Glutamatergic and GABAergic Control of Monkey Pallidal Neuronal Activity during Performing a Motor Task

Nobuya Kaneko

Department of Physiological Sciences, School of Life Science,
The Graduate University for Advanced Studies

Introduction

The basal ganglia (BG) are a group of nuclei composing loop circuits with cerebral cortex (Cx) and thalamus, and are essential for control of voluntary movements and motor leaning. Lesions in the BG result in severe disturbance in the execution of voluntary movements as typically observed in movement disorders such as Parkinson's disease. The striatum (Str) and subthalamic nucleus (STN) are input nuclei of the BG. On the other hand, the internal segment of the globus pallidus (GPi) and the substantia nigra pars reticulata (SNr) are the output stations, and the external segment of the globus pallidus (GPe) is a connecting nucleus that relays information from the input nuclei to the output stations. Thus, it is a key to analyze the mechanism controlling GPi and GPe activity especially during voluntary movements in order to understand the functions of the BG.

There are following three pathways connecting inputs nuclei with output stations of the BG: (1) *direct* pathway, Str neurons containing gamma-aminobutyric acid (GABA) and substance P project monosynaptically to the GPi/SNr; (2) *indirect* pathway, Str neurons containing GABA and enkephalin project polysynaptically to the GPi/SNr by way of sequential connections with the GPe and STN; (3) *hyperdirect* pathway, STN neurons receiving cortical inputs project monosynaptically to the GPi/SNr. In addition, the GPe-GPi projections and the GPe-GPe projections with local axon collaterals are also suggested. Thus, GPi/GPe activity is controlled by excitatory glutamatergic inputs from the STN and inhibitory GABAergic inputs from the Str and GPe. Actually, cortical stimulation induces a triphasic response consisting of early excitation, inhibition and late excitation in the GPi/GPe, and intensive studies have revealed that each component is mediated by the Cx-STN-GPi/GPe, Cx-Str-GPi/GPe and Cx-Str-GPe-STN-GPi/GPe pathways, respectively.

GPi and GPe neurons either increase or decrease their activity during voluntary limb movements. These changes during movements are likely to be induced by phasic glutamatergic and GABAergic inputs, which are transferred through the Cx-BG pathways indicated above. However, these contributions have not been studied yet. Previous studies showed that local injection of glutamatergic and GABAergic antagonists successfully block excitatory glutamatergic and inhibitory GABAergic inputs to the GPi/GPe, respectively. In the present study, I applied similar methods to GPi/GPe neurons of monkeys during performing a motor task and examined how excitatory glutamatergic and inhibitory GABAergic inputs contributed the movement-related GPi/GPe activity.

Materials and methods

Two Japanese (*Macaca fuscata*) and one Rhesus (*Macaca mulatta*) monkeys were used in this experiment. The experimental protocols were approved by the Institutional Animal Care and Use Committee 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.*

Each animal was trained to be seated in a primate chair and perform a goal-directed reaching task with delay. Three slots (Left, Center and Right) were aligned horizontally in a panel that was placed at a distance of 30 cm in front of the animal. Three slots were separated from each other by 10 cm. A two-color (red and green) light-emitting diode (LED) was installed in the bottom of each slot. Each trial was initiated after the animal placed its hand at the resting position that was located below the panel for at least 1,500 ms. In Go trials, one of three LEDs was lit with a red color for 150 ms as an instruction stimulus. A random delay period of 550–1,800 ms followed the instruction stimulus.

During the instruction stimulus and delay period, the monkey was required to keep its hand at the resting position. After a delay period, all three LEDs were lit with a green color for 1,200 ms as a triggering stimulus. Upon the presentation of a triggering stimulus, the monkey was required to reach out its forelimb, using its index finger, and touch the LED inside the slot that had been directed previously by the instruction stimulus. The onset timings of the instruction stimulus and the triggering stimulus are denoted as S1 and S2, respectively. The timings of hand release (HR) from the resting position and finger in (FI) the slot were detected by infrared photoelectric sensors (Keyence, Osaka, Japan), installed in the resting position and slots. If the monkey touched the correct LED within 1,200 ms, it was rewarded with juice. The onset timing of reward delivery is denoted as R. If the monkey released its hand from the resting position during the instruction stimulus and delay period, touched the wrong LED, or touched the LED after 1,200 ms, it was not rewarded, and the trial with same task conditions was repeated. In No-go trials, all three LEDs were lit simultaneously with a red color for 150 ms as an instruction stimulus (S1). After a delay period of 550–1,800 ms, all three LEDs were lit with a green color for 1,200 ms as a triggering stimulus (S2). If the monkey kept its hand at the resting position during the entire delay and triggering-stimulus periods, it was rewarded with juice (R). If the monkey released its hand from the resting position during entire periods, it was not rewarded, and the No-go trial was repeated. Left, Center and Right targets (appearance probability of each target, 29%) and No-go (13%) trials were presented randomly. Intertrial intervals were 2,000–3,000 ms.

After learning the behavioral task, the monkeys underwent surgical operations to fix their head painlessly in a stereotaxic frame attached to a primate chair under general anesthesia with sodium pentobarbital (25 mg/kg body wt, iv) or propofol (7 μg/mL blood

concentration, iv) with ketamine hydrochloride (10 mg/kg, im) and xylazine hydrochloride (1–2 mg/kg, im). Bipolar stimulating electrodes were implanted chronically into the forelimb regions of the motor cortex (MI) and supplementary motor area (SMA).

Single-unit recordings of GPi and GPe neurons in combination with local applications of drugs were performed with an electrode assembly consisting of a glass-coated Elgiloy microelectrode (0.6–0.9 MΩ at 1 kHz) for unit recording and two silica tubes (OD, 147 μm; ID, 74 μm; Polymicro Technologies Inc., Phoenix, AZ, USA) for drug delivery. The silica tubes were connected to two 25-μL Hamilton microsyringes, which contained following drugs dissolved in saline: (1) a mixture of 1,2,3,4-tetrahydro-6-nitro-2,3-dioxo-benzo[f]quinoxaline-7-sulphonamide disodium (NBQX, AMPA/kainate receptor antagonist, 0.5mM; Sigma, St Louis, MO, USA) and (±)-3-(2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid (CPP, NMDA receptor antagonist, 0.5mM; Sigma) (NBQX+CPP); (2) gabazine (the $GABA_A$ receptor antagonist, 0.5mM; Sigma).

First, cortically evoked responses in the GPi/GPe were examined using stimulating electrodes implanted chronically in the forelimb regions of the primary motor cortex (MI) and supplementary motor area (SMA). Next, GPi/GPe neuronal activity during the performance of the task was recorded. Then, one of the above drugs was injected (0.2–0.6 μL) through a silica tube in the vicinity of recoding neurons. After confirming the effects of the injected drug by observing the changes in the cortically evoked responses, GPi/GPe neuronal activity during the performance of the task was recorded again. Finally, the other drug was injected through the other silica tube, and recordings were performed again. By comparing neuronal activity before and after NBQX+CPP/gabazine

injections, glutamatergic and GABAergic components during the task performance could be calculated. The present study was designed to induce activity changes of a limited number of neurons without behavioral changes.

Results

Among 298 GPi/GPe neurons sampled from six hemispheres of three monkeys, activity of 55 (36 GPi and 19 GPe) neurons was successfully recorded during the task performance before and after the first drug injection. Among them, activity of 32 (22 GPi and 10 GPe) neurons was successfully recorded after the second drug injection.

The present study revealed the following results: 1) Both glutamatergic and GABAergic inputs contributed to the movement-related GPi/GPe activity, and their weights were different among neurons; 2) Phasic changes of glutamatergic and GABAergic inputs preceded the onset of movements in more than half of GPi/GPe neurons; 3) Phasic changes of both glutamatergic and GABAergic inputs were dependent on the directions of reaching movements; 4) In addition to incremental glutamatergic and decremental GABAergic components, decremental glutamatergic and incremental GABAergic components were also observed, although their contribution was small. They were considered to be caused by disfacilitatory and disinhibitory mechanisms; 5) Sustained glutamatergic inputs in the GPi were observed during delay periods.

Discussion

The present study has clearly shown that both glutamatergic and GABAergic inputs transfer specific neuronal information to the GPi/GPe in similar timing and contribute to GPi/GPe activity. Observed activity changes of GPi/GPe neurons are the results of

competition between glutamatergic and GABAergic inputs. The main origins of glutamatergic inputs are considered to be the Cx-STN-GPi/GPe and Cx-Str-GPe-STN-GPi/GPe pathways, while GABAergic inputs seem to be brought by the Cx-Str-GPi/GPe pathway (The pathways targeting the GPi are referred to as the *hyperdirect*, *indirect* and *direct* pathways, respectively). Other minor pathways, such as the Cx-Str-GPe-GPi/GPe, Cx-STN-GPe-STN-GPi/GPe and Cx-STN-GPe-GPi/GPe pathways, may also contribute to GPi/GPe activity.

Previous studies have reported that GPi and GPe neurons either increase or decrease their activity during voluntary limb movements. The increase/decrease ratio of GPi and GPe neurons, the number of neurons that increase their activity during movements over the number of neurons that decrease their activity, were always more than 1. The present study showed that activity increase of GPi and GPe neurons was largely suppressed by NBQX+CPP injections, suggesting that such activity increase reported previously is considered to be caused by excitatory glutamatergic inputs from the STN. The time course and amplitude of glutamatergic and GABAergic components were compared, and amplitude of glutamatergic components was larger than that of GABAergic components in the GPi and GPe, explaining the higher increase/decrease ratio.

Some inhibitory responses disappeared after NBQX/CPP injection and some excitatory responses disappeared after gabazine injection in the present study, which were observed as decremental glutamatergic and incremental GABAergic components, respectively. The decremental glutamatergic changes in the GPi are considered to be caused by disfacilitatory mechanism, such as disfacilitation through the net inhibitory Cx-STN-GPe-STN-GPi pathways. Similarly, the incremental GABAergic components in the GPi are considered to be caused by disinhibitory mechanism, such as disinhibition

through the net excitatory Cx-Str-GPe-GPi pathway. However these components were small comparing excitatory glutamatergic and inhibitory GABAergic components.

The present results showed that phasic glutamatergic and GABAergic changes preceded the onset of movements in more than half of GPi and GPe neurons, suggesting that both glutamatergic inputs through the net excitatory Cx-STN-GPi/GPe and Cx-Str-GPe-STN-GPi/GPe pathways and GABAergic inputs through the net inhibitory Cx-Str-GPi/GPe and Cx-STN-GPe-GPi/GPe pathways contribute to early activity changes of GPi and GPe neurons. In addition to activity changes during movements, glutamatergic components during delay the periods were observed. This component may be transferred through the Cx-STN-GPi *hyperdirect* pathway, because such activity changes were not observed in the GPe.

Activity of GPi and GPe neurons showed different activity changes depending on the reaching directions, indicating the directional selectivity. The present analyses showed that glutamatergic and GABAergic components showed similar directional selectivity. These observations suggest that both inputs have similar information regarding to movement directions. Such directional selectivity may be transferred to the GPi and GPe directly through the Cx-Str-GPi/GPe pathway and indirectly through the Cx-Str-GPe-STN-GPi/GPe pathway. It is also probable that Cx-STN-GPi/GPe pathway also transfers such specific movement related information. Activity changes in the GPi are finally transferred to the motor cortices through the thalamus and may control voluntary movements. Further analyses are necessary to determine which pathways exemplified above transfer specific information and contribute to glutamatergic/GABAergic inputs.