Neural Organization of the Pathways From the Superior Colliculus to Trochlear Motoneurons

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Izawa Y, Sugiuchi Y, Shinoda Y. Neural organization of the pathways from the superior colliculus to trochlear motoneurons. J Neurophysiol 97: 3696–3712, 2007; doi:10.1152/jn.01073.2006. The neural organization of the pathways from the superior colliculus (SC) to trochlear motoneurons was analyzed in anesthetized cats using intracellular recording and transneuronal labeling techniques. Stimulation of the ipsilateral or contralateral SC evoked excitation and inhibition in trochlear motoneurons with latencies of 1.1–2.3 ms. Stimulation of the contralateral SC facilitated contralateral INC-evoked monosynaptic inhibition. These results revealed a reciprocal input pattern from the SC to trochlear motoneurons on both sides. The FFH and INC contain MLBNs that disinhibit trochlear motoneurons with latencies of 1.1–2.3 ms, respectively, suggesting that the earliest components of excitation and inhibition were disynaptic. A midline section between the two SCs revealed that ipsi- and contralateral SC stimulation evoked disynaptic excitation and inhibition in trochlear motoneurons, respectively. Premotor neurons labeled transneuronally after application of wheat germ agglutinin-conjugated horseradish peroxidase into the trochlear nerve were mainly distributed ipsilaterally in the Forel’s field H (FFH) and bilaterally in the interstitial nucleus of Cajal (INC). Consequently, we investigated these two likely intermediaries between the SC and trochlear nucleus electrophysiologically. Stimulation of the FFH evoked ipsilateral mono- and disynaptic excitation and contralateral disynaptic inhibition in trochlear motoneurons. Preconditioning stimulation of the ipsilateral SC facilitated FFH-evoked monosynaptic excitation. Stimulation of the INC evoked ipsilateral monosynaptic excitation and inhibition, and contralateral monosynaptic inhibition in trochlear motoneurons. Preconditioning stimulation of the contralateral SC facilitated contralateral INC-evoked monosynaptic inhibition. These results revealed a reciprocal input pattern from the SCs to vertical oculomotor motoneurons in the saccadic system; trochlear motoneurons received disynaptic excitation from the ipsilateral SC via ipsilateral FFH neurons and disynaptic inhibition from the contralateral SC via contralateral INC neurons. These inhibitory INC neurons were considered to be a counterpart of inhibitory burst neurons in the horizontal saccadic system.

INTRODUCTION

The superior colliculus (SC) plays a prominent role in controlling eye and head movements. The neural organization of the pathways from the SC to motoneurons in the horizontal oculomotor system has been extensively analyzed (see reviews in Grantyn and Moschovakis 2004; Scudder et al. 2002; Sparks 1999). Electrical stimulation of the SC evokes saccades with a contraversive horizontal component (Guitton et al. 1980; Robinson 1972), and neurons in the deeper layers of the SC show a burst of spikes before contraversive saccades (Schiller and Körner 1971; Wurtz and Goldberg 1972). Medium-lead burst neurons (MLBNs) in the paramedian reticular formation that burst before and during horizontal saccades include both excitatory neurons (Cohen and Henn 1972; Keller 1974; Luschei and Fuchs 1972) and inhibitory neurons (Hikosaka and Kawakami 1977; Yoshida et al. 1982). MLBNs in the paramedian reticular formation rostral and caudal to the abducens nucleus are activated monosynaptically from the contralateral SC in alert cats (Chimoto et al. 1996). The shortest excitatory pathway from the contralateral SC to abducens motoneurons is a disynaptic circuit via the ipsilateral paramedian pontine reticular formation (PPRF), whereas the shortest inhibitory pathway from the ipsilateral SC to abducens motoneurons is a disynaptic circuit via the contralateral paramedian pontomedullary reticular formation (Izawa et al. 1999). In contrast, the connections between the SC and motoneurons in the vertical oculomotor system have not yet been analyzed in detail. The trochlear nucleus, which supplies the contralateral superior oblique muscle, is separate from the oculomotor nucleus (Warrick 1953) and provides a convenient model for investigation of the vertical gaze system, as its major pulling direction is downward with lesser lateral and intortion components (Fuchs and Luschei 1971; Tokumasu et al. 1965). The effects of stimulation of the SC on trochlear motoneurons were first examined by Precht et al. (1974). However, consistent postsynaptic potentials (PSPs) were not detected in trochlear motoneurons in this study, even after strong and multiple shock stimulation of the SC, in contrast to the presence of clear SC-evoked PSPs in abducens motoneurons in the same preparations. Since this report, to our knowledge, inputs from the SC to trochlear motoneurons have not been investigated.

The best-studied premotor neurons in the vertical oculomotor system are found in the Forel’s field H (FFH) (Graybiel 1977), the homologue of the rostral interstitial nucleus of the medial longitudinal fasciculus (riMLF) in the monkey (Büttner-Ennever and Büttner 1978) and in the interstitial nucleus of Cajal (INC) and adjacent mesencephalic reticular formations (MF). Schwinding et al. (1974) examined the effects of stimulation of the latter area on trochlear motoneurons and found that stimulation of the INC evoked a monosynaptic excitatory and inhibitory postsynaptic potential (EPSP-IPSP) sequence in trochlear motoneurons on both sides. Later, Nakao and Shiraiishi (1985) reported direct synaptic connections between the FFH and trochlear motoneurons; FFH stimulation evoked monosynaptic EPSP-IPSPs or EPSPs in trochlear motoneurons on both sides. The FFH and INC contain MLBNs that discharge most vigorously for saccades with vertical components and have either upward or downward on-directions (Büttner et al. 1977; King and Fuchs 1979; Moschovakis et al. 1991a,b; Shimizu and Takahashi 1993). The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
Nakao et al. 1988). In addition to MLBNs, neurons that exhibit a burst- tonic discharge pattern are also found in the INC and appear to provide position signals for vertical and torsional gaze (Chimoto et al. 1999; Fukushima et al. 1990; King et al. 1981). Furthermore, the FFH and INC contain neurons that project to the spinal cord that may be related to gaze shifts (Fukushima et al. 1981; Isa and Sasaki 2002). In addition to the discharge patterns of neurons in the FFH and INC, anatomical experiments in the cat suggested that the FFH/MLF contains overlapping populations of neurons that are immunoreactive to putative inhibitory and excitatory neurotransmitters (Spencer and Wang 1996). In spite of the wealth of knowledge that has been obtained about premotor neurons in the vertical saccadic system, the input-output organization of the FFH and INC in the pathways from the SC to vertical ocular motoneurons is not fully understood.

We performed the present study to determine the neural organization of circuits connecting the SC to trochlear motoneurons by using electrophysiological and morphological techniques in anesthetized cats. In trochlear motoneurons, intracellular responses were examined following electrical stimulation of the SC, FFH, and INC. To locate last-order premotor neurons terminating on trochlear motoneurons, we labeled them transneuronally with wheat germ agglutinin-conjugated horseradish peroxidase (WGA-HRP). The results showed that a reciprocal input pattern from the SCs to trochlear motoneurons existed in the vertical saccadic system; the shortest excitatory pathway from the ipsilateral FFH, whereas the shortest inhibitory pathway from the contralateral INC to trochlear motoneurons was a disynaptic one via the ipsilateral FFH, whereas the shortest inhibitory pathway from the contralateral SC to trochlear motoneurons was a disynaptic one via the contralateral INC. These results were previously reported briefly (Izawa et al. 1997).

**Methods**

Experiments were performed in 19 cats weighing 2.4–4.4 kg. The surgery and animal care conformed to the “Guide for the Care and Use of Laboratory Animals” (Institute of Laboratory Animal Resources, National Research Council 1996), and also to the “Guiding Principles for the Care and Use of Animals in the Field of Physiological Sciences” (The Physiological Society of Japan, revised in 2001). All surgical and experimental protocols were approved by the Committee for Animal Experimentation of Tokyo Medical and Dental University. The animals were initially anesthetized with an intramuscular injection of ketamine hydrochloride (Ketalar, Parke-Davis; 25 mg/kg) followed by an intravenous injection of α-chloralose (40–45 mg/kg initial dose, supplemented with an additional dose of 10–25 mg/kg as required). The body temperature was kept between 37.5 and 39.0°C by a heating pad. Heart rate was monitored constantly by electrocardiogram. The trochlear nerve was detached from the superior oblique muscle, and its peripheral end was mounted on a bipolar hook electrode for electrical stimulation or to record nerve discharge. One silver ball electrode was placed on the oval window and the other on the round window for stimulation of the primary vestibular nerves on each side, and single or double shock stimuli of 0.2 ms in duration were delivered between them at a maximum intensity of 500 μA (Shinoda and Yoshida 1974; Shinoda et al. 1986a, 1992). The bone over the parietal and occipital cortex was removed and the cerebral cortex overlying the SC was aspirated unilaterally to introduce stimulating or recording electrodes into the SC. Three pairs of monopolar stimulating electrodes (epoxy-coated acupuncture needle electrodes; diameter, 0.3 mm) or four concentric bipolar stimulating electrodes (0.1 mm ID; 0.3 mm OD; interelectrode distance, 0.5 mm) were positioned in the intermediate or deep layer (1.5–2.0 mm deep from the surface) of the SC on either side. Stimulation sites in the SC were determined by monitoring nerve discharge of the trochlear nerve that was elicited by stimulating the deeper layers of the SC. Two to 10 negative pulses of 0.2 ms in duration were applied at an interval of 1.5 ms to evoke nerve discharge in trochlear nerves at a maximum of 100 μA by use of a constant current generator. Similar concentric stimulating electrodes were placed bilaterally at A7.0–7.5 in the FFH and also bilaterally at A5.0–5.5 in the INC stereotaxically. The final depths of the electrode tips were determined by monitoring negative field potentials or unit spikes evoked by stimulation of the ipsilateral SC. The vermis overlying the fourth ventricle was aspirated for intracellular recording from trochlear motoneurons. In three experiments, muscimol, which was used as a solution in saline with a concentration of 1 μg/μl, was injected with a Hamilton syringe into the FFH contralateral to the recorded trochlear nerve. Muscimol was injected in steps of 1 μl at 10-min intervals to give a total volume of 2.0–4.0 μl, and its effect on SC-evoked trochlear nerve discharge was examined. To examine axonal projections of single tectal output neurons, a concentric stimulating electrode of the same type as that used to stimulate the SC was placed in the FFH on each side, another electrode was placed in the contralateral PPRF, and two other electrodes were placed in the descending medial longitudinal fasciculus (MLF) at the level of the obex (Verhaart 1964) on the side contralateral to the SC in two animals. During recording, the animals were paralyzed by the intravenous administration of pancuronium bromide (Mioblock, Organon, The Netherlands) and artificially ventilated with the end-tidal CO2 held at −37 mmHg.

Glass microelectrodes for intracellular recording were filled with 3 or 0.4 M KCl and had a resistance of 8–15 MΩ. A tungsten electrode insulated in a glass microelectrode was used to record extracellular spikes in the SC (Shinoda et al. 1976, 1986b). Negative pulses of 0.2-ms duration were delivered at 100–500 μA to stimulate the SC, FFH, and INC, at <200 μA (usually <100 μA) to stimulate the PPRF, and at a maximum of 500 μA to stimulate the MLF. At the end of each experiment, the positions of the stimulating electrodes were marked by passing negative currents of 20 μA for 20 s. The animals were then deeply anesthetized with pentobarbital sodium (45 mg/kg, Nembutal, Abbott, Switzerland) and perfused with 2 l of saline followed by 2.1 l of 10% formalin solution. Stimulated sites in the SC, FFH, INC, PPRF, and MLF were histologically confirmed in serial transverse sections stained with thionin. In two experiments in which the PPRF was stimulated, we confirmed that the stimulating electrode was located in the PPRF where last-order interneurons were labeled retrogradely by injecting HRP into the ipsilateral abducens nucleus. This procedure has been described by Izawa et al. (1999).

To determine the location of last-order premotor neurons terminating on trochlear motoneurons, 2% WGA-HRP (Toyobo) in 0.05 M Tris-HCl buffer (pH 8.6) was injected into the trochlear nerve for transneuronal labeling in three animals. For the first two animals, after 4–6 days, the brain was removed and serial transverse sections of 50 or 75 μm were reacted to reveal the presence of HRP by use of the tetramethyl benzidine method (Mesulam 1978) following previously described procedures (Sugiuchi et al. 1995). For the last animal, 12.5% dextran-biotin (Molecular Probes) was also injected into the SC contralateral to the WGA-HRP-injected trochlear nerve to confirm that axon terminals of tectofugal neurons terminate on last-order premotor neurons, which, in turn, terminate on trochlear motoneurons. After 4–6 days, the brain was removed and serial transverse sections of 75 μm thickness were treated for WGA-HRP and biotin according to the double-labeling procedure described previously (Izawa et al. 1999).

**Results**

To determine the neural pathways from the SC to trochlear motoneurons, intracellular potentials were recorded from 151 trochlear motoneurons in the cat, and the effects of electrical...
stimulation of the SC, FFH and INC on these neurons were examined. All lateralities in this study are described with reference to the recording side.

Effects of stimulation of the SC on trochlear motoneurons

Trochlear motoneurons were identified by their antidromic responses to stimulation of the trochlear nerve in the contralateral orbit (Fig. 1B). Of the 151 trochlear motoneurons examined, SC-evoked PSPs were identified in 89 trochlear motoneurons, and therefore these motoneurons were used for later analysis. Their resting membrane potentials ranged from −40 to −76 mV (−56 ± 15 (SD) mV). In every motoneuron examined, we always examined vestibular inputs. This procedure helped differentiate EPSPs from reversed IPSPs due to spontaneous diffusion of Cl⁻ into a penetrated cell because we used KCl-filled micropipettes. Stimulation of the ipsilateral vestibular nerve evoked hyperpolarization (Fig. 1Ca), and stimulation of the contralateral vestibular nerve evoked depolarization in every trochlear motoneuron (Fig. 1Da) (Baker and Berthoz 1974; Highstein 1973; Precht and Baker 1972). In the same motoneuron, single shock stimuli applied to the SC on either side usually did not evoke any response, and double or triple shock stimuli were required to evoke PSPs. Stimulation of the ipsilateral SC evoked a depolarization (Fig. 1Ea), which was followed by a hyperpolarization in some cases. Stimulation of the contralateral SC evoked a hyperpolarization (Fig. 1Fa) that was followed or preceded by a depolarization in some motoneurons. Latencies and amplitudes of these PSPs fluctuated, suggesting that these responses were induced polysynthetically. To determine the nature of these depolarizing and hyperpolarizing PSPs, we injected Cl⁻ into the cell or passed hyperpolarizing or depolarizing currents through a recording microelectrode. To check the effects of the intracellular injection of Cl⁻ or the passage of current through a motoneuron membrane, PSPs evoked by stimulation of the contra- and ipsilateral primary vestibular nerves were compared before and after the injection of Cl⁻ or before and during the passage of current. Injection of Cl⁻ into the cell did not affect the polarity of the depolarizations evoked by stimulation of the contralateral vestibular nerve (Fig. 1Db), whereas the hyperpolarizations evoked by stimulation of the ipsilateral vestibular nerve were reversed in a depolarizing direction (Fig. 1Cb). Therefore the depolarizations evoked by contralateral vestibular stimulation were considered to be EPSPs, and the hyperpolarizations evoked by ipsilateral vestibular stimulation were considered to be IPSPs (Baker and Berthoz 1974; Coombs et al. 1955; Eccles 1964). Under the same conditions as those with which the vestibular-evoked disynaptic IPSPs were reversed after Cl⁻ injection, the polarity of the depolarizations evoked by stimulation of the ipsilateral SC was not affected (Fig. 1Eb), whereas the hyperpolarizations evoked by stimulation of the contralateral SC were reversed to depolarizing potentials (Fig. 1Fb). Therefore the depolarizations evoked by ipsilateral SC stimulation were considered to be EPSPs, and the hyperpolarizations evoked by contralateral SC stimulation were considered to be IPSPs (Coombs et al. 1955; Eccles 1964). Because these PSPs were usually evoked by the second or third stimulus, their latencies were measured from the first effective stimulus, which was most often the second stimulus. One additional SC stimulus more than the first effective stimulus usually decreased such latencies of the PSPs by 0.1–0.3 ms. The onsets of EPSPs and IPSPs were determined by superimposing the EPSPs and IPSPs on field potentials recorded just outside a cell. When the IPSPs followed EPSPs, the onsets of the IPSPs were obscure and were determined by superimposing the reversed IPSPs on the hyperpolarizing EPSPs, and the point of divergence of these two IPSPs was considered to be the onset of the IPSPs. Ipsilateral SC stimulation evoked IPSPs at latencies of 1.1–2.3 ms (1.6 ± 0.3 (SD) ms, n = 72; Fig. 2Aa), and IPSPs at latencies of 1.5–3.9 ms (2.7 ± 0.7 ms, n = 13; Fig. 2Ab). Contralateral SC stimulation evoked IPSPs at latencies of 1.1–3.8 ms (1.8 ± 0.6 ms, n = 92; Fig. 2Bb).
ms, \( n = 50; \) Fig. 2A), and EPSPs at latencies of 1.2–2.7 ms (1.9 ± 0.4 ms, \( n = 17; \) Fig. 2B).

The effects of the stimulation site in the SC on PSPs were examined by recording PSPs from a trochlear motoneuron and stimulating various parts of the SC at identical intensities (Fig. 3). Three pairs of monopolar electrodes were arranged in the medial and lateral sites of the SC on either side in six experiments; one pair in the rostral, another pair in the middle, and the last pair in the caudal site of the SC. The lateral electrodes were cathodal in the ipsilateral SC and anodal in the contralateral SC. As shown in Fig. 3, D and E, stimulation of each site in the ipsi- and contralateral SC, respectively, evoked EPSPs and IPSPs in a trochlear motoneuron. Similar input patterns from the lateral and medial SC to trochlear motoneurons were usually observed when the stimulus polarity was reversed between the lateral and medial electrodes in the same preparation. However, larger PSPs with shorter latencies and lower thresholds were evoked when the lateral electrode of the ipsilateral SC (Fig. 3F), and the medial electrode of the contralateral SC were cathodal (Fig. 3G). This finding is consistent with the motor map in which the lateral electrodes would activate parts of the SC producing gaze changes with downward components, and the medial electrodes would activate parts of the SC producing gaze changes with upward components (McIlwain 1986; Roucoux and Crommelinck 1976). In a rostrocaudal direction, evoked EPSPs became larger and their rising phase became sharper (Fig. 3D), and evoked IPSPs also became larger and their falling phase became sharper as the stimulation sites were shifted more caudally in the SC in this example (Fig. 3E). However, depending on the arrangement of stimulating electrodes in the SC, stimulation of the more rostral site evoked larger EPSPs on the ipsilateral side (9/36 trochlear motoneurons) and larger IPSPs on the contralateral side at shorter latencies (8/40 trochlear motoneurons). In eight experiments, four concentric bipolar electrodes were arranged in the rostrocaudal and mediolat-
eral directions of the SC on either side. Figure 4, A and B, shows examples of the spatial distribution of inputs from the ipsi- and contralateral SC, respectively, to a trochlear motoneuron. In the ipsilateral SC, stimulation of lateral sites evoked larger EPSPs with shorter latencies (Fig. 4A, d2 and d4), whereas stimulation of rostral or medial sites evoked smaller EPSPs (Fig. 4A, d1 and d3). In the contralateral SC, stimulation of medial sites evoked larger IPSPs (top traces in Fig. 4B, d5 and d6), whereas stimulation of lateral sites did not evoke IPSPs but rather evoked small EPSPs (top traces in Fig. 4B, d7 and d8).

Distribution of last-order premotor neurons terminating on trochlear motoneurons

Next we attempted to determine the location of interneurons that mediated excitation and inhibition from the SC to trochlear motoneurons. Last-order premotor neurons terminating on trochlear motoneurons were identified by transneuronal labeling after the injection of WGA-HRP into the trochlear nerve. Retrogradely labeled neurons were darkly stained in the trochlear nucleus contralateral to the injected trochlear nerve. Lightly stained neurons were considered to be transneuronally labeled on the assumption that the WGA-HRP they contained was present because their axon terminals contacted retrogradely labeled trochlear motoneurons. These cells were mainly distributed in the FFH on the same side as the stained trochlear motoneurons and were densely distributed in the INC on both sides (Fig. 5). These findings are consistent with the observations that have been made using transneuronal tracers in the monkey (Horn and Büttner-Ennever 1998). Transneuronally labeled neurons were also observed in the nucleus of the posterior commissure mainly ipsilateral to retrogradely labeled trochlear motoneurons and bilaterally in the vestibular nuclei. Based on these anatomical data, we analyzed the supranuclear pathways that mediate excitation and inhibition from the SC to trochlear motoneurons electrophysiologically.

Effect of muscimol injection into the FFH on SC-evoked trochlear nerve discharge

We examined the effects of blocking FFH activity on the trochlear nerve discharge recorded following SC stimulation. Lateralis in these experiments are described with reference to the side of the recording in the trochlear nerve, which is contralateral to its corresponding cell bodies. Stimulation of the ipsilateral primary vestibular afferents evoked large responses in the trochlear nerve (Fig. 6b), which confirmed the viability of trochlear nerve recordings. In contrast, stimulation of the contralateral vestibular nerve did not evoke any response (Fig. 6d) due to type I inhibition as shown in Figs. 1C and 3B. Stimulation of the SC with single or double pulses did not evoke any trochlear nerve discharge, and multiple stimulus pulses were always required to evoke visible nerve responses. Multiple stimulation of the contralateral SC evoked a large response in the trochlear nerve (Fig. 6Bb), but multiple stimulation of the ipsilateral SC evoked responses with much smaller amplitudes (Fig. 6Ba). After these responses were recorded as controls, muscimol solution (3 μl; concentration, 1 μg/μl) was injected into the FFH contralateral to the trochlear nerve that was being recorded from (Fig. 6A). Responses of the trochlear nerve evoked by stimulation of the ipsilateral (Fig. 6Ca) and contralateral SC (Fig. 6Cb) gradually decreased and disappeared ~5 min after muscimol injection. However, even after SC-evoked responses had disappeared, a large response was still evoked by vestibular stimulation, although its amplitude was slightly decreased (Fig. 6Cc). Similar results obtained in three experiments indicated that the response of the trochlear nerve evoked by stimulation of the SC on either side was
mediated via the contralateral FFH (the FFH ipsilateral to cell bodies of the corresponding trochlear nerve).

**Tectofugal neurons projecting to the FFH**

To examine the projection of tectofugal neurons to the FFH and their spike conduction times, extracellular spikes were recorded in the SC, and tectofugal neurons projecting to the FFH and/or the PPRF were sought in the SC following stimulation of the FFH and the PPRF. Figure 7 shows examples of tectofugal neurons activated from the ipsilateral FFH. These neurons were further examined to determine whether they also sent their axons to the contralateral PPRF and spinal cord. For the neuron in Fig. 7B, extracellular spikes were evoked by stimulation of the ipsilateral FFH at a fixed latency of 1.0 ms. These spikes were regarded as antidromic because they had a fixed latency even at threshold and followed double shock stimuli at 0.7-ms intervals in half of the trials (Fig. 7Bb) and at

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**FIG. 5.** Distribution of transneuronally labeled last-order premotor neurons terminating on trochlear motoneurons. Wheat germ agglutinin-conjugated horseradish peroxidase (WGA-HRP) was injected into the left trochlear nerve. Sections at the mesencephalic level are arranged from the rostral (A) to the caudal order (J). Labeled neurons observed in 3 consecutive 50-μm-thick sections are plotted in representative transverse sections at 300-μm intervals at the level of the FFH and INC (A–G), and the trochlear nucleus (H–J). Large dots, transneuronally labeled neurons; small dots, retrogradely labeled trochlear motoneurons. III Nucl., oculomotor nucleus; III n., oculomotor nerve; CP, cerebral peduncle; IC, inferior colliculus; NPC, nucleus of posterior commissure; OT, optic tract; PAG, periaqueductal gray; RF, retroflex bundle; RN, red nucleus; SN, substantia nigra; ST, subthalamic nucleus. Lt. and Rt., left and right.
0.8-ms intervals in all trials (Fig. 7Bc). This neuron could not be activated by stimulation of the contralateral FFH (Fig. 7Bd), the contralateral PPRF (Fig. 7Be) or the descending MLF at the level of the obex (Fig. 7Bf), even with a stimulus intensity of 500 μA. For the neuron in Fig. 7D, spikes were evoked by stimulation of the ipsilateral FFH at a fixed latency of 0.6 ms at a threshold intensity of 225 μA (Fig. 7Da) and followed double shock stimuli (300 μA) at an interval of 0.6 ms (Fig. 7Db). This neuron was also activated by stimulation of the contralateral PPRF at a latency of 0.7 ms (Fig. 7Dc) and the contralateral descending MLF at a latency of 0.6 ms (Fig. 7De) but was not activated by stimulation of the contralateral FFH (not illustrated). Whenever spikes were evoked from both the PPRF and the descending MLF, we examined the possibility that the activation was due to current spread from the PPRF stimulation site to a stem axon descending as far as the obex by using a spike collision test that was carried out between spikes activated from the contralateral PPRF and MLF (Shinoda et al. 1976, 1977) (Fig. 7D, g–o). Using the values obtained in this spike collision test, the conduction time was calculated along an axon collateral from a stimulation site in the PPRF to a branching point of a stem axon descending as far as the obex, using an equation reported by Shinoda et al. (1976). To be brief, assume that a stem axon of a neuron is divided into two branches projecting to sites A and B, respectively. When this neuron is antidromically activated from sites A and B, the latencies of extracellular spikes evoked from sites A (L_A) and B (L_B) may be calculated as follows

\[
L_A = X_A + X_s
\]

\[
L_B = X_B + X_s
\]

where \(X_A\) and \(X_B\) are the conduction times between the branching point of the stem axon and the stimulation sites A and B, respectively; \(X_s\) is the conduction time between the branching point of the stem axon and its cell body. Using the values obtained in a spike collision test (I_AB), the conduction times of \(X_B\) and \(X_s\) may be calculated as follows (Shinoda et al. 1976)

\[
X_B = \frac{1}{2}(I_{AB} + L_B - L_A - R_B)
\]

\[
X_s = L_A - X_A
\]

where \(I_{AB}\) is the maximal conditioning-test interval when the spikes evoked from site B are blocked by the spikes evoked from site A; \(R_B\) is the refractory period measured by double shock stimuli at site B. Using this equation, spike collision tests were performed between spikes evoked from different pairs of stimulation sites (Fig. 7D, g–i, j–l, and m–o). The calculated conduction times along individual parts of the axon are shown in Fig. 7C. For example, the conduction time along the axon collateral in the PPRF from its stem axon was 0.1 ms, indicating that an axon collateral was stimulated in the PPRF rather than its stem axon due to current spread. If a stem axon had been activated by current spread from the PPRF, this conduction time along the axon collateral should have been zero (see the details of the calculation of spike conduction time along an axon collateral and its associated problems in Shinoda et al. 1976, 1977, 1986b). In a similar way, neurons were considered to have an axon collateral to the PPRF when conduction times along their axon collaterals were ≥0.1 ms. The stimulating electrode tips were histologically confirmed to be located in the PPRF (Izawa et al. 1999). The latencies of antidromic spikes were 0.5–2.9 ms from the ipsilateral FFH (1.0 ± 0.4 ms, \(n = 107\); Fig. 7F), 0.5–2.8 ms from the contralateral FFH (1.2 ± 0.5 ms, \(n = 132\); Fig. 7G), and 0.5–2.7 ms from the MLF (1.1 ± 0.4 ms, \(n = 92\); Fig. 7H). These tectofugal neurons were recorded at depths of 1.5–3.0 mm from the SC surface. Of the 159 SC neurons that were antidromically activated from the FFH or the PPRF, 19 were antidromically activated only from the FFH, 29 were activated from both the FFH and the PPRF, and 51 were activated from the FFH, the PPRF and the MLF. Of the 114 tectofugal neurons with ascending branches, only 17 (~15%) were activated antidromically from the contralateral FFH, and 10 of these (~59%) were activated from the FFH on both sides. Based on a comparison of the antidromic latencies of tectofugal neurons projecting to the FFH (Fig. 7F) and the latencies of PSPs evoked by SC stimulation (Fig. 2, A

![FIG. 6. Effects of muscimol injection into the FFH on trochlear nerve discharge. A: experimental setup. Nerve discharge was recorded from the left trochlear nerve. Muscimol solution (3 μl; concentration, 1 μg/μl) was injected into the right FFH, which was contralateral to the recording site. B and C: discharge of the left trochlear nerve evoked by stimulation of the left (a) and right SC (b), and the left (c) and right vestibular nerve (d) before (B) and after injection of muscimol into the right FFH (C). Stimulation of the right SC evoked much larger nerve discharge of the trochlear nerve than that of the left SC in control. Note that trochlear nerve discharge evoked by stimulation of the right and left SC disappeared after muscimol injection into the right FFH, whereas large nerve responses were still evoked by stimulation of the left vestibular nerve. →, the points of divergence of the traces before and after muscimol injection. Stimulus intensities: B, a and b, and C, a and b, 100 μA; B, c and d, and C, c and d, 500 μA.](http://jn.physiology.org/Downloadedfrom)
and B), the earliest components of both the EPSPs and IPSPs evoked in trochlear motoneurons by ipsi- and contralateral SC stimulation were considered to be disynaptic.

To confirm excitatory inputs from the SC to FFH neurons, we recorded extracellular spike potentials from neurons in the FFH and examined the effects of stimulation of the SC on FFH neurons. FFH neurons were identified as such by their antidromic responses evoked by stimulation of their axons within the ipsilateral trochlear nucleus (Fig. 7Ec). Latencies of the antidromic spikes ranged from 0.3 to 1.0 ms (0.7 ± 0.2 ms, n = 13; Fig. 7J). Stimulation of the ipsilateral SC evoked short-latency spikes in FFH neurons (Fig. 7Ea), and the latency of these spikes was 0.5 ms (0.5 ± 0.2 ms, n = 13; Fig. 7J). Stimulation of the contralateral SC evoked short-latency spikes in FFH neurons (Fig. 7Ea), and the latency of these spikes was 1.0 ms (1.0 ± 0.2 ms, n = 13; Fig. 7J). Therefore inputs from the SC to the FFH are considered to be monosynaptic.
Fig. 8. Synaptic inputs from the FFH to trochlear motoneurons. A: photomicrograph of the frontal section of the midbrain showing the stimulation sites in the FFH on both sides (arrowheads). B and C: PSPs in a trochlear motoneuron evoked by stimulation of the ipsilateral (B) and contralateral FFH at 500 μA (C) before (Ba, C, a and b) and after Cl− injection into the cell (Bb and Cc). B and C: juxtacellular field potentials. D: effects of stimulus intensity on FFH-evoked PSPs in a trochlear motoneuron. Stimulus intensities: a, 100 μA; b, 200 μA; c, 300 μA; d and e, 500 μA. e, juxtacellular field potentials. E: effects of stimulus number on ipsilateral FFH-evoked PSPs in a trochlear motoneuron. Single (a) and double shock stimuli at 500 μA (b and c). e, juxtacellular field potentials. Dashed line, algebraic sum of the EPSPs evoked by single shock stimulation. Note that the dashed line is occluded by the solid lines of intracellular records, indicating the absence of temporal facilitation. F: 2 components of EPSPs evoked by stimulation of the ipsilateral FFH. Arrow indicates the 2nd component of the EPSPs. b, juxtacellular field potentials. G: spatial facilitation of ipsilateral FFH-evoked monosynaptic EPSPs by preconditioning stimulation of the ipsilateral SC. Records were obtained from the same trochlear motoneuron as in D. a: stimulation of the ipsilateral SC at almost the threshold for evoking disynaptic EPSPs (400 μA). b: small monosynaptic EPSPs evoked by test FFH stimulation at 150 μA. c: preconditioning SC stimulation facilitated FFH-evoked monosynaptic EPSPs, when the SC stimuli preceded the FFH stimuli by 2.0 ms. Dashed line, algebraic sum of the response in G, a and b; vertical dashed line indicates the onset of EPSPs. Bottom traces in individual records, juxtacellular field potentials. d: time course of the facilitation of FFH-evoked EPSPs produced by preconditioning SC stimulation. Abscissa: interval between the preconditioning stimuli of the SC and the test FFH stimuli. Ordinate: means and SDs of the difference in amplitude between the control and conditioned FFH-evoked EPSPs.

Effects of stimulation of the FFH on trochlear motoneurons

The results so far showed that trochlear motoneurons were excited through the ipsilateral FFH from the ipsilateral SC. To confirm the monosynaptic connection between the FFH and trochlear motoneurons, we recorded intracellular potentials from trochlear motoneurons while stimulating the FFH on either side. Stimulation of the ipsilateral FFH evoked depolarization in trochlear motoneurons even with a single shock stimulus (Fig. 8Aa). Intracellular injection of Cl− (Fig. 8Ab) or the passage of a hyperpolarizing current did not change the polarity of the depolarization evoked from the ipsilateral FFH, indicating that the depolarization was an EPSP (Coombs et al. 1955; Eccles 1964). However, late components of the depolarization became larger after Cl− injection (Fig. 8Bb), indicating that IPSPs were involved in the later part of the depolarization (Fig. 8Bd). Latencies of EPSPs evoked by ipsilateral FFH stimulation ranged from 0.6 to 1.7 ms (1.0 ± 0.2 ms, n = 68; Fig. 8Ca), and latencies of IPSPs evoked by ipsilateral FFH stimulation ranged from 0.9 to 2.4 ms (1.6 ± 0.4 ms, n = 12; Fig. 2Cc). When the stimulus intensities were increased from 100 to 500 μA, EPSPs increased in size and their latencies decreased by 0.2–0.3 ms (Fig. 8Da–d). Double shock stimulation of the ipsilateral FFH did not produce temporal facilitation of the early EPSPs (Fig. 8Ea and b), indicating that these EPSPs were monosynaptic. Because the latencies of trochlear nucleus-activated antidromic spikes in FFH neurons were 0.3–1.0 ms (Fig. 7J), the latencies of the early components of FFH-evoked EPSPs were considered to be monosynaptic. In some motoneurons, however, the depolarization had a second component (arrow in Fig. 8Fa) and double shock stimulation of the FFH facilitated this second component. The latencies of the second components of these EPSPs ranged from 1.3 to 2.3 ms (1.7 ± 0.3 ms, n = 8; Fig. 2Cb). Because these latencies were ~0.8 ms longer than those of the early components of the EPSPs, the second components of the EPSPs were considered to be disynaptic from the ipsilateral FFH.

The results described so far suggested that EPSPs evoked by ipsilateral SC stimulation were most likely mediated via the ipsilateral FFH. To confirm this possible disynaptic excitatory pathway from the ipsilateral SC to trochlear motoneurons, the interaction of SC-evoked disynaptic EPSPs and FFH-evoked monosynaptic EPSPs was examined in a preconditioning-test paradigm (Fig. 8G). The stimulus intensity for the ipsilateral SC was adjusted to just subthreshold for evoking EPSPs in a trochlear motoneuron (Fig. 8Ga), and the stimulus intensity for the ipsilateral FFH was reduced to evoke small early EPSPs in the same trochlear motoneuron (Fig. 8Gb). When preconditioning stimulation of the ipsilateral SC was given 2.0 ms before test stimulation of the ipsilateral FFH, the preconditioning stimulation increased the amplitudes and rising slopes of the EPSPs evoked by the test FFH stimulation in the trochlear motoneuron (Fig. 8Gc). The existence of this facilitation of the FFH-evoked monosynaptic EPSPs indicated that SC stimulation activated neurons in the FFH that terminated on the trochlear motoneurons. The time course of this facilitation indicated that facilitation started when the test FFH stimuli were given at 0.8 ms after the preconditioning SC stimuli, reached its peak at ~2.0 ms and then gradually decreased (Fig. 8Gd). Because the preconditioning stimulation of the ipsilateral SC facilitated the FFH-evoked monosynaptic EPSPs at 0.8 ms, these results confirmed that tectofugal neurons directly terminated on FFH neurons that terminated on the ipsilateral
trochlear motoneuron. Similar facilitation was observed in all of the 11 trochlear motoneurons examined.

To confirm the disynaptic excitatory pathway from the SC to trochlear motoneurons morphologically, we injected WGA-HRP into the left trochlear nerve and dextran-biotin into the right SC in the same animal. Labeled tectofugal axon terminals were observed on the cell bodies and proximal dendrites of many transneuronally labeled FFH neurons contralateral to the injected left trochlear nerve. In the section shown in Fig. 9A, 4 of 12 transneuronally labeled neurons (both and ) in the right FFH had labeled tectofugal axon terminals on them. Figure 9B shows a photomicrograph of such axon terminals of tectofugal fibers that made contact with the cell body and proximal dendrites of a transneuronally labeled premotor neuron in the FFH. Figure 9C shows a photomicrograph of such an association of dextran-biotin-labeled tectofugal axon terminals and the cell body and proximal dendrites (arrowheads) of a transneuronally labeled premotor neuron in the INC.

Effects of stimulation of the INC on trochlear motoneurons

To determine the pathway that mediates inhibition from the contralateral SC to trochlear motoneurons, synaptic inputs from the INC to trochlear motoneurons were examined by recording intracellular potentials from them while stimulating the INC on either side. Stimulation of the ipsilateral INC evoked depolarization at a latency of 0.8 ms in a trochlear motoneuron, even with a single shock stimulus (Fig. 10A). The amplitudes of the depolarizations increased and their latencies decreased by 0.2–0.3 ms when the stimulus intensities were increased from 50 to 500 μA (Fig. 10A, a–e). In contrast, single shock stimulation of the contralateral INC evoked hyperpolarization at a latency of 1.0 ms in the same trochlear motoneuron (Fig. 10Ba). Double shock stimulation of the contralateral INC did not produce temporal facilitation of the hyperpolarization (dashed line in Fig. 10Bb), suggesting that this hyperpolarization was monosynaptic. Intracellular injection of Cl– or the passage of a hyperpolarizing current reversed the hyperpolarization evoked from the contralateral INC (Fig. 10Ba) in a depolarizing direction (Fig. 10Bc), although the polarity of the depolarization evoked from the ipsilateral INC remained unchanged (Fig. 10Af), indicating that the depolarization was an EPSP and the hyperpolarization was an IPSP (Coombs et al. 1955; Eccles 1964). The latencies of EPSPs evoked by ipsilateral INC stimulation ranged from 0.6 to 1.5 ms (0.9 ± 0.2 ms, n = 51; Fig. 2Fa), whereas those of IPSPs evoked by contralateral INC stimulation ranged from 0.9 to 1.8 ms (1.2 ± 0.2 ms, n = 55; Fig. 2Fa), indicating that the earliest components of both EPSPs and IPSPs were monosynaptic. In 29 trochlear motoneurons, stimulation of the ipsilateral INC evoked IPSPs following EPSPs (Figs. 2Ea and 10Cb). These IPSPs were confirmed by their reversal to the depolarizing direction in response to passing hyperpolarizing current (Fig. 10Ca). Double shock stimulation of the ipsilateral INC did not produce temporal facilitation of the IPSPs (dashed line in Fig. 10Cc), suggesting that these IPSPs were monosynaptic. The latencies of the IPSPs evoked by stimulation of the INC on the ipsilateral side were shorter than those on the contralateral side by 0.1–0.3 ms. This finding suggested that ipsilateral INC stimulation might activate passing fibers originating from inhibitory neurons in the contralateral INC because injection of a tracer into the INC labeled many neurons in the contralateral INC (Kokkoroyannis et al. 1996; Onodera 1997). In contrast, stimulation of the contralateral INC evoked an EPSP–IPSP complex in only five trochlear motoneurons (Fig. 2Fb).

To determine whether IPSPs that were evoked in trochlear motoneurons by stimulation of the contralateral FFH were actually mediated via the contralateral INC, we examined the interaction between FFH- and INC-evoked IPSPs in a pre-conditioning-test paradigm (Fig. 10D). The stimulus intensity of the contralateral FFH was adjusted to just subthreshold to evoke IPSPs in a trochlear motoneuron (Fig. 10Da). The stimulus intensity of the contralateral INC was also reduced to evoke small early IPSPs in the same trochlear motoneuron (Fig. 10Db). When the same stimulation of the contralateral INC was given as test stimulation 1.0 ms after the preconditioning stimulation of the contralateral FFH, the preconditioning stimulation increased the amplitudes and falling slopes of the INC-evoked IPSPs in the trochlear motoneuron (Fig. 10Dc). Facilitation occurred when the INC stimuli were given at 0.6 ms after the FFH stimuli, reached a peak at ~1.0 ms, and then gradually decreased (Fig. 10Dd). Because preconditioning stimulation of the contralateral FFH facilitated the early monosynaptic component of the INC-evoked IPSPs, these results support the interpretation that FFH neurons project onto INC neurons on the same side, and those INC neurons terminate on a trochlear motoneuron on the opposite side. Similar facilitation was observed in all five of the trochlear motoneurons examined.

To determine whether disynaptic inhibition from the contralateral SC to a trochlear motoneuron is mediated by the
showing the stimulation sites in the INC on both sides (arrowheads). Inputs from the SC and FFH onto common INC neurons occurred at were given at learm motoneurons. Facilitation occurred when the FFH stimuli conditioning stimulation of the contralateral SC in three troch- toneurons tested. In addition, disynaptic IPSPs evoked by contralateral SC was mediated via the contralateral INC. Sim- stimulation indicated that the disynaptic inhibition from the INC-induced monosynaptic IPSPs by the preconditioning SC and a b. The FFH stimuli preceded the INC stimuli by 1.0 ms. Dashed line, algebraic sum of the responses in the contralateral INC evoked small monosynaptic IPSPs at 450 ms. Dashed line indicates the algebraic sum of the responses in D, a and b. d: time course of facilitation of INC-evoked IPSPs produced by preconditioning FFH stimulation. Abscissa: interval between the preconditioning FFH stimuli and the test INC stimuli. Ordinate: means and SDs of the difference in amplitude between the control and conditioned INC-evoked IPSPs.

Effects of the midline section between the SCs on SC-evoked PSPs in trochlear motoneurons

As previously described, stimulation of the ipsilateral and contralateral SC evoked mainly EPSPs and IPSPs in trochlear motoneurons, respectively (Fig. 1). However, stimulation of the SC evoked an EPSP-IPSP complex in some trochlear motoneurons; i.e., stimulation of the ipsilateral SC evoked IPSPs after the EPSPs, and stimulation of the contralateral SC evoked EPSPs prior to the IPSPs. The earliest latencies of such IPSPs and EPSPs were in the disynaptic range, but the later latencies might be in the trisynaptic range (Fig. 2, Ab and Bb). As previously mentioned in relation to Fig. 7, the projection from the SC to the FFH was mainly ipsilateral in agreement with the previous findings (Grantyn and Grantyn 1982; Kawamura et al. 1974; Moschovakis and Karabelas 1985). In addition, the projection from the FFH to the trochlear nucleus was also mainly ipsilateral because last-order premotor neu- rons in the FFH terminating on trochlear motoneurons identified by transneuronal transport of WGA-HRP injected in the trochlear nerve were mainly distributed ipsilaterally (Fig. 5). As shown in Fig. 10, INC neurons exert, for the most part, excitation on ipsilateral trochlear motoneurons and inhibition on contralateral trochlear motoneurons. Therefore if excitatory FFH neurons project to the INC on the opposite side and activate the excitatory and inhibitory INC neurons there, this pathway from the FFH to the INC can explain excitation of
contralateral trochlear motoneurons and inhibition of ipsilateral trochlear motoneurons by the SC. However, this pathway seems highly unlikely because the injection of biocytin into the INC revealed that retrogradely labeled cell bodies in the riMLF were located mainly ipsilaterally and few cells were observed contralaterally (Kokkoroyannis et al. 1996). On the other hand, there is a commissural connection between the two SCs (Behan and Kime 1996; Edwards 1977; Grantyn and Grantyn 1982; Kawamura et al. 1974; Moschovakis and Karabelas 1985; Munoz and Istvan 1998; Olivier et al. 1998). This commissural connection was considered to be inhibitory (Maeda et al. 1981; Moschovakis and Karabelas 1985). However, our recent study showed that strong commissural excitation exists in the rostral SC, and that tectal output neurons projecting to the ipsilateral FFH provide commissural axon collaterals to the contralateral SC (Takahashi et al. 2005). Therefore the most likely explanation for the excitation of trochlear motoneurons by the contralateral SC and inhibition by the ipsilateral SC is that this activity is due to direct activation of commissural axon collaterals by axon reflex or to commissural synaptic excitation of tectal output neurons that project to the FFH and/or INC. To examine this possibility, a midline section was made between the SCs on both sides and the effects of stimulation of the SC on either side were investigated in 28 trochlear motoneurons in three cats. The midline section extended for 4.0–5.5 mm rostrocaudally, included the rostral half of the SC, and was 4–5 mm deep from the surface of the SC (Fig. 11D). In the control, stimulation of the ipsilateral SC evoked an EPSP-IPSP complex (Fig. 11Bc), and stimulation of the contralateral SC also evoked an EPSP-IPSP complex in a trochlear motoneuron (Fig. 11Bd). After the midline section, stimulation of the ipsilateral SC evoked EPSPs but not IPSPs (Fig. 11Cc), and stimulation of the contralateral SC evoked IPSPs but not EPSPs in the trochlear motoneuron (Fig. 11Cd). Similar tectal inputs were observed in 26 of the 28 trochlear motoneurons tested. Stimulation of the ipsilateral and contralateral vestibular nerves evoked IPSPs and EPSPs in the same trochlear motoneurons in the control case (Fig. 11B, a and b) and after midline section (Fig. 11C, a and b). These vestibular-evoked PSPs in the same trochlear motoneurons assured that IPSPs were not reversed to depolarizations due to the spontaneous diffusion of Cl⁻. Therefore this midline-section experiment showed that excitation of contralateral trochlear motoneurons and inhibition of ipsilateral trochlear motoneurons evoked by stimulation of the SC were mediated by tectal output neurons in the SC opposite to the one stimulated, i.e., tectal output neurons opposite to the stimulated SC were antidromically activated through their commissural collaterals or synthetically activated by stimulation of excitatory commissural neurons. These tectal output neurons in turn orthodromically activated excitatory neurons in the FFH and inhibitory neurons in the INC on the same side as the nonstimulated SC. After the midline section between the SCs, the latencies of SC-evoked PSPs were almost equal to or shorter than those with the normal commissural connection between the SCs, and the thresholds for evoking PSPs in trochlear motoneurons by stimulation of the SC tended to be lower than those in the control. These changes may be ascribed to the abolition of commissural inhibition between the SCs (Munoz and Istvan 1998; Takahashi et al. 2005) by midline section, although experiments were performed under anesthesia. In the remaining 2 of the 28 trochlear motoneurons tested, 1 neuron displayed an EPSP-IPSP complex at a long latency by stimulation of the ipsilateral SC and the other neuron displayed PSPs by stimulation of the contralateral SC. These tectal inputs might be due to some bilaterality in the projection of the SC to the FFH and in the projection of the FFH to the trochlear nucleus.

**DISCUSSION**

The present study has revealed the most direct pathways from the SC to trochlear motoneurons in the cat. The main excitatory pathway from the ipsilateral SC to trochlear motoneurons is a disynaptic one via the ipsilateral FFH, whereas...
the main inhibitory pathway from the contralateral SC to trochlear motoneurons is a disynaptic one via the contralateral INC (Fig. 12). In addition, there are probably trisynaptic pathways via the FFH and the INC for both excitatory and inhibitory pathways. In contrast to the tectal inputs to horizontal ocular motoneurons (Izawa et al. 1999), PSPs evoked by SC
stimulation in trochlear motoneurons tended to be small. This finding is consistent with a previous report by Precht et al. (1974) and might be related to the strength of the action of the superior oblique muscle. In addition, in contrast to the reciprocal inputs from the SCs on both sides to horizontal ocular motoneurons (Izawa et al. 1999), stimulation of the SC on either side sometimes evoked both disynaptic EPSPs and IPSPs in trochlear motoneurons. However, these EPSP-IPSP sequences disappeared after midline section of the commissural fibers between the SCs. Therefore the EPSP-IPSP complexes were most likely due to direct activation of tectofugal neurons by way of their commissural collaterals and/or synaptic activation of tectofugal neurons by stimulation of the SC on the opposite side (Takahashi et al. 2005). Therefore trochlear motoneurons receive reciprocal inputs from the SCs on both sides; i.e., excitation from the ipsilateral SC and inhibition from the contralateral SC. This reciprocal pattern is the reverse of that observed in abducens motoneurons; i.e., excitation from the contralateral SC and inhibition from the ipsilateral SC, as might be expected due to the crossed nature of the trochlear nerve.

Effective stimulation sites in the SC for evoking PSPs in trochlear motoneurons depended on the location of stimulating electrodes in the SC, although the stimulation sites in the SC tended to have less clear effect on the size of PSPs in trochlear motoneurons than in abducens motoneurons. When stimulating electrodes were arranged mediolaterally, stimulation of the lateral region of the ipsilateral SC, which represents downward eye movements in the SC motor map, evoked larger EPSPs in trochlear motoneurons, whereas stimulation of the medial region of the contralateral SC, which represents upward eye movements in the motor map, was more effective for evoking IPSPs in trochlear motoneurons. Therefore excitatory inputs from the lateral part of the SC and inhibitory inputs from the medial part of the opposite SC may have reciprocal effects on trochlear motoneurons. In the rostrocaudal plane, evoked EPSPs and IPSPs often became larger, and their rising or falling phase became sharper as the stimulation sites were shifted more caudally in the SC as shown in Fig. 3. These larger PSPs in trochlear motoneurons may reflect the vertical components of large oblique saccades that are evoked by stimulation of the more caudal part of the SC (McIlwain 1986; Rouxou and Crommelinck 1976), although the location of the stimulating electrodes with respect to the motor map in the SC, especially in terms of the vertical components of eye movements, can only be roughly guessed at. However, in other cases, stimulation of more rostral sites evoked larger EPSPs and IPSPs at shorter latencies. These large PSPs may reflect the vertical components of pure vertical saccades that are represented more rostrally, in the region around the vertical meridian of the motor map (McIlwain 1986; Rouxou and Crommelinck 1976).

The crucial feature of vertical gaze, when compared with horizontal gaze, is that vertical conjugacy is generally thought to require co-contraction of homonymous muscles in both eyes, whereas horizontal gaze requires excitation of one muscle and inhibition of the homonymous muscle on the other side. So it is not surprising that the present study revealed that trochlear motoneurons receive reciprocal inputs from the SCs on both sides, as has been observed in abducens motoneurons (Fig. 12). To look down and to the left, the lateral portion of the right SC will presumably activate downward premotor neurons in the right FFH, and these neurons in turn will activate right trochlear motoneurons that cause the left eye to move down and out. As the left eye needs to move down and to the right, the lateral portion of the left SC would have no effect on the right trochlear nucleus-left superior oblique muscle but probably would activate the left inferior rectus instead. When the medial portion of the left SC signals an eye movement up and to the right and the left eye moves up and to the right, the right trochlear nucleus-left superior oblique muscle would be actively inhibited. As the left eye needs to move up and to the left, the medial portion of the right SC would have no effect on the right trochlear nucleus-left superior oblique muscle in agreement with the fact that this is not the plane of the primary action of the left superior oblique. This reciprocal input pattern from the SCs to trochlear motoneurons is very similar to that connecting the semicircular canals to trochlear motoneurons; trochlear motoneurons receive disynaptic excitation from the contralateral posterior canal nerve and disynaptic inhibition from the ipsilateral anterior canal nerve (Ito et al. 1976a,b).

**FFH connections**

The FFH in the cat is believed to be a homologue of the riMLF in the monkey. MLBNs, which show bursting neuronal activity at the onset of vertical saccades and supply saccadic eye-velocity signals, have been found in the FFH in the cat (Nakao and Shiraishi 1985; Nakao et al. 1988). The present study showed that stimulation of the FFH evoked monosynaptic excitation in trochlear motoneurons. In an early study, Schwindt et al. (1974) reported that stimulation of the FFH had no effect on trochlear motoneurons, but Nakao and Shiraishi (1985) later reported that stimulation of the FFH evoked monosynaptic excitation and inhibition in trochlear motoneurons. However, this does not necessarily indicate that neurons in the FFH have direct synaptic connections with trochlear motoneurons because other pathways might be activated by stimulation of the FFH either due to current spread to nearby passing fibers or by axon reflex activation in collaterals. In the present study, the transneuronal-labeling experiment revealed that neurons in the ipsilateral FFH terminated on trochlear motoneurons, so at least a portion of the PSPs observed in trochlear motoneurons must be due to monosynaptic input from neurons in the FFH. Furthermore the disappearance of SC-evoked trochlear nerve discharge after inactivation by muscimol injection into the FFH (Fig. 6) indicates that trochlear motoneurons receive excitatory input from the ipsilateral SC via the ipsilateral FFH. This excitatory pathway was further confirmed by the existence of facilitation of FFH-evoked monosynaptic EPSPs in trochlear motoneurons by preconditioning stimulation of the ipsilateral SC (Fig. 8G).

In addition to the monosynaptic EPSPs, stimulation of the ipsilateral FFH evoked disynaptic EPSPs in some trochlear motoneurons (Fig. 8F). FFH output neurons and their presynaptic excitatory fibers might be activated in the FFH, so that directly activated and synaptically activated spikes were generated in FFH output neurons. However, stimulation of stem axons of FFH neurons at a site just caudal to the retroflex bundle still evoked disynaptic EPSPs in trochlear motoneurons, indicating that activation of presynaptic excitatory fibers terminating on output neurons in the FFH was not essential to
evoke disynaptic EPSPs in trochlear motoneurons. This finding indicates that stimulation of only output fibers from the FFH could evoke disynaptic EPSPs. Therefore in addition to direct projections, the FFH is most likely to supply an excitatory drive to interneurons that, in turn, activate ipsilateral trochlear motoneurons. The transneuronal labeling data showed that many INC neurons terminate on trochlear motoneurons on the same side, and stimulation of the ipsilateral INC evoked monosynaptic excitation in trochlear motoneurons. In addition, FFH neurons project to the ipsilateral INC (Moschovakis et al. 1991a,b). Therefore it is most likely that FFH neurons exert not only a direct monosynaptic effect on trochlear motoneurons but also an indirect disynaptic effect via the ipsilateral INC.

Stimulation of the ipsilateral FFH also evoked IPSPs after EPSPs in some trochlear motoneurons, and their early components were considered to be monosynaptic (Fig. 8B). The FFH/rMLF is believed to contain an admixture of neurons representing both upward and downward eye movements (Büttner et al. 1977; King and Fuchs 1979; Moschovakis et al. 1991a,b) and presumably representing both inhibitory and excitatory burst neurons (Nakao and Shiraishi 1985; Spencer and Wang 1996; Wang and Spencer 1996a), although Wang and Spencer (1996b) have argued for a tendency of upward neurons lying more caudally and downward neurons more rostrally in the cat. Therefore these ipsilateral FFH-evoked IPSPs might be conveyed via GABA-immunoreactive neurons in the rMLF reported in the cat by Spencer and Wang (1996), although the laterality of their projection to the oculomotor and trochlear nuclei was not described. However, such a GABAergic projection was not seen in the monkey (Carpenter et al. 1992), and Chen and May (2002) also found primarily excitatory outputs from the FFH/rMLF to levator palpebrae motoneurons in the cat. Therefore it may be that ipsilateral FFH stimulation activates axon collaterals of inhibitory neurons located in the INC on the opposite side that project to trochlear motoneurons on the stimulated side because other studies have shown that injection of a tracer into the INC labels axon terminals in the contralateral FFH (Kokkoroyannis et al. 1996; Onodera 1997).

Stimulation of the contralateral FFH evoked IPSPs, which were most likely disynaptic, in trochlear motoneurons. This finding is compatible with the present anatomical findings. Specifically, the transneuronally labeled, last-order premotor neurons terminating on trochlear motoneurons were primarily distributed in the ipsilateral FFH and in the INC on both sides. Similar observations have been made using transneuronal tracers in the monkey (Horn and Büttner-Ennever 1998). Most likely, inhibitory neurons in the INC activated by FFH neurons on the same side terminate on trochlear motoneurons on the opposite side (see following text). In fact, this pathway was demonstrated by the presence of spatial facilitation of INC-evoked monosynaptic IPSPs in trochlear motoneurons by preconditioning stimulation of the FFH (Fig. 10D).

INC connections

Contralaterally projecting INC neurons were considered to be the most likely candidate for mediating disynaptic inhibition from the contralateral SC to trochlear motoneurons among the last-order premotor neurons because output neurons of the SC projecting to the midbrain are mainly ipsilateral. Typically, stimulation of the contralateral INC evoked monosynaptic IPSPs in trochlear motoneurons, whereas stimulation of the ipsilateral INC evoked monosynaptic EPSPs in the same trochlear motoneurons. In some trochlear motoneurons, however, stimulation of the ipsilateral and, occasionally, the contralateral INC evoked both the EPSP and IPSP components. In this respect, our result is consistent with the finding by Schwindt et al. (1974) in that stimulation of both ipsi- and contralateral INC could evoke an EPSP-IPSP sequence in trochlear motoneurons. However, our data showed that stimulation of the contralateral INC usually caused monosynaptic IPSPs in trochlear motoneurons, and furthermore these monosynaptic IPSPs were facilitated by preconditioning stimulation of the SC on the same side as the stimulated INC. Therefore it is safe to conclude that disynaptic IPSPs in trochlear motoneurons evoked by stimulation of the contralateral SC are mediated via the contralateral INC.

Although tectal input has been reported to be weak for interstitiospinal neurons in the INC (Fukushima et al. 1981), anatomical studies do show the presence of projections from the SC to the INC, mainly on the same side (Altman and Carpenter 1961; Grantyn and Grantyn 1982). In addition, the INC contains vertical MLBNs (Moschovakis et al. 1991a,b). Although monosynaptic inhibitory input from the contralateral INC to trochlear motoneurons may be conveyed via these MLBNs and mediate reciprocal inhibition from the contralateral SC. On the other hand, stimulation of the ipsilateral INC evoked monosynaptic EPSPs in trochlear motoneurons. These EPSPs may be induced by stimulation of descending axons of FFH neurons that pass near the INC. However, premotor neurons in the INC also may have been stimulated directly because transneuronally labeled neurons were also found in the INC ipsilateral to retrogradely labeled trochlear motoneurons (Fig. 5). In addition to MLBNs, the INC contains burst-tonic neurons that appear to provide position signals for vertical and torsional gaze developed through presumed integrator circuits between the INC and the vestibular nuclei (Chimoto et al. 1999; Fukushima et al. 1990; King et al. 1981). These INC neurons receive excitatory inputs from the vertical semicircular canals via the contralateral vestibular nucleus and project to the ipsilateral vestibular nucleus. These excitatory INC neurons, although their oculomotor targets have not yet been identified, most likely convey the disynaptic activation evoked in trochlear motoneurons by stimulation of the ipsilateral FFH. Therefore this pathway may be involved in the transformation of a burst signal into a tonic one for position and subserve neural integration (Fukushima et al. 1992). Although the MLF was not sectioned in the present study, Schwindt et al. (1974) sectioned the MLF chronically and still observed INC-evoked EPSP-IPSP complexes in trochlear motoneurons. Thus their findings indicated that at least a portion of INC-evoked EPSPs and IPSPs were not due to axon reflex of vestibular nucleus neurons.

Overview of connections

During vestibular horizontal nystagmus, abducens motoneurons receive strong excitation at the ipsilateral quick phase and strong inhibition at the contralateral quick phase (Maeda et al. 1972). This strong inhibition is caused by contralateral inhibitory burst neurons (IBNs) in the paramedian pontomedullary...
reticular formation (Hikosaka and Kawakami 1977; Hikosaka et al. 1977). During vestibular vertical nystagmus, trochlear motoneurons also receive strong excitation at the downward quick phase and strong inhibition at the upward quick phase (Baker and Berthoz 1974). However, presynaptic fibers to trochlear motoneurons that fire only during the quick inhibitory phase, which are a vertical homologue of horizontal IBNs, were not observed with vertical vestibular nystagmus. Instead the inhibitory vestibular nucleus neurons started firing at the onset of the quick inhibitory phase (Baker and Berthoz 1974).

Therefore these inhibitory vestibular nucleus neurons were considered to be equivalent to IBNs for horizontal vestibular nystagmus. In the vertical saccadic system, Moschovakis et al. (1991a) reported one MLBN with an upward on-direction in the riMLF that projected to the ipsilateral inferior rectus and superior oblique subdivisions of the oculomotor complex. Based on this pattern of connections, the neuron was presumed to be inhibitory. Later, Shiraishi and Nakao (1995) used spike-triggered averaging of field potentials in awake cats to demonstrate that upward MLBNs in the mesodiencephalic junction make inhibitory connections with inferior rectus motoneurons. The present results revealed for the first time that a reciprocal input pattern connecting the SCs to oculomotor neurons exists in the vertical saccadic system, as well as in the horizontal saccadic system (Izawa et al. 1999); i.e., the shortest excitatory pathway from the ipsilateral lateral SC to trochlear motoneurons was disynaptic via the ipsilateral FH, whereas the shortest inhibitory pathway from the contralateral medial SC to trochlear motoneurons was disynaptic via the contralateral INC. In the present anesthetized preparation, inhibitory INC neurons terminating on contralateral trochlear motoneurons could not be determined to be burst neurons. However, the similarities of the connections of the SCs with trochlear motoneurons to those with abducens motoneurons strongly suggest that these inhibitory INC neurons are most likely inhibitory burst neurons. Therefore inhibitory INC neurons terminating on contralateral trochlear motoneurons are considered to be a vertical counterpart for IBNs in the horizontal saccadic system (Hikosaka and Kawakami 1977). The present results may then represent the first demonstration of the existence of IBNs in the vertical saccadic system mediating tectal input to vertical oculomotor motoneurons. The activity of vertical oculomotor motoneurons has not been systematically analyzed in relation to different directions of vertical saccades. It is tacitly assumed that the SC on one side produces co-contraction of homonymous vertical oculomotor muscles in both eyes for vertical gaze. However, the present results indicate that the SC activates one vertical oculomotor muscle and inhibits the homonymous muscle on the other side presumably because of their minor pulling directions. Instead the SC most likely co-activates ipsilateral superior oblique and inferior rectus motoneurons to produce an oblique downward conjugate eye movement. The SCs on both sides must simultaneously function to produce pure vertical upward or downward gaze. Nevertheless further study is required to understand neural mechanisms for generating vertical saccades by the SCs.

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