo), and neuronal activity during task performance was recorded. When penetrating the dura, lidocaine was applied as the local anesthetics. Signals from each channel was amplified, sampled at 25 kHz, and stored using a multichannel recording system (The RZ2 BioAmp Processor, Tucker Davis Technologies, USA). Following data were obtained from each neuron: (1) spontaneous activity, (2) activity in response to cortical stimulation (0.5-0.7 mA, 0.3 ms duration, single pulse at 0.7 Hz) through the chronically implanted electrodes in the M1 and SMA, and (3) activity during performance of a hand reaching task.

6. Data analysis

Multi-channel recording data were analyzed off-line to isolate spike events of individual neurons using OpenSorter software (Tucker-Davis Technologies, TDT Co., FL, USA). Autocorrelogram was constructed from the digitized data to evaluate isolation quality. If evidence of multiple cells or inclusion of noise was found, the unit was re-isolated or excluded from analysis. For analysis of response to cortical stimulation, peri-stimulus time histograms (PSTHs, bin width of 1 ms) were constructed for the 60 stimulation trials. The mean value and standard deviation (SD) of the firing rate (FR) during 100 ms preceding the onset of stimulation were calculated from a PSTH. Changes in the firing activity in response to the stimulation (i.e. excitation and inhibition) were judged to be significant if the FR during

at least two consecutive bins (2 ms) reached the statistical level of $p < 0.05$ (one tailed *t*-test).

For analysis of peri-event time histogram (PETH), GPe/GPi unit activity during task performance was aligned with the task events, such as "LED on", "hand release" (from the home position) and "touch" (the corrected target LED). The mean FR was calculated from the 100 ms before LED on and the confidential interval was calculated assuming the z-distribution.

The correlated activity and spectral properties of neural spiking were analyzed by expressing a spike train as a sequence of 0s and 1s where 1 represents an action potential in 1 ms bin. Then, the binary sequences were used for cross-correlation analysis. Emergence of the spiking correlation of neuronal pairs during the task performance was examined with permutation tests, where a 2-step statistical method was applied under the null hypothesis of no correlated activity.

In the first step, the cross correlation of 100 ms duration before and after the task event timings, that is, from -100 ms to 0 ms ("pre-event period") or from 0 ms to 100 ms ("post-event period"), were calculated and averaged across trials. The expected cross correlation under the null hypothesis was obtained by randomly rearranging the trial number of one neuron. Repeating the rearrangement for 1000 times and sorting correlation scores at each lag time from the smallest to the largest, the confidence interval under the null hypothesis was estimated; the neuronal pair was considered to have a significant negative correlation at a lag time if the original correlation score was smaller than the 3rd of the expected cross

correlations, and a significant positive correlation if larger than the 998th, roughly corresponding to $p < 0.005$.

Since the statistical analysis was performed each lag time from -3 to 3 ms, false positives due to multiple comparisons were predicted. Hence, neuronal pairs were further examined at the lag time and the event period with which the significant correlation appeared. In the second step, correlated spiking events constituting the significant correlation score in the first step were visualized as a PETH with 1 ms bin. If the lag time was 0 ms, coincident spiking events were used for the PETH; for the non-zero lag time, spiking events in which spikes of neuron 1 preceding those of neuron 2 by the lag time were used. Similar to the first step, trial number of one neuron was randomly rearranged for 1000 times to calculate the permuted PETH, and the significant deviation of the original PETH was examined during the same task period, that is, 100 ms before or after the task event timing. Only neuronal pairs that rejected the null hypothesis in both the first and second steps were considered to have the correlated activity during the task performance.

Results

1. Neuronal activity evoked by cortical stimulation

Among well-isolated neurons, M1 and/or SMA stimulation induced responses in 139 GPe (monkey H, 98; monkey L, 41) and 79 GPi (monkey H, 39; monkey L, 40) neurons. The mean spontaneous firing rate of GPe neurons was 71 ± 29 and 91 ± 28 Hz (monkey H and L respectively), and that of GPi neurons was 75 ± 28 and 100 ± 26 Hz. As both the GPe and GPi have a clear somatotopic organization (Nambu 2011). The responses evoked by the stimulation of the forelimb regions of the M1 and/or SMA ensure that the recorded GP neurons receive inputs from these cortical areas. The response pattern was typically a triphasic response composed of early excitation followed by inhibition and late excitation as previously reported (Fig.6) (Nambu et al. 2000). These components are mediated by the *hyperdirect, direct* and *indirect* pathways of the BG circuit, respectively (References; Nambu et al. 2000, 2002).

2. Neuronal modulation during task performance

Neuronal activity of GPe and GPi neurons was observed during task performance and represented as PETHs (Figs.7 and 8). These neurons showed either increase or decrease of their firing rate during reaching to both ipsilateral and contralateral LED targets. While both GPe and GPi showed movement-related modulations (Figs.9 and 10), more GPe neurons were modulated during hand release in both ipsilateral LED target trials (47% and

56% in GPe versus 31% and 35% in GPi for monkey H and L, respectively) and contralateral LED target trials (58% and 54% in GPe versus 41% and 40% in GPi); the same tendency was observed during touch in both ipsilateral LED target trials (59% and 59% in GPe versus 51% and 53% in GPi) and contralateral LED target trials (63% and 51% in GPe versus 41% and 58% in GPi). When noticed the majority of the modulation, the FR increasing was obviously both during hand release and touch.

3. Cross-correlogram during task performance

The simultaneously recorded GPe/GPi neuronal pairs (187 GPe-GPe, 62 GPe-GPi and 71 GPi-GPi pairs) from the same recording sessions were examined for the possible correlated activity during each task performance period. Figure 11 shows an example of a GPe-GPe neuronal pair from monkey H simultaneously recorded from channels 2 and 9 during reaching to the ipsilateral target LED. Cross-correlograms between this neuronal pair were constructed for each period of the reaching task. Small but significant correlation was observed around lag time -3 ms before the touch period. Then correlated spike events of this neuronal pair were further examined with the specific lag time (Fig.12). The correlated spike events were increased above the chance level before touch period ($p < 0.005$, a permutation test; see methods for details). This neuronal pair was considered to have significant positive correlated activity before touching the target.

Among of all neuronal pair types, only small numbers revealed the correlated activity during task events (Fig.13) either before LED on (7, 3, 3 pairs for positive versus 1, 0, 0 pairs for negative correlation, respectively),

LED on (10, 0, 3 pairs for positive versus 1, 0, 1 pairs for negative correlation, respectively), hand release (6, 5, 1 pairs for positive versus 1, 1, 0 pairs for negative correlation, respectively) or touch (14, 8, 3 pairs for positive versus 1, 0, 1 pairs for negative correlation, respectively).

Discussion

Correlated neuronal activity among populations of neurons has been observed in many brain regions and is suggested to play crucial role in information processing through the neural networks (Rosenbaum et al. 2014; Salinas & Sejnowski 2001; Singer 1993). For example, frontal cortical neurons altered their correlated activity without apparent changes of firing rates during motor task performance especially between the neighboring neurons (Vaadia et al. 1995). In the cerebellum, synchronous inhibitory postsynaptic potentials of small number of Purkinje cells successfully generated time-locked action potentials of cerebellar nuclear neurons, whereas asynchronous inputs suppressed their spiking (Person & Raman 2011). Thus, not only glutamatergic neurons in the cortex but also GABAergic neurons in the cerebellum present the correlated activity during transaction.

In the GP, a previous study has examined the correlation of neuronal activity during resting state in non-human primate and has reported lack of significant correlated activity. (Nini et al. 1995). In the present study, I examined whether correlated neuronal activity of GPe/GPi neurons increases during hand reaching task. I found that a limited number of GP neuronal pairs exhibited correlated activity either at rest or during movement periods even they received common cortical inputs and showed movement related activity. The following mechanisms may be underlying correlated GP activity: 1) Common inputs from the striatum or STN, 2) the local axon collaterals in the

GPe or the GPe-GPi projection. The weak correlated activity in the GP suggests the parallel information processing that movement-related neuronal information is parallely and independently processed in the GPe and GPi.

Based on the anatomical connections, GPe/GPi neurons received the afferent from the striatum and STN. The number of neurons in the striatum far exceeds that in the GPe/GPi, thus each GPe/GPi neuron receives inputs from different striatal neurons (Flaherty & Graybiel 1993). This mechanism may underlie the low correlated activity among the GPe/GPi neurons.

Figures and legends

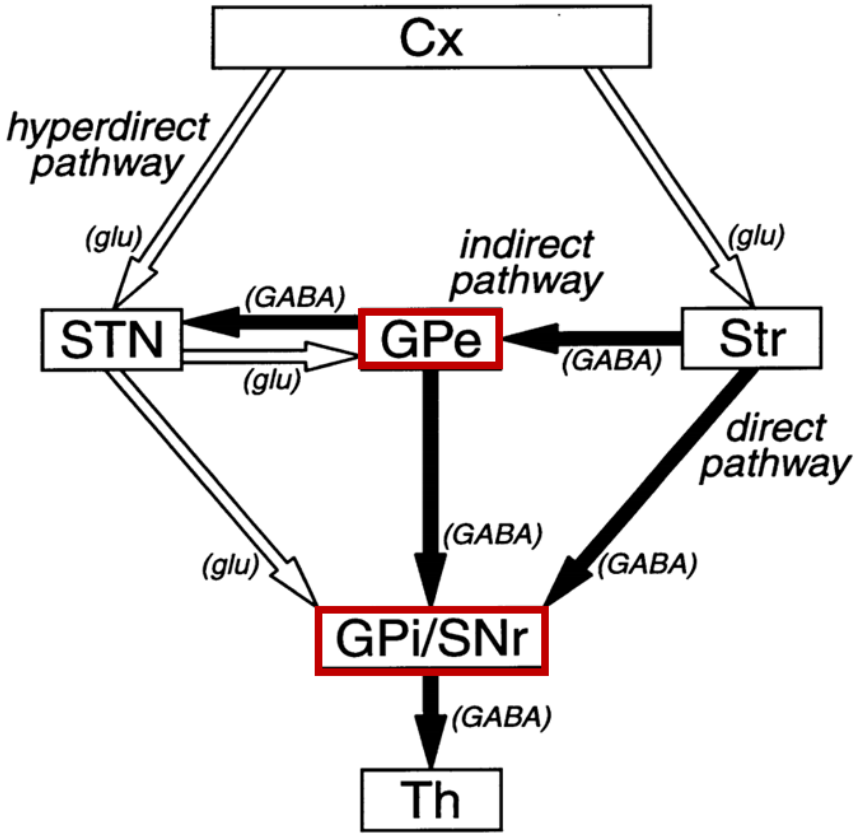


Figure 1: Schematic illustration of the basic circuitry of the basal ganglia. The basal ganglia circuit consists of the *hyperdirect* (Cx-STN-GPi/SNr), *direct* (Cx-Str-GPi/SNr) and *indirect* (Cx-Str-GPe-STN-GPi/SNr) pathways. Open and filled arrows represent excitatory glutamatergic (glu) and inhibitory GABAergic (GABA) projections, respectively. The red boxes represent GPe/GPi in the pathways. Cx, cerebral cortex; GPe, external segment of the globus pallidus; GPi, internal segment of the globus pallidus; SNr, substantia nigra pars reticulata; STN, subthalamic nucleus; Str, striatum; Th, thalamus (Nambu et al., 2000).

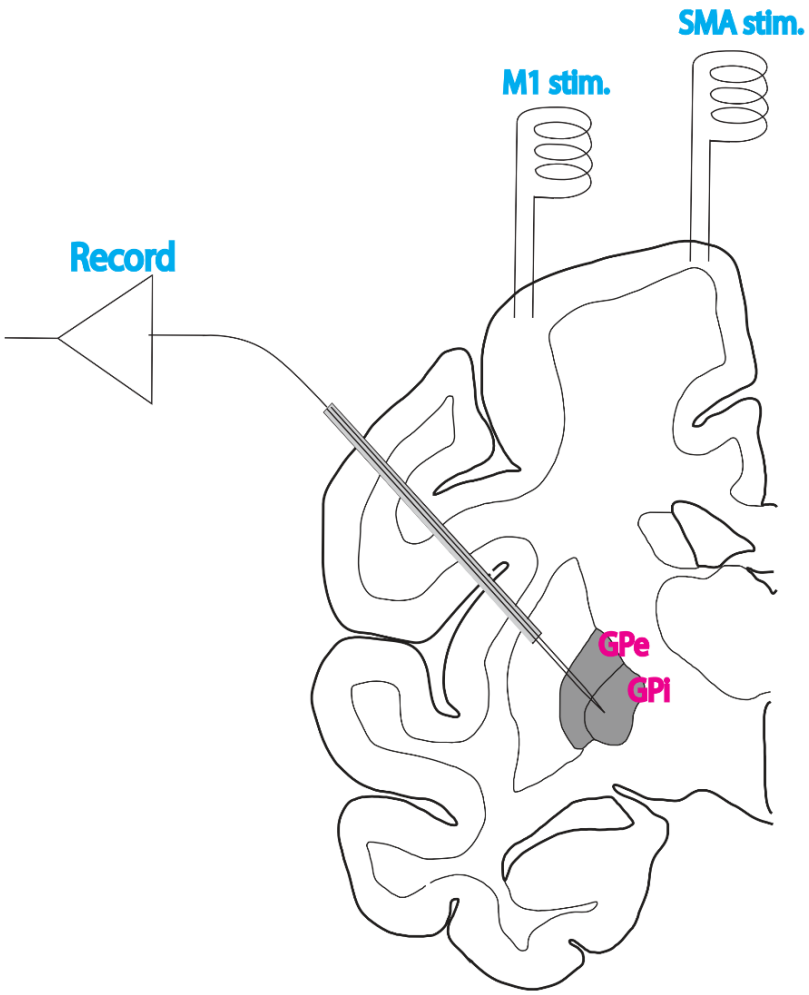


Figure 2: Schematic representation of the experimental setup for recording neuronal activity in the external (GPe) and internal (GPi) segments of the globus pallidus. The skull over the primary motor cortex (M1) and the supplementary motor area (SMA) was removed. Bipolar stimulating electrodes were implanted chronically in the forelimb regions of M1 and SMA. A multiple channel recording electrode was inserted obliquely (40 degrees from vertical) through the guide tube into the GPe or GPi for extracellular recording during the task performance.

Figure 3: Schematic representation of a hand reaching task. A Japanese macaque monkey was trained to sit on a monkey chair quietly and perform a hand reaching task. The task was initiated when monkey placed its hand on the home position for 300 ms (Start Period). Then, the green LED was lit in the left or right side of the touch screen (LED On Timing). The monkey was required to release its hand from the home position and touch the target indicated by LED for 40 ms. If the monkey touches the target within 6000 ms, it received water (Reward) after a delay period of 200 ms (Holding Period, Reward Timing).

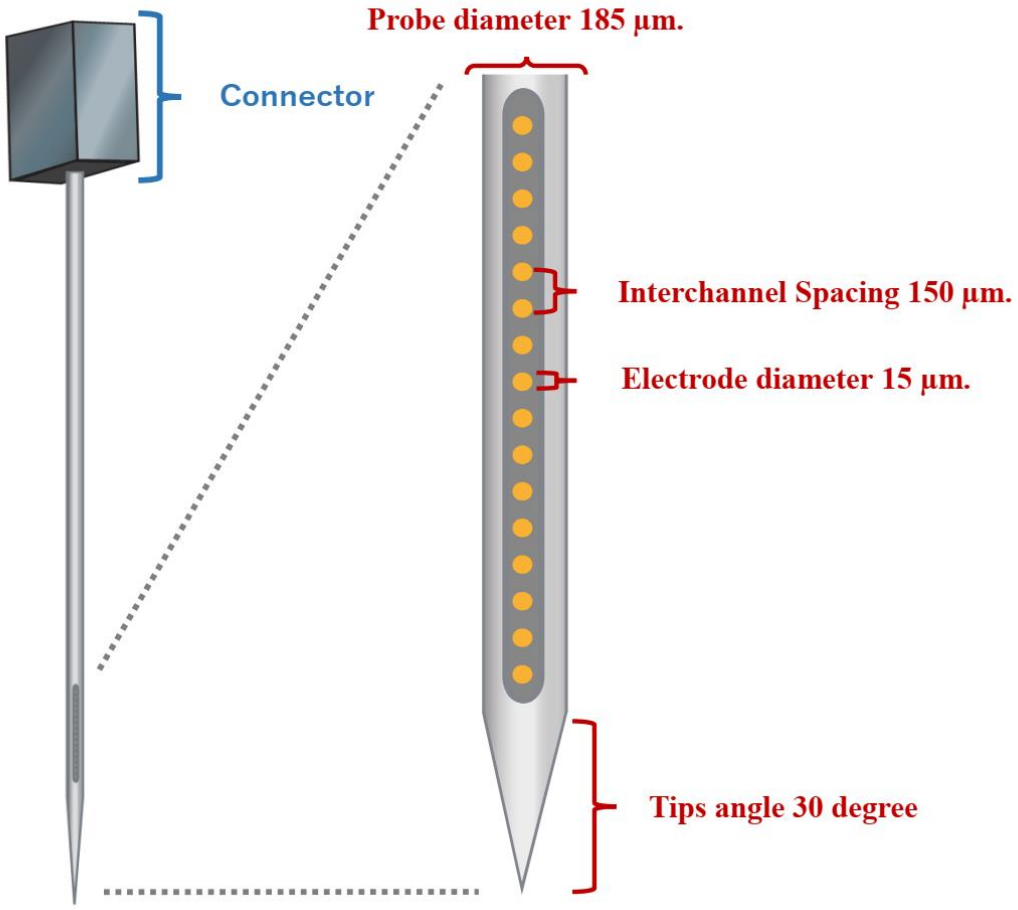


Figure 4: Schematic representation of the multi-channel electrode. This electrode was used to record pallidal activity during task performance. The electrode had 16 contacts linearly arranged with 150 µm inter-channel spacing. (Electrode diameter = 15 µm, Probe diameter = 185 µm, Impedance 275 ± 50 kΩ, distance from tip to the first channel = 500 µm, tip angle = 30 degree).

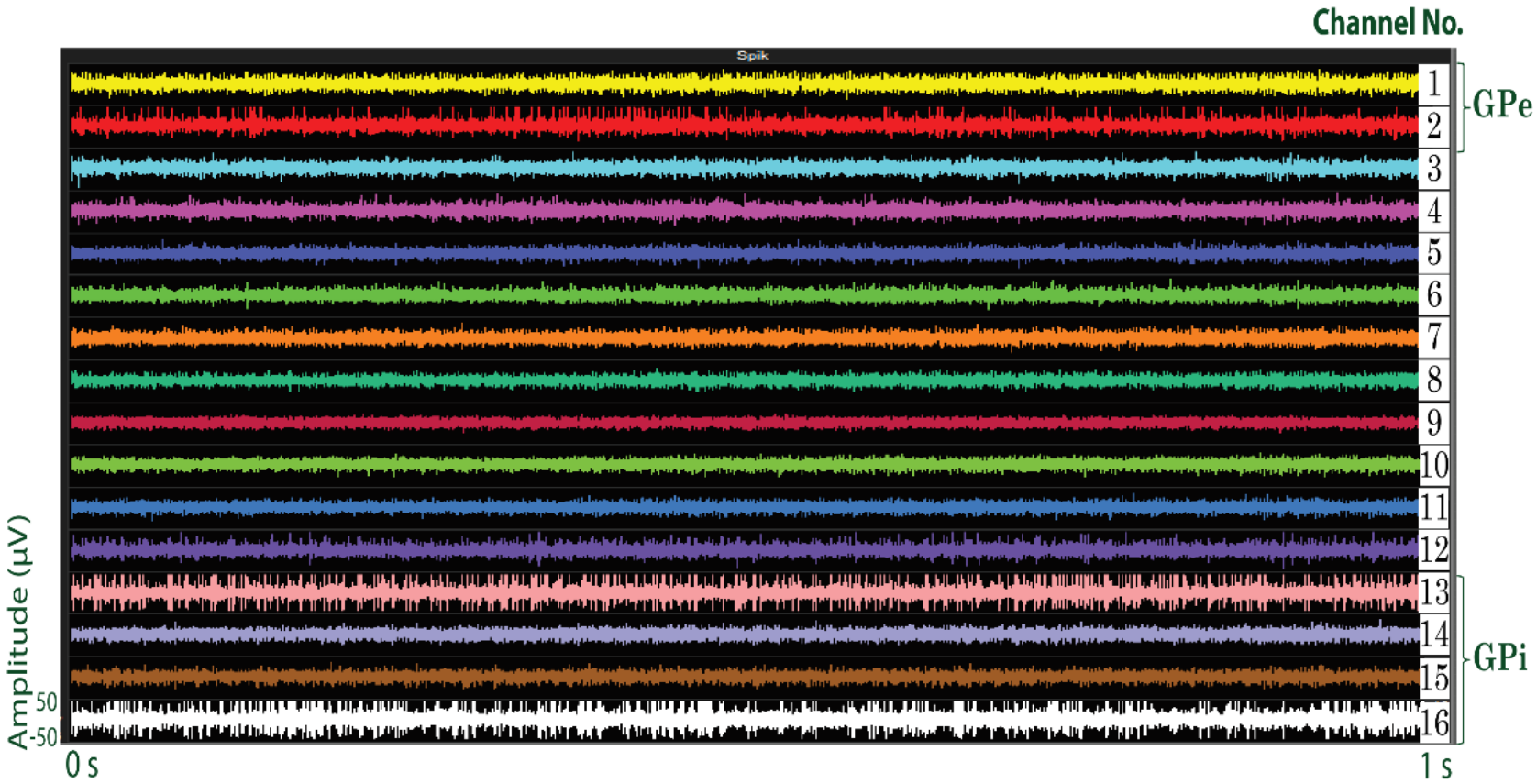


Figure 5: An example of raw data of GPe-GPi activity recorded simultaneously from 16 channels electrode. Ch 1-2 were located in the GPe, and Ch 13-16 were in the GPi. Data from channel numbers 2, 13 and 16 show significant neuronal activity.

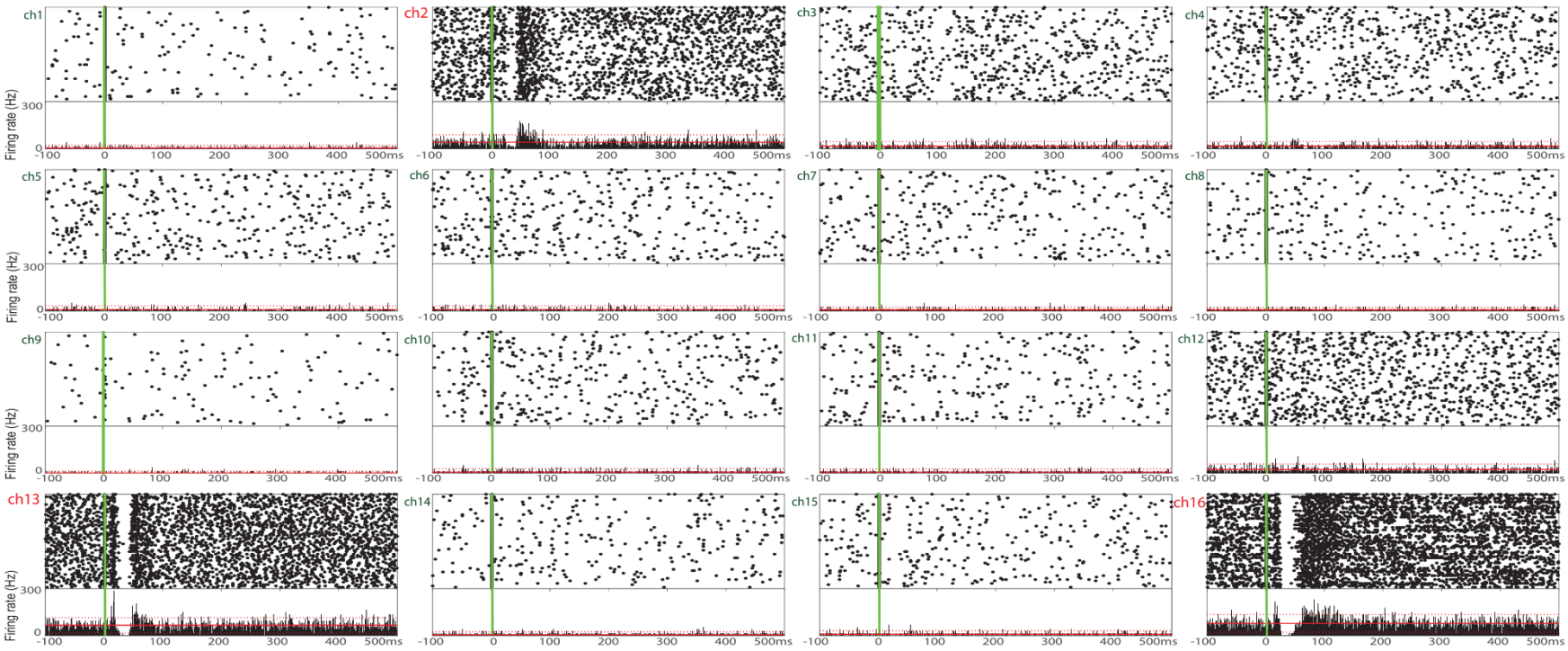


Figure 6: An example of cortically evoked responses. SMA stimulation was delivered (at time 0, green vertical line, 0.3 ms duration, 0.5 mA, single pulse) to neurons shown in Figure 5. Neurons in channel numbers 2, 13 and 16 exhibited a triphasic response composed of early excitation, inhibition and late excitation. The blue horizontal continuous and dotted lines represent the mean firing rate and the statistical level of p = 0.05, respectively.

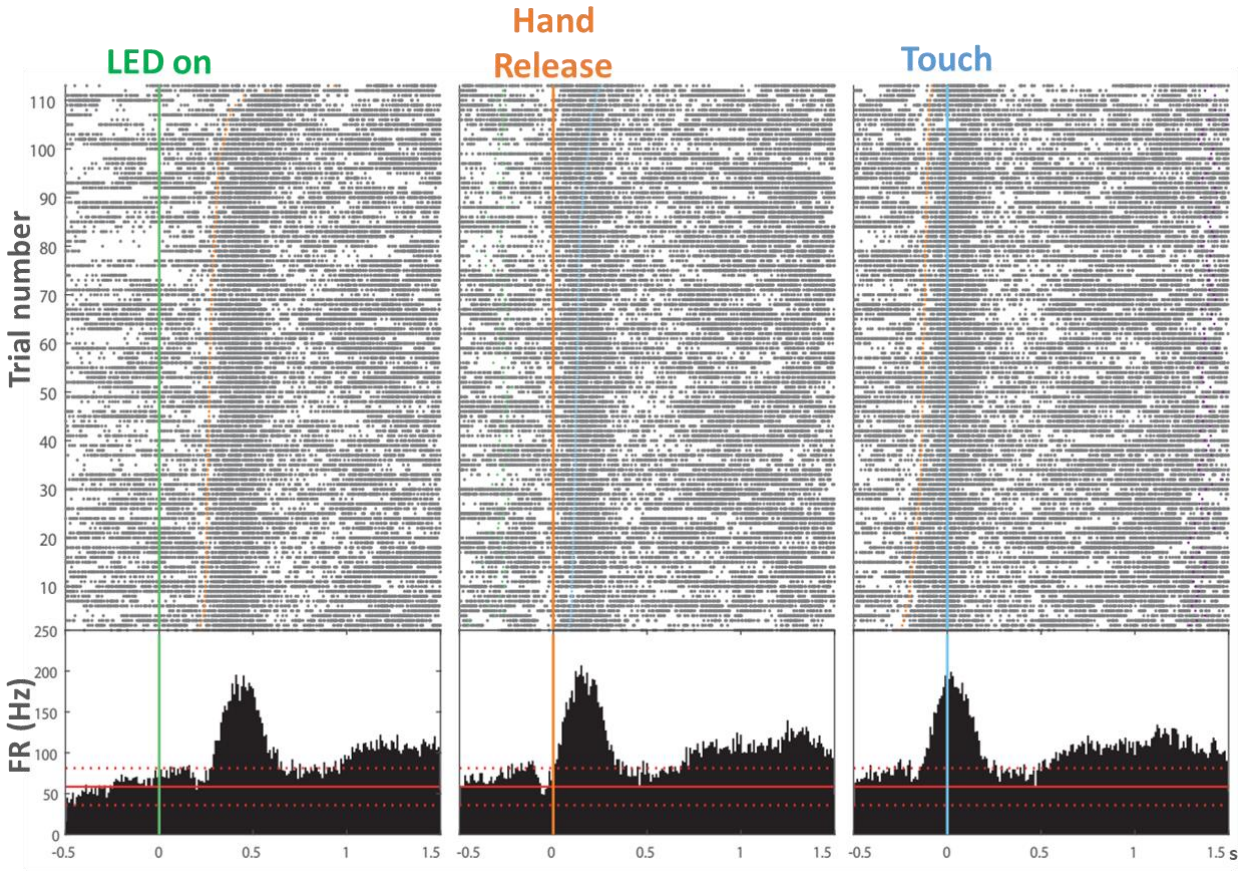


Figure 7: An example of a GPe neuron from monkey H, which increased their firing rates during hand release (orange vertical line) until touch event (blue vertical line) to the ipsilateral LED target. The green vertical line represents LED on timing. The red horizontal continuous and dotted lines represent the mean firing rate and the statistical level of p = 0.05, respectively.

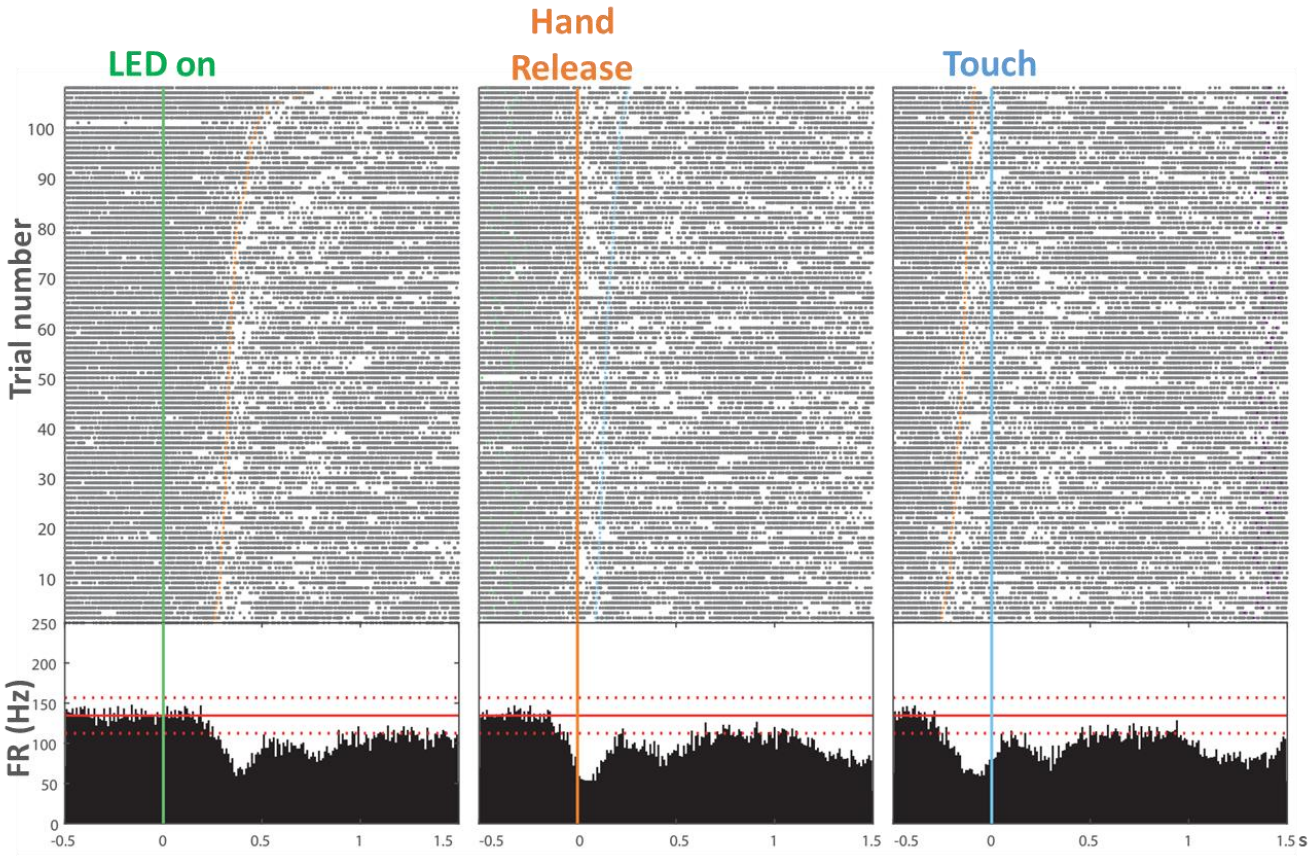


Figure 8: An example of a GPe neuron from monkey H, which decreased their firing rates during hand release (orange vertical line) until touch event (blue vertical line) to the ipsilateral LED target.

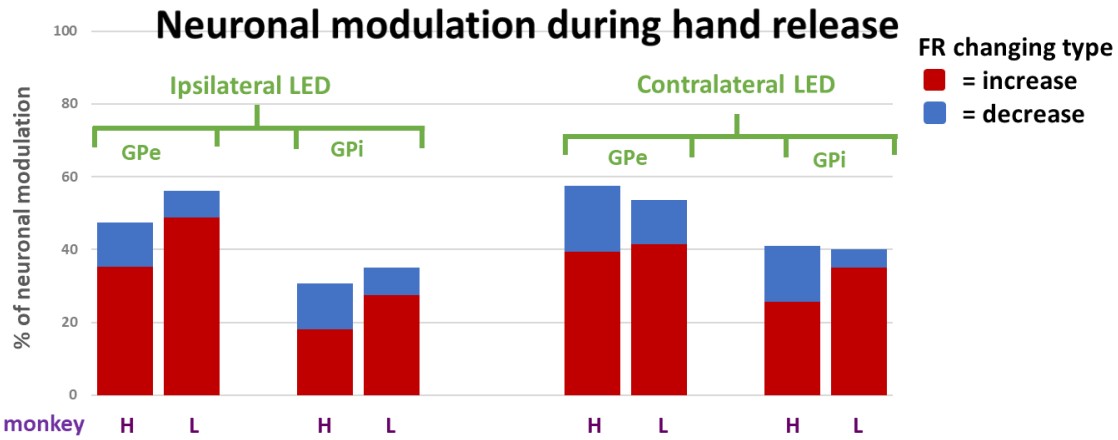


Figure 9: Percent of GPe and GPi neurons of both monkeys showing significant increase (red) or decrease (blue) during hand release to ipsilateral or contralateral targets. Half of GPe neurons showed FR modulation during hand release to both targets. Around 30-40% of GPi neurons showed FR modulation. FR mostly increased during hand release. The red and blue bar represent FR increase or decrease changing type respectively.

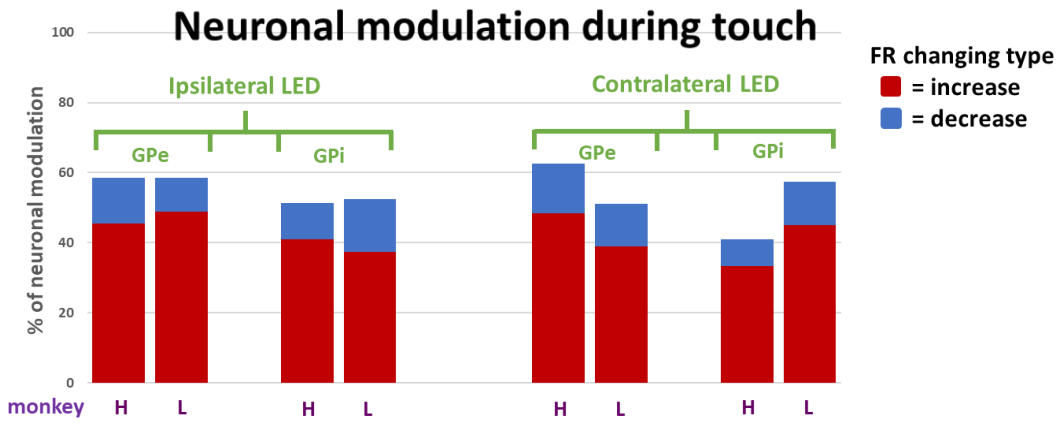


Figure 10: Percent of GPe and GPi neurons of both monkeys showing significant increase or decrease during touch to ipsilateral or contralateral targets. Half of GPe and GPi neurons showed FR modulation during touch to both targets. FR mostly increased during touch.

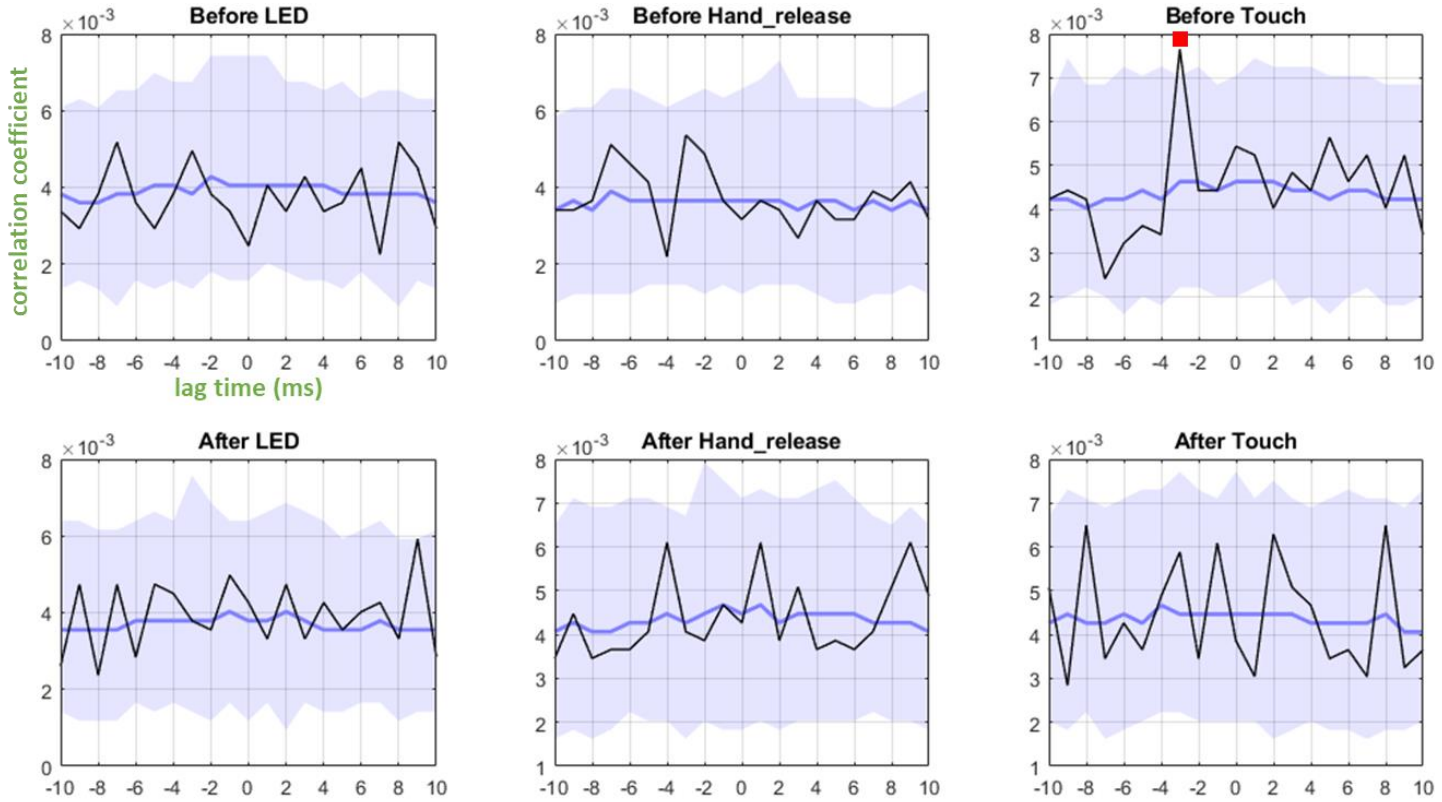


Figure 11: An example of cross-correlogram a GPe-GPe pair during task performance from monkey H. This neuronal pair showed significant positive correlation at lag -3 ms before touch timing. The black horizontal line represents the correlation of neuronal pairs. The blue line and shade represent a permutation test with 99.5% confidential interval. The square red represents positive correlation. Significant correlation was observed within ± 3 ms lag time. This specific lag time was used for further correlated spike analysis.

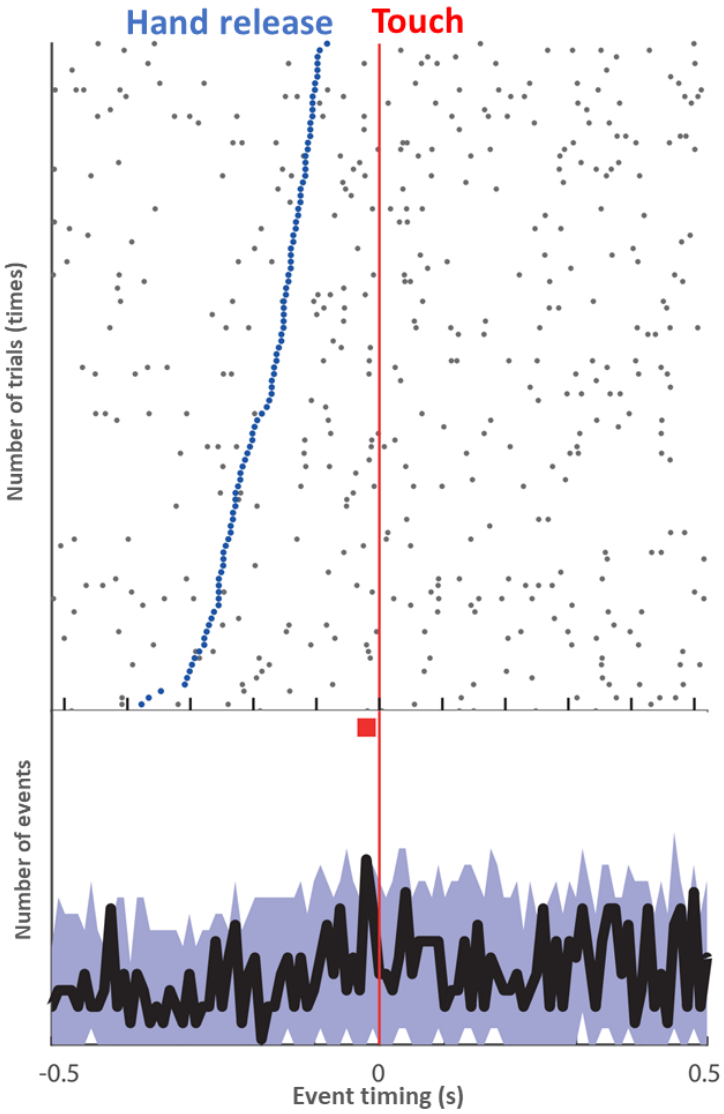


Figure 12: An example of coincident correlated spike event in the specific lag time. The neuronal pair from Fig. 10 during touch timing with lag -3 ms was used for this analyzed. This GPe-GPe pair showed significant coincidence firing during before touch period. The blue and red vertical line represent the hand release and touch timing respectively. The red square represents the significant positive correlation. The black line represents the correlated event of the neuronal pair, and the blue shade represents the confidential interval 99%.

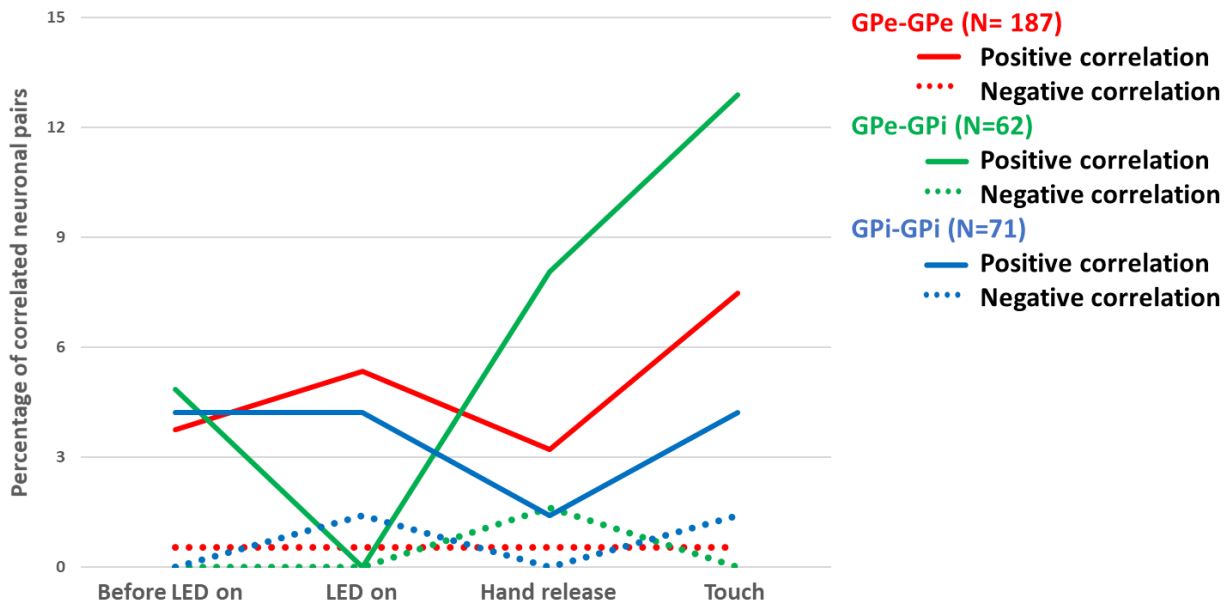


Figure 13: Percentage of correlated neuronal pairs during task. Only small percentage of GPe and GPi neurons showed correlated activity. Positive correlation was dominant. The red, green and blue lines represent the GPe-GPe, GPe-GPi and GPi-GPi pairs respectively. The continuous and dotted lines represent the positive and negative correlation respectively.

Part II

Correlation of GP neuronal activity

during task performance in mild PD monkey

Abstract

The impairment of the basal ganglia (BG) circuit results in movement disorders such as Parkinson's disease (PD) and dystonia. PD is caused by progressive loss of dopaminergic neurons in the substantia nigra pars compacta (SNc) which project to the striatum, and is characterized by severe motor symptoms such as bradykinesia, rigidity and tremor. In 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated PD monkeys, oscillatory and synchronized activity have been reported, previously. However, these studies were performed only during resting state in severe PD monkeys, and changes in neuronal activity during movements remain unclear. In the present study, I used monkey L (continued from Part I experiment) and make the mild PD condition by MPTP injections (total dose 3.9 mg/kg). The performance of a hand reaching task was affected but the monkey could still control the affected limb. Then, I performed multi-channel recordings of globus pallidus (GP) neurons during the task performance and analyzed the spike correlation among GP neurons. Following results have been obtained. (1) The spontaneous firing rates of GPe/GPi neurons decreased in PD state compared to normal state. (2) Reaction time and reaching time of the task were changed in PD state. (3) GP neurons modulated their firings during the task performance in normal and PD states. Especially the firing rate changes of GPi neurons during reaching period to the contralateral LED target in PD state were significantly different from those in normal state. (4) However, only a limited number of neurons showed significant correlations after the

MPTP treatment. These results suggest that GPe/GPi neurons in mild PD monkeys as well as those in normal monkeys independently fire and convey motor signals parallelly, which is essential to control voluntary movements.

Introduction

The functional abnormality in the basal ganglia (BG) circuit is related to movement disorders such as Parkinson's disease (PD) and dystonia. The pathological hallmark of PD is the loss of dopaminergic neurons in the substantia nigra pars compacta (SNc). The SNc projects to the striatum through the nigrostriatal dopaminergic pathway. The PD patients show movement alterations including bradykinesia/akinesia, rigidity, postural abnormalities and tremor. To understand pathophysiological mechanism of PD symptoms, it is essential to observe neuronal activity changes in PD models. The gold standard to make monkey PD models is applying neurotoxins, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), which induces selective neuronal death in dopaminergic neurons in the SNc. The MPTP-treated Japanese macaque monkeys show the major parkinsonian motor symptoms with the exception of resting tremor.

The electrophysiological studies using MPTP monkeys have shown oscillatory and synchronized activity in the GPe and GPi (Bergman et al. 1998; Nini et al. 1995). GPe and GPi neurons exhibit non-synchronized independent activity in normal monkeys and their firing patterns during resting state change to the oscillatory in PD states. The emergence of GPe/GPi oscillation could be due to the changes in the intrinsic properties and/or the alternated network connection of the BG circuit. However, previous experiments were conducted in the severe PD condition such that monkeys cannot perform behavioral tasks, and the correlation of GPe/GPi

neuronal activity during movements has not been studied yet. In the present study, I would like to examine whether correlated activity is increased during task performance in mild PD states. Activity of multiple neurons in the GPe/GPi was simultaneously recorded during hand reaching movement in the MPTP-treated monkey with mild PD condition, and the cross-correlation of spike trains was analyzed.

Methods

1. Operation (change hemisphere)

One female Japanese monkey (monkey L), which was used in Part I experiment, was used. I re-trained the monkey to perform the reaching task using the opposite upper limb in Part I experiment. Cortical stimulation in the M1 and SMA and electrophysiological recording in the GPe and GPi was performed on the opposite hemisphere from the side used in Part I. The surgical operation processes were the same as those described in Part I method section.

2. MPTP injections

After recording of GPe/GPi neurons in normal state, the administration of neurotoxic MPTP (Sigma-Aldrich, St. Louis, MO, USA) was performed. Under general anesthesia with propofol TCI (6-9 μg/mL) and fentanyl (2-5 μg/kg, i.m.) (see Part I Methods for details), the common carotid artery together with the internal and external carotid arteries were dissected at its bifurcation point in the neck region ipsilateral to the side of recording. MPTP was dissolved in saline (2 mg/ml) and injected into the common carotid artery with the external carotid clumped (Tachibana et al. 2011). The monkey received carotid artery injections twice (1.0 and 0.5 mg/kg, respectively) and additional intravenous injections eight times (0.3 mg/kg every 3 days, 1 week after the last carotid injection). The total doses of MPTP was 3.9 mg/kg. Around 4 weeks after the last MPTP injection, the

monkey's condition was stabilized, and the motor deficits were assessed with the parkinsonian rating scales (the maximum score was 53) (Schneider et al. 2003). The monkey was scored 13 and considered as a mild PD condition. Then, GP neuronal recordings during task performance in parkinsonian state were started. During recording, the monkey showed stable parkinsonian scores.

3. Data analysis

The neuronal firing rate, neuronal activity changes during task performance and cross-correlation were analyzed following the same steps as Part I.

The reaction time was defined as the time from the LED target on to the beginning of release from the home position, and the reaching time ("movement time" is commonly used) was the time from onset of release from the home position to the target touch. These movement parameters were compared between normal and PD states in the same monkey.

Power spectral density (PSD) of GPe and GPi activity was calculated using Welch's method (Tachibana et al. 2011). The spike train of a neuron was segmented to 2048 ms length with 50% overlap, Hann windowed, and transformed into the frequency domain by the fast Fourier transform (FFT) algorithm. The PSD was calculated as the average frequency power for the segments and compensated for the effect of refractory period with the local shuffling method (Rivlin-Etzion et al. 2006). The spike train was shuffled within 250- to 300-ms length such that the inter-spike intervals were preserved. The shuffled PSD was obtained by repeating the local shuffling

50 times and averaging the resulting PSDs, and then the original PSD was divided with the shuffled. Cross spectral density (CSD) was calculated similarly (Rivlin-Etzion et al. 2006); the cross correlation of a neuron pair was calculated and transformed into the frequency domain using Welch's method (2048 points, 50 % overlap). The resulting CSD was compensated for refractory period by applying the local shuffling method to both spike trains (250-300 ms length, 50 repetitions).

Results

1. Spontaneous firing after MPTP treatment

In normal state, the GPe/GPi neurons of the monkey L fired at high discharge rate (91 ± 28 Hz for GPe, n = 41 ;100 ± 26 Hz for GPi, n = 40). After the MPTP treatment, monkey L developed mild PD condition which was 13 out of 53 total scores (Schneider et al. 2003). The firing rates of both GPe and GPi neurons significantly decreased after MPTP treatment (69 ± 28 Hz for GPe, n = 35, $p < 0.001$, *t*-test; 71 ± 29 Hz for GPi, n = 28, $p < 0.001$) (Fig. 14).

2. Changes in task performance after MPTP treatment

The PD monkey showed significant performance changes compared to normal conditions (Fig.15). The reaction time was defined as the time from the target LED presentation to the beginning of hand release from the home position. In normal state, the monkey took around 280 ms to react to both ipsilateral (mean 286 ± 27 ms) and contralateral (mean 284 ± 28 ms) LED targets (Fig.15A). However, in PD state, the reaction times for both ipsilateral and contralateral LED targets were significantly longer than those in normal state (404 ± 76 ms for ipsilateral, $p < 0.001$, *t*-test; 430 ± 76 ms for contralateral, $p < 0.001$). In normal state, the reaching time, time from the beginning of hand release to the target touch, was 183 and 202 ms for ipsilateral and contralateral target LEDs, respectively (Fig.15B). In the PD state, the reaching time was significantly different from that in normal state

and between target LED sides. The monkey spent significantly longer time (279 ms; $p < 0.001$) for reaching to the ipsilateral LED target, whereas significantly shorter time for the contralateral target (157 ms; $p < 0.001$) after MPTP treatment. Detailed observations showed that touch position was more accurate and the success rate is higher in the ipsilateral LED trials, compared in the contralateral LED trials (Supple Fig.1). Hence, the monkey exhibited slower and accurate reaching to the ipsilateral LED, while faster but inaccurate reaching to the contralateral LED target.

3. Modulation of firing during task performance

Modulation of firing of GPe and GPi neurons during task performance was represented as PETHs. After the MPTP treatment, neurons both in the GPe and GPi showed either increase or decrease in their firing rates during task performance as in normal state (Fig.16). The GPi neurons that showed the significant decreased activity in the contralateral LED trials became dominant both during hand release (from 5% in normal to 25% in PD; $p < 0.001$, Fisher's exact test) and during touch (from 13% in normal to 36% in PD; $p < 0.001$) in PD conditions, while other responses of GPe and GPi neurons showed similar tendency between normal and PD conditions.

4. Cross-correlation during task performance

The recorded GPe/GPi neuronal pairs in PD state (66 GPe-GPe, 31 GPe-GPi and 70 GPi-GPi pairs) were examined for possible correlated activity during task performance (Fig.17). Among of all neuronal pair types, the significantly correlated pairs did not increase from normal state (before

LED on: 3 GPe-GPe pairs, 1 GPe-GPi pair, 1 GPi-GPi pair for positive correlation and 0, 0, 0 pair for negative correlation, respectively; LED on: 2, 0, 0 pairs for positive and 2, 0, 1 pairs for negative correlation; hand release: 4, 2, 4 pairs for positive and 1, 0, 1 pairs for negative correlation; and touch: 4, 1, 1 pairs for positive and 0, 0, 2 pairs for negative correlation, respectively; $p > 0.05$, χ^2 test). Therefore, these results demonstrate that GPe and GPi neurons had independent activity during task performance even after the MPTP treatment.

5. Cross spectrum density (CSD)

The CSD was constructed to examine the oscillatory correlation between two groups of neurons at the normal and PD states. An example of the CSD of GPe-GPe neuronal pairs in PD states is shown in Fig.18. Most of the GPe/GPi neuronal pairs did not show any peak in the CSD, indicating that the oscillatory coupling did not occur among GPe/GPi neurons even after the MPTP treatment. Therefore, the low-dose MPTP injection was not sufficient to change the neuronal firing pattern in the GPe/GPi.

Discussion

Spontaneous firing rate changes in the GPe and GPi after the induction of parkinsonism have been reported. The GPe neurons tend to decrease their firing rate whereas the GPi neurons tend to increase (DeLong & Wichmann 2007). The GPe/GPi neuronal firing pattern also changes. Both GPe and GPi neurons show oscillatory pattern in MPTP-treated PD monkeys (Nini et al. 1995; Raz et al. 2000), whereas in the normal state GPe neurons fire spontaneously at high frequencies with pause, whereas GPi neurons fire continuously without any pause (DeLong 1971).The neuronal correlated activity was also reported in the PD state (Nini et al. 1995; Raz et al. 2000). It was hypothesized that the loss of independent activity in GPe/GPi neurons affects the motor control, resulting in the PD symptoms. However, all the previous studies were conducted during resting state in PD animals. GP neuronal activity during movements in PD state remains to be elucidated. The present study is the first report examining the neuronal correlated activity of the GPe/GPi during task performance in a PD monkey.

Even though the low dose of MPTP was injected (3.9 mg/kg), the task performances were disturbed. The reaction time to target LEDs in both sides was significantly increased in PD state. On the other hand, the reaching time to the ipsilateral target LED increased but that to the contralateral target decreased. The apparent decrease of the reaching time seemed to be caused by technical limitation; the monkey tended to touch the panel at the nearest position and then move to the target along the surface of the panel because

of motor disturbance, and the infrared photoelectric sensor detected the timing of the first touch to the panel.

The monkey, which exhibited mild PD symptoms after MPTP treatment, showed the significant firing rate decrease in both GPe and GPi neurons (68 ± 28 and 71 ± 29 Hz, respectively, $p < 0.001$, *t*-test). Firing rate decrease in the GPi contradicts classical firing rate model of PD, but was also reported previously (DeLong & Wichmann 2007). Neuronal modulation during task performance was similar to that in the normal state except for GPi activity in contralateral LED trials: GPi neurons tended to show the decrease in their firing rate during hand release and touch.

For neuronal cross-correlation, I examined whether number of correlated GP pairs increased in PD state. However, the present study clearly demonstrated independent GPe/GPi activity in task events during task performance in PD state as in normal state. Moreover, the monkey did not show any oscillatory firing activity as observed in the CSD analysis. These results suggest that independent GPe/GPi activity is essential to control voluntary movements even in mild PD state.

Figures and legends

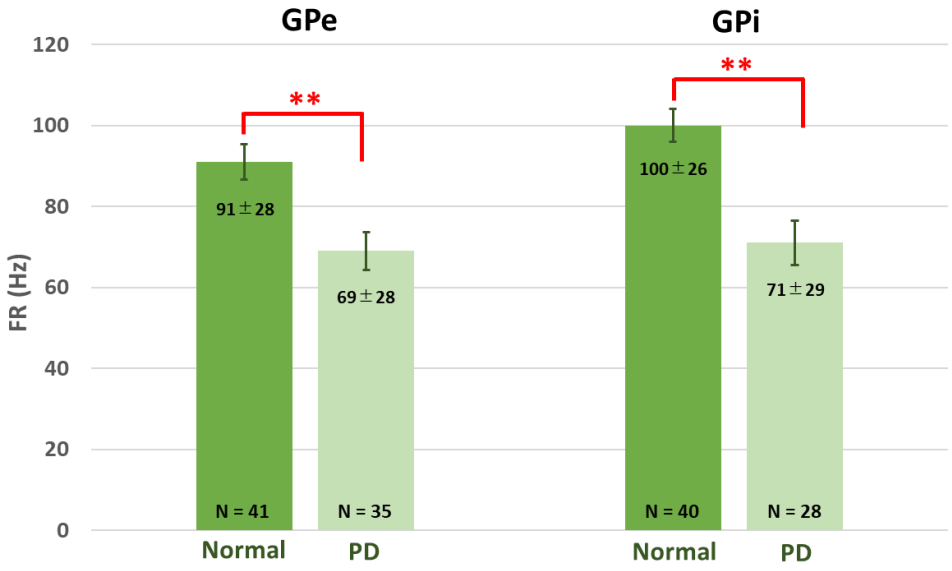


Figure 14: Mean spontaneous firing rates of GPe and GPi neurons in normal and PD states. Both GPe and GPi neurons show the significant decrease of their firing rates in PD state (, $p < 0.001$). Error bars represent SD.**

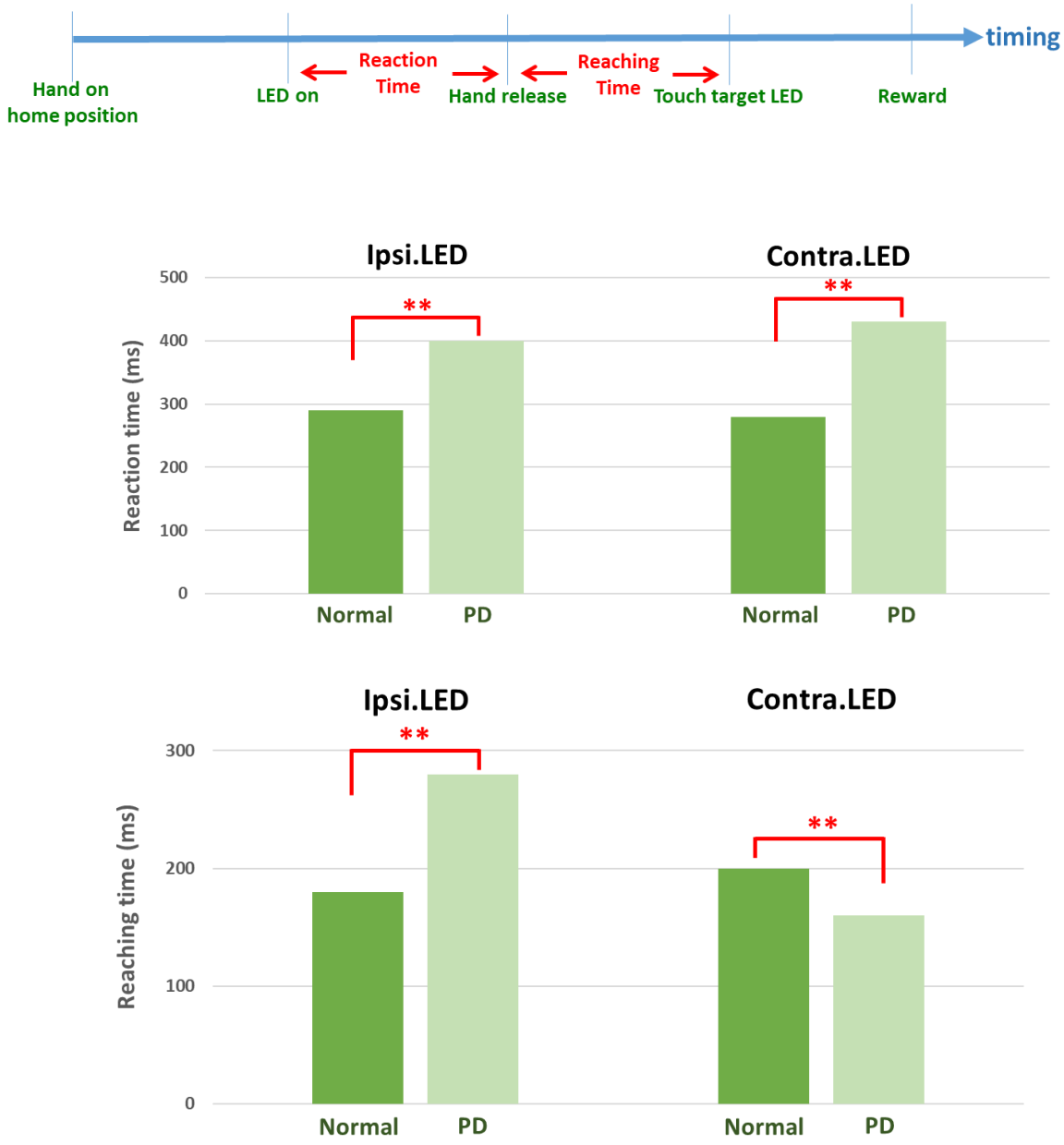


Figure 15: Mean reaction time (A) and reaching times (B) in normal and PD states. **, $p < 0.001$ significantly different.

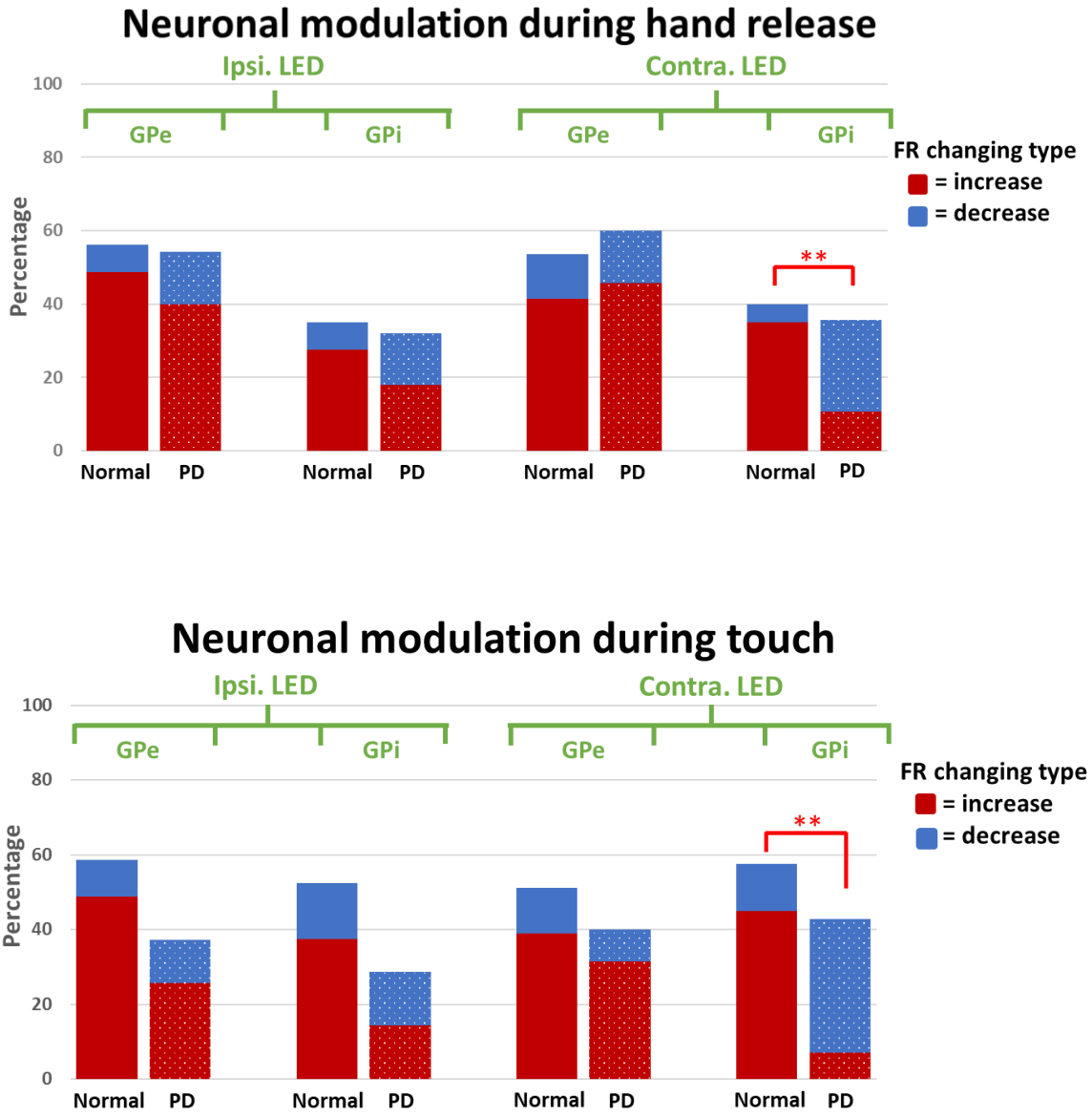


Figure 16: Neuronal modulation during hand release (A) and touch (B) periods in normal and PD states. The red and blue bars represent GP neurons firing rate increase and decrease, respectively. The decrease of the firing rate of GPi neurons in the contralateral LED trials became dominant (, $p < 0.001$).**

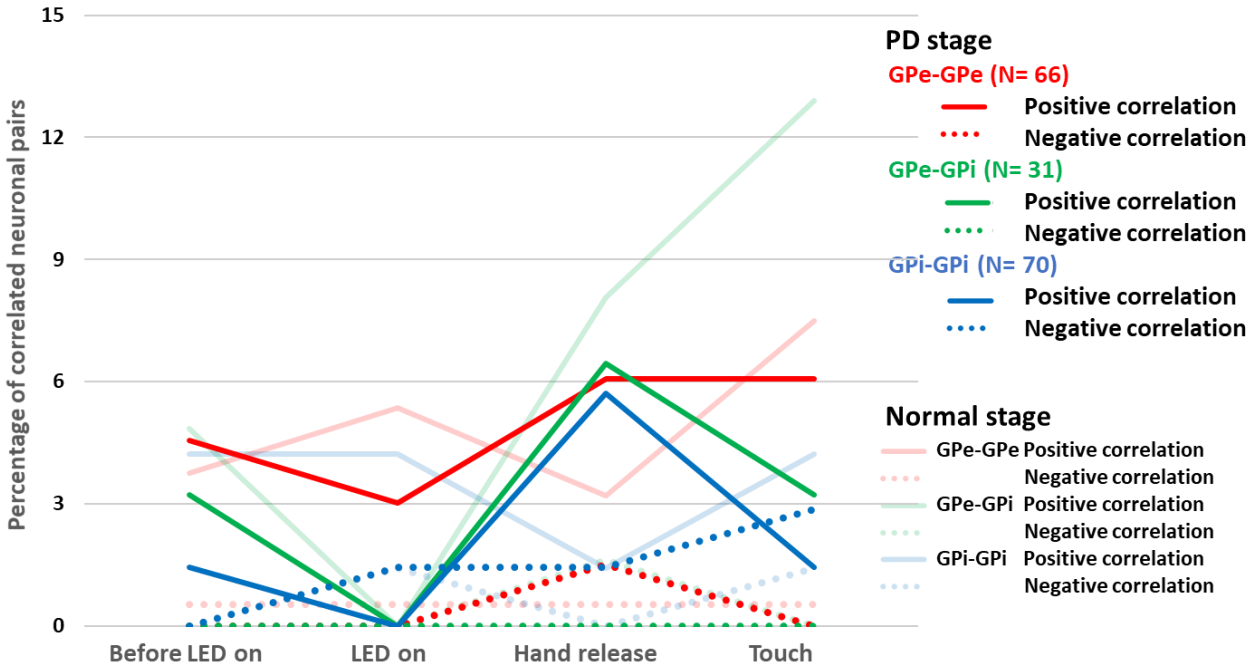


Figure 17: Percentages of correlated neuronal pairs during task performance in PD state. Only small percentage of GPe and GPi neurons showed correlated activity and it was not significantly different from normal state. The red, green and blue lines represent the GPe-GPe, GPe-GPi and GPi-GPi pairs, respectively. The continuous and dot lines represent the positive and negative correlation, and dark-colored and light-colored represent PD and normal states, respectively.

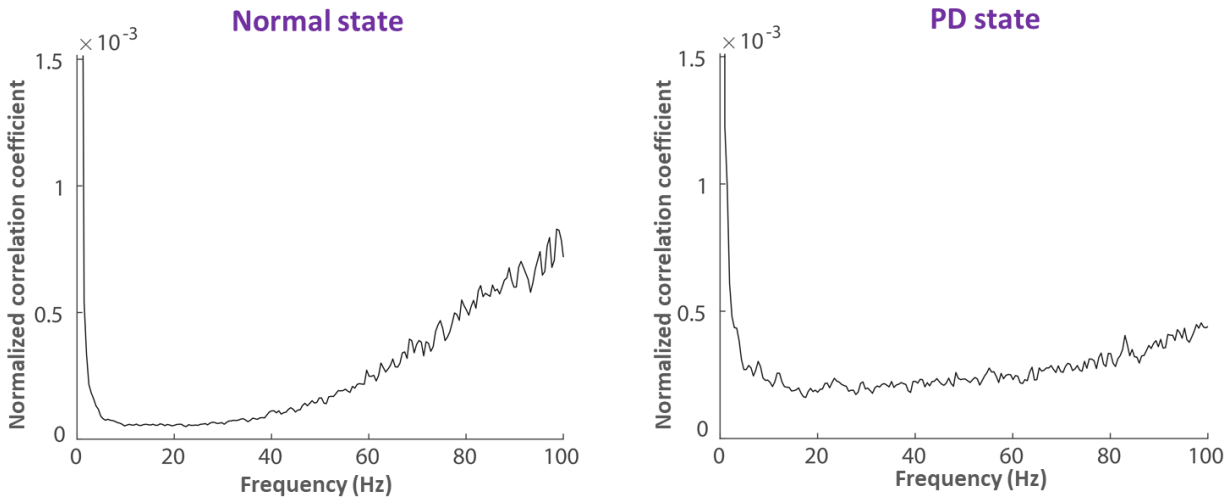
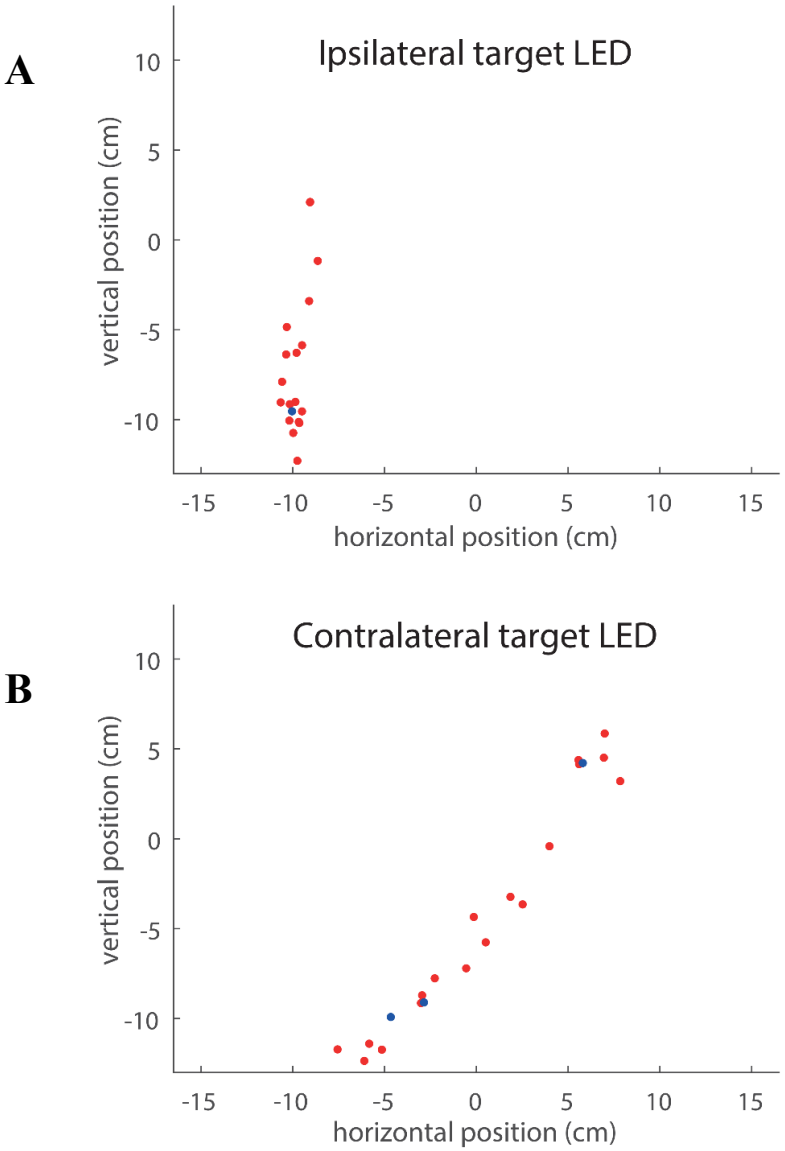


Figure 18: An example of cross-spectral density (CSD) between a GPe-GPe pair in normal (left) and PD (right) states. The CSD in both normal and PD states showed no significant peak, suggesting no correlated oscillation in mild PD state.



Supplementary figure 1: An example of positions where a monkey first touched the panel in the ipsilateral (A) and contralateral (B) target LED trials. The target LEDs were located at -9 cm in the horizontal position and 0 cm in the vertical position in the ipsilateral side and at 9 cm and 0 cm in the contralateral side. The red and blue dots represent success and failure trials, respectively. Touch positions were largely distributed in the ipsilateral trials than the contralateral trials.

Part III

Correlation of GP neurons in a severe PD monkey before and after L-dopa treatment

Abstract

The pathological hallmark of Parkinson's disease (PD) is the loss of dopaminergic neurons in the substantia nigra pars compacta (SNc). Dopamine replacement therapy using dopamine precursor is the main standard treatment. L-dihydroxyphenylalanine or L-dopa is the primary choice for PD patients. On the other hand, electrophysiological studies have shown that the significant phenomenon of oscillatory and synchronized firing in GP neurons in PD state, especially at beta band frequency (10-30Hz) (Bergman et al. 1998; Brown 2007; Nini et al. 1995). In the present study, I generated a MPTP-treated PD monkey with severe symptoms, and observed changes in oscillatory and synchronized firing of GP neurons before and after the treatment with L-dopa application. The following results have been obtained 1) GPe/GPi neurons exhibited abnormal oscillatory and synchronized activity at beta range in severe PD state, 2) L-dopa treatment improved PD symptoms and decreased oscillation and synchronized activity of GPe/GPi neurons. These results suggest that independent neuronal firings in the GPe/GPi is necessary for proper control of voluntary movements, and that their abnormal oscillation and synchronization disturb it and lead to PD symptoms.

Introduction

The pathological hallmark of PD is the loss of dopaminergic neurons in the substantia nigra pars compacta (SNc) which sends the projection to the striatum. The denervation of dopaminergic neurons causes the multiple changes in the basal ganglia circuit such as the compensation mechanisms to normalize the function of glutamatergic and GABAergic synapses (Blandini et al. 2000). The pharmacological therapies using the dopamine precursor is the main standard treatment. L-dihydroxyphenylalanine or L-dopa, the dopamine precursor, was discovered to alleviate PD symptoms in early 1960's (Ehringer & Hornykiewicz 1960) and still being the primary choice for PD patients.

On the other hand, electrophysiological studies have shown that GP neurons exhibit prominent oscillatory and synchronized firing at beta band frequency (10-30 Hz) in PD state, whereas they do not show correlated activity in normal state (Bergman et al. 1998; Brown 2007; Nini et al. 1995). However, it is not clear if abnormal neuronal firing in the BG circuitry return to normal when dopamine replacement therapy alleviates PD symptoms.

In the present study, I would like to focus on the effect of L-dopa treatment on the oscillatory firing and cross correlation. The MPTP was injected to the monkey to make a severe PD condition, and neuronal activity was recorded before and after L-dopa administrations.

Methods

1. Operation (change the hemisphere)

A female Japanese monkey (monkey H) in Part I experiment was used. Cortical stimulation and electrophysiological recording were performed in the contralateral hemisphere to that used in the Part I experiment. The operation processes were the same as describe in Part I method section.

2. MPTP injections

After recording of GPe/GPi activity in the normal state, the administration of neurotoxic MPTP (Sigma-Aldrich, St Louis, MO, USA) was conducted using the same methods as described in Part II method section. The monkey received bilateral carotid artery injections 1.5 mg/kg to the left carotid artery and 1.2 and 1.0 mg/kg to the right carotid artery, respectively; total three times) and intravenous injection once (0.5 mg/kg). The total dose of MPTP was 4.2 mg/kg. The monkey H showed obvious motor alteration. After the monkey's symptom was stabilized, the motor deficits were assessed with the parkinsonian rating scales (for more information, see Part II method). The monkey showed 35 score, and was considered in severe PD condition.

3. L-dopa treatments

To examine effects of dopamine replacement therapy on neuronal activity, dopamine precursor L-dopa (DOPASTON, OHARA Pharmaceutical, Japan) was applied during neuronal recording. L-dopa (1 mg／kg) was manually injected through the great saphenous vein followed by the infusion of electrolyte fluid. Around 5 min after L-dopa injection when motor symptoms were alleviated, GP neuronal recoding were resumed. The L-dopa experiment was conducted only once a day.

4. Data analysis

The neuronal firing rates and cortically evoked responses were analyzed with the same processes as Part I experiment. The firing rates were compared between before and after the L-dopa treatment and were statistically analyzed (paired *t*-test).

The power spectrum density (PSD) and cross spectrum density (CSD) were analyzed with the same processes as Part II experiment and classified into the specific frequency ranges (1-4 Hz, delta; 4-10 Hz, theta; 10-15 Hz, low beta; 15-30 Hz, high beta; 30-50 Hz, low gamma and 50-80 Hz, high gamma range, respectively).

Results

1. Spontaneous firing rates before and after L-dopa treatment

The firing rates of GPe/GPi neurons in PD state were (58 ± 28 Hz 14 GPe neurons and 53 ± 24 Hz 9 GPi neurons) and were not significantly changed after L-dopa treatment (57 ± 30 Hz and 55 ± 20 Hz). Thus L-dopa administration did not affect spontaneous firing rates of GPe/GPi neurons in the PD monkey.

2. Neuronal activity evoked by cortical stimulation

The response pattern evoked by cortical stimulation of M1 and SMA was typically a triphasic response composed of early excitation followed by inhibition and late excitation in normal monkeys. These components are mediated by the *hyperdirect*, *direct* and *indirect* pathways of BG circuit, respectively. However, in the PD monkey, the response pattern was changed in both the GPe and GPi neurons. Figure 20 showed an example of GPi neuron of a PD monkey responding to M1 stimulation. Cortically evoked triphasic pattern was modulated in the PD monkey: the inhibition, which is conveyed through the *direct* pathway, was mostly lost. However, triphasic response pattern was recovered after L-dopa treatment, suggesting that L-dopa administration normalize information flow through the BG circuit.

3. Power spectrum density (PSD) before and after L-dopa treatment

The PSDs of GPe/GPi neurons were compared before and after the L-dopa treatment (Fig.21). Figure 21A represents an example of GPe neurons. The significant peak around 10-20 Hz before L-dopa administration indicates oscillatory activity of GPe neuron (blue line). However, the peak was greatly diminished after L-dopa treatment (red line). Detailed PSD analysis for each frequency range revealed that L-dopa significantly decreased the oscillatory activity at low beta range (10-15 Hz) in the GPe/GPi ($p < 0.05$, paired *t*-test) and increased at delta range (1-4 Hz) in the GPi (Fig.21B) ($p < 0.05$, paired *t*-test).

4. Cross spectrum density (CSD) before and after L-dopa treatment

The CSD of GPe-GPe and GPi-GPi pairs were compared before and after the L-dopa treatment (Fig.22). Figure 22A represents an example of GPe-GPe pair. The significant peak around 10-20 Hz which before L-dopa treatment indicates correlated firing of the neuronal pair (blue line). However, the peak was greatly diminished after the L-dopa administration (red line). Detailed CSD analysis for each frequency range revealed that L-dopa significantly decreased the correlated firing both at low beta range (10-15 Hz, both GPe-GPe and GPi-GPi pairs) and at high beta range (15-30 Hz, GPe-GPe pairs) ($p < 0.05$, paired *t*-test), whereas oscillatory coupling at delta increased (1-4 Hz, both GPe-GPe, and GPi-GPi pairs) ($p < 0.05$, paired *t*-test) (Fig.22B).

Discussion

The neuronal firing in the GP of normal monkeys is uncorrelated as showed in Part I. On the other hand, in the PD state, the oscillatory activity emerges and the neuronal firing becomes correlated (Bergman et al. 1998; Nini et al. 1995). Since the standard treatment for PD is the dopamine replacement therapy, I injected L-dopa to the PD monkey and investigated the change in the activity pattern. The results in the current study showed that the MPTP-treated monkey had abnormal oscillations in both GPi and GPe neurons and their firing pattern returned to normal after L-dopa administration.

Neither GPe nor GPi neurons of the PD monkey showed any significant change in the firing rate after the L-dopa treatment (Fig.19). However, the responses to the cortical stimulations were normalized to be triphasic-responses after the treatment (Fig.20). Before the treatment, GPi neurons responding to the M1stimulation show only early and late excitations, but it showed clear inhibitory response after the treatment. The inhibitory response is conveyed through the direct pathway of BG circuit, suggesting that L-dopa facilitated direct information flow from the striatum to the GPi.

Then I examined the PSD of GPe/GPi neurons (Fig.21). The PSD of GPe/GPi neurons exhibited a significant peak at beta range and the peak was diminished after the L-dopa treatment. The peak of PSD is related to the oscillatory activity of the neurons. Furthermore, the CSD of GPe-GPe and

GPi-GPi pairs were also analyzed. The PD monkey showed significant peak of CSD around the beta frequency for both GPe-GPe and GPi-GPi pairs, and the height of peaks decreased after L-dopa administration. The results from Part I showed parallel and independent processing of movement-related information in the GPe/GPi was essential for normal function. Whereas the PD monkey showed the correlated activity, and the L-dopa treatment recovered GPe/GPi activity to normal state. The beta frequency neuronal activity is observed not only in the GP but also in the STN of PD animals (Gatev et al. 2006). The interactions among different nuclei of the BG have been proposed as oscillatory mechanisms in PD state (Brown 2007; Kumar et al. 2011; Tachibana et al. 2011) such as the interaction between the GPe and STN. Actually, the application of muscimol into the GPe diminished the oscillatory activity in the STN of PD monkeys, suggesting the involvement of the reciprocal connection between the GPe and STN (Nambu & Tachibana 2014).

The relevance of abnormal neuronal firing in the GP to abnormal motor control is an essential story to describe PD symptoms. The GPi, the output station of the BG, sends movement-related information to the thalamus through inhibitory GABAergic projections. The oscillatory and correlated neuronal firing in the GPi affects thalamic activity which sends movement-related information back to the motor cortex. The previous study showed the rebound firing of thalamus was induced by the activation of GPi-thalamic inhibitory synapses (Kim et al. 2017). In the cerebellum, synchronous inhibitory inputs from Purkinje cells effectively generated time-locked action potentials of cerebellar nuclear neurons (Person & Raman

2011). The same mechanism may exist in GPi-thalamic synapses. Oscillatory correlated inhibitory inputs from the GPi induces rhythmic firing of thalamic neurons, which is sent to the cortex. Finally, normal information processing through the cortico-BG-thalamo-cortical circuit is disturbed, resulting in abnormal motor control. In the present study, L-dopa treatment abolished oscillatory and correlated activity of GPi neurons when PD symptoms were alleviated. The results support the hypothesis that correlated activity in the BG disturb information flow and disturbs normal control of movements.

Figures and legends

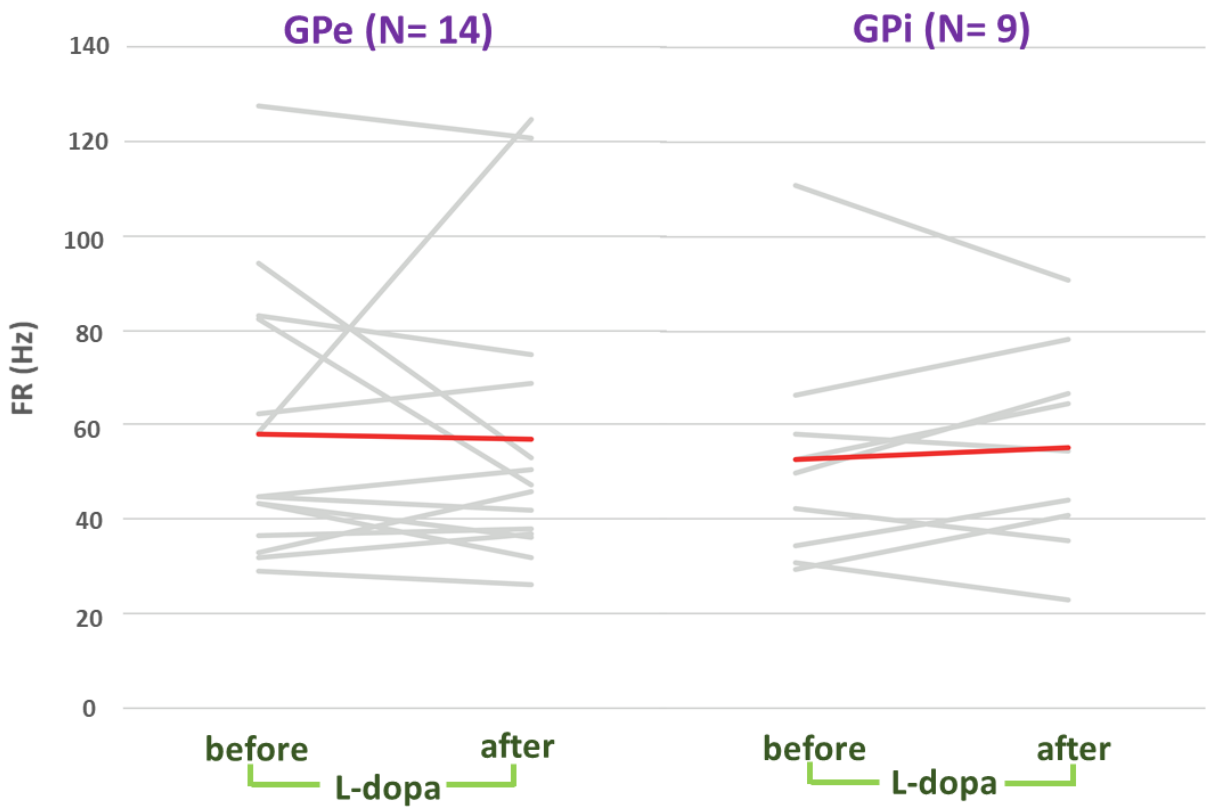


Figure 19: spontaneous firing rates of GPe and GPi neurons before and after L-dopa treatment in PD state. Both GPe and GPi neurons did not show significant different firing rates after L-dopa treatment. The gray and red lines represent the individual and mean firing rate of GPe/GPi neurons, respectively.

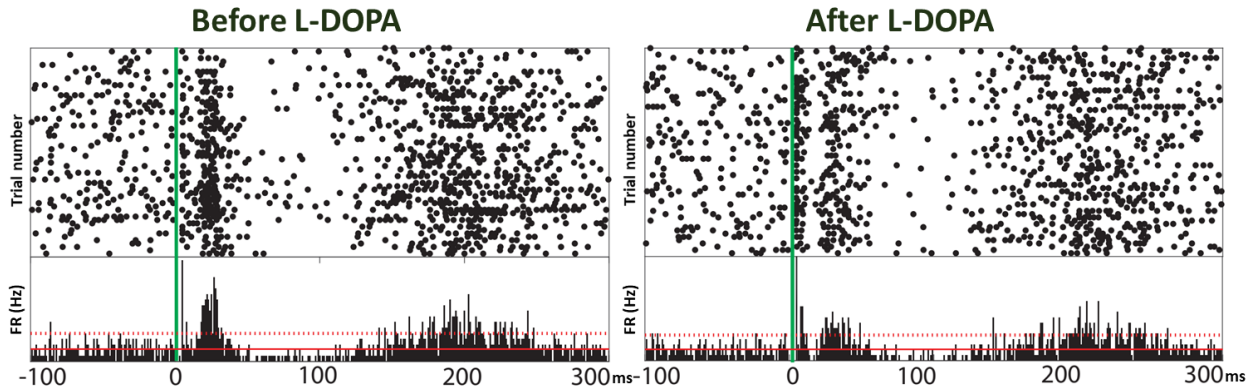


Figure 20: Raster and peri-stimulus time histograms (PSTHs) showing cortically evoked responses of a GPi neuron before (left) and after (right) L-dopa treatment. M1 stimulation (0.3 ms duration, 0.5 mA, single pulse) was delivered at time 0 (green vertical line). Biphasic excitation was induced before L-dopa treatment, whereas triphasic response which is typically observed in normal state was induced after L-dopa treatment. The red horizontal continuous and dotted lines represent the mean firing rate and the statistical level of $p = 0.05$, respectively.

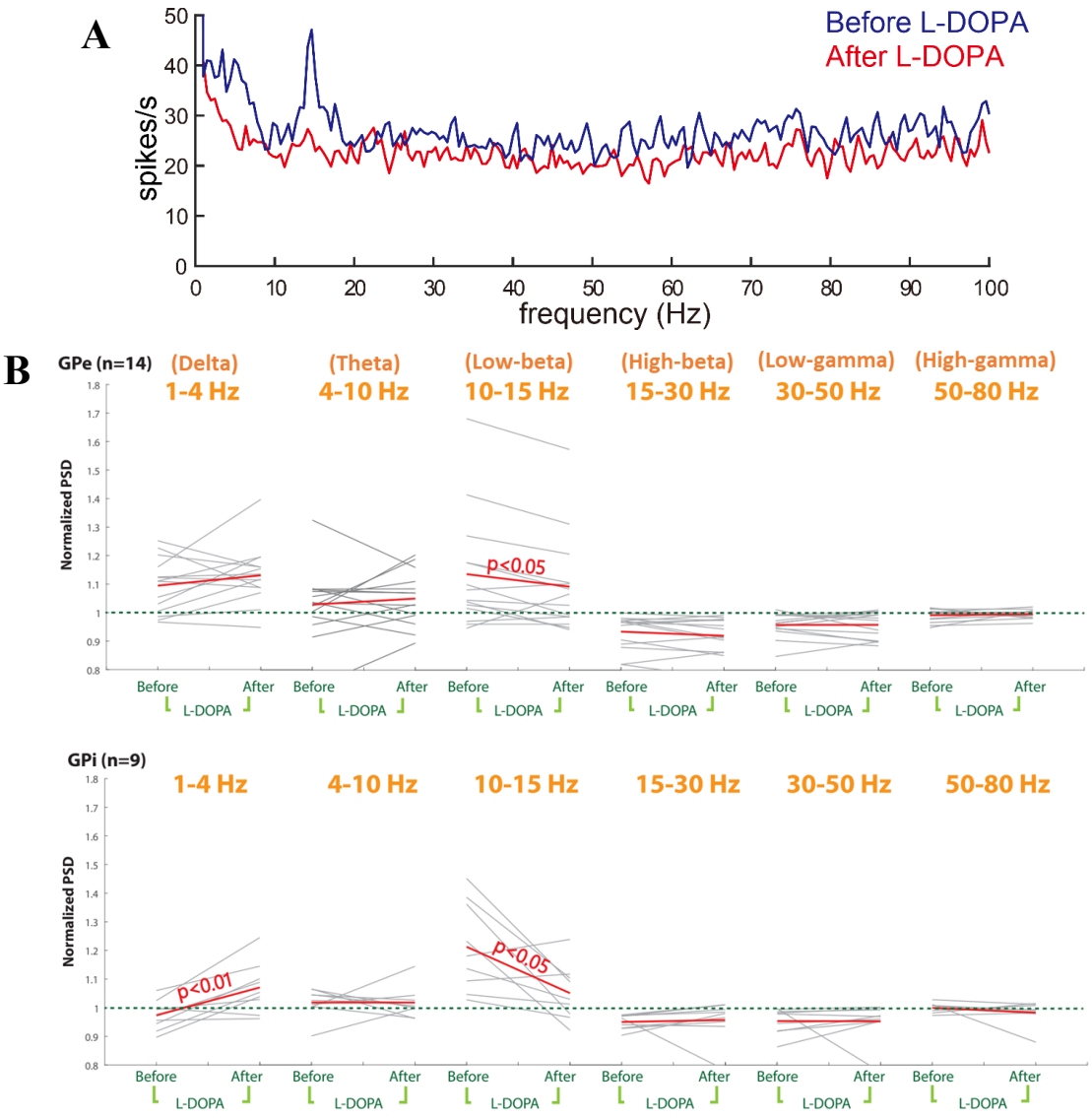


Figure 21: Power spectral density (PSD) of GPe/GPi neurons before and after L-dopa treatment. A; PSD of a GPe neuron showing significant peak around 10-20 Hz before L-dopa treatment (blue line). The peak was disappeared after the treatment (red line). B; Each PSD was normalized and divided based on the specific frequency range of GPe/GPi neurons. Both GPe and GPi neurons show significant PSD decreasing at low-beta range after L-dopa treatment. Moreover, GPi showed significant increase of delta range after the treatment. Gray and red lines represent the individual and mean values. Significant difference was indicated by P value.

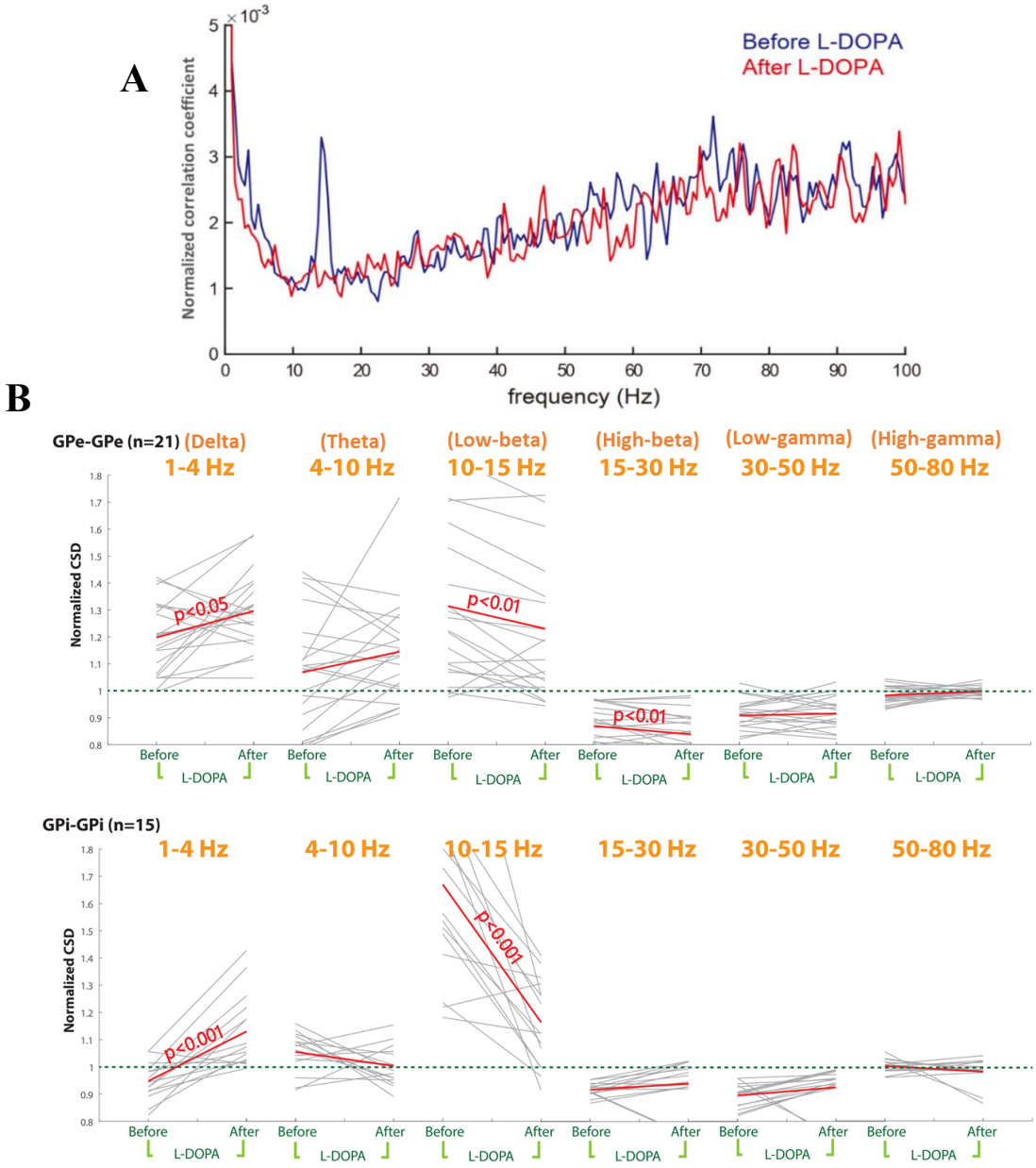


Figure 22: Cross spectral density (CSD) of GPe/GPi neurons before and after L-dopa treatment. A; CSD of a GPe-GPe neuronal pair showing significant peak around 10-20 Hz before L-dopa treatment (blue line). The peak disappeared after the treatment (red line). B; Each CSD was normalized and divided based on the specific frequency ranges. Both GPe-GPe and GPi-GPi neuronal pairs show significant CSD decrease at the low-beta range and significant increase of delta range after the treatment. Gray and red lines represent the individual and mean values. Significant difference was indicated by P value.

Conclusion

In the Part I, I examined the cross-correlation of GPe/GPi neuronal activity when monkeys performed a hand reaching task. Limited number of GPe/GPi neurons showed correlated activity even during hand reaching movements. Together with previous studies, GPe/GPi neurons function independently either at rest or during movement periods.

In Part II, I examined cross-correlation of GPe/GPi neuronal activity in a mild PD monkey during performance of the hand reaching task. The number of correlated GPe/GPi pairs did not increase during task performance in PD state compared with normal state. These results suggest that independent GPe/GPi activity is essential to control voluntary movements even in mild PD state.

In Part III, I examined oscillatory firing and cross-correlated activity of GPe/GPi neurons before and after L-dopa treatment in a severe PD monkey. Even though L-dopa did not change their firing rate, cross-correlation of GP pairs was significantly decreased after L-dopa treatment. The results support the hypothesis that correlated activity disturbs information flow in the BG and normal control of movements.

These results suggest that the GPe/GPi neurons could be activated independently and that this independent activity is necessary for normal information processing within the basal ganglia. Moreover, the L-dopa treatment in PD state may targets the oscillation and correlated activity but not firing rates. Further studies are required to investigate the neural

mechanism of correlated activity within the BG, leading to exploration of more effective therapeutic targets for PD.

References

Bergman H, Feingold A, Nini A, Raz A, Slovin H, et al. 1998. Physiological aspects of information processing in the basal ganglia of normal and parkinsonian primates. *Trends in Neurosciences*. 21(1):32–38

Blandini F, Nappi G, Tassorelli C, Martignoni E. 2000. Functional changes of the basal ganglia circuitry in Parkinson's disease. *Progress in Neurobiology*. 62(1):63–88

Brown P. 2007. Abnormal oscillatory synchronisation in the motor system leads to impaired movement. *Current Opinion in Neurobiology*. 17(6):656–64

de la Rocha J, Doiron B, Shea-Brown E, Josić K, Reyes A. 2007. Correlation between neural spike trains increases with firing rate. *Nature*. 448(7155):802–6

DeLong MR. 1971. Activity of pallidal neurons during movement. *Journal of Neurophysiology*. 34(3):414–27

DeLong MR, Wichmann T. 2007. Circuits and Circuit Disorders of the Basal Ganglia. *Arch Neurol*. 64(1):20–24

Ehringer H, Hornykiewicz O. 1960. [Distribution of noradrenaline and dopamine (3-hydroxytyramine) in the human brain and their behavior in diseases of the extrapyramidal system]. *Klin. Wochenschr.* 38:1236–39

Flaherty AW, Graybiel AM. 1993. Output architecture of the primate putamen. *J. Neurosci.* 13(8):3222–37

Gatev P, Darbin O, Wichmann T. 2006. Oscillations in the basal ganglia under normal conditions and in movement disorders. *Movement Disorders*. 21(10):1566–77

Hamada I, DeLong MR, Mano N. 1990. Activity of identified wrist-related pallidal neurons during step and ramp wrist movements in the monkey. *Journal of Neurophysiology*. 64(6):1892–1906

Hornykiewicz O. 2010. A brief history of levodopa. *J Neurol*. 257(2):249–52

Kim J, Kim Y, Nakajima R, Shin A, Jeong M, et al. 2017. Inhibitory Basal Ganglia Inputs Induce Excitatory Motor Signals in the Thalamus. *Neuron*. 95(5):1181-1196.e8

Kumar A, Cardanobile S, Rotter S, Aertsen A. 2011. The Role of Inhibition in Generating and Controlling Parkinson's Disease Oscillations in the Basal Ganglia. *Front Syst Neurosci*. 5:

Miguelez C, Morin S, Martinez A, Goillandeau M, Bezard E, et al. 2012. Altered pallido-pallidal synaptic transmission leads to aberrant firing of globus pallidus neurons in a rat model of Parkinson's disease. *J Physiol*. 590(Pt 22):5861–75

Mushiake H, Strick PL. 1995. Pallidal neuron activity during sequential arm movements. *Journal of Neurophysiology*. 74(6):2754–58

Nambu A. 2011. Somatotopic Organization of the Primate Basal Ganglia. *Front Neuroanat*. 5:

Nambu A, Tachibana Y. 2014. Mechanism of parkinsonian neuronal oscillations in the primate basal ganglia: some considerations based on our recent work. *Front Syst Neurosci*. 8:

Nambu A, Tokuno H, Hamada I, Kita H, Imanishi M, et al. 2000. Excitatory cortical inputs to pallidal neurons via the subthalamic nucleus in the monkey. *J. Neurophysiol.* 84(1):289–300

Nambu A, Tokuno H, Takada M. 2002. Functional significance of the cortico–subthalamo–pallidal 'hyperdirect' pathway. *Neuroscience Research*. 43(2):111–17

Nini A, Feingold A, Slovin H, Bergman H. 1995. Neurons in the globus pallidus do not show correlated activity in the normal monkey, but phase-locked oscillations appear in the MPTP model of parkinsonism. *Journal of Neurophysiology*. 74(4):1800–1805

Person AL, Raman IM. 2011. Purkinje neuron synchrony elicits time-locked spiking in the cerebellar nuclei. *Nature*. 481(7382):502–5

Person AL, Raman IM. 2012. Synchrony and neural coding in cerebellar circuits. *Front Neural Circuits*. 6:

Raz A, Vaadia E, Bergman H. 2000. Firing Patterns and Correlations of Spontaneous Discharge of Pallidal Neurons in the Normal and the Tremulous 1-Methyl-4-Phenyl-1,2,3,6-Tetrahydropyridine Vervet Model of Parkinsonism. *J. Neurosci.* 20(22):8559–71

Rivlin-Etzion M, Ritov Y, Heimer G, Bergman H, Bar-Gad I. 2006. Local Shuffling of Spike Trains Boosts the Accuracy of Spike Train Spectral Analysis. *Journal of Neurophysiology*. 95(5):3245–56

Rosenbaum R, Tchumatchenko T, Moreno-Bote R. 2014. Correlated neuronal activity and its relationship to coding, dynamics and network architecture. *Front. Comput. Neurosci.* 8:

Salinas E, Sejnowski TJ. 2001. Correlated neuronal activity and the flow of neural information. *Nature Reviews Neuroscience*. 2(8):539

Schneider JS, Gonczi H, Decamp E. 2003. Development of levodopa-induced dyskinesias in parkinsonian monkeys may depend upon rate of symptom onset and/or duration of symptoms. *Brain Research*. 990(1):38–44

Singer W. 1993. Synchronization of Cortical Activity and its Putative Role in Information Processing and Learning. *Annual Review of Physiology*. 55(1):349–74

Tachibana Y, Iwamuro H, Kita H, Takada M, Nambu A. 2011. Subthalamo-pallidal interactions underlying parkinsonian neuronal oscillations in the primate basal ganglia. *European Journal of Neuroscience*. 34(9):1470–84

Vaadia E, Haalman I, Abeles M, Bergman H, Prut Y, et al. 1995. Dynamics of neuronal interactions in monkey cortex in relation to behavioural events. *Nature*. 373(6514):515