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RESEARCH ARTICLE

Nobuo Miyashita · Okihide Hikosaka

Minimal synaptic delay in the saccadic output pathway of the superior colliculus studied in awake monkey

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Abstract The synaptic organization of the saccade-related neuronal circuit between the superior colliculus (SC) and the brainstem saccade generator was examined in an awake monkey using a saccadic, midflight electrical-stimulation method. When microstimulation (50–100 μA, single pulse) was applied to the SC during a saccade, a small, conjugate contraversive eye movement was evoked with latencies much shorter than those obtained by conventional stimulation. Our results may be explained by the tonic inhibition of premotor burst neurons (BNs) by omnipause neurons that ceases during saccades to allow BNs to burst. Thus, during saccades, signals originating from the SC can be transmitted to motoneurons and seen in the saccade trajectory. Based on this hypothesis, we estimated the number of synapses intervening between the SC and motoneurons by applying midflight stimulation to the SC, the BN area, and the abducens nucleus. Eye position signals were electronically differentiated to produce eye velocity to aid in detecting small changes. The mean latencies of the stimulus-evoked eye movements were: 7.9±1.0 ms (SD; ipsilateral eye) and 7.8±0.9 ms (SD; contralateral eye) for SC stimulation; 4.8±0.5 ms (SD; ipsilateral eye) and 5.1±0.7 ms (SD; contralateral eye) for BN stimulation; and 3.6±0.4 ms (SD; ipsilateral eye) and 5.2±0.8 ms (SD; contralateral eye) for abducens nucleus stimulation. The time difference between SC- and BN-evoked eye movements (about 3 ms) was consistent with a disynaptic connection from the SC to the premotor BNs.

Key words Superior colliculus · Brainstem saccade generator · Eye movements · Saccadic midflight stimulation · Awake monkey

N. Miyashita (✉) · O. Hikosaka
Department of Physiology, School of Medicine,
Juntendo University, 2–1–1 Hongo, Bunkyo-ku, Tokyo 113, Japan;
Fax: +81-3-3813-4954, e-mail: mnobuo@med.juntendo.ac.jp

Introduction

The superior colliculus (SC) in mammals is thought to play a key role in the generation of saccadic eye movements (Sparks and Hartwich-Young 1989). Electrical stimulation of the SC evokes saccadic eye movements (Robinson 1972). Neurons in its intermediate and deep layers show a burst of spikes before a saccade (Schiller and Koerner 1971; Wurtz and Goldberg 1971, 1972) and project their axons to the saccade-generating mechanisms in the reticular formation (Moschovakis et al. 1988; Guitton and Munoz 1991). Reversible blockade of the neural activity in the SC virtually abolishes the monkey's ability to generate saccades (Hikosaka and Wurtz 1985a).

However, it remains controversial as to how many neurons are interposed between the outputs of the SC and extraocular motoneurons. Earlier electrophysiological studies in the cat suggested that the SC-output neurons connect to extraocular motoneurons disynaptically (Grantyn and Grantyn 1976; Grantyn and Berthoz 1977). Anatomical studies indicated that axon terminals of SC-output neurons were distributed in the pontine and medullary reticular formation (Grantyn and Grantyn 1982) where saccadic burst neurons (BNs) are located that project in turn to abducens motoneurons directly in the cat (Hikosaka and Kawakami 1977; Sasaki and Shimazu 1981) and in the monkey (Strassman et al. 1986a,b).

On the other hand, some studies suggested that the SC-output pathway is polysynaptic in the cat (Precht et al. 1974). Raybourn and Keller (1977) suggested that long-lead BNs, not short-lead BNs, receive direct inputs from the SC in the monkey. Assuming that the short-lead BNs, not long-lead BNs, are presynaptic to motoneurons (Hepp et al. 1989), their results support the indirect theory, although it remains unknown how indirect the SC-outputs are.

The indirect theory seems even more likely if we compare the latencies of stimulus-evoked eye movements. Both in the monkey (Robinson 1972) and in the cat (Munoz et al. 1991), stimulation of the SC evokes

saccades with latencies of about 20 ms or more. This might be taken to suggest polysynaptic connections intervening between the SC and the saccade generators, especially because the conduction velocities of SC output neurons are known to be quite high (Grantyn and Grantyn 1982). The presaccadic neural activities observed in these areas are consistent with these results. The saccade-lead time (time between onset of neural activity and onset of saccade) is usually about 20 ms or more in SC output neurons (Sparks et al. 1976; Moschovakis et al. 1988; Munoz et al. 1991); it is around 10 ms in brainstem BNs (Strassman et al. 1986a,b).

A key element behind the controversy may be the omnipause neurons (Keller 1974; Büttner-Ennever et al. 1988). They tonically inhibit the premotor BN most of the time but pause occasionally in a transient manner, thus allowing BNs to fire to generate a saccade (Nakao et al. 1980; Curthoys et al. 1984). It is likely, therefore, that during fixation the signals from the SC induced by its stimulation could be blocked at the level of BNs. This in turn suggests that, if we stimulate the SC during a saccade, the SC-originated signals may be transmitted to motoneurons with no blockade or delay. The time required in this process would then be close to the sum of synaptic delays that intervene between the SC and motoneurons.

This is in fact what Munoz et al. (1991) demonstrated in the alert cat: when stimulation was applied to the SC during a saccade, the eye reaccelerated after 10–11 ms. In the present study, we adopted this method for the alert monkey. We found that such saccadic midflight stimulation evoked an eye velocity change with latencies of about 8 ms – a value that was much shorter than the latencies obtained by conventional stimulation. Based on this result together with the results obtained by brainstem stimulation, we suggest that the SC connection to premotor BNs is most likely to be disynaptic.

Materials and methods

Material

We used one male Japanese monkey (*Macaca fuscata*) weighing 8 kg. The monkey was housed in an individual primate cage in an air-conditioned room and brought to the experimental room at each experimental session. Though the monkey could have access to its food freely in its home cage, water supply was restricted during periods of training and the experiment. Health was checked and supplementary water and fruits were provided daily. Animal care and experiments were in accordance with *Principles of laboratory animal care* (NIH publication No. 86–23, revised 1985).

Surgical procedures

Under general anesthesia with i.v. pentobarbital sodium (4.5–6.0 mg/kg per hour), the monkey was placed in a stereotaxic frame. Acrylic screws, which acted as anchors for dental resin and a head stabilization device, made of Delrin, were implanted on the skull with dental acrylic resin. Use of metal was avoided to permit magnetic resonance imaging (MRI). Eye coils were implanted around both eyes to measure eye movements using the method of Matsumura et al. (1992), which was modified from Judge et al. (1980). After recovering from the operation, brain MR images (Hitachi Laboratory MRIS, 2.1 T; National Institute for Physiological Sciences, Okazaki) were taken to determine the mounting position of two recording chambers, one for the SC and the other for the brainstem. Two recording chambers made of Delrin were implanted based on the MR images. For the SC, the chamber was tilted posterior by 30° from the frontal plane, and centered over the midline. For the brainstem, the chamber was tilted leftward by 35° from the sagittal plane, and the center of the chamber was aimed at the abducens nucleus.

Behavioral tasks

The monkey was trained to perform two basic saccade tasks before experiments: a *saccade task*, which was designed to elicit visually guided saccades, and a *delayed saccade task*, to elicit memory-guided saccades. Details of those tasks were described in previous papers (Hikosaka et al. 1989; Matsumura et al. 1992; Kori et al. 1995). We mainly used the delayed saccade task for the stimulation experiment.

The monkey sat in a primate chair in a dimly lit and sound-attenuated room with his head fixed. In front of him was a tangent screen (57 cm from his face) onto which small red spots of light (diameter 0.2°) were backprojected using two light-emitting diode (LED) projectors. The first projector was used for a fixation point, the second for a target point. The positions of the stimuli were controlled by reflection of the lights via two orthogonal (horizontal and vertical) mirror galvanometers.

Stimulation method

We used glass-insulated Elgiloy microelectrodes (Suzuki and Azuma 1976) for single-unit recording as well as electrical stimulation. The electrode was driven by a micromanipulator (Narishige, MO-95) attached to the recording chamber. After identifying the SC or the brainstem saccade area by single-unit recording, we used the same electrode for stimulation.

The stimulation experiment consisted of two steps. First, we applied a train of five pulses (negative square pulses, duration 0.2 ms, interval 2 ms) to determine the threshold for evoking a saccade, while the monkey was not performing a task. We defined the threshold as the intensity with which a saccade was evoked in more than half of stimulation trials. The threshold was between 20 and 100 μA at the intermediate and deep layers of the SC.

We then applied single-pulse stimulation (negative square pulse; intensity 25–100 μA; duration 0.1–0.2 ms) midflight during a saccade (midflight stimulation). For this purpose, we used the delayed saccade task to induce memory-guided saccades, which are slower and longer in duration than visually guided saccades (Hikosaka and Wurtz 1985b; Smit et al. 1987) to facilitate midflight stimulation. We set the stimulation delay at 210–310 ms after the fixation point went off, because memory-guided saccades typically began between 200 and 250 ms and ended between 300 and 350 ms.

Position of both eyes were simultaneously measured by the magnetic search coil method and signals were electronically differentiated for detecting small changes of eye velocity. The velocity data following stimulation (duration 100 ms) were sampled at 10 kHz and were stored in a computer (NEC, PC9801RA; Tokyo).

Within a block of experiments consisting of 20 or 40 trials, the saccade target was selected randomly from 2, 4, or 8 directions with the same eccentricity (typically 20°). The target directions were spaced by 180°, 90°, and 45°, respectively, when there were 2, 4, or 8 possible target locations. By averaging these data, the velocity changes due to voluntary saccades were mostly eliminated while exposing the stimulus-evoked eye velocity changes.

The electrode was moved down or up by 500 μm around the regions of interest and these stimulation procedures were repeated. For brainstem stimulation, we sometimes applied the same single-pulse stimulation while the monkey was fixating, in addition to the midflight stimulation.

Identification of the SC and the brainstem saccade area

The electrode penetration toward the SC was roughly orthogonal to its surface. With this approach, the SC was identified by visual responses in its superficial layers followed by saccadic responses in the intermediate and deep layers (Schiller and Stryker 1972; also see Fig. 4). Before the stimulation experiment we determined visual receptive fields and saccade movement fields of neurons recorded along a given electrode penetration.

The penetration to the brainstem saccade area was tilted laterally so that we were able to stimulate three groups of neurons in one penetration: abducens nucleus neurons, BNs, and pause neurons (see Fig. 5). Along the penetration, we first identified the abducens nucleus by its burst-tonic activities observed in all isolated neurons. The stimulation there evoked a robust eye movement with short latencies in the ipsilateral eye (see Fig. 5), thus confirming the identification of the abducens nucleus. Beneath the abducens nucleus (ventromedial to it) we found BNs in the reticular formation and then omnipause neurons close to the midline (Scudder et al. 1988). The identification of these areas was confirmed by later histological examination.

Data analysis

The eye velocity data (horizontal component for each eye) were averaged to yield the stimulus-evoked velocity changes. The latency of the stimulus-evoked velocity change from the onset of stimulation was determined by eye by moving a cursor on a computer display. If it was difficult to determine the latency because of noise or base line fluctuation, we discarded the data. For statistical analysis, we used the nonparametric Mann-Whitney U-test; statistical difference was assessed at a level of $P<0.05$.

Histology

We made several marking lesions at sites of stimulation by passing current through the recording/stimulation electrode (5 μA, 200 s, electrode positive). At the end of the experiments, the monkey was deeply anesthetized with pentobarbital and perfused with mixture of 4% formaldehyde, 1% glutaraldehyde, in 0.1 M phosphate buffer, pH 7.4. The brain was removed and placed into postfixative solution. After a cryoprotection procedure, the tissue was cut into 50-μm-thick coronal sections on a freezing microtome and stained with cresyl violet.

Results

Stimulation of the SC

Conventional stimulation

In agreement with previous studies (Robinson 1972; Schiller and Stryker 1972), stimulation of the SC (outside the rostral pole) while the monkey was fixating evoked contraversive saccades with similar amplitudes and directions regardless of the initial eye position (Fig. 1A). The latencies from stimulation onset to the saccade onset were 18–20 ms (Fig. 1B). A train of pulses (usually more than five pulses) was required to evoke a saccade; single-pulse stimulation never evoked a saccade.

Midflight stimulation

Stimulation of the SC during a saccade (midflight stimulation) perturbed the on-going saccades with very short latencies (less than 10 ms; Fig. 2B). There was no effect when the same stimulation was applied either just before the initiation of saccades (Fig. 2A) or just after the termination of saccades (Fig. 2C). The midflight stimulation was effective even with single-pulse stimulation.

The direction of perturbation was always contralateral to the stimulation regardless of the direction and amplitudes of on-going saccades (Fig. 3). However, the magnitude of the perturbation was dependent on the direction of the saccade: it was largest when the saccade was directed to the side ipsilateral to the stimulation, as evident in the velocity traces (Fig. 3B). The peak velocity change was 307.9±88.0°/s (mean±SD) when the saccade was directed to the right (Fig. 3B, top); it was 123.1±35.8°/s with vertical saccades (Fig. 3B, middle) and 98.7±30.4°/s with leftward saccades (Fig. 3B, bottom). Similar differences were observed consistently

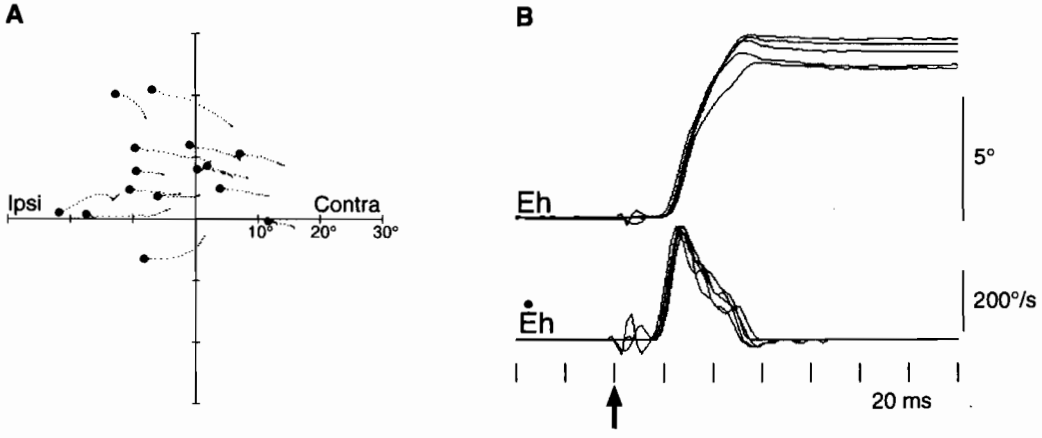


Fig. 1A, B Contraversive saccades induced by conventional electrical stimulation of the left superior colliculus (SC). **A** Two-dimensional display of evoked saccades (rightward), their starting points indicated by *small dots*. Stimulation (40 μA, 5 pulses, 500 Hz) was applied when the monkey's gaze was directed to different locations. For each saccade trajectory, eye positions are shown at 500 Hz. **B** Horizontal traces of evoked saccades (*top*, *Eh*, eye position; *bottom*, *Ėh*, eye velocity) were aligned on the onset of stimulation (indicated by *arrow*); they are superimposed as if starting from the same position

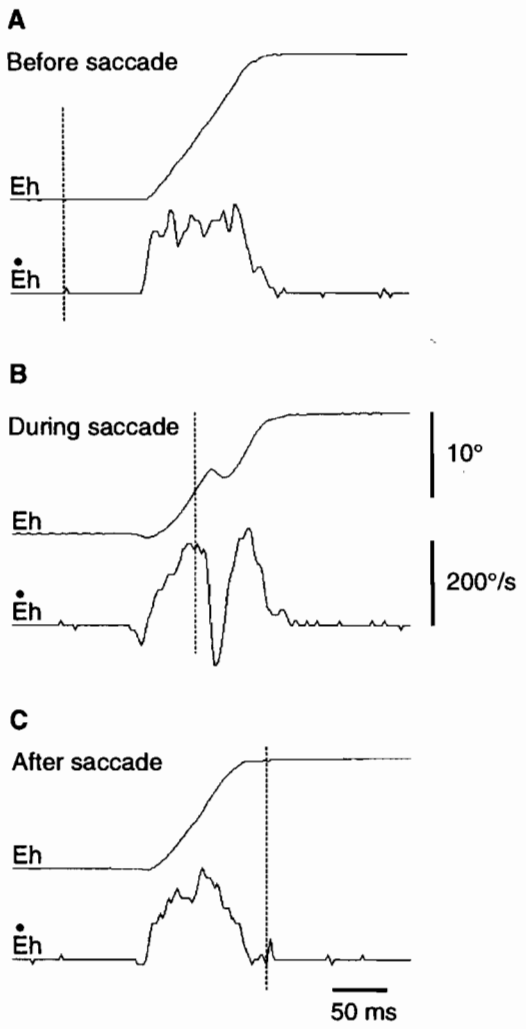


Fig. 2A–C SC stimulation was most effective during saccades. **A** No effect was produced when stimulation (30 μA, 3 pulses, 500 Hz) was applied to the right SC before the onset of a saccade. **B** Contraversive eye movements was evoked when the same stimulus was applied midflight. **C** The same stimulus just after the saccade offset evoked no eye movement. *Broken vertical lines* indicate the onset of stimulation. *Upper trace*, horizontal eye position; *lower trace*, horizontal eye velocity

across different experiments. The perturbation was conjugate in that it was evoked in both eyes in a similar manner (Fig. 4, right).

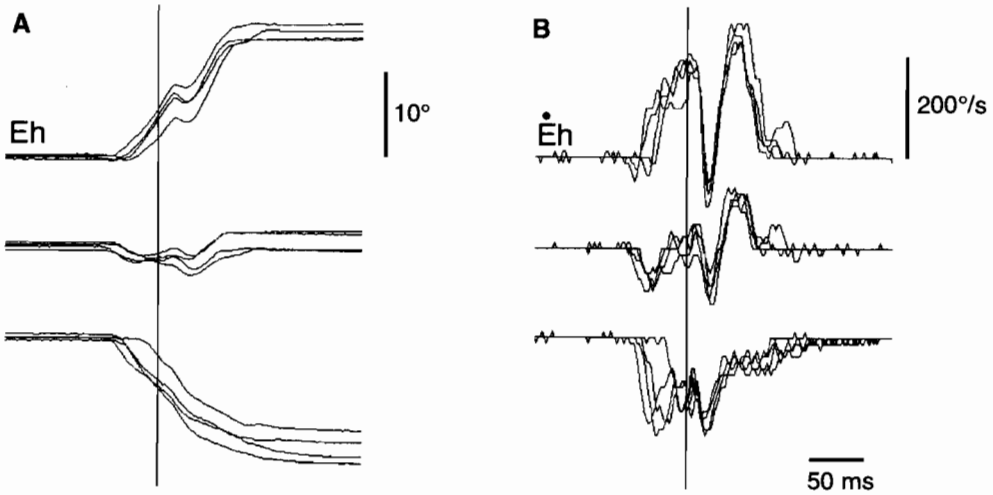
Effects of midflight stimulation depend on the depth in the SC

Figure 4 shows an example of electrode penetration in which we systematically varied the depth of recording/stimulation. In the superficial layer in which we could record only visual responses (Fig. 4, left; depth 0–900 μm), the saccade-evoking threshold was very high (≥100 μA; Fig. 4, middle) and no eye movement was evoked by the midflight stimulation (Fig. 4, right). When the electrode was moved down to the intermediate-deep layer in which pure saccadic neurons appeared (Fig. 4, left; depth 2400–2900 μm), the saccade-evoking threshold became lower (Fig. 4, middle) and the effects of the midflight stimulation became larger (Fig. 4, right).

As the electrode was advanced further into the deep layers (Fig. 4, left; depth 3400–3900 μm), the threshold became higher and midflight stimulation was slightly less effective. When the electrode reached the most ventral portion of the deep layer, the threshold again became low and the largest effect was obtained by the midflight stimulation, although no neural activity was recorded. By histological examination, it was confirmed that the deepest site was out of the SC and probably at the bundle of efferent fibers of the SC beside the periaqueductal gray (Moschovakis and Karabelas 1985; Fig. 4, left).

To determine the latency of the SC-evoked eye velocity changes, we chose 37 stimulating sites in ten penetrations. These sites were thought to be within the output layer of the SC based on the following criteria: (1) presaccadic neural activities were recorded at the site, (2) saccade-evoking threshold during fixation was less than 100 μA using a train of five pulses. A total of 160 experiments were performed, as the data were collected several times at each site.

Fig. 3A, B SC-evoked eye movements during saccades in different directions. **A** Horizontal eye position. **B** Horizontal eye velocity. Traces are rightward saccades (*top*), vertical (either upward or downward) saccades (*middle*), and leftward saccades (*bottom*). Stimulation (30 μA, 3 pulses, 500 Hz) was applied to the right SC, its onset indicated by a *vertical line*. The evoked eye movements were always to the left, but their amplitudes and velocities depended on the saccade directions



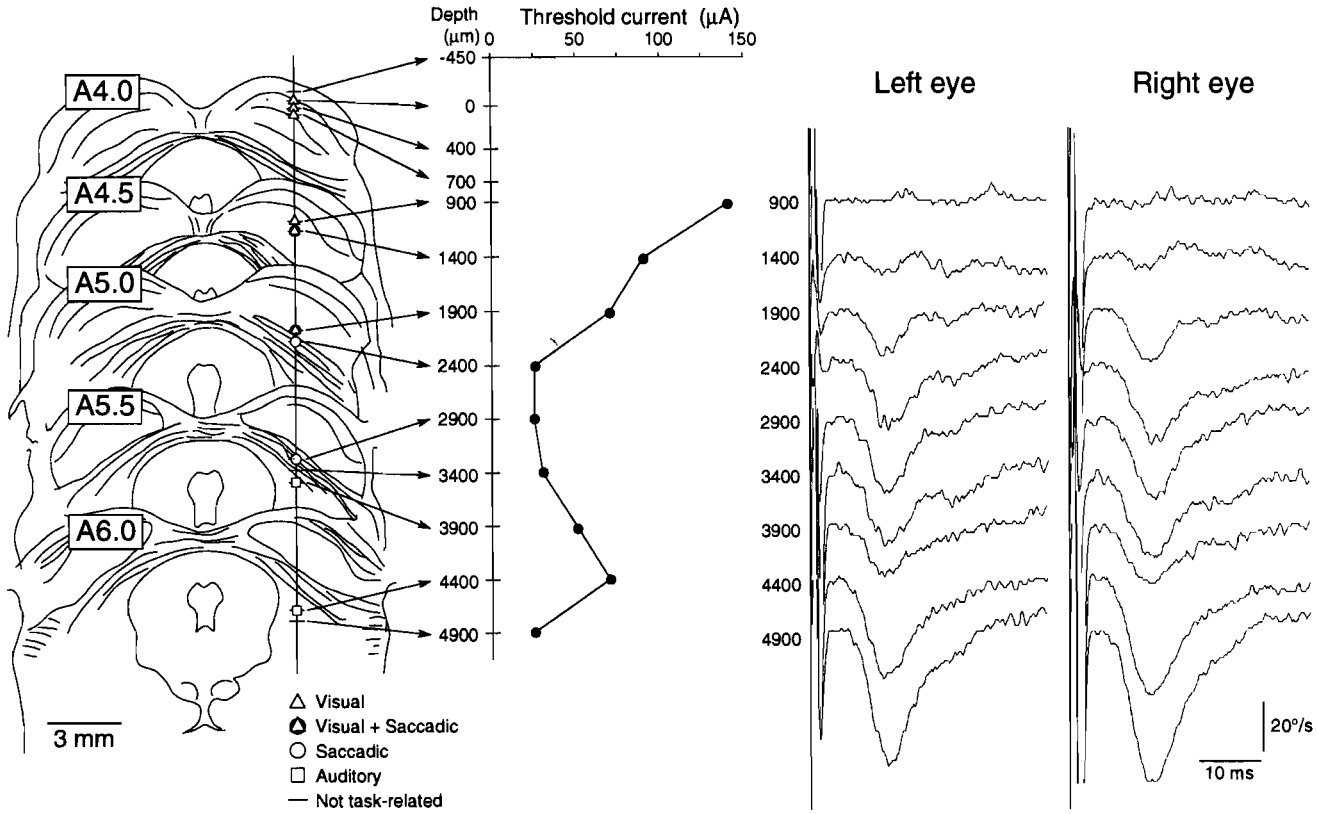


Fig. 4 Sites of recording/stimulation in the SC and stimulus-evoked eye velocity changes. *Left* An example of electrode penetration for recording/stimulation. Since SC penetrations were tilted by 30° posteriorly from the frontal plane in which histological sections were made, the penetration appeared in many sections from *A4.0* to *A6.0*. *Each mark* indicates the type and location of a neuron recorded along the penetration. *Middle* Thresholds for evoking saccades at different depths. The depths were measured from the first visual response. *Right* Velocity changes of the left and right eyes elicited by stimulation (100 μA, single pulse, duration 100 μs) of the right SC at different depths. Similar leftward velocity changes occurred in both eyes. Velocity changes due to the monkey's voluntary saccades have been subtracted out (see Materials and methods)

The results are shown in Fig. 6A. The mean latencies of the SC-evoked eye velocity changes were 7.9±1.0 ms (SD) and 7.8±0.9 ms (SD) for the eye ipsilateral and contralateral to the stimulation side, respectively (Fig. 6A). There was no statistically significant difference between the ipsilateral and the contralateral latencies (P=0.21).

One penetration was thought to be in the rostral pole of the right SC because fixation-related cells were recorded (Munoz and Wurtz 1993a). Stimulation at this site reduced both horizontal and vertical eye velocities regardless of saccadic directions. The latencies of the eye velocity changes were between 9 and 10 ms (data are not

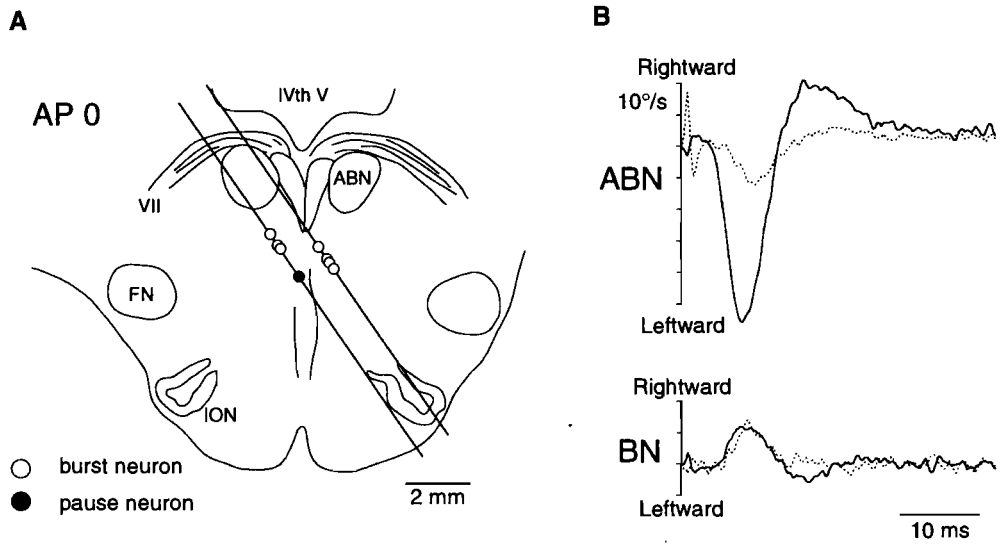


Fig. 5A, B Eye velocity changes evoked from the brainstem. **A** Locations of burst neurons (*open circles*) and a pause neuron (*filled circle*) along two penetrations. **B** Velocity changes evoked from the left abducens nucleus (*top*) and the right burst neuron area (*bottom*). The data for the two eyes are shown separately with respect to the site of stimulation: ipsilateral (*solid line*) and contralateral (*dotted line*). Single-pulse stimulation (duration 200 μs) was used (75 μA for ABN, 100 μA for BN). (*ABN* abducens nucleus, *BN* burst neuron, *FN* facial nucleus, *ION* inferior olive, *IVth V* fourth ventricle, *VII* facial nerve)

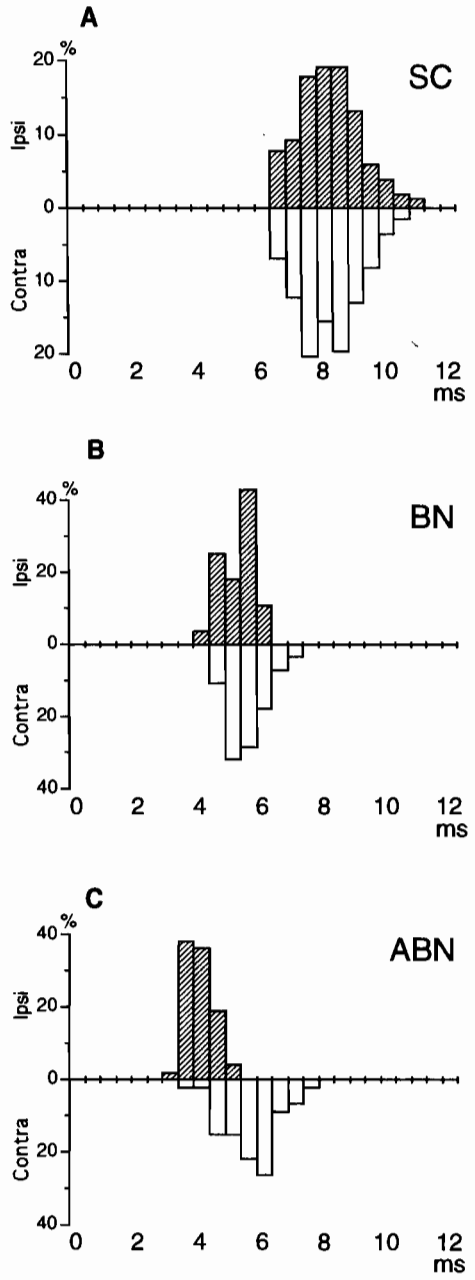


Fig. 6A–C Distribution of latencies of eye velocity changes evoked from the **A** SC (n=160), **B** burst neuron area (n=28), and **C** abducens nucleus (n=48). *Upward hatched bar*, data from the eye ipsilateral to the stimulation side; *downward white bar*, data from the contralateral eye

shown). Similar results were obtained by Munoz and Wurtz (1993b).

Stimulation of the brainstem

Location of the abducens nucleus and the BN area

Along the tilted penetration, we first encountered the abducens nucleus followed by BNs and omnipause neurons, as shown in Fig. 5A. The recorded BNs were of short-lead type (Hepp et al. 1989). Histological examination showed that they were located in the pontomedullary reticular formation. This area roughly corresponded to the location of inhibitory burst neurons (IBNs), which is caudoventromedial to the abducens nucleus (Hikosaka and Kawakami 1977; Yoshida et al. 1982; Strassman et al. 1986b; Scudder et al. 1988), not the location of excitatory burst neurons (EBNs), which is rostral to the abducens nucleus (Igusa et al. 1980; Sasaki and Shimazu 1981; Strassman et al. 1986a). The pause neurons were located close to the midline raphe, as indicated by Büttner-Ennever et al. (1988).

Stimulation of the BNs

Unlike the SC, conventional stimulation (train of pulses) at the BN area usually evoked smooth eye movements in both eyes to the side ipsilateral to the stimulation. Unlike the SC, the speed of the evoked eye movements increased by increasing the strength and frequency of stimulation, in agreement with Cohen and Komatsuzaki (1972). Occasionally, however, ipsilateral saccades were evoked by the same stimulation.

Single-pulse stimulation at the BN area evoked an eye movement in both eyes to the ipsilateral side (Fig. 5B, bottom). This was observed even when the stimulation was applied during eye fixation, unlike the SC stimulation. Data were obtained from 28 stimulation sessions at the BN area. The mean latency was 4.8±0.5 ms (SD) for the ipsilateral eye and 5.1±0.7 ms (SD) for the contralateral eye (Fig. 6B). There was no statistically significant difference in latencies between the eyes (P=0.18).

Stimulation of the abducens nucleus

Single-pulse stimulation in the abducens nucleus, whether or not it is applied during fixation, evoked a prominent eye movement in the ipsilateral eye (Fig. 5B, top). The contralateral eye also showed leftward eye movement, but the effects were smaller and delayed. A total of 48 stimulation sessions were performed in the abducens nucleus. The mean latencies of eye velocity changes were 3.6±0.4 ms (SD) and 5.2±0.8 ms (SD) for the ipsilateral eye and the contralateral eye, respectively (Fig. 6C). Latencies for the ipsilateral eye were significantly shorter than those for the contralateral eye (P<0.0001).

Discussion

Connections from abducens neurons to extraocular muscles

The mean latencies of eye movements evoked by abducens nucleus stimulation were 3.6 ms in the ipsilateral eye and 5.2 ms in the contralateral eye. This difference

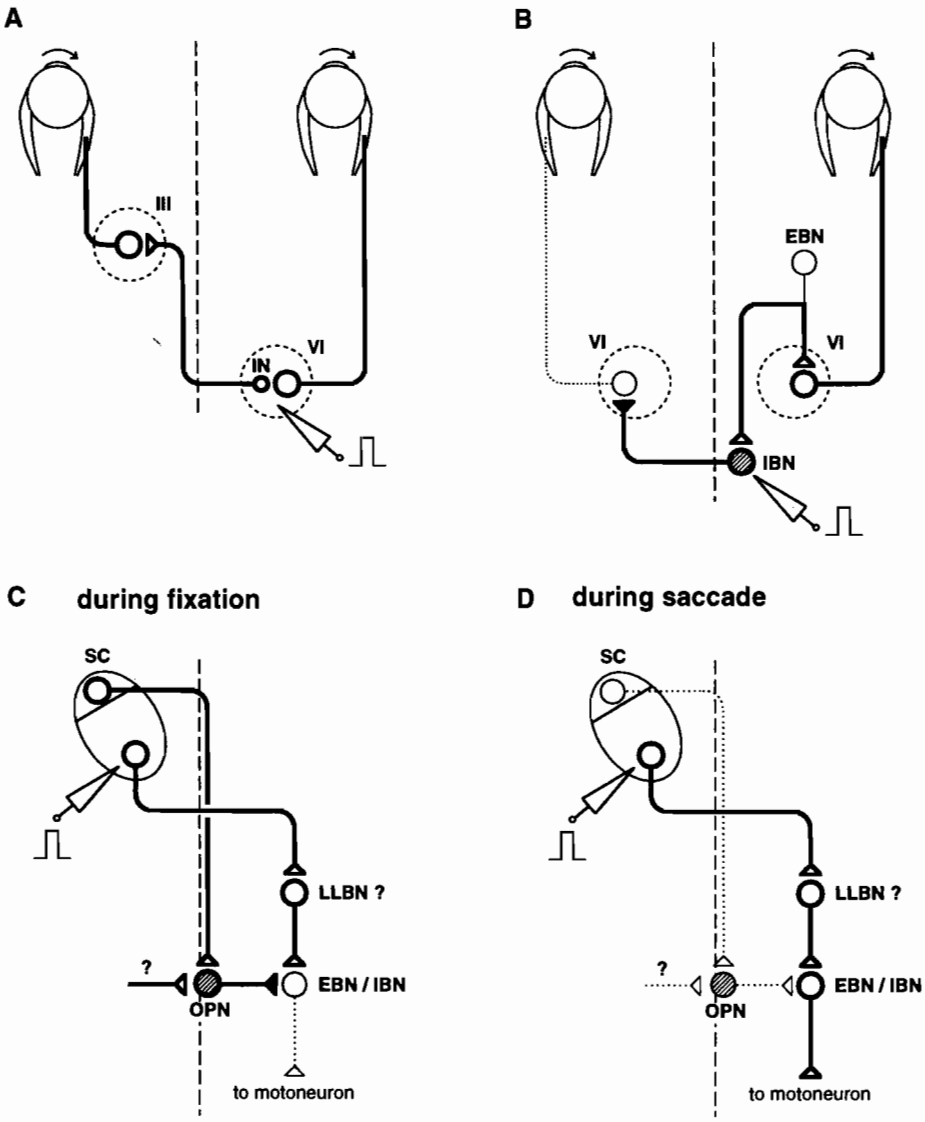


Fig. 7A–D Hypothesized connections from each stimulation site to target neurons/muscles. **A** Stimulation of the abducens nucleus (*VI*) activates the ipsilateral lateral rectus muscle monosynaptically, whereas the contralateral medial rectus muscle is excited disynaptically via the interneurons (*IN*) in the ipsilateral VI and motoneurons in the contralateral oculomotor nucleus (*III*). **B** Stimulation of inhibitory burst neurons (*IBN*) inhibits contralateral VI motoneurons monosynaptically and activates ipsilateral VI motoneurons monosynaptically through the axonal reflex of the ipsilateral excitatory burst neurons (*EBN*). **C** Single-pulse stimulation of the SC during fixation cannot evoke a saccade, because the activity of burst neurons in the brainstem is strongly inhibited by omnipause neurons (*OPN*). The tonic activity of OPN during fixation might be maintained by neurons in the rostral pole of the SC and unknown other sources. **D** During a saccade, the tonic inhibition of OPN on the burst neurons ceases temporarily. Single-pulse stimulation of the SC can then activate burst neurons and evoke a contraversive eye movement with a minimum synaptic delay. *Thick lines* indicate excited connections; *dotted lines* indicate inhibited connections. *White circle*, excitatory neuron; *hatched circle*, inhibitory neuron; *vertical dashed line*, midline

should reflect the fact that the ipsilateral control is monosynaptic while the contralateral control is disynaptic (Fig. 7A). Motoneurons in the abducens nucleus connect to the ipsilateral lateral rectus muscle directly (Baker et al. 1969), whereas interneurons in the abducens nucleus connect to the motoneurons in the contralateral oculomotor nucleus that innervate the medial rectus muscle (Highstein and Baker 1978).

The ipsilateral delay of 3.6 ms is considered to be the earliest neural effect on the contraction of the lateral rectus muscle. The contralateral delay of 5.2 ms is longer than the ipsilateral delay by 1.6 ms, which is most likely explained by the intervention of abducens interneurons. The latencies of unitary excitatory postsynaptic potentials (EPSPs) evoked in medial rectus motoneurons caused by single spikes of abducens interneurons were between 0.8 and 1.0 ms (Nakao and Sasaki 1980). Although the contralateral/ipsilateral difference obtained in the present study (1.6 ms) was somewhat longer, there are no other synapses.

Connections from premotor BNs to abducens motoneurons

Single-pulse stimulation at the BN area evoked an ipsiversive eye movement in both eyes. The mean latency was 4.8 ms for the ipsilateral eye and 5.1 ms for the contralateral eye. These values roughly correspond to the shortest saccade-lead times of EBNs (Strassman et al. 1986a) and IBNs (Yoshida et al. 1982; Strassman et al. 1986b; Scudder et al. 1988), although other studies reported somewhat different values (see Hepp et al. (1989)).

The time difference between the BN-evoked effect (4.8–5.1 ms) and the ipsilateral abducens-evoked effect (3.6 ms) was 1.2–1.5 ms. This is compatible with the data obtained by Scudder et al. (1988), who compared the latencies of EMG responses in the lateral rectus muscle in the monkey: the latency of the IBN-evoked response (2.7 ms) was longer than the latency of abducens nucleus-evoked response (1.4 ms) by 1.3 ms. The delay times

of 1.2–1.5 ms suggest that BNs we stimulated were monosynaptic to abducens motoneurons.

Given that activation of the medial rectus requires an additional synaptic transmission compared with activation of the lateral rectus, why did the BN-evoked eye movement have comparable latencies for both eyes? It is well known that horizontal BNs are divided into two groups – excitatory burst neurons (EBNs) and inhibitory burst neurons (IBNs). EBNs are located in the pontine reticular formation anterior to the abducens nucleus (Igusa et al. 1980; Sasaki and Shimazu 1981), while IBNs are located in the pontomedullary reticular formation posterior to the abducens nucleus (Hikosaka and Kawakami 1977).

According to the histological examination (Fig. 5A), the BNs we stimulated in the present study were more likely to be IBNs (Scudder et al. 1988). Stimulation of IBNs on one side should reduce the activity of contralateral abducens motoneurons. This would allow the contralateral eye to rotate to the ipsilateral side, because the medial rectus of this eye would maintain its contracting force.

Why then would stimulation of the IBN area lead to the ipsilateral rotation of the ipsilateral eye? One possible mechanism would be the axonal reflex of EBNs. It has been demonstrated that EBNs project to the abducens nucleus as well as to the IBN area in the cat (Sasaki and Shimazu 1981) and in the squirrel monkey (Strassman et al. 1986a). Therefore, it was likely that stimulation of the IBN area activated ipsilateral abducens motoneurons monosynaptically via the axonal reflex of ipsilateral EBNs. Consequently, stimulation of the unilateral IBN area can produce ipsiversive movements of both eyes with approximately the same latencies (Fig. 7B).

Though we stimulated the area where BN activities were recorded, we cannot eliminate the possibility of stimulating passing fibers, including axons of vestibular neurons. We think, however, the contribution of vestibular axons is weak. For example, vestibular axons projecting to the contralateral abducens motoneurons are known to be excitatory. Stimulation of such vestibular axons should produce contraversive eye movements of the contralateral eye, unlike the data in the present study.

Connection from the SC to premotor BNs

In agreement with Munoz et al. (1991), single-pulse stimulation of the SC (except the rostral pole), when applied during a saccade (midflight stimulation), evoked contraversive eye movements that perturbed the on-going saccade. The same stimulation never evoked eye movements when the monkey was fixating (either with or without visual targets).

This may be explained by the gating mechanism of omnipause neurons. During fixation, BNs are strongly inhibited by omnipause neurons (Nakao et al. 1980; Fig. 7C) and therefore strong stimulation is required to activate them. During a saccade, however, BNs are released from tonic inhibition (Evinger et al. 1982; Strassman et al. 1987; Fig. 7D) and therefore are more susceptible to excitatory inputs from the SC.

What interested us most was that the midflight stimulation evoked short-latency effects (eye velocity changes), especially from the SC. Whereas the latency of saccades evoked by conventional stimulation of the SC was about 18 ms, the latency of eye movements by the saccadic midflight stimulation was only 8 ms. Apparently, the 10-ms delay reflects the delay in the discharge of BNs that is caused by tonic inhibition exerted by omnipause neurons. Assuming that, during a saccade, the SC output pathway is devoid of significant inhibitory effects, the short latency obtained by the midflight stimulation would be close to the minimum time required for the SC outputs. Thus, we can estimate the number of synapses interposed between the SC and motoneurons by using approximately 3 ms as the time corresponding to the signal transmission time from the SC to BNs. This result makes it most likely that the SC-BN connection is disynaptic, for the following reasons.

First, it is unlikely to be monosynaptic, because the conduction velocities of SC output neurons are generally high and thus the axonal conduction time to the periabducens area is mostly less than 1 ms (Grantyn and Grantyn 1982). Even if we add the synaptic delay of about 0.5 ms, the total transmission time is less than 1.5 ms, which is significantly smaller than our value of 3 ms. The delay of 3 ms may be explained by including one more synapse, considering that the monosynaptic delay between premotor BNs and abducens motoneurons was estimated to be 1.2–1.5 ms in the present study. Extending the same argument, it seems unlikely to be trisynaptic. The estimated time delay would certainly be more than 3 ms if we add the time for another synapse. Our conclusion agrees with that of Raybourn and Keller (1977), who used awake monkeys, but not Chimoto et al. (1996), who used awake cats.

Of course, there remains the slight possibility that the SC-BN connection is either monosynaptic or trisynaptic, depending on whether the intervening neurons have unusually slow or fast signal transmission.

Which neurons mediate SC-originated saccadic signals to the premotor BNs?

If we suppose that the SC-BN connection is disynaptic, there must be one intermediary group of neurons. An obvious candidate is the so-called long-lead BNs (LLBNs), which start firing more than 15 ms before a saccade occurs (Scudder et al. 1988; Hepp et al. 1989). Raybourn and Keller (1977) showed that SC outputs were transmitted to LLBNs monosynaptically, but not directly to short-lead BNs (EBN or IBN) in the monkey. However, it is still unknown whether LLBNs are actually connected to EBNs or IBNs.

This question is very important, because the nature of saccadic information is different in the SC and the brain-

stem saccade generators. For example, the amplitude of a saccade is encoded by the locations of active neurons in the SC (spatial-encoding; Sparks and Hartwich-Young 1989) but by the amount of activity in individual neurons in the brainstem BNs (temporal-encoding; Hepp et al. 1989). Some LLBNs show temporal-encoding (like EBNs and IBNs), while other LLBNs show spatial-encoding (like SC neurons; Hepp and Henn 1983). However, it is unlikely that these two types of LLBNs are interposed in a sequential manner between the SC and EBNs/IBNs, because it would make the SC-BN connection trisynaptic.

Saccadic midflight stimulation can reveal saccadic neuronal networks in behaving animals

Although extensively studied, the investigation into the saccadic neural mechanisms has been hampered by the discrepancy between the data obtained in anatomical/electrophysiological studies and the data obtained in behavioral experiments. For the studies of skeletal movements, on the other hand, a refined technique, such as spike-triggered averaging, has been successfully applied to reveal the relationship between higher motor areas and the muscular activities (Fetz et al. 1976).

The saccadic midflight stimulation we used in the present study may be one way to reduce the discrepancy between the behavioral and anatomical studies. It will facilitate the investigation into the neuronal organizations of more complicated structures such as the basal ganglia, cerebellum, and cerebral cortex in behaving animals.

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