Release from $GABA_A$ receptor-mediated inhibition unmasks interlaminar connection within superior colliculus in anesthetized adult rats

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Abstract

The superior colliculus (SC) contains two major subdivisions, the superficial layers (sSC) and the deeper layers (dSC). sSC receives visual information from the retina and visual cortex, while dSC sends descending projections to the brainstem and spinal cord. It has not been clear whether and how sSC directly activates dSC, however recent studies in slice preparations reported that electrical stimulation of sSC induces burst firing in dSC neurons after application of bicuculline. In the present study, we tested whether sSC directly activates dSC *in vivo*. In isoflurane-anesthetized rats, electrical stimulation of the optic nerve (ON) induced negative field responses mainly in sSC, but not as much in dSC, under control conditions. However, after injection of bicuculline into dSC, ON stimulation induced long-lasting negative field responses in dSC. dSC neurons including the tectofugal neurons exhibited burst firing during the long-lasting negative field responses. The burst responses were largely suppressed by local injection of the NMDA-receptor antagonist, D-2-amino-5-phosphopenovalerate. The burst responses remained after ablation of the cerebral cortex ipsilateral to the SC recordings. These results suggest that retinal inputs induce burst responses in tectofugal neurons in dSC via subcortical pathways when the SC circuit is released from $GABA_A$ receptor-mediated inhibition. We propose that sSC is the main candidate for

the source of the excitatory synaptic inputs to dSC among the subcortical regions.

Introduction

The mammalian superior colliculus (SC) consists of several layers with distinct organizations that are generally grouped into two major subdivisions. The superficial layers (sSC) [stratum zonale (SZ), stratum griseum superficiale (SGS), and stratum opticum (SO)] receive visual information directly from the retina and indirectly from the visual cortex. Neurons in sSC respond to visual stimulation, and their receptive fields are arranged to form a retinotopic map of the contralateral visual field (Siminoff and Schwassmann 1966; Cynader and Berham 1972; Tiao et al. 1976). The deeper layers (dSC) [stratum griseum intermedium (SGI), stratum album intermedium (SAI), stratum griseum profundum (SGP), and stratum album profundum (SAP)] receive non-visual sensory inputs (Meredith and Stein 1986) as well as inputs from the cerebral cortex (Cadusseau and Roger, 1985; Harting et al. 1992). The dSC--which sends descending projections to the brainstem (BS) and spinal cord (SP) that generate orienting behaviors of various body parts (including eyes, head, trunk, and limbs) (Huerta et al. 1982; Redgrave et al. 1987)--has a topographic representations of a vectorial map of these orienting behaviors (Robinson 1972; Schiller and Stryker 1972; Sahibzada et al. 1986). Because the spatial representations of the visual map in sSC and the motor map in dSC are coincident with each other, Robinson (1972) and Sprague

(1975) proposed the existence of vertical connection from sSC to dSC. However, this proposal was opposed by Edwards (1980) who, following careful anatomical inspection, concluded that no significant connection exists between the two layers. On the other hand, Maeda et al. (1979) showed that in anesthetized cats, short latency EPSPs could be induced in dSC neurons following electrical stimulation of the optic nerve (ON). Later, the existence of an interlaminar connection was unequivocally demonstrated anatomically by Moschovakis and colleagues in primates (Moschovakis et al. 1988a,b) and by Rhoades and colleagues in the hamster (Mooney et al. 1988; Rhoades et al. 1989; Mooney et al. 1992), and this was then confirmed in other species (Behan and Appell 1992; Hall and Lee 1993; Lee and Hall 1995). More recently, by using the whole-cell patch-clamp recording technique in slice preparations, Lee et el. (1997) and Isa et al. (1998) succeeded in showing the existence of an excitatory connection from sSC to dSC in young tree shrews and young rats, respectively. In the latter study, it was found that signal transmission through the interlaminar pathway is much enhanced when the circuit is disinhibited from $GABA_A$ receptor-mediated inhibition by application of bicuculline (BIC) to the slices (for review, see Isa and Saito 2001; Isa 2002). It has also been found that stimulation of the SGS or optic tract induces long-lasting burst responses (duration sometimes exceeding 1s) in dSC neurons.

Previous studies had shown that neurons that send tonic inhibitory signals to burst neurons in SC decrease their firing rate before the initiation of orienting behaviors (Hikosaka and Wurtz 1983; Munoz and Wurtz 1993). Thus, a disinhibition of the SC circuit actually occurs prior to the initiation of movements. Furthermore, a disinhibition of the SC circuit by injection of BIC has been shown to facilitate the initiation of orienting behaviors *in vivo* (Hikosaka and Wurtz 1985; Dean et al. 1989). In the present study, we investigated *in vivo* whether inputs from the retina can directly activate dSC neurons via an interlaminar connection through sSC. For this purpose we used isoflurane-anesthetized adult rats. We stimulated the optic nerve (ON) and recorded field and unit responses in various layers of SC. The main aim was: (1) to investigate the synaptic responses of SC neurons in various layers before and after disinhibition caused by injection of BIC into dSC, (2) to examine whether sSC can directly activate dSC by observing whether the field and unit responses remain after ablation of the cerebral cortex ipsilateral to the side used for SC recordings, (3) to investigate the retinal axon subtypes that induce excitatory synaptic responses in dSC neurons, and (4) to assess the contribution made by NMDA-type glutamate receptors to synaptic responses in dSC neurons.

Materials and methods

All experimental procedures were approved by the *Committee for Animal Experiments at Okazaki National Institutes*. Fifteen male Long-Evans hooded rats (8-12 weeks old), weighting 200-400 g, were made to inhale isoflurane and then given an intramuscular injection of atropine sulfate (0.02 mg/kg). After orotracheal intubation, artificial ventilation was instituted and anesthesia was maintained with 2.0-3.0 % isoflurane in a 1:1 mixture of N_2O and O_2 during the surgical preparation. An intravenous cannula was inserted into a great saphenous vein, and an intraarterial cannula into a femoral artery. The animal was fixed into a stereotaxic head holder and all pressure points were infiltrated with 1% lidocaine. During the experiment, end-tidal CO_2 was continuously monitored, and blood pressure directly from the femoral artery. Body temperature was monitored using a rectal probe and maintained at 36-7 $^{\circ}$C with the aid of a feedback-controlled infrared lamp. During recording, the isoflurane was reduced to 1.0-2.0 % and the animal was immobilized by means of intravenous injection of pancuronium bromide (Mioblock, Organon; 0.1 mg/kg/hr). We changed isoflurane concentration to keep arterial blood pressure stable between 80 and 120 mmHg and end-tidal CO_2 between 3.5 and 4.0 %, respectively to confirm the adequate depth of anesthesia. At the end of the experiment, the animal was given a bolus of

pentobarbital sodium and perfused intravenously with 10 % formalin. The brain was immediately removed and cut into blocks along the stereotaxic axis in the coronal plane. Sections through the SC and BS stimulation sites were cut at 100 μm thickness, mounted directly onto glass slides, and stained with cresyl violet to determine the position of the tips of the electrode and injection needle.

Electrophysiological recording and drug injection

Fig. 1*A* schematically illustrates the arrangement of the present experiments.

The right eyeball was separated from the surrounding tissue to allow dissection of the ON. The nerve was hooked onto a pair of J-shaped bipolar silver wire electrodes, and stimulation was applied in the paraffin pool (square wave pulses: duration, 0.2 ms; interval between individual stimuli, 3.3 ms during train stimulation). The intensity of the current pulse was kept below 2 mA.

Since the retinotectal fibers mainly project to the contralateral SC, the right ON was stimulated, and field and unit responses were recorded from the left SC. A square opening (4 mm x 4 mm) was made in the skull overlying the parieto-occipital region of the left hemisphere, and the dura was cut and retracted. The cerebral cortex overlying the left SC was preserved except in 5 rats in which the left cortex was entirely ablated

(see below). Recordings of field and unit responses from the left SC were made using a tungsten electrode (impedance, 5-10 MO) or a glass pipette filled with 2M NaCl (impedance, 0.5-3 MO). Recordings were made at 100 μm intervals in depth with the aid of a stepping motor drive (individual step, 2 μm). Evoked responses were recorded using MEZ-8301 amplifier (Nihon Koden, Tokyo, Japan), digitized using Axon 1200 series Digidata A/D board with pCLAMP8 software (Axon Instruments, Foster City, CA), and analyzed using Matlab 6.1 software (The Mathworks, Natick, MA). Field and unit responses were obtained with the band-pass filter set at 0.08 Hz-3 kHz and 100 Hz-10 kHz, respectively. Field responses were examined by averaging 5 or 10 records. Microinjection of bicuculline (BIC, 0.2-1.0 μl, 10 mM) or D-2-amino-5-phosphopenovalerate (APV, 0.2-1.0 μl, 50 mM) was made through a Hamilton syringe fitted with a 30G needle at depths of 1.2-1.6 mm from the dorsal surface of the SC, aiming at the center of dSC (Fig. 1*B*). BIC and APV (RBI, Natick, MA) were dissolved in saline and administered over a period of 1 min. Since the effect of BIC lasted from 10 to 40 min, recordings were made throughout this period (see Discussion). The tip of the injection needle was set within 200 μm of the recording electrode track.

To investigate whether the tectofugal neurons in dSC showed burst firing following ON

stimulation after BIC injection into dSC, tectofugal neurons in dSC were identified by antidromic activation from the contralateral predorsal bundle (PDB), where the descending axons of the tectofugal neurons pass on their way to the brainstem and spinal cord. Single constant-current rectangular pulses (0.1 ms duration) were delivered via bipolar concentric tungsten electrodes at positions 0.14-0.30 mm lateral to the midline, at 1.0-1.2 mm caudal to bregma and 0.8-1.5 mm ventral to the bottom of the fourth ventricle. The criteria for antidromic activation were that the spike should: (1) have a constant latency with threshold below 500 μA and (2) follow high frequency stimulation (300 Hz) with constant latency at suprathreshold stimulus intensities.

In 5 experiments, after the skull overlying the left hemisphere had been opened and the dura cut and retracted, the entire left cerebral cortex was removed using a vacuum pump.

Results

Depth profile of field responses evoked by stimulation of Y- and W-fibers

(1) Single ON stimulation

First, we examined the depth profile of field responses evoked by applying stimuli to the contralateral ON. In 10 penetrations in 10 rats, field responses of two major distinct types were evoked by such stimulation. One comprised negative field responses that reached maximum at depths of 300-500 μm from the dorsal surface of the SC and had a latency to peak of 2.6-3.3 ms (Fig. 2 *A* and *E*). These responses appeared to be reversed at more dorsal positions (depth: 0-200 μm), while at more ventral positions the negativity remained but the response became smaller. Because this component had a relatively low threshold (50-500 μA) and a short latency, it was assumed to be evoked by the fast conducting Y-group retinotectal fibers, a notion in accord with the findings of Sefton (1969) and Fukuda et al. (1978). The other type of field response comprised negative field responses recorded at depths of 0-200 μm from the dorsal surface of the SC and with a latency to peak of 6.0-7.4 ms (Fig. 2 *C* and *G*). This negativity was reversed at positions deeper than 400 μm. This type had a higher threshold for activation and a longer latency than the first type, and was thus assumed to be evoked by activation of the slowly conducting W-group retinotectal fibers, in line

with Sefton (1969) and Fukuda et al. (1978).

(2) Repetitive ON stimulation

Even if we increased the intensity of the above single ON stimulation, the sink of negative field responses could not be identified in dSC. We therefore tested whether repetitive ON stimulation could evoke negative field responses with their sink in dSC (in 10 penetrations in 10 rats). Repetitive ON stimulation at low stimulus intensity (20 pulses at 300 Hz), which activated Y-fibers alone, evoked short latency field responses whose sink was in sSC linked to individual stimuli, and small negative field responses also in dSC (Fig. 2 *B* and *F*, Fig. 3*A*). Repetitive ON stimulation at high stimulus intensity (5 pulses at 300 Hz), which activated Y- and W-fibers, also evoked only short latency field responses in sSC linked to individual stimuli and no negative field responses of longer duration with their sink in dSC (Fig. 2 *D* and *H*, Fig. 3*B*).

These results suggested that under control conditions, activation of Y- and W-fibers induced excitatory synaptic responses mainly in sSC. In addition, activation of Y-fibers induced weak direct excitatory effect in dSC. However, these retinal inputs were not strong enough to induce polysynaptic excitation in dSC, even if they were intensively activated.

Effect of BIC injection on depth profile of field responses

We injected BIC into dSC to reduce $GABA_A$ receptor-mediated inhibition. The effect of ON stimulation was investigated in 10 rats, although a complete depth profile was obtained in only 5 penetrations. Before injection of BIC, negative field responses were found mainly in sSC (Fig. 2 *A-H*, Fig 3 *A* and *B*), whereas after the injection ON stimulation induced long-lasting negative field responses in dSC (Fig. 3 *C*, *D* and *E*). These long-lasting negative field responses were evoked not only by high intensity stimulation (5 pulses at 300 Hz), which activated both Y- and W-fibers (Fig. 3*D*), but also by applying a larger number of repetitive stimuli at lower intensity (20 pulses at 300 Hz), which activated Y-fibers alone (Fig. 3*C*). The duration of the long-lasting negative field responses was longer than 100 ms, sometimes exceeding 500 ms (Fig. 3*E*). Depth profiles (peak amplitude of the long-lasting negative field responses at individual depths) are plotted in Fig. 3*F*. Amplitude was the greatest at 500-700 μm, depths corresponding to the ventral portion of sSC and the dorsal portion of dSC. The duration of the long-lasting negative field responses was longer in the ventral portion of dSC than in the more dorsal portion, as indicated in Fig. 3*G* in which the half-maximum duration of negative field responses at individual depths was shown. These negative field responses were assumed to represent either EPSPs or spike firings generated in

neurons located in the ventral portion of sSC or in dSC (see below). These results suggested that release from $GABA_A$ receptor-mediated inhibition facilitated excitatory synaptic transmission to dSC. Fig. 4 shows the fast (A) and slow (B) sweep records of depth profile of field responses recorded after BIC injection into dSC. The onset latency of the long-lasting negative field responses was 3.3-3.7 ms from the last (third) stimuli in sSC, and 5.6-7.7 ms in dSC (Fig. 4 *B* and *C*). Thus, the onset latency of the long-lasting negative field responses in dSC was longer than that in sSC. Moreover, the long-lasting negative field responses in dSC started as long as 2.8-4.9 ms after the peak of monosynaptic field responses of the Y-fibers origin (arrow in Fig. 4*C*). Thus, in this situation the long-lasting negative field responses did not appear to be directly triggered by the monosynaptic retinotectal inputs to the dSC neurons (see Discussion).

Effect of BIC injection on unit responses

As described above, after injection of BIC long-lasting negative field responses in dSC were evoked by ON stimulation. To try to determine whether these responses represent EPSPs alone or include spike firings of dSC neurons, extracellular unit activities were investigated in 27 isolated single units and 14 multi-units in dSC following BIC injection into dSC. Since most dSC neurons exhibited no spontaneous

firing before injection of BIC, we searched for unit responses evoked by ON stimulation only after BIC had been injected. We found that neurons in dSC exhibited burst firing (Fig. 5 *A2*, and *B2*) during long-lasting negative field responses (Fig. 5 *A1* and *B1*), with the maximum instantaneous firing frequency of single units often exceeding 500 Hz during these burst responses (Fig. 5 *A3*, *A4*, *B3* and *B4*). Not only repetitive stimulation at high stimulus intensity which activated both Y- and W-fibers, but also low intensity stimulation, which activated Y-fibers alone, induced burst firing in all the 23 dSC neurons tested. Single stimuli with the high intensities sometimes evoked burst firing in dSC neurons as well as long-lasting negative field responses (data not shown). These results suggested that the spike firing contributed to the long-lasting negative field responses.

Antidromic identification of tectofugal neurons in dSC

We investigated whether the dSC neurons responding with burst firing to ON stimulation (after BIC injection) included the tectofugal neurons. We recorded from 8 identified tectofugal neurons in dSC that were antidromically activated from the contralateral PDB (Fig. 6*D*). The antidromic nature of the firing was confirmed by the fixed latency of the responses (Fig. 6*A*) and the fact that the firing response was able to

follow high frequency stimulation (500 Hz in this case) with a fixed latency (Fig. 6*B*). All the antidromically identified neurons exhibited burst firing responses to ON stimulation after BIC injection, as exemplified in Fig. 6*C*. This result showed that dSC neurons projecting to the brainstem and spinal cord were activated by ON stimulation after BIC injection.

Involvement of NMDA-type glutamate receptors in long-lasting field and unit responses in dSC

In slice preparations from young rats (Isa et al. 1998), application of APV abolishes the late components of the long-lasting depolarization and burst firing responses in SGI neurons evoked by stimulation of SGS or optic tract. To test whether this also holds true in adult rats in *in vivo* preparations, we investigated the effect of injection of APV into dSC on 13 field responses and 7 isolated single unit and 5 multi unit responses at a particular depth in dSC and on single depth profile of field responses in 8 rats. Injection of APV abolished the late components of the long-lasting negative field responses (Fig 7 *B* and *C*) and also the burst firings (Fig. 8 *A* and *B*). This was observed in all cases tested. These results suggest that NMDA-type glutamate receptors are essential for the maintenance of long-lasting depolarization and burst

firing in dSC neurons.

Ablation of the cerebral cortex ipsilateral to the side used for SC recordings

As described above, ON stimulation induced long-lasting negative field responses and burst firing of dSC neurons following injection of BIC into dSC. However, it was not clear whether or not ON stimulation activated dSC neurons through an interlaminar connection from sSC. Besides sSC, a main candidate for the excitatory inputs to dSC was the ipsilateral cerebral cortex including sensorimotor cortex and visual cortex. An alternative explanation was that dSC neurons were being activated by the inputs via these cortical regions. To clarify this, we investigated the effect of ablation of the cerebral cortex ipsilateral to the side used for SC recordings (5 tracks involving field recording, 7 isolated single unit and 12 multi-units responses in 5 rats). As shown in Fig. 9*A*, virtually the entire cerebral cortex ipsilateral to the SC recording site was ablated. After ablation, ON stimulation evoked both short latency negative field responses (2.5-3.3 ms latency to peak) of Y-fiber origin mainly in the ventral portion of sSC, and longer latency negative field responses (6.3-7.5 ms latency to peak) of W-fiber origin in the most dorsal portion of sSC (Fig. 9 *B* and *C*) as in the intact animals. After injection of BIC into dSC, ON stimulation evoked long-lasting negative field responses

in dSC that were quite similar to what had been observed in the intact animals (Fig. 9*D* and Fig. 11*A*) as to their time course (duration > 100 ms; Fig. 9*E,G*), the depth profiles of their peak amplitude (Fig. 9*F*), and the onset latencies (sSC: 3.1-3.8 ms, dSC: 5.4-7.8 ms; Fig. 10). ON stimulation evoked burst firing in dSC neurons during the long-lasting negative field responses after the ablation as in the intact animals (Fig. 11*B*). Not only the high intensity stimulation, which activated Y- and W-fibers, but also the low intensity stimulation which activated Y-fibers alone evoked long-lasting negative field responses and burst firing of single units in dSC (data not shown). Thus, most of these characteristics of the long-lasting negative field responses and burst firings in dSC induced by ON stimulation in the intact animals were also observed after the cortical ablation.

Discussion

Methodological considerations

Spread of injectate

In the present experiments, we investigated the effects of injection of 0.2-1 μl BIC (10 mM) on field responses. Estimation of the spread of injectate is critical for the interpretation of the results. For this purpose, Fast Green was dissolved at a saturated concentration in 1 μl BIC solution and injected into dSC. After 2 h, we fixed the brain using 4 % paraformaldehyde, and we examined it 48 h later. The diameter of the spread did not exceed 1.5 mm. Actually, the injectate presumably did not travel more than 0.75 mm in any direction away from the injection site. Since the injections were made at depths of 1200-1400 μm from the dorsal surface of the SC, the spread of BIC was assumed to be within the ventral portion of sSC (mainly SO) and dSC. Furthermore, since the tip of the recording electrode was set within 200 μm of the recording electrode track, most recording would have been within direct reach of the injected BIC or APV. Injection of saline alone into dSC did not cause any change in the field responses (data not shown), so damage and movement of the tissue caused by the injection was assumed to be minimal at the recording sites.

Duration of BIC effect

The duration of the BIC effects is also a critical factor for the interpretation of the present results. We usually started recording field responses 10 min after the injection, when the amplitude of the long-lasting negative field responses had become stable. To test the possibility that the effect of BIC might have declined during the recording of a depth profile of field responses in a single electrode track, we repeated the recording from the same track as soon as we finished recording in that track. In this way, we confirmed that the depth profile remained almost unchanged during the 10-40 min after the injection, and only after that did the effect of BIC gradually decline. Thus, all the records shown in this study were obtained at a time when the BIC effect was stable. In this way, we confirmed that the depth profiles remained almost unchanged during the 10-40 min after the injection, and only after that did the effect of BIC gradually decline. In the experiment to test the effect of APV, which was injected 20 min after BIC, we observed that the effect of BIC was largely abolished by the APV injection (Fig. 7 *A-C*). To exclude the possibility that this was simply due to a decline in the BIC effect, we gave a second injection of BIC, which had been confirmed to be effective again (in the absence of APV) in a preliminary study. When it was given in the presence of APV, we could not observe any enhancement of the long-lasting negative field responses in

dSC (data not shown). Thus, all the records shown in this study were obtained at a time when the BIC effect was stable.

Neural pathway evoking long-lasting negative field responses and burst firings in dSC

Many areas of the CNS send excitatory projections to dSC (Cadusseau et al. 1985). However, the areas that could mediate short latency visual responses are limited. Besides sSC, which has been proposed as the main candidate structure in this study, the sensorimotor cortex in the frontal region can be listed as a major candidate. A projection from the visual cortex in the occipital lobe cannot be excluded, since it is well known that although the projection from the visual cortex terminates mainly in sSC, many neurons in dSC have dendritic arborizations projecting into sSC (Lopez-Barneo and Llinás 1988; Hall and Lee 1997). In addition, there are reports showing that the ventral portion of the lateral geniculate nucleus (Ribak et al 1975; Swanson et al 1974; Moore et al. 2000) and some retinal fibers project directly to dSC (Beckstead et al 1983). As to cerebral cortical regions, it has been reported that only the ipsilateral sensorimotor cortex and visual cortex project to SC (Cadusseau et al 1985). Therefore, the present observation that long-lasting negative field responses and burst firings remained after

ablation of the entire ipsilateral cerebral cortex (Fig. 9, 10 and 11) strongly suggests that they could be induced via a route that bypasses the cerebral cortex. As to the retinal projection to the ventral portion of the lateral geniculate nucleus, it has been reported that only W-fibers project to this nucleus (Sumitomo et al. 1979). Accordingly, the present observation that activation of Y-fibers alone can elicit long-lasting negative field responses and burst firings cannot be explained by invoking a pathway through that nucleus. A direct retinal input to dSC has been described anatomically (Beckstead et al 1983) and physiologically (Berson and McIlwain 1982). In sSC, the onset latency of long-lasting negative field responses was 3.1-3.8 ms (Fig. 10). Therefore, the long-lasting burst responses in sSC appeared to be triggered by direct retinotectal inputs. Since Y-fibers induced monosynaptic field responses in dSC with latencies of 2.5-2.8 ms, long-lasting negative field responses in dSC whose latencies were 5.4-7.8 ms did not appear to be triggered by direct retinotectal inputs to dSC (Fig. 10). Further, even after BIC injection, ON stimulation did not induce spike firing in most (59/60) of the dSC neurons within the latency range of the monosynaptic negative field responses. Only one dSC neurons exhibited orthodromic firing with a monosynaptic latency (3.7-4.0 ms, overriding the monosynaptic negative field response caused by Y-fiber stimulation), but this firing did not immediately trigger burst firing in the same neuron

(data not shown). Accordingly, monosynaptic inputs to dSC, originating from the Y-fibers, were supposed not to be potent enough to induce burst firing in dSC neurons. Taken together, these results strongly suggest that retinal inputs activated dSC neurons via the interlaminar connection from sSC when the SC circuit was released from $GABA_A$ receptor-mediated inhibition.

Contribution of NMDA-type glutamate receptor to burst firing in dSC

Our previous study showed that NMDA-type glutamate receptors are essential for the generation of long-lasting depolarization in SGI neurons in slice preparations from young rats. Since NMDA-type glutamate receptors tend to be expressed in the CNS more abundantly in younger animals than in adults, one possible explanation might be that the contribution of NMDA receptors to long-lasting depolarization is confined to the period in which the neural circuitry is still developing and that such a contribution may not be observed in the adult brain. However, we have now shown that NMDA-receptor activation plays a role in the generation of long-lasting depolarization in dSC neurons in the adult rats. Binns and Salt (1996) suggested NMDA receptors are involved in associating sensory inputs from different modalities. Recently, we hypothesized that in addition to such a role, NMDA receptors might be involved in the

generation of movement-related burst activities (Isa and Saito 2001). Experiments in slice preparations have shown that NMDA receptors are involved in the generation of long-lasting depolarization with threshold properties of an all-or-none nature and also in the synchronous depolarization of a neuronal population in dSC (Saito and Isa 2000). These characteristics are shared with the movement-related burst activities of dSC neurons in behaving animals. The present observations in *in vivo* preparations strengthen the hypothesis proposed by Isa and Saito (2001).

Amplitude and time course of long-lasting negative field responses

In the present study, the amplitude of the long-lasting negative field responses was the greatest at depths of 500-800 μm, and this was consistent throughout the study (Fig.3*F* and Fig.9*F*). These depths corresponded to the ventral portion of sSC (mainly SO) and the dorsal portion of dSC. We should point out that injection of BIC was performed at the depth of 1200-1400 μm, which suggests that the maximum response was not obtained at the center of injection. In even more ventral portions, the negative field responses had smaller peak amplitudes and a longer duration, and the peak response was delayed (Fig. 3*C, D* and Fig.9*D*). Below we will discuss the excitatory signal flow within SC based on these electrophysiological observations and several anatomical

studies. Fig. 11*C* shows the schematic diagram of the excitatory local circuits in the SC that we propose. As shown in Fig. 2 and Fig. 9, W-group retinotectal terminate directly in the dorsal portion of sSC, while Y-group retinotectal fibers terminate in the ventral portion of sSC (Fukuda et al. 1978) with minor projection also in dSC (Berson and McIlwain 1982). Projection neurons in sSC terminate mainly in the dorsal portion of dSC, while they have smaller amount of termination in the ventral portion of dSC. (Mooney et al. 1988). Several studies have reported that a population of SGI neurons has dendritic arborizations projecting to the ventral portion of sSC (Mooney et al. 1984, Moschovakis et al. 1988, Hall and Lee 1997; Isa et al. 1998). Axons of dSC neurons that project dendrites in the dorsal direction terminate in areas slightly ventral to the cell soma (Hall and Lee 1997). These anatomical and physiological observations suggest that the retinal inputs evoke burst firings in neurons in the dorsal portion of dSC mainly via sSC neurons with virtually the same latencies (Fig.11*B*). Therefore, the amplitude of the long-lasting negative field responses was greatest and the duration was shorter at this depth (Fig. 11*A*). Because neurons in more ventral portion of dSC receive polysynaptic excitatory inputs via the dorsal portion of dSC and rarely receive monosynaptic inputs directly from sSC, burst firings were evoked with various timings (Fig. 11*B*). Therefore, the amplitude of the long-lasting negative field responses was

smaller and the duration was longer than those in more dorsal portion (Fig. 11*A*). Thus, the present observation suggests a tandem-like polysynaptic flow of signals from sSC to the ventral portion of dSC (Fig. 11*C*). Such tandem-like polysynaptic excitatory circuit may underlie the signal amplification mechanism which has been suggested to be implemented in the intrinsic circuit of dSC (Bozis and Moschovakis 1998, Isa et al. 1998) and actually demonstrated in slice studies (Saito and Isa, unpublished observation).

Functional implications of the "gating" of the interlaminar connection by disinhibition

The present results show that release from $GABA_A$ receptor-mediated inhibition facilitates signal transmission through an interlaminar connection within SC in anesthetized rats. In awake rats, injection of BIC into SC increased frequency of contraversive orientating behaviors (Imperato and Chiara 1981; Dean et al. 1989) suggesting that the BIC injection lowered the threshold for command generation leading to such behaviors. To judge from the present results, it is likely that visual inputs triggered orienting behaviors via the interlaminar connections within SC in these animals. However, in the above studies the latencies to onset of the orienting

behaviors were not measured. On the other hand, injection of BIC into SC induced short latency contraversive saccades in non-human primates (Hikosaka and Wurtz 1985), the latencies being within the range reported for extremely short latency "express" saccade (Fischer and Boch 1983). The existence of an interlaminar connection within SC has been shown anatomically in primates (Moschovakis et al. 1988a,b). If signal transmission through this interlaminar connection is gated in primates in a manner similar to that demonstrated here in rats, then the express saccades observed by Hikosaka and Wurtz (1985) might well have been induced by signal transmission through this shortcut circuit.

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Legends

Figure 1

Schematic diagram of experimental arrangement (A) and laminar organization of the rat SC (B). A indicates the experimental arrangement drawn on a sagittal plane showing stimulation of optic nerve (ON), recording from SC, antidromic stimulation of PDB, and BIC injection into dSC. The right ON was hooked onto a pair of J-shaped bipolar silver wire electrodes. A recording electrode vertically penetrates in the SC. The stimulating electrode for antidromic activation in the PDB penetrates the SC with the angle of approximately 20° to the vertical axis of the stereotaxic coordinates inclined to the caudal directions. The 30G injection needles attached to Hamilton syringes of BIC and APV approached the SC with the angle of approximately 15° and 20° to the vertical axis of the stereotaxic coordinates inclined to the rostral and caudal directions, respectively. B shows laminar organization of the SC. The sSC is divided into three layers (SZ, SGS, SO) and the dSC into larger number of layers (SGI, SAI, SGP etc). An arrow and an asterisk indicate track of the injection needle and actual injection site, respectively.

Figure 2

Depth profile of field responses recorded in SC following ON stimulation. Field responses are shown for every 100 μm step. Left, field responses evoked by single ON stimuli at low (A, 0.5 mA x 1) or high (C, 2 mA x 1) intensity. Right, field responses evoked by repetitive ON stimulation at low (B, 0.5 mA x 20) or high (D, 2 mA x 5) intensity. Bottom, typical negative field responses evoked by stimulation of Y-fibers (E,F) or W-fibers (G,H). Voltage calibrations were 0.2 mV in A, 0.5 mV in B, and 1 mV in C and D, respectively. Field responses were obtained with the band-pass filter set at 0.08 Hz-3 kHz.

Figure 3

Field responses to ON stimulation recorded after injection of BIC into dSC. Top, depth profile of field responses to repetitive ON stimulation before (A,B) and after (C,D) BIC (10 mM, 1 μl) injection into dSC. ON stimulation was at low (A,C, 0.5 mA x 20) or high (B,D, 2 mA x 5) intensity. In E, records obtained at low and high stimulus intensities before and after BIC injection at a depth of 700 μm are superimposed (E). F shows peak amplitude of negative field responses at individual depths. G shows half-maximum duration of negative field responses at individual

depths. (F, G: n = 5) The responses with two short durations were obtained in case of BIC injection of 0.5 μl and the others were in case of 1.0 μl injection. Field responses were obtained with the band-pass filter set at 0.08 Hz-3 kHz.

Figure 4

Depth profile of field responses induced by ON stimulation, especially focused on their onset. Field responses are indicated every 200 μm step. A shows slow sweep and B fast sweep records, respectively. In C, fast sweep records of negative field responses at depths of 400, 800, and 1200 μm are superimposed. A vertical arrow shows the monosynaptic field responses of the Y-fiber origin. Field responses were obtained with the band-pass filter set at 0.08 Hz-3 kHz.

Figure 5

Long-lasting field and unit responses recorded simultaneously from dSC (using different filter settings). A and B show long-lasting field and single unit responses to ON stimulation at a depth of 1220 μm after BIC (10 mM, 1 μl) injection. Stimulus intensities were low (A, 0.5 mA x 20) and high (B, 2 mA x 5), respectively. A3 and B3 show rastergrams and A4 and B4 averaged histograms of burst firings aligned with

respect to the timing of the stimulus. Bold lines at bottom of A3 and B3 indicate duration of stimulus. Field and unit responses were obtained with the band-pass filter set at 0.08 Hz-3 kHz and 100 Hz-10 kHz, respectively

Figure 6

Antidromic spikes in a dSC neuron evoked in an all-or-none fashion at threshold intensity (A, 0.3 mA). Note the constant spike latency. The antidromic spikes followed the stimuli at 500 Hz (B, 0.35 mA). This neuron exhibited burst firing responses to ON stimulation after injection of BIC (10 mM, 1 μl) into dSC (C, 2 mA x 5). D shows stimulation site in contralateral PDB (*). Unit responses were obtained with the band-pass filter set at 100 Hz-10 kHz.

Figure 7

Effect of APV on long-lasting negative field responses in dSC. Left depth profile of field responses to ON stimulation (2 mA x 5) under control conditions (A), after BIC (10 mM, 1 μl) injection (B), and after additional injection of APV (50 mM, 1μl) (C). D shows examples of recordings made at depth of 700 μm (D1: slow time sweeps, D2: fast time sweeps). Field responses were obtained with the band-pass filter set at 0.08 Hz-3 kHz.

Figure 8

Effect of APV on burst firing responses in dSC. Effects of BIC (10 mM, 1μl) and additional injection of APV (50 mM, 1 μl) on burst firing responses to ON stimulation (1 mA x 5) recorded from a dSC neuron at a depth of 1172 μm. A1 and B1 show records of single unit responses. A2 and B2 show rastergrams and A3 and B3 averaged histograms of burst firings aligned with respect to the timing of the stimulus. Bold lines at bottom of A2 and B2 indicate duration of stimulus. Unit responses were obtained with the band-pass filter set at 100 Hz-10 kHz.

Figure 9

Ablation of the cerebral cortex ipsilateral to the side used for SC recordings. A shows ablation of the cerebral cortex, levels 1-4 being coronal sections taken at the bottom. Right depth profiles for field responses to ON stimulation before (B,C) and after (D) BIC (10 mM, 1 μl) injection into dSC. Stimulus intensity was 2 mA x1 (B) or 2 mA x3 (C,D). In E, field responses recorded before and after BIC injection at a depth of 800 μm are superimposed. F shows peak amplitude of negative field responses at individual depths. G shows half-maximum duration of negative field responses at individual depths. (F, G: n = 6) Volume of BIC injection was 1.0 μl in all cases.

Field responses were obtained with the band-pass filter set at 0.08 Hz-3 kHz.

Figure 10

Depth profile of field responses induced by ON stimulation after ablation of the cerebral cortex ipsilateral to the side used for SC recordings. Field responses are shown for every 200 μm step. A shows slow sweep and B fast sweep records, respectively. In C, fast sweep records of negative field responses at depths of 400, 800, and 1200 μm are superimposed. A vertical arrow indicates the monosynaptic field responses of the Y-fiber origin. Field responses were obtained with the band-pass filter set at 0.08 Hz-3 kHz.

Figure 11

Depth profile of field (A) and multi-unit (B) responses recorded simultaneously (using different filter settings) following ON stimulation after BIC (10 mM, 1 μl) injection into dSC. Stimulus intensity was 2 mA x 3. Field and unit responses were obtained with the band-pass filter set at 0.08 Hz-3 kHz and 100 Hz-10 kHz, respectively. C shows a schematic diagram of the structure of the local excitatory circuits of the SC that we propose.