

Brainstem Circuits Triggering Saccades and Fixation

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Omnipause neurons (OPNs) in the nucleus raphe interpositus have tonic activity while the eyes are stationary (“fixation”) but stop firing immediately before and during saccades. To locate the source of suppression, we analyzed synaptic inputs from the rostral and caudal superior colliculi (SCs) to OPNs by using intracellular recording and staining, and investigated pathways transmitting the inputs in anesthetized cats of both sexes. Electrophysiologically or morphologically identified OPNs received monosynaptic excitation from the rostral SCs with contralateral dominance, and received disynaptic inhibition from the caudal SCs with ipsilateral dominance. Cutting the tectoreticular tract transversely between the contralateral OPN and inhibitory burst neuron (IBN) regions eliminated inhibition from the caudal SCs, but not excitation from the rostral SCs in OPNs. In contrast, a midline section between IBN regions eliminated disynaptic inhibition in OPNs from the caudal SCs but did not affect the monosynaptic excitation from the rostral SCs. Stimulation of the contralateral IBN region evoked monosynaptic inhibition in OPNs, which was facilitated by preconditioning SC stimulation. Three-dimensional reconstruction of HRP-stained cells revealed that individual OPNs have axons that terminate in the opposite IBN area, while individual IBNs have axon collaterals to the opposite OPN area. These results show that there are differences in the neural circuit from the rostral and caudal SCs to the brainstem premotor circuitry and that IBNs suppress OPNs immediately before and during saccades. Thus, the IBNs, which are activated by caudal SC saccade neurons, shut down OPN firing and help to trigger saccades and suppress (“latch”) OPN activity during saccades.

Key words: eye movement; inhibitory burst neuron; oculomotor; omnipause neuron; saccade trigger; superior colliculus

Significance Statement

Saccades are the fastest eye movements to redirect gaze to an object of interest and bring its image on the fovea for fixation. Burst neurons (BNs) and omnipause neurons (OPNs) which behave reciprocally in the brainstem, are important for saccade generation and fixation. This study investigated unsolved important questions about where these neurons receive command signals and how they interact for initiating saccades from visual fixation. The results show that the rostral superior colliculi (SCs) excite OPNs monosynaptically for fixation, whereas the caudal SCs monosynaptically excite inhibitory BNs, which then directly inhibit OPNs for the initiation of saccades. This inhibition from the caudal SCs may account for the omnipause behavior of OPNs for initiation and maintenance of saccades in all directions.

Introduction

Saccades are fast eye movements that redirect gaze to an object of interest in the visual field by bringing its image onto the fovea for visual fixation. The superior colliculus (SC) is involved in

saccade generation (Wurtz and Albano, 1980; Sparks and Hartwich-Young, 1989; Sparks and Mays, 1990; Leigh and Zee, 1991; Moschovakis and Highstein, 1994; Soetedjo et al., 2002; May, 2006). It has been proposed that the SC is composed of the following two zones: a fixation zone located in its most rostral pole and a saccade zone located more caudally (Munoz and Guitton, 1989, 1991; Munoz and Wurtz, 1993a, b). Fixation zone neurons fire tonically to help maintain foveal fixation on the target, whereas saccade zone neurons fire a burst before saccades to generate gaze shifts toward peripheral targets. However, subsequent studies cast doubt on this view. These studies found that the rostral SC contains neurons with movement field properties similar to those observed in the more caudal SC. Specifically, these rostral neurons increase firing during microsaccades, the very small, fast eye movements made during foveation (Hafed et al., 2009; Hafed and Krauzlis, 2012). This supports an alternative view of collicular organization in which the SC forms a

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continuous map from caudal to rostral SC sites, and where the population of the active neurons encodes the target location in oculocentric coordinates (Sparks et al., 1976; Krauzlis et al., 1997).

If the rostral and caudal SCs have different functional roles, connections from these sites to the brainstem premotor circuitry should be different. Robinson (1973, 1975) proposed a model of the saccade control system in which two command signals from a higher center were postulated to act in parallel. One was a saccade driving signal to activate oculomotor burst neurons (BNs) and the other was a saccade-triggering signal to suppress omnipause neurons (OPNs) in the brainstem. OPNs in the nucleus raphe interpositus (Büttner-Ennever et al., 1988) have tonic activity during fixation, and cease firing before and during saccades (Cohen and Henn, 1972; Luschei and Fuchs, 1972; Evinger et al., 1977, 1982; Keller, 1977; King et al., 1978, 1980; Kaneko and Fuchs, 1982). Consequently, OPNs have been considered to be at the center of saccade suppression (Gandhi and Keller, 1999a, b) and initiation in many computational models of saccade generation. However, a neural substrate for the inhibition of OPN activity immediately before and during saccades still remains unsolved. One possible source is inhibitory BNs (IBNs). However, there is a timing problem; IBNs are thought to receive disynaptic or trisynaptic excitation from the SC, whereas OPNs can be inhibited disynaptically from the SC (King et al., 1980; Kamogawa et al., 1996; Yoshida et al., 2001). Furthermore, OPNs start decreasing their activity 7.6 ms before the onset of IBN burst activity (Yoshida et al., 1999). Accordingly, these findings suggested that inhibitory interneurons other than IBNs are responsible for the inhibition of OPNs at the saccade onset. Instead, IBNs are believed to suppress OPN activity during saccades (Scudder et al., 1988), although there is a lack of axonal projections by IBNs to OPNs (Yoshida et al., 1982; Strassman et al., 1986).

We have previously described neural circuits of saccade driving systems for horizontal (Izawa et al., 1999; Sugiuchi et al., 2005; Takahashi et al., 2005a) and vertical saccades (Izawa et al., 2007; Takahashi et al., 2010; Sugiuchi et al., 2013; Takahashi and Shinoda, 2018). In the present study, we examined neural pathways from the rostral and caudal SCs onto OPNs and IBNs by using intracellular recording and staining techniques with electrical stimulation in anesthetized cats. We specifically explored how saccade driving and suppressing systems interact with each other by examining connections between IBNs and OPNs. The results show that the rostral SCs excite OPNs monosynaptically for fixation, whereas the caudal SCs monosynaptically excite IBNs, which then directly inhibit OPNs for saccade initiation. Parts of this study have been briefly reported previously (Takahashi et al., 2005b).

Materials and Methods

Experiments were performed in 14 cats weighing 2.8–4.3 kg (6 male, 8 female). Animal experimentation was performed in accordance with “Policies on the Use of Animals and Humans in Neuroscience Research” revised and approved by the Society for Neuroscience in 1995, and “Guiding Principles for the Care and Use of Animals in the Field of Physiologic Sciences” (The Physiologic 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, i.m.) and xylazine (1 mg/kg) followed by α -chloralose (initial dose, 40–45 mg/kg, i.v.; supplemental additional doses, 10–

25 mg/kg, i.v.) or pentobarbital sodium (10–15 mg/kg, i.v.), when necessary, throughout the duration of the experiment. During recording, animals were artificially ventilated with the end-tidal CO₂ held at 35–40 mmHg. Heart rate was constantly monitored by electrocardiogram. Body temperature was kept at 37.0–38.5°C by a heating pad. Animals were mounted in a stereotaxic frame fixed on a horizontal turntable, which could be rotated very smoothly by hand. For intracellular recording, the experimental setup was essentially the same as reported in a previous article (Sugiuchi et al., 2005; Fig. 1A). Briefly, the bone over the parietal and occipital cortices was removed, and the cerebral cortex was removed by aspiration bilaterally to allow the introduction of stimulating electrodes into the SCs on both sides under direct visual observation. Four concentric bipolar stimulating electrodes were arranged rostrocaudally at 1.0–1.2 mm intervals along the presumed horizontal meridian of the motor map in the SC (McIlwain, 1986) on either side, and their tips were positioned in the intermediate or deep layer (1.5–2.0 mm deep from the surface) of the SC (Kawamura and Hashikawa, 1978; Moschovakis and Karabelas, 1985; Moschovakis et al., 1998; Izawa et al., 1999; May, 2006).

For identification of neurons in the OPN region by antidromic activation, two concentric bipolar electrodes (interelectrode distance, 1.0 mm) were placed in the field of Forel (FFH) on either side to activate projections to the vertical gaze center. The rostrocaudal level of the FFH electrode tips was determined stereotactically (A 7.0–7.5 mm; Nakao and Shiraishi, 1985), with the aid of a stereotaxic atlas of the brainstem (Snider and Niemer, 1961), and their dorsoventral position was fixed at the level where the maximal field potentials were evoked by electrical stimulation of the ipsilateral SC (Sugiuchi et al., 2013). Glass microelectrodes for intracellular recording were filled with 0.4 M KCl or 2 M potassium-citrate and had a resistance of 15–25 M Ω . Before starting intracellular recording, the location of the abducens nucleus was identified by mapping type II responses to horizontal rotation of the turntable (Shinoda and Yoshida, 1974). The location of the abducens nucleus was used as a landmark for recording sites in the OPN region, and for placement of the stimulating electrode in the contralateral IBN region. Wheat-germ agglutinin–horseradish peroxidase (HRP; Toyobo) was injected into the excitatory burst neuron area in the paramedian pontine reticular formation (PPRF; Sugiuchi et al., 2005), and retrogradely labeled neurons were plotted in the nucleus raphe interpositus (RIP) for determining the distribution of OPNs in the brainstem. To identify OPNs by their antidromic spikes, an array of four electrodes arranged at 1 mm intervals in a dorsoventral direction was placed in the contralateral paramedian pontomedullary reticular formation (PPMRF) where IBNs are located (Izawa et al., 1999; Sugiuchi et al., 2005). The same electrode arrangement was also used for investigating the presence or absence of direct connections of IBNs with OPNs. Negative pulses of 0.2 ms in duration were delivered at <500 μ A (usually <300 μ A) for stimulation of the SC and the FFH, and <200 μ A (usually <100 μ A) for stimulation of the PPMRF. Ranck (1975) estimated an effective current spread of 1.0–1.5 mm around an electrode tip when using monopolar stimulation at 500 μ A (duration pulse, 200 μ s) in the mammalian CNS. Since we used bipolar stimulation with a concentric electrode, the effective current spread should be much less than the values estimated by Ranck (1975) and Shinoda et al. (1977). We estimated that 500 μ A could not activate fibers or cells beyond 1.0 mm from an electrode tip in the cat, when a concentric bipolar electrode is used (Sasaki et al., 1970, 1972). The positions of the stimulating electrodes in the FFH, SCs, and the brainstem were marked by passing negative currents of 20 μ A for 20 s after each experiment, and were histologically verified in sections stained with thionine.

To identify recording sites histologically, a glass microelectrode used for intracellular recording was left in the OPN region for a marker at the end of each experiment. The position of a broken tip of the glass microelectrode was identified histologically on serial sections and used for reference to reconstruct the other electrode tracks. Stimulation sites in the SCs and the IBN and FFH regions were also reconstructed histologically on serial sections in every experiment. To morphologically confirm electrophysiologically identified cells as OPNs, recorded neurons were injected iontophoretically with HRP

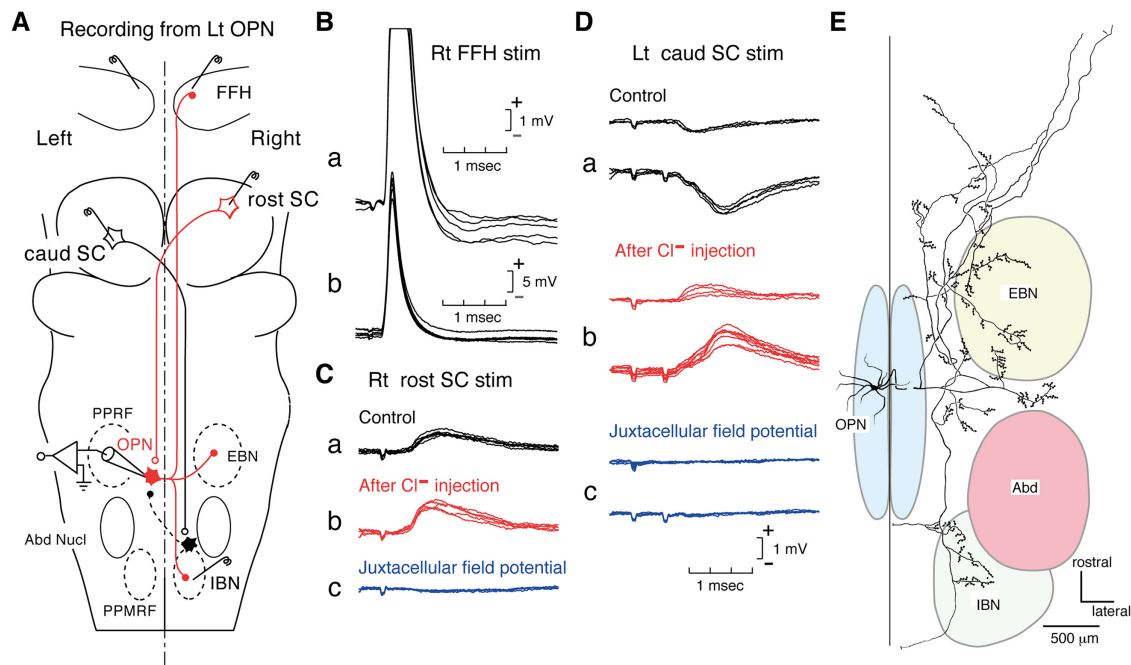


Figure 1. Morphologic and electrophysiological identification of OPNs. **A**, Experimental setup viewed from above showing the locations of stimulating electrodes placed bilaterally in the FFH and rostral and caudal sites in the SC, as well as contralaterally in the IBN region. Left (Lt) OPNs, located just off the midline and medial to the Abd and EBN region, were recorded intracellularly. rostral SC, Rostral SC; caudal SC, caudal SC; Nucl, nucleus. **B**, Antidromic spikes in a left OPN evoked by stimulation of the contralateral [right (Rt)] FFH (200 μ A) at low (b) and high (a) magnification. **C**, **D**, Intracellular PSPs in the OPN evoked by stimulation of the contralateral (right) rostral SC (300 μ A; **C**) and the ipsilateral (left) caudal SC (300 μ A; **D**). a, Control (black traces); b, after injection of Cl^- (red traces); c, juxtacellular field potentials (blue traces). The same color scheme is used to show synaptic inputs in the following figures. Hyperpolarizing PSPs (**D**, a) were reversed to depolarizing potentials (**D**, b) after intracellular iontophoretic injection of Cl^- into the cell. Top and bottom traces in **D** show PSPs evoked by single-pulse and double-pulse stimulation, respectively. Calibration in **D**, c, applies to **C** and **D**, and positive potentials are represented upward in all traces in the following figures. **E**, Dorsal view of the axonal trajectory of an HRP-stained OPN reconstructed from 74 serial coronal sections of the brainstem (80 μ m thick). HRP was iontophoretically injected through a recording micropipette (15 nA, 14 min) into the electrophysiologically identified OPN.

(Toyobo) after taking electrophysiological data. Electrophysiologically identified cells as IBNs were also morphologically confirmed with the same method (Sugiuchi et al., 2005). Positive pulse currents (200 ms duration) of 10–20 nA were passed through the HRP-filled recording microelectrode at 2.5 pulses/s for 3–20 min for both OPNs and IBNs. At the end of the experiments after a survival time of 5–11 h, animals were deeply anesthetized with ketamine hydrochloride (25 mg/kg, i.m.) followed by pentobarbital sodium (50 mg/kg, i.v.) and perfused with 2 L of 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 coronal serial sections (80 μ m thick) were cut on a freezing microtome. The axonal trajectories of the stained neurons were reconstructed from serial transverse sections of the brainstem under a microscope equipped with a drawing tube. Details of the intracellular iontophoretic staining method with HRP, staining histochemistry, and the approach used for reconstruction of the axonal trajectory of a single stained neuron have been described previously (Shinoda et al., 1981, 1982, 1986, 1992).

All quantitative data in this study are presented as the mean $\pm$ SD; an unpaired *t* test was used to characterize the statistical significance when comparing different mean values. The significance level was set at *p* < 0.05.

Results

Electrophysiological and morphologic identification of OPNs

To examine synaptic potentials evoked from the SCs, we penetrated cells in the medial part of the pontine reticular formation from the middle level of the abducens nucleus to 2.5 mm rostral to it, from 1.0–4.0 mm deep from the floor of the fourth ventricle, and within 0.5 mm from the midline in cats (Keller, 1977; King et al., 1978, 1980; Nakao et al., 1980; Curthoys et al., 1981;

Evinger et al., 1982; Langer and Kaneko, 1983; Ohgaki et al., 1987, 1989). The location of the abducens nucleus was identified by recording type II unit activity around the genu facialis in response to horizontal rotation of the turntable at the beginning of each experiment (Maeda et al., 1972; Shinoda and Yoshida, 1974; Baker and Highstein, 1975). All lateralities reported herein are described with reference to the recording site, if not stated otherwise.

From previous studies, it is known that OPNs send axons to the FFH (Langer and Kaneko, 1983; Nakao et al., 1988; Ohgaki et al., 1989), the excitatory BN (EBN) region in the PPRF, and the IBN region in the PPMRF (King et al., 1980; Nakao et al., 1980, 1988; Furuya and Markham, 1982; Langer and Kaneko, 1983; Curthoys et al., 1984; Ohgaki et al., 1987; Strassman et al., 1987). Since we could not examine the firing properties of neurons recorded in the nucleus raphe interpositus during saccades or the quick phases of nystagmus in the present anesthetized preparations, we systematically searched for neurons in the midline pontine region, and identified them as OPNs when they satisfied the following four criteria: (1) neurons were located in the OPN region mentioned above based on reconstructions; (2) neurons were activated antidromically by stimulation of the FFH [Fig. 1B (see also Fig. 10B)], and/or the IBN region on either side (see Fig. 10C); (3) neurons received monosynaptic excitation from the ipsilateral or contralateral SC as previously reported (Fig. 1C; Raybourn and Keller, 1977; Paré and Guitton, 1994; Chimoto et al., 1996; Yoshida et al., 2001; Takahashi et al., 2005a); and (4) neurons should satisfy characteristic morphologic features of OPNs.

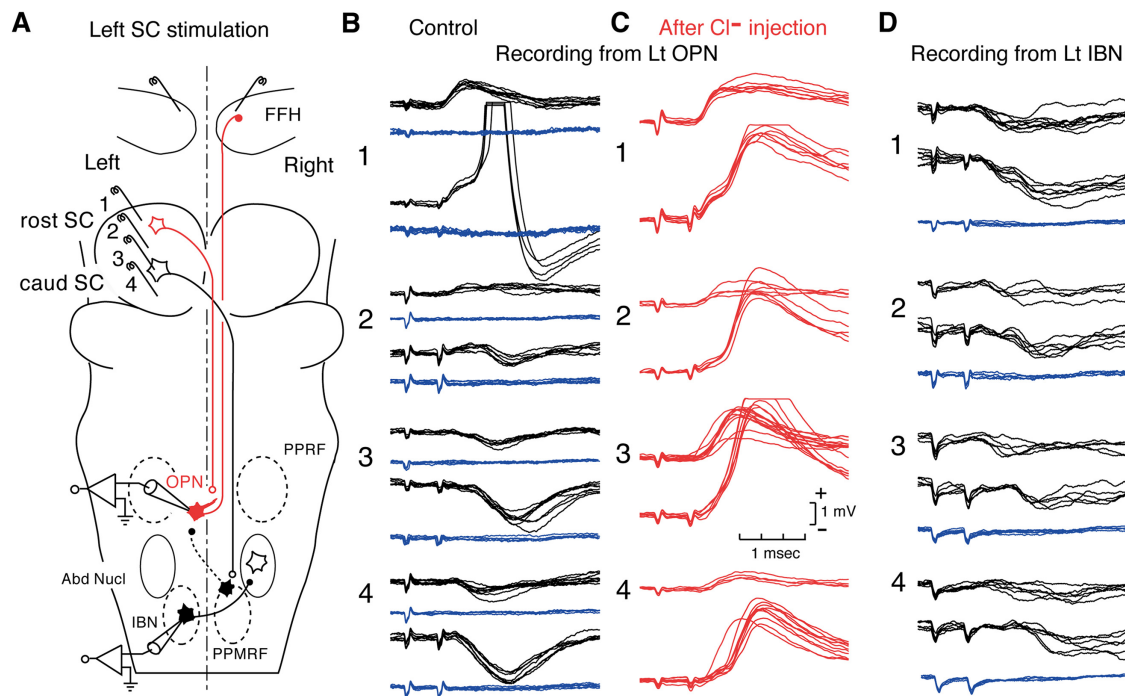


Figure 2. Pattern of synaptic input from the ipsilateral [left (Lt)] SC recorded in a left OPN (**B**, **C**) and that in an IBN (**D**) in the same cat. **A**, Experimental setup. **B**, **C**, PSPs in a left OPN evoked by single (top traces) and double-pulse (bottom traces) stimulation (500 μ A) of the ipsilateral SC before (**B**) and after Cl^- injection into the cell (**C**). Calibration in **C**, 3, applies to all traces in **B**, **C**, and **D**. In **B**, 1, early depolarizations were EPSPs, since they did not change their polarity (**C**, 1), but later PSPs contained IPSPs, since Cl^- injection increased later depolarizations in their falling phase (**C**, 1). Hyperpolarizations in **B**, 2–4, were IPSPs, since they changed their polarity after Cl^- injection (**C**, 2–4). **D**, PSPs recorded in a left IBN evoked by double-pulse stimulation (500 μ A) of the ipsilateral SC. Disynaptic IPSPs were evoked from all four stimulation sites in the left SC (**D**, 1–4). Calibration in **C**, 3, applies to all traces in **B**–**D**. For details of the pattern of synaptic input from the ipsilateral SC to IBNs, see the study by Sugiuchi et al. (2005). rostr, Rostral; caud, caudal.

Neurons that satisfied the first three criteria were tentatively presumed to be OPNs and were further examined as to the detailed nature of synaptic inputs from individual stimulating sites in the SCs on both sides. However, after the spike-generating mechanism deteriorated or a lesion was made in the brainstem for determining neural circuits, the second criterion of antidromic spikes could no longer be used. In such cases, the third criterion by itself was sufficient for reliable identification, because IBNs receive disynaptic inhibition (see Figs. 4D, 5), and abducens motoneurons (Abd MNs) receive disynaptic excitation from the contralateral rostral SC (Sugiuchi et al., 2005), whereas OPNs receive monosynaptic excitation from the contralateral rostral SC (see Figs. 4B, 5).

The fourth criterion was used to further confirm the recorded cells as OPNs. We injected HRP iontophoretically into cell bodies or proximal axons of presumed OPNs that satisfied the first three criteria, then reconstructed their axonal trajectories using serial sections at the early stage of the present experiments. Of 20 neurons injected with HRP, 15 neurons were recovered, and their main axons and cell bodies could be identified in the RIP ipsilateral to the injection sites. Among the 15 neurons, 10 neurons were stained well enough to trace their axonal trajectories for some distance, although all of the axon branches of each neuron could not be traced to their terminals. Figure 1E shows a typical example of the morphology of such a stained neuron. The cell body of this neuron was located 0.5 mm rostral to the rostral end of the abducens nucleus, 0.4 mm lateral to the midline and 2.5 mm ventral from the floor of the fourth ventricle. As in this example, individual stained neurons usually had 10–15 dendrites that lacked spines extending from their cell bodies, and individual dendrites bifurcated once or twice. These dendrites spread more or less in all directions within a radius of $\sim 500 \mu\text{m}$. When

their cell bodies were close to the midline, some of their dendrites crossed the midline to the opposite side and their dendritic field extended into the medial reticular formation on both sides. The stem axon of this stained neuron ran horizontally and medially to cross the midline almost at a right angle. It then bifurcated into main ascending and descending branches. The ascending branch that entered into the PPRF ramified extensively and gave rise to axon terminals there. Some of their axon collaterals seemed to extend further rostrally, but they could not be traced further because of the insufficient labeling. Without any axonal collaterals to the abducens nucleus, the descending branch that passed the middle level of the abducens nucleus gave rise to fine axon branches in the PPMRF. In addition to these typical OPNs with axonal projections to the EBN and IBN regions contralaterally, some OPNs projected ipsilaterally and some others located near the midline projected bilaterally. This axonal projection pattern of the presumed OPNs in the PPMRF and the PPRF described above is consistent with the previous morphologic findings on OPNs (Ohgaki et al., 1987; Strassman et al., 1987). In addition, many of these neurons were inhibited by stimulation of the caudal SCs (Fig. 1D). After identifying OPNs using the above criterion, we conducted further electrophysiological analysis.

Synaptic inputs from the SCs to OPNs

To understand properties of synaptic inputs from the posited rostral “fixation zone” and caudal “saccade zone” of the SCs to OPNs, we analyzed intracellular potentials evoked in OPNs by stimulation of the rostral and caudal parts of the SCs on both sides [Fig. 2 (see also Figs. 4, 5)]. In most penetrated OPNs, the spike-generating mechanism deteriorated very rapidly after an electrode penetration probably because of the small soma size, noted in previous intracellular recording from

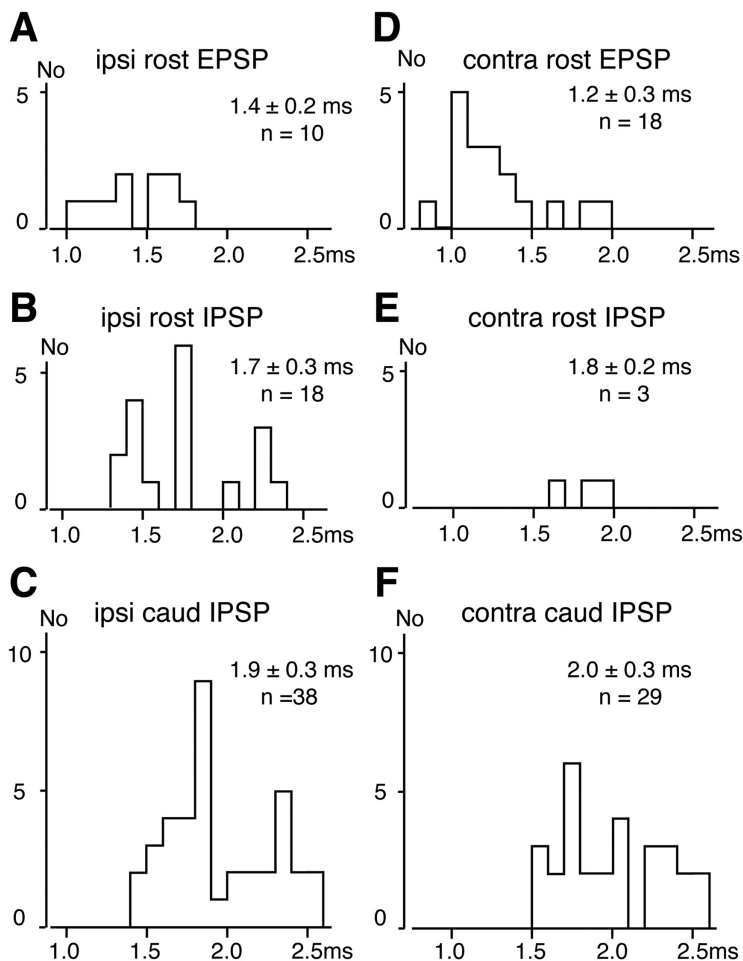


Figure 3. *A–F*, Latency histograms of PSPs in OPNs evoked from the ipsilateral (ipsi) SC (*A–C*) and from the contralateral (contra) SC (*D–F*). *A, D*, EPSPs evoked from the rostral (rostr) SCs. *B, E*, IPSPs evoked from the rostral SCs. *C, F*, IPSPs evoked from the caudal (caud) SCs. Abscissas, Latencies of PSPs; ordinates, number of cells recorded. Numerical values in each panel indicate the mean $\pm$ SD and the total number of cells. Latencies of PSPs were determined by comparing control traces with juxtacellular field potentials or comparing control traces with traces after injection of Cl^- . The shortest latencies are plotted among PSPs evoked from the rostral two electrodes or from the caudal two electrodes of the four electrodes placed in each SC. When double-pulse stimulation was used, the latencies of PSPs were determined by comparing the PSPs evoked by single-pulse and double-pulse stimulation.

OPNs (Ohgaki et al., 1987; Yoshida et al., 2001). As a consequence, only small spike-like depolarizations often remained in response to stimulation of the FFH and the IBN region (data not shown). These spike-like sharp depolarizations were antidromic spikes, since they were activated in an all-or-none manner at the same thresholds for activating antidromic full spikes in the same cells before their deterioration. Therefore, these small spikes were considered to be either axon spikes or spikes generated at an initial segment, after the spike-generating mechanism in cell bodies deteriorated (Eccles, 1964). The resting membrane potentials of OPNs ranged from -40 to -70 mV (mean $\pm$ SD, -48 ± 12 mV, $n = 52$). The properties of evoked postsynaptic potentials (PSPs) were identified by passing depolarizing or hyperpolarizing currents or by injecting Cl^- into the cell (Coombs et al., 1955a, b; Eccles, 1964). All lateralities in this article are described with reference to the recording site, if not stated otherwise.

As to the effects of SC stimulation on OPNs, we will first describe synaptic inputs from the ipsilateral SC to OPNs, because OPNs tended to receive clear synaptic inputs from the ipsilateral

SC. Figure 2 shows an example of intracellular potentials evoked in a left OPN by either one or two stimulating pulses to the ipsilateral SC at four positions within the SC from the rostral (1) and moving caudally (4). In the example neuron, stimulation of the most rostral site in the ipsilateral SC evoked depolarizations (Fig. 2*B*, 1). On the other hand, double pulse stimulation of caudal parts of the ipsilateral SC evoked larger hyperpolarizations (Fig. 2*B*, 2–4, lower traces), and single stimuli evoked smaller hyperpolarizations (Fig. 2*B*, 3, 4, top traces). The size of the hyperpolarizations increased as more caudal SC stimulation sites were activated (Fig. 2*B*, 2–4). After injection of Cl^- into the cell through the recording electrode, the polarity of the depolarizations from the most rostral site was not changed (Fig. 2*C*, 1), whereas the polarity of the hyperpolarizations from the remaining caudal parts of the SC was changed to the depolarizing direction (Fig. 2*C*, 2–4). Therefore, the depolarizations from the rostral SC were regarded as EPSPs, and the hyperpolarizations as IPSPs (Coombs et al., 1955b; Eccles, 1964). Comparison of the depolarizing potentials in Figure 2, *B*, 1, and *C*, 1, showed that the falling phase of the depolarizations in Figure 2*C*, 1, was more depolarized than in Figure 2*B*, 1, indicating that the IPSPs were also involved in later PSPs evoked from the most rostral SC. This pattern of input from the ipsilateral rostral and caudal SCs was typical of OPNs observed in this study.

To compare patterns of input from the ipsilateral SC to OPNs and IBNs, PSPs evoked in an IBN in the same preparation are shown in Figure 2*D*. IPSPs were evoked from all the rostrocaudal sites in the ipsilateral SC. These IPSPs in IBNs are classified as disynaptic based on their latencies of 1.4–2.4 ms (mean, 1.8 ± 0.2 ms; $n = 69$), and are shown to be transmitted via input from contralateral IBNs (Sugiuchi et al., 2005). In OPNs in the present

study, the latencies of the EPSPs from the most rostral SC stimulation were 1.0–1.7 ms (mean, 1.4 ± 0.2 ms; $n = 10$; Fig. 3*A*), and are 0.4 ms longer than the latencies of antidromic spikes in rostral SC tectoreticular neurons (TRNs) evoked from the OPN region (mean, 1.0 ± 0.4 ms; $n = 26$; $p < 0.01$, t test; Takahashi et al., 2005b, their Fig. 5*D*). Taking into consideration a 0.3–0.4 ms delay for synaptic transmission (Eccles, 1964), these EPSPs were regarded as monosynaptic from the ipsilateral rostral SC. In contrast, the latencies of the IPSPs from the caudal SC in the OPNs ranged from 1.4 to 2.5 ms (median, 1.9 ± 0.3 ms; $n = 38$; Fig. 3*C*). These IPSP latencies were 1.2 ms longer than the latencies of antidromic spikes of caudal SC TRNs evoked by stimulation of the OPN region (mean, 0.7 ± 0.2 ms; $n = 44$; $p < 0.001$, t test; Takahashi et al., 2005b, their Fig. 5*Aa*). This latency difference was much longer than a 0.3–0.4 ms synaptic delay, indicating that these IPSPs in OPNs were regarded as disynaptic from the ipsilateral caudal SC. These IPSPs are very similar to those reported previously for disynaptic IPSPs evoked in Abd MNs (1.4–2.4 ms; mean, 1.8 ± 0.3 ms; $n = 57$; Izawa et al., 1999) and IBNs (1.3–2.4 ms;

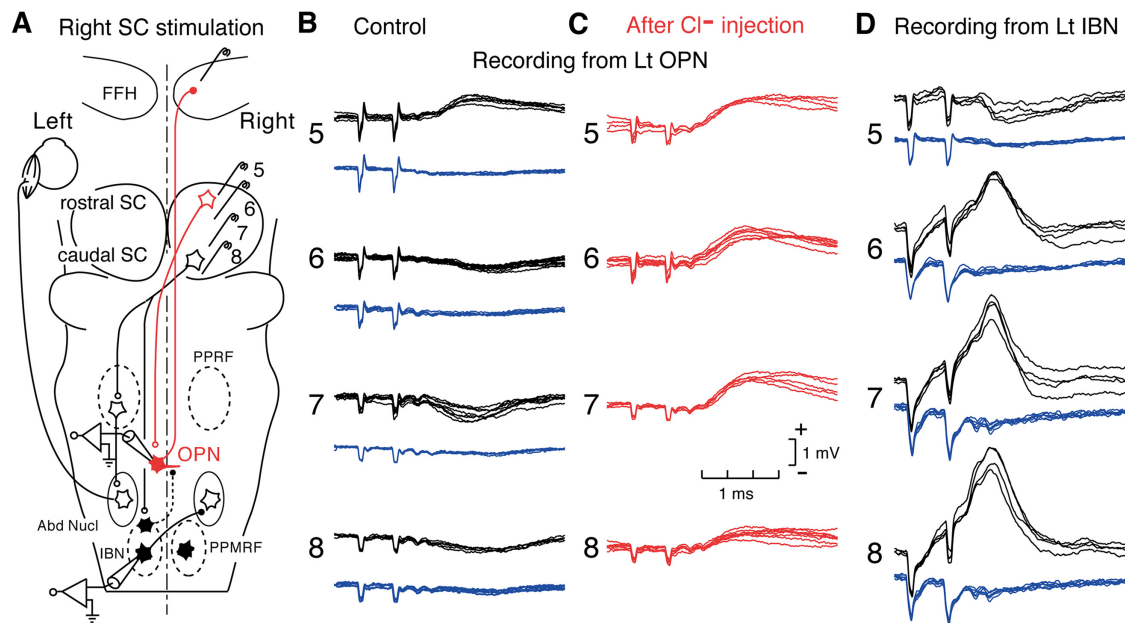


Figure 4. Comparison of patterns of synaptic input from the contralateral (right) SC recorded in a left OPN (**B**, **C**) and a left IBN (**D**) in the same cat. **A**, Experimental setup. **B**, **C**, PSPs in a left OPN evoked by double-pulse stimulation (500 μ A) of the contralateral SC (sites 5–8) before (**B**) and after (**C**) Cl^- injection into the cell. Depolarizations in **B**, 5, were EPSPs, since they had no component whose polarity was reversed in **C**, 5, whereas hyperpolarizations in **B**, 6–8, were IPSPs, since their polarity was reversed to depolarizations. Calibration in **C**, 7, applies to all traces in **B**–**D**. **D**, PSPs recorded in a left IBN evoked by double-pulse stimulation (500 μ A) of the right SC. Disynaptic IPSPs were evoked from the most rostral part (**D**, 5) and monosynaptic EPSPs from the remaining caudal parts of the SC (**D**, 6–8). For details of the pattern of input from the contralateral SC to IBNs, see Sugiuchi et al. (2005). Nucl, Nucleus; Lt, left.

mean, 1.8 ± 0.3 ms; $n = 52$; Sugiuchi et al., 2005, their Fig. 6H) by stimulation of the ipsilateral caudal SC. The disynaptic IPSPs were also evoked by stimulation of the rostral SC (Fig. 3B). These IPSPs are most likely because of the activation of TRNs located in the rostral SC that cause small macrosaccades or because of current spread to the more caudal TRNs.

Next, we describe the effect of stimulation of the contralateral SC on a left OPN (Fig. 4B). Again, stimulation of the most rostral site evoked depolarizations (Fig. 4B, 5), whereas stimulation of the more caudal sites evoked hyperpolarizations (Fig. 4B, 6–8). Injection of Cl^- into the cell reversed the hyperpolarizations to depolarizing potentials (Fig. 4C, 6–8), but the depolarizations from rostral SC stimulation remained almost unchanged (Fig. 4C, 5). Accordingly, we again conclude that this OPN received EPSPs from the most rostral site, and IPSPs from the more caudal sites. A similar pattern of input from the rostrocaudal sites in the contralateral SC was commonly observed. However, while the EPSPs evoked in OPNs from the ipsilateral and contralateral rostral sites were consistent and similar, but with contralateral dominance (compare Fig. 3A and Fig. 3D), the amplitude of the IPSPs from the contralateral caudal sites was usually not as large, and their falling slopes were not as sharp, compared with those from the ipsilateral SC. For comparison of input from the contralateral SC to OPNs and IBNs, PSPs evoked in an IBN are shown in Figure 4D. IPSPs were evoked from the rostral SC (Fig. 4D, 5), whereas large EPSPs were evoked from the caudal SC (Fig. 4D, 6–8). These IPSPs were classified as disynaptic and the EPSPs as monosynaptic (Sugiuchi et al., 2005). As shown in Figure 4, B and D, the patterns of input were reversed from the rostral and caudal parts of the contralateral SC in OPNs and IBNs. IBNs receive the strongest monosynaptic inputs from the most caudal SC and gradually decreased inputs from the more rostral SC (Fig. 4D). Assuming that SC projections to EBNs and IBNs are similar, this finding shows the opposite trend from the SC to PPRF (Moschovakis et al., 1998). The

latencies of the EPSPs evoked from the rostral SC site in OPNs ranged from 0.8 to 1.9 ms (mean, 1.2 ± 0.3 ms; $n = 18$; Fig. 3D). The latencies of antidromic spikes of TRNs in the rostral SC evoked by stimulation of the OPN region ranged from 0.4 to 2.0 ms (mean, 1.0 ± 0.4 ms; $n = 26$; Takahashi et al., 2005b, their Fig. 5D) in cats and from 0.7 to 2.3 ms (mean, 1.13 ± 0.38 ms; $n = 30$) in monkeys (Gandhi and Keller, 1997). The latencies of the rostral SC-evoked EPSPs were ~ 0.2 ms longer than those of antidromic spikes of rostral TRNs. Taking into consideration a 0.3–0.4 ms delay for synaptic transmission (Eccles, 1964), the EPSPs were monosynaptic from the rostral SC. The latencies of the IPSPs from the contralateral caudal SC sites were 1.5–2.5 ms (mean, 2.0 ± 0.3 ms; $n = 29$; Fig. 3F). These latencies were ~ 0.8 ms longer than those of the monosynaptic EPSPs evoked from the rostral SC site (Fig. 3D; $p < 0.001$, t test), and very similar to those of disynaptic IPSPs in Abd MNs (Izawa et al., 1999) and IBNs from the ipsilateral SC sites (Sugiuchi et al., 2005, their Fig. 6H). Therefore, these IPSPs from the contralateral SC to OPNs were regarded as disynaptic. As shown in Figures 2 and 4, inhibition from the ipsilateral caudal SC was large and its falling phase was sharp, while inhibition from the contralateral caudal SC was not so clear in many OPNs (compare Fig. 3, C, F), and IPSPs were not evoked from the rostral SC (Fig. 3E). However, in some OPNs, inputs from the two SCs were very similar as shown in an example of Figure 5, although the IPSPs evoked from the ipsilateral SC (Fig. 5B, 2–4) were larger and sharper in falling phases than those from the contralateral SC (Fig. 5C, 6–8).

To summarize, the input patterns from four rostrocaudal sites in the SCs to OPNs varied, depending on individual OPNs. OPNs receive disynaptic inhibition from bilateral caudal SCs, with more likely, clearer, and stronger inputs from ipsilateral SC stimulation. OPNs receive monosynaptic excitation from bilateral rostral SCs, with contralateral input being slightly stronger.

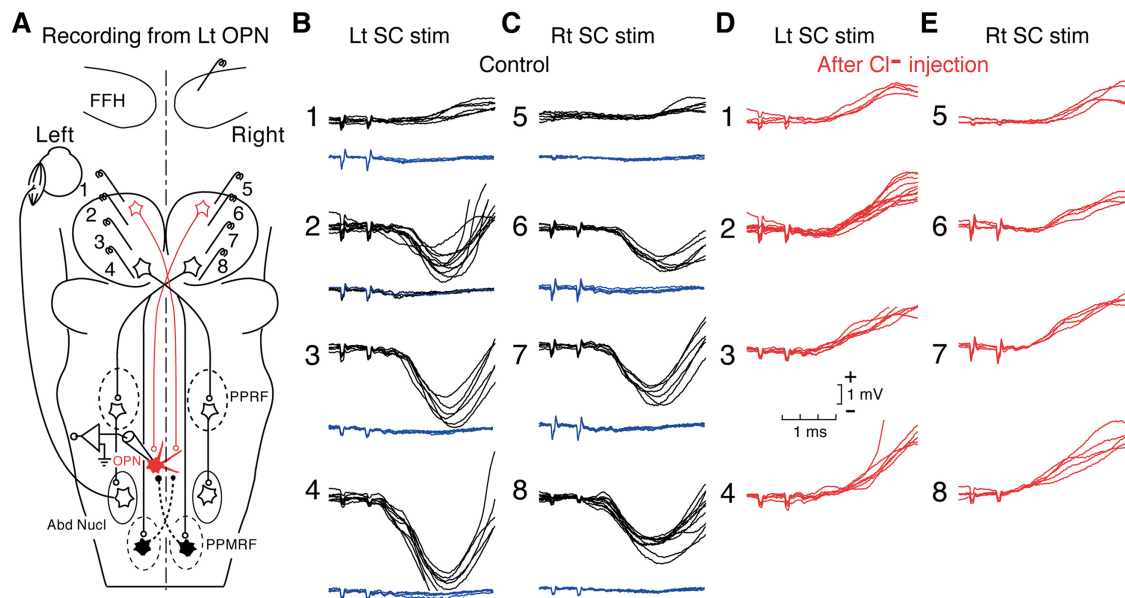


Figure 5. Patterns of synaptic input evoked bilaterally from the SCs in a left OPN. **A**, Experimental setup. **B**, **C**, PSPs evoked by left (Lt; **B**, 1–4) and right (Rt) SC stimulation (stim; **C**, 5–8), and PSPs by the same left (**D**, 1–4) and right (**E**, 5–8) SC stimulation after intracellular Cl^- injection into the cell. Stimulus intensity, 500 μA . Top and bottom traces in each set of records in **B** and **C** show PSPs (black traces) and juxtacellular field potentials (blue traces), respectively. Note sharper and larger falling phases of the IPSPs evoked by ipsilateral stimulation. Calibration in **D**, 3, applies to all traces in **B**–**E**. Nucl, Nucleus.

Sectioning of tectoreticular fibers to determine pathways from the SCs to OPNs

Having determined disynaptic IPSPs evoked in OPNs in response to both ipsilateral and contralateral stimulation of the caudal SC, the next step is to elucidate the specific pathways by which these signals reach the OPNs. Our previous study showed that transverse section of the tectoreticular fibers at the level rostral to the OPN region eliminated IPSPs evoked by ipsilateral SC stimulation in Abd MNs and IBNs (Sugiuchi et al., 2005), indicating that the inhibitory interneurons responsible are located caudal to the section level. To narrow the location of the inhibitory interneurons for suppressing OPN activity, we sectioned tectoreticular axons between the OPN and IBN regions, and examined SC-evoked PSPs in OPNs in two cats. To interrupt tectoreticular fibers projecting to OPNs and IBNs, we transversely sectioned the right tectoreticular tract, which contains fibers of the predorsal bundle and is located in the ventral part to the medial longitudinal fasciculus (Verhaart, 1964), just rostral to the IBN region (a lesion at the middle rostrocaudal level of the abducens nucleus, up to 1.5 mm lateral from the midline and 6.0–6.5 mm deep from the floor of the fourth ventricle; Fig. 6A, red thick bar). Figure 6 shows an example of intracellular records from a left OPN after the section (Fig. 6B,C). If the inhibitory interneurons are located rostral to the level of the caudal end of the OPN region and their axons do not traverse the sectioned area, the inhibition from the caudal SCs should remain after this transverse section. However, disynaptic inhibition from the left caudal SC (Fig. 6B, 3, 4) and the right caudal SC (Fig. 6C, 7, 8) disappeared in a left OPN, but excitation from the left rostral SC (Fig. 6B, 1) and right rostral SC (Fig. 6C, 5, 6) remained. Similar results were obtained in five OPNs tested. Therefore, the inhibitory interneurons for mediating disynaptic inhibition from both caudal SCs should be located more caudally than OPNs. However, very small late inhibition with a latency of 3.0 ms was observed (Fig. 6B, 3). This late inhibition might be mediated ipsilaterally via the central mesencephalic reticular formation (Wang

et al., 2017). Previous studies have demonstrated that the SC projections to OPNs are strongest from the most rostral SC and less dense from increasing caudal SC sites (Büttner-Ennever and Horn, 1994; Paré and Guitton, 1994; Gandhi and Keller, 1997). The present preparation (Fig. 6) is suitable for assessing how the amplitude of SC-evoked monosynaptic EPSPs varies along the rostrocaudal extent of the SCs, because the disynaptic IPSPs, which obscured the exact amplitude of the monosynaptic EPSPs in the control, were eliminated by the transverse section. From the ipsilateral SC, the monosynaptic EPSPs with slow rising phase were evoked by stimulation of the most rostral site (Fig. 6B, 1), whereas from the contralateral SC the sharp rising and short-latency large EPSPs with spikes were evoked from the most rostral site (Fig. 6C, 5) and smaller but reasonably large EPSPs with spikes were constantly evoked by stimulation of the second rostral site (Fig. 6C, 6). These findings indicate that the SC projections to OPNs are localized in the rostral SC and strongest from the most rostral SC, and are stronger from the contralateral SC than from the ipsilateral SC. The next step is to determine the crossing pathway by which inhibitory neuron signals reach the OPNs. To do that, we investigated the effects of a midline section.

Effects of midline section between the bilateral IBN regions on SC-evoked PSPs in OPNs

Our previous study showed that IBNs received, in addition to monosynaptic excitation from the contralateral caudal SC, disynaptic inhibition from the ipsilateral caudal SC via contralateral IBNs (Sugiuchi et al., 2005). The present study showed that the same input pattern from the ipsilateral caudal SC was also observed in OPNs (Fig. 2B, 6–8). Therefore, if IBNs on the opposite side directly terminate on OPNs, as well as on IBNs, it would account for the pathway for disynaptic inhibition in OPNs from the ipsilateral caudal SC. To explore this possibility, we sectioned the midline between the IBN regions on both sides, and examined SC-evoked IPSPs in OPNs in 2 cats (Fig. 7). Based on

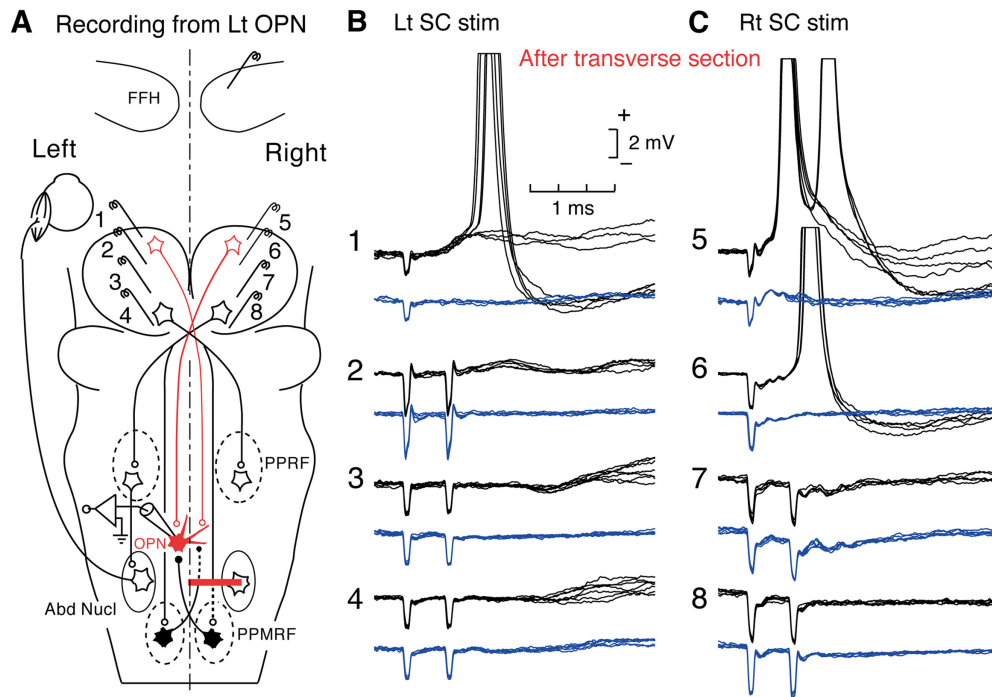


Figure 6. Effects of the sectioning of tectoreticular axons on SC-evoked PSPs in an OPN. **A**, Experimental setup. A transverse section of tectoreticular axons (red horizontal bar) was made in the right brainstem at a rostrocaudal level of the center of the abducens nucleus (width, ~2 mm from the midline; depth, ~5–6 mm). The OPN was penetrated on the left side rostral to the section. **B**, **C**, Synaptic inputs from the left (Lt; **B**) and right (Rt; **C**) SC in the left OPN. EPSPs were evoked from the right rostral SC at 0.8 ms (**C**, 5) and from the left rostral SC at a longer latency with single stimuli (stim; **B**, 1). In **B**, 1, EPSPs with and without action potentials were observed since the stimulus strength was just at the threshold for the action potentials. In **C**, 5, double action potentials were evoked with single stimulus in some traces, and single action potentials were evoked at a longer latency in **C**, 6. In contrast, disynaptic IPSPs could not be evoked from the caudal SCs even with double stimuli of 500 μ A (**B**, 3, 4, **C**, 7, 8), indicating that inhibitory interneurons for suppressing OPN activity should be located more caudally than the transverse section level. Calibration in **B**, 1, applies to **B** and **C**.

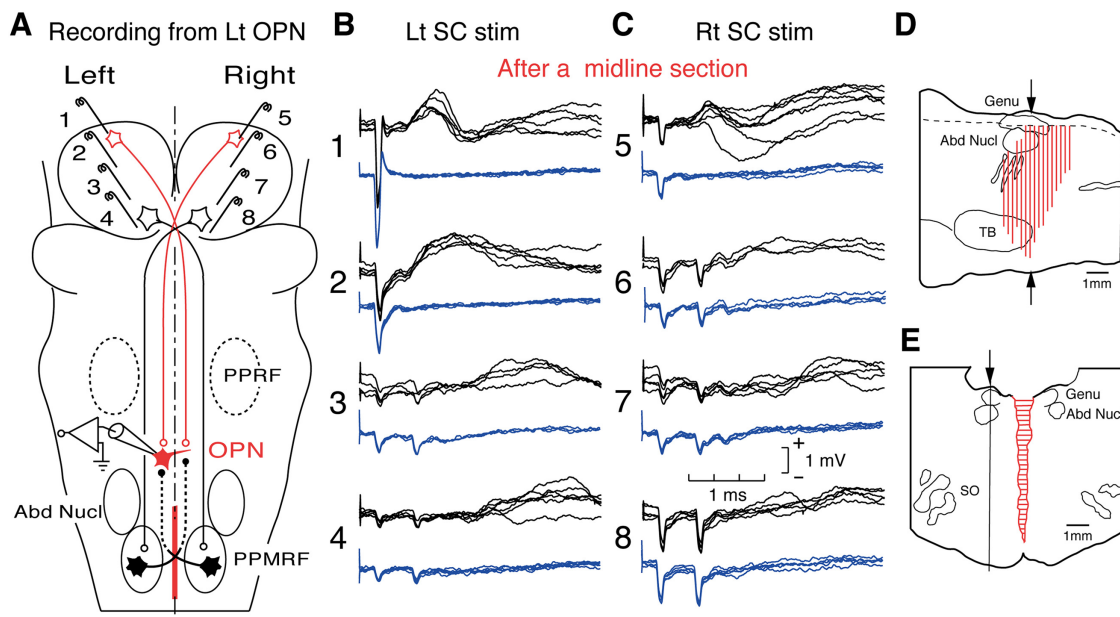


Figure 7. Effects of a midline section between the bilateral IBN regions on SC-evoked PSPs in an OPN. **A**, Experimental setup. The midline section (red bar) was made at the level from the middle of the abducens nucleus to ~3 mm caudally. **B**, **C**, Inputs from the ipsilateral (left; **B**, 1–4) and contralateral (right) SC (**C**, 5–8). Note that the disynaptic IPSPs from the caudal SCs disappeared for the first 2.5 ms in **B**, 3, 4, and **C**, 7, 8, while monosynaptic EPSPs from the rostral SCs remained in **B**, 1, and **C**, 5. Calibration in **C**, 7, also applies to **B**, **D**, **E**. **D**, Lateral view of the lesion reconstructed from coronal sections of the brainstem. Vertical lines indicate the extent of the lesion in each section. **E**, Coronal section of the brainstem showing the depth of the midline cut at the rostrocaudal level (**D**, arrows) between the IBN regions. The vertical line and arrows indicate the laterality of the drawing of **D**. Nucl, Nucleus; Lt, left; Rt, right.

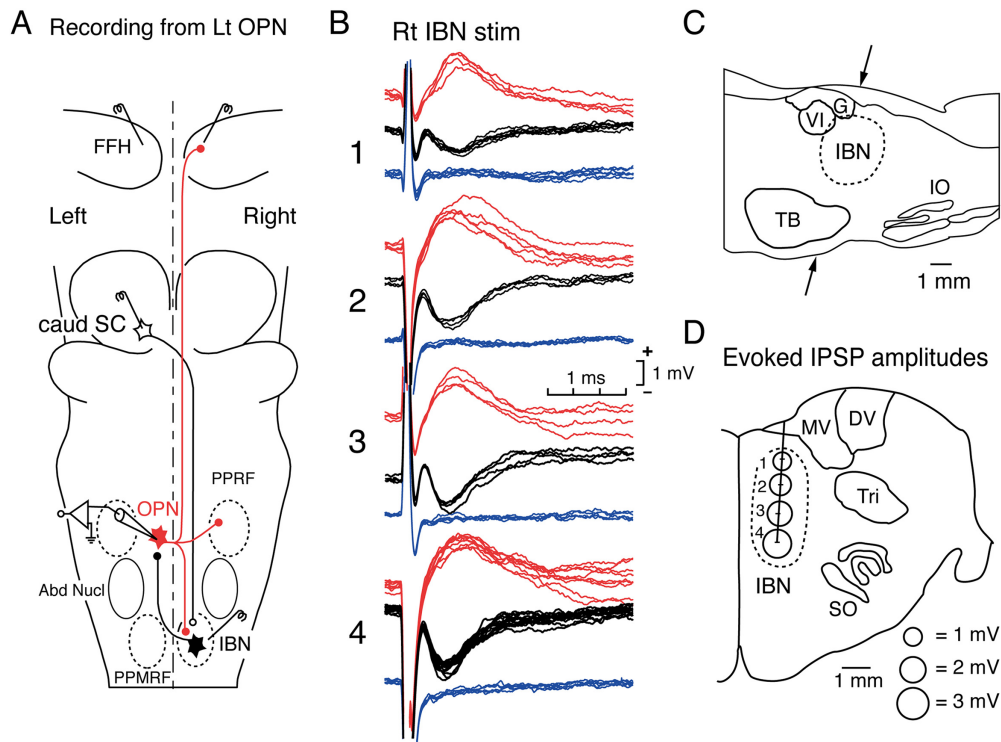


Figure 8. Effects of stimulation of the IBN region on an OPN. **A**, Experimental setup. An array consisting of four electrodes arranged in a dorsoventral direction were placed in the right IBN region (**D**, 1–4). **B**, Monosynaptic IPSPs evoked in a left (Lt) OPN by stimulation (stim) of the right (Rt) IBN region at 100 μ A. This neuron received disynaptic IPSPs from the left caudal SC. PSPs evoked from stimulation sites in the IBN region are shown in their order of depth (1–4) from the surface of the fourth ventricle. Sites 1–4 correspond to stimulation sites shown in a histology drawing of **D**. Hyperpolarizing PSPs (black traces) were reversed to depolarizing potentials after Cl^- injection (red traces), indicating that these hyperpolarizations were IPSPs. **C**, Lateral view of the brainstem to indicate the transverse plane (arrows) shown in **D**, including an array of the stimulating electrodes in the right IBN region. IO, Inferior olive; TB, trapezoid body; G, genu facialis; VI, abducens nucleus. **D**, Distribution of effective stimulation sites in the IBN region. The diameter of circles in **D** is proportional to the amplitude of the IPSPs (**B**, 1–4) evoked at 100 μ A from each IBN stimulation site. MV, Medial vestibular nucleus; DV, descending vestibular nucleus; Tri, trigeminal nucleus; SO, superior olive; Nucl, nucleus.

previous anatomic data of the trajectories of IBN axons (Yoshida et al., 1982; Strassman et al., 1986; Sugiuchi et al., 2005), a mid-line section was made 5.0–6.5 mm deep from the floor of the fourth ventricle and 4–6 mm caudal from the middle of the abducens nucleus (Fig. 7D,E). In this preparation, we first recorded PSPs in two Abd MNs and three IBNs to confirm the absence of disynaptic inhibition from the ipsilateral caudal SC. Then, in the same preparation, stimulation of the rostral SC on either side evoked monosynaptic depolarizations in an OPN (Fig. 7B, 1, 2, C, 5), but even double-pulse stimulation of the caudal SC on either side did not evoke disynaptic IPSPs in the same OPN (Fig. 7B, 3, 4, C, 7, 8). We could conclude that stimulation of the caudal SCs did not evoke IPSPs in OPNs ($n = 4$) because no PSPs were observed up to 2.5 ms after the second stimulus of the SC. This finding was consistent with the interpretation that the IPSPs in OPNs evoked by stimulation of the ipsilateral caudal SC were mediated by contralateral IBNs, and those evoked by stimulation of the contralateral caudal SC were likely to be mediated by ipsilateral IBNs, since the tectoreticular fibers project to the contralateral side (Verhaart, 1964).

Effects of stimulation of the IBN region on OPNs

To provide further evidence that IBNs directly inhibit OPNs, we investigated the effects of microstimulation of the IBN region on OPNs. Stimulation of the contralateral IBN region evoked hyperpolarizations at a latency of 0.8 ms in OPNs [Fig. 8B, middle black traces (numbers correspond to IBN stimulation sites in Fig. 8D)]. These hyperpolarizations were reversed to depolarizations by Cl^- injection (Fig. 8B, top red traces), indicating that the

hyperpolarizations were IPSPs (Coombs et al., 1955b; Eccles, 1964). Since the latencies of these IPSPs ranged from 0.7 to 1.2 ms (mean, 0.8 ± 0.2 ms; $n = 23$) in OPNs, the IPSPs were considered to be primarily monosynaptic. To map effective stimulation sites in the IBN region for evoking monosynaptic IPSPs in OPNs, we stimulated four sites with an array of electrodes arranged in a dorsoventral direction in the contralateral IBN region at the same stimulus intensity and examined the amplitudes of the evoked IPSPs at individual stimulating sites. Figure 8D shows the distribution of the amplitudes of monosynaptic IPSPs in the OPN evoked from individual stimulation sites in the IBN region. Since the stimulus intensity was 100 μ A, the stimulus effect was considered to be well localized within 1 mm from a stimulus electrode (Abzug et al., 1974; Shinoda et al., 1976). However, these IPSPs might be evoked by activating passing fibers of neurons other than IBNs in and around the IBN region.

Determination of the pathway from the caudal SC to OPNs via IBNs

To confirm that stimulation of the IBN region really activates cell bodies of IBNs and disynaptic inhibition from the ipsilateral caudal SC to an OPN is mediated via the IBNs, we examined the interaction between SC-evoked IPSPs and IBN region-evoked IPSPs in a conditioning test manner (Fig. 9). Stimulation of the left caudal SC evoked IPSPs at a latency of 1.7 ms in an OPN (Fig. 9B), and stimulation of the right IBN region evoked IPSPs at a latency of 0.7 ms in the same OPN (Fig. 9C). Stimulus intensities for both stimulation sites were decreased and adjusted, so that the stimuli for the IBN region were weak enough to evoke

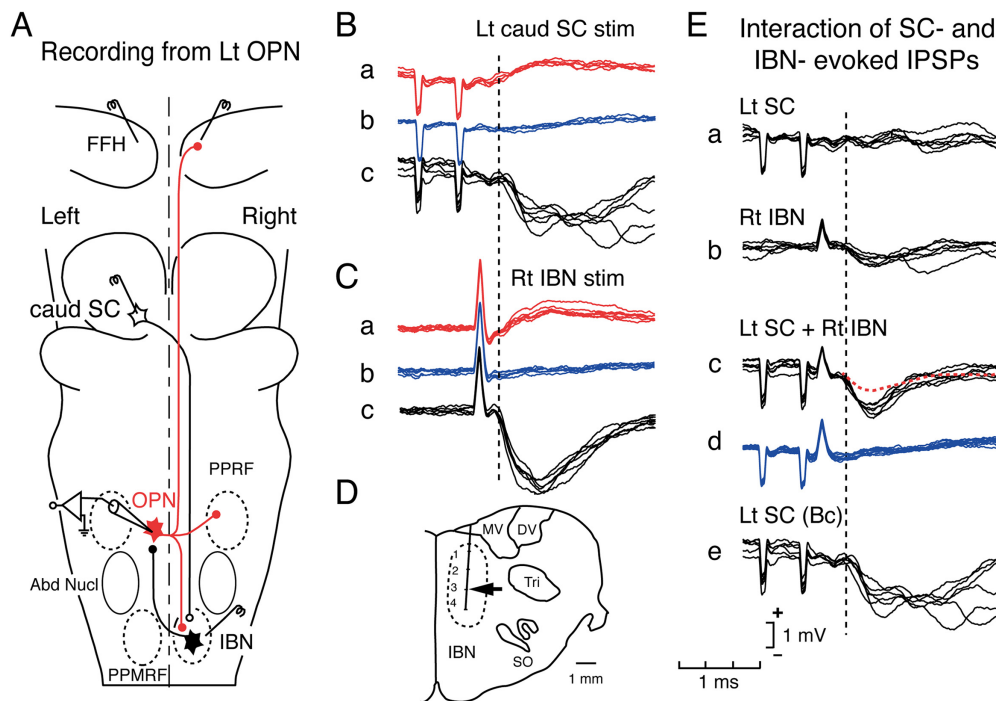


Figure 9. Interaction between caudal SC-evoked IPSPs and IBN-evoked IPSPs in an OPN. **A**, Experimental setup. **B**, Disynaptic IPSPs evoked by double-pulse stimulation of the left (Lt) caudal (caud) SC (300 μ A). **C**, **D**, Monosynaptic IPSPs evoked by stimulation (stim) of the right (Rt) IBN region (100 μ A; **C**) shown by an arrow in the histology drawing (**D**). Red (a), blue (b), and black (c) traces; traces after Cl^- injection, juxtacellular field potentials, and control, respectively. Vertical dashed line indicates the onsets of the SC-evoked and IBN-evoked IPSPs. **E**, Spatial facilitation of the IBN-evoked monosynaptic IPSPs in the OPN by preconditioning stimulation of the left caudal SC. **E**, a, b, Stimulus intensities of the same stimulus sites as in **B** and **C** were decreased to evoke smaller IPSPs in the same OPN. **E**, a, 250 μ A. **E**, b, 40 μ A. **E**, c, Combined stimulation of the caudal SC (**E**, a) and the IBN region (**E**, b). Note that the IPSPs evoked by the combined stimulation (**E**, c) were much larger than the algebraic sum (red broken line) of individual IPSPs evoked by the stimulation of the SC (**E**, a) and the IBN area (**E**, b), indicating that the left caudal SC induces disynaptic IPSPs in the OPN via the right IBNs. **E**, e, and vertical dashed line represent the onset of the disynaptic IPSPs evoked from the left caudal SC. Calibration in **E**, e, applies to **B**, **C**, and **E**. MV, Medial vestibular nucleus; DV, descending vestibular nucleus; Tri, trigeminal nucleus; SO, superior olive; Nucl, nucleus.

small IPSPs (Fig. 9E, b), and the stimuli for the SC were just below the threshold for the IPSPs (Fig. 9E, a). When the preconditioning stimuli of the left caudal SC preceded the test stimuli of the right IBN region by 1.0 ms, the preconditioning stimuli for the left caudal SC facilitated monosynaptic IPSPs evoked by the right IBN stimuli in the OPN (Fig. 9E, c). Similar facilitation was observed in four of six OPNs tested. This facilitation of the monosynaptic IPSPs by the preconditioning SC stimulation supports the proposition that the monosynaptic inhibition in OPNs was induced by activating cell bodies of IBNs in the IBN region rather than passing fibers of other neurons, and ensured that the disynaptic inhibition in OPNs evoked by ipsilateral SC stimulation was mediated by contralateral IBNs.

Reciprocal inhibition between IBNs and OPNs

In addition to OPN inputs via IBNs from the SC, there are also recurrent connections between OPNs and IBNs. We investigated the interactions between IBNs and OPNs by recording from an OPN while microstimulating the contralateral IBN region. In Figure 10, the cell was penetrated slightly rostral to the rostral end of the abducens nucleus. Antidromic spikes were evoked from the contralateral FFH at a latency of 1.0 ms (Fig. 10B). The same neuron was also antidromically activated from the contralateral IBN region at a latency of 0.8 ms at suprathreshold stimuli for antidromic spikes (Fig. 10C). We carefully adjusted the stimulation strength so that antidromic spikes appeared in about half of all stimulation trials. In those trials where IBN stimulation failed to elicit antidromic spikes, we could clearly see hyperpolarized potentials (Fig. 10D). These responses were evoked from the dorsoventral stimulation sites in the contralateral IBN region

(Fig. 10F). Gradual spontaneous diffusion of Cl^- from a recording microglass electrode into the cell reversed the hyperpolarizations to the depolarizing potentials (Fig. 10E, a), indicating that these hyperpolarized potentials were monosynaptic IPSPs with a latency of 0.9 ms (Fig. 10E, b). This finding shows that OPNs that project to IBNs also receive inhibition from the IBNs. We frequently observed this type of reciprocal inhibition between OPNs on one side and IBNs on the opposite side. This reciprocal negative feedback loop is comparable to a positive feedback loop in function, and could work to quickly suppress OPNs for initiating saccades.

Anatomical re-examination of the axonal trajectory of single IBNs to the OPN region

The above electrophysiological investigation clearly shows that IBNs directly terminate on contralateral OPNs. However, previous studies of the morphology of single IBNs have shown that IBNs project to the contralateral abducens nucleus, and EBN and IBN regions, but not to the OPN region (Yoshida et al., 1982; Strassman et al., 1986; Sugiuchi et al., 2005). Since the length of stained axon collaterals of single cells depends on the amount of intracellular injection of HRP into the cells and the time allowed for transport, we re-examined the axonal trajectory of individual IBNs with intracellular iontophoretic injection of HRP and three-dimensional reconstruction of axonal trajectory from coronal serial sections of the stained cells. The HRP injection was performed in 21 electrophysiologically identified IBNs, and 16 neurons were recovered. Cell bodies, dendrites, and a part of their main axons were stained in these cells. In the eight cells that were well stained, the axonal trajectory could be traced beyond

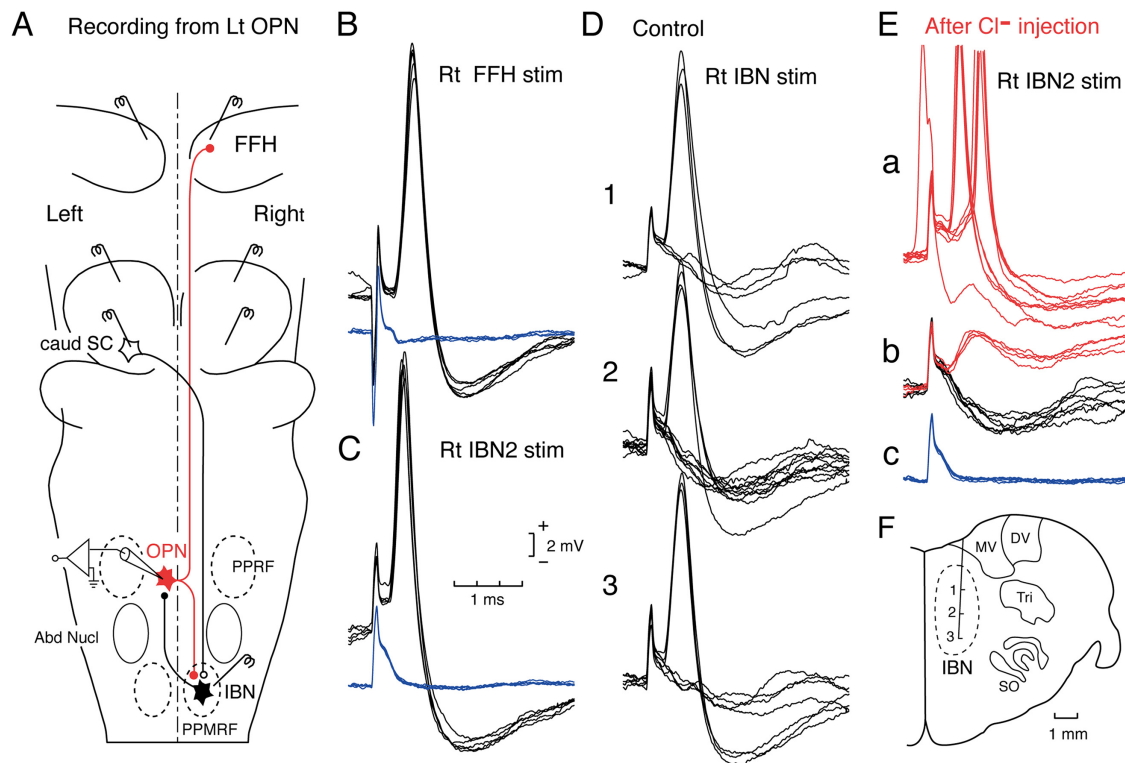


Figure 10. Reciprocal inhibitory connections between OPNs and IBNs. **A**, Experimental setup. An intracellular recording was made in a left OPN, and the contralateral (right) IBN region and FFH were stimulated. This neuron received disynaptic inhibition at a latency of 1.8 ms from the left caudal SC (data not illustrated). **B**, Antidromic spikes evoked from the right FFH region at 200 μ A. **C**, Antidromic spikes with a latency of 0.8 ms evoked from site 2 in the right IBN region (in **F**; IBN2) at 100 μ A. Calibration in **C** applies to all traces in **B–E**. **D**, Stimulus intensities of the IBN region were adjusted to threshold intensity for the antidromic spikes, so that monosynaptic IPSPs appeared in about half of the traces (**D**, 1, 70 μ A; **D**, 2, 85 μ A; **D**, 3, 75 μ A). Numbers (1–3) correspond to the stimulating locations in the right IBN region shown in **F**. **E**, Reversed IPSPs evoked at 100 μ A from the same stimulus site as in **D**, 2, after gradual spontaneous diffusion of Cl^- into the cell (**E**, a). Note that orthodromic spikes caused by the reversed IPSPs and antidromic spikes appeared in different traces. **E**, b, Superposition of hyperpolarizing IPSPs in control (black traces) and their reversed depolarizing potentials after Cl^- diffusion (red traces). Stimulus intensity, 80 μ A at the stimulus site as in **D**, 2. **E**, c, Juxtacellular potential. **F**, Histologic identification of the location of the stimulating electrodes in the IBN region. stim, Stimulation; Rt, right; Nucl, nucleus.

the midline to the contralateral abducens nucleus and to the IBN and/or EBN region. The axon collaterals could be traced into the OPN region in four of these eight cells. Figure 11 shows an example of the axonal trajectory of a single well stained IBN. The stem axon extends to the contralateral side at the same level of its cell body and bifurcates into descending and ascending branches (Fig. 11, left). The descending branch projects to the area ventral to the nucleus prepositus hypoglossi (Fig. 11E). The main ascending branch projects to the abducens nucleus (Fig. 11B,C) and from there to the EBN region (Fig. 11A). On its way, an axon collateral is given to the IBN region and the vestibular nucleus (Fig. 11D). In the abducens nucleus, three axon collaterals are issued medially and ventrally, and spread into the OPN region (Fig. 11A,B). They terminate extensively within the rostrocaudal extent of the nucleus (Fig. 11, left, medial blue area). Their axon terminals are most dense in the medial OPN region adjacent to the midline, but no terminals are observed on the opposite side (no recrossing of axon collaterals was observed; Fig. 11B). These collaterals into the OPN region from the ascending branch in the abducens nucleus were observed only in the well stained cells. It is not clear whether all IBNs have an axonal projection to the OPN region or only a portion of IBNs have such a projection.

Discussion

The present study provides evidence that IBNs in the horizontal saccadic system have strong monosynaptic connections with

OPNs, and that the caudal SCs inhibit OPNs via these IBNs. Figure 12 illustrates a schematic summary of our current understanding of the neural circuit in which the rostral and caudal SCs trigger versus suppress OPNs. Thus, IBNs likely inhibit OPNs before saccades to allow their initiation, and during saccades for their maintenance (latch function). Since the original model proposed by Robinson (1975), OPNs have been considered to play a role in the initiation of saccades by gating signals from a higher center in many computational models of horizontal saccade generation. However, several serious points remained undetermined in this hypothesis. These will be discussed on the basis of the present experimental findings. Here we demonstrated that OPNs receive monosynaptic excitation from the rostral SCs with contralateral dominance (Figs. 2, 4, 5, 6). TRNs send their axons through the predorsal bundle to the contralateral brainstem and terminate there on cell bodies of OPNs (Büttner-Ennever and Horn, 1994; Büttner-Ennever et al., 1999). Since OPNs near the midline have dendrites extending to the bilateral RIP (Ohgaki et al., 1987; Strassman et al., 1987; Fig. 1E), TRNs likely terminate on dendrites extending to the opposite side (Fig. 12). By the same token, intracellular staining of single IBNs shows that their axons cross the midline to terminate on cell bodies of OPNs on the opposite side. Again, they can target crossing dendrites of OPNs on the same side as IBNs.

As mentioned in the Introduction, there are different views in regard to the function of the rostral SC. The present study could not disambiguate between the fixation cell hypothesis and the

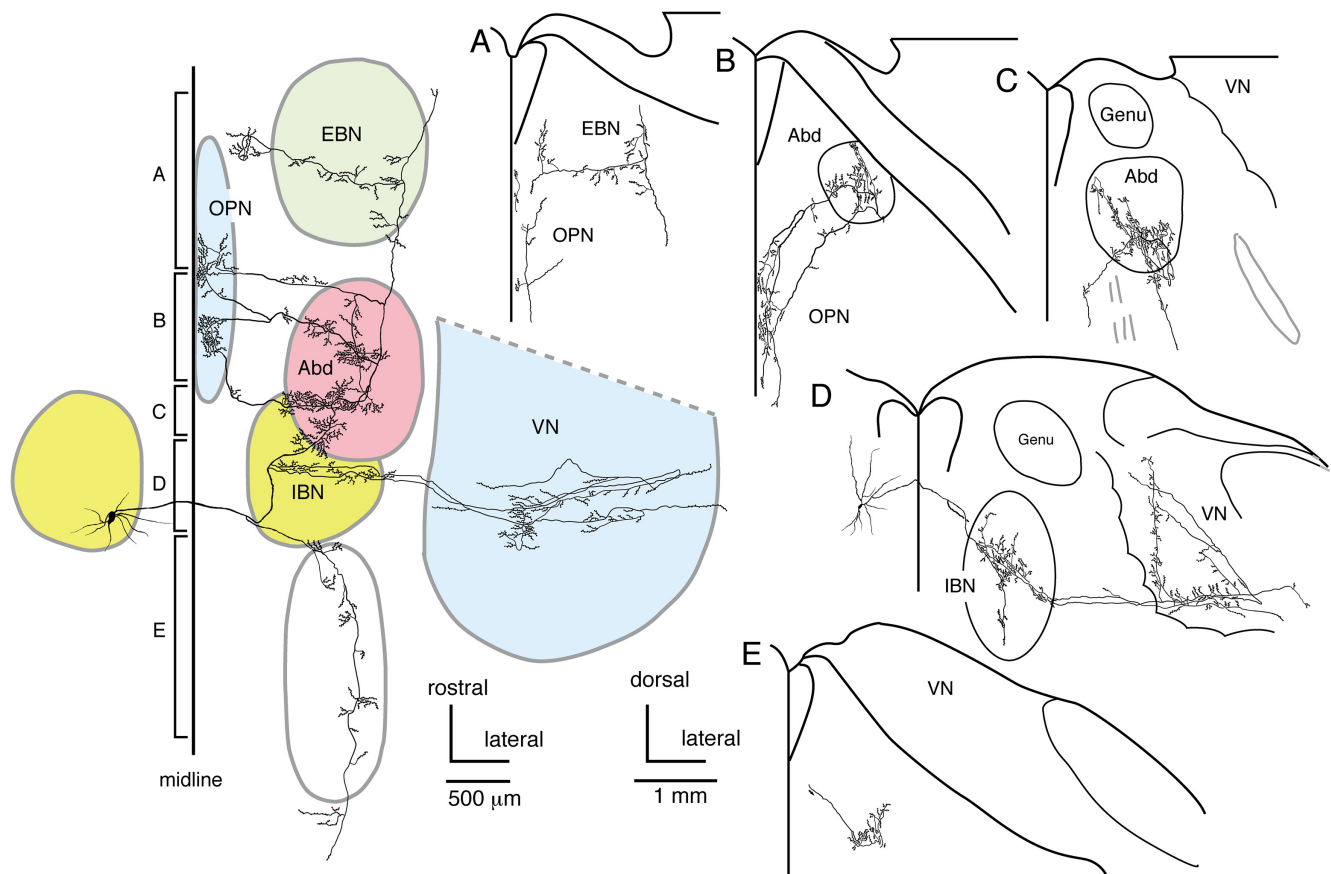


Figure 11. The morphology of the axonal trajectory of a single IBN stained with intracellular injection of HRP. This IBN received monosynaptic excitation from the contralateral caudal SC at a latency of 1.0 ms and disynaptic inhibition from the ipsilateral caudal SC at a latency of 1.6 ms. HRP was injected iontophoretically (15 nA, 16 min) into the electrophysiologically identified neuron. The axonal trajectory was reconstructed from 82 serial coronal sections of the brainstem (80 μ m thick). Left, Dorsal view of the serially reconstructed axonal trajectory of the stained IBN. **A–E**, Right panels, Serial reconstructions of sets of serial section projected onto a representative frontal view of the set to demonstrate axon collateralization in specific areas. These areas are indicated in **A–E** in the dorsal reconstruction of the left panel. Note that the stem axon crossed the midline at the same level of its cell body and that all of the axon collaterals terminated on the side opposite the cell, especially with extensive terminals in the opposite OPN region. VN, Vestibular nucleus; Genu, genu facialis.

continuous hypothesis, since it is possible that “fixation cells” and “microsaccade cells” are intermixed in the most rostral SC, and our electrical stimulation excited both of them. However, it is evident that rostral SC neurons, fixation cells, and microsaccade cells reported previously have two common features in contrast to caudal SC saccade neurons with only phasic activity during saccades, as follows: (1) sustained activity during fixation; and (2) cessation of their sustained activity for large saccades. Stimulation of the most rostral SCs evoked monosynaptic EPSPs in OPNs (Fig. 2*B*, 1), but disynaptic IPSPs in IBNs (Fig. 2*D*, 1), whereas caudal SC stimulation evoked monosynaptic EPSPs in IBNs (Fig. 4*D*; Sugiuchi et al., 2005), but disynaptic IPSPs in OPNs (Fig. 2*B*, 4). Therefore, it is evident that TRNs in rostral and caudal sites in the SC have differential connections with OPNs and IBNs. These neural connections are compatible with the view that the rostral and caudal parts of the SC have different functions.

Our major unsolved question regards the identity of the inhibitory interneurons that suppress tonic firing of OPNs, and thus trigger saccades. The present study reveals that horizontal IBNs are one of these inhibitory interneuron populations. Specifically, microstimulation of the IBN region evoked monosynaptic IPSPs in OPNs, which were facilitated by preconditioning stimulation of the caudal SC (Fig. 9*E*). Moreover, our

electrophysiological finding following lesions confirmed that disynaptic IPSPs evoked in OPNs from the caudal SC are mediated by IBNs. In fact, IBNs, Abd MNs, and OPNs are all disynaptically inhibited by the ipsilateral caudal SC, and this inhibition is caused by the very same contralateral IBNs, as we have presented convincing morphologic evidence that single IBNs have axon collaterals terminating on the OPN region in addition to the IBN region and Abd nucleus (Fig. 11). A previous study argued that interneurons in the EBN region inhibit OPNs, because stimulation of the EBN region evoked short-latency inhibition in OPNs (Kamogawa et al., 1996). However, IBNs have axon collaterals projecting to EBNs in the PPRF and OPNs in the RIP on the contralateral side (Fig. 11, left). Accordingly, stimulation of the EBN portion of the PPRF could evoke monosynaptic inhibition in OPNs by axon reflex, or by activating excitatory axons that terminate on IBNs.

OPNs that project to the IBN region also receive monosynaptic inhibition from the IBN region (Fig. 10*C–E*). This reciprocal inhibitory connection between IBNs and OPNs can work in such a way that an increase in IBN activity may inhibit OPN tonic activity, which in turn disinhibits IBN activity. This reciprocal negative loop, effectively a positive feedback between IBNs and OPNs, will cause depolarization in IBNs from the hyperpolarized state that is caused by tonic OPN inhibition during fixation. Such abrupt disinhibition may accelerate activation of low-

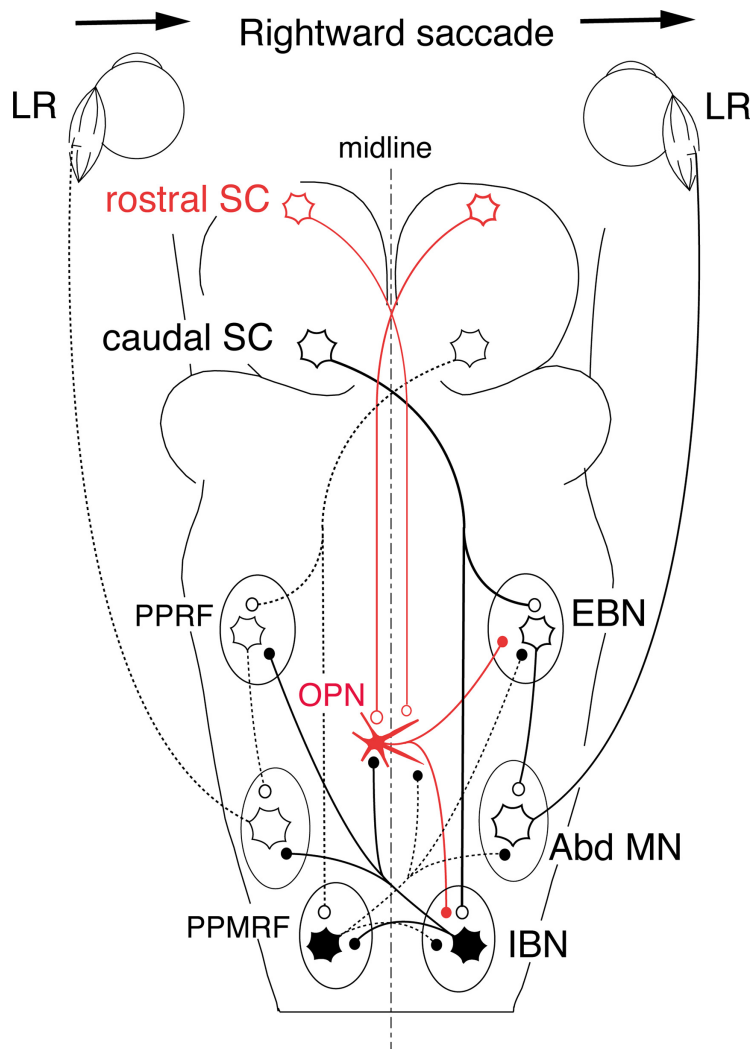


Figure 12. Schematic summary diagram showing the neural circuit for triggering and suppressing of OPNs by the rostral and caudal SCs. Filled neurons; inhibitory neurons, open neurons; excitatory neurons. Black filled lines indicate saccade driving pathways for rightward saccades, and dashed black lines indicate saccade driving pathways that are suppressed during rightward horizontal saccades. Red filled lines indicate pathways for fixation. LR, Lateral rectus muscle.

threshold Ca^{2+} channels for spike burst in IBNs (Serafin et al., 1990; Miura and Optican, 2006). Under this proposal, the onset of burst spikes in IBNs should be earlier than the onset of reduced sustained spike activity in OPNs.

IBNs were not considered as candidates for suppressing OPNs at the onset of saccades until now for several reasons. First, it has been tacitly assumed that IBNs received disynaptic or trisynaptic excitation from the SC (Takahashi and Shinoda, 2018). Meanwhile, OPNs were known to be inhibited disynaptically by the SC (King et al., 1980; Kaneko and Fuchs, 1982; Yoshida et al., 2001). Under these assumptions, it was argued that the pathway from the SC to OPNs could not be mediated by IBNs. Furthermore, Yoshida et al. (1999) analyzed the relationship between the onset of IBN burst spikes and the cessation of OPNs during spontaneous saccades in the cat and concluded that OPNs begin decreasing their spike activity 7.6 ms on average before the onset of IBN burst activity [i.e., the lead time of spike activity in horizontal IBNs relative to the onset of saccades was 8.3 ± 2.0 ms ($n = 10$), and the lead time of cessation of spike activity in OPNs was 15.9 ± 3.8 ms (range, 7.5–23.9 ms; $n = 23$)]. These findings strongly suggested that inhibitory interneurons other than IBNs should be responsible for the inhibition of

OPNs at the onset of saccades. Accordingly, it was generally believed that IBNs did not initiate cessation of OPN firing. Instead, IBNs were proposed to suppress only OPN activity during saccades (Scudder et al., 1988). Even this hypothesis lacked morphologic evidence to support it, as axonal projection by IBNs to OPNs had not been observed (Yoshida et al., 1982; Strassman et al., 1986). Thus, the pathway from the SC to IBNs was considered to be trisynaptic, via long-lead EBNs and short-lead EBNs (Fuchs et al., 1985). However, our previous study revealed that IBNs receive strong monosynaptic excitation from the contralateral SC (Sugiuchi et al., 2005; Takahashi and Shinoda, 2018), and the present study shows that IBNs are, in fact, the dominant source for inhibition of OPNs from the caudal SCs.

If IBNs are the dominant source for inhibition of OPNs before saccades, there should be some IBNs that show burst activity before the cessation of tonic activity in OPNs. If a subset of IBNs start burst firing before the onset of decreased OPN activity, OPNs will be quickly suppressed by the positive feedback connection between IBNs and OPNs. Some anecdotal data have shown much earlier onsets of burst activity in IBNs that precede the decrease in OPN activity in the cat [Hikosaka and Kawakami, 1977 (their Fig. 1); Nakao et al., 1980 (their Fig. 6)], although the authors did not refer to the earlier onset of burst activity of such IBNs. In fact, early-bursting IBNs were later reported by experimental studies in awake monkeys. Scudder et al. (1988) and Cullen and Guitton (1997) reported two types of IBNs, short-lead IBNs (SLIBNs) and long-lead IBNs (LLIBNs). Depending on the period between the first spike leading a saccade and the onset of saccade velocity, horizontal IBNs were categorized as SLIBNs

if their latency was 11 ± 2.8 ms, and LLIBNs if it was 21 ± 7.9 ms (Cullen and Guitton, 1997). In the monkey, it was reported that the time interval between the beginning of the pause in OPNs and the beginning of the quick eye movement is between 12 and 20 ms (Cohen and Henn, 1972), and OPNs start pausing on average 10.1 ± 0.9 and 11.7 ± 0.9 ms before leftward and rightward saccades, respectively (Everling et al., 1998). Together, these data clearly show the existence of such a subset of IBNs that start firing earlier than the onset of decrease in OPN activity and support our interpretation that some early-bursting horizontal IBNs cause a decrease in tonic activity of OPNs before and during saccades. The present results provide compelling evidence that the suppression of OPN activity at the onset of horizontal saccades is because of brainstem mechanisms for initiating and maintaining saccades (latch function), without requiring inhibitory neurons other than IBNs. It has been suggested that EBNs and IBNs, which are tonically inhibited by OPNs during visual fixation, must be released by inhibiting OPNs before the onset of saccades. However, this study has provided evidence that such a supranuclear “trigger pulse” command is not required to initiate

saccades. Instead, the same SC saccade neurons for encoding the amplitude and direction of saccades can directly activate a subset of IBNs to inhibit OPN activity, thus releasing EBNs and IBNs from the tonic inhibition.

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