Input-output organization of inhibitory neurons in the interstitial nucleus of Cajal projecting to the contralateral trochlear and oculomotor nucleus

Y. Sugiuchi, M. Takahashi, and Y. Shinoda

Department of Systems Neurophysiology, Graduate School of Medicine, Tokyo Medical and Dental University, Tokyo, Japan

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Neurons in the interstitial nucleus of Cajal (INC) that are known to be involved in eye and head movements are excitatory. We investigated the input-output organization of inhibitory INC neurons involved in controlling vertical saccades. Intracellular recordings were made in INC neurons activated antidromically by stimulation of the contralateral trochlear or oculomotor nucleus, and their synaptic input properties from the superior colliculi (SCs) and the contralateral INC were analyzed in anesthetized cats. Many INC neurons projected to the contralateral trochlear nucleus, Forel’s field H, INC, and oculomotor nucleus, and mainly received monosynaptic excitation from the ipsi- and contralateral SCs. After sectioning the commissural connections between the SCs, these neurons received monosynaptic excitation from the ipsilateral medial SC and disynaptic inhibition via the INC from the contralateral lateral SC. Another group of INC neurons were antidromically activated from the contralateral oculomotor nucleus, INC, and Forel’s field H, but not from the trochlear nucleus, and received monosynaptic excitation from the ipsilateral lateral SC and disynaptic inhibition from the contralateral medial SC. The former group was considered to inhibit contralateral trochlear and inferior rectus motoneurons in upward saccades, whereas the latter was considered to inhibit contralateral superior rectus and inferior oblique motoneurons in downward saccades. The mutual inhibition existed between these two groups of INC neurons for upward saccades on one side and downward saccades on the other. This pattern of input-output organization of inhibitory INC neurons suggests that the basic neural circuits for horizontal and vertical saccades are similar.

The neural substrates for generating horizontal saccades have been extensively analyzed (Grantyn and Moschovakis 2004; Isa and Hall 2009; May 2006; Schiller and Körner 1971; Scudder et al. 2002; Sparks 1999; Wurtz and Goldberg 1972). Excitatory and inhibitory medium-lead burst neurons (MLBNs) that have shown burst activity before and during horizontal saccades have been identified in the paramedian reticular formation rostral and caudal to the abducens nucleus, respectively (Cohen and Henn 1972; Hikosaka and Kawakami 1977; Keller 1974; Luschei and Fuchs 1972; Yoshida et al. 1982). Our previous studies showed that the shortest excitatory pathway from the superior colliculus (SC) to contralateral abducens motoneurons was disynaptic via contralateral excitatory burst neurons (EBNs), whereas the shortest inhibitory pathway from the SC to ipsilateral abducens motoneurons was disynaptic via contralateral inhibitory burst neurons (IBNs) (Izawa et al. 1999; Sugiuchi et al. 2005).

On the other hand, the neural substrates for generating vertical saccades are only poorly understood, although they have been the focus of many clinical, anatomical, and physiological studies (for reviews, see Büttner-Ennever and Büttner 1988; Büttner-Ennever et al. 1982; Fukushima 1987, 1991; Fukushima et al. 1990a, 1990b, 1991, 1995; Hassler 1972; Henn 1992; Hepp et al. 1989; Hess 1954). The premotor structure for vertical eye movements was first investigated anatomically by Szentágothai (1943). He showed that the interstitial nucleus of Cajal (INC) projects to the extraocular motor nuclei for vertical eye movements, but not to the abducens nucleus. This finding has been confirmed by many anatomical studies (Büttner-Ennever et al. 1981; Carpenter et al. 1970; Graybiel and Hartweg 1974; Kokkoroyannis et al. 1996; Steiger and Büttner-Ennever 1979).

The rostral interstitial nucleus of the medial longitudinal fasciculus (rMLF), another premotor structure for vertical eye movements, was defined by Büttner-Ennever and Büttner (1978) as a distinct group of cells that are related to vertical eye movements. The rMLF contains MLBNs related to vertical saccades, and the characteristics of their responses suggested that they are output neurons of a pulse generator responsible for the vertical components of saccades (Büttner et al. 1977; Hepp et al. 1988, 1989; King and Fuchs 1979). Neurons that showed saccade-related-activity with similar characteristics were reported by Shiraishi and Nakao (1995) in the Forel’s field H (FFH) (equivalent to the rMLF in monkeys) in awake cats. Projection from the rMLF to the ocular motor nuclei related to vertical eye movements has been analyzed anatomically (Büttner-Ennever et al. 1981; Moschovakis et al. 1991a, 1991b; Steiger and Büttner-Ennever 1979; Wang and Spencer 1996). These findings suggested that burst neurons in the rMLF could be considered the equivalent of EBNs with horizontal on-directions in the paramedian pontine reticular formation.

The activity of neurons in the INC has also been examined in awake monkeys and cats. While most of the eye movement-related neurons in the rMLF were burst neurons (Büttner et al. 1977), most of the INC neurons with activities that were related to upward and downward eye movements were either burst-tonic or tonic neurons (Fuchs 1977; Fukushima 1991; Fukushima et al. 1990a, 1990b; Kaneko and Fukushima 1998; King et al. 1981). Since neurons in the rMLF encode saccade velocity, the saccadic signal has to be integrated in the mathematical sense to provide an eye position signal for oculomotor motoneurons. Although many studies have shown that the INC...
is an essential component of the neural integrator for vertical eye movements (Chimoto et al. 1999; Crawford and Vilis 1993; Fukushima 1987, 1991; Fukushima and Kaneko 1995; Fukushima et al. 1990a, 1990b, 1992; Nakamagoe et al. 2000), the INC has not specifically been considered to play an important role in generating the saccadic movement itself, and little is known about saccade-related burst neurons in the INC. Helmchen et al. (1996) first analyzed saccade-related burst neurons in the INC with three-dimensional eye movement recordings. In contrast to previous findings, they found many burst neurons in the INC and reported that such INC neurons encoded vertical saccades in an ipsiversional direction and have burst characteristics similar to riMLF cells. Moschovakis et al. (1991a, 1991b) analyzed projection patterns of single MLBNs using intracellular staining with horseradish peroxidase (HRP) in the alert monkey. They reported that MLBNs within the riMLF projected to the oculomotor nuclei, but those located outside the riMLF did not. Therefore, they concluded that MLBNs in the INC are involved in an INC-riMLF reciprocal feedback loop rather than in providing inputs to extracocular motoneurons.

Monosynaptic inhibition of trochlear motoneurons by electrical stimulation of the INC was first reported by Schwindt et al. (1974). Effective stimulation sites for evoking synaptic effects on motoneurons or evoking field potentials in the subnuclei of individual eye muscles were systematically analyzed by Nakao and Shiraishi (1983, 1985) in the cat. They reported that input patterns from the FFH areas on both sides to motoneurons of vertical eye muscles were mostly symmetrical. The effective sites of stimulation for inducing effects in the inferior oblique subdivision of the oculomotor nuclei and the trochlear nucleus were also located in the INC, the adjacent reticular nucleus, the posterior commissure, and in and around the medial part of the FFH (Nakao and Shiraishi 1985). These findings indicated that not only neurons in the FFH, but also those in the rostral midbrain outside the FFH are likely to project to vertical ocular motoneurons.

While the INC and the FFH represent the source of premotor input to vertical ocular motoneurons, the source of the saccade vector lies in the SC. The connections between the SCs and motoneurons of vertical eye muscles have not been analyzed in as much detail as those in the horizontal oculomotor system. The effects of stimulation of the SC on trochlear motoneurons were first investigated by Precht et al. (1974). However, consistent postsynaptic potentials (PSPs) were not detected in trochlear motoneurons in their study, even after strong multiple-shock stimulation of the SC. This contrasted with the presence of clear PSPs in abducens motoneurons in the same preparations. In a previous study, our laboratory examined the tectal input to trochlear motoneurons in the cat (Izawa et al. 2007). In contrast to the tectal inputs to horizontal oculomotor neurons (Izawa et al. 1999), PSPs in trochlear motoneurons evoked by SC stimulation tended to be small. This finding was consistent with that in a previous report by Precht et al. (1974). However, our laboratory clearly showed that trochlear motoneurons received disynaptic excitation from the ipsilateral SC via the ipsilateral FFH and disynaptic inhibition from the contralateral SC via the contralateral INC (Izawa et al. 2007). Takahashi et al. (2005, 2007, 2010) have shown some fundamental aspects of the functional organization of the SC regarding the spatial coding of vertical saccades, but little is known about the neural substrate for this spatiotemporal transformation. Furthermore, the mechanism by which the SC controls the activity of premotor neurons involved in vertical saccades remains to be determined. In the present study, to reveal the basic neural circuits in the midbrain that are responsible for vertical saccades, we analyzed the properties of synaptic inputs from the SC to inhibitory INC neurons that-projected to the contralateral trochlear and oculomotor nuclei with electrophysiological and morphological methods in the cat.

METHODS

Experiments were performed in 16 cats weighing 2.6–4.4 kg. Animal experimentation was conducted in accordance with the “Policies on the Use of Animals and Humans in Neuroscience Research,” as revised and approved by the Society for Neuroscience in 1995, and the “Guiding Principles for the Care and Use of Animals in the Field of Physiological Sciences” (The Physiological Society of Japan, revised in 2001). The experimental protocol was approved by the Animal Care Committee of Tokyo Medical and Dental University. The animals were initially anesthetized with ketamine hydrochloride (Ketalar, Parke-Davis; 25 mg/kg im) followed by α-chloralose (40–45 mg/kg iv, initial dose, supplemented with additional doses of 10–25 mg/kg iv, throughout the remainder of the experiment). During recording, the animals were paralyzed by the intravenous administration of pancuronium bromide (Mioblock, Organon, The Netherlands), and artificially ventilated with end-tidal CO2 held at 35–40 mmHg. The heart rate was continuously monitored by an electrocardiogram. The body temperature was kept at 37.0–38.5°C by a heating pad.

The bone over the parietal and occipital cortex was removed, and the cerebral cortex was removed by suction on both sides to introduce stimulating electrodes into the SCs, FFH, INC, and oculomotor and trochlear nuclei, and facilitate intracellular recording from the left INC under direct visual observation. The tips of the stimulating electrodes were positioned in the intermediate or deep layer (1.5–2.0 mm from the surface) of the SC (Izawa et al. 1999; Kawamura and Hashikawa 1978; Moschovakis and Karabelas 1985; Sugiuichi et al. 2005; Takahashi et al. 2005, 2007). Two concentric bipolar stimulating electrodes were placed in the FFH (A: 7.0–7.5, L: 1.5–2.5), and one or two were placed in the INC (A: 5.0–5.5, L: 0.8–1.2), according to the locations of the premotor neurons determined by anatomical methods (see the following section). In some preparations, an electrode array consisting of four dorsoventrally-arranged monopolar electrodes that were insulated except at the tip (100 μm in diameter, 1-mm intervals) was used to stimulate the INC (Sugiuichi et al. 2005). Negative pulses with a duration of 0.2 ms were delivered at less than 500 μA for stimulation of the SC, FFH, and INC. Ranck (1975) estimated an effective current spread of 1.0–1.5 mm around an electrode tip by monopolar stimulation at 500 μA (0.2-ms-duration pulse) in the mammalian central nervous system. However, since we used bipolar stimulation, the effective current spread should be much less than the values estimated by Ranck (1975) (Shinoda et al. 1977). Sasaki et al. (1970, 1972) estimated that 500 μA could not activate fibers or cells beyond 1.0 mm from an electrode tip when a concentric bipolar electrode of the same type as in the present study was used. The positions of the stimulation sites in the SC, FFH, INC, and oculomotor and trochlear nuclei were confirmed histologically on sections stained with thionin.

Glass microelectrodes for intracellular recording were filled with 0.4 M KCl or 2M potassium-citrate and had a resistance of 15–25 MΩ. Neurons in the INC area were recorded in the midbrain region around the stereotaxic coordinates (A 5.0–5.5 and L 0.8–1.2). At the end of each experiment, a recording electrode was left as a marker track, and recording tracks were reconstructed on serial sections stained with thionin in relation to the location of the marker track and

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the recording depths, and we confirmed that recording sites were located in the INC and its close vicinity (see Fig. 2K). All procedures for recording intracellular potentials from trochlear motoneurons have been described previously (Izawa et al. 2007).

The locations of premotor neurons in the midbrain that terminated on trochlear motoneurons were determined using the transneuronal labeling method in two cats (Izawa et al. 2007). Briefly, 2% wheat germ agglutinin (WGA)-HRP (Toyobo, Osaka, Japan) in 0.05 M Tris·HCl buffer (pH 8.6) was injected into the left trochlear nerve. After 4–6 days, the brain was removed, and serial transverse sections of 75 or 100 μm were reacted to reveal the presence of HRP by the tetramethyl benzidine method (Mesulam 1978), as described previously (Izawa et al. 2007). Labeled neurons were plotted under a microscope using a camera lucida system and a computer-assisted plotting and reconstruction program (Neurolucida, MicroBrightField, Colchester, VT).

To confirm and support the findings obtained by the transneuronal labeling method, and examine the distribution of axon terminals of
premotor neurons that terminated on trochlear motoneurons, we made three tracks by the iontophoretic injection (2-μA positive current pulses of 1-s duration at 0.5 Hz for 30 min) of 12.5% dextran-biotin (DB; Invitrogen) into the trochlear nucleus and determined the distribution of retrogradely labeled neurons that terminated in the trochlear nucleus in one cat. The injection site in the trochlear nucleus was determined by recording antidromic negative field potentials evoked by stimulation of the contralateral trochlear nerve. After a survival time of 5 days, the brain was removed, and serial coronal sections with a thickness of 75 μm were incubated in an avidin-biotinylated HRP complex (ABC, Vector Laboratories) and then treated for HRP, as described previously (Takahashi et al. 2010).

RESULTS

Distribution of last-order premotor neurons in the midbrain terminating on trochlear motoneurons. Before the electrophysiological experiments, we determined the exact locations of premotor neurons that terminated on trochlear motoneurons using a transneuronal-labeling method in two cats. After WGA-HRP was injected into the left trochlear nerve, many heavily labeled neurons were found almost exclusively in the right trochlear nucleus, but a small number (<5% of the labeled neurons in the right trochlear nucleus) of labeled neurons were scattered in the left trochlear nucleus (Fig. 1). In addition to these retrogradely labeled motoneurons (Fig. 1D, g–j), many lightly labeled neurons were found in the midbrain (Fig. 1), and they were considered to be transneuronally labeled last-order premotor neurons. As shown in Fig. 1, A–C and D, a and b, labeled neurons in the FFH were exclusively on the right side and were distributed rostrocaudally at the level of and slightly rostral to the retroflexus bundle. The transitional region between the FFH and INC near the caudal end of the retroflexus bundle was almost free of transneuronally labeled neurons. Mediolaterally, the labeled neurons were mainly located 0.7–2.0 mm lateral from the midline.

In the INC region, many labeled neurons were found bilaterally throughout the rostrocaudal extent of the INC, with a slight predominance on the left side (Fig. 1A and D, c–f). The labeled neurons were relatively well confined to the typical INC region, but some were distributed ventrolaterally in the adjacent midbrain reticular formation, and dorsally among axons from the posterior commissure. Based on these anatomical findings, we recorded intracellular potentials from neurons in the INC region (A 5.0–5.5, L 0.8–1.2). Labeled neurons were also found bilaterally in the nucleus of the posterior commissure (PCN), but with predominance on the right side (Fig. 1D, d–f). Most of them were distinctly larger than the labeled neurons in the FFH and INC. Populations of labeled neurons in the INC and PCN were generally well segregated, but were continuous at some level (Fig. 1Df). Based on the recording depth along the recording track in the present study, our sample was considered to include neurons in the INC and PCN (see Fig. 4B).

Synaptic inputs to trochlear motoneurons from the SCs. Before we describe electrophysiological data regarding the input-output organization of premotor neurons in the INC that terminate on trochlear motoneurons, we show a typical pattern of inputs to a trochlear motoneuron from the SCs on both sides (Fig. 2, A and C) to supplement the data and support their interpretation in the present study (see Izawa et al. 2007). As shown in this example, stimulation of the ipsilateral and contralateral SC evoked depolarizations (Fig. 2A) and hyperpolarizations in trochlear motoneurons (Fig. 2C), respectively. By injecting CI–, the depolarizations were identified as excitatory PSPs (EPSPs) and the hyperpolarizations as inhibitory PSPs (IPSPs) (Eccles 1964) (see Izawa et al. 2007, Fig. 1, E and F). The latencies of EPSPs and IPSPs in trochlear motoneurons from the ipsilateral and contralateral SC ranged from 1.1 to 2.3 ms (Fig. 2D, see Fig. 2A in Izawa et al. 2007), and from 1.1 to 3.8 ms (Fig. 2E, see Fig. 2Ba in Izawa et al. 2007), respectively. Based on the difference between the latencies of these PSPs and the antidromic latencies of tectofugal neurons projecting to the FFH, the earliest components of these PSPs were considered to be disynaptic (Izawa et al. 2007). These disynaptic EPSPs and IPSPs in trochlear motoneurons from the ipsilateral and contralateral SC were conveyed via the ipsilateral FFH and contralateral INC, respectively (Izawa et al. 2007). Based on this finding that INC neurons with monosynaptic EPSPs evoked from the ipsilateral SC exert an inhibitory effect on contralateral trochlear motoneurons, we analyzed the input-output organizations of inhibitory premotor neurons in the INC that mediated the output of the SC to trochlear and oculomotor motoneurons.

Identification of INC neurons. The INC is known to consist of neurons of heterogeneous groups with different functions (Büttner-Ennever and Büttner 1988; Büttner-Ennever et al. 1982; Chen and May 2007; Chimoto et al. 1992; Crawford and Vilis 1993; Fukushima 1987, 1991; Fukushima and Kaneko 1995; Fukushima et al. 1990a, 1990b, 1991, 1992, 1995; Hassler 1972; Helmschen et al. 1996, 1998; Herr 1992; Hepp et al. 1989; Hess 1954). To analyze the neural mechanisms that underlie vertical saccades, we focused on INC neurons that projected to the contralateral trochlear and/or oculomotor nucleus. Seventy-two neurons were identified as INC neurons that projected to the contralateral trochlear nucleus, since they were antidromically activated by stimulation of the contralat-
eral trochlear nucleus (Fig. 2G). We used these INC neurons for later analysis, if not stated otherwise. The latencies of antidromic spikes evoked by stimulation of the contralateral trochlear nucleus were 0.3–1.1 ms (mean ± SD, 0.5 ± 0.2, n = 72). Their resting membrane potentials ranged from −40 to −65 mV (−55 ± 16 mV, n = 72). All lateralities in this study are described with reference to the recording site. Of the 72 INC neurons, antidromic spikes were also evoked by stimulation of the contralateral INC in 43 of 68 neurons examined (see Fig. 6Ba), and by stimulation of the contralateral FFH in 36 of 72 neurons examined. In 27 of 68 neurons examined, antidromic spikes were evoked from all three of these sites: the contralateral trochlear nucleus, INC, and FFH. The presence or absence of projections to the ipsilateral FFH, trochlear nucleus, and the contralateral oculomotor nucleus was also examined in four preparations. In 17 of 24 neurons examined, antidromic responses were observed from the contralateral oculomotor nucleus.

**Typical pattern of synaptic effects on INC neurons evoked by stimulation of the SC.** The effects of stimulation of the SCs on both sides were examined in the 72 INC neurons that were identified as projecting to the contralateral trochlear nucleus. A typical example of the effects of stimulation of the ipsilateral and contralateral SCs on an INC neuron is shown in Fig. 2, I and J. In this INC neuron, stimulation of the contralateral trochlear nucleus evoked spikes at 0.3 ms (Fig. 2G), and the threshold for evoking spikes was 290 µA. These spikes were considered to be evoked antidromically, since spikes occurred in an all-or-none manner at a fixed latency at the stimulus intensity of the threshold for evoking spikes, and no EPSPs were evoked at a stimulus intensity just below the threshold (Fig. 2G). This INC neuron was also antidromically activated

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**Fig. 2.** Typical examples of the effects of stimulation of the bilateral superior colliculi (SCs) on intracellular potentials recorded in a trochlear motoneuron (A–C) and an INC neuron (G–J) that projected to the contralateral trochlear nucleus. A: postsynaptic potentials (PSPs) evoked by stimulation of the ipsilateral SC with single- (a), double- (b), and triple-shock stimuli at 400 µA (c). B: antidromic spikes evoked by stimulation of the contralateral trochlear nerve (IVth nerve) at 120 µA. C: PSPs evoked by stimulation of the contralateral SC with single- (a) and double-shock stimuli at 400 µA (b). The calibration in H also applies to A–C, G, I, and J. In this and the following figures, the bottom traces are field potentials recorded just outside the penetrated cells, and upward deflection in all potential traces represents positivity. D and E: latency histograms of excitatory PSPs (EPSPs) and inhibitory PSPs (IPSPs) recorded in trochlear motoneurons evoked by stimulation of the ipsilateral (D) and contralateral SC (E), respectively. These two histograms include cells reported in a previous paper (see Fig. 2, Aa and Bc, in Izawa et al. 2007). F: schematic drawing of the experimental setup for intracellular recording from INC neurons. Intracellular potentials were recorded in neurons in the INC. Stimulation electrodes were placed in the bilateral SCs and FFH, contralateral INC and oculomotor nucleus, and the bilateral trochlear nuclei. Open and filled neurons indicate excitatory and inhibitory neurons, respectively, in this and the following figures. SO, superior oblique muscle. G and H: antidromic spikes in an INC neuron evoked by stimulation of the contralateral trochlear nucleus (G) and oculomotor nucleus (H) at a stimulus intensity of 300 µA. Thresholds for activation of antidromic spikes were 290 (G) and 220 µA (H), respectively. I and J: PSPs recorded in the same INC neuron showing typical responses evoked by stimulation of the ipsi- (I) and contralateral SCs (J) before (Ia and J, a and b) and after the injection of Cl⁻ into the cell (Ib and Jc). Dotted lines in Ia and Ib indicate the superimposition of reversed IPSPs in Ib and Jc after Cl⁻ injection, respectively. The stimulus intensity was 200 µA. K: photomicrograph of the coronal section of the midbrain showing a recording glass electrode tip (arrow) in the INC. The glass microelectrode that was left in the recording site was sectioned with the brain stem for identification of the location of the recorded neuron in the INC. L–O: latency histograms of EPSPs (L) and IPSPs (M) in INC neurons evoked by stimulation of the ipsilateral SC and latency histograms of EPSPs (N) and IPSPs (O) by stimulation of the contralateral SC, respectively.
from the contralateral oculomotor nucleus at a latency of 0.5 ms (Fig. 2H). Single-shock stimulation of the ipsilateral SC evoked orthodromic spikes at 500 μA (not shown). When the stimulus intensity was decreased to 200 μA, spikes were observed less frequently, and clear depolarizations were observed (Fig. 2A). After Cl⁻ was injected into the cell, the rising phase of depolarizations did not change, but the slope of the falling phase became a little less steep (compare Fig. 2A, a and b), suggesting that the synaptic effects from the ipsilateral SC were mainly EPSPs, but that small IPSPs were superimposed on the falling phase of the preceding EPSPs. With regard to the inputs from the contralateral SC, single-shock stimulation at 200 μA showed only very small PSPs, mainly hyperpolarizations (Fig. 2A), but double-shock stimuli at 200 μA evoked clear depolarizations followed by hyperpolarizations (Fig. 2B). After Cl⁻ was injected into the cell, the early depolarizations did not change, but the later hyperpolarizations were reversed into depolarizations (Fig. 2Bc), indicating that these PSPs consisted of early EPSPs followed by late IPSPs.

As seen in the example in Fig. 2, G–J, the pattern of synaptic inputs in an INC neuron evoked by stimulation of the SC on each side could be summarized as follows. In 30 of the 72 INC neurons that projected to the contralateral trochlear nucleus, when the ipsilateral SC was stimulated at 500 μA, early EPSPs with occasional spike generation were followed by small IPSPs. When the stimulus intensity was decreased to 200–300 μA, the EPSPs remained, but the presence of IPSPs on the falling phase of the EPSPs became less obvious (see Fig. 2I). When the contralateral SC was stimulated at 500 μA, the pattern of responses seemed to be similar to that with stimulation of the ipsilateral SC, i.e., early EPSPs followed by late IPSPs that curtailed the falling phase of the preceding EPSPs in 35 of the 72 INC neurons. When the stimulus strength was decreased to 200–300 μA, the IPSPs remained, and the preceding EPSPs became small (see Fig. 2J). The overall time course of PSPs evoked by stimulation of each SC tended to be more complex when the stimulation site was in the rostral one-third vs. the more caudal part of the SC. This tendency was due to the fact that larger EPSPs and IPSPs were evoked by stimulation of the rostral part of the SC.

Latencies of synaptic inputs from the SC to INC neurons. The latencies of synaptic inputs from the SC to INC neurons were determined by superimposing EPSPs and IPSPs on field potentials recorded just outside the penetrated neurons. When EPSPs followed IPSPs, the onsets of IPSPs were not clear. Therefore, by superimposing the reversed potentials after Cl⁻ injection on the hyperpolarizing IPSPs before Cl⁻ injection, the point of divergence of these two IPSPs was considered to be the onset of the IPSPs. Latency histograms of PSPs evoked by stimulation of the ipsi- and contralateral SCs recorded in INC neurons are shown in Fig. 2, L–O. Although PSPs were usually evoked from more than one electrode in each SC, the minimal value among latencies of the PSPs evoked from each SC was adopted for each INC neuron. The latencies of EPSPs evoked by ipsilateral SC stimulation ranged from 0.7 to 2.0 ms (1.2 ± 0.3 ms, n = 49) (Fig. 2L). Judging from the latencies of antidromic spikes of tectoreticular neurons (TRNs) in the SC evoked by stimulation of the FFH (0.5–2.9 ms, see Fig. 7F in Izawa et al. 2007), and the short distance difference between the FFH and the INC, it is most likely that many of the EPSPs evoked in INC neurons from the ipsilateral SC were monosynaptic. Such EPSPs were observed in 49 of the 72 INC neurons examined (68.1%). Of these 49 INC neurons in which EPSPs were evoked by stimulation of the ipsilateral SC, 35 (71.4%) showed IPSPs superimposed on the falling phase of EPSPs, as shown in Fig. 2I. The latencies of these IPSPs evoked by stimulation of the ipsilateral SC ranged from 1.3 to 2.0 ms (1.6 ± 0.2 ms, n = 30) (Fig. 2M) and were considered to be disynaptic. On the other hand, the latencies of EPSPs and IPSPs evoked from the contralateral SC ranged from 0.7 to 2.1 ms (1.2 ± 0.3 ms, n = 35) (Fig. 2N), and from 1.1 to 2.7 ms (1.7 ± 0.3 ms, n = 40) (Fig. 2O), respectively. Their latencies were distributed within a range similar to those of the EPSPs and IPSPs evoked by stimulation of the ipsilateral SC, respectively. Therefore, the EPSPs and IPSPs evoked by stimulation of the contralateral SC were considered to be monosynaptic and disynaptic, respectively.

Effects of stimulation of different mediolateral sites in the SC on PSPs in INC neurons. To examine the effects of stimulating different mediolateral sites in the SCs on evoking PSPs in INC neurons, four concentric bipolar electrodes were placed in the rostral parts of the SCs along the vertical meridian of the motor map (Fig. 3B). A typical pattern of PSPs in an INC neuron evoked by stimulation of different mediolateral sites in the ipsilateral and contralateral SC is shown in Fig. 3. In this neuron, stimulation of all four sites in the ipsilateral SC evoked monosynaptic EPSPs (Fig. 3C, sites 1–4). There was a clear tendency for the amplitudes of EPSPs evoked from the more medial sites to be larger than those from the more lateral sites in the ipsilateral SC. In the same neuron as in Fig. 3C, the amplitudes of EPSPs evoked from the contralateral SC clearly showed a similar tendency; i.e., EPSPs from the more medial site were larger than those from the more lateral site in the contralateral SC (Fig. 3D, sites 5–8). Similar tendencies of the EPSPs evoked from the contralateral SC were observed in 21 of 35 INC neurons.

With regard to IPSPs in INC neurons evoked by stimulation of either the ipsilateral or contralateral SC, it was often difficult to measure the amplitudes of disynaptic IPSPs because of the presence of preceding EPSPs, as shown in Fig. 3, C and D. However, when only IPSPs were evoked or IPSPs were reversed by the injection of Cl⁻, the amplitudes of the IPSPs could be compared, as in the example shown in Fig. 4, C–F. In this INC neuron, stimulation of each of the four sites in the ipsilateral SC evoked early EPSPs followed by IPSPs (Fig. 4C, sites 1–4), and among the EPSPs, those evoked from the most medial site (Fig. 4C, site 1) had the largest amplitude and the shortest latency (1.1 ms), and those evoked by stimulation of the other sites had smaller amplitudes and longer latencies (Fig. 4C, sites 2–4). After the injection of Cl⁻, clearly reversed IPSPs were evoked by stimulation of all sites in the ipsilateral SC (Fig. 4D, sites 1–4). Comparison of the PSPs evoked by stimulation of different mediolateral sites revealed that the amplitudes were larger and the latencies were shorter for the medial site than for the lateral site of the ipsilateral SC. In the neuron shown in Fig. 4, stimulation of the four sites in the contralateral SC evoked clear disynaptic IPSPs (Fig. 4E, sites 5–8). After the injection of Cl⁻, they were reversed to depolarizations (Fig. 4F), and their amplitudes and latencies could be measured reliably. The rising phases of reversed IPSPs were steeper from the lateral part (compare Fig. 4F, sites 5–8), indicating stronger inhibition from the lateral part of the
contralateral SC. In addition to these disynaptic IPSPs, clear monosynaptic EPSPs were evoked by stimulation of the most medial site of the contralateral SC (Fig. 4E, site 5).

The general characteristics of synaptic inputs from the SCs to INC neurons projecting to the contralateral trochlear nucleus can be summarized as follows. First, the overall pattern of responses consisted of early depolarizations followed by hyperpolarizations, and this was observed when each site in either SC was stimulated. However, their amplitudes and relative contributions to the amplitude of the overall response varied from site to site along the mediolateral axis in the SC in a graded manner. As a consequence, several characteristic features related to stimulation sites in the SC were observed. Monosynaptic EPSPs with larger amplitudes were usually evoked by stimulation of the ipsilateral vs. the contralateral SC, whereas IPSPs that curtailed EPSPs were more clearly evoked by stimulation of the contralateral vs. the ipsilateral SC. The amplitudes of EPSPs evoked by stimulation of individual sites were different in a graded manner in the mediolateral direction: the EPSPs from the medial part were larger than those from the lateral part, because, of the 28 INC neurons in which clear EPSPs were evoked from more than 3 sites in the ipsilateral SC, 17 received larger EPSPs from the more medial site than the more lateral site, 3 neurons did not show a graded difference in the amplitudes of EPSPs, and 8 neurons received slightly larger EPSPs from the more lateral site in the ipsilateral SC. On the other hand, the lateral part of the contralateral SC tended to inhibit INC neurons more strongly than the medial part, because, of the 19 INC neurons in which clear IPSPs were evoked from more than 3 sites in the contralateral SC, 11 received larger IPSPs from the more lateral site than the more medial site, 2 neurons did not show a graded difference in the amplitudes of IPSPs, and 6 neurons received slightly larger IPSPs from the more medial site in the contralateral SC.

Effects of a midline section between the SCs on SC-evoked PSPs in INC neurons. Commisural inputs from the opposite SC are important sources of inputs to TRNs in the SC (Hoffman and Straschill 1971; Infante and Leiva 1986; Maeda et al. 1979; Moschovakis and Karabelas 1985). Single TRNs that project to the FFH and INC have axon collaterals projecting to the contralateral SC (Takahashi et al. 2005, 2007, 2010). Therefore, it was most likely that SC-evoked PSPs in INC neurons might involve effects exerted via axon reflex of these tectal commissural collaterals. To exclude the components evoked via tectal commissural fibers from PSPs evoked by the stimulation of individual mediolateral sites in the SC, the effects of sectioning the tectal commissural sites in the SC, the effects of sectioning the tectal commissure on synaptic inputs from the SC were examined in two cats. Before the tectal commissure was sectioned, intracellular potentials were recorded from some INC neurons to confirm the stimulating and recording conditions in each preparation. A section was made approximately on the midline at the level from the rostral to caudal end of the SC under direct visual observation (Fig. 5D). In coronal sections, the sectioned part began from the rostral...
end of the SC and coursed for 3.8 and 3.4 mm caudally in each preparation. The partial section began about 0.3 mm in front of the rostral end of the SC and ended 0.3 and 0.6 mm more caudally than the completely sectioned part in the inferior colliculus. In one animal, the section was almost in the midline, but in the other animal it was displaced to the contralateral side by 0.3–0.4 mm from the midline. The ventral border of the lesion was at the level of the central canal (Fig. 5D).

An example of SC-evoked PSPs in an INC neuron after the tectal commissure was sectioned is shown in Fig. 5, B and C. In this neuron, antidromic spikes were evoked at 0.6 ms by stimulation of the contralateral trochlear nucleus (not shown).

Fig. 5. Effects of a midline section between the bilateral SCs on SC-evoked PSPs in an INC neuron. A midline section was made between the bilateral SCs as shown in D. A–C: PSPs in an INC neuron evoked by stimulation of the contralateral INC (A) and contralateral (B) and ipsilateral SC (C) after the tectal commissure was sectioned. A: IPSPs evoked by stimulation of the contralateral INC at 200 μA before (a) and after Cl⁻ injection (b) with field potentials (c). B: PSPs evoked by single- (a) and double-shock stimulation (c and d) of the contralateral SC at 100 μA. The dotted line indicates the reversed IPSPs shown in d. Traces in b and e are field potentials recorded just outside the penetrated cell. C: PSPs evoked by stimulation of the ipsilateral SC at 100 μA before (a) and after Cl⁻ injection (b). Antidromic spikes were evoked in this neuron at 0.6 ms by stimulation of the contralateral trochlear nerve at 140 μA (not shown). D: photomicrograph of the coronal section of the SC embedded in gelatin showing the midline section between the bilateral SCs and the stimulating electrode lesion on the left.

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injection, the falling phases of these depolarizations did not change their polarity, indicating that the depolarizations were EPSPs, and these PSPs did not involve IPSPs (Fig. 5Cb). As shown in this example, monosynaptic EPSPs were still evoked, but subsequent IPSPs were not evoked by stimulation of the ipsilateral SC after the midline section in seven of eight INC neurons examined. In the same INC neuron, single-shock stimulation of the contralateral SC evoked very small PSPs (Fig. 5Ba), and double-shock stimuli at 100 μA still evoked relatively small IPSPs. These IPSPs had a latency of 1.9 ms and lacked preceding EPSPs (Fig. 5B, c and d). As shown in this example, monosynaptic EPSPs were not evoked by stimulation of the contralateral SC, but disynaptic IPSPs were still evoked in five of eight neurons examined after the midline section. These results indicated that monosynaptic EPSPs evoked by stimulation of the contralateral SC and disynaptic IPSPs evoked by stimulation of the ipsilateral SC in INC neurons before sectioning the tectal commissure (Figs. 2, I and J, 3, and 4) could be ascribed to axon reflex via commissural axon collaterals of TRNs projecting to INC neurons.

Synaptic inputs from the contralateral INC region to INC neurons. As mentioned above, stimulation of the contralateral INC region evoked antidromic spikes in many INC neurons (see Fig. 6Ba). However, lower stimulus intensities revealed large short-latency hyperpolarizations in most of the INC neurons (Fig. 6Bb, see also Fig. 5Aa) (48 of 68 neurons, 75.0%) (Fig. 7G). These hyperpolarizations were reversed to depolarizations after the injection of Cl⁻ into penetrated cells (Fig. 6Bc), indicating that they were IPSPs. The latencies of the IPSPs ranged from 0.5 to 1.8 ms (1.0 ± 0.3 ms, n = 48) (Fig. 7G). Since antidromic spikes of INC neurons evoked by stimulation of the contralateral INC had latencies of 0.3–1.1 ms (0.5 ± 0.3 ms, n = 43) (Fig. 7E), most of these IPSPs were considered to be monosynaptic. These findings strongly suggest that disynaptic IPSPs from the contralateral SC to INC neurons were mediated by contralateral INC neurons. To confirm that the contralateral INC mediates disynaptic IPSPs from the contralateral SC to INC neurons that projected to the contralateral trochlear nucleus, we examined whether the monosynaptic IPSPs evoked by stimulation of the contralateral INC region were facilitated by preconditioning stimulation of the contralateral SC. In the INC neuron shown in Fig. 6, B–D, stimulation of the contralateral SC evoked disynaptic IPSPs at a latency of 1.8 ms (Fig. 6Cb). In the same INC neuron, stimulation of the contralateral INC region (Fig. 6E) evoked monosynaptic IPSPs at 200 μA (Fig. 6Bb) at a latency of 1.1 ms. The stimulus intensities for both the contralateral SC and INC region were decreased, so that small IPSPs were evoked from both of them (Fig. 6D, a and b). When preconditioning stimulation of the contralateral SC was applied 1.2 ms before test stimulation of the contralateral INC (Fig. 6Dc), the evoked IPSPs were larger in amplitude and steeper in the falling phase than the algebraic sum of individual responses evoked by the preconditioning SC (Fig. 6Da) and the test INC stimuli (Fig. 6Db). The facilitation was most obvious at the interval of 1.2 ms and less clearly observed at other preconditioning-test intervals of 1.0–1.5 ms. Such spatial facilitation caused by the preconditioning SC stimuli indicated that SC stimulation activated cell bodies of INC neurons that mediated disynaptic IPSPs to the penetrated INC neuron. Similar facilitation was observed in three of five INC neurons tested. These findings indicate that a considerable part of contralateral INC neurons mediate disynaptic inhibition from the contralateral SC to INC neurons.

In addition to IPSPs, stimulation of the contralateral INC region often induced depolarizations in INC neurons (Fig. 7C). Synaptic effects evoked by stimulation of the contralateral INC with two different electrodes are shown in Fig. 7, C and D. In this INC neuron, stimulation with one electrode in the contralateral INC region at 300 μA evoked large depolarizations

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**Fig. 6.** Disynaptic IPSPs in an INC neuron conveyed via contralateral INC neurons from the contralateral SC. A: experimental setup with the intact tectal commissure. B: antidromic spikes (a) and IPSPs in an INC neuron evoked by stimulation of the contralateral INC before (b) and after Cl⁻ injection (c). The stimulus intensity was 400 (a) and 200 μA (b and c), respectively. The lower traces in a and c are field potentials. C: small EPSPs followed by IPSPs evoked from the contralateral SC with single- (a) and double-shock stimuli at 500 μA (b). Note the clear temporal facilitation of IPSPs in b. D: spatial facilitation of contralateral INC-evoked monosynaptic IPSPs in the INC neuron by preconditioning stimulation of the contralateral SC. Stimulation of the contralateral SC did not evoke clear IPSPs at 420 μA (a), but stimulation of the contralateral INC region evoked small monosynaptic IPSPs at 125 μA in the same neuron (b). Combined stimulation of the INC region (b) and the contralateral SC (a) evoked much larger and steeper IPSPs (c) than the algebraic sum of the IPSPs evoked by stimulation of the contralateral INC region (b) and the SC (a). The existence of such facilitation indicated that the disynaptic inhibition from the contralateral SC was mediated by contralateral INC neurons. E: photomicrograph of the coronal section of the midbrain showing the stimulation site (arrow) in the contralateral INC for B and D.
with orthodromic spikes in some traces (Fig. 7C) at a latency of 0.8 ms. These depolarizations were not reversed IPSPs, but rather EPSPs, since, just after stimulation of this INC region with this electrode, stimulation with the other electrode evoked hyperpolarizations in the same INC neuron (Fig. 7D). The exact location of the most effective stimulation site in the INC region was not determined, but in many other INC neurons, EPSPs followed by IPSPs were often evoked with the same stimulating electrode in the contralateral INC region, suggesting that the neurons of origin for these EPSPs and IPSPs were located close to each other. The latencies of INC-induced EPSPs ranged from 0.4 to 1.9 ms (0.9 ± 0.4 ms, n = 40) (Fig. 7F) and were considered to be monosynaptic. When EPSPs were not curtailed by subsequent IPSPs, these EPSPs were usually large, and their steep rising phases were powerful enough to generate orthodromic spikes in INC neurons with single-shock stimuli, as shown in Fig. 7C.

**Synaptic inputs from the SCs to INC neurons projecting to the contralateral oculomotor nucleus.** Some INC neurons that were antidromically activated from the contralateral trochlear nucleus were also antidromically activated from the contralateral oculomotor nucleus (Fig. 2, G and H). When the latencies of the antidromic spikes activated from the oculomotor nucleus were equal to or longer than those activated from the trochlear nucleus, it was safe to conclude that the electrical stimulation within the contralateral oculomotor nucleus activated axon collaterals of INC neurons that projected to the contralateral trochlear nucleus, but not their passing stem axons. However, in other cases, it was not possible to exclude the possibility of activating the passing fibers of stem axons of INC neurons that projected to the contralateral trochlear nucleus. Such cases were excluded from our sample of INC neurons that were identified as projecting to the contralateral oculomotor nucleus. These INC neurons that projected to both the trochlear and oculomotor nuclei on the contralateral side usually received monosynaptic excitation and disynaptic inhibition preferentially from the ipsilateral medial SC and the contralateral lateral SC, respectively.

There was another group of INC neurons that projected to the contralateral oculomotor nucleus (Fig. 8). These INC neurons were not antidromically activated from the contralateral trochlear nucleus (Fig. 8Ab) or the ipsilateral trochlear nucleus (Fig. 8Ac). Instead, they were antidromically activated from the contralateral oculomotor nucleus (Fig. 8Ac) and in some cases from the contralateral INC region (not in the example in Fig. 8) and the contralateral FFH (not in the example in Fig. 8). Stimulation of the ipsilateral and contralateral SC evoked monosynaptic EPSPs followed by disynaptic IPSPs in these INC neurons (Fig. 8B and C, and D and E, respectively). This pattern of synaptic inputs from the SCs was basically similar to, but the mediolateral distribution of the effective stimulating sites in the SCs for evoking EPSPs and IPSPs was very different from, those in INC neurons that projected to the contralateral trochlear nucleus. The INC neuron shown in Fig. 8, A–E, clearly had larger EPSPs from the lateral part of the ipsilateral SC than from its medial part (compare Fig. 8, B and C, sites 1–4). These early EPSPs were curtailed by later IPSPs, since the falling phases of the depolarizations became steeper and hyperpolarized after double-shock stimuli (Fig. 8, B and C). As for the PSPs evoked by stimulation of the contralateral SC, stimulation of the most medial site evoked small, monosynaptic EPSPs (Fig. 8E, site 5), and stimulation of the other three more lateral sites evoked clear disynaptic IPSPs (Fig. 8E, sites 6–8). The slopes of the falling phase of the EPSPs were steepest and the latencies were shortest from the medial part (Fig. 8E, site 6). Although we did not systematically examine the INC neurons with this pattern of antidromic responses, we confirmed a similar tendency in the mediolateral distribution of the effective stimulating sites in the ipsi- and contralateral SCs for evoking EPSPs and IPSPs, respectively, in seven such INC neurons. We could not determine the exact distributions of

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**Fig. 7.** EPSPs and IPSPs evoked by stimulation of the contralateral INC region in an INC neuron. A: experimental setup. Two stimulating electrodes were placed in the contralateral INC. B: antidromic spikes evoked in an all-or-none manner by stimulation of the contralateral trochlear nucleus at 210 μA. C: monosynaptic EPSPs and orthodromic spikes evoked by stimulation of the lateral part (site 2 in A) of the contralateral INC at 300 μA before the injection of Cl⁻ into the cell. D: monosynaptic IPSPs evoked by stimulation of the medial part (site 3 in A) of the contralateral INC at 250 μA before (top traces) and after the injection of Cl⁻ into the cell (middle traces). E–G: latency histograms of antidromic spikes and PSPs recorded in INC neurons. E: latency histogram of antidromic spikes evoked by stimulation of the contralateral INC. F and G: latency histograms of EPSPs (F) and IPSPs (G) evoked by stimulation of the contralateral INC region recorded in INC neurons that projected to the contralateral trochlear nucleus.
these INC neurons that projected to the contralateral oculomotor nucleus, but not to the trochlear nuclei.

Midbrain distribution of labeled neurons and axon terminals after iontophoretic injection of DB into the trochlear nucleus. To confirm and support the morphological findings obtained from transneuronal labeling with WGA-HRP, we injected DB iontophoretically into the right trochlear nucleus and mapped retrogradely labeled neurons in the midbrain (Fig. 9E, open circles).

The distribution of retrogradely labeled neurons in the midbrain showed a pattern that was mostly similar to that shown in Fig. 1, although there were much fewer labeled neurons. They were found in the ipsilateral FFH and bilaterally in the INCs. A smaller number of labeled cells were also present in the regions surrounding these nuclei. In addition to these retrogradely labeled neurons, a large number of axon terminals were labeled in the midbrain, and they were also plotted in Figs. 9E and 10B (dots). A large number of terminals were distributed in the FFH, including the area around the retrolflexus bundle (Fig. 9E, a–e), the INC and its surrounding area (Fig. 9E, g–j), the PCN (Fig. 9E, g–j), and the oculomotor nucleus (Figs. 9Ek and 10B) on the ipsilateral side. Within the oculomotor nucleus, most terminals were distributed ventrolaterally in the rostral two-thirds of it. This area corresponds to the subnucleus of the inferior rectus motor nucleus (Fig. 10B) (compare Fig. 10, B and C). The neurons of origin of these labeled axon terminals and the significance of the results will be addressed in the DISCUSSION.

DISCUSSION
The present study showed that neurons in the INC that projected to the contralateral trochlear and/or oculomotor nucleus received monosynaptic excitation from the ipsilateral SC and disynaptic inhibition via the contralateral INC from the contralateral SC. Many of them also projected to the contralateral INC and FFH. One group of these INC neurons projected to the contralateral trochlear and oculomotor nucleus and received monosynaptic excitation predominantly from the ipsilateral medial SC and disynaptic inhibition from the contralateral lateral SC. Our previous study showed that trochlear motoneurons receive disynaptic inhibition from the contralateral SC via the contralateral INC (Izawa et al. 2007). Therefore, the synaptic nature of these last-order premotor INC neurons that projected to the contralateral trochlear nucleus is considered to be inhibitory (see DISCUSSION below), and their on-direction is most likely upward. Judging from the pattern of input-output organization of these INC neurons, they are considered to be equivalent to IBNs in the horizontal saccade system. The second group of INC neurons projected to the contralateral oculomotor nucleus, but not to the trochlear nuclei, and received monosynaptic excitation predominantly.
from the ipsilateral lateral SC and disynaptic inhibition from the contralateral medial SC. This pattern of input-output organization of these INC neurons suggested that they were most likely IBNs for downward saccades. The mutual inhibition between these two groups of INC neurons on both sides is considered to form the reciprocal inhibition between the presumed IBNs for upward oblique saccades on one side and those for downward oblique saccades on the other (Fig. 11).

**Distribution of premotor neurons that terminated on trochlear motoneurons.** The localization of premotor neurons in the vertical eye movement system has been described previously (Büttner-Ennever et al. 1981; Steiger and Büttner-Ennever 1979). Wang and Spencer (1996) reported the localization of premotor neurons in the FFH that projected to motoneurons of different vertical eye muscles in the cat, but the location of premotor neurons in the INC has not been studied. Horn and Büttner-Ennever (1998) reported the distribution of premotor neurons in the riMLF and the INC following injection of fragment C of tetanus toxin into each eye muscle. They noted that there was always some spread to neighboring eye muscles, as judged from the direct labeling pattern of motoneurons. In the present study, we injected WGA-HRP into the nerve trunk, but not into the muscle, and neurons labeled by direct retrograde labeling were observed only in the trochlear nucleus. This guarantees that the transneuronal labeling in the present study was exclusively caused by uptake of the tracer from trochlear motoneurons. Since labeled neurons were preferentially located in the dorsolateral INC and also extended into the PCN, these neurons in the PCN and INC might form a single entity from the perspective of oculomotor function. This interpretation is consistent with the finding that the recording sites of the neurons analyzed in the present study were found not only in the INC but also in the PCN (Fig. 4B).

**INC neurons in the present study and previously known populations of INC neurons.** Many different targets of projections from the INC have been reported so far, such as the vestibular nucleus (Chimoto et al. 1999; Fukushima 1987, 1991; Fukushima et al. 1992; Kokkoroyannis et al. 1996; Markham et al. 1966; Pompeiano and Walberg 1957), spinal cord (Fukushima 1987), inferior olive (Kokkoroyannis et al. 1996; Onodera 1984; Zuk et al. 1982), and nucleus prepositus hypoglossi (Carpenter et al. 1970; Kokkoroyannis et al. 1996; Mabushi and Kusama 1970; Panneton and Martin 1979; Pompeiano and Walberg 1957). All of these projections are reported to be excitatory and exclusively or mainly ipsilateral. The INC neurons in the present study are different from those described previously, since the INC neurons were identified by their antidromic responses to stimulation of the contralateral trochlear and/or oculomotor nucleus.

Helmchen et al. (1996) fist analyzed characteristics of saccade-related burst neurons in the INC quantitatively. Since then, some different kinds of neurons related to saccades in the INC have been reported. Fukushima et al. (1991, 1995) reported burster driving neurons (BDNs) (later called vestibular and saccade neurons) in the cat, which showed characteristic discharges during pitch rotation (gradually increasing activity during upward/downward slow phases of nystagmus and a burst of spikes during downward/upward fast phases, respectively). Since they were activated at short latencies by stimulation of the contralateral vestibular nerve, these vestibular and saccade neurons were very similar to the BDNs reported by Ohki et al. (1988). If these INC neurons are the counterpart of the BDNs in the horizontal system, they should provide excitatory input to burst neurons in the vertical system on the opposite side. In contrast, the present INC neurons inhibited them instead, and therefore the present finding indicated that these two groups of INC neurons belonged to different functional groups.

**Origin of synaptic inputs to INC neurons evoked by electrical stimulation of the SC.** The presence of projection from the SC to the INC is has been controversial. Although early anatomical studies reported projection from the SC to the INC (Altman and Carpenter 1961; Edwards 1977), later anatomical studies did not confirm this finding (Edwards and Henkel 1978; Graham 1977; Harting et al. 1980). Grantyn and Grantyn (1982) demonstrated that tectal neurons projecting to the bulbar brain stem or the spinal cord in cats had thin axon collaterals that terminated in the INC. Mainly negative electrophysiological findings have been reported regarding inputs from the SC to the INC (Fukushima 1987; Grantyn and Grantyn 1982; King et al. 1980).

In contrast to the controversial projection from the SC to the INC, the projection from the pretectum (Benevento et al. 1977; Itoh 1977; Weber and Harting 1980) and PCN (Bernard 1977) to the INC seems to be accepted. Therefore, we had to ensure that synaptic inputs in INC neurons evoked by stimulation of the SC in the present study actually originated in the SC. During our recording session, each stimulus site in the SC was usually stimulated first at 500 μA to determine the presence or absence of synaptic effects. When some responses were obtained, the stimulus intensities were usually lowered, and we checked whether qualitatively similar responses were evoked. In many cases, the latencies became slightly longer by about 0.2–0.3 ms, but even bipolar stimulation at 100–150 μA still evoked clear PSPs in INC neurons. These findings indicated that the PSPs evoked at 500 μA represented the effect of stimulation of a rather restricted region around each SC stimulating electrode. Sasaki et al. (1970, 1972) estimated that 500 μA could not activate fibers or cells beyond 1.0 mm from an electrode tip when a concentric bipolar electrode was used. According to this estimation and the electrode positions that were confirmed histologically after each experiment, the effects of electrical stimulation of the SC in our preparation could be safely considered to be ascribed to the SC, and not to the pretectum (see Takahashi et al. 2005).

**Effects of tectal commissural connections of the SCs on synaptic inputs to INC neurons.** Stimulation of the SC on either side evoked EPSPs followed by IPSPs in INC neurons, although the EPSPs were predominant from the ipsilateral SC and the IPSPs from the contralateral SC. The presence of monosynaptic excitation of INC neurons from the SC on either side suggested that TRNs on one side projected to INC neurons on both sides. However, this was not likely, because the vast majority of projection of TRNs is ipsilateral above the midbrain regions (Grantyn and Grantyn 1982). Our previous study showed that excitatory commissural connections existed between the two SCs, especially in their rostral parts (Takahashi et al. 2007). These tectal excitatory commissural connections were conveyed via intratectal commissural neurons and/or commissural axon collaterals of TRNs projecting to the ipsilateral FFH and INC (Takahashi et al. 2010). Therefore, stimulation of the SC might activate commissural collaterals...
originating from TRNs in the contralateral SC, and these activated TRNs might exert effects on INC neurons on the contralateral side. The involvement of the effect of such axon reflex on SC-evoked PSPs in INC neurons was tested by a midline section of the tectal commissural connections. After the section, monosynaptic excitation from the ipsilateral SC and disynaptic inhibition from the contralateral SC were still present in INC neurons, but monosynaptic excitation from the
contralateral SC and disynaptic inhibition from the ipsilateral SC disappeared, indicating that they were evoked by activation of contralateral TRNs via axon reflex through their commissural axon collaterals.

King et al. (1980) showed that no synaptic potentials were observed in trochlear motoneurons, but clear synaptic potentials were observed in abducens motoneurons after stimulation of the SC under pentobarbital anesthesia. The present study showed that INC neurons that projected to contralateral trochlear motoneurons received clear synaptic inputs from the SC, but they were less powerful and less constant than those observed in IBNs in the horizontal saccade system (Sugiuchi et al. 2005), despite the similar experimental conditions, including anesthesia. The present study suggested that the difference in strength of synaptic inputs from the SCs to motoneurons and premotor neurons in the horizontal and vertical saccade systems was not due to weaker inputs from the SCs to neurons in the vertical saccade system per se, but rather due to the fundamental anatomical organization of the SCs. In the motor map of the SC, the medial part represents upward saccades, and the lateral part represents downward saccades (McIlwain 1986; Roucoux and Crommelinck 1976). Takahashi et al. (2005, 2007) reported the detailed properties of commissural inhibition and excitation of the SC. Reciprocal commissural inhibition exists between the SCs: from the medial part of one SC to the lateral part of the opposite SC, and vice versa. These tectal commissural inhibitory projections connect TRNs of the entire rostrocaudal extent of the SC for both horizontal and vertical saccades. In contrast, the commissural excitation exists between point-to-point symmetrical parts of the rostral SCs, i.e., between the medial parts or between the lateral parts of the two rostral SCs, and influences mainly rostral TRNs related to vertical or oblique saccades, but not TRNs related to horizontal saccades. The existence of such excitatory commissural connections between the rostral SCs would cause complex responses in premotor neurons in the vertical saccade system, since stimulation of the rostral SC should activate not only ipsilateral TRNs but also contralateral TRNs via commissural collaterals. Activation of these TRNs in the contralateral SC would evoke inhibition in INC neurons via commissural inhibitory INC neurons. As a result, stimulation of the ipsilateral SC might evoke monosynaptic EPSPs followed by disynaptic IPSPs in INC neurons (see Figs. 3 and 4), and hence action potentials would not be easily induced in these INC neurons. As a consequence, only small IPSPs might be evoked in trochlear motoneurons. In a similar way, it is also likely that, when the rostral SC is stimulated, excitatory inputs to EBns in the FFH would also be affected by inhibition via the axon reflex of TRNs. As a consequence, only small EPSPs might be evoked from the ipsilateral SC in trochlear motoneurons. In contrast, EBns and IBns in the horizontal saccade system are not significantly affected by inhibition caused by the axon reflex of TRNs (Sugiuchi et al. 2005), since TRNs with commissural axon collaterals do not exist in the caudal SC, where large horizontal saccades are represented (Takahashi et al. 2005, 2007, 2010). This difference of the tectal commissural connections may explain the apparent weaker synaptic inputs from the SC to trochlear motoneurons than to abducens motoneurons (Izawa et al. 2007; King et al. 1980).

Distribution of effective sites in the SC for evoking excitation and inhibition in INC neurons. Synaptic inputs to INC neurons were more easily evoked by stimulation of the rostral SC than the caudal SC. This finding was consistent with the anatomical finding that retrogradely labeled TRNs were mainly distributed in the rostral half of the SC after injection of a tracer into the FFH or INC, whereas label was distributed more evenly throughout the rostrocaudal extent of the SC after tracer injection into the IBN area in the ponto-medullary reticular formation (Takahashi et al. 2010). The pattern of synaptic inputs in INC neurons evoked by stimulation of the caudal SCs was simpler: monosynaptic excitation from the ipsilateral SC and disynaptic inhibition from the contralateral SC. This was most likely due to the absence of commissural axon collaterals and the presence of commissural inhibition in caudal TRNs (Takahashi et al. 2005, 2007). In contrast, the pattern of synaptic inputs evoked by stimulation of the rostral SCs was complex. In the present INC neurons projecting to the contralateral trochlear motoneurons, major excitatory inputs from the ipsilateral SC originated from its medial upward saccade area, whereas major inhibitory inputs from the contralateral SC originated from its lateral downward saccade area. In our previous study, we showed that SC-evoked disynaptic IPSPs were conveyed via the INC to trochlear motoneurons (Izawa et al. 2007). Therefore, the pattern of inputs from the SCs mentioned above strongly supports that these INC neurons are inhibitory in the synaptic nature, although it was impossible to completely exclude the possibility of the presence of very small EPSPs which might completely overlap the IPSPs.

Projection pattern of single INC neurons: basis for maintaining conjugacy in the vertical saccade system. The INC neurons in the present study that were antidromically activated from the contralateral trochlear nucleus were also often antidromically activated from the contralateral INC, FFH, and the oculomotor nucleus, but not from the ipsilateral FFH and trochlear nucleus (see also Sugiuchi et al. 2011). These findings indicated that axon collaterals of single INC neurons that projected to the contralateral trochlear nucleus might inhibit EBns in the contralateral FFH, similar to how single IBns in the horizontal saccade system inhibit both contralateral EBns and abducens motoneurons (Hikosaka et al. 1977). Such projection of single INC neurons with their axon collaterals to both trochlear motoneurons and EBns in the FFH on the
contralateral side can explain the presence of monosynaptic IPSPs in trochlear motoneurons from the ipsilateral FFH reported by Nakao and Shiraishi (1985). The presence of antidromic responses from the contralateral oculomotor nucleus in some of these INC neurons indicated that axon collaterals of the same INC neurons might inhibit neurons in the contralateral oculomotor nucleus and trochlear nucleus. Their target subnucleus of the oculomotor complex could not be determined electrophysiologically, since the subnuclei of different eye muscles are closely packed. However, the anatomical data shown in Fig. 10 suggested that the target subnucleus was the nucleus of the inferior rectus muscle, according to the topography of the subnucleus for each extraocular muscle reported by Akagi (1978) and Evinger (1988). Since no efferent neurons projecting to the midbrain from the trochlear nucleus have been found, the axon terminals labeled in the oculomotor nuclei after DB injection into the trochlear nucleus must have been labeled via axon collaterals of the retrogradely labeled neurons. There are several possible candidates for these neurons, including ipsilateral inhibitory and contralateral excitatory vestibular nucleus neurons (Graf et al. 1983; Iwamoto et al. 1990), contralateral inhibitory INC neurons, and ipsilateral excitatory FFH neurons. Indeed, all of these candidate neurons were retrogradely labeled, as shown in Fig. 9, B, C, and E, and consequently the labeled axon terminals may arise from all of

Fig. 10. Distribution of orthogradely labeled terminals in the oculomotor nucleus in the same animal as shown in Fig. 9 after injection of DB into the right trochlear nucleus (A and B), and retrogradely labeled inferior rectus motoneurons in another animal (C). A: photomicrograph of the orthogradely labeled axon terminals in the boxed area shown in the inset diagram of section b in B. B: magnified drawings of the area around the oculomotor nucleus of the same animal as shown in Fig. 9, A–E, showing the distribution of orthogradely labeled terminals (dots) in the subnuclei of the oculomotor nucleus. The dotted line indicates the subnucleus of the inferior rectus muscle as determined based on the distribution of retrogradely labeled motoneurons of the inferior rectus muscle shown in C. Note that most of the terminals were distributed in the area corresponding to the subnucleus of the inferior rectus. The calibration in B also applies to C. C: coronal sections of the oculomotor nuclei showing the distribution of retrogradely labeled motoneurons after WGA-HRP was injected into the inferior rectus muscle in another cat. IR, subnucleus of the inferior rectus muscle.

Fig. 11. Summary diagram of the input-output organization of inhibitory INC neurons for upward and downward saccades. d-EBN, an excitatory burst neuron (EBN) with a downward on-direction; d-IBN, an inhibitory burst neuron (IBN) with a downward on-direction; down-MN, a motoneuron involved in downward eye movements; d-TRN, a tectoreticular neuron in the downward saccade area in the SC (see Fig. 14B in Takahashi et al. 2007); u-EBN, an EBN with an upward on-direction; u-IBN, an IBN with an upward on-direction; up-MN, a motoneuron involved in upward eye movements; u-TRN, a tectoreticular neuron in the upward saccade area in the SC (see Fig. 14A in Takahashi et al. 2007).
these neurons. This pattern of projection of single last-order premotor neurons in the vertical saccade system is considered to be important for the formation of a functional synergy between a pair of eye muscles in both eyes and for maintaining conjugacy.

**Excitatory input from the INC to inhibitory INC neurons.** Stimulation of the contralateral INC often evoked large monosynaptic EPSPs in INC neurons that projected to the contralateral trochlear nucleus (Fig. 7C). Since these EPSPs were usually curtailed by monosynaptic IPSPs as an EPSP-IPSP complex following stimulation of the identical sites in the INC at low stimulus intensities, they were considered to originate from the INC and its adjacent structure. The precise source of this strong excitation was not identified in the present study, but the most likely candidate of this input is upward BDNs in the INC (Fukushima et al. 1991, 1995). Although the target of BDNs has not yet been identified, they might supply excitatory inputs to both EBNs and IBNs, judging from their characteristic activity pattern during vestibular quick phases and the similarity of these BDNs to horizontal BDNs (Ohki et al. 1988).

**INC neurons projecting to the contralateral oculomotor but not trochlear nucleus (presumed downward IBNs).** During our recording session, we encountered INC neurons that were not antidromically activated by stimulation of the contralateral trochlear nucleus, but the contralateral oculomotor nucleus (Fig. 8, A–E). In some of these INC neurons, antidromic responses were also evoked by stimulation of the contralateral FFH and INC. These INC neurons received monosynaptic EPSPs from the ipsilateral SC and disynaptic IPSPs from the contralateral SC (Fig. 8, B–E), the EPSPs were larger from the ipsilateral lateral SC (downward saccade area), and the IPSPs were larger from the contralateral medial SC (upward saccade area), which is the reverse of what was observed in INC neurons projecting to the contralateral trochlear nucleus. Therefore, these INC neurons may comprise a second group of INC neurons for vertical saccades. Based on this pattern of synaptic inputs from the SCs, they are most likely to be downward IBNs and terminate on motoneurons of eye muscles related to elevation of the eyes: the inferior oblique and superior rectus muscle.

**Mutual inhibition between inhibitory INC neurons on both sides.** The interaction between INC-evoked monosynaptic and SC-evoked disynaptic IPSPs in INC neurons showed that the monosynaptic IPSPs evoked by stimulation of the contralateral INC were facilitated by preconditioning stimulation of the contralateral SC (Fig. 6D). This finding indicated that the disynaptic inhibition in INC neurons from the contralateral SC was mediated by the contralateral INC neurons. INC neurons that projected to the contralateral trochlear nucleus were almost always strongly inhibited by stimulation of the contralateral INC, and the same INC neurons were often activated antidromically from the same stimulation site in the contralateral INC. This commissural inhibition between INC neurons on both sides most likely constitutes the reciprocal inhibition between the saccade systems for upward and downward directions with torsional components in opposite directions (Helmchen et al. 1996) (Fig. 11) and is similar to the reciprocal inhibition between IBNs for rightward and leftward saccades in the horizontal saccade system (Sugiuchi et al. 2005).

The inhibitory commissural connection exists between the medial SC representing upward oblique saccades on one side and the lateral SC representing downward oblique saccades on the other side (Takahashi et al. 2007, 2010). Takahashi et al. (2011) suggested that the SC output saccade system uses the same coordinate system as the semicircular canal-induced eye movement system, because this pattern of the reciprocal inhibition between the two SCs is similar to that seen in the oblique eye movements evoked by head rotation in the plane of the anterior semicircular canal on one side and the posterior semicircular canal on the other side. The present finding of the reciprocal inhibition between the presumed IBNs in the upward and downward oblique saccade systems further supports the suggestion that the SC saccade system and the semicircular canal-induced eye movement system use the common coordinate system (Takahashi et al. 2011).

**REFERENCES**


**AUTHOR CONTRIBUTIONS**

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**DISCLOSURES**

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