Excitatory Cortical Inputs to Pallidal Neurons Via the Subthalamic Nucleus in the Monkey

ATSUCHI NAMBU, HIRONOBU TOKUNO, IKUMA HAMADA, HITOSHI KITA, MICHIKO IMANISHI, TOSHIKAZU AKAZAWA, YOKO IKEUCHI, and NAOMI HASEGAWA

1Department of Neurobiology, 2Department of Cell Biology, and 3Department of Neurophysiology, Tokyo Metropolitan Institute for Neuroscience, Tokyo Metropolitan Organization for Medical Research, Fuchu, Tokyo 183-8526, Japan; and 4Department of Anatomy and Neurobiology, College of Medicine, University of Tennessee, Memphis, Tennessee 38163

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INTRODUCTION

In the current model of the basal ganglia organization, the striatum receives direct excitatory cortical inputs and projects to the output nuclei, i.e., the internal segment (GPI) of the globus pallidus (GP) and the substantia nigra pars reticulata (SNr) through two major projection systems: “direct” and “indirect” pathways (Albin et al. 1989; Alexander and Crutcher 1990). The direct pathway arises from GABAergic striatal neurons containing substance P and projects monosynaptically to the GPI/SNr. Activation of this pathway tends to suppress the activity of the GPi/SNr. The indirect pathway arises from GABAergic striatal neurons containing enkephalin and projects polysynaptically to the GPI/SNr by way of a sequence of connections involving the external segment (GPe) of the GP and the subthalamic nucleus (STN). Activation of this pathway tends to increase the activity of the GPi/SNr because this pathway comprises an inhibitory GABAergic projection from the striatum to the GPe, an inhibitory GABAergic projection from the GPe to the STN, and an excitatory glutamatergic projection from the STN to the GPi/SNr. The two pathways thus have opposing effects on the basal ganglia output nuclei and are considered to be simultaneously involved in the control of voluntary movements. Imbalance between the activity of the two pathways has been proposed to account for the hypo- and hyperkinetic features of basal ganglia disorders (DeLong 1990).

Recently there has been growing evidence that the STN should be regarded as another input stage of the basal ganglia (Kita 1994; Levy et al. 1997; Mink 1996; Mink and Thach 1993). The monkey STN receives somatotopically organized inputs from the primary motor cortex (MI), supplementary motor area, and premotor cortex (Hartmann-von Monakow et al. 1978; Nambu et al. 1996, 1997) and, in turn, sends outputs to the GPe and GPi/SNr. Through this cortico-subthalamic-pallidal pathway, the motor-related areas of the cerebral cortex exert a direct influence on the output nuclei of the basal ganglia, bypassing the striatal afferent and efferent fibers, which have slow conduction velocity (Bauswein et al. 1989; Yoshida et al. 1971). Previous electrophysiological experiments have shown that cortical stimulation evokes an early excitation, an inhibition, and a late excitation in globus pallidus (GPi of primates) and SNR neurons in anesthetized rats (Fujimoto and Kita 1992; Kita 1992, 1994; Mauric et al. 1999; Ryan and Clark 1991). The early excitation disappeared after either permanent lesions of the STN (Kita 1992, 1994; Ryan

Address for reprint requests: A. Nambu, Dept. of Neurobiology, Tokyo Metropolitan Institute for Neuroscience, Tokyo Metropolitan Organization for Medical Research, 2-6 Musashidai, Fuchu, Tokyo 183-8526, Japan (E-mail: nambu@minin.ac.jp).

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and Clark 1991) or blockade of cortico-subthalamic transmission from the prefrontal cortex (Maurice et al. 1999), suggesting that it is mediated by the STN. A similar excitation-inhibition-excitation pattern in response to cortical stimulation was also evoked in GPe, GPi, and SNR neurons in monkeys (Kitano et al. 1998; Nambu et al. 1990; Yoshida et al. 1993), but the origin of each phase of such a response pattern in GP neurons still remains unclear.

To understand the motor control mechanisms of the forelimb movements by the basal ganglia, the present study was undertaken to characterize the influence of the MI and primary somatosensory cortex (SI) on the activity of GP neurons through the cortico-subthalamic-pallidal pathway in unanesthetized monkeys. Stimulating electrodes were chronically implanted into the forelimb region of the MI and SI after electrophysiological mapping of these areas. Responses of GP and STN neuronal pairs to the stimulation in the MI and SI were then recorded simultaneously. Changes in the response pattern of GP neurons to cortical stimulation were further examined by means of continuous recording of single neurons before and after injection of drugs into the STN.

**Method**

**Surgery**

Two (K1 and K2) female Japanese monkeys (Macaca fuscata), weighing 5.0 and 6.0 kg, were used in this study. The use of animals in the present study was approved by the animal care committee of the Tokyo Metropolitan Institute for Neuroscience. Before experiments, monkeys were trained to sit on a monkey chair quietly. Under general anesthesia with pentobarbital sodium (25 mg/kg body wt iv) after induction with ketamine hydrochloride (10 mg/kg im), the monkeys received a surgical operation to fix their heads painlessly in a stereotaxic frame attached to a monkey chair: each monkey was positioned in a stereotaxic apparatus, the skull was widely exposed, small stainless screws were attached to the skull as anchors, the exposed skull and screws were completely covered with transparent acrylic resin, two stainless steel pipes were mounted in parallel over the frontal and occipital areas for head fixation, and a small pin was fixed on the skull at A0, L0, H +40 as a stereotaxic reference point. All surgical procedures were performed under aseptic conditions.

**Implantation of stimulating electrodes in the cerebral cortex**

A few days after the surgery, stimulating electrodes were implanted chronically in the forelimb regions of the MI and SI after electrophysiological mapping. Each monkey was anesthetized with ketamine hydrochloride (10 mg/kg im) and xylazine hydrochloride (1–2 mg/kg im), and two holes (10–15 mm diam) were made in the skull over the midline and the orificial area of the MI. A rectangular chamber (40 mm × 50 mm) covering both holes was fixed with transparent acrylic resin. In the first part of the experiment, the activity of GP and STN neurons was observed simultaneously. Glass-coated Elgiloy-alloy microelectrodes (0.5–1.5 MΩ at 1 kHz) were inserted obliquely (45° from vertical in the frontal plane) through the dura into the GP, and vertically into the STN to record neuronal activity using hydraulic microdrives (Narishige Scientific Instrument, Tokyo). When penetrating the dura, the dura was anesthetized with local application of lidocaine. The unitary activity of GP and STN neurons was amplified, converted into digital data using a window discriminator, and then sampled at 2 kHz using a microcomputer for on-line data analysis. The unitary activity and converted digital data were also stored on videotapes using a Neurocorder (Neurodata, Delaware Water Gap, PA) for further analysis. Pairs of GP and STN neurons that showed activity change in response to cortical stimulation were sought. The following were measured routinely: 1) responses of both GP and STN neurons to the cortical electrical stimulation (0.3 ms duration single pulse, 0.3–0.7 mA strength at 0.4–0.8 Hz), as assessed by constructing peri-stimulus time histograms (PSTHs; binwidth, 1 ms); 2) spontaneous activity patterns of GP and STN neurons; 3) responses of GP neurons to STN stimulation (0.1–0.3 ms duration single cathodal pulse, 50 μA strength at 0.4–0.8 Hz); and 4) somatosensory responses of GP neurons to passive joint movement.

**Recording of GP and STN neuronal activity**

During experimental sessions, the monkey was seated in a monkey chair with its head restrained. For accessing GP and STN, the monkeys were anesthetized with ketamine hydrochloride (10 mg/kg im) and xylazine hydrochloride (1–2 mg/kg im), and two holes (10–15 mm diam) were made in the skull over the midline and the orificial area of the MI. A rectangular chamber (40 mm × 50 mm) covering both holes was fixed with transparent acrylic resin. In the first part of the experiment, the activity of GP and STN neurons was observed simultaneously. Glass-coated Elgiloy-alloy microelectrodes (0.5–1.5 MΩ at 1 kHz) were inserted obliquely (45° from vertical in the frontal plane) through the dura into the GP, and vertically into the STN to record neuronal activity using hydraulic microdrives (Narishige Scientific Instrument, Tokyo). When penetrating the dura, the dura was anesthetized with local application of lidocaine. The unitary activity of GP and STN neurons was amplified, converted into digital data using a window discriminator, and then sampled at 2 kHz using a microcomputer for on-line data analysis. The unitary activity and converted digital data were also stored on videotapes using a Neurocorder (Neurodata, Delaware Water Gap, PA) for further analysis. Pairs of GP and STN neurons that showed activity change in response to cortical stimulation were sought. The following were measured routinely: 1) responses of both GP and STN neurons to the cortical electrical stimulation (0.3 ms duration single pulse, 0.3–0.7 mA strength at 0.4–0.8 Hz), as assessed by constructing peri-stimulus time histograms (PSTHs; binwidth, 1 ms); 2) spontaneous activity patterns of GP and STN neurons; 3) responses of GP neurons to STN stimulation (0.1–0.3 ms duration single cathodal pulse, 50 μA strength at 0.4–0.8 Hz); and 4) somatosensory responses of GP neurons to passive joint movement.

**Drug injection into the STN and recording of GP neuronal activity**

In the second part of the experiment, the activity of GP neurons was observed before and after injection of drugs into the STN (experimental setup shown in Fig. 1). Recording of GP neuronal activity was performed as described above. For recording and injection of drugs in the STN, a small incision of the dura was made with local application of lidocaine, and a tungsten wire electrode attached to the needle of a 10-μl Hamilton microsyringe (Tokuno et al. 1998) was inserted vertically into the STN using a hydraulic microdrive. Pairs of GP and STN neurons that showed activity change in response to cortical stimulation were sought, and spontaneous activity and responsiveness of these neurons were examined. When observing responses of GP neurons to STN stimulation, stimuli (0.3 ms duration single pulse, 0.2–0.4 mA strength, sometimes up to 0.7 mA, at 0.4–0.8 Hz) were delivered using the tip of the electrode and the needle of a Hamilton microsyringe as a bipolar stimulating electrode. Then the recording microsyringe was advanced by the distance between the tip of the electrode and the center of the orifice of the needle (approximately 900 μm) to position the orifice of the needle at the target site (inset in Fig. 1). This sometimes made it impossible to record STN neuronal activity during and after drug injections. Through a needle, 0.5–2.0 μl of one of the following drugs was injected: 0.5–1.0 μg/μl muscimol (GABA A receptor agonist; Sigma, St Louis, MO), 20 mM N- methyl-d-aspartate (NMDA) receptor antagonist; RBI, Natick, MA), 10 mM 1,2,3,4-tetrahydro-6-nitro-2,3-dioxo-benzo[f]quinoxaline-7-sulfonamide disodium (NBQX, non-NMDA receptor antagonist; RBI), 10 mM bicuculline methiodide (GABA A receptor antagonist; Sigma), each of which was dissolved in saline. The activity of GP neurons was continuously monitored before and after injection of drugs into the STN. The spontaneous activity and responsiveness of these neurons were then examined.
data analysis

Mean values and standard deviations of the discharge rate during the prestimulus period (usually the 100 ms preceding the stimulation) were calculated from PSTHs and were considered to be the values for spontaneous discharge. Change in the neuronal activity in response to cortical stimulation was judged to be significant if the discharge rate during at least two consecutive bins (2 ms) reached the statistical level of $P < 0.05$ (1-tailed $t$-test). The latency of the response was defined as the time at which the discharge rate first exceeded this level. Spontaneous activity patterns of GP neurons were analyzed by calculating autocorrelograms (binwidth, 0.5 ms). The relationship between GP and STN neuronal discharges was analyzed by calculating cross-correlograms (binwidth, 0.5 ms). The mean values and standard deviations of coefficients long before time 0 (usually during the 50 ms beginning at −100 ms) were calculated. The correlation of the neuronal pair of GP and STN neurons was judged to be significant if the coefficient during at least two consecutive bins (1 ms) exceeded the 99% confidence limits ($t$-test).

Responses of GP and STN neurons

The activities of 55 pairs of GP and STN neurons (23 GPe and 32 GPi) were simultaneously recorded. Stimulation of the forelimb region of the MI induced activity changes in 51 pairs of GP and STN neurons (21 GPe and 30 GPi). Typical responses of GP and STN neurons evoked by MI stimulation are shown in Fig. 3A. Cortical stimulation induced an early, short-latency excitation, followed by an inhibition, and then a late excitation in both GPe and GPi neurons (Fig. 3A, top; for mean ± SD, see Table 1). The latency of the inhibition in GPe neurons was shorter than that in GPi neurons (Table 1, *), while the latencies of the early excitation and the late excitation were comparable in the two segments (Table 1). The same stimulation induced an early excitation and a late excitation, which were interrupted by a brief period of inhibition, and a subsequent long-lasting inhibition in STN neurons (Fig. 3A, bottom).
The onset of the early excitation in STN neurons preceded that in GP neurons by 2–6 ms in most cases (mean, 2.8 ms), as shown in Fig. 3B.

The remaining four pairs of GP and STN neurons (2 GPe and 2 GPi) responded to the stimulation of the forelimb region of the SI, not to that of the MI. Pairs of GP and STN neurons that responded to MI stimulation often responded to SI stimulation (7 GPe and 6 GPi of 15 GPe and 16 GPi tested). SI stimulation caused similar responses in GP and STN neurons as MI stimulation, except that the latency of the early excitation in GPe neurons was longer with SI stimulation (Table 1, †).

The response of GP neurons to STN stimulation was observed in 33 pairs of GP and STN neurons (15 GPe and 18 GPi). Twenty-one GP neurons (9 GPe and 12 GPi) showed an excitatory response (Fig. 3C, Table 1). The latency of the excitation was 2–10 ms (5.3 ± 2.1 ms) among GP neurons that responded to MI stimulation. Four GP neurons (1 GPe and 3 GPi) showed an inhibitory response to STN stimulation (Fig. 3D). One GPe neuron was antidromically activated by STN stimulation with a latency of 1.0 ms (Fig. 3E). The crosscorrelograms of 38 pairs of GP and STN neurons (16 GPe and 22 GPi) were constructed among 55 pairs. A few pairs (1 GPe and 3 GPi) showed small but significant correlation. Among them, only one pair of GPi and STN neurons showed excitation of the GP neuron following STN neuronal firing (Fig. 3F), which may have resulted from the excitatory subthalamo-pallidal projection.

Change in cortically evoked responses of GP neurons after drug injection into the STN

Muscimol was injected into the STN to block the neuronal activity, and its effect on the cortically evoked responses of GP neurons was examined in 12 of the 55 pairs of GP and STN neurons. Stimulation of the forelimb region of the MI evoked an early, short-latency excitation, an inhibition and a late excitation in GP neurons (Fig. 4, A1 and B1). Muscimol injection instantly blocked the spontaneous activity of STN neurons, then abolished the early and late excitations of GP neurons and made the inhibition longer (Fig. 4, A2 and B2) in a total of eight GPe and GPi neurons (Table 2). In two of three such GPe neurons, the duration of the inhibition was enor-
mously increased (50–500 ms) after muscimol injection (Fig. 4A2), while no such long inhibition was observed in any of the five GPi neurons (Fig. 4B2, Table 2). Since suppression of the STN neuronal activity continued throughout the day’s experimental session after muscimol injection, cortically evoked responses of another 51 GP neurons (33 GPe and 18 GPi) were analyzed after muscimol injection without recording before injection. A similar response pattern of inhibition without excitation was observed in 35 GP neurons (25 GPe and 10 GPi). Most GPe neurons showed a long-lasting inhibition (23/25), while far fewer GPi neurons showed such a response (3/10). These additional observations further support the consistency of the results obtained in 12 pairs of GP and STN neurons described above.

CPP or NBQX was injected into the STN to block the glutamatergic cortico-subthalamic transmission (Bevan et al. 1995; Rouzaire-Dubois and Scarnati 1987) with little effect on the GABAergic pallido-subthalamic transmission (the indirect pathway). The effect of CPP injection on the cortically evoked responses of GP neurons was examined. CPP injection abolished most of the early excitation and part of the late excitation without a significant change in the duration of the inhibition (Fig. 5A) in most cases (6/8, Table 2). On the other hand, injection of NBQX, sometimes up to 6 μl, had no effect on the response pattern of GP neurons tested (0/5, Table 2).

Bicuculline was injected into the STN to interrupt the GABAergic pallido-subthalamic transmission (Rouzaire-Dubois et al. 1980; Smith et al. 1990) with little effect on the cortico-subthalamic transmission. The effect of bicuculline injection on the cortically evoked responses of GP neurons was examined. Bicuculline injection had little effect on the early excitation, but increased the duration of the inhibition and

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<th>Table 1. The latency of the responses evoked in GP and STN neurons by MI, SI, or STN stimulation</th>
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<td><strong>Recording Location</strong></td>
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<tr>
<td></td>
</tr>
<tr>
<td>GP</td>
</tr>
<tr>
<td>GPe</td>
</tr>
<tr>
<td>GPe</td>
</tr>
<tr>
<td>STN</td>
</tr>
</tbody>
</table>

Values are means ± SD expressed in ms. GP, globus pallidus; STN, subthalamic nucleus; MI, primary motor cortex; SI, primary somatosensory cortex; GPe and GPi, external and internal segments of GP, respectively. * P < 0.05, † P < 0.02 (ANOVA with Bonferroni/Dunn post hoc tests; * and † are significantly different from each other).
slightly decreased the magnitude of the early phase of the late excitation (Fig. 5; 2/2, Table 2).

**Change in spontaneous activity of GP neurons after drug injection into the STN**

The time course of the effect of muscimol injection into the STN on the spontaneous discharge of GP neurons was examined for 21–89 min after injection in eight GP neurons (3 GPe and 5 GPi) that exhibited the abolishment of the early and late excitations by cortical stimulation. The spontaneous discharge rate of all the GP neurons decreased to 0–78% of the control level within 15 min after muscimol injection (Fig. 6). Two GP neurons showed a rebound increase of the spontaneous discharge rate following the decrease, while the others remained at low discharge rate levels throughout the recording period. The effect of CPP injection into the STN on the spontaneous discharge rate of GP neurons was examined in six GP neurons (4 GPe and 2 GPi) that exhibited attenuation of the early and late excitations. The spontaneous discharge rate of two GPe neurons decreased to 31 and 41% of the control level after CPP injection, while the other GP neurons did not show any manifest change.

Muscimol injection into the STN changed not only the spontaneous discharge rate but also the spontaneous discharge pattern of GP neurons (Fig. 7). One change in the discharge pattern was that pauses between the groups of spikes became frequent (Fig. 7, A and B). In high-frequency-discharge-with-pause type GP neurons (Fig. 7A1), typically observed in the GPe (DeLong 1971), muscimol injection increased the duration of pauses and induced grouped discharges (Fig. 7A3). Groups of discharges became periodic, as observed as humps in the autocorrelogram (arrowheads in Fig. 7A4). In high-frequency-discharge type GP neurons (Fig. 7B1), typically found in the GPi (DeLong 1971), muscimol injection induced pauses and grouped discharges (Fig. 7B3). The grouping of discharges was observed as a shoulder in the autocorrelogram (arrowhead in Fig. 7B4). These activity changes were observed in six of eight GP neurons (GPe, 3/3; GPi, 3/5) that exhibited the abolishment of the early and late excitations by cortical stimulation after muscimol injection into the STN. Another change was that the firing pattern of GP neurons became regular and oscillatory after muscimol injection (Fig. 7C1 and C3). This change could be observed more clearly in the autocorrellogram (Fig. 7, C1 and C4). These activity changes were observed in four of eight GP neurons examined (GPe, 2/3; GPi, 2/5). Similar oscillatory activity was also induced in some GP neurons by CPP (GPe, 2/4; GPi, 0/2) or bicuculline (GPe, 1/1; GPi, 0/1) injection into the STN.

**TABLE 2. Number of GP neurons that showed change in the responses evoked by cortical stimulation after drug injection into the STN**

<table>
<thead>
<tr>
<th>Recording Location</th>
<th>Drugs Injected Into the STN</th>
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<tr>
<td></td>
<td>Muscimol</td>
</tr>
<tr>
<td>GPe</td>
<td>3/5 (2)</td>
</tr>
<tr>
<td>GPi</td>
<td>5/7 (0)</td>
</tr>
<tr>
<td>Total</td>
<td>8/12</td>
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Each cell indicates the number of GP neurons that showed change in the responses evoked by cortical stimulation after drug injection into the STN over the number of GP neurons tested. Numbers in parentheses indicate the number of GP neurons showing a long-lasting inhibition after muscimol injection, as shown in Fig. 4A. CPP, (±)-3-(2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid; NBQX, 1,2,3,4-tetrahydro-6-nitro-2,3-dioxo-benzo[f]quinoxaline-7-sulfonamide disodium; other abbreviations, see Table 1.

**Other findings after drug injection into the STN**

Passive joint movements of the forelimb often changed the activity of the GP neurons studied. The effect of STN blockade on somatosensory responses to passive joint movements was examined in GP neurons that exhibited clear somatosensory responses and abolishment of cortically evoked excitation after
drug injection into the STN. All GP neurons studied increased their activity in response to joint movements before drug injection into the STN. Somatosensory responses disappeared after muscimol injection in four of five GP neurons tested (GPe, 3/3; GPi, 1/2). Similarly, somatosensory responses disappeared after CPP injection in two of two GP neurons tested (GPi, 2/2). On the other hand, bicuculline injection had little effect on somatosensory responses of the one GPe neuron tested.

It has been reported that hemiballismus was induced by STN lesion or muscimol injection into the STN (Hamada and DeLong 1992a; Hamada and Hasegawa 1994). In the present study, similar abnormal movements were observed in the contralateral limbs after muscimol injection into the STN in three of eight trials in which cortically evoked excitation of GP neurons was abolished.

**Locations of recorded GP neurons and injection sites in STN**

After histological examination, the locations of GP and STN neuronal pairs were plotted with different symbols according to the drugs injected (Fig. 8). GP neurons were located in the sector along the dorso-lateral to ventro-medial direction in both the GPe and GPi (Fig. 8, A1 and B1). This distribution corresponds well to the forelimb area of the GPe and GPi reported previously (DeLong 1971; DeLong et al. 1985; Hamada et al. 1990; Hoover and Strick 1993, 1999; Mink and Thach 1991a; Zemanick et al. 1991). Indeed, GP neurons with response to MI stimulation have been reported to show movement-related activity (Nambu et al. 1990). STN neurons were located mainly in the lateral part of the STN (Fig. 8, A2 and B2). This distribution corresponds well to the terminal zone from the MI (Hartmann-von Monakow et al. 1978; Nambu et al. 1996) and the locations of STN neurons showing movement-related activity (DeLong et al. 1985; Wichmann et al. 1994). The GP neurons and STN injection sites that showed change of cortically evoked responses in GP neurons after drug injection into the STN (●, ●, and ■) were intermingled with those without effect (○, △, and ▽). In the same area, GP neurons and STN injection sites with no change in the responses after NBQX injection into the STN were found (▽).

**DISCUSSION**

The present study emphasizes the prominent cortical influence through the cortico-subthalamo-pallidal pathway on the output nuclei of the basal ganglia, in addition to that through the direct and indirect pathways (Fig. 9A). Cortical stimulation evoked an early excitation, followed by an inhibition and a late excitation in GP neurons. In the present series of experiments, we demonstrated that the early excitation was mediated by the cortico-subthalamo-pallidal pathway.

**Origin of the early excitation in GP neurons by cortical stimulation**

The early excitation evoked in STN neurons by cortical stimulation is likely to be caused by the cortico-subthalamic projections because 1) the pattern and latency of the cortically evoked responses in this study are similar to those of the previously reported cortico-subthalamic responses (Fujimoto and Kita 1993; Kita 1994; Kitai and Deniau 1981; Maurice et al. 1998; Ryan and Clark 1992), and 2) the distribution of recorded STN neurons in this study corresponds well to the terminal zone of cortico-subthalamic projections from the MI (Hartmann-von Monakow et al. 1978; Nambu et al. 1996). The direct projection from the SI to the STN has also been reported in rats (Canteras et al. 1988) and cats (Noda and Oka 1993).
The early excitation evoked in GP neurons by cortical stimulation is considered to be caused by the early excitation in STN neurons through the excitatory glutamatergic subthalamo-pallidal projection (Nakanishi et al. 1991) based on the following findings. The early excitation in GP neurons was preceded by that in STN neurons, and the latency difference was 2–6 ms (mean, 2.8 ms), which is comparable to the latency of the STN-induced excitation of GP neurons in rats (Kita and Kitai 1991; Nakanishi et al. 1991) and to the latency of the GP-induced antidromic response of STN neurons in monkeys (0.5–3.5 ms) (A. Nambu, S. Yoshida, I. Tanibuchi, and K. Jin-nai, unpublished observations) and in rats (Kita et al. 1983). GP neurons were orthodromically activated by STN stimulation in this study (Fig. 3C), although the latency (5.3 ± 2.1 ms) was rather long. The early-phase responses might not have been adequately sampled because of stimulus artifacts. Finally, the effects of the injection of drugs into the STN support the idea that the early excitation of GP neurons is derived from the STN. Indeed, blockade of neuronal activity of the STN by muscimol injection abolished the early and late excitations in GP neurons (Fig. 4). In addition, blockade of the cortico-subthalamic transmission by CPP injection into the STN suppressed the early excitation in GP neurons (Fig. 5A), whereas interference with the pallido-subthalamic transmission by bicuculline injection had little effect on the early excitation (Fig. 5B).

The cortico-subthalamic transmission has been considered to be mediated by both NMDA and non-NMDA type glutamate receptors based on the receptor localization in the STN (Clarke and Bolam 1998) and an in vitro study (Nakanishi et al. 1988). A recent physiological study showed that the early excitation evoked in SNr neurons by stimulation in the prelimbic and medial orbital areas of the rat frontal cortex was decreased by 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, non-NMDA receptor antagonist) application in the STN (Maurice et al. 1999). In contrast, injection of 20 mM CPP, but not 10 mM NBQX, into the STN suppressed the early excitation evoked by MI stimu-
lation in awake monkeys in this study (Table 2). The concentration of NBQX in the STN was likely to have been sufficient to block non-NMDA receptors, because 8 μM NBQX and 50 μM CPP in the superfusion medium were reported to abolish most of the non-NMDA and NMDA receptor–mediated responses, respectively, of striatal spiny neurons in slice preparations (Kita 1996). Thus the cortico-subthalamic glutamatergic transmission directly related to motor control in the monkey is considered to be mediated mainly by NMDA receptors. The in vitro study mentioned above (Nakanishi et al. 1988) also showed that repetitive firing of STN neurons by internal capsule stimulation was largely abolished after blockade of NMDA receptor–mediated transmission. STN neurons usually fire at high frequency and may be sufficiently depolarized to activate NMDA receptors by removing the Mg$^{2+}$ blockade (Nowak et al. 1984).

**Origin of other responses in GP neurons**

The inhibition evoked in GP neurons by cortical stimulation is considered to be mediated by the direct cortico-striato-pallidal pathway, according to previous studies. Stimulation of the striatum evoked inhibitory responses in GP neurons, and the difference between the latency of the inhibition evoked by cortical stimulation and that of the inhibition evoked by striatal stimulation corresponds well to the cortico-striatal conduction time in the monkey (Yoshida et al. 1993). Inhibitory postsynaptic potential is.
aptic potential (IPSP) or inhibition evoked by cortical stimulation in the rat was abolished by systemic injection of a GABAergic blocker (Kita 1992) or by blocking cortico-striatal neurotransmission (Maurice et al. 1999). The distribution of recorded GP neurons in this study (Fig. 8) corresponds well to the distribution of GP neurons receiving cortical inputs from the forelimb region of the MI via the striatum, which was revealed by an anatomical study using transneuronal transport (Zemanick et al. 1991). The fact that the latency of the inhibition evoked by cortical stimulation in GP neurons was shorter than that in GPi neurons in this study (Table 1) could be explained by the striatal origin of the inhibition and by the difference of distance of GPe and GPi neurons from the striatum.

GPe neurons showed a prolonged lengthening of the inhibition after muscimol injection into the STN, while GPi neurons did not (Fig. 4, Table 2). Our preliminary results showed that local application of bicuculline in the GPe decreased the duration of the long inhibition (unpublished observations), suggesting that the long inhibition was triggered by striato-pallidal GABAergic transmission. GABA release from the axon terminals of striato-GPe fibers might be controlled by the subthalamo-GPe projection and increased and prolonged by some unknown mechanism after inactivation of STN neuronal activity.

The late excitation evoked in GP neurons by cortical stimulation could be ascribed to the late excitation in STN neurons, because blockade or lesion of the STN abolished the late excitation as well as the early excitation in GP or SNr neurons in the present (Fig. 4) and previous studies (Kita 1992, 1994; Ryan and Clark 1991). The late excitation in STN neurons has been considered to be the late component of a single long cortico-subthalamic excitatory response interrupted by a brief inhibition, because lesion of the striatum or the GPe did not change the pattern of excitatory response to cortical stimulation (Fujimoto and Kita 1993; Kita 1994; Ryan and Clark 1992). A recent study, however, suggested the alternative possibility that the late excitation in STN and SNr neurons resulted from a disinhibitory process through the indirect cortico-striato-pallido-subthalamo-nigral circuit, because the late excitatory response in STN and SNr neurons was markedly reduced after the blockade of the cortico-striatal or striato-pallidal transmission (Maurice et al. 1998, 1999). Injection of CPP into the STN attenuated the late excitation in the present study (Fig. 5A), suggesting the involvement of the cortico-subthalamo-pallidal pathway in the late excitation of GP neurons. Injection of bicuculline into the STN also decreased the late excitation (Fig. 5B). Bicuculline removes continuous GABAergic inhibition from the GPe on STN neurons, and therefore the indirect cortico-striato-pallido-subthalamic transmission would not induce an excitatory response in STN neurons through disinhibitory mechanism, leading to the attenuation of the late excitation in GP neurons. The result suggests the involvement of the indirect pathway in the late excitation of GP neurons in addition to that of the cortico-subthalamo-pallidal pathway. A contribution of rebound firing after IPSPs via the direct pathway cannot be neglected, because striatal stimulation caused rebound firing after IPSPs in GP neurons in brain slice preparations (Nambu and Llinás 1994). The present study showed orthodromic activation and inhibition of GP neurons by STN stimulation (Fig. 3, C and D). Possible origins of the inhibitory response of GP neurons to STN stimulation include the orthodromic or antidromic activation of GP neurons with local axon collaterals on other GP neurons and/or with projection to GPi (Shink et al. 1996), and/or the orthodromic activation of GPi neurons with local axon collaterals on other GPi neurons. Current spread of STN stimulation to other structures, such as pallido-thalamic fibers, appeared to be negligible, because no GPi neurons were antidromically activated in this study.

A previous study in the monkey showed no correlation of activity between GP and STN neurons (Bergman and DeLong 1989). The authors of that study argued that the subthalamo-pallidal projection was highly topographic and specific. In the present study, only a few pairs of GP and STN neurons showed correlation of activity, among pairs of GP and STN neurons that responded to stimulation of the same cortical sites. The subthalamo-pallidal projection may be diffuse and continuously active to maintain the membrane potentials of GP neurons over the firing threshold. Thus each spontaneous firing of STN neurons may contribute slightly to evoking the spontaneous firing of GP neurons.

Cortical stimulation produced an early excitation, then an inhibition, and a late excitation in many GP neurons. This suggests that inputs conveyed through the cortico-subthalamo-pallidal, direct and indirect pathways may converge on a single GP neuron. Such input convergence is also supported by a previous double-labeling anatomical study that showed that the subthalamo-pallidal and striato-pallidal projections converged onto the same GP neurons in the monkey (Hazrati and Parent 1992a).

Spontaneous activity change in GP neurons and abnormal limb movements after STN blockade

Blockade of the STN decreased the firing rate and altered the firing pattern of GP neurons in this study (Figs. 6 and 7). Similar changes of activity were reported in patients with hemiballismus (Suarez et al. 1997), monkeys with STN lesion (Hamada and DeLong 1992b), and rats with STN lesion (Robledo and Fèger 1990; Ryan and Sanders 1993). GP neurons have their own membrane properties capable of high-frequency discharges (Nakashiba et al. 1990; Nambu and Llinás 1994). Continuous excitatory inputs from the STN are considered to maintain the firing rate and pattern of GP neurons. Thus the STN could be considered to be a driving force of the GP (Kita and Kita 1987).

Somatosensory responses of GP neurons disappeared after muscimol or CPP injection into the STN in this study. This suggests that somatosensory responses observed in GP neurons may be mediated by the cortico-subthalamo-pallidal pathway. In addition, the observation that the increase of activity in response to somatosensory stimuli was dominant before drug injection into the STN in the present study is also consistent with this mechanism, which has been shown to function in the case of movement-related activity of GP neurons, as will be discussed below.

The present observations lead us to consider the pathophysiological mechanism of hemiballismus. Indeed, abnormal movements of the contralateral limbs were observed after muscimol injection into the STN in this study. While the
mechanism of hemiballismus may be explained by decreases in pallidal inhibition on the thalamus, it is paradoxical that GPi lesions per se did not induce dyskinesias (DeLong and Georgopoulos 1981). Abnormal activity of GP neurons, such as the grouping of discharges and oscillatory activity observed in the present experiment, might be transmitted to the thalamus and disturb the mechanism of inhibition of unwanted motor programs (see next section).

Functional significance of the cortico-subthalamo-pallidal pathway

Recent anatomical studies showed that the subthalamo-pallidal fibers were more widely arborized and terminated on more proximal neuronal elements than the striato-pallidal fibers (Hazrati and Parent 1992a,b). These findings suggest the “center-surround model” of basal ganglia function (Mink 1996; Mink and Thach 1993). The present study clearly shows that the cortico-subthalamo-pallidal pathway exerts powerful excitatory effects on the output nuclei, and is faster in signal conduction than the direct and indirect pathways (Fig. 9A). Moreover, cortico-pallidal neuronal discharge began near the onset of or after the movement and lagged behind the onset times of pyramidal tract neurons (Bauswein et al. 1989), whose axon collaterals were suggested to project to the STN (Giuffrida et al. 1985). These previous findings together with our present findings support the center-surround model and emphasize its aspects in the time domain (Fig. 9B). When a voluntary movement is about to be initiated by cortical mechanisms, a corollary signal is sent simultaneity from the motor cortex to the GPi through the cortico-subthalamo-pallidal pathway to activate GPi neurons extensively, thereby resulting in the inhibition of their targets related to both the selected motor program and other competing programs (Fig. 9B, middle). Then another corollary signal through the direct cortico-striato-pallidal pathway is sent to the GPi to inhibit a specific population (center) of pallidal neurons. Finally, such pallidal neurons in the center area disinhibit their target neurons in the thalamus (Fig. 9B, bottom). Thus only the selected motor program is executed at the selected timing, whereas other competing programs mediated by pallidal neurons in the surrounding area are canceled.

This model may explain discrepancy between increased pallidal activity during voluntary limb movements and the “disinhibition model” of the basal ganglia function based on the SNr neuronal activity. SNr neurons show a reduction in discharge during saccadic eye movements, and the reduced SNr activity is believed to disinhibit superior collicular neurons and finally evoke saccadic eye movements (Hikosaka and Wurtz 1983a,b). In contrast, pallidal activity during voluntary limb movements has always shown mainly an increase in discharge, and the incidence ratio of increase to decrease has been reported to be 1.6–5.8 (Anderson and Horak 1985; Georgopoulos et al. 1983; Hamada et al. 1990; Mink and Thach 1991b; Mitchell et al. 1987; Nambu et al. 1990; Turner and Anderson 1997). In addition, neurons in the lateral STN (DeLong et al. 1985; Georgopoulos et al. 1983; Wichmann et al. 1994) and STN neurons projecting to the GP (Jinnai et al. 1990) show movement-related activity. Thus it is more likely that neuronal activity of most GP neurons during limb movements are mediated by the net excitatory cortico-subthalamo-pallidal pathway than by the net inhibitory direct cortico-striato-pallidal pathway, and the former pathway is faster than the latter.

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