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

運動課題遂行中のサルにおける淡蒼球ニューロン活動の

グルタミン酸および GABA 作動性調節

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Abstract

The basal ganglia are a group of nuclei composing loop circuitry with cerebral cortex (Cx) and thalamus, and are essential for control voluntary movements and motor leaning. Lesions in the basal ganglia 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 basal ganglia. 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 basal ganglia.

Actually GPi and GPe neurons either increase or decrease their activity during voluntary limb movements. They receive excitatory glutamatergic inputs from the STN and inhibitory GABAergic inputs from the Str and GPe. To analyze how these glutamatergic and GABAergic inputs contribute to the movement-related GPi/GPe activity, neuronal activity of these neurons in behaving monkeys was recorded after blocking these inputs in the present study. Three macaque monkeys were trained to perform a goal-directed reaching task with delay. An electrode assembly consisting of a glass-coated Elgiloy microelectrode for unit recording and two silica tubes for drug delivery was inserted into the GPi/GPe. After cortically evoked responses were examined using stimulating electrodes implanted chronically in the forelimb

regions of the primary motor cortex (MI) and supplementary motor area (SMA), neuronal activity during the performance of the task was recorded. Then, one of the following drugs (0.2–0.6 μL) was injected through a silica tube in the vicinity of recoding neurons: (1) a mixture of the AMPA/kainite receptor antagonist 1,2,3,4-tetrahydro-6-nitro-2,3-dioxo- benzo[f]quinoxaline-7-sulphonamide disodium (NBQX, 0.5mM) and the NMDA receptor antagonist (±)-3-(2-carboxypiperazin-4-yl)-propyl-1- phosphonic acid (CPP, 0.5mM) (NBQX+CPP); (2) the $GABA_A$ receptor antagonist gabazine (0.5mM). The effects of the injected drug were confirmed by changes of cortically evoked responses, and neuronal activity during the performance of the task was recorded again. Finally the other drug was injected through a silica tube, and similar procedures were repeated. By comparing neuronal activity before and after NBQX+CPP/gabazine injections, glutamatergic and GABAergic components during task performance can be calculated.

The present study showed the following results: 1) Both glutamatergic and GABAergic inputs contributed to the movement-related GPi/GPe activity, and their weights are different among neurons; 2) Phasic glutamatergic and GABAergic changes preceded the onset of movements in more than half of GPi/GPe neurons; 3) Both phasic glutamatergic and GABAergic inputs were dependent on directions of 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 are considered to be caused by disfacilitatory and disinhibitory mechanism; 5) Glutamatergic changes in the

GPi were observed during delay periods; 6) No behavioral changes were observed after drug injections.

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

Introduction

The basal ganglia (BG) are a group of nuclei composing loop circuits with cerebral cortex (Cx) and thalamus, and are essential for control voluntary movements and motor leaning (Evarts et al., 1981; Alexander and Crutcher, 1990; DeLong MR, 1990; Graybiel, 2005). 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 (Fig. 1): (1) Direct pathway, striatal neurons containing gamma-aminobutyric acid (GABA) and substance P project monosynaptically to the GPi/SNr; (2) Indirect pathway, striatal 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 (Smith et al., 1994; Shink and Smith, 1995). Thus, GPi/GPe activity is controlled by excitatory glutamatergic inputs from

the STN and inhibitory GABAergic inputs from the striatum and GPe. Actually, cortical stimulation induces triphasic responses consisting of early excitation, inhibition and late excitation in the GPi/GPe, and intensive studies have reveled that each component is mediated by the Cx-STN-GPi/GPe, Cx-Str-GPi/GPe and Cx-Str-GPe-STN- GPi/GPe pathways, respectively (Nambu et al., 2000; Kita et al., 2004; Tachibana et al., 2008).

GPi and GPe neurons either increase or decrease their activity during voluntary limb movements (DeLong, 1971; Georgopoulos et al., 1983; Anderson and Horak, 1985; Hamada et al., 1990; Nambu et al., 1990; Mink and Thach, 1991; Nini et al., 1995; Mitchell et al., 1987; Turner and Anderson, 1997). 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 (Kita et al., 2004; Tachibana et al., 2008). 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

Behavioral task

Two Japanese (*Macaca fuscata*, named A and K) and one Rhesus (*Macaca mulatta*, named M) monkeys of either sex, weighing 5.2–9.5 kg, 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 (Fig.2A). Three slots (Left, Center and Right; 18 mm in height, 6 mm in width, and 11 mm in depth) 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 (Fig.2A), 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 right 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 (repeat of the error conditions). In No-go trials (Fig.2A), 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 (between the end of Reward and the beginning of the following trial) were 2,000–3,000 ms.

Surgery

After learning the behavioral task, the monkeys underwent surgical

operations to fix their head painlessly in a stereotaxic frame attached to a primate chair (for details, see Nambu et al. 2000, 2002) 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). After full recovery from the operation, the skull over the left primary motor cortex (MI) and supplementary motor area (SMA) on the side contralateral to the hand for the task was removed under light anesthesia with ketamine hydrochloride (10 mg/kg im) and xylazine hydrochloride (1–2 mg/kg im). The forelimb regions of the MI and SMA were identified by electrophysiological methods (Fig.2B; for details, see Nambu et al. 2000, 2002). According to this mapping, three pairs of bipolar stimulating electrodes (made of 200-μm-diameter, enamel-coated, stainless steel wires; intertip distance, 2 mm) were implanted chronically into the MI and SMA: one into the distal forelimb region of the MI (MId), another into the proximal forelimb region of the MI (MIp), and the other into the forelimb region of the SMA (Fig.3A). Exposed areas were covered with transparent acrylic resin, except for the orofacial area of the MI (10–15 mm diameter), for access to the GPi and GPe. A rectangular plastic chamber covering the hole was fixed onto the skull with acrylic resin.

Injecting microelectrode and receptor blockers

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 (see Kita et al., 2004; Tachibana et al., 2008). The silica tubes were connected to two 25-μL Hamilton microsyringes, which contained following drugs dissolved in saline: (1) a mixture of the AMPA/kainite receptor antagonist 1,2,3,4-tetrahydro-6-nitro-2,3-dioxo-benzo[f]quinoxaline-7-sulphonamide disodium (NBQX, 0.5mM; Sigma) and the NMDA receptor antagonist (±)-3-(2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid (CPP, 0.5mM; Sigma, St Louis, MO, USA) (NBQX+CPP); (2) the $GABA_A$ receptor antagonist gabazine (0.5mM; Sigma). Plungers of Hamilton microsyringes were mechanically controlled (Ultramicropump II, World Precision Instruments, Sarasota, FL).

Single-unit recording of GPi and GPe neurons

Recording neuronal activity of the GPi and GPe was initiated after full recovery from the surgery and was performed 2 or 3 days/wk. During the experimental sessions, each monkey was seated in the monkey chair with head fixation. The electrode assembly was penetrated obliquely (45° from vertical in the frontal plane) into the GPe and GPi using a hydraulic microdrive (Narishige Scientific Instrument Laboratory, Tokyo, Japan).

The neuronal activity recorded from the microelectrode was amplified (×10,000), filtered (100–2,000 Hz), and displayed on an oscilloscope. Activity of the GPi and GPe neuron was isolated and converted into digital pulses using a time-amplitude window discriminator. The firing rate of GPi and GPe neurons were characterized by their high firing rates (80–100 Hz). GPi and

GPe neurons were discriminated by 1) the depth profile, 2) firing pattern difference, such as firing pauses of GPe neurons and continuous firings of GPi neurons, and 3) "border neurons" that are located in the border between GPe and GPi, and fire at regular low frequency rates (Tachibana et al., 2008).

The responses to cortical stimulation (300 μs duration single pulse, strength < 0.6 mA) were observed by constructing peristimulus time histograms (PSTHs; bin width of 1 ms; summed for 100 stimulus trials) using a computer. During constructing PSTHs, the monkey sat quietly without performing any tasks. MI stimulation induced movements of corresponding body parts, while SMA stimulation rarely induced. The typical response pattern of GPi/GPe neurons is triphasic consisting early excitation, inhibition and late excitation (Nambu et al., 2000; Kita et al., 2004; Tachibana et al., 2008). The forelimb region of the GPi/GPe can be identified by the cortically evoked responses. Only GPi/GPe neurons responding to the MI and/or SMA stimulation were recorded.

Then, the neuronal activity during the performance of a goal-directed reaching task with delay was recorded as control condition. Timings of neuronal firings and task events (S1, S2, HR, FI, and R) were stored on a computer at a time resolution of 1 ms. These data, along with raw neuronal activity, were also stored on videotapes using a pulse-code modulation recorder (Cygnus Technology, Delaware Water Gap, PA).

After recording under control condition, the first drug, NBQX+CPP or gabazine, was injected in the vicinity of recording neuron (a total volume of 0.2–0.6 μL, at the rate of 0.03 μL/min), and cortically evoked responses were

recorded. NBQX+CPP is expected to interrupt excitatory ionotropic glutamatergic inputs to GPi/GPe neurons, decrease spontaneous firing rates and diminish cortically evoked early and late excitation (Kita et al., 2004; Tachibana et al., 2008). On the other hand, gabazine is expected to interrupt inhibitory ionotropic GABAergic inputs to GPi/GPe neurons, increase spontaneous firing rates and diminish cortically evoked inhibition (Kita et al., 2004; Tachibana et al., 2008). After confirming these changes in PSTHs, the neuronal activity during the performance of the task was recorded

Finally, the second drug, gabazine or NBQX+CPP, which was different from the first one, was additionally injected in the vicinity of recording neuron (a total volume of 0.2–0.6 μL, at the rate of 0.03 μL/min), and cortically evoked responses were recorded. Additional injection is expected to interrupt both excitatory ionotropic glutamatergic and inhibitory ionotropic GABAergic inputs, induce regular firings and diminish all components of cortically evoked responses (Kita et al., 2004; Tachibana et al., 2008). After confirming these changes in PSTHs, the neuronal activity during the performance of the task was recorded. Injection sites were located at least 1 mm apart because the effective radius of the drugs was estimated to be ~1 mm (see Kita et al., 2004).

Data analysis

Neuronal activity during task performance was aligned with the task events (S1, S2, HR, and R) separately, according to the S1 conditions (Left,

Center, and Right targets and No-go trials) and shown in raster display. Then, spike-density functions (σ = 10 ms) were calculated.

Glutamatergic and GABAergic components were calculated as follows:

Glutamatergic component = (spike-density function before NBQX+CPP) - (spike-density function after NBQX+CPP)

GABAergic component = (spike-density function before gabazine) - (spike-density function after gabazine)

For detecting event-related changes of glutamatergic and GABAergic components, mean value and standard deviation (SD) during 1,000 ms, preceding the S1, were calculated and were considered to be the value for baseline. Activity changes during delay (0–900 ms after S1) and HR periods (from 300 ms before HR to 700 ms after HR) were judged to be significant if the neural response during at least 10 ms reached a significance level $p <$ 0.001 (mean ± 3.09 SD). The start and end points of the neural response were defined as the time at which the firing rate first exceeded a significance level $p < 0.05$ (mean ± 1.65 SD). If neurons showed delay-related changes and reached a significance level ($p < 0.001$) 300 ms before HR, mean value and SD during 500 ms, preceding the S2, were calculated and used instead.

Amplitude of incremental responses related to the delay or HR was defined as the total numbers of spikes above the baseline minus that of the

baseline (i.e., the areas above the baseline) during the delay (0–900 ms after S1) or HR periods (from 300 ms before the HR to 700 ms after the HR), respectively. Similarly, amplitude of decrement responses was defined as the total numbers of spikes below the baseline minus that of the baseline (i.e., the areas below the baseline). Then these amplitudes were normalized as the number of spikes per one second. In addition, amplitudes of significant activity changes related HR was also defined as the number of spikes from the start point to the end point minus that of the baseline (i.e., the area of the response).

Glutamatergic and GABAergic components were modulated by target directions. Amplitude of responses in Left, Center and Right targets was plotted as a vector in 3-D space. Directional selectivity was defined as an angle θ (degree) between that vector and the vector (1, 1, 1) ($0 \leq \theta \leq 54.7$). Large θ value indicates high directional selectivity.

Histology

At the end of the final experiment, several recording sites were marked by passing cathodal direct current (20 μA for 30 s) through the electrode. The monkeys were then anesthetized deeply with sodium pentobarbital (50 mg/kg, iv) and perfused transcardially with 2 L of PBS, pH 7.3, followed by 5 L of 8% formalin in 0.1 M phosphate buffer (PB), pH 7.3, and 3 L of 0.1 M PB containing 10% sucrose. The brains were removed, kept in 0.1 M PB containing 30% sucrose at 4°C, and then cut serially into 50 μm-thick frontal sections on a freezing microtome. The sections were mounted onto gelatin

coated glass slides and stained with cresyl violet. The recording sites were reconstructed according to the lesions made by current injection and the traces of electrode tracks. The reconstruction helps to classify recorded neurons into GPe or GPi neurons (see Tachibana et al., 2008). The positions of cortical stimulation electrodes were also confirmed histologically.

Results

Database

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. These neurons can be classified based on the cortical inputs by examining PSTHs (Fig. 4A, Table 1). Most of them received convergent inputs from the SMA and MI (27/36 GPi and 14/19 GPe).

All of these GPi/GPe neurons changed their activity during the HR periods (from 300 ms before the HR to 700 ms after the HR), and activity increases (28/36 GPi and 13/19 GPe) were major changes comparing activity decreases. Some neurons showed delay-related activity, i.e., gradual increase or decrease during delay periods (activity increase, 4 GPi with SMA inputs, 6 GPi with SMA+MI inputs; activity decrease, 2 GPe with SMA+MI inputs, 2 GPi with SMA+MI inputs). Some (13 GPi and 7 GPe) neurons showed activity changes in no-go trials in addition to go-trials around the S2 (1000 ms periods centered at the S2), however amplitude of changes in no-go trials were smaller than that in go trials. Finally, GPi/GPe neurons with SMA inputs and those with convergent inputs from MI and SMA showed similar activity changes, thus these neurons were examined together in further analyses.

A mixture of NBQX+CPP was usually injected first in most cases (31/35 GPi and 12/19 GPe, Table 2). Among them, 21 GPi and 8 GPi neurons were examined after additional gabazine injection. We also tried gabazine injection first. Gabazine depolarized neurons, and it became difficult to keep recording

in good conditions. Thus, we succeeded in limited cases.

Changes of cortically evoked responses and task related activity by drug injections

Figures 4 and 5 show an example of GPi neurons. MIp stimulation induced a triphasic response composed of early excitation, inhibition and late excitation, while SMA stimulation induced a biphasic response composed of inhibition and late excitation (Fig. 4A), indicating that this neurons received convergent inputs from the MIp and SMA. Figure 5A shows activity changes during performing a goal-directed reaching task with delay. This neuron increased its activity with two peaks during the HR periods: one peak preceded the HR and the other lagged the HR. Its response patterns were different between Left, Center and Right target conditions. Then, 0.2 μL of NBQX+CPP was injected at the rate of 0.03 μL/min, and cortical evoked responses were examined 15 min after the completion of injection. The early and late excitation was abolished, and the inhibition was prolonged (Fig. 4B), indicating that excitatory glutamatergic inputs were blocked (Kita et al., 2004, Tachibana et al., 2008). Figure 5B shows activity changes after blocking excitatory glutamatergic inputs. Base firing rates and movement-related activity changes were decreased. Finally 0.2 μL of gabazine was additionally injected at the rate of 0.03 μL/min, and cortical evoked responses were examined 15 min after the completion of injection. The inhibition was largely suppressed (Fig. 4C), indicating that excitatory glutamatergic and inhibitory GABAergic inputs were blocked (Kita et al., 2004, Tachibana et al., 2008).

Figure 5C shows activity changes after blocking excitatory glutamatergic and inhibitory GABAergic inputs. Base firing rates were increased, however movement-related activity changes were mostly lost. By calculating the difference between normal and NBQX+CPP conditions, and between NBQX+CPP and additional gabazine conditions, glutamatergic (Fig. 5D) and GABAergic (Fig. 5E) components were obtained, respectively. Incremental glutamatergic and decremental GABAergic changes are evident (Fig. 5D and 5E. Left target), however other changes, such as decremental glutamatergic (Fig. 5D, Center target) and incremental GABAergic (Fig. 5E, Center and Right targets) were also observed.

Figure 6 shows another example of GPi neurons. This neuron received convergent inputs from the SMA, MIp and MId. This neuron showed activity increase during the HR periods in all target conditions (Fig. 6A), and increase preceded the HR and was prolonged after the FI. After blocking excitatory glutamatergic inputs by 0.2 μL NBQX+CPP injection, which was confirmed by PSTHs (data not shown), the activity increase mostly disappeared (Fig. 6B). After blocking excitatory glutamatergic and inhibitory GABAergic inputs by additional gabazine injection, no further changes were observed (Fig. 6C). Only incremental glutamatergic changes were evident (Fig. 6D, E).

Figure 7 shows an example of GPe neurons. This neuron received convergent inputs from the SMA and MIp. This neuron showed small activity decrease with two troughs during the HR periods, which was evident in Left target conditions. After NBQX+CPP injection, large activity decrease appeared during the HR periods (Fig. 7B). After gabazine injection,

movement-related activity changes were mostly diminished (Fig. 7C). Both incremental glutamatergic and decremental GABAergic components were evident (Fig. 7D, E).

Glutamatergic and GABAergic components of GPi/GPe neurons

To understand overall changes of glutamatergic and GABAergic components in go-trials, gland averages of each component were obtained in GPi and GPe neurons (Fig. 8). Gradual increase during delay period was observed in glutamatergic component of GPi neurons (Fig. 8A), while other components did not show delay-related changes. Glutamatergic components temporally decreased 200 ms before the HR, and decremental GABAergic changes appeared (Fig. 8A, B). Incremental glutamatergic changes continued after the HR, decremental GABA changes disappeared 100 ms after the HR and reappeared 200 ms after the HR. On the other hand, incremental glutamatergic changes and decremental GABAergic changes in GPe neuron began around 100 ms before the HR and showed similar time profiles (Fig 8C, D). Gland averages of glutamatergic and GABAergic component in no-go trials were also calculated (Fig. 9). Small fluctuations might exist, however significant and steady changes were not observed in the GPi and GPe neurons.

Timing of glutamatergic and GABAergic component changes in GPi/GPe neurons

The timing of incremental/decremental changes of glutamatergic and GABAergic component was compared in reference to the HR (Fig. 10). In more

than half of neurons, changes started before the HR in GPi and GPe neuron except for incremental GABAergic changes in GPe neurons. The distributions of change timing were similar among these four components in GPi and GPe neuron (Table 3), indicating that all these components were similarly activated.

Amplitude of glutamatergic and GABAergic component changes in GPi/GPe neurons

Amplitude of changes of glutamatergic and GABAergic components during the delay periods was compared in GPi and GPe neurons (Fig. 11). The incremental glutamatergic changes were significantly larger than the others in GPi neurons (Fig. 11 A; $p < 0.01$, Bonferroni test). On the other hand, incremental glutamatergic and decremental GABAergic changes showed comparable amplitude in GPe neurons (Fig. 11 B).

Amplitude of changes of glutamatergic and GABAergic components during the HR periods was also compared in GPi and GPe neurons (Fig. 12A, B). Incremental glutamatergic and decremental GABAergic changes were evident in GPi neurons, and incremental glutamatergic changes were significantly larger than decremental glutamatergic and incremental GABAergic changes (Fig. 12A, $p < 0.01$, Bonferroni test). Incremental glutamatergic changes were also evident in GPe neurons.

Relations between glutamatergic and GABAergic components during the HR periods in each neuron were investigated (Fig. 12C, D). Incremental glutamatergic and decremental GABAergic changes in different reaching

directions were plotted in each GPi (Fig. 12C) and GPe (Fig. 12D) neuron. These scatter plots showed that each neuron received different contribution of glutamatergic and GABAergic components. Circles connected with lines, indicating amplitude in different reaching directions of single neurons, had a tendency to concentrate, suggesting that single neurons received comparable glutamatergic and GABAergic inputs.

Neurons with early glutamatergic and early GABAergic changes, i.e., neurons whose glutamatergic and GABAergic changes preceded the HR, showed comparable amplitude of glutamatergic and GABAergic components (red circles in Fig 12C, D). Neurons with late glutamatergic and early GABAergic changes showed similar distribution (orange circle) in GPi and GPe neurons. On the other hand, neurons with early glutamatergic and late GABAergic changes were dominated by glutamatergic components (green circle) in GPi and GPe neurons ($p < 0.01$, Bonferroni test). Neurons with late glutamatergic and late GABAergic changes (blue circle) showed small glutamatergic and GABAergic components.

Directional selectivity of glutamatergic and GABAergic component changes in GPi/GPe neurons

To investigate the specificity of glutamatergic and GABAergic components, the modulation of glutamatergic and GABAergic changes by reaching direction were analyzed. The amplitudes of incremental/decremental glutamatergic and GABAergic component changes were plotted as a vector in 3-D space (Fig. 13 A). Directional selectivity was

defined as an angle θ (degree) between that vector and the vector (1, 1, 1) ($0 \leq \theta \leq 54.7$). Directional selectivity of incremental/decremental glutamatergic and GABAergic changes were plotted in GPi (Fig. 13B) and GPe (Fig. 13C) neurons. There are no significant differences among incremental/ decremental glutamatergic and GABAergic changes (Table 4). Thus, incremental/decremental glutamatergic and GABAergic component changes showed similar directional selectivity, suggesting that these components covey similar movement related information.

Behavioral changes after drug injections

Abnormal movements, such as tremor, bardykinesia and dyskinesia were not observed after drug injections. Reaction time (time between the S2 and the HR) and movement time (time between the HR and the FI) were measured before and after drug injections. NBQX+CPP and Gabazine injections (0.2–0.6 μL) into the GPi and GPe did not obviously change reaction and movement time.

Discussion

To analyze how excitatory glutamatergic and inhibitory GABAergic inputs contribute to the movement-related GPi and GPe activity, glutamatergic and GABAergic antagonists were locally injected, and activity of GPi/GPe neurons was recorded during performance of a reaching task with delay. The present study showed the following results: 1) Both glutamatergic and GABAergic inputs contributed to the movement-related GPi/GPe activity, and their weights are different among neurons; 2) Phasic glutamatergic and GABAergic changes preceded the onset of movements in more than half of GPi/GPe neurons; 3) Both phasic glutamatergic and GABAergic inputs were dependent on directions of 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 are considered to be caused by disfacilitatory and disinhibitory mechanism; 5) Glutamatergic changes in the GPi were observed during delay periods; 6) No behavioral changes were observed after drug injections.

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.

Technical considerations

Cortical stimulation typically induces a triphasic response composed of early excitation, inhibition and late excitation in the GPi/GPe (Nambu et al., 2000; Kita et al., 2004; Tachibana et al., 2008). Previous intensive studies have shown that the early excitation, inhibition and late excitation are mediated by the Cx-STN-GPi/GPe, Cx-Str-GPi/GPe and Cx-Str-GPe-STN-GPi/GPe pathways, respectively. Previous studies and the present study showed that NBQX+CPP and gabazine injections diminished early and late excitation, and inhibition, respectively (Fig. 4). In the present study, combining local drug injections and cortically evoked responses, activity of GPi and GPe neurons was observed during performing a motor task, and contribution of glutamatergic and GABAergic inputs were dissected.

Dissecting glutamatergic and GABAergic components

Previous studies have reported that GPi and GPe neurons either increase or decrease their activity during voluntary limb movements (DeLong, 1971; Georgopoulos et al., 1983; Anderson and Horak, 1985; Hamada et al., 1990; Nambu et al., 1990; Mink and Thach, 1991; Nini et al., 1995; Mitchell et al., 1987; Turner and Anderson, 1997). 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 (Fig 8, Fig 12A, B), 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 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 (see Fig. 1). Similarly, 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. However these components were small comparing excitatory glutamatergic and inhibitory GABAergic components (Fig. 11m Fig 12A, B).

Comparison between glutamatergic and 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 (Fig 10), 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 (see Fig. 1). In addition to activity changes during movements,

glutamatergic components during delay the periods were observed (Fig. 8). 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 (Fig. 13). These observations suggest that both inputs have similar information regarding to movement directions. A previous study showed that striatal neurons showed directional selectivity (Takara et al., 2011). Such directional selectivity may be transferred to the GPi and GPe directly through the Str-GPi/GPe pathway and indirectly through the Str-GPe-STN-GPi/GPe pathway. It is also probable that Cx-STN-GPi/GPe pathway also transfers such specific movement related information.

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Figure legends

Fig. 1. A schematic diagram of the basal ganglia circuitry. White and black arrows represent excitatory glutamatergic (glu) and inhibitory GABAergic (GABA) projections, respectively. Cx, cerebral cortex; GPe, GPi, external and internal segments of the globus pallidus; STN, subthalamic nucleus; Str, striatum; SNr, substantia nigra pars reticulata; Th, thalamus.

Fig. 2. **A**: goal-directed reaching task with delay. Three slots (Left, Center, and Right) with 2-color (red and green) light-emitting diode (LED) were aligned horizontally in a panel that was placed in front of the animals. The animal kept its hand on the resting position for at least 1,500 ms to start trials. In Go trials, 1 of 3 LEDs (Left, Center, or Right) was lit with red color for 150 ms as an instruction stimulus (S1). After a random delay period of 550–1,800 ms, all 3 LEDs were lit with a green color for 1,200 ms as a triggering stimulus (S2). Upon the presentation of the S2, the monkey was required to reach out its forelimb and touch, using its index finger, the LED inside the slot that had been instructed previously by the S1. The timings of hand release (HR) from the resting position and of finger in (FI) the slot were detected by the infrared photoelectric sensors. If the monkey touched the correct LED within 1,200 ms, it was rewarded (R) with juice. In No-go trials, all 3 LEDs were lit simultaneously with a red color for 150 ms (S1). After a delay period of 550–1,800 ms, all 3 LEDs were lit with a green color for 1,200 ms (S2). If the monkey kept its hand at the resting position during these

periods, it was rewarded with juice. **B**: cortical mapping (Monkey A) for implantation of stimulating electrodes. B1: top view of the monkey brain. Gray squares indicate mapped areas in 2 and 3. B2 and B3: mapping of the supplementary motor area (SMA) and primary motor cortex (MI), respectively. Each letter indicates the somatotopic body part: D, digit; E, elbow; F, foot; H, hip; J, jaw; S, shoulder; Ta, tail; Tr, trunk; V, visual response; W, wrist. Somatotopic arrangements in the medial surface and the rostral bank of the central sulcus are also shown, along with depths from the cortical surface. Three pairs of bipolar-stimulating electrodes were implanted into the loci, indicated by small gray circles: the forearm region of the SMA and the proximal (MIp) and distal (MId) forelimb regions of the MI.

Fig. 3. **A**: Schematic representation of the experimental setup. Bipolar stimulating electrodes were chronically implanted in the SMA, MIp and MId. The injection and recoding electrode was penetrated obliquely (45 degrees from vertical in the frontal plane) into the GPe and GPi. **B**: The injection and recoding electrode was consisted of a glass-coated Elgiloy microelectrode for unit recording and 2 silica tubes (outside diameter, 147 μm; inside diameter, 74 μm) for drug delivery (see Kita et al., 2004).

Fig. 4. Effects of local injection of 1,2,3,4-tetrahydro-6-nitro-2,3-dioxo-benzo[f]quinoxaline-7-sulphonamide disodium (NBQX) and (±)-3- (2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid (CPP), and gabazine on cortically evoked responses of a GPi neuron. Peristimulus time histograms

(PSTHs; 1ms bin, 100 time) show neuronal responses to MIp (0.6 mA, left column) and SMA (0.6 mA, right column) stimulation. **A:** MIp-stimulation induced a triphasic response consisting of early excitation, inhibition and late excitation, and SMA-stimulation induced a biphasic response consisting of inhibition and late excitation, before drug injections. **B:** The early and late excitation was abolished, and the inhibition was prolonged, 15 min after NBQX+CPP injection (total volume of 0.4 µL at speed of 0.03 µL/min). **C:** The inhibition also diminished, 15 min after additional gabazine injection (total volumes of 0.4 µL at speed of 0.03 µL/min). Cortical stimulation was given at time = 0. The mean firing rate and statistical levels of $p < 0.05$ (one-tailed t-test) calculated during the 100-ms period preceding the stimulation onset are indicated in PSTHs by red solid and dotted lines, respectively.

Fig. 5. An example of a GPi neuron. **A:** Raster display showing the neuronal firings (red dots) during the performance of a goal-directed reaching task with delay. Neuronal activity was aligned separately according to the S1 condition (Left, Center and Right targets and No-go trials, from left to right) with S1, HR (in Go-trials) or S2 (in No-go trials), and R. Short, blue vertical lines indicate the timing of the task events, such as S2 and FI. Each plot of go-trials was sorted according to the reaction time (S2-HR). Continuous green traces indicate spike density functions (σ = 10 ms) for associated raster. Activity increases were observed before and after HR. **B:** Raster display and spike density functions 18 min after 0.2 µL NBQX+CPP injection. Base firing rates and movement-related activity changes were decreased. C: Raster

display and spike density functions 15 min after additional 0.2 µL gabazine injection. Base firing rates increased, while movement-related changes were mostly lost. Note that the reaction times are comparable between control, NBQX+CPP and gabazine conditions. **D:** Glutamatergic components calculated as algebraic differences between spike density functions in control conditions and ones after NBQX+CPP injection. The solid, broken and dotted lines indicate mean value, statistical levels of $p < 0.05$ and $p < 0.001$ calculated during 1000 ms periods preceding S1, respectively. **E:** GABAergic components calculated as algebraic differences between spike density functions after NBQX+CPP injection and ones after additional gabazine injection.

Fig. 6. Another example of a GPi neuron. This neuron shows activity increase in relation to movement in control condition (**A**). The activity increase disappeared after of 0.2 µL NBQX+CPP injection (**B**). No further changes were observed during movements after additional 0.2 µL gabazine injection (**C**). Glutamatergic (**D**) and GABAergic (**E**) components were calculated.

Fig. 7. An example of a GPe neuron. This neuron shows small activity decrease during movements in control conditions (**A**). NBQX+CPP injection (0.2 µL) unmasked activity decrease during movements (**B**). Additional gabazine injection (0.2 µL) diminished activity decrease (**C**). Glutamatergic (**D**) and GABAergic (**E**) components were calculated.

Fig. 8. Grand average of glutamatergic (**A, C**) and GABAergic (**B, D**) components of GPi (**A, B**) and GPe (**C, D**) neurons in go trials aligned with the S1 (left) and HR (right). Green lines indicate standard errors. Black dotted line represents mean values calculated 1000 ms periods preceding the S1. In the GPi, delay- and movement-related glutamatergic components (**A**) and movement-related GABAergic components (**B**) were observed. In the GPe, movement-related glutamatergic (**C**) and GABAergic (**D**) components were mainly observed.

Fig. 9. Grand average of glutamatergic (**A, C**) and GABAergic (**B, D**) components of GPi (**A, B**) and GPe (**C, D**) neurons in no-go trials aligned with the S1 (left) and S2 (right). No significant changes were observed.

Fig. 10. Cumulative histograms of the latencies of movement-related glutamatergic and GABAergic components of GPi (**A**) and GPe (**B**) neurons. The abscissa represents the timing in reference to the HR. Thick and thin red lines represent incremental and decremental changes of glutamatergic components, while thick and thin blue lines represent decremental and incremental changes of GABAergic components. Colored triangles indicate mean values corresponding colored traces.

Fig. 11. Histograms showing the amplitude of glutamatergic and GABAergic components during delay periods in GPi (**A**) and GPe (**B**) neurons.
Incremental and decremental glutamatergic components and decremental

and incremental GABAergic components are separately shown. Black vertical lines represent SE. *, $p < 0.001$ significantly different each other (Bonferroni test).

Fig. 12. Movement-related changes of glutamatergic and GABAergic components. **A, B**: Histograms showing the amplitude of glutamatergic and GABAergic components during HR periods (300 ms before HR to 700 ms after HR) in GPi (**A**) and GPe (**B**) neurons. Incremental and decremental glutamatergic components and decremental and incremental GABAergic components are separately shown. $p < 0.001$ significantly different each other (Bonferroni test). **C, D**: Scatter plots showing the amplitude of significant activity changes during HR periods in each reaching direction (Left, Center and Right) in GPi (**C**) and GPe (**D**) neurons. Data of single neurons are connected each other. The ordinate represents incremental glutamatergic changes, while the abscissa represents decremental GABAergic changes. Red circles, neurons with early glutamatergic and early GABAergic changes, i.e., neurons whose glutamatergic and GABAergic changes preceded the HR; Blue circles, neurons with late glutamatergic and late GABAergic changes; Green circles, neurons with early glutamatergic and late GABAergic changes; Orange circles, neurons with late glutamatergic and early GABAergic changes. Colored circles with crosses indicate mean values and SDs of corresponding colored circles, respectively.

Fig. 13. Directional selectivity of glutamatergic and GABAergic components.

A: Amplitude of incremental glutamatergic and decremental GABAergic components in Left, Center and Right targets of each neuron was plotted as a vector in 3-D space. Directional selectivity was defined as an angle θ (degree) between that vector and the vector (1, 1, 1) ($0 \leq \theta \leq 54.7$). **B, C**: Cumulative histogram of the directional selectivity θ of GPi (B) and GPe (C) neurons. Thick and thin red lines represent incremental and decremental changes of glutamatergic components, while thick and thin blue lines represent decremental and incremental changes of GABAergic components. Colored triangles indicate mean values corresponding colored traces.

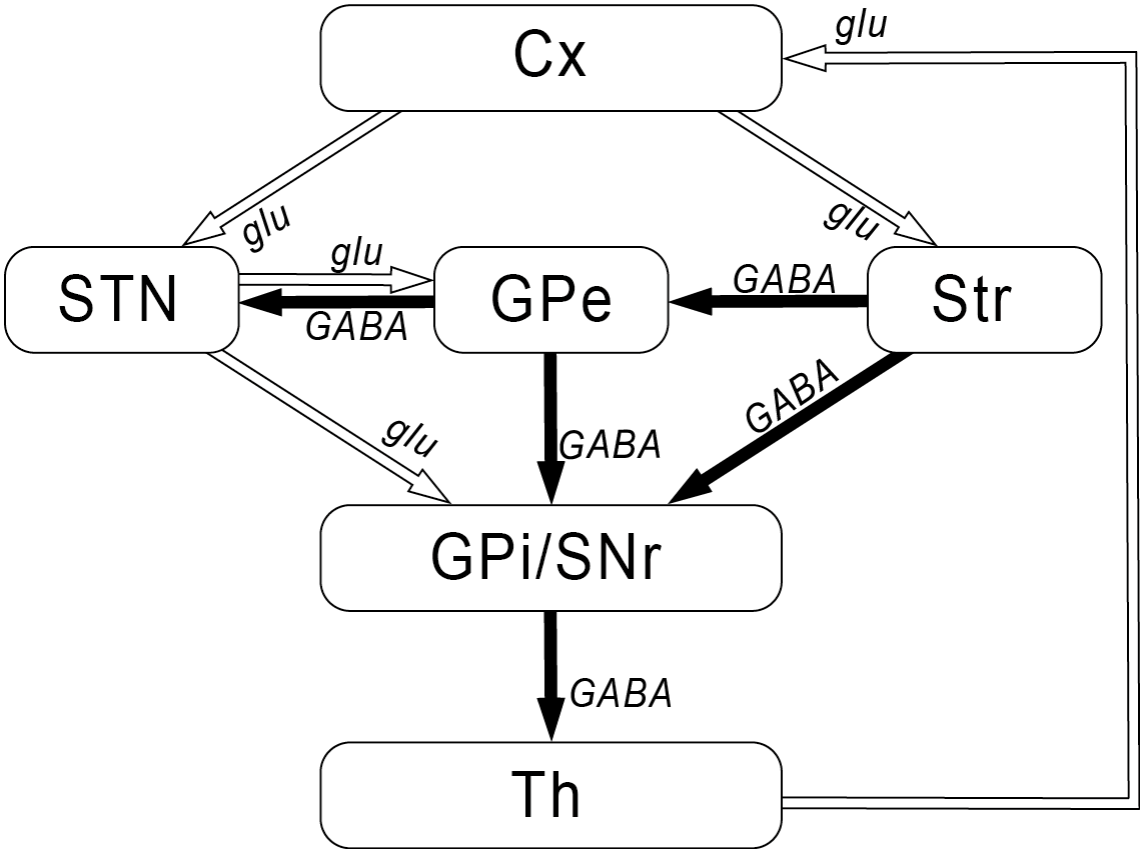
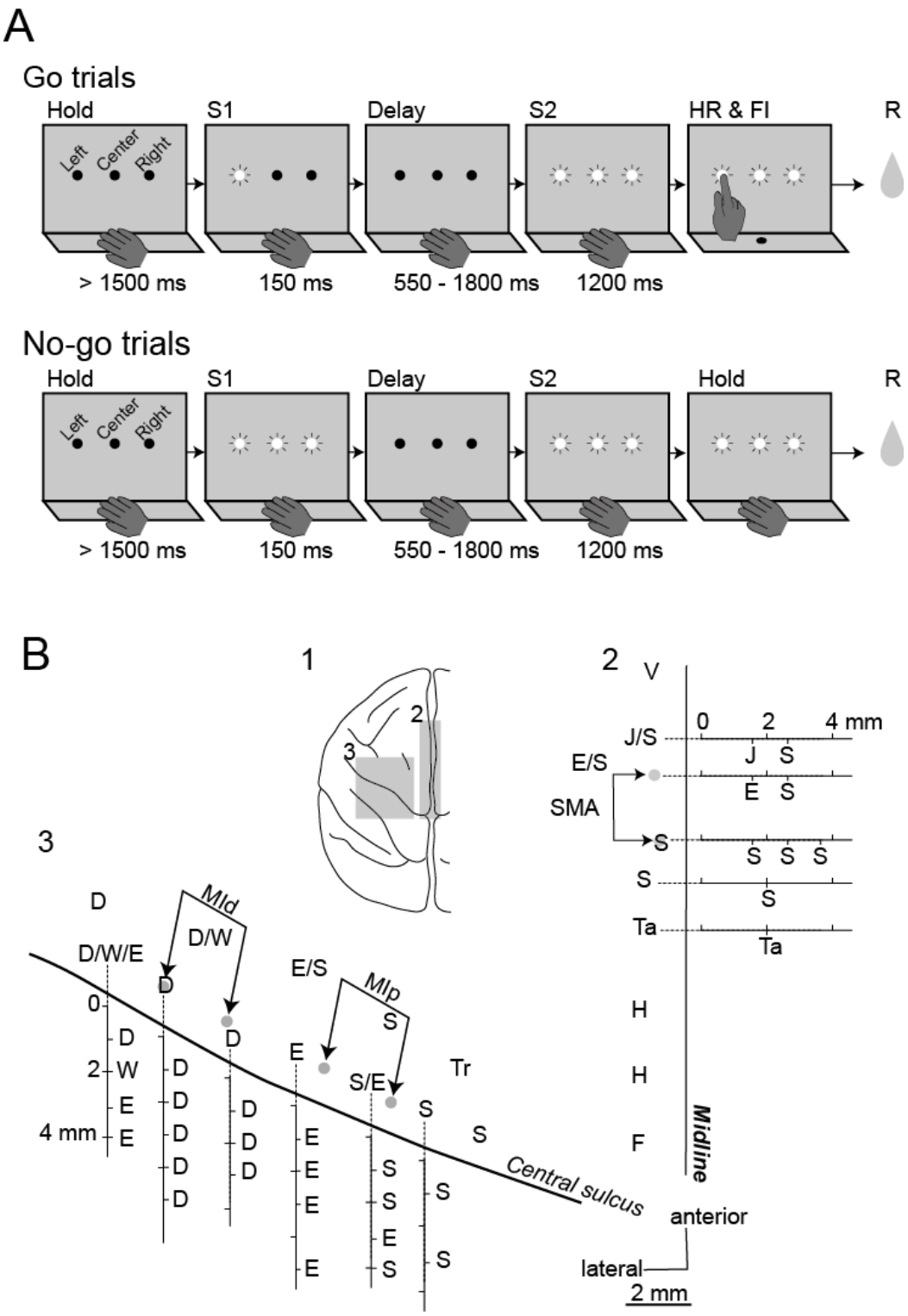

Cx
glu
glu
glu
STN
glu
GPe
GABA
Str
GABA
glu
GABA
GABA
GPi/SNr
GABA
Th

Fig1


A
Go trials
Hold
S1
Delay
S2
HR & FI
R
Left
Center
Right
> 1500 ms
150 ms
550 - 1800 ms
1200 ms
No-go trials
Hold
S1
Delay
S2
Hold
R
Left
Center
Right
> 1500 ms
150 ms
550 - 1800 ms
1200 ms
B
1
2
3
2
V
0
2
4 mm
J/S
J
S
E/S
E
S
SMA
S
S
S
S
S
S
Ta
Ta
H
H
F
Midline
anterior
lateral
2 mm
3
D
MId
D/W
D/W/E
D
E/S
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W
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4 mm
E
E
S/E
Tr
S
S
Central sulcus

Fig2

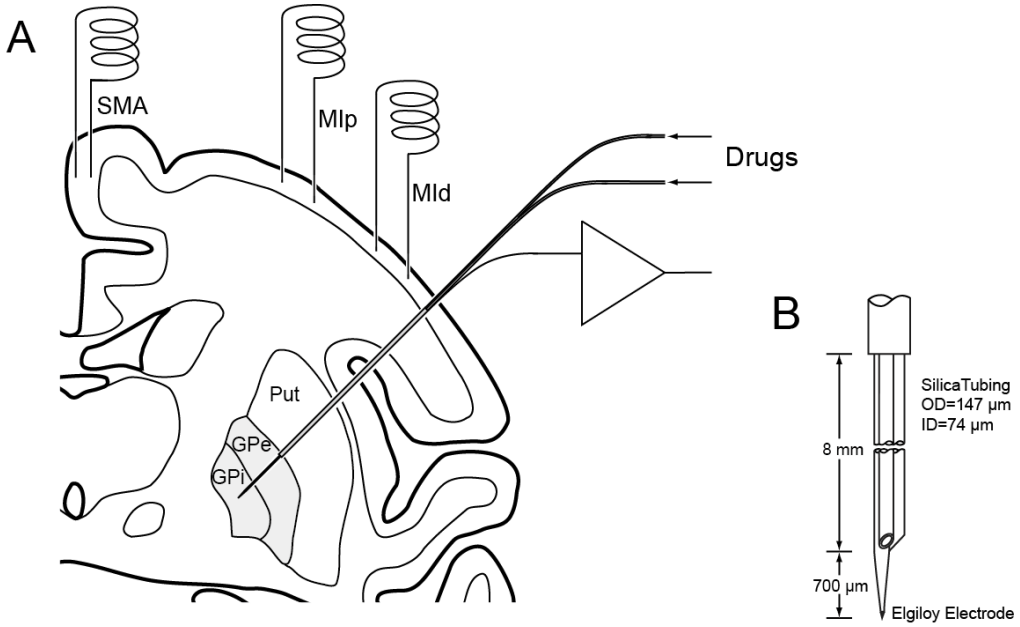

A
SMA
MIp
MId
Drugs
Put
GPe
GPi
B
SilicaTubing
OD=147 µm
ID=74 µm
8 mm
700 µm
Elgiloy Electrode

Fig3

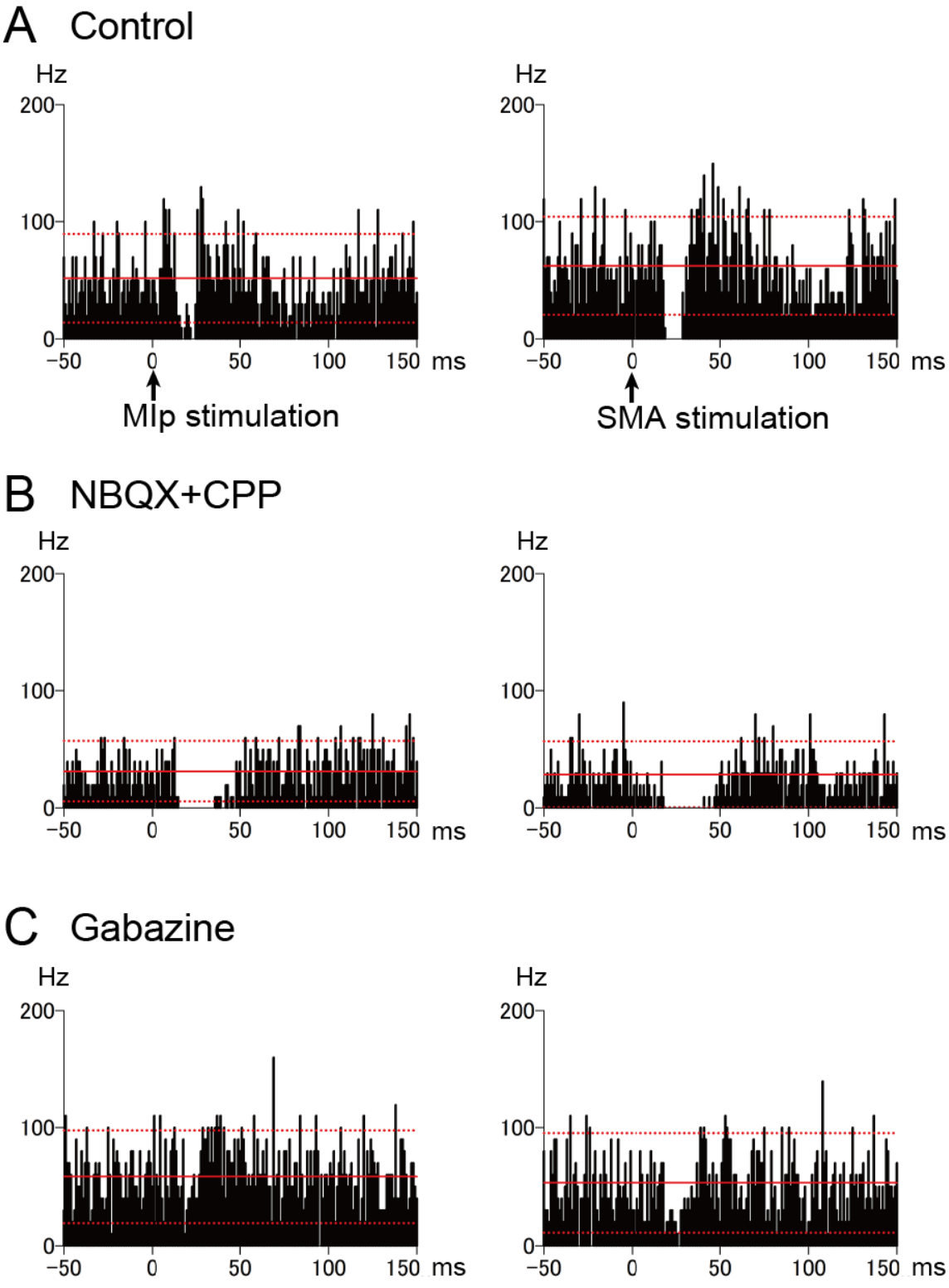

A Control
Hz
200
100
0
−50
0
50
100
150
ms
MIp stimulation
SMA stimulation
B NBQX+CPP
C Gabazine

Fig4

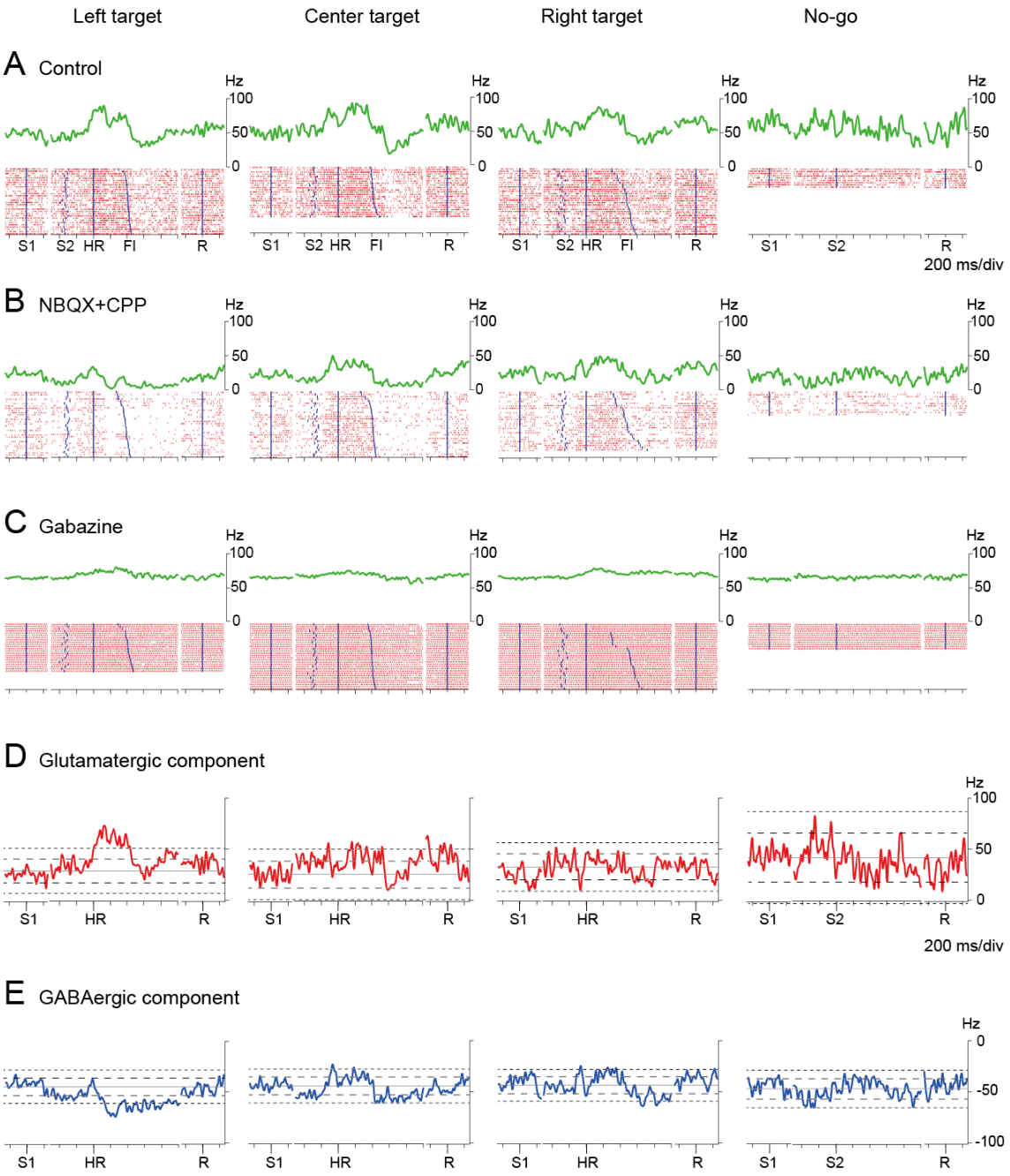

Left target
Center target
Right target
No-go
A Control
B NBQX+CPP
C Gabazine
D Glutamatergic component
E GABAergic component
Hz
100
50
0
-50
-100
S1
S2
HR
FI
R
200 ms/div

Fig5

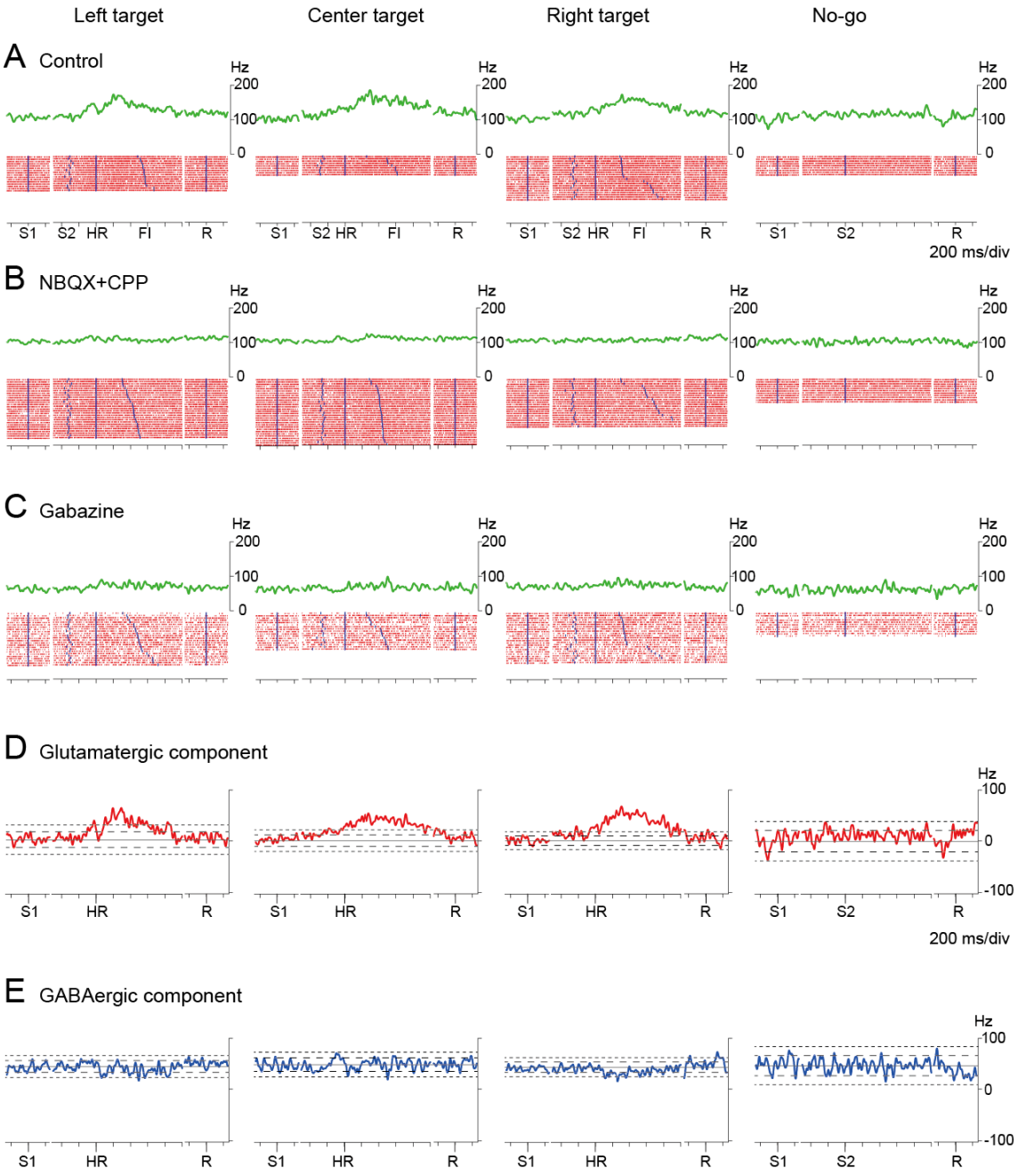

Left target
Center target
Right target
No-go
A Control
B NBQX+CPP
C Gabazine
D Glutamatergic component
E GABAergic component
Hz
200
100
0
-100
S1
S2
HR
FI
R
200 ms/div

Fig6

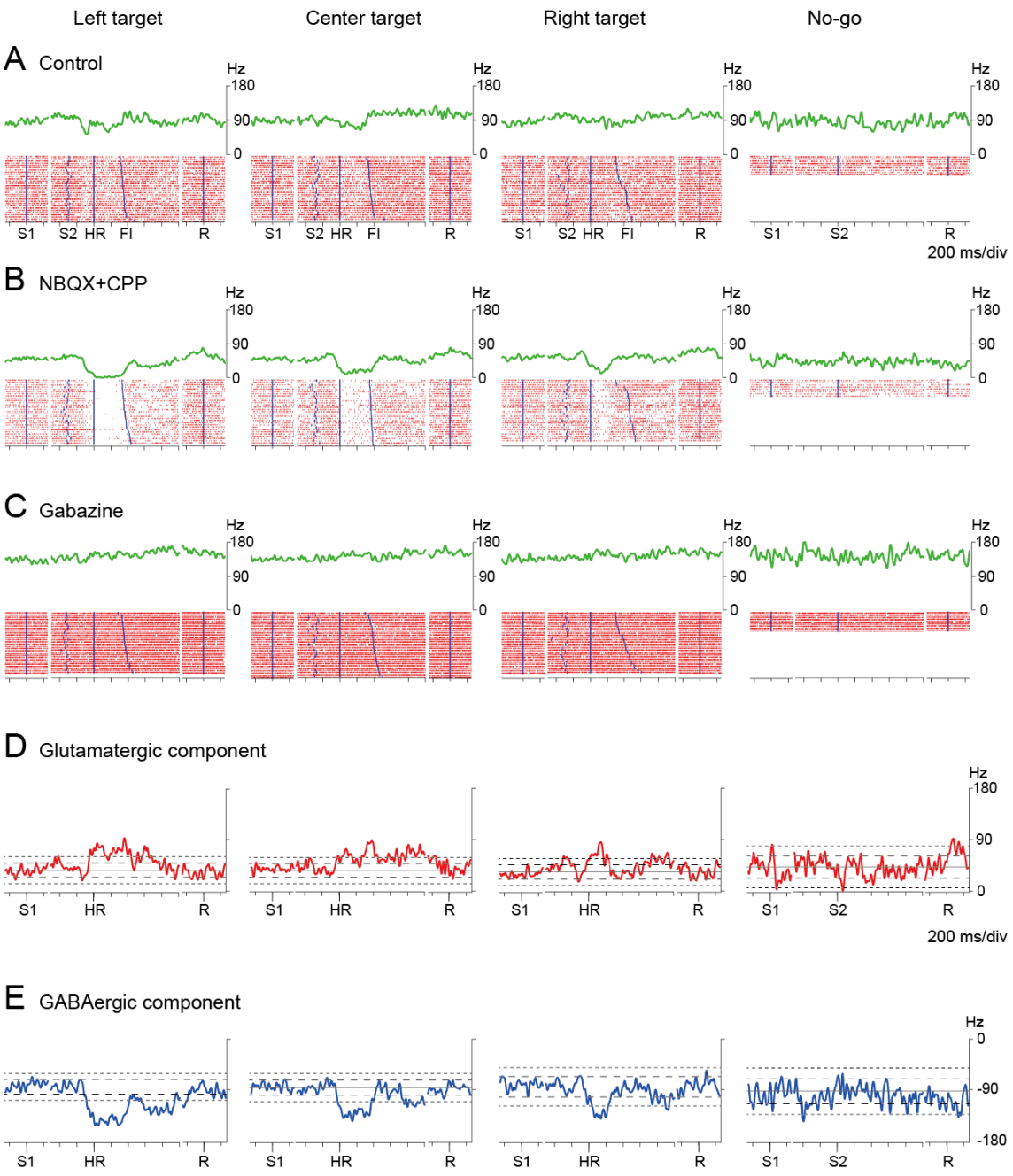

Left target
Center target
Right target
No-go
A Control
B NBQX+CPP
C Gabazine
D Glutamatergic component
E GABAergic component
Hz
180
90
0
-90
-180
S1
S2
HR
FI
R
200 ms/div

Fig7

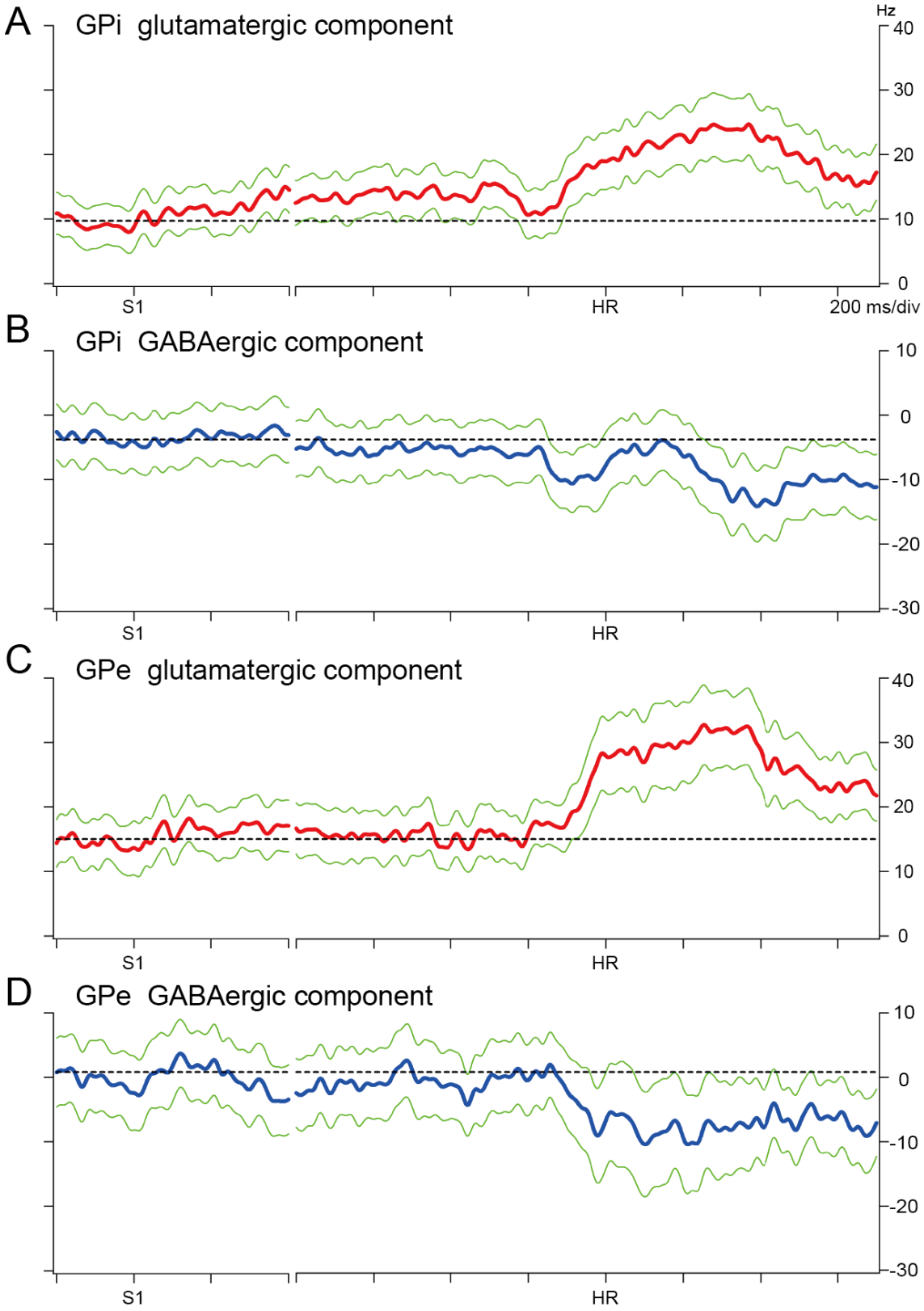

A
GPi glutamatergic component
Hz
40
30
20
10
0
S1
HR
200 ms/div
B
GPi GABAergic component
10
0
-10
-20
-30
S1
HR
C
GPe glutamatergic component
40
30
20
10
0
S1
HR
D
GPe GABAergic component
10
0
-10
-20
-30
S1
HR

Fig8

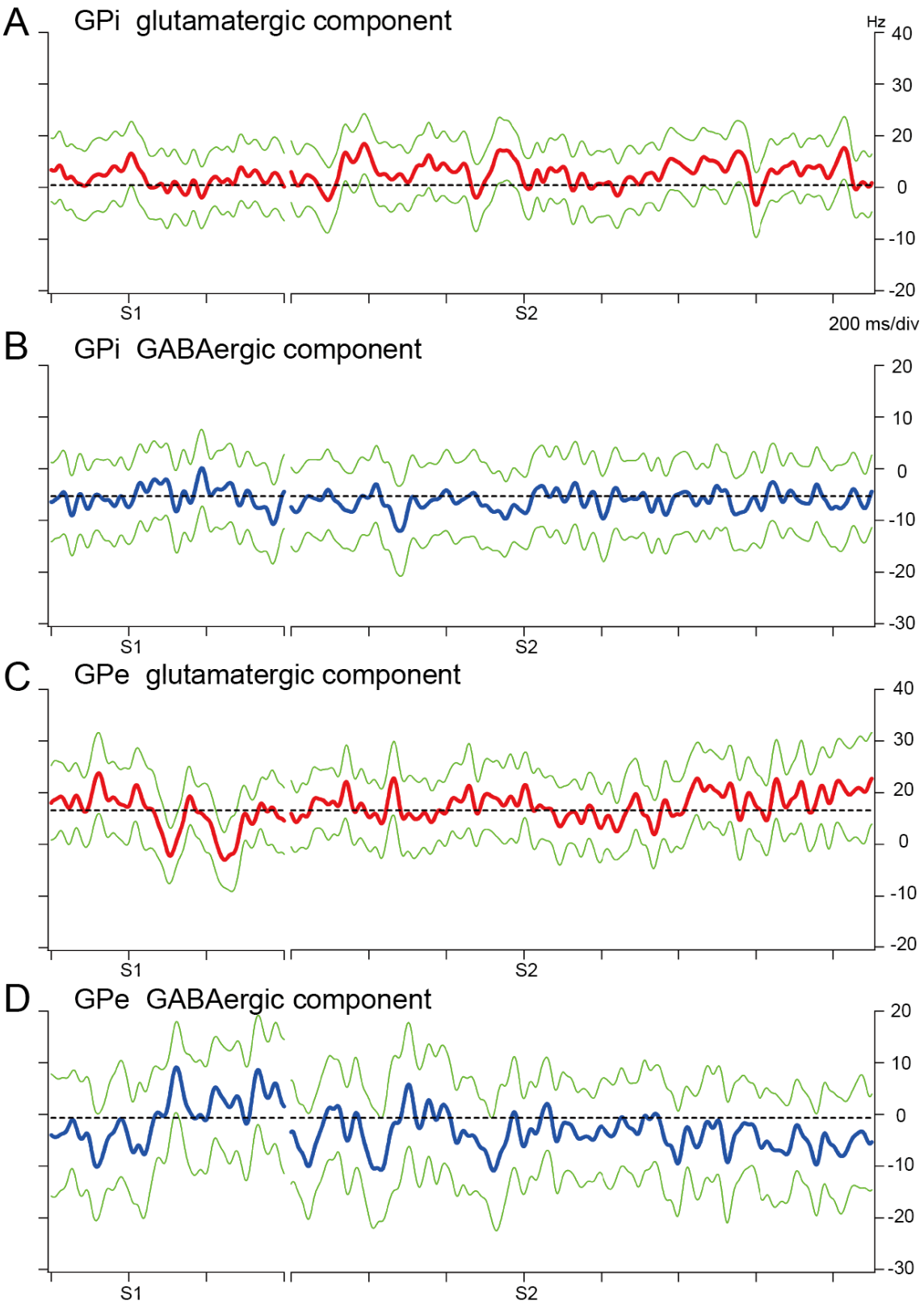

A
GPi glutamatergic component
Hz
40
30
20
0
-10
-20
S1
S2
200 ms/div
B
GPi GABAergic component
20
10
0
-10
-20
-30
S1
S2
C
GPe glutamatergic component
40
30
20
0
-10
-20
S1
S2
D
GPe GABAergic component
20
10
0
-10
-20
-30
S1
S2

Fig9

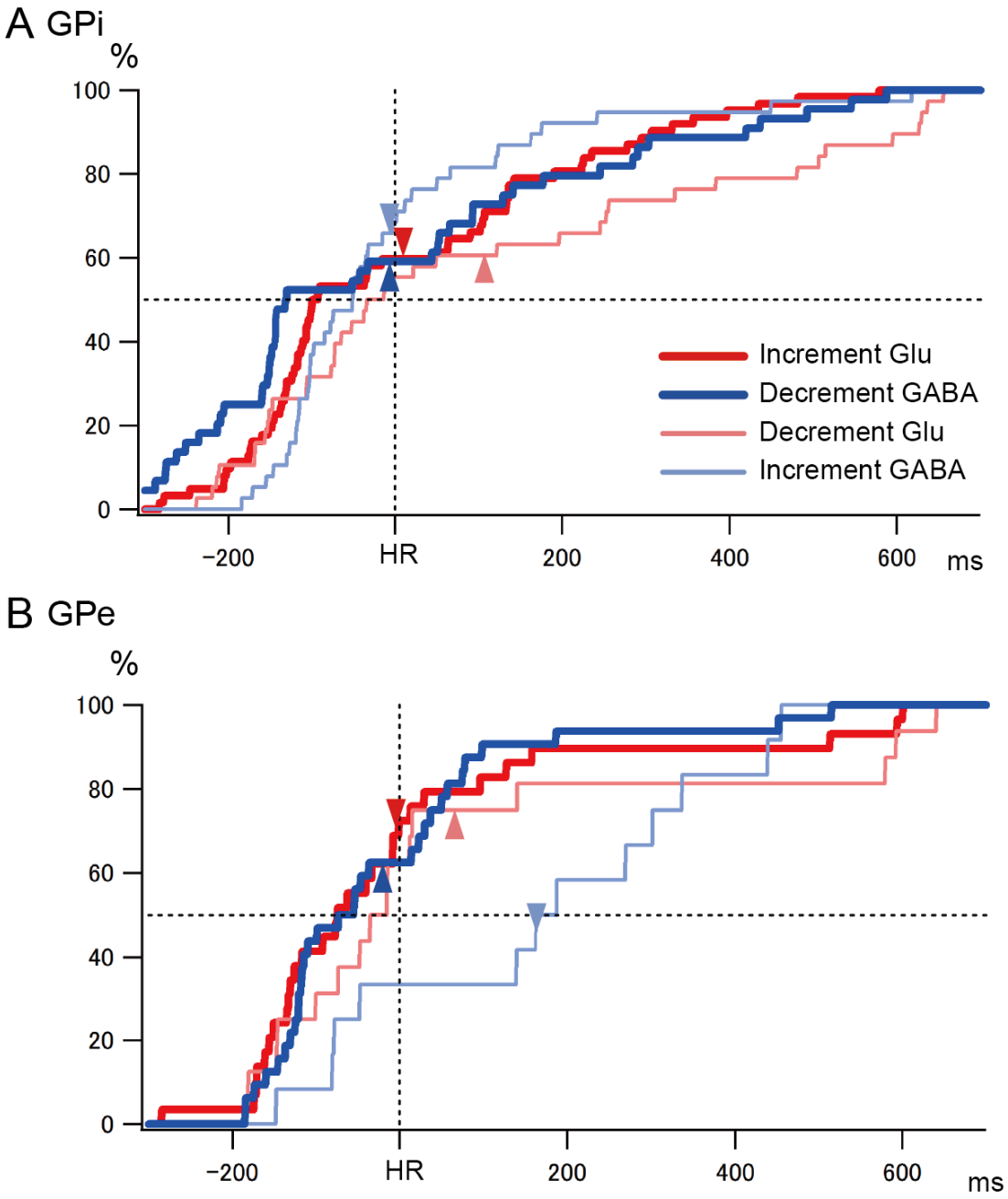
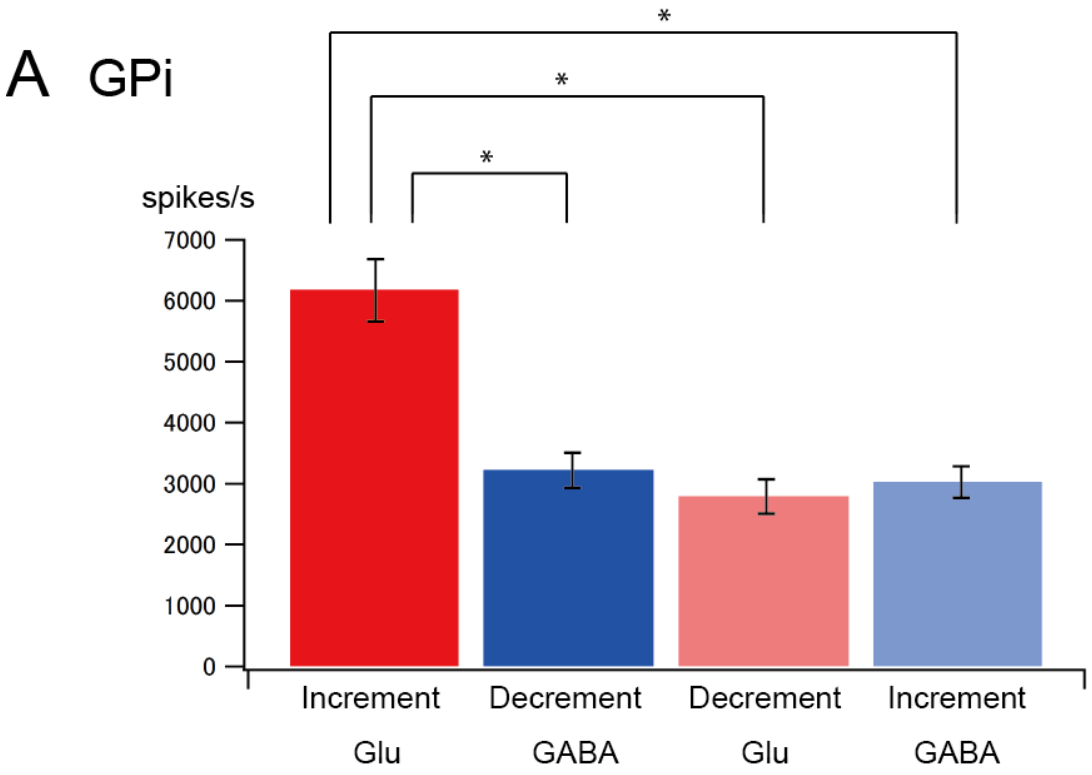

A GPi
%
100
80
60
40
20
0
−200
HR
200
400
600
ms
Increment Glu
Decrement GABA
Decrement Glu
Increment GABA
B GPe
%
100
80
60
40
20
0
−200
HR
200
400
600
ms

Fig10


A GPi
*
*
*
spikes/s
7000
6000
5000
4000
3000
2000
1000
0
Increment
Glu
Decrement
GABA
Decrement
Glu
Increment
GABA

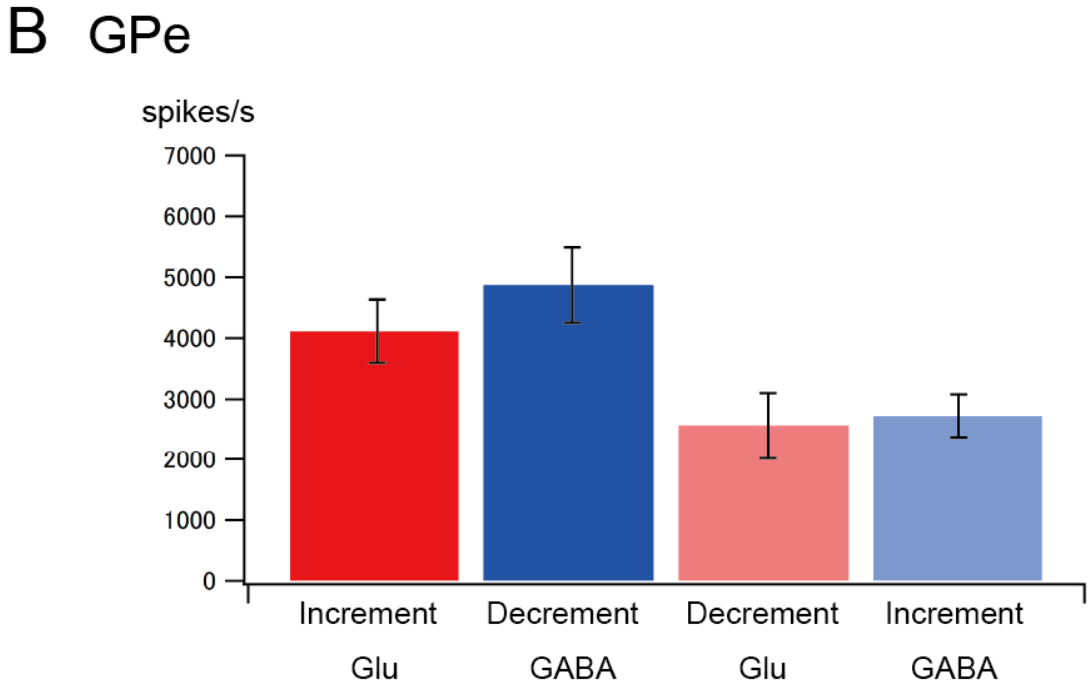

B GPe
spikes/s
7000
6000
5000
4000
3000
2000
1000
0
Increment
Glu
Decrement
GABA
Decrement
Glu
Increment
GABA

Fig11

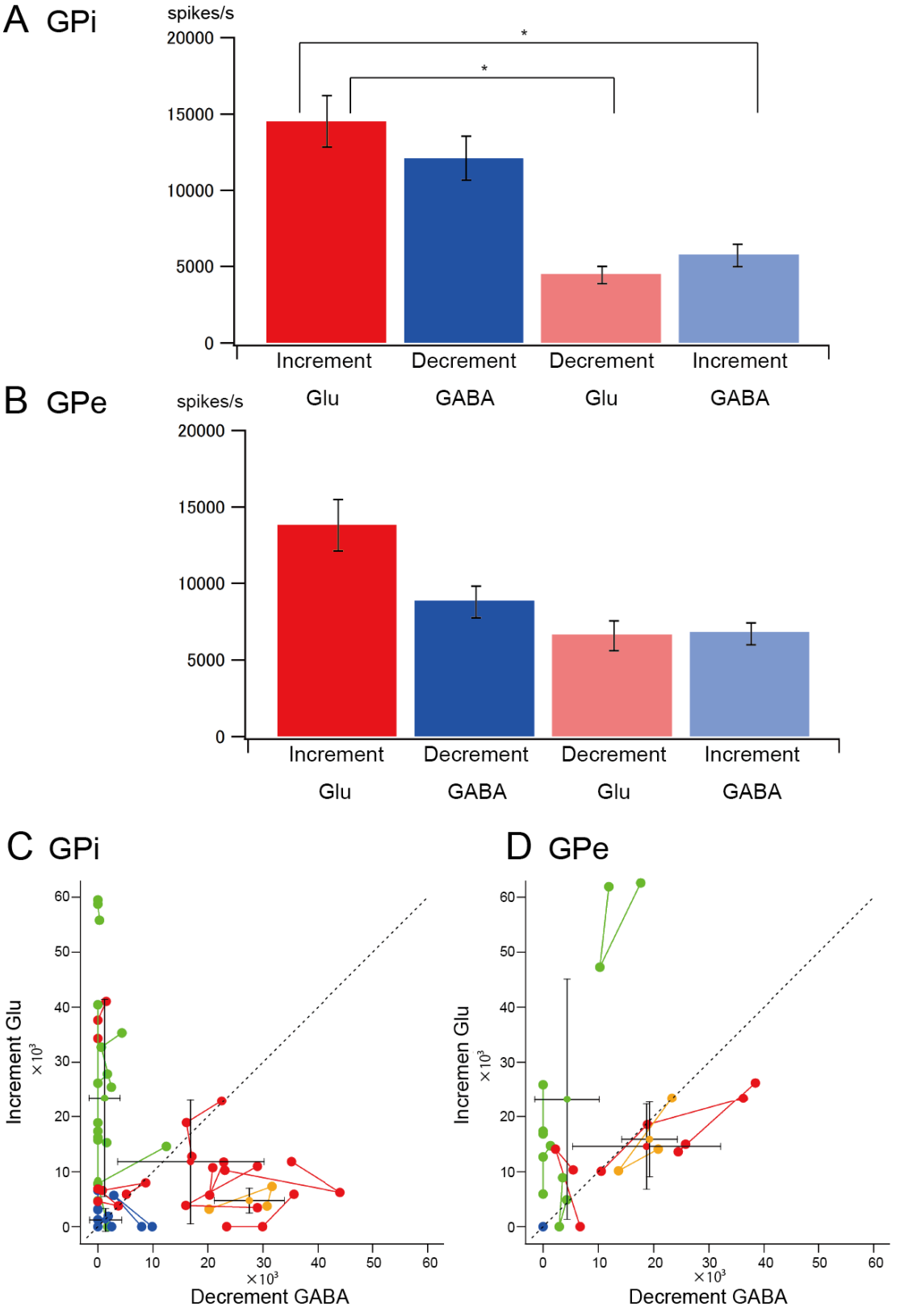

A GPi
spikes/s
20000
15000
10000
5000
0
*
*
Increment
Glu
Decrement
GABA
Decrement
Glu
Increment
GABA
B GPe
spikes/s
20000
15000
10000
5000
0
Increment
Glu
Decrement
GABA
Decrement
Glu
Increment
GABA
C GPi
Increment Glu
×10³
Decrement GABA
×10³
D GPe
Incremen Glu
×10³
Decrement GABA
×10³

Fig12

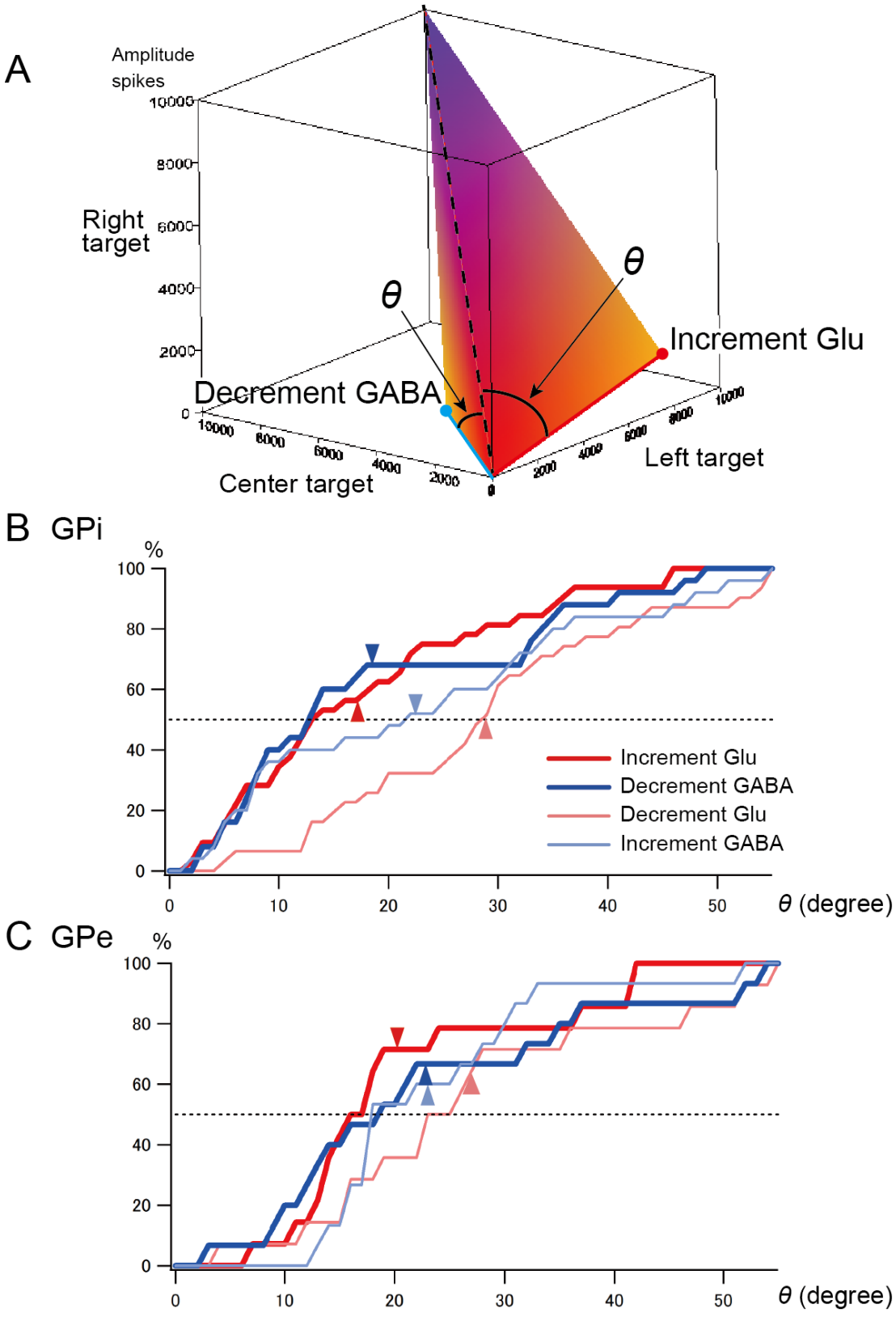

A
Amplitude
spikes
10000
8000
6000
4000
2000
Right
target
θ
θ
Increment Glu
Decrement GABA
0
10000
8000
6000
4000
2000
Center target
2000
4000
6000
8000
10000
Left target
B GPi
%
100
80
60
40
20
0
0
10
20
30
40
50
θ (degree)
Increment Glu
Decrement GABA
Decrement Glu
Increment GABA
C GPe
%
100
80
60
40
20
0
0
10
20
30
40
50
θ (degree)

Fig13

Table 1. Number of neurons classified according to cortical inputs

	SMA	MIp	MId	SMA+MId	SMA+MId	MIp+d	SMA+MIp+d	Total
GPi	9	0	0	4	9	0	14	36
GPe	4	1	0	5	0	4	5	19
Total	13	1	0	9	9	4	19	55

Table 2. Number of neurons tested with drug injection

GPi	NBQX+CPP, then gabazine	21
	NBQX+CPP only	10
	Gabazine, then NBQX+CPP	1
	Gabazine only	4
	Total	36
GPe	NBQX+CPP, then gabazine	8
	NBQX+CPP only	4
	Gabazine, then NBQX+CPP	2
	Gabazine only	5
	Total	19

Table3. Latency of glutamatergic and GABAergic component changes (ms, mean ± SD)

GPi	Incremental glutamatergic	9.4 ± 204.8
	Decremental GABAergic	-7.2 ± 251.4
	Decremental glutamatergic	106.6 ± 294.1
	Incremental GABAergic	-9.2 ± 165.2
GPe	Incremental glutamatergic	-5.7 ± 221.9
	Decremental GABAergic	-21.2 ± 162.8
	Decremental glutamatergic	64.9 ± 280.6
	Incremental GABAergic	161.7 ± 209.2

Table4. Directional selectivity of glutamatergic and GABAergic component changes (degree, mean ± SD)

GPi	Incremental glutamatergic	17.2 ± 12.6
	Decremental GABAergic	18.5 ± 14.7
	Decremental glutamatergic	28.8 ± 14.0
	Incremental GABAergic	22.4 ± 16.4
GPe	Incremental glutamatergic	20.2 ± 11.3
	Decremental GABAergic	22.9 ± 15.4
	Decremental glutamatergic	27.0 ± 15.0
	Incremental GABAergic	23.0 ± 10.2