INTRODUCTION

The superior colliculus (SC) plays an important role in generation of saccades (Sparks and Hartwich-Young 1989). Electrical stimulation of the SC evokes saccades with a con-
were shown to convey a horizontal canal input to MR motoneurons (Baker and Highstein 1975; Furuya and Markham 1981; Highstein and Baker 1978). Despite the numerous studies to elaborate the neural organization of saccade generating mechanisms in the brain stem, the exact tectoabducens connections still remain controversial, and the tectooculomotor connections remain to be identified.

The present study was performed to reexamine the synaptic connections from the SC to horizontal ocular motoneurons in the anesthetized cat using intracellular recordings from LR and MR motoneurons and AINs, and electrical stimulation of the SC. To locate last-order premotor neurons that receive direct input from the SC and terminate on LR motoneurons, we labeled last-order premotor neurons with wheat germ agglutinin–conjugated horseradish peroxidase (WGA-HRP) transneuronally and tectoreticular axon terminals with dextran-biotin in the same preparations. The results show that excitation from the contralateral SC to LR motoneurons is mainly disynaptic via last-order premotor neurons in the ipsilateral SC. After these two experiments, 0.05 μl of 30% horseradish peroxidase (HRP) (Toyobo, Osaka, Japan) was injected into the abducens nucleus ipsilateral to the stimulated PPRF for retrograde labeling of last-order interneurons terminating in the abducens nucleus. After a survival time of 16 h, the animals were deeply anesthetized with pentobarbital sodium (45 mg/kg, Nembutal, Abbott, Switzerland) and perfused with 2 l of 10% sucrose phosphate buffer (pH 7.4) followed by 2 l of a fixative solution containing 2% paraformaldehyde and 1% glutaraldehyde in 4% sucrose phosphate buffer. Frozen sections of 75 μm were cut from the brain stem and the midbrain and treated for HRP by the tetramethylbenzidine method (Mesulam 1978).

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 for recording extracellular spikes in the SC. Negative pulses of 0.2 ms were delivered at 100–500 μA for stimulation of the SC, at <200 μA (usually <100 μA) for stimulation of the PPRF and at a maximum of 500 μA for stimulation of the MLF. The positions of the stimulating electrodes were marked by passing negative currents of 20 μA for 20 s after each experiment, and stimulated sites in the SC, PPRF, and MLF were histologically confirmed in sections stained with thionin. 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 concentration held at ~37 mmHg. The body temperature was kept between 37.0 and 38.5°C by a heating pad. Heart rate was constantly monitored by electrocardiogram.

To examine whether axon terminals of tectoreticular neurons terminate on last-order premotor neurons that, in turn, terminate on LR motoneurons, 12.5% dextran-biotin (Molecular Probes) was injected into the SC, and WGA-HRP (Toyobo, Japan) was injected into the abducens nerve in the same preparations in each of five animals (Sugiuchi et al. 1995). After 4–6 days, the animals were anesthetized with pentobarbital sodium (45 mg/kg) and perfused with 10% sucrose phosphate buffer (pH 7.4) followed by 2 l of a fixative solution containing 4% paraformaldehyde and 0.05% glutaraldehyde with 0.2% picric acid in 4% sucrose phosphate buffer. Frozen sections of 50 or 75 μm were cut from the brain stem and the midbrain, incubated in anti-WGA antibody and avidin-biotin complex, and then treated for HRP by the heavy metal intensification method of Adams (1981).

RESULTS

To determine the neural pathways from the SC to horizontal ocular motoneurons, intracellular potentials were recorded from LR and MR motoneurons and AINs in cats, and the effects of bilateral electrical stimulation of the superior colliculi 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 LR motoneurons

The resting membrane potentials of antidiromically identified LR motoneurons (Fig. 1A) ranged from −45 to −80 mV (−59 ± 12 mV, mean ± SD, n = 66). Single stimuli applied to the SC usually evoked no response or a very small response (Fig. 1, Ba and Ca). Double or triple stimuli of the contralateral SC evoked depolarization (Fig. 1Bb), and those of the ipsilateral SC evoked hyperpolarization (Fig. 1Cb) in a LR motoneuron. Latencies and amplitudes of these postsynaptic potentials (PSPs) fluctuated, suggesting that these responses were induced polysynthetically. The behavior of these PSPs on passing hyperpolarizing or depolarizing currents through the recording microelectrode confirmed that the depolarization evoked by contralateral SC stimulation was an excitatory postsynaptic potential (EPSP; Fig. 1D), and the hyperpolarization evoked by ipsilateral SC stimulation was an inhibitory postsynaptic potential (IPSP; Fig. 1E) (Eccles 1964).
As the stimulus intensity at the most effective site in the contralateral SC was increased, the size of early EPSPs increased, and their latencies decreased by 0.2–0.3 ms, whereas late EPSPs appeared on the falling phase of the early EPSPs (Fig. 2Aa). This indicates that stronger stimuli recruit a larger number of tectofugal neurons over a wider SC area. When double stimuli were used (Fig. 2Ab), multiple EPSPs of various latencies became prominent in addition to the facilitation of early EPSPs. This spatial facilitation in SC-evoked EPSPs and IPSPs was found in all of the examined LR motoneurons.

Increasing the number of stimuli produced a significant temporal facilitation of EPSPs (Fig. 2B). The increased number of preceding stimuli produced a greater facilitatory effect on the EPSPs evoked by the last stimulus in each trial in Fig. 2, Bb–Be, suggesting that more last-order excitatory interneurons were recruited. Similar temporal facilitation was also remark-

Fig. 1. Effects of stimulation of the superior colliculus (SC) on a lateral rectus (LR) motoneuron. A: antidromic spikes evoked at 200 μA. B and C: contralateral (B) and ipsilateral SC (C) stimulation-evoked intracellular potentials. a and b: single and double stimulation at 250 μA, respectively. D and E: intracellular responses evoked by stimulation of the contralateral (D) and ipsilateral SC (E). a: control records at rest. b: while passing hyperpolarizing current (3 nA). Bottom traces in individual records indicate field potentials recorded just extracellular. Calibration for A applies to B–E.

Fig. 2. Effects of stimulus intensity (A) and number (B and C) on postsynaptic potentials (PSPs) in LR motoneurons. A: single (a) and double stimulation (b) with increasing stimulus intensity (indicated on the left) of the contralateral SC. B: 1–5 stimuli (a–e) at 1.5-ms intervals given to the contralateral SC. Records were obtained from the same LR motoneuron as in A. In about 1/2 of the trials in a–e, the last stimulus was not applied, so that the effects of the last stimulus were given by the difference between the traces obtained with and without it. C: effects of stimuli on inhibitory postsynaptic potentials (IPSPs) in a LR motoneuron. a: IPSPs evoked by double stimulation (500 μA) of the ipsilateral SC. b: excitatory postsynaptic potentials (EPSPs) and IPSPs evoked by stimulation of the ipsilateral vestibular nerve (200 μA) before injecting Cl⁻ into the cell. c: reversed vestibular-evoked IPSPs at the same intensity but after injection of Cl⁻ into the same cell. d–f: effects of the number of stimuli on the reversed IPSPs in the same condition as in c. d: single stimulus. e: double stimuli. f: double stimuli in 4 trials and triple stimuli in 5 trials. g: extracellular field potentials. Calibrations in Aa and Ab apply to C and B, respectively.
able in IPSPs (Fig. 2C). To avoid the saturation of hyperpolarized IPSPs, IPSPs were reversed to depolarized IPSPs by injecting Cl\textsuperscript{−} into a motoneuron (Fig. 2, Cb–Cf). With an increase of stimuli, late IPSPs with an additional latency of ~1.0 ms were superimposed on the early IPSPs (Fig. 2Cf). The temporal facilitation in SC-evoked EPSPs and IPSPs was observed in all of the examined LR motoneurons. In each motoneuron examined, the number of stimuli was changed between one and five, while keeping the stimulus intensity constant, and the least number of effective stimuli for significant PSPs was determined, and then the number of the stimuli was further increased. The PSP latencies were measured by comparing PSPs evoked by the preceding stimuli with PSPs evoked by one more additional stimuli to the preceding stimuli (see Fig. 2, B and C). One to three additional SC stimuli to the first effective stimuli usually decreased such PSP latencies by 0.1–0.3 ms, but further increasing the number of the stimuli did not shorten their latencies. Therefore these shortest latencies were regarded as latencies for the PSPs. The latencies of contralateral SC-evoked EPSPs ranged from 0.9 to 1.9 ms (1.6 ± 0.2 ms, n = 56; Fig. 3A), and those of ipsilateral SC-evoked IPSPs ranged from 1.4 to 2.4 ms (1.8 ± 0.3 ms, n = 57; Fig. 3B).

The effect of the stimulation site in the SC on the size of PSPs was examined by recording PSPs from LR motoneurons while stimulating different parts of the SC with pulses of the same intensity. In the contralateral SC (Fig. 4, A1–A4), stimulation of the caudomedial site evoked the largest EPSPs at the shortest latency, and stimulation of the central site evoked smaller EPSPs at longer latencies, whereas rostral and rostro-lateral stimulation evoked small or no EPSPs. In the ipsilateral SC (Fig. 4, A5–A8), stimulation of the caudomedial and caudolateral sites evoked the largest IPSPs, whereas stimulation of the rostral and rostro-lateral sites evoked only small or no IPSPs. Similar tendencies were observed in all of the experiments. In three experiments, seven stimulating electrodes were arranged in the rostrocaudal direction of the SC along the horizontal meridian of the motor map (Fig. 4B). Evoked EPSPs became larger and their rising phase became sharper as the stimulation sites were shifted more caudally in the SC, but the most caudal stimulation usually evoked slightly smaller EPSPs. In general, stimulation at either the caudomedial or caudolateral site evoked large EPSPs.

The effect of electrode depth was examined by recording EPSPs from a LR motoneuron while a single stimulating electrode was moved vertically and stimuli of the same intensity were applied at different depths in the contralateral SC (Fig. 4C). The size of evoked EPSPs was small when the electrode was located in the superficial layers and increased together with its distance from the surface of the SC.

Inputs from the SC to internuclear neurons in the abducens nucleus

Inputs from the SC to AINs were examined by recording intracellular potentials from them while stimulating the SC. AINs received disynaptic EPSPs from the contralateral vestibular nerve (Fig. 5C) and IPSPs from the ipsilateral vestibular nerve (Fig. 5D) (cf. Baker and Highstein 1975). Single pulse stimulation of the SC evoked small PSPs more often in AINs than in LR motoneurons (Fig. 5, Ea and Fa). Increasing the number of stimuli always produced larger PSPs, with shorter latencies, with depolarizations from the contralateral SC (Fig. 5, Eb and Ec) and hyperpolarizations from the ipsilateral SC (Fig. 5, Fb and Fc). Intracellular injection of Cl\textsuperscript{−} reversed the depolarization evoked from the contralateral side (Fig. 5G) but did not affect the polarity of the depolarization evoked from the contralateral side, thus indicating that the depolarization was an EPSP and the hyperpolarization was an IPSP. The latencies of contralateral SC-evoked EPSPs ranged from 0.9 to 1.7 ms (1.4 ± 0.2 ms, n = 14; Fig. 3C), whereas those of ipsilateral SC-evoked IPSPs ranged from 1.3 to 2.1 ms (1.8 ± 0.3 ms, n = 12; Fig. 3D). The effects of stimulating various sites in the SC on AINs were similar to those on LR motoneurons.

Inputs from the SC to MR motoneurons

Intracellular potentials were recorded from MR motoneurons to investigate their inputs from the SC. Their resting
membrane potentials ranged from $-40$ to $-70$ mV ($-56 \pm 12$ mV, $n = 22$). MR motoneurons were identified by their antidromic responses to stimulation of the MR muscle nerve (Fig. 6A). Because the MR nerve is short from where it separates from the third nerve trunk, current sometimes spread from a stimulating electrode for the MR nerve to the IR or IO nerve in some preparations. To confirm that intracellular potentials were recorded from MR motoneurons, we always checked that the motoneurons activated by MR nerve stimulation were not activated by stimulation of the IR or IO nerve at lower stimulus intensities. MR motoneurons were also reliably distinguished from IR and IO motoneurons by examining their vestibular inputs. For example, stimulation of the ipsilateral vestibular nerve evoked small disynaptic EPSPs (1.2–1.8 ms, $1.4 \pm 0.2$ ms, $n = 10$) followed by larger trisynaptic EPSPs (1.9–3.1 ms, $2.4 \pm 0.3$ ms, $n = 10$) in MR motoneurons; contralateral stimulation of the vestibular nerve produced EPSPs (1.7–2.5 ms, $2.0 \pm 0.3$ ms, $n = 5$). In contrast, stimulation of the vestibular nerve evoked ipsilateral disynaptic inhibition and contralateral disynaptic excitation in IR and IO motoneurons (Ito et al. 1976).

In MR motoneurons, single stimulation of the ipsilateral SC often evoked small depolarization (Fig. 6Da), and double or triple stimuli always evoked larger depolarization at shorter latencies (Fig. 6B, Db, and Dc). This depolarization was an EPSP because injection of Cl$^-$ into the cell had no effect and injection of small depolarizing currents decreased the depolarization but did not reverse it. The latencies of these EPSPs ranged from 1.7 to 2.8 ms ($2.1 \pm 0.3$ ms, $n = 22$; Fig. 3E) and were longer by 0.7 ms on average than those of the EPSPs in AINs ($t$-test, $P < 0.001$). Stimulation of the contralateral SC did not evoke any potentials even with double or triple stimuli in most MR motoneurons (Fig. 6C), although EPSPs were evoked in some neurons from stimulation of the medial or rostral part of the contralateral SC. To ensure that EPSPs were not canceled by IPSPs or vice versa in such cases, either depolarizing or hyperpolarizing currents were always passed through the recording electrode, but no hidden PSPs were revealed. In some MR motoneurons, stimulation of the medial or rostral part of the contralateral SC evoked EPSPs, whereas caudal stimulation never evoked potentials even in such cases.

Excitation from the ipsilateral SC to MR motoneurons may be mediated by way of the contralateral AINs. These interneurons have been previously shown to relay excitation from the ipsilateral vestibular nerve to MR motoneurons (Baker and Highstein 1975), and, as the present results have shown, they
receive an excitatory input from the SC 0.7 ms before excitation is observed in MR motoneurons. To confirm that AINs relay excitation from the SC to MR motoneurons, the interaction of SC-evoked EPSPs and vestibular-evoked EPSPs was examined in a conditioning-test paradigm (Fig. 7). Stimulation of the ipsilateral vestibular nerve (Fig. 7 Eb) and that of the ipsilateral SC (Fig. 7 Ea) were adjusted to near threshold for evoking EPSPs in the same MR motoneuron. The SC conditioning stimulation given at 1.5 ms before the test vestibular stimulation did not affect the early disynaptic component of the vestibular-evoked EPSPs but increased the amplitude and rising slope of the later trisynaptic component in the MR motoneuron (Fig. 7Ec). The time course of this facilitation was examined by changing the intervals between the conditioning and test stimuli. Facilitation occurred when the vestibular stimuli were given at the same time as the SC stimuli (at 0 ms) reached its peak at 1.0 ms and then gradually decreased after 2.0 ms (Fig. 7F). Because conditioning SC stimulation facilitated the late trisynaptic component of the vestibular-evoked EPSPs, but not the early disynaptic component, these results suggest that an input from the SC and a vestibular input converge onto common AINs terminating on MR motoneu-
rons. Similar facilitation was observed in all of the eight MR motoneurons tested.

**Tectoreticular neurons projecting to the PPRF**

To determine the location of interneurons mediating excitation from the SC to LR motoneurons, last-order interneurons terminating on LR motoneurons were identified in the brain stem by transneuronal labeling after injection of WGA-HRP into the abducens nerve (Figs. 8–10). The details of the distribution of transneuronally labeled neurons will be reported separately (unpublished observations). Briefly, those neurons were mainly distributed ipsilaterally in the PPRF just rostral to the abducens nucleus (Fig. 9), the vestibular nuclei, and contralaterally in the PPMRF just caudomedial to the abducens nucleus (Fig. 10), the vestibular nuclei, and the prepositus hypoglossi nucleus. Among them, the PPRF just rostral to the abducens nucleus and contralateral to the SC most likely corresponds to the location where stimulation produced and lesion eliminated horizontal conjugate eye movements (Cohen et al. 1968), and where MLBNs that burst at the onset of a horizontal saccade were found (Cohen and Henn 1972; Luschei and Fuchs 1972).

To examine the projection of tectofugal neurons to this PPRF area, extracellular spikes were recorded from neurons in the SC. One group of tectoreticular neurons \((n = 19)\) was antidromically activated from the contralateral PPRF, but not from the descending MLF (Fig. 8B), and the other group \((n = 27)\) was activated from both the contralateral PPRF and the contralateral descending MLF (Fig. 8C). In the neuron in Fig. 8B, spikes activated by PPRF stimulation were regarded as antidromic, because they had a fixed latency of 1.9 ms even at threshold and followed double shock stimuli at a 0.9-ms interval. This neuron could not be activated by MLF stimulation at 500 \(\mu A\) (Fig. 8Bc). In the neuron in Fig. 8C, spikes were evoked by PPRF stimulation at a fixed latency of 0.8 ms at threshold and followed double shock stimuli at a 0.5-ms interval (Fig. 8Cb). This neuron was also activated by MLF stimulation at a latency of 0.8 ms (Fig. 8Cc). To examine the possibility of current spread from the PPRF stimulus to the nearby MLF, a spike collision test was carried out between spikes activated from the contralateral PPRF and MLF (Fig. 8, Ce–Ch) (Shinoda et al. 1976, 1986). Spikes evoked by the preceding PPRF stimulation had no effects on spikes evoked by the following MLF stimulation at latencies longer than 1.1 ms, but blocked MLF-evoked spikes at an interval of 1.0 ms (Fig. 8Cf). On the other hand, spikes evoked by the preceding MLF stimulation had no effect on PPRF-evoked spikes at intervals of 1.0 ms, but blocked PPRF-evoked spikes at intervals of 0.9 ms (Fig. 8Ch). By using the values (spike latencies, refractory periods, and maximal intervals for spike collision) obtained in this spike collision test, the additional conduction time along the axon collateral in the PPRF was calculated (Shinoda et al. 1976) to be 0.3 ms (Fig. 8F). If the stem axon had been activated by current spread from the PPRF, this...
FIG. 8. Antidromic activation of tectoreticular and tectoreticulospinal neurons in the SC from the contralateral paramedian pontine reticular formation (PPRF). A: experimental setup. Stimulating electrodes were placed in the left PPRF at a depth of 2.0 mm ~1 mm rostral to the abducens nucleus, and in the left descending medial longitudinal fascicle (MLF) near the obex. B: a tectoreticular neuron. Ba: antidromic spikes activated by PPRF stimulation at 75 μA (spike threshold, 50 μA). Bb: spikes activated by double shock stimulation at 0.9-ms intervals. Bc: MLF stimulation at 500 μA. C: a tectoreticulospinal neuron. Ca: PPRF stimulation at 45 μA (spike threshold, 30 μA).Cb: double-shock PPRF stimulation (45 μA) at 0.5-ms intervals.Cc: MLF stimulation at 150 μA. Cd: double-shock MLF stimulation (150 μA) at 0.5-ms intervals. Ce–Ch: spike collision experiment. Ce and Cf: PPRF stimulation (45 μA) preceded MLF stimulation (150 μA) by 1.1 (Ce) and 1.0 ms (Cf). Cg and Ch: the MLF stimulation preceded the PPRF stimulation by 1.0 (Cg) and 0.9 ms (Ch). D: latency histogram of antidromic spikes of tectoreticular neurons evoked by contralateral PPRF stimulation. E: latency histograms of antidromic spikes of tectoreticulospinal neurons evoked by contralateral PPRF (top histogram) and MLF stimulation (bottom histogram). F: conduction times along axon collaterals calculated based on the values obtained by the collision test in Ce–Ch. G and H: distribution of retrogradely labeled neurons projecting to or through the abducens nucleus relative to the position of the stimulating electrode in the PPRF (small arrow). Horseradish peroxidase (HRP) was injected into the center of the abducens nucleus ipsilateral to the stimulated PPRF, which was electrophysiologically identified by mapping antidromic field potentials in the abducens nucleus. G: lateral view of the brain stem (parasagittal section indicated by large arrows in H). Dots, retrogradely labeled neurons projecting to the ipsilateral abducens nucleus; hatched area, injection site of HRP. H: frontal view of the PPRF on the transverse plane indicated by large arrows in G. Retrogradely labeled neurons located 300 μm rostral and caudal to the stimulating electrode tip (small arrow) were plotted. IO and SO, inferior and superior olive; PN, pontine nucleus; TB, trapezoid body; VI n, abducens nerve. Calibration in H applies to G.
Conduction times along presumed axon collaterals in the PPRF were calculated in all of the neurons examined, and neurons were discarded from the sample when calculated conduction times were <0.1 ms (see the details of the calculation of axonal conduction time and associated problems in Shinoda et al. 1976, 1986).

At the conclusion of these experiments, the PPRF was identified by injecting HRP into the ipsilateral abducens nucleus. We confirmed that the electrolytic lesion marking the location of the stimulating electrode was located in the PPRF where retrogradely labeled neurons were abundant (Fig. 8, G and H) in both animals. Latencies of antidromic spikes from the PPRF were 0.5–2.8 ms (1.6 ± 0.6 ms, n = 19) for tectoreticular neurons (Fig. 8D) and 0.5–2.3 ms (1.0 ± 0.4 ms, n = 27) for tectoreticulospinal neurons (Fig. 8E). Tectoreticular neurons tended to have slower conducting axons, which is consistent with the finding that smaller spikes tended to be recorded from them. These tectofugal neurons were recorded at depths of 1.5–3.0 mm from the SC surface.

By comparing the latencies of PSPs evoked by SC stimulation (Fig. 3, A–E) and the antidromic latencies of tectoreticular neurons projecting to the PPRF, SC-evoked EPSPs and IPSPs in LR motoneurons, and EPSPs and IPSPs in AINs were most likely disynaptic. SC-evoked EPSPs in MR motoneurons were most likely trisynaptic, because their latencies were ~0.7 ms longer than those of disynaptic EPSPs in AINs.

**Morphological evidence of disynaptic excitatory and inhibitory pathways from the SC to LR motoneurons**

To confirm that tectoabducens connections are mainly disynaptic and to identify last-order premotor neurons in these connections, we anatomically examined the relationship between tectoreticular axons (identified by injecting dextran-biotin injection into the right SC at the same level of the brain stem in the identical animal). Labeled neurons and terminals on 3 serial sections (75 μm thick) were plotted on representative transverse sections.

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**FIG. 9.** Distributions of transneuronally labeled premotor neurons in the PPRF area ipsilateral to the injected left abducens nerve and tectoreticular axon terminals labeled after dextran-biotin injection into the right SC at the same level of the brain stem in the identical animal. Labeled neurons and terminals on 3 serial sections (75 μm thick) were plotted on representative transverse sections.

**FIG. 10.** Distributions of transneuronally labeled premotor neurons in the PPMRF area contralateral to the injected left abducens nerve and tectoreticular axon terminals labeled after dextran-biotin injection into the left SC at the same level of the brain stem in the identical animal. Same arrangement as in Fig. 9.
biotin into the SC) and last-order premotor neurons terminating on LR motoneurons (identified by transneuronal transport of WGA-HRP injected in the abducens nerve). Areas showing both the presence of tectoreticular axon terminals and transneuronally labeled neurons were restricted to the PPRF, PP-MRF, and to some extent in the abducens nucleus. Terminal axons in these areas were traced proximally, from serial sections, and they were seen to join the predorsal bundle after crossing the midline. Labeled tectoreticular axon terminals were distributed in the same PPRF area as transneuronally labeled neurons, but they spread more widely in the ventromedial direction (Fig. 9). Many of these labeled neurons seemed to have the labeled tectoreticular axon terminals on their cell bodies and proximal dendrites. Figure 11A shows a photomicrograph of such synaptic contacts of labeled tectoreticular axon terminals with a transneuronally labeled neuron in the PPRF ipsilateral to the abducens nucleus. Figure 12 shows a reconstruction of labeled tectoreticular axons terminating on transneuronally labeled neurons in the PPRF. In this example, 0.59 µl of dextran-biotin was relatively widely injected into the right SC, and 91 of 285 transneuronally labeled neurons in the PPRF ipsilateral to the injected abducens nerve had labeled tectoreticular axon terminals on them. In the other two experiments (0.1 µl and 3.3 µl injected), 24 of 177 and 104 of 262 transneuronally labeled neurons in the PPRF had labeled tectoreticular axon terminals on them, respectively. These morphological data show that the shortest main pathway from the contralateral SC to LR motoneurons is disynaptic via the ipsilateral last-order premotor neurons in the PPRF rostral to the abducens nucleus. Synaptic contacts of a small number
of labeled terminals observed in the abducens nucleus with retrogradely labeled LR motoneurons were scarcely observed in each preparation.

The distributions of contralateral last-order premotor neurons terminating on LR motoneurons and tectoreticular axon terminals were examined in a similar way (Fig. 10). Labeled premotor neurons were fairly localized in a vertical column in the PPMRF caudomedial to the caudal part of the abducens nucleus and at a depth of 0.5–2.5 mm from the surface of the fourth ventricle. Labeled tectoreticular axon terminals were distributed in this PPMRF area, although they spread more widely than the location of the premotor neurons. Many of the labeled premotor neurons in this area were contacted by labeled tectoreticular terminals (Fig. 11). Figure 13 shows a reconstruction of labeled tectoreticular axon terminals terminating on transneuronally labeled neurons in the PPMRF contralateral to the injected left abducens nerve. In this example, 3.1 μl of dextran-biotin was widely injected into the SC including its caudal half, and 42 of 112 transneuronally labeled neurons in the PPMRF contralateral to the injected abducens nerve had labeled tectoreticular terminals on their cell bodies and proximal dendrites. In another experiment, 2.5 μl of dextran-biotin was injected into the left SC widely including its caudal half, and 190 of 490 transneuronally labeled neurons in the PPMRF were contacted by labeled tectoreticular axon terminals on their cell bodies and proximal dendrites. These morphological data show that the shortest main pathway from the ipsilateral SC to LR motoneurons is disynaptic via the last-order premotor neurons located in the contralateral PPMRF caudomedial to the abducens nucleus. By comparing the electrophysiologically identified disynaptic excitatory and inhibitory connections and the above morphologically identified disynaptic connections between the SC and LR motoneurons, we conclude that the main connections between the SC and LR motoneurons are disynaptic. The excitatory pathway from the contralateral SC is relayed through PPRF neurons ipsilateral to the LR motoneurons, whereas the inhibitory pathway from the ipsilateral SC is relayed through PPMRF neurons contralateral to the LR motoneurons.

**DISCUSSION**

The present study has revealed that the main excitatory pathway from the contralateral SC to LR motoneurons is disynaptic via the ipsilateral PPRF, whereas the main inhibitory pathway from the ipsilateral SC to LR motoneurons is disynaptic via the contralateral PPMRF. In contrast to these reciprocal inputs to LR motoneurons, only excitation was observed in MR motoneurons from the ipsilateral SC; inhibition from the contralateral SC was not observed.

Precht et al. (1974) first reported that the average latency of EPSPs from the contralateral SC to LR motoneurons was 1.6
ms (1.2–2.2 ms) and concluded that the main connection from the contralateral SC to LR motoneurons was at least trisynaptic, although some disynaptic connections were present. However, Grantyn and Grantyn (1976) reanalyzed these pathways and concluded that EPSP latencies in the range of 0.8–1.0 ms were monosynaptic, whereas the most frequently observed latencies of 1.4–2.0 ms were disynaptic. In the present study, the latencies of the EPSPs ranged from 0.9 to 1.9 ms (1.6 ± 0.2 ms) and were very similar to the latency ranges reported by Precht et al. (1974) and Grantyn and Grantyn (1976). Multiple SC stimulation induced temporal facilitation and decreased latencies of EPSPs by 0.1–0.3 ms in virtually all LR motoneurons examined, indicating that these EPSPs were at least disynaptic. Comparison of conduction times of tectofugal fibers from the SC to the PPRF with the EPSP latencies indicate that most of the EPSPs are likely to be disynaptic, although some of the longer latency responses could involve an additional synapse. This electrophysiological conclusion is supported by our morphological findings that axon terminals of tectofugal fibers made direct contact with many transneuronally labeled neurons in the PPRF terminating on LR motoneurons.

The latencies of SC-evoked IPSPs in ipsilateral LR motoneurons in the present study (1.4–2.4 ms, 1.8 ± 0.3 ms) are similar to those reported by Precht et al. (1974) and Grantyn and Grantyn (1976). The latter group reasoned that because IPSP latencies were usually in the range of 1.8–2.4 ms, which is substantially longer than the EPSP latencies, the shortest inhibitory pathway from the ipsilateral SC to LR motoneurons had essentially one more synapse than the excitatory pathway (Grantyn and Grantyn 1976). Our results confirm that the IPSP latency is, on average, 0.2 ms longer than that of the contralaterally evoked EPSPs, but this seems insufficient time for an additional synapse. Instead, we ascribe this latency difference to the longer conduction distance for the inhibitory pathway and conclude that the inhibitory connection from the ipsilateral SC to LR motoneurons is predominantly disynaptic. Our anatomic data support the conclusion because axon terminals of tectofugal fibers made direct contact with transneuronally labeled neurons in the contralateral PPMRF terminating directly on injected LR motoneurons. With increased stimulus intensity or number of stimuli, SC-evoked disynaptic excitation and inhibition in LR motoneurons were greatly enhanced, and late polysynaptic components of PSPs were extensively recruited. The former phenomenon indicated the existence of spatial and/or temporal facilitation at the level of interneurons in the tectobulbocerebral pathways. Recruitment might be due to either of at least two underlying neural mechanisms: 1) intervening pontine reticular interneurons mediating disynaptic excitation to LR motoneurons fire repetitively, and 2) additional interneurons involved between the above interneurons and LR motoneurons are recruited. The latter mechanism has been discussed (see Fuchs et al. 1985 for references), but reliable experimental evidence has not been provided yet. Burst neurons in the PPRF do not continue to burst for a long time after cessation of repetitive stimulation of the SC (Grantyn and Grantyn 1976). Therefore long-lasting EPSPs are most likely mediated by additional interneurons such as vestibular nucleus neurons or prepositus hypoglossi neurons. Repetitive stimulation of the ipsilateral SC evoked late components of IPSPs in LR motoneurons, suggesting that excitatory reticular neurons in the PPRF may exert their influence on inhibitory reticular neurons in the PPRF. This possible pathway needs to be further investigated, although some burst neurons in the PPRF have a collateral near the abducens nucleus (Strassman et al. 1986).

The present study has shown that the patterns and latencies of inputs from the SC to LR motoneurons and AINs are similar; the main connection is disynaptic excitation from the contralateral SC and disynaptic inhibition from the ipsilateral SC, although the latencies of EPSPs recorded from AINs were slightly shorter. Grantyn and Grantyn (1976) reported EPSPs with latencies of 0.8–1.0 ms in a very small fraction of LR motoneurons, suggesting the existence of a monosynaptic connection. Axon terminals of tectofugal axons were scattered in the contralateral abducens nucleus as reported by Grantyn and Grantyn (1982) and Olivier et al. (1993). Direct contact of these terminals on retrogradely labeled LR motoneurons was rarely observed, suggesting that they might contact AINs, distal dendrites of LR motoneurons, or distal dendrites of other neurons.

SC stimulation evoked EPSPs in ipsilateral MR motoneurons at latencies of 1.3–2.6 ms, described as disynaptic (Grantyn and Berthoz 1977). In the present study, the latencies of EPSPs ranged from 1.7 to 2.8 ms (2.1 ± 0.3 ms), which were ~0.7 ms longer than in AINs. This suggests that the main pathway from the SC to ipsilateral MR motoneurons is trisynaptic. We have shown that the pathway from the ipsilateral SC to MR motoneurons is mediated via contralateral AINs, because conditioning stimulation of the ipsilateral SC facilitated the late component but not the early component of EPSPs evoked by ipsilateral vestibular stimulation in MR motoneurons. The discrepancy of the presence or absence of the short-latency EPSPs (<1.7 ms) in the previous and present studies remains to be explained. One possible explanation is that their SC stimulation might activate a part of the SC or an adjacent tegmental structure that is not related to saccades, because their stimulation depths were deeper than ours. The latencies of SC-evoked EPSPs in MR and LR motoneurons slightly overlapped each other, despite the fact that MR motoneurons receive excitation via one more intervening interneuron than LR motoneurons. This is partly because AINs have slightly stronger and shorter-latency inputs from the SC. Because MR motoneurons on one side and LR motoneurons on the other side must be synchronously activated for conjugate horizontal saccades, this neural mechanism is functionally significant and may explain why AINs start discharging earlier and have a higher velocity sensitivity than LR motoneurons (Delgado-Garcia et al. 1986). MR motoneurons received reciprocal inputs from the two superior colliculi, excitation from the ipsilateral SC and inhibition from the contralateral SC, and the latencies of contralateral SC-evoked IPSPs were 2.0–3.5 ms (Grantyn and Berthoz 1977). The inhibitory SC action on MR motoneurons appeared to be weaker under pentobarbital anesthesia than in “encephale isole” preparations (Grantyn et al. 1979). The ipsilateral dorsomedial reticular formation between the abducens and the trochlear nuclei was suggested as a source for monosynaptic inhibition of MR motoneurons (Grantyn et al. 1980). In the present study under chloralose anesthesia, contralateral SC stimulation usually did not evoke any short-latency IPSPs in MR motoneurons, but sometimes evoked
EPSPs. The pathway responsible for these EPSPs remains
underdetermined but may be related to a vergence eye movement.

Precht et al. (1974) first reported that the excitatory and
inhibitory tectoabducens pathways ran from the tectum through
the ipsilateral midbrain reticular formation to LR motoneurons.
Grantyn et al. (1979) showed that the pathways underlying the
transmission of oligosynaptic excitatory and inhibitory effects
from the SC to LR motoneurons decussate in the mesencephalic
tegmentum, and the second decussation of the inhibitory
tectoabducens pathway occurs at the level of the abducens
nucleus. Grantyn and Berthoz (1977) first analyzed the anatomic
paths from the SC to MR motoneurons by comparing
responses in MR motoneurons before and after transecting the
pontobulbar structures and speculated that reticulospinal
eurons in the paramedian pontine tegmentum receiving monosynaptic
input from the contralateral SC would exert their monosynaptic
excitation on MR motoneurons. Later, the “abducens region”
involved interneurons within or adjacent to the abducens
nucleus was thought to be responsible for disynaptic excitatory
connection between the SC and MR motoneurons (Grantyn et al.
1979), but Grantyn and co-workers had difficulty in explain-
ing disynaptic connections between the SC and MR moto-
neurons without assuming that tectofugal fibers establish
monosynaptic connections with AINs. The present analysis
revealed that AINs mainly received disynaptic excitation from
the contralateral SC and disynaptic inhibition from the ipsilat-
eral SC. Conditioning stimulation of the ipsilateral SC did not
exert any effect on the disynaptic vestibuloculomotor re-
sponse but had a facilitatory effect on the trisynaptic vestibu-
loculomotor response. Accordingly, AINs were regarded as
last-order excitatory premotor neurons from the ipsilateral SC
to MR motoneurons, whereas vestibular nucleus neurons do
not appear to be part of this pathway.

The combination of electrophysiological and morphological
methods we employed allowed us to overcome some of the
drawbacks of previous lesion and microstimulation studies and
to specify last-order interneurons that directly receive SC out-
puts and terminate on LR motoneurons. In the present study,
30% of the transneuronally labeled reticular neurons in the
PPRF or the PPMRF were directly contacted by tectoreticular
axon terminals. This value is probably an underestimation,
because the small amounts of dextran-biotin we injected into
the SC could label only a small portion of tectofugal neurons.
With this reservation in mind, it seems safe to conclude that
tectoreticular axons contact the somata and proximal dendrites
of the vast majority of PPRF neurons that terminate directly on
ipsilateral LR motoneurons and of PPMRF neurons that ter-
minate directly on contralateral LR motoneurons, and that
disynaptic connections between the SC and LR motoneurons
are substantial. Together with the electrophysiological find-
ings, it is safe to conclude that the reticuloabducens neurons
with direct tectoreticular contacts in the ipsilateral PPRF and in
the contralateral PPMRF are excitatory and inhibitory, respec-
tively. The question arises as to whether all PPRF neurons
contacted by contralateral tectofugal neurons and terminating
on ipsilateral LR motoneurons are excitatory, and all PPMRF
neurons contacted by contralateral tectofugal neurons and ter-
minating on contralateral LR motoneurons are inhibitory.
Because tectal stimulation evoked only disynaptic EPSPs in
contralateral LR motoneurons, we conclude that all transneu-
ronally labeled PPRF neurons contacted by tectofugal axons
exert an excitatory influence on ipsilateral LR motoneurons.
On similar grounds, we conclude that all transneuronally la-
beled PPMRF neurons contacted by tectofugal axons exert an
inhibitory influence on contralateral LR motoneurons.

The PPRF contains MLBNs that exhibit a high-frequency
burst of spikes before and during saccades in monkeys and cats
(Cohen and Henn 1972; Hikosaka and Kawakami 1977; Keller
1974; Luschei and Fuchs 1972; Yoshida et al. 1982). These
MLBNs are considered to be premotor neurons that directly
terminate on LR motoneurons (Hikosaka and Kawakami 1977;
Hikosaka et al. 1978; Yoshida et al. 1982). Raybourn and
Keller (1977) examined the effect of SC stimulation on MLBNs
to determine the pathway from the SC to LR motoneu-
rons and showed that MLBNs could not be activated at a short
latency by SC stimulation in alert monkeys, whereas long-lead
burst neurons (LLBNs) received short-latency excitatory input
from the SC with about one-third of the responses being in the
monosynaptic range. In contrast, Chimmoto et al. (1996) showed
that MLBNs in the paramedian reticular formation rostral and
caudal to the abducens nucleus were activated monosynapti-
cally from the contralateral SC in alert cats. It seems likely that,
in the cat, these MLBNs in the ipsilateral PPRF are the relay
neurons that are responsible for disynaptic excitation from the
contralateral SC to LR motoneurons and those in the contralat-
eral PPMRF are responsible for disynaptic inhibition from the
ipsilateral SC to LR motoneurons.

The effects of stimulation at various depths in the SC on
single LR motoneurons showed that deeper stimulation in-
duced steeper-rising and larger EPSPs, whereas superficial-
layer stimulation induced small EPSPs. Tectoreticular neurons
activated antidromically from the PPRF were located at 1.5–
3.0 mm from the SC surface, depths that corresponded to the
intermediate and deep layers of the SC (Kawamura and
Hashikawa 1978; Moschovakis and Karabelas 1985). Stimula-
tion of different tectal sites evokes characteristic saccadic eye
movements in the awake cat (Guitton et al. 1980; McIlwain
1986), and the spatial distribution of effective SC stimulating
sites for evoking EPSPs in horizontal ocular motoneurons is
generally similar to the “motor map” obtained for horizontal
saccadic components in awake animals. The longer the dis-

tance from the rostral pole of the SC, the larger the EPSP evoked
in LR motoneurons. Anatomical data show that neurons in the
SC extensively project to the contralateral nucleus reticularis
ponsis caudalis and the rostral part of the nucleus reticularis
gigantocellularis (Grantyn and Grantyn 1982; Kawamura et al.
1974; Scudder et al. 1996). In agreement with previous results
(Grantyn and Grantyn 1982), there are two groups of tectore-
ticular and tectoculospinal neurons. In the present study,
60% (27/46) of tectoreticular neurons projecting to the con-
tralateral PPRF were found to send their axons to the spinal
cord. Our recent morphological study showed that tectospinal
neurons emit multiple axon collaterals in the cervical cord, and
spinal interneurons receiving monosynaptic excitation from the
contralateral SC directly terminate on neck motoneurons (Muto
et al. 1996). Therefore the SC could influence neck muscles
through such tectoculospinal neurons as well as reticulospinal
neurons that receive monosynaptic input from the SC.
(Kakei et al. 1994; Sasaki 1992). The SC of the cat can be divided into three anteroposterior zones from a functional point of view: the anterior, intermediate, and posterior zones (Guitton et al. 1980). Tectal output neurons projecting to the spinal cord predominantly exist in the intermediate zone (Muto et al. 1996). Therefore tectoreticulospinal neurons in this zone contribute to gaze control by a large-amplitude eye movement and synchronous neck movement to fixate a visual target in the periphery of the visual field (Guitton et al. 1980).

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