Optogenetic Activation of the Sensorimotor Cortex Reveals “Local Inhibitory and Global Excitatory” Inputs to the Basal Ganglia

Mitsunori Ozaki¹,², Hiromi Sano¹,³, Shigeki Sato¹, Mitsuhiro Ogura², Hajime Mushiake⁴, Satomi Chiken¹,³, Naoyuki Nakao² and Atsushi Nambu¹,³

¹Division of System Neurophysiology, National Institute for Physiological Sciences, Okazaki 444-8585, Japan, ²Department of Neurological Surgery, Wakayama Medical University, Wakayama 641-0012, Japan, ³Department of Physiological Sciences, SOKENDAI (The Graduate University for Advanced Studies), Okazaki 444-8585, Japan and ⁴Department of Physiology, Tohoku University School of Medicine, Sendai 980-8575, Japan

Address correspondence to Atsushi Nambu, Division of System Neurophysiology, National Institute for Physiological Sciences, 38 Nishigonaka, Myodaiji, Okazaki 444-8585, Japan. Email: nambu@nips.ac.jp; Naoyuki Nakao, Department of Neurological Surgery, Wakayama Medical University, 811-1 Kimiidera, Wakayama 641-0012, Japan. Email: nnakao@wakayama-med.ac.jp; Satomi Chiken, Division of System Neurophysiology, National Institute for Physiological Sciences, 38 Nishigonaka, Myodaiji, Okazaki 444-8585, Japan. Email: chiken@nips.ac.jp

Abstract

To understand how information from different cortical areas is integrated and processed through the cortico-basal ganglia pathways, we used optogenetics to systematically stimulate the sensorimotor cortex and examined basal ganglia activity. We utilized Thy1-ChR2-YFP transgenic mice, in which channelrhodopsin 2 is robustly expressed in layer V pyramidal neurons. We applied light spots to the sensorimotor cortex in a grid pattern and examined neuronal responses in the globus pallidus (GP) and entopeduncular nucleus (EPN), which are the relay and output nuclei of the basal ganglia, respectively. Light stimulation typically induced a triphasic response composed of early excitation, inhibition, and late excitation in GP/EPN neurons. Other response patterns lacking 1 or 2 of the components were also observed. The distribution of the cortical sites whose stimulation induced a triphasic response was confined, whereas stimulation of the large surrounding areas induced early and late excitation without inhibition. Our results suggest that cortical inputs to the GP/EPN are organized in a "local inhibitory and global excitatory" manner. Such organization seems to be the neuronal basis for information processing through the cortico-basal ganglia pathways, that is, releasing and terminating necessary information at an appropriate timing, while simultaneously suppressing other unnecessary information.

Key words: channelrhodopsin, cortico-basal ganglia pathway, entopeduncular nucleus, globus pallidus, mouse (up to 5)
Introduction

The basal ganglia play a crucial role in controlling voluntary movements, and their dysfunction causes severe movement disorders, such as Parkinson’s disease and dystonia (DeLong 1990; Obeso et al. 2000). The basal ganglia receive inputs from a wide area of the cerebral cortex including motor-related areas and send processed information back to the original cortex via the thalamus (Alexander et al. 1986; Alexander and Crutcher 1990). To understand the mechanism of control of voluntary movements by the basal ganglia, it is important to clarify how information originating from cortical areas is integrated and processed through the cortico-basal ganglia pathway. Electrical stimulation of the cerebral cortex, which mimics cortical excitation, typically induces a triphasic response composed of early excitation, inhibition, and late excitation in both the external and internal segments of the globus pallidus (GPe and GPi) in primates, which are also referred to as the globus pallidus (GP) and the entopeduncular nucleus (EPN) in rodents; these structures are the relay and output nuclei of the basal ganglia, respectively (Nambu et al. 2000; Chiken et al. 2008, 2015; Sano et al. 2013). Each component of the cortically evoked responses in the GPe/GPi (GP/EPN) is mediated by a different basal ganglia pathway, that is, “early excitation” by the cortico-subthalamic (STN)-GPe/GPi (GP/EPN) pathway, “inhibition” by the corticostriato-GPe/GPi (GP/EPN) pathway, and “late excitation” by the cortico-striato-GPe (GP)-STN-GPe/GPi (GP/EPN) pathway (Maurice et al. 1999; Nambu et al. 2000; Kita et al. 2004; Tachibana et al. 2008). Electrical stimulation also induces other response patterns that lack 1 or 2 of these components, suggesting that not all cortical sites uniformly send information to a single GPe/GPi (GP/EPN) neuron. However, discrete sites in the cortical areas that exert excitatory or inhibitory effects on a single GPe/GPi (GP/EPN) neuron are distributed remains to be studied, because conventional electrical stimulation through implanted electrodes is not suitable for stimulating discrete multiple sites of the cerebral cortex. Recent developments in optogenetic tools have enabled us to stimulate a discrete population of neurons with light and to precisely control the timing of light stimulation. The white light from the projector (Fig. 1A) was sent to the stereomicroscope (Fig. 1A2) through the shutter (Fig. 1A3), and was focused on the surface of the mouse cerebral cortex (Fig. 1B, C). The stereotoxic apparatus could be swiveled so that the light was projected perpendicularly to the cortical surface (Fig. 1A4). The size, shape, color, location, and intensity of light stimulation were controlled by a computer connected to the LCD projector, and a circular spot of light (diameter, 1.0 mm; intensity, 8–12 mW/mm²) was used. The opening and closing of the shutter were controlled by a transistor–transistor logic (TTL) pulse (duration, ≥5 ms) fed by a stimulator (SEN-7203, Nihonkohden). The timing of light stimulation was monitored by a photodiode placed on the cortical surface. Light stimulation was applied every 1.4 s.

Methods

Animals

A total of 10 Thy1-ChR2-YFP transgenic mice (line 9, The Jackson Laboratory; 5 males and 5 females) older than 6 weeks were used in the present study. The experimental protocols were approved by the Institutional Animal Care and Use Committee of National Institutes of Health, and all experiments were conducted according to the guidelines of the National Institutes of Health “Guide for Care and Use of Laboratory Animals”. Before experiments, mice were handled daily to allow them to become familiar with the experimenters.

Surgery

Under general anesthesia with ketamine hydrochloride (100 mg/kg body weight, i.p.) and xylazine hydrochloride (5 mg/kg body weight, i.p.), each mouse underwent a surgical operation to painlessly fix its head in a stereotatic frame. The skull was widely exposed, and the periosteum and blood on the skull were carefully removed. The exposed skull was completely covered with bone-adhesive resin (Bistite II; Tokuyama Dental) and acrylic resin (Unifast II; GC), and then a small polycetal U-frame holder was mounted and fixed with acrylic resin. After recovery from the first surgery (2 or 3 d later), under light anesthesia with ketamine hydrochloride (30–50 mg/kg, i.p.), the mouse was positioned in a stereotactic apparatus with its head restrained using the U-frame head holder, and a large area of the anterior part of the skull over the right hemisphere was removed to access the sensorymotor cortex and GP/EPN. After full recovery from the craniotomy, light or electrical stimulation and recording of neuronal responses were started.

Light Stimulation System

For optical stimulation, we refined the Multi-independent Light Stimulation System (MiLSS, Aska Company) (Sakai et al. 2013), which was originally designed for in vitro slice experiments. This system consists of a conventional digital light processing (DLP) projector (MX514P, BenQ) and an upright microscope. We replaced the DLP projector with a brighter liquid crystal display (LCD) projector (EB-1960, EPSON) and the upright microscope with a stereo microscope (SZX7, Olympus) for in vivo experiments (Fig. 1A). We also inserted a mechanical shutter (EN-609, COPAL) between the projector and the stereomicroscope to precisely control the timing of light stimulation. The white light from the projector (Fig. 1A1) was sent to the stereomicroscope (Fig. 1A2) through the shutter (Fig. 1A3), and was focused on the surface of the mouse cerebral cortex (Fig. 1B, C). The stereotoxic apparatus could be swiveled so that the light was projected perpendicularly to the cortical surface (Fig. 1A4). The size, shape, color, location, and intensity of light stimulation were controlled by a computer connected to the LCD projector, and a circular spot of light (diameter, 1.0 mm; intensity, 8–12 mW/mm²) was used. The opening and closing of the shutter were controlled by a transistor–transistor logic (TTL) pulse (duration, ≥5 ms) fed by a stimulator (SEN-7203, Nihonkohden). The timing of light stimulation was monitored by a photodiode placed on the cortical surface. Light stimulation was applied every 1.4 s.

Recording of Cortical Activity Evoked by Cortical Light Stimulation

Two mice were used to assess the effects of cortical light stimulation on cortical neuronal activity. Each mouse was kept awake and quiet in the stereotoxic apparatus with its head restrained painlessly using the U-frame head holder. A glass-coated Elgiloy-alloy microelectrode (0.5–1.5 Ω at 1 kHz) was obliquely inserted into the sensorimotor cortex (30° posteriorly from the vertical) using a hydraulic microdrive (Narishtige) to record cortical activity (Fig. 1C). Signals through the recording electrode were amplified (±10 000), filtered (0.3–5.0 kHz), monitored with an oscilloscope, and stored at 50 kHz on a computer for off-line analysis. Light stimulation was delivered through the light stimulation system described above. The location of the light spot was controlled by a computer, and the horizontal distance between the center of the light spot and the tip of the recording electrode was adjusted (Fig. 1C).
Figure 1. Light stimulation of the sensorimotor cortex. A, Refined light stimulation system. The white light (blue–white line) from a liquid crystal display projector (1) was sent to a stereomicroscope (2) through a mechanical shutter (3) and was focused on the target. The stereotaxic apparatus was placed on a swivel system (4). B, Cortical stimulation spots (inset), their precise location in reference to bregma (left), and cortical maps of the primary motor (M1), secondary motor (M2), and primary sensory (S1) cortices (right) are shown. C, Experimental setup for cortical light stimulation and recording of cortical activity through an electrode inserted obliquely (30° posteriorly from the vertical). Cx, cortex. D, Cortical activity induced by light stimulation. A TTL pulse (top), light (diameter, 1.0 mm; intensity, 12 mW/mm²; 5718 | Cerebral Cortex, 2017, Vol. 27, No. 12 Downloaded from https://academic.oup.com/cercor/article-abstract/27/12/5716/4209666 by guest on 26 July 2018
Recording of GP/EPN Activity Evoked by Cortical Light and Electrical Stimulation

Ten mice were used to record GP/EPN neuronal activity in response to cortical light stimulation. To record single unit activity, using the hydraulic microdrive, a glass-coated Elgiloy-alloy microelectrode (0.5–1.5 MΩ at 1 kHz) was inserted vertically into the sensorimotor area of the GP or EPN, which received inputs from the motor and sensory cortices (Chiken et al. 2008, 2015; Sano et al. 2013). Signals through the recording electrode were amplified (×10,000), filtered (0.3–5.0 kHz), and continuously monitored with an oscilloscope. The GP and EPN were identified by the depth profile and their firing characteristics. Unit activity was isolated and converted to digital pulses using a homemade time-amplitude window discriminator. Digital pulses sampled at 2 kHz and analog data sampled at 50 kHz were stored on a computer for off-line analysis. A rectangular area of 3 mm (medio-lateral; from 0.5 mm to 3.5 mm to the midline) × 4 mm (antero-posterior; from +2.5 mm to −1.5 mm relative to bregma) that covered the orofacial, forelimb, and hindlimb regions of the primary motor cortex (M1) and the primary sensory cortex (S1), and the secondary motor cortex (M2) was targeted for light stimulation (Fig. 1B). A light spot was projected on the cortical surface (diameter, 1.0 mm; intensity, 12 mW/mm²; duration, 10 ms; inter-stimulus intervals, 1.4 s) every 1 mm in a 3 × 4 grid pattern using the light stimulation system (Fig. 1B).

For comparison, 2 mice were also used to record the responses of GP/EPN neurons to electrical stimulation of the sensorimotor cortex. Electrical stimulation (monophasic; strength, 50 μA; pulse width, 200 μs; inter-stimulus intervals, 1.4 s) was applied through bipolar stimulating electrodes consisting of a pair of Teflon-coated tungsten wires with a 50-μm diameter (tip distance, 300–400 μm) inserted in the forelimb and orofacial regions of the M1 (Chiken et al. 2008, 2015; Sano et al. 2013).

Analysis of Neuronal Responses

Cortical and GP/EPN responses induced by cortical light/electrical stimulation were assessed by constructing peristimulus time histograms (PSTHs; bin width of 1 ms; 50 stimulus trials for cortical neurons; 100 stimulus trials for GP/EPN neurons). Cortical activity induced by light stimulation sometimes exhibited large deflections from baseline that may be composed of evoked local field potentials and/or population spikes of multiple neurons. After removing the deflections off-line, single or multi-neuronal firings were detected and converted to digital pulses using a software window discriminator to construct PSTHs. PSTHs for GP/EPN neurons were constructed on-line from recorded digitized spikes. The mean and standard deviation (SD) of the discharge rate during the 100-ms period preceding the onset of stimulation were calculated for each PSTH and considered the baseline activity. Activity changes in response to cortical stimulation (i.e., excitation and inhibition) were judged significant if the discharge rate reached a significance level of $P < 0.05$ (tailed t-test) during at least 2 consecutive bins (2 ms) in GP/EPN neurons or during at least 1 bin (1 ms) in cortical neurons (Nambu et al. 2000; Chiken et al. 2008, 2015; Tachibana et al. 2008; Chiken and Nambu 2013; Sano et al. 2013). The latency of each component was defined as the time at which the first bin exceeded this level. The responses were judged to end when 2 consecutive bins fell below the significance level. The end point was determined as the time of the last bin exceeding this level. The amplitude of each component was defined as the difference between the number of spikes during significant changes and the number of spikes of baseline activity (i.e., the area of the response).

Contour Map of the Sensorimotor Cortex

The contribution of cortical sites to each response component (i.e., early excitation, inhibition, and late excitation) was analyzed by constructing a contour map for each GP/EPN neuron. Twelve PSTHs corresponding to the light stimulation of a 3 × 4 grid pattern were constructed. Neurons that exhibited a triphasic response composed of early excitation, inhibition, and late excitation to at least 1 of the 12 stimulating spots were used for further analysis. We defined the stimulation spot that induced a triphasic response with the largest amplitude of inhibition as “the best spot” (“light green filled circles” in Figs 2B and 3B). The amplitude of each response component was calculated in 12 PSTHs and plotted on a 3 × 4 grid pattern. A contour map was constructed by connecting the same amplitude in each response component. The contribution of cortical sites to each response component was further analyzed by plotting the amplitude of each response component against the distance from the “best spot”. The distance was defined as the number of squares from “the best spot” in the 3 × 4 grid (see Fig. 4B, lower). The amplitude at each distance was averaged and expressed as the relative value of the amplitude at “the best spot”.

Population contour maps of each response component were also constructed for GP and EPN neurons. The contour maps for each neuron were standardized by expressing response components as the relative amplitude to the maximum amplitude among all response components (early excitation, inhibition, and late excitation of 12 PSTHs), centered at “the best spot”, and averaged among all GP or EPN neurons examined. Population contour maps were constructed by connecting the same amplitude. Peripheral areas in which 3 or fewer values were averaged were discarded.

Which cortical sites induced triphasic responses in GP and EPN neurons was also analyzed. Ratio of number of GP/EPN neurons showing a triphasic response to number of all GP/EPN neurons was plotted on a 3 × 4 grid pattern. A contour map was constructed by connecting the same ratio for the GP and EPN.

Histology

After the final recording, several sites of neuronal recording were marked by passing a cathodal DC current (15 μA for 20 s) through the recording electrodes. The mice were anesthetized deeply with sodium pentobarbital (80 mg/kg, i.p.), and perfused...
transcardially with 0.1 M PB (pH 7.3) followed by 10% formalin in 0.1 M PB, and then 0.1 M PB containing 10% sucrose. The brains were removed immediately and saturated with the same buffer containing 30% sucrose. Frontal sections (40 μm) were cut with a freezing microtome, mounted onto gelatin-coated glass slides, stained with 0.5% cresyl violet, dehydrated, and coverslipped. The sections were observed under a light microscope, and the recording sites were reconstructed according to the lesions made by current injection and the traces of electrode tracks with reference to the mouse brain atlas (Franklin and Paxinos 2007).

Results

Responses of Cortical Neurons to Light Stimulation

By using a refined light stimulation system, we first examined the response of cortical neurons (Fig. 1C–F). The TTL pulse opened and closed the mechanical shutter rapidly (rise or fall time, i.e., the time from the beginning of open to full open or from the beginning of close to full close, ≤1 ms) with a 2.4-ms delay (TTL and Light in Fig. 1D). Because the shutter had a minimum open time of 10 ms, a 5-ms TTL pulse precisely triggered a 10-ms duration of light stimulation. Light stimulation (diameter, 1.0 mm; intensity, 8 and 12 mW/mm²; duration, 10 ms) successfully activated cortical neurons located within the circular light spot in all cases as shown in the raw data (Cx in Fig. 1D) and PSTH (Fig. 1E). The mean latency of cortical activity from light stimulation was 5.0 ± 1.9 ms (n = 10). We then examined the size of the cortical areas activated by the light spots (Fig. 1F). Cortical neurons were strongly activated at the center of the light spot and at ±0.25 mm from the center. Excitation was reduced at ±0.5 mm, that is, at the border of the light spot, and no activation was observed at ±0.75 mm. Thus, the range of cortical areas activated by the light spot was confined to within the illumination range. Each light spot stimulated the cortical site roughly corresponding to the M2, M1, or S1 (Franklin and Paxinos 2007), however some light spots were on the boundaries between the M2 and M1 and between the M1 and S1 (Fig. 1B). When a light spot was applied to the M1, it occasionally induced muscle twitches in the orofacial, forelimb, or hindlimb. However, it was difficult to stimulate somatotopic details because of the large size of a light spot.

Responses of GP and EPN Neurons to Cortical Light Stimulation

Light stimulation of the sensorimotor cortex (diameter, 1.0 mm; intensity, 12 mW/mm²; duration, 10 ms) typically induced a
triphasic response composed of early excitation, inhibition, and late excitation (Fig. 1G), nearly identical to the response observed with electrical stimulation (Fig. 1H) in both GP and EPN neurons, except that the latency of each component was longer with light stimulation (Table 1, cf., Chiken et al. 2008; Sano et al. 2013).

Among GP and EPN neurons whose responses to cortical light stimulation were examined by constructing PSTHs corresponding to each stimulation spot in a 3 × 4 grid pattern, 82 GP and 43 EPN neurons that exhibited a triphasic response to at least 1 cortical spot were further analyzed (Figs 2 and 3). Cortical light stimulation induced various response patterns in GP and EPN neurons, that is, a triphasic response composed of early excitation, inhibition, and late excitation; biphasic excitation; early excitation and inhibition; inhibition and late excitation; monophasic excitation; and monophasic inhibition (Figs 2A and 3A). The number of cortical spots whose stimulation induced a triphasic response was small (2.4 ± 1.7 spots for the GP; 1.8 ± 1.7 spots for the EPN), whereas stimulation of other spots induced early and/or late excitation without inhibition. We then examined the distribution of cortical spots that induced each component of the response by constructing contour maps (Figs 2B and 3B). “The best spots” whose stimulation induced a triphasic response with the largest amplitude of inhibition are indicated by light green circles in Figures 2B and 3B. Cortical areas inducing early excitation, inhibition, and late excitation largely overlapped. However, a limited cortical area only in the vicinity of “the best spot” induced inhibition (Figs 2B and 3B, Inh), whereas a broader cortical area surrounding “the best spot” induced early excitation and late excitation with a large amplitude (Figs 2B and 3B, Early ex and Late ex) in both GP and EPN neurons.

This tendency was further examined quantitatively by analyzing the same 82 GP and 43 EPN neurons (Fig. 4). The numbers of cortical spots that induced inhibition (3.7 ± 2.3 spots for the GP; 2.8 ± 1.7 spots for the EPN) were significantly smaller than the numbers of cortical spots that induced early excitation (7.1 ± 2.3 for the GP; 6.1 ± 2.3 for the EPN; 1-way ANOVA with Tukey’s post hoc test, P < 0.001 for the GP and EPN) and late excitation (5.9 ± 2.9 for the GP; 7.7 ± 2.4 for the EPN; 1-way ANOVA with Tukey’s post hoc test, P < 0.001 for the GP and EPN). We then plotted these numbers for each GP and EPN neuron as shown in Figure 4A. Most plots were V-shaped, that is, fewer cortical spots induced inhibition than early or late excitation, displaying the above mentioned tendency. Plots of the averaged amplitude of each response component against the distance from “the best spot” also reflected this tendency (Fig. 4B). Light stimulation of “the best spot”
Inhibition in GP and EPN neurons, and its amplitude was abruptly reduced at "distance 1" (next to "the best spot") (25% for the GP; 20% for the EPN). On the other hand, the amplitudes of early and late excitation gradually decreased with increasing distance from "the best spot", that is, an amplitude of nearly half was still observed at "distance 2" (49% for the GP and 42% for the EPN).
late excitation). These distributions were also evident in the population contour maps (Fig. 4C): Stimulation of only a confined cortical area induced inhibition, whereas stimulation of larger surrounding cortical areas induced early and late excitation in GP and EPN neurons. Finally, which cortical sites induced triphasic responses in each neuron are indicated by different colors and the corresponding locations inside the rectangle (inset). Distances from bregma are indicated in mm. ic, internal capsule; op, optic tract; Str, striatum.

**Table 1 Parameters of responses induced by cortical light stimulation**

|                | GP          | EPN         |
|----------------|-------------|-------------|
| No. of neurons | 82          | 43          |
| Early excitation |             |             |
| Latency (ms)   | 7.1 ± 1.3   | 7.3 ± 2.1   |
| Duration (ms)  | 7.2 ± 2.1   | 7.9 ± 3.1   |
| Amplitude (spikes) | 107.0 ± 52.7 | 95.5 ± 61.6 |
| Inhibition     |             |             |
| Latency (ms)   | 16.5 ± 2.5  | 18.2 ± 2.9  |
| Duration (ms)  | 6.7 ± 3.0   | 5.6 ± 3.6   |
| Amplitude (spikes) | 31.0 ± 20.1 | 21.4 ± 21.2 |
| Late excitation |             |             |
| Latency (ms)   | 27.5 ± 5.2  | 27.6 ± 4.9  |
| Duration (ms)  | 26.9 ± 25.8 | 11.4 ± 11.3 |
| Amplitude (spikes) | 261.0 ± 288.0 | 108.0 ± 144.0 |

Values are mean ± SD. Latency, duration, and amplitude of early excitation, inhibition, and late excitation induced by cortical light stimulation at “the best spot” are shown for GP and EPN neurons.

Locations of Recorded GP and EPN Neurons

Recording sites in the GP and EPN are shown in frontal sections with rectangles (Fig. 5). Cortical stimulating spots that induced a triphasic response in an individual GP or EPN neuron are indicated by different colors and the corresponding locations inside the rectangle. GP neurons that responded to stimulation of the posterior cortex (green and dark green) were found in the dorsal part of the GP, whereas those that responded to stimulation of the anterior cortex (orange, red, and violet) were located in the ventrolateral part of the GP (Fig. 5A). GP neurons with inputs from the M2 (orange and light violet) were located in the central part. Most EPN neurons responded to stimulation of the posterior and medial cortex (green and blue) and were found in the central part of the EPN, and a small number of EPN neurons responded to stimulation of the anterior cortex (orange and violet) and were located in the ventrolateral part of the EPN (Fig. 5B).

**Discussion**

The present study revealed the following results: 1) Light stimulation of the sensorimotor cortex of Thy1-ChR2-YFP transgenic mice typically induced early excitation, inhibition, and late excitation in GP/EPN neurons. 2) Stimulation of only a confined cortical area induced inhibition, whereas stimulation of larger surrounding cortical areas induced early and late excitation in individual GP/EPN neurons. 3) Cortical stimulation sites and the responsive GP/EPN sites were topographically related.

**Optogenetic Activation of Cortical Neurons in Thy1-ChR2-YFP Transgenic Mice**

In the present study, we used Thy1-ChR2-YFP line 9 transgenic mice, in which ChR2-YFP is highly expressed in layer V pyramidal neurons (Arenkiel et al. 2007; Wang et al. 2007). Direct or transcranial light stimulation induces firing of cortical neurons and limb movements (Arenkiel et al. 2007; Ayleing et al. 2009; Hira et al. 2009). We used a refined light stimulation system that can project light on arbitrary locations in an arbitrary pattern with an accurate timing. Compared to the original system for in vitro experiments (Sakai et al. 2013), the refined system in the present study can deliver stronger light in in vivo experiments with more accurate timing. We carefully examined the local effects of light stimulation and showed that a circular light spot successfully induced cortical neuronal firing (Fig. 1D, E). Cortical light stimulation also induced a triphasic response in GP/EPN neurons that was nearly identical to the response induced by electrical stimulation of the cortex (Fig. 1G, H). Latencies of response components evoked by light stimulation in GP/EPN neurons were 4–5 ms longer than those evoked by electrical
stimulation (Chiken et al. 2008). The difference can be explained by the latency of cortical activity induced by cortical light stimulation (5.0 ± 1.9 ms; Fig. 1D, E). This latency was shorter than that reported in a previous study (9.9 ms in Arenkiel et al. 2007), probably because 1) the parameters for light stimulation, such as the intensity and size of a light spot, were different, and 2) neuronal activity was recorded in awake mice in the present study, as distinct from the previous study with general anesthesia.

“Local Inhibitory and Global Excitatory” Inputs from the Cortex to the GP/EPN

Cortical electrical stimulation induces a triphasic response composed of early excitation, inhibition, and late excitation in GP/EPN (GPe/GPi) neurons; these components are mediated by the cortico-STN-GP/EPN (GPe/GPi), cortico-striato-GP/EPN (GPe/GPi), and cortico-striato-GP (GPe)-STN-GP/EPN (GPe/GPi) pathways, respectively (Maurice et al. 1999; Nambu et al. 2000; Kita et al. 2004; Tachibana et al. 2008). In this study, we showed that cortical light stimulation also induced the same triphasic response, and each component seemed to be mediated by the same pathway. These responses in the GP/EPN are considered to be mediated by direct cortical inputs to the basal ganglia, not by the cortico-cortical and subsequent cortico-basal ganglia projections, because the cortical area activated by the light stimulation was confined and did not extend beyond the margin of the light spot (Fig. 1F).

The cortical areas whose stimulation induced early and/or late excitation in each GP/EPN neuron extended broadly in the sensorimotor cortex, whereas only stimulation of a confined cortical area in the vicinity of "the best spot" induced a triphasic response with early excitation, inhibition, and late excitation in each GP/EPN neuron (Figs 2–4). These results suggest that information from global areas of the sensorimotor cortex (Cx_{n-1}, n, n+1) in Fig. 6A) is integrated and conveyed through the "global excitatory" cortico-STN-GP/EPN and cortico-striato-GP-STN-GP/EPN pathways (red lines in Fig. 6A) to excite a local area of the GP/EPN (GPe/GPi) in Fig. 6A) for a certain period of time. On the other hand, information from a local area of the sensorimotor cortex (Cx, in Fig. 6A) is conveyed through the "local inhibitory" cortico-striato-GP/EPN pathway (blue line in Fig. 6A) to transiently suppress a local area of the GP/EPN neuron (GP/EPN_{n-1}, n, n+1) at a specific timing. The observations indicate convergent "local inhibitory and global excitatory" inputs from the cortex to the GP/EPN (Fig. 6A). If we reasonably assume that this relation between cortical and GP/EPN neurons in Figure 6A is repetitively represented, a local area of sensorimotor cortex (Cx, in Fig. 6B) would exert inhibitory effects on a local area of the GP/EPN neuron (GP/EPN_{n-1}, n, n+1) through the "local inhibitory" cortico-striato-GP/EPN pathway (blue line in Fig. 6B) and excitatory effects on global areas of the GP/EPN (GP/EPN_{n-1}, n, n+1) in Fig. 6B) through the "global excitatory" cortico-STN-GP/EPN and cortico-striato-GP-STN-GP/EPN pathways (red lines in Fig. 6B), creating divergent "local inhibitory and global excitatory" inputs from the cortex to the GP/EPN (Fig. 6B) (for proof, see Appendix). Previous anatomical studies suggested the “center-surround” organization similar to the model shown in Figure 6B by demonstrating that STN-GPe/GPi projections send axons to a large number of GPe/GPi neurons in a uniform manner, whereas striato-GPe/GPi projections send axons to selected sets of the same GPe/GPi neurons in monkeys (Hazrati and Parent 1992b, c; Mink 1996). Our present study has electrophysiological confirmed, for the first time, both convergent (Fig. 6A) and divergent (Fig. 6B) "local inhibitory and global excitatory" inputs from the cortex to the GP/EPN (GPe/GPi). The cortical area whose stimulation induction inhibition in the GP/EPN was confined in the small area, whereas that inducing early and late excitation extended broadly to other somatotopic representations and/or other cortices (Figs 2B, 3B, and 4C). As it was difficult to stimulate somatotopic details in this study, the detailed relationship between somatotopic representations and induced response patterns remains to be studied.

Anatomical Basis of the “Local Inhibitory and Global Excitatory” Inputs from the Cortex to the GP/EPN (GPe/GPi)

Information processing through the net inhibitory cortico-striato-GP/EPN (GPe/GPi) pathway seems to maintain specificity. The somatosensory and motor cortices project to the striatum in a somatotopic manner, and information from different body parts terminates in distinct areas of the striatum without overlapping in rodents (Alloway et al. 2000; Hoover et al. 2003) and primates (Flaherty and Graybiel 1991, 1993; Inase et al. 1999). In addition, striato-GPe/GPi projections also maintain very precise topographical organization without convergence: Axons of striatal neurons from 2 small adjacent populations do not converge on the same GPe/GPi neurons, but instead project to several distinct subsets of GPe/GPi neurons (Hazrati and Parent 1992b, c; Mink 1996).

Figure 6. Schematic drawings of the convergent (A) and divergent (B) “local inhibitory and global excitatory” inputs from the cortex (Cx) to the GP/EPN (GPe/GPi). A, information from Cx_{n-1}, n, n+1 is integrated and conveyed through the “global excitatory” Cx-subthalamo (STN)-GP/EPN (GPe/GPi) and Cx-striato (Str)-GP-STN-GP/EPN (GPe/GPi) pathways (red lines) to excite GP/EPN_{n}, (GPe/GPi), while information from Cx_{n}, is conveyed through the “local inhibitory” Cx-Str-GP/EPN (GPe/GPi) pathway (blue line) to transiently suppress GP/EPN_{n} (GPe/GPi). B, Cx_{n} would exert inhibitory effects on GP/EPN_{n} (GPe/GPi) through the “local inhibitory” pathway (blue line) and excitatory effects on GP/EPN_{n-1}, n, n+1 (GPe/GPi_{n-1}, n, n+1) through the “global excitatory” pathways (red lines).
6

Functional Considerations

We would like to discuss the divergent “local inhibitory and global excitatory” inputs from the cortex to the EPN (GPi) (Fig. 6B). Suppose that Cx, is excited. The Cx, activation excites, inhibits, and excites EPN, (GPi,) at the target center through the excitatory cortico-STN-EPN (GPi) “hyperdirect” (Nambu et al. 2002), inhibitory cortico-striato-EPN (GPi) “direct”, and excitatory cortico-striato-GP (GPi)-STN-EPN (GPi) “indirect” pathways, respectively. The Cx, activation also excites EPN,1 and EPN,n+1 (GPi,1 and GPi,n+1) in the surrounding area through the excitatory “hyperdirect” and “indirect” pathways. The EPN, (GPi,) at the center increases thalamocortical activity via a disinhibitory mechanism and releases necessary movements. On the other hand, the EPN,1 and EPN,n+1 (GPi,1 and GPi,n+1) in the surrounding area inhibit thalamocortical activity and suppress unnecessary movements (“center-surround model”; Mink 1996; Nambu 2007).

The present study also suggests the convergent “local inhibitory and global excitatory” inputs from the cortex to the EPN (GPi) (Fig. 6A). Suppose that Cx−1 and Cx+1 are simultaneously excited in addition to Cx, the inhibition of EPN, (GPi,) induced by the input from Cx, through the “direct” pathway is reduced by excitatory inputs from Cx−1 and Cx+1 through the “hyperdirect” and “indirect” pathways, and movements that should be released by EPN, (GPi,) will be canceled or stopped (Aron and Poldrack 2006; Isoda and Hikosaka 2008; Sano et al. 2013; Schmidt et al. 2013).

Therefore, the convergent (Fig. 6A) and divergent (Fig. 6B) “local inhibitory and global excitatory” inputs from the cortex to the GP/EPN (GPi/GPi) may be the neuronal basis for proper information processing through the cortico-basal ganglia pathways, that is, releasing and canceling/stopping necessary information with appropriate timing, while suppressing unnecessary information at the same time.

Notes

We thank I. Tsuda for discussion on the model of cortico-pallidal relations, Y. Tachibana for discussion on experimental design, and K. Awamura, H. Isogai, M. Goto and N. Suzuki for technical assistance. Conflict of Interest: None declared.

Funding

This work was supported by CREST (to A.N.) from Japan Science and Technology Agency; a Grant-in-Aid for Scientific Research (A) (26 250 009 to A.N.) from the Ministry of Education, Culture, Sports, Science and Technology (MEXT) of Japan, a Grant-in-Aid for Scientific Research on Innovative Areas, “Non-linear Neurooscillometry” (15H05873 to A.N., 15H05879 to H.M.), and Platforms for Advanced Technologies and Research Resources (16H06276) from the MEXT.

Appendix

If the local inhibition and global excitation in Figure 6B were not true, local excitation or global inhibition could exist. However, it contradicts the present results shown in Figure 6A. Therefore, the initial assumption that negates the “local inhibition and global excitation” must be false, providing that only the connections in Figure 6A exist.

References

Alexander GE, Crutcher MD. 1990. Functional architecture of basal ganglia circuits: neural substrates of parallel processing. Trends Neurosci. 13:266–271.

Alessandro GE, DeLong MR, Strick PL. 1986. Parallel organization of functionally segregated circuits linking basal ganglia and cortex. Annu Rev Neurosci. 9:357–381.

Alloway KD, Mutic JJ, Hoffer ZS, Hoover JE. 2000. Overlapping corticostraital projections from the rodent vibrissal representations in primary and secondary somatosensory cortex. J Comp Neurol. 428:51–67.

Arenkiel BR, Peca J, Davison IG, Feliciano C, Deisseroth K, Augustine GJ, Ehlers MD, Feng C. 2007. In vivo light-induced activation of neural circuitry in transgenic mice expressing channelrhodopsin-2. Neuron. 54:205–218.

Aron AR, Poldrack RA. 2006. Cortical and subcortical contributions to stop signal response inhibition: role of the subthalamic nucleus. J Neurosci. 26:2424–2433.

Ayling OG, Harrison TC, Boyd JD, Goroshkov A, Murphy TH. 2009. Automated light-based mapping of motor cortex by photoactivation of channelrhodopsin-2 transgenic mice. Nat Methods. 6:219–224.

Bevan MD, Clarke NP, Bolam JP. 1997. Synaptic integration of functionally diverse pallidal information in the entopeduncular nucleus and subthalamic nucleus in the rat. J Neurosci. 17:308–324.

Chiken S, Nambu A. 2013. High-frequency pallidal stimulation disrupts information flow through the pallidum by GABAergic inhibition. J Neurosci. 33:2268–2280.

Chiken S, Shashidharan P, Nambu A. 2008. Cortically-evoked long-lasting inhibition of pallidal neurons in a transgenic mouse model of dystonia. J Neurosci. 28:13967–13977.

Chiken S, Sato A, Ohta C, Kurokawa M, Arai S, Maeshima J, Sunayama-Morita T, Sasaoka T, Nambu A. 2015. Dopamine D1 receptor-mediated transmission maintains information flow through the cortico-striato-entopeduncular direct pathway to release movements. Cereb Cortex. 25:4885–4897.

DeLong MR. 1990. Primate models of movement disorders of basal ganglia origin. Trends Neurosci. 13:281–285.

Flaherty AW, Graybiel AM. 1991. Corticostriatal transformations in the primate somatosensory system. Projections from physiologically mapped body-part representations. J Neurophysiol. 66:1249–1263.

Flaherty AW, Graybiel AM. 1993. Two input systems for body representations in the primate striatal matrix: experimental evidence in the squirrel monkey. J Neurosci. 13:1120–1137.
Franklin KBJ, Paxinos G. 2007. The mouse brain in stereotaxic coordinates. 3rd ed. New York (NY): Academic.

Hammond C, Yelnik J. 1983. Intracellular labelling of rat subthalamic neurones with horseradish peroxidase: computer analysis of dendrites and characterization of axon arborization. Neuroscience. 8:781–790.

Haynes WI, Haber SN. 2013. The organization of prefrontal-subthalamic inputs in primates provides an anatomical substrate for both functional specificity and integration: implications for Basal Ganglia models and deep brain stimulation. J Neurosci. 33:4804–4814.

Hazrati LN, Parent A. 1992a. The striatopallidal projection displays a high degree of anatomical specificity in the primate. Brain Res. 592:213–227.

Hazrati LN, Parent A. 1992b. Convergence of subthalamic and striatal efferents at pallidal level in primates: an anterograde double-labeling study with biocytin and PHA-L. Brain Res. 569:336–340.

Hazrati LN, Parent A. 1992c. Differential patterns of arborization of striatal and subthalamic fibers in the two pallidal segments in primates. Brain Res. 598:311–315.

Hira R, Honkura N, Noguchi J, Maruyama Y, Augustine GJ, Kasai H, Matsuzaki M. 2009. Transcranial optogenetic stimulation for functional mapping of the motor cortex. J Neurosci Methods. 179:258–263.

Hoover JE, Hoffer ZS, Alloway KD. 2003. Projections from primary somatosensory cortex to the neostriatum: the role of somatotopic continuity in corticostriatal convergence. J Neurophysiol. 89:1576–1587.

Inase M, Tokuno H, Nambu A, Akazawa T, Takada M. 1999. Corticostriatal and corticosubthalamic input zones from the presuppementary motor area in the macaque monkey: comparison with the input zones from the supplementary motor area. Brain Res. 833:191–201.

Isoda M, Hikosaka O. 2008. Role for subthalamic nucleus neurons in switching from automatic to controlled eye movements. J Neurosci. 28:7209–7218.

Kita H, Nambu A, Kaneda K, Tachibana Y, Takada M. 2004. Role of ionotropic glutamatergic and GABAergic inputs on the firing activity of neurons in the external pallidum in awake monkeys. J Neurophysiol. 92:3069–3084.

Kolomiets BP, Deniau JM, Mailly P, Ménétrey A, Glowinski J, Thierry AM. 2001. Segregation and convergence of information flow through the cortico-subthalamic pathways. J Neurosci. 21:5764–5772.

Lim DH, Mohajerani MH, Ledue J, Boyd J, Chen S, Murphy TH. 2012. In vivo large-scale cortical mapping using channelrhodopsin-2 stimulation in transgenic mice reveals asymmetric and reciprocal relationships between cortical areas. Front Neural Circuits. 6:11.

Maurice N, Deniau JM, Glowinski J, Thierry AM. 1999. Relationships between the prefrontal cortex and the basal ganglia in the rat: physiology of the cortico-nigral circuits. J Neurosci. 19:4674–4681.

Mink JW. 1996. The basal ganglia: focused selection and inhibition of competing motor programs. Prog Neurobiol. 50:381–425.

Nambu A. 2007. Globus pallidus internal segment. Prog Brain Res. 160:135–150.

Nambu A. 2011. Somatotopic organization of the primate basal ganglia. Front Neuroanat. 5:26.

Nambu A, Tokuno H, Hamada I, Kita H, Imanishi M, Akazawa T, Ikeuchi Y, Hasegawa N. 2000. Excitatory cortical inputs to pallidal neurons via the subthalamic nucleus in the monkey. J Neurophysiol. 84:289–300.

Nambu A, Tokuno H, Takada M. 2002. Functional significance of the cortico-subthalamo-pallidal ‘hyperdirect’ pathway. Neurosci Res. 43:111–117.

Obeso JA, Rodríguez-Oroz MC, Rodríguez M, Lanciego JL, Artieda J, Gonzalo N, Olanow CW. 2000. Pathophysiology of the basal ganglia in Parkinson’s disease. Trends Neurosci. 23:S8–S19.

Sakai S, Ueno K, Ishizuka T, Yawo H. 2013. Parallel and patterned optogenetic manipulation of neurons in the brain slice using a DMD-based projector. Neurosci Res. 75:59–64.

Sano H, Chiken S, Hikida T, Kobayashi K, Nambu A. 2013. Signals through the striatopallidal indirect pathway stop movements by phasic excitation in the substantia nigra. J Neurosci. 33:7586–7594.

Schmidt R, Leventhal DK, Mallet N, Chen F, Berke JD. 2013. Canceling actions involves a race between basal ganglia pathways. Nat Neurosci. 16:1118–1124.

Tachibana Y, Kita H, Chiken S, Takada M, Nambu A. 2008. Motor cortical control of internal pallidal activity through glutamatergic and GABAergic inputs in awake monkeys. Eur J Neurosci. 27:238–253.

Yelnik J, Percheron G. 1979. Subthalamic neurons in primates: a quantitative and comparative analysis. Neuroscience. 4:1717–1743.

Wang H, Peca J, Matsuzaki M, Matsuzaki K, Noguchi J, Qiu L, Wang D, Zhang F, Boydén E, Deisseroth K, et al. 2007. High-speed mapping of synaptic connectivity using photostimulation in Channelrhodopsin-2 transgenic mice. Proc Natl Acad Sci USA. 104:8143–8148.