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

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Introduction

The basal ganglia (BG) play an important 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 the 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). In the hyperdirect pathway, the subthalamic nucleus (STN) relays the 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 the 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 shown the importance of synchronized neuronal activity in the brain circuits, such as the cerebral cortex and cerebellum (Person & Raman 2011; Rosenbaum et al. 2014; Salinas & Sejnowski 2001; Singer 1993). On the other hand, the 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 the neuronal discharge changing related to the 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; de la Rocha et al. 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 GPe/GPi neurons in normal state became the oscillatory and synchronized activity in PD state. The emergence of GPe/GPi 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.

Material and Methods

1. Animal preparation

Two female Japanese macaque monkeys 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 the Care and Use of Laboratory Animals.

2. Hand reaching task

Monkeys were trained daily to sit in the monkey chair quietly and perform a hand reaching task. Monkey used a hand contralateral side to the recorded brain hemisphere. An infrared optical imaging touch panel was placed in front of the monkey, and two light-emitting diodes (LEDs) were arranged horizontally on the touch panel. Each trial was initiated after the monkey placed its hand onto the home position at least for 300 ms. The monkey was required to release its hand from the home position and reach out to the target area indicated by the LED within 6000 ms and hold at least for 40 ms. If the monkey touched the target area, the trial was considered

successful and a small amount of juice was dispensed as reward. 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

The monkeys received surgical operation to fix their heads painlessly in a stereotaxic frame that was attached to a monkey chair. Under general anesthesia with propofol TCI (6-9 μg/mL blood concentration, i.v.) and fentanyl (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 (for more details see Nambu et al. 2000, 2002). 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

Under anesthesia with ketamine hydrochloride and xylazine hydrochloride, the skull over the primary motor cortex (M1) and the supplementary motor area (SMA) was removed. According to the electrophysiological mapping, two pairs of bipolar stimulating electrodes were chronically implanted into the forelimb region of the M1, and one pair into the forelimb region of the SMA (for detail, see Tachibana et al. 2011 and 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.

5. Multi-channel recordings of GPe/GPi activity

The multi- channel recording electrodes (Plextrode U-Probe; Plexon Inc), consisting of 16 contacts in linear formation (the inter-contact spacing was 150 μm) were used. The electrode was inserted obliquely (40 degrees from the vertical in the frontal plane) 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.

6. 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 blood concentration, i.v.) and fentanyl (2-5 μg/kg, i.m.), 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 and additional intravenous injections. The total doses of MPTP was 3.9 mg/kg for a mild MPTP state's monkey and 4.2 mg/kg for a severe MPTP state's monkey. 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). A mild MPTP state's monkey was scored 13 and a severe MPTP state's monkey was scored 35. Then, GPe/GPi neuronal recordings during parkinsonian state were started.

7. 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 further 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 histograms (PETHs), 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.

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 and Discussions

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). In the GPe/GPi, 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 GPe/GPi 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 GPe/GPi suggests the parallel information processing that movement-related neuronal information is parallelly and independently processed in the GPe and GPi.

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, and 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. The monkey, which exhibited mild PD symptoms after MPTP treatment, showed the significant firing rate decrease in both GPe and GPi neurons. 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. For neuronal cross-correlation, 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.

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. Then I examined the PSD of GPe/GPi neurons. The PSD of GPe/GPi neurons exhibited a significant peak at beta range and the peak was largely 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 markedly decreased after L-dopa administration. The beta frequency neuronal activity is observed not only in the GPe/GPi 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. The relevance of abnormal neuronal firing in the GPe/GPi to abnormal motor control is an essential story to describe PD symptoms. 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.

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