Substantia Nigra Output to Prefrontal Cortex Via Thalamus in Monkeys. I. Electrophysiological Identification of Thalamic Relay Neurons

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INTRODUCTION

Cortico–basal ganglia (BG)–thalamic loop circuitry arising from the prefrontal cortex (PFC) and returning to the same cortical areas is generally considered to play important roles in higher-order functions of the mammalian brain (Alexander et al. 1986). In particular, nigro-thalamo-cortical pathways from the substantia nigra pars reticulata (SNr) to the PFC via the mediodorsal (MD) and ventral anterior (VA) thalamic nuclei form key connections in the cortico-BG-thalamic-cortical positive feedback network (Goldman-Rakic and Friedman 1991). In nonhuman primates, although some anatomical studies were done on the nigrothalamic (Beckstead and Frankfurter 1982; Carpenter et al. 1976; Francois et al. 2002; Parent et al. 1982; Carpenter et al. 1976; Francois et al. 2002; Parent et al. 1982) and thalamocortical projections (Barbas et al. 1991; Ilinsky et al. 1985; Jürgens 1984; Kievit and Kuypers 1977; Miyata and Sasaki 1983), only one study has addressed the nigro-thalamo-cortical pathways (Ilinsky et al. 1985); it clarified the outline of the topographical organization of the nigro-thalamo-cortical pathways from the SNr via the MD and VA to widespread areas of the frontal cortex (FRC). Also, transneuronal tracing studies (Lynch et al. 1994; Middle-}

Two Japanese monkeys (Macaca fuscata, male: 5.7–6.4 kg) that served as subjects in this study were the same as those in our previous study (Kitano et al. 1998). They were trained to perform an operant conditional task, the details of which are described in the companion paper (Tanibuchi et al. 2009). All procedures in training, surgery, recording, and housing of the monkeys were done in accordance with the Guidelines for Animal Experimentation at Shiga University of Medical Science and National Institutes of Health Guide for the Care

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and Use of Laboratory Animals. This experimental protocol was approved by the Animal Care Committee of Shiga University of Medical Science.

Surgical procedures

Prior to surgery, the borders of the MD, VA, ventral lateral nucleus pars medialis (VLm), and SNr, as well as the outline of the FRC, were conjectured based on magnetic resonance imaging (MRI) of the monkeys in both coronal and horizontal planes. Before initiating anesthesia, the monkeys were pretreated with atropine sulfate [0.05 mg/kg, administered intramuscularly (im)]. Under general anesthesia with sodium pentobarbital (30 mg/kg, im) after induction with ketamine hydrochloride (10 mg/kg, im), the monkeys were placed in a stereotaxic apparatus. The surgery was conducted under aseptic conditions and pentobarbital anesthesia (initial dose: 30 mg/kg; additional dose: 5–7 mg/kg/h, im). Their body temperature was maintained at 36–37°C throughout surgical procedures by using a heating pad. They were injected with antibiotics (isepamicin sulfate: 8 mg/kg, im) after the surgery. Based on the MRI findings, a hole was made in the skull overlying the right thalamic region of interest and an acrylic recording cylinder was placed over the opening. Using stereotaxic guidance, the cylinder was tilted laterally by 5.7° (tangent value of 0.1) to prevent a recording electrode, directed to the medial part of the MD, from puncturing the superior sagittal sinus. The cortical surface of the right hemisphere was partly exposed by excising the bone fragment and incising the dura mater to implant cortical and subcortical stimulating electrodes. Bipolar stimulating electrodes (2-mm intertip distance; 0.3-mm exposed tip) made of enamel-coated silver wire (0.2 mm in diameter), for antidromic activation of thalamocortical neurons, were implanted in various areas of the FRC (19 and 20 pairs in the two monkeys, respectively); these areas included the ventral (PSv) and dorsal (PSd) parts of the principal sulcus (PS), the dorsal part of the dorsolateral PFC (PFD), the medial PFC (PFm), the orbitofrontal area (OF), the dorsal part (FEFd, Walker’s area 8a) of the frontal eye field.
(FEF), the supplementary motor area (SMA), the ventral and dorsal parts (PMv and PMd, respectively) of the premotor area (PM), and the face and arm parts of the primary motor area (M) (Fig. 1A). Caudal pairs (PSvC) of PSv electrodes were located in the rostral part of Walker’s area 45, according to the cytoarchitectonic criteria of Barbas and Pandya (1989). Rostral (PSvr) and intermediate (PSvi) pairs of the PSv electrodes were positioned in the middle and caudal thirds of the PS rostrocaudally, respectively. An array of concentric stimulating electrodes (outer diameter, 0.5 mm; central core extruding by ~0.2 mm) for activating SNr neurons was stereotaxically introduced into the SNr through the occipital lobe with a caudal tilt angle of 36.9° (tangent value of 3/4). The tips of nine stimulating electrodes were located in various portions of the SNr (circles in Fig. 2). Those of two other electrodes were placed in the cerebral peduncle (CP) and in the medial lemniscus (ML); they were used as control electrodes to nigral stimulation (asterisks in Fig. 2).

After thalamic recording we conducted another experiment in one of the two monkeys, to confirm that the inhibitory responses of thalamocortical neurons to SNr stimulation were due to monosynaptic transmission and to determine the exact location of SNr neurons projecting to the thalamic regions where thalamocortical neurons were located. An array of five stimulating electrodes (enamel-coated stainless needles with 0.5-mm outer diameter and ~0.3-mm exposed tip) was implanted in the nuclear subdivisions of the thalamus where clusters of the thalamocortical neurons responding orthodromically to SNr stimulation were recorded; this array was stereotaxically introduced from the caudal FRC with a lateral tilt of 45°. Implantation of the additional electrodes was also performed under deep anesthesia and aseptic conditions.

Recording procedures

We used an extracellular unit recording technique applying the methods of antidromic and orthodromic stimulation. Recording sessions began after the training session of the operant conditional task. Single-neuron activity was recorded while the monkeys performed the task sitting in a primate chair with their head restrained. Recording sessions of several hours were held every 3 or 4 days. After the experimental session, the monkeys were returned to their home cages and given unrestricted access to water, food, and fruit. Their body weights were regularly monitored.

THALAMIC RECORDING. A glass-coated Elgiloy microelectrode was advanced through the recording cylinder to the thalamic portions by a micromanipulator (Narishige, MO-951). We first surveyed thalamic regions of interest (the MD, VA, and VLm), with reference to the MRI photographs, to determine the location of thalamocortical neurons with SNr input. Thereafter, neuronal recording was concentrated in the thalamic portions where thalamocortical neurons with nigral afferents were clustered.

Identification of thalamocortical neurons. Thalamocortical neurons were identified by their antidromic responses to cortical stimulation \[ \leq 0.55 \text{ mA strength, 0.3-ms duration, tip negative, 0.8- to 1.0-Hz frequency; see Lipski (1981) for details.} \] To examine whether all spikes were generated by an antidromically activated neuron, we applied “spike-triggered averaging” method to “collision test”; cortical stimuli were delivered at a constant delay interval after each spike discharge, by which 10 sweeps of prestimulus and stimulation-evoked action potentials were averaged. We then measured the current threshold for its antidromic activation (i.e., current that elicited the response on half of the stimulation trials). Whenever a thalamocortical neuron was identified by stimulation of a pair of cortical electrodes, we tested whether the neuron was antidromically activated by stimulation of all other pairs of the cortical electrodes.

Identification of nigrothalamic input. After a single thalamocortical neuron was isolated, the effect of SNr stimulation on its spike discharge was observed between 50 ms preceding the initiation of the stimulus and 100 ms after its initiation, using peristimulus time histograms (PSTHs) with 0.2- or 0.5-ms bin widths. Action potentials were converted into digital data using a window discriminator. The count level of the discriminator was set to the midpoint between the negative peak of spikes and the baseline. Single current pulses (0.5-mA strength, 0.3-ms duration, tip negative) were delivered at a low frequency of 0.7 Hz, to avoid the effect of the preceding stimulus, and 40–140 stimulus trials were summated on the PSTHs. After the

![Figure 2](http://jn.physiology.org/)

**FIG. 2.** The location of SNr stimulation sites. Open and filled circles show the tips of 4 and 3 electrodes in 2 monkeys, respectively; 8 were located within the SNr and one on the border between the SNr and the substantia nigra pars compacta (SNc). Asterisks represent 2 electrodes in the cerebral peduncle (CP) and in the medial lemniscus (ML) of the 2 monkeys, respectively. Numbers above coronal sections indicate the distance (in mm) from the interaural plane. Photomicrographs show 2 marking sites in the rostral extremity of the SNr (filled circle in AP +16) and its caudal part (filled circle in AP +13), respectively. Three oval-shaped lesions medial to the iron deposits in the right photograph are trajectories of other nigral electrodes. See RESULTS for description of arrowheads in AP +13 plane. P, pons; Stth, subthalamus.
nigral input was examined, the “spike-triggered collision test” was again applied to the thalamocortical neurons to confirm that the counted spikes were generated by the identical thalamocortical neurons. The procedures for identifying thalamocortical neurons with nigral input are schematized in Fig. 1B.

NIGRAL RECORDING. Two electrodes in the VA–VLm were stimulated as a pair of bipolar electrodes and three in the rostrolateral MD and in its laterally neighboring structures were used as two pairs of bipolar electrodes (Fig. 3); the intertip distance of these three pairs of bipolar electrodes was 2.5 to 3.0 mm. SNr neurons projecting to the MD and VA–VLm were identified in a manner similar to that used to identify thalamocortical neurons—i.e., antidromic responses of SNr neurons to thalamic stimulation (≤0.5-mA strength, 0.3-ms duration, 0.8- to 1.0-Hz frequency) and their collision with prestimulus action potentials were examined. A recording electrode was lowered to the SNr through the same cylinder that was used for the thalamic recording. As with the thalamic recording, neuronal recording in the SNr was first broadly surveyed and then focused on subdivisions where nigrothalamic neurons were clustered.

On completion of the recordings we electrically stimulated each electrode in the FEFd and PSv to examine whether the stimulation would evoke any eye movement. Stimulation (0.1- to 0.5-mA strength, eight pulses, 0.3-ms pulse duration, 3-ms interpulse interval, 1.0-Hz repetitive frequency) of FEFd electrodes induced large contraversive saccades (0.1 threshold ≤ 0.2 mA), whereas that of PSvc electrodes by much higher current yielded small contraversive saccades (0.3 ≤ threshold ≤ 0.5 mA). We also examined whether their body movements were elicited by electrical stimulation. Electromyographic (EMG) activity to stimulation (0.5-mA strength, single pulse with 0.3-ms duration, 1.0-Hz frequency) of all cortical electrodes was tested using surface electrodes for musculus (m.) masseter, m. orbicularis oculi, m. orbicularis oris, dorsal neck muscles, shoulder muscles, paravertebral muscles, and upper limb muscles (m. brachioradialis, wrist flexors, m. triceps brachii, and m. biceps brachii). Stimulation of the face part of the M yielded the activity of m. masseter, m. orbicularis oculi, and m. orbicularis oris. Ventral pairs of PMv electrodes produced the activity of m. orbicularis oris. The arm part of the M induced the movement of the upper limb muscles.

Data analysis

Latency measurement of antidromic responses. Antidromic response latencies of thalamocortical neurons to cortical stimulation and those of nigrothalamic neurons to thalamic stimulation were determined by measuring the time from the start of the stimulus artifact to the onset of the antidromically evoked response.

Statistical analysis and latency and duration determination of orthodromic responses. Inhibitory SNr input to thalamocortical neurons recorded in the PSTHs was statistically evaluated by t-test; if the firing rate of a thalamocortical neuron during a 5-ms interval starting 1 ms after the initiation of SNr stimulation (to avoid the effect of the SNr-stimulus artifacts) was significantly (P < 0.01) lower than that during a 50-ms control period preceding nigral stimulation, the neuron was considered to receive inhibitory nigral input. Latency of thalamic inhibitory responses to SNr stimulation was determined by PSTHs. Spikes were
The time between the stimulus onset and the first appearance of a 3-ms interval without action potentials was measured in the PSTHs with 0.2 and 0.5 ms/bin, respectively, and then subtracted 0.08 ms from the time measured in the PSTHs. Duration of the orthodromic inhibitory responses was defined as the time period without action potentials persisting after the onset in the PSTHs.

**Histological verification of stimulating and recording sites**

Anterior–posterior (A-P), medial–lateral (M-L), and dorsal–ventral (D-V) coordinates were registered for the recording sites of all neurons. Elgiloy microelectrodes were kept attached to the microdrive over 7–15 sessions, depending on their viability. After each electrode was exhausted, iron deposits were made by passing DC current (10–20 μA for 30–60 s, tip positive). After perfusion, they were used as landmarks for the reconstruction of recording sites. On completion of the recording sessions, marking lesions were made at the tips of stimulating electrodes implanted in the cortical and nigral subdivisions by passing DC anodal and cathodal current (10 V for 3–6 s each). The monkeys were then deeply anesthetized with an overdose of sodium pentobarbital (60 mg/kg, im) and then perfused intracardially with 10% formalin containing 3% potassium ferrocyanide. The brains were stereotaxically cut into two blocks in the coronal plane. The blocks were removed from the skull and then soaked in 0.1 M phosphate buffer containing 30% sucrose at 4°C for 10–14 days. The blocks were cut into frontal sections (90 μm thick) on a freezing microtome stage. Every section was stained with neutral red. The positions of the cortical and nigral stimulating electrodes were histologically verified from the electrolytic lesions (see photomicrographs in Figs. 1A and 2). The location of individual units recorded by each electrode was reconstructed on the coronal sections of the thalamus and SNr, on the basis of both their distance from the iron deposits with registered A-P, L-M, and D-V coordinates and the trajectories of electrode penetrations.

**Nomenclature, delineation, and subdivision**

Nomenclature and delineation of the thalamic nuclei and SNr followed the atlas of Kusama and Mabuchi (1970). Delineation of the cerebral cortex was drawn by standardizing coronal sections of the brains used in this study. Cytoarchitectural division of the macaque frontal lobe was on the basis of the criteria of Walker (1940) and Barbas and Pandya (1989). The subdivisions of the MD and VA were determined on the basis of their morphological features (Jones 1985). Because the borders between the MD pars multifomis (MDmf) and the MD pars parvocellularis (MDpc) and also between the VA pars magnocellularis (VAmc) and the VA pars parvocellularis (VApc) were scarcely discernible, some neurons in the vicinity of the boundaries could have belonged to the neighboring subnuclei.

**FIG. 4.** Histological reconstruction of thalamocortical neurons with SNr input. Six drawings illustrate the location of thalamic [MD pars multifomis (MDmf), MD pars parvocellularis (MDpc), VA pars magnocellularis (VAmc), and VLm] neurons recorded between AP +12 and AP +17. As shown at top right, thalamic relay neurons were sorted in terms of both cortical projection areas and inhibitory nigral sourcing portions. Circles, neurons projecting to the PSv (A); squares, neurons projecting to other PFC (B); triangles, neurons projecting to motor areas (C). Neurons projecting to 2 cortical areas are signified by combinations of their corresponding symbols. Open and filled symbols represent relay neurons receiving inhibitory input with short latencies (<5 ms) from the rostral and caudal SNr, respectively. a, b, and c refer to neurons discussed in the text and shown in Fig. 6.
RESULTS

Thalamocortical neurons with inhibitory nigral input

Thalamic recording was performed in thalamic nuclei spanning from AP +10 to +17 mm in front of the interaural line and extending from about 0.5 to 7 mm lateral to the midline. In particular, it was concentrated in the rostral half of the lateral MD (corresponding to the MDmf and MDpc, i.e., the MDmf/pc) and the VAmc where thalamocortical neurons with SNr input were clustered (Figs. 4 and 5). We identified 246 thalamocortical neurons and examined them using PSTHs to see whether they received inhibitory input from the SNr in two monkeys. A total of 70 thalamocortical neurons exhibited suppression in discharge rate at short latencies (<5.0 ms) to SNr stimulation (Table 1). Three examples of the thalamocortical neurons with nigral input are shown in Fig. 6.

CORTICAL PROJECTIONS. Of the 70 thalamocortical neurons with SNr input, 3 were each antidromically activated by stimulation of two pairs of electrodes in different cortical areas; 2 responded to stimulation of the OF and the PMd and one to stimulation of the PfD and the PMd (Table 1). Of the 70 thalamocortical neurons, 58 projected to the PFC, while 15 projected to motor areas (the M, PM, and SMA) (Fig. 1A, Table 1). Of the 58 thalamo-PFC neurons, 40 projected exclusively to the PSv and the remainder (n = 18) projected to the Psd, PfD, PfM, or OF (Fig. 1A, Table 1). Of the 40 PSv-projection neurons, almost all (n = 39) were antidromically activated by stimulating electrodes in the PSvc (n = 31) or PSvi (n = 8) (Fig. 1A, Table 1). No thalamo-FEFd neurons with inhibitory SNr input were identified, although 9 thalamo-FEFd neurons were found in the rostrolateral MD (n = 8) and central lateral nucleus (CL; n = 1). We classified the cortical areas where the 70 neurons project into three groups: the PSv, other PFC (the Psd, PfD, PfM, and OF), and motor areas (the M, PM, and SMA) (Table 1). The 70 neurons were then examined with respect to the electrophysiological properties of their antidromic responses. To cortical stimulation, antidromic responses of the 70 neurons had a mean latency of 3.4 ± 1.7 ms (range: 0.7 to 10.1 ms, n = 73; Fig. 7A). Latencies to

TABLE 1. Nigral sourcing portions, thalamic recording sites, and cortical projecting areas of 70 nigrothalamocortical neurons

<table>
<thead>
<tr>
<th>Nigral Source</th>
<th>Thalamic Nuclei</th>
<th>Cortical Projection Areas</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MDmf/pc</td>
<td>Total</td>
</tr>
<tr>
<td>Rostral SNr</td>
<td>1*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>VAmc</td>
<td></td>
</tr>
<tr>
<td></td>
<td>VApc</td>
<td></td>
</tr>
<tr>
<td>Caudal SNr</td>
<td>24 (from caudolateral SNr)</td>
<td>6 (from caudolateral SNr)</td>
</tr>
<tr>
<td></td>
<td>Vm</td>
<td>2 (from caudolateral SNr)</td>
</tr>
<tr>
<td></td>
<td>Vzm</td>
<td>6 (from caudolateral SNr)</td>
</tr>
<tr>
<td></td>
<td>PSm</td>
<td>16 (from caudolateral SNr)</td>
</tr>
<tr>
<td></td>
<td>PsV</td>
<td>11 (from caudolateral SNr)</td>
</tr>
<tr>
<td></td>
<td>Other PFC</td>
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</tr>
<tr>
<td></td>
<td>total</td>
<td>40</td>
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<tr>
<td></td>
<td>Motor Areas</td>
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<tr>
<td></td>
<td>Multiple Areas</td>
<td></td>
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<tr>
<td></td>
<td>total</td>
<td>30</td>
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*R, **: Three neurons projecting to PSvc, PFd, and SMA, respectively, responded orthodromically to stimulation by both rostral and caudal SNr electrodes.
stimulation of the PSv (mean ± SD = 2.7 ± 1.6 ms, n = 40) and motor areas (3.3 ± 0.9 ms, n = 15) were significantly shorter than those to stimulation of other PFC (4.7 ± 1.9 ms, n = 18; Tukey–Kramer’s post hoc test, P < 0.05; Fig. 7B). The thresholds of the pulse current inducing the antidromic responses in the 70 thalamocortical neurons averaged 0.24 ± 0.12 mA (range: 0.03 to 0.53 mA).

INHIBITORY NIGRAL INPUT. The 70 thalamic relay neurons exhibited 73 suppressive responses to stimulation of one or two SNr electrodes. The majority (n = 67) of the 70 neurons were each inhibited by only one stimulating electrode in the rostral (n = 28; Fig. 2, AP +14 to +16) or caudal (n = 39; Fig. 2, AP +11 to +13) portions of the SNr, while 3 others were each inhibited by two electrodes in the rostral and caudal portions. The 73 inhibitory responses were usually followed by excitatory ones (e.g., Fig. 6, right column). Latencies of the inhibitory responses averaged 2.1 ± 0.9 ms (n = 73; range: 0.9 to 4.1 ms), 84% of which were < 3.0 ms; those of MD neurons and of VA–VLm neurons had mean latencies of 2.3 ± 0.8 ms (n = 32) and 1.8 ± 0.9 ms (n = 41), respectively (Fig. 8, A-I and A-2). The inhibitory responses had a mean duration of 6.0 ± 2.1 ms. All nine stimulating electrodes in the SNr (circles in Fig. 2) elicited inhibitory responses in the thalamic relay neurons to varying degrees. Of four electrodes in the caudal SNr, two in the caudolateral SNr (Fig. 2, circles with arrowheads in AP +13) had more effect (39 of 42 inhibitory responses induced by four electrodes in the caudal SNr) on eliciting the orthodromic inhibition than two others in the caudomedial SNr (Fig. 2, circles in AP +12 to +13). Two electrodes in the CP and in the ML (asterisks in Fig. 2) elicited no inhibitory response with short latency (< 5.0 ms) in the 246 thalamocortical neurons. Taken together with the location of the effective stimulating electrode tips, the intranigral source of the nigro-thalamo-cortical projections could be localized in the rostral and caudolateral portions of the SNr.

LOCATION OF THALAMOCORTICAL NEURONS WITH SNR INPUT. The 70 thalamocortical neurons with SNr input were located in the rostralateral MD, medial VA, and VLm, spanning between AP +12 and +17 (Fig. 4). The distribution of the 70 thalamic neurons showed some topographical features, with regard to both nigral...
sourcing portions (the rostral and caudal SNr) and cortical projection areas (the PSv, other PFC, and motor areas) (Fig. 5).

**MD neurons.** The vast majority (29/31) of neurons in the rostromedial MD, inhibited by caudal SNr stimulation, projected exclusively to the PSv (filled and half-filled circles in Figs. 4 and 5). In contrast, no thalamocortical neurons with nigral input were found in the medial and caudal MD.

**VA and VLM neurons.** In contrast to the MD relay neurons, neurons in the VAmc, VAp, and VLM projected to widespread areas of the FRC. Most (25/29) neurons in the VAmc were inhibited by stimulation of the rostral SNr (open and half-open symbols in Figs. 4 and 5A), whereas a small number (n = 5) of them were inhibited by stimulation of the caudal SNr (filled and half-filled symbols in Figs. 4 and 5A). Neurons (n = 9) in the medial VAp were inhibited by stimulation of the rostral SNr (n = 4) and/or caudal SNr (n = 6) (Figs. 4 and 5A). Neurons in the vicinity of the VAmc–VAp border mostly projected to motor areas (triangles in Fig. 4), while neurons more medially distributed in the VAmc projected to the PSv (circles in Fig. 4) and other PFC (squares in Fig. 4). One neuron in the VLM, inhibited by stimulation of the caudal SNr, projected to the face part of the M (filled triangle in Fig. 4).

**Nigrothalamic neurons**

Recording sites of nigral neurons were distributed throughout the whole medial–lateral extent of the SNr, spanning from AP +12 to +16 mm in front of the interaural line (Fig. 9A). We identified 51 nigrothalamic neurons projecting to the portions of the MD, VA, and VLM where the 70 thalamocortical neurons with SNr input were recorded. They were antidromically activated by stimulation of five electrodes in the rostral (VA–VLM) and caudal (rostromedial MD, and its laterally neighboring portions) thalamic regions in one of the two monkeys (see Fig. 9B for response examples); 30 responded to stimulation of the rostromedial thalamic portions, 15 to that of the caudal thalamic portions and 6 to that of both the rostral and caudal thalamic portions (Figs. 3 and 9A, left). The laterally neighboring portions on the rostromedial MD included the CL and the medial portions of the X and VL pars caudalis (VLc).

**ANTIDROMIC RESPONSES.** Latencies of a total of 57 antidromic responses evoked in the 51 SNr neurons ranged from 0.9 to 3.5 ms (mean ± SD = 1.6 ± 0.5 ms); those to MD—neighboring nuclear regions and to VA–VLM stimulation averaged 1.6 ± 0.5 ms (n = 21) and 1.5 ± 0.4 ms (n = 36), respectively (Fig. 8, B-1 and B-2). The mean current threshold for eliciting the antidromic responses was 0.19 mA (SD = 0.10 mA; range: 0.01 to 0.48 mA). Because one of the major goals in this study was to examine whether the orthodromic responses of thalamic neurons were monosynaptically produced by nigrothalamic fibers, we compared antidromic activation latencies of SNr neurons with orthodromic inhibition latencies of thalamic neurons. Taking together the synaptic delay, which was nearly 0.5 ms in the CNS (Eccles 1964; Ito and Yoshida 1966), we statistically compared antidromic latencies from the MD—neighboring nuclei to the SNr with orthodromic ones from the SNr to the MD and also antidromic latencies from the VA–VLM to the SNr with orthodromic ones from the SNr to the VA–VLM (Fig. 8). When 0.5 ms was added to the antidromic latencies, neither was significantly different (t-test, P > 0.20 and >0.17, respectively).

**TOPOGRAPHY.** Twenty-one nigral neurons projecting to the thalamic region in and adjacent to the rostromedial MD were located densely in the caudal lateral SNr, while 36 nigral neurons projecting to the VA–VLM complex were positioned throughout the whole rostrocaudal extent along the rostromedial–caudolateral axis of the SNr (Fig. 9A). The connectional findings of the 51 nigrothalamic neurons, along with those of the 70 thalamocortical neurons with nigral input, are schematized in Fig. 10.

**DISCUSSION**

The present study investigated the nigro-thalamo-cortical pathways from the SNr to the FRC via the MD, VA, and VLM in the macaque monkey. Thalamocortical neurons receiving inhibitory SNr input with short latencies (<5.0 ms) and projecting mainly to the PFC were found in the MD, VA, and VLM; MDmf/pc neurons with inhibitory afferents from the caudal lateral SNr projecting to the PSv composed the densest nigro-thalamo-cortical projections. Also, nigrothalamic neurons projecting to the portions of the MD, VA, and VLM, where the thalamocortical neurons with nigral input were clustered were topographically organized in the SNr.

**Inhibitory SNr input**

The present study is the first to reveal that SNr signals have an inhibitory effect on spike discharge of primate thalamic neurons, which is in accord with previous data in nonprimates (Chevalier and Deniau 1982; Deniau et al. 1978; Miyamoto and Jinnai 1994; Ueki 1983). The nigral inhibitory input to primate thalamocortical neurons was suggested by histological data that nigral presynaptic boutons form symmetric contacts with thalamocortical neurons in the primate VAmc (Kultas-Illinsky and Illinsky 1990).

**ORIGIN OF INHIBITORY INPUT.** Nine stimulating electrodes in the SNr elicited orthodromic inhibitory responses with short latencies (<5.0 ms) in 70 thalamocortical neurons, but two electrodes in the CP and in the ML yielded no inhibitory responses with short latencies in 246 thalamocortical neurons (Fig. 2), indicating that
all inhibitory responses in the 70 neurons were highly likely to
derive from the SNr but not from other structures (such as the CP
and ML) surrounding the SNr. Stimulation of the caudolateral
SNr was effective in eliciting inhibitory responses in VA–VLm
and MD neurons, while that of the rostral SNr was so only in
VA–VLm neurons. Because nearly all (96%, \( n = 67 \)) of the 70
thalamic neurons were each inhibited by only one of the nigral
stimulating electrodes, the spread of the current delivered from the
concentric electrodes appears to be confined to the vicinity of their
tips. Accordingly, VA–VLm neurons may receive nigrothalamic
fibers from the rostral and caudolateral SNr, whereas MD neurons
may receive those only from the caudolateral SNr. However,
there still remained the possibility that the inhibition was
caused by activation of fibers passing through the SNr. To
exclude this possibility and to clarify the finer topography of
the nigrothalamic projections, we recorded SNr neurons
responding antidromically to thalamic stimulation. We ob-
tained the topographic findings corresponding to those from
the thalamic recordings (Fig. 9A).

TERMINAL REGIONS OF NIGROTHALAMIC FIBERS. Caudal thalamic
stimulating electrodes, which were supposed to be implanted in
the rostrolateral MD, were indeed positioned not only in the
rostrolateral MD but also in its laterally neighboring portions (i.e.,
CL, medial X, and medial VLc). Fifty-one nigrothalamic axons
were estimated to be directly activated within 0.9 mm from the
effective electrode tips when the mean threshold of 0.19 mA (see
RESULTS) was applied to the equation

\[
\text{Distance} = \left(\frac{\text{Current}}{K}\right)^{0.5}
\]

\( (K \approx 0.227 \text{ mA/mm}^2 \) for 0.3-ms duration cathodal pulses in
low-threshold axons) (Tehovnik 1996), suggesting that nigrotha-
lamic axons were antidromically activated in the VA–VLm and in
the rostrolateral MD and its laterally neighboring portions. Ac-
cording to prior histological studies (Carpenter et al. 1976; Fran-
cois et al. 2002; Ilinsky et al. 1985), nigrothalamic fibers project
to the MD, VA, VLm, and parafascicular nucleus. In addition,
nigrothalamic fibers destined for the MD mostly cross the in-
tralaminar nuclei (CL and centromedian nucleus), enter the ven-
trolateral MD, and then terminate massively in the MDmr/pc but
sparsely in the MD pars magnocellularis (MDmc), although no

FIG. 8. Onset latencies of orthodromic in-
hibition and antidromic activation conveyed
by nigrothalamic fibers. A: orthodromic la-
tencies. Latency distributions of orthodromic MD responses to SNr stimulation ranged
from 0.9 to 4.1 ms (\( n = 32 \); A-1), and those
of orthodromic VA–VLm responses to SNr
stimulation ranged from 0.9 to 4.1 ms (\( n = 41 \); A-2). B: antidromic latencies. Latency
distributions of antidromic SNr responses to
MD stimulation ranged between 1.1 and 3.5
ms (\( n = 21 \); B-1), and those of antidromic
SNr responses to VA–VLm stimulation
ranged between 0.9 and 2.4 ms (\( n = 36 \); B-2).
nigrothalamic fibers project to thalamic nuclei (such as the CL, X, and VLc) lateral to the MD (Francois et al. 2002; Ilinsky et al. 1985). Therefore the nigrothalamic neurons antidromically activated by rostral thalamic electrodes may project to the VA–VLM complex, whereas those antidromically activated by caudal thalamic electrodes may project to the rostro-lateral MD but not to its laterally neighboring portions (the CL, medial X, and medial VLc).

**POSSIBILITY OF INDIRECT SNR INPUT.** Orthodromic SNR inhibition in the thalamic relay neurons is most likely to be monosynaptically produced. SNR neurons, however, also project to
the superior colliculus (SC) (Beckstead 1983; Hikosaka and Wurtz 1983; Parent et al. 1983) and SC neurons project to the MD and VA (Harting et al. 1980; Russchen et al. 1987; Sommer and Wurtz 2004). Thus there remains the possibility that, if SC neurons with SNr input project to the MD and VA, SNr stimulation could suppress spontaneous firing of the SC neurons and thus disynaptically decrease spontaneous discharge of thalamic neurons via the SC. In practice, however, SC neurons with SNr input show low spontaneous firing rates because they are tonically inhibited by SNr neurons firing at very high frequencies (Hikosaka et al. 2000), whereupon SNr stimulation cannot significantly decrease the spike discharge of SC neurons and thus has little disfacilitatory effect on thalamic neurons. The thalamic reticular nucleus (R) and thalamic local circuit (LC) neurons are also not involved in the orthodromic nigral inhibitory effect. Because the R does not receive SNr input, the nigral inhibition could not be induced by SNr-R-MD/VA pathways. Nigral presynaptic boutons form symmetric contacts with thalamocortical and LC neurons (Kultas-Ilinsky and Ilinsky 1990) and LC neurons showed positive glutamic acid decarboxylase immunoreactivity (i.e., they seem to have inhibitory axon terminals) (Ilinsky and Kultas-Ilinsky 1990). Thus even if the LC neurons on which nigrothalamic fibers make synapses terminate on thalamocortical and LC neurons, nigral input to thalamocortical neurons would not result in inhibitory effect on the thalamocortical neurons. Accordingly, we consider that all SNr input to the thalamic neurons is carried through nigrothalamic fibers.

Cortical terminal zone

Because the current threshold for eliciting the antidromic responses had a mean of 0.24 mA (see results), we estimated that axons with low and high thresholds were directly activated only within 1.0 and 0.3 mm, respectively, of the effective electrode tips when the mean current value (0.24 mA) was applied to the equation Distance = (Current/K)^0.5 (K = 0.227 and 2.883 mA/mm^2 for 0.3-ms duration cathodal pulses in axons with low and high thresholds, respectively) (Tehovnik 1996). Accordingly, it appears that the current spread was mostly confined to the vicinity (<1.0 mm for the low-threshold axons) of the effective electrode tips. Cortical stimulation could therefore not activate axons outside of the cortical subdivisions where the stimulating electrodes were implanted.

Topography of nigro-thalamo-cortical projections

We compared the present results to previous data obtained by Ilinsky et al. (1985), the only previous anatomical study of the primate nigro-thalamo-cortical system. We discuss two points in respect to both findings. First, the great majority (30/31; Fig. 5B) of MDmf/pc neurons with SNr input projected exclusively to the PSv in our study, while those projected to widespread regions of the dorsal and lateral FRC in the previous study. In the present experiments tracer injections into different parts of the dorsal and lateral FRC retrogradely labeled thalamocortical neurons in the regions of the MDmf/pc where nigrothalamic terminals were anterogradely labeled. The difference between both findings might be caused by their anterograde and retrograde labeling experiments performed in different subjects. Meanwhile, we identified single thalamic neurons with both nigral afferents and cortical efferents in identical subjects. We thus consider that SNr efferent fibers have sparse projections to the lateral and dorsal FRC other than the PSv via the MDmf/pc. Second, in the present study, the MDmf/pc received input from the caudal SNr, whereas the VAmc, VAp, and VLm received afferents from the longitudinal band along the rostromedial–caudalateral axis of the SNr (Fig. 9A). On the other hand, according to Ilinsky et al. (1985), the medial VAmc received afferents from the medial SNr, while the caudalateral VAmc and the lateral MD (the MDmf, MDpc, and MD pars densocellularis) received afferents from the lateral SNr. However, taking together the anatomical configuration that the SNr expands laterally in its caudal portion, tracer injections into the lateral and medial SNr in the previous study appear to have been located mainly in the caudalateral and rostromedial SNr, respectively. Consequently there is no substantial difference between both results in this respect.

Lynch et al. (1994) reported that distinct thalamocortical projections with different subcortical afferents (nigrothalamic pathways to the VAmc, tectothalamic afferents to the MDmf, and cerebellothalamic afferents to area X) converge on the FEF (corresponding to areas 8a and 45) of the cebus monkey, by observing retrograde transneuronal transport of herpes virus injected into the FEF. Regarding the centripetal pathways via the VAmc, the previous findings that efferents from the caudalateral SNr project to the FEF via the VAmc are partly congruent with our data; a few (n = 3) VAmc neurons receiving input from the caudalateral SNr and projecting to the PSvc, the ventral part of the FEF, were found in the present study (Table 1). With respect to the centripetal pathways via the MD, in addition to the tecto-MDmf-FEF pathways revealed by the previous study, we observed that nigral signals (n = 24) travel to the ventral part of the FEF (i.e., PSvc) via the MDmf/pc (Table 1).

Middleton and Strick (2002) examined BG projections to the dorsal and lateral PFC [medial and lateral area 9 (9m and 9l, respectively), dorsal and ventral area 46 (46d and 46v, respectively), and lateral area 12 (12l)] in the cebus monkey, using herpes virus. They found that nigral output targets the dorsal
and lateral PFC in various degrees in topographical organization. Specifically, efferents from the caudal lateral SNr were preferentially directed to 46v and 12i, whereas those from the rostral SNr were directed to areas 9m and 9l. The anatomical findings are generally congruent with SNr-recipient territories of the dorsal and lateral PFC via the MDmf/pc and VAmc in the present results.

**BG-THALAMO-CORTICAL PROJECTIONS.** The internal segment of the globus pallidus (GPi), which is the other major output nucleus of the BG, as well as the SNr, have an inhibitory effect on primate thalamic neurons (Nambu et al. 1988; Yamamoto et al. 1983). As shown in the present study, however, nigral efferents projected primarily to the PFC (particularly to the PSv) via the rostral lateral MD and medial VA, whereas pallidal efferents projected mostly to motor areas via the ventral lateral nucleus pars oralis (VLo) and VAp (Jinnai et al. 1993; Nambu et al. 1988; Sakai et al. 1999, 2000; Tokuno et al. 1992); thalamic relay and cortical terminal subdivisions of the nigrothalamo-cortical projections were positioned medial and rostral to those of the pallido-thalamo-cortical projections, respectively. These findings indicate that SNr-recipient subdivisions of the thalamus and cerebral cortex are mostly segregated from GPi-recipient ones, although both pathways slightly overlapped with each other.

**CORTICO-BG-THALAMIC LOOPS.** Among the 51 nigrothalamic neurons reported in the present study, some neurons in the caudal lateral SNr were identified to receive inhibitory input from the PSv via the caudate nucleus (Cd) and to send inhibitory output to the rostral lateral MD (Kitano et al. 1998). Accordingly, the nigro-thalamo-prefrontal projections that we identified in the present study may form the feedback limb of the prefronto-caudato-nigro-thalamic loop circuitry; signals emanating from the PSVs, via inhibitory caudatonigral and nigrothalamic pathways, have a disinhibitory effect on thalamic neurons in the rostral lateral MD, wherefrom they may eventually return to the same cortical area as positive feedback signals. The present study, together with our previous report (Kitano et al. 1998), is the first to substantiate the corticothalamo-nigral-thalamic loops arising from the PSv and return ing to the same cortical area via the Cd, caudal lateral SNr, and then rostral lateral MD at the single-neuron level using identical subjects. This neural network seems to be one of multiple parallel organizations of the cortico-BG-thalamic loop circuitry hypothesized by Alexander et al. (1986).

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**REFERENCES**


