Laminar Specific Distribution of Lateral Excitatory Connections in the Rat Superior Colliculus

Yasuhiro Saito1,2 and Tadashi Isa1

1Department of Integrative Physiology, National Institute for Physiological Sciences, Myodaiji, Okazaki 444-8585; and 2Department of Neurophysiology, Gunma University Graduate School of Medicine, Maebashi, Gunma 371-8511, Japan

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Saito, Yasuhiko and Tadashi Isa. Laminar specific distribution of lateral excitatory connections in the rat superior colliculus. J Neurophysiol 92: 3500–3510, 2004. First published June 2, 2004; doi:10.1152/jn.00033.2004. Premovement activities in neurons in the intermediate gray layer [stratum griseum intermediale (SGI)] of the mammalian superior colliculus (SC) are essential for initiation of orienting behaviors such as saccades. Our previous study demonstrated that burst activities are induced by synchronous activation of SGI neurons communicating within a local excitatory network, which depends on NMDA-receptor–dependent synaptic transmission and release from GABAergic inhibition. Furthermore, dual whole cell recordings from adjacent neurons in SGI revealed that application of 10 μM bicuculline (Bic) and reduction of extracellular Mg2+ concentration (to 0.1 mM) induce spontaneous depolarization that is synchronous between neuron pairs, suggesting the recruitment of a large number of neurons communicating through intense excitatory connections. In the present study, we investigated the properties of synchronous depolarization and the fundamental structure of the lateral excitatory network that recruits a neuronal population in SC to synchronous activation, by analyzing the synchronicity of spontaneous depolarization induced in the presence of Bic plus low Mg2+. We found that 1) spontaneous depolarization exhibits bidirectional horizontal propagation among the SGI neuron pairs; 2) induction of spontaneous depolarization is not caused by activation of intrinsic voltage-dependent conductances; 3) neurons exposed to low Mg2+ alone exhibit spontaneous depolarization, although in this case the depolarization is less synchronous; and 4) neurons exposed to Bic alone exhibit synchronous depolarization, but less frequently than those exposed to both Bic and low Mg2+. Analysis of the synchronicity of spontaneous depolarization indicates that the distribution of lateral excitatory connections is markedly different among layers of SC; the SGI neurons form extensive lateral excitatory connections, whereas they are sparse or limited within subsets of neurons in the stratum griseum superfi ciale (SGS). Widespread vertical neurons in the stratum opticum have features intermediate between neurons in the SGS and SGI. Such differences in the structure of lateral excitatory connections may reflect the different way signal processing is achieved in each layer of SC.

INTRODUCTION

Neurons in the deeper layers (intermediate and deep layers) of the mammalian superior colliculus (SC) exhibit high-frequency burst firing preceding orienting behaviors such as saccades (Dean et al. 1989; Sparks 1986; Wurtz and Albano 1980). The premovement (presaccadic) activity in the deeper layers may represent a determinant of decision making in the initiation of saccades after target selection or prediction (Dorris et al. 1997; Glimcher and Sparks 1992) and predict both the metrics and timing of saccades (Munoz and Wurtz 1995; Schiller and Koerner 1971; Schiller and Stryker 1972; Sparks and May 1980; Sparks et al. 1976; Wurtz and Goldberg 1972). Our previous in vitro study demonstrated that neurons in the intermediate gray layer [stratum griseum intermediale (SGI)] exhibit prolonged burst discharges in response to the stimulation of the optic fibers, after application of a GABA receptor antagonist [bicuculline meth bromide (Bic) or SR95531], in slices obtained from both young (17–22 postnatal days) and adult (7–8 wk old) rats (Saito and Isa 2003). The bursting responses were not caused by intrinsic membrane properties of SGI neurons and they were induced in a small rectangular piece of tissue punched out from the SGI. Furthermore, bursting responses were abolished by application of an NMDA-receptor antagonist [2-amino-5-phosphonovalerate (d-APV), 50 μM] and tetrodotoxin (TTX, 0.25 μM). Taken together, these results suggest that activation of local excitatory networks within the SGI and NMDA-receptor–dependent synaptic transmission under disinhibition are fundamental mechanisms for the generation of bursting responses. These findings also support the proposal that local excitatory interactions underlie the generation of the presaccadic burst (Bozis and Moschovakis 1998; Moschovakis et al. 1988a,b).

The excitatory network-based, and NMDA-receptor–dependent, mechanism for burst generation in the SGI under disinhibition is further supported by the induction of repetitions of spontaneous burst discharges in the presence of 10 μM Bic and low Mg2+ (0.1 mM) (Saito and Isa 2003). Dual whole cell recordings from 2 adjacent SGI neurons revealed that the spontaneous burst discharges occurred almost simultaneously between the 2 neurons. When the recordings were performed using an intracellular solution containing a sodium channel blocker, QX-314, spontaneous depolarization without spikes that is highly synchronous between the 2 neurons was observed. These findings suggest that a neuronal population communicating through excitatory connections is synchronously activated (Saito and Isa 2003). Previous in vivo studies demonstrated that pairs of adjacent saccadic burst neurons exhibited synchronous firing during presaccadic burst activities (Istvan and Munoz 1997). Therefore presaccadic bursts may involve synchronous activation of a population of adjacent SGI neurons.

It has been demonstrated that the movement fields of deeper layer neurons are spatially large and coarsely tuned (Sparks and May 1980; Sparks et al. 1976). Focal pharmacological
inactivation of a local area in the SC influences the metrics of a wide range of saccades (Lee et al. 1988). These findings imply that saccades are initiated by activity across a spatially distributed neuronal population in the SC (Lee et al. 1988; McIlwain 1991; Sparks et al. 1990). For recruitment of a large number of neurons that use a population code, the lateral excitatory network may be essential. Thus exploration of excitatory networks in the SC may clarify the structural basis for population coding, although inhibitory circuits may also be involved (Munoz and Istvan 1998). In the present study, we aimed to clarify the fundamental structure of the excitatory network that recruits a neuronal population in SC to synchronous activation, by analyzing the synchronicity of spontaneous depolarization induced by application of Bic plus low Mg2+.

We also compared excitatory intralaminar connections in the superficial and intermediate layers of the SC. Some of these data were previously presented in abstract form (Saito and Isa 2000; Saito et al. 1999).

METHODS

Slice preparations

Frontal slices of SC (350–400 μm in thickness) were obtained from young Wistar rats (17–22 postnatal days) using procedures similar to those described previously (Isa et al. 1998; Saito and Isa 1999, 2003). In brief, the brain was quickly removed after decapitation under deep ether or isoflurane anesthesia (adequacy judged by the absence of reflex movements to toe pinches). The slices were cut using a Microslicer (DTK-2000, Dosaka EM, Kyoto, Japan) and then incubated in oxygenated standard Ringer solution containing (in mM): 145 NaCl, 2.5 KCl, 2 CaCl2, 1 MgCl2, 5 HEPES, and 10 glucose, for more than 1 h before the recording at room temperature (21–24°C). These procedures were approved by the Animal Research Committee of the Okazaki National Institutes.

Dual whole cell recordings

After incubation, a slice was placed in a submersion-type recording chamber under continuous superfusion with oxygenated standard Ringer solution. Simultaneous whole cell recordings were obtained from a pair of neurons in the stratum griseum superficiale (SGS), the stratum opticum (SO), or the stratum griseum intermediale (SGI). We roughly divided the frontal SC slice into the medial, intermediate, and lateral regions. Pairs of neurons, which were separated by a distance of <200 μm, were recorded in the medial or intermediate regions. When the recordings were performed from pairs of neurons that were separated by more than 200 μm, one neuron was always selected in the medial region and another was recorded in the intermediate or lateral regions. The distance between 2 recorded neurons was estimated from measurement of the distance between the tips of recording electrodes. Patch pipettes were filled with a solution containing (in mM): 140 K-glucuronate, 20 KCl, 0.2 EGTA, 2 MgCl2, 2 Na2ATP, 10 N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid (HEPES), 0.1 spermine, and 5 hiodocaine N-ethyl bromide quaternary salt (XQ-314) (pH 7.3).

In some experiments, biocytin (5 mg/ml) was added to the solution to verify the location and morphology of recorded neurons. The resistance of the recording pipettes was 4–7 MΩ in the bath solution, and the series resistance during recording was 10–30 MΩ. The liquid junction potential between the patch pipette solution and the standard Ringer solution was estimated to be ~10 mV, and the data were corrected for this voltage. Spontaneous membrane potentials were recorded using an EPC-9 patch-clamp amplifier (Heka, Lambrecht, Germany) in fast current-clamp mode. All recordings were performed at a bath temperature of 32–33°C controlled with a thermostat (Ecoline, Lauda Dr. R. Wobser GmbH KG, Lauda-Königshofen, Germany). Pharmacological agents were dissolved in distilled water to make a concentrated stock solution (1,000 times the final concentration; see RESULTS). They were diluted to the final concentration in the external solution just before the experiments and were bath-applied. Spontaneous depolarization (see RESULTS) was usually induced within 1 min after the bath solution containing Bic and/or low concentration of Mg2+ reached the recording chamber. To obtain stable recordings after application of drugs, we waited for 1 min after complete exchange of the bath solution and then started recording. It has been shown that incubation in the bath solution containing low or no concentration of Mg2+ for more than 20 min induces spontaneous epileptic discharge in neurons in vitro preparations such as the hippocampus (Anderson et al. 1986; Mody et al. 1987; Schneiderman and MacDonald 1987) and the neocortex (Silva et al. 1991; Tsau et al. 1998). To minimize such presumed plastic change in neuronal circuits after long-lasting exposure to low Mg2+ solution, we limited the recording time to <10 min; therefore spontaneous membrane potentials were recorded for 2–8 min in each pair of neurons. Voltage signals were filtered at 3 kHz and digitized at 1 kHz. Data were acquired and stored using PULSE/PULSEFIT software (Heka). Offline analysis was performed with Axograph (Axon Instruments, Foster City, CA), Igor Pro (WaveMetrics, Lake Oswego, OR), and MATLAB (The MathWorks, Natick, MA).

The GABA A receptor antagonist CGP 55845A was a kind gift from Novartis Pharma (Basel, Switzerland). The GABA A receptor antagonists 2-(3-carboxypropyl)-3-amino-6-(4-methoxyphenyl)-pyridazinum bromide (SR95531) and (--)bicuculline methobromide, as well as biocytin and QX-314, were purchased from Sigma-RBI (St. Louis, MO). The GABA C receptor antagonist (1,2,5,6-tetrahydropyridine-4-yl)methylphosphinic acid (TPMPA) was purchased from Tocris Cookson (Bristol, UK), and other drugs from Wako Pure Chemicals (Osaka, Japan).

When the experiment was carried out using a pipette solution containing biocytin, patch pipettes were carefully detached from the recorded cells, and the slices were then fixed with 4% paraformaldehyde in 0.05 M phosphate buffer (pH 7.4) for 2 to 3 days at 4°C. The procedures for visualization of the biocytin-filled neurons were previously described in detail elsewhere (Isa et al. 1998; Saito and Isa 1999, 2003).

Data analysis

Analysis was performed on data from neuron pairs that satisfied the following criteria: during recordings, 1) a drift of baseline membrane potential ≤5 mV, and 2) the amount of injected steady-state current needed to maintain the membrane potential at −60 mV was ≤50 pA. As described above, recordings in the presence of low Mg2+ were limited to <10 min. The small number of synchronous depolarizations from each pair of neurons made it difficult to perform a cross-correlation analysis for detecting synchronicity. Instead, we analyzed the percentage of synchronous depolarization (PSD; see following text) and the correlation coefficient (CC) of spontaneous membrane potentials from the phase plots of membrane potentials (Fig. 1B). Because amplitudes of depolarization were not always identical between a pair of neurons, the phase plots were made from plots of amplitudes normalized to the maximum amplitude of depolarization of each neuron (Fig. 1B1). The PSD was defined as the sum of the number of plots with the values of normalized amplitude of both neurons >0.5 (plots in area II of Fig. 1B2) divided by the sum of the number of plots in areas I, II, and III (Fig. 1B2). Although the plots shown in the figures are presented at 20-ms intervals for 1–2 min, the PSD and the CC were analyzed from plots obtained at 1-ms intervals for 2–6 min. The time difference in the onset of depolarization between the 2 neurons was analyzed for events in which the normal-
FIG. 1. Spontaneous membrane potentials of a pair of stratum griseum intermediale (SGI) neurons (cells 1 and 2). A: spontaneous fluctuations of membrane potentials in control (A1) and in the presence of 10 μM bicuculline (Bic) and low (0.1 mM) Mg\(^{2+}\) (A2). B: phase plots of normalized membrane potentials for cell 1 against cell 2 (B1). Plots were obtained from recordings for 1 min. B2: division of the area of plots. Plots in area I indicate that the normalized membrane potentials of cell 1 are <0.5 and those of cell 2 >0.5, implying that depolarization occurred only in cell 2. Plots in area II indicate normalized membrane potentials >0.5 in both cell 1 and cell 2, implying that depolarization occurred simultaneously in both cells. Plots in area III indicate that normalized membrane potentials of cell 1 are >0.5 and those of cell 2 <0.5, implying that depolarization occurred only in cell 1. C: spontaneous membrane potentials of another pair of SGI neurons (cells 3 and 4) in the presence of Bic plus low Mg\(^{2+}\). C1: slow-sweep records. C2 and C3: faster-sweep records of the segments underlined in C1. D: histogram illustrating the distribution of the time difference in the onset of depolarization between cell 3 and cell 4. Negative values mean that the onset of depolarization in cell 3 precedes that of cell 4. E: histogram illustrating the distribution of the time difference in the onset of depolarization obtained from 11 pairs of SGI neurons for a total of 37 min of recording time (a total of 176 events). F: histogram illustrating the distribution of the average time difference for each SGI pair.

ized amplitudes were larger than 0.5 for both neurons. The onset of a depolarization was defined as the time at which the membrane potential was greater than 3 times the SD of the mean membrane potential recorded 50–200 ms before the event. In the case of a cluster of depolarizations (see RESULTS), in which each depolarization did not return to the baseline, the onset of the first depolarization was analyzed. The time difference in onset was obtained by subtracting the onset of a depolarization of a neuron located laterally from that of a neuron located medially. A normalized duration of synchronous depolarization during the recording time was defined as the sum of the number of plots >0.5 in both recorded neurons (plots in the area II of Fig. 1B2), divided by the total recording time. All values are shown as the means ± SD. Statistical significance was analyzed using Student’s t-test (paired or unpaired data), Friedman test, or a one-way ANOVA with a post hoc Scheffé test with StatView software (version 5.0, Hulinks, Tokyo, Japan).

RESULTS

Spontaneous depolarization in adjacent SGI neurons

Before comparing spontaneous depolarization induced in the different layers of the SC, we performed dual whole cell recordings from pairs of SGI neurons separated by <100 μm to investigate the properties of spontaneous depolarization in the SGI. Figure 1A exemplifies a recording obtained from a pair of SGI neurons (cells 1 and 2, distance = 71.7 μm). In control solution, the membrane potentials of these neurons were mostly stable (Fig. 1A1), although small fluctuations of membrane potentials were occasionally observed. When 10 μM Bic was applied and the extracellular Mg\(^{2+}\) concentration was reduced to 0.1 mM, these neurons exhibited spontaneous depolarization, in which the amplitude was usually >20 mV (Fig. 1A2). Clusters of depolarizations, some of which continued for several seconds, were often observed. Figure 1B1 shows phase plots of the normalized membrane potentials of cell 1 against those of cell 2. The phase plots illustrate that these 2 neurons changed their membrane potentials synchronously (PSD = 69.8, CC = 0.91). The mean PSD and CC values of 11 adjacent SGI neuron pairs (distance <100 μm) were 57.9 ± 11.8 and 0.84 ± 0.07, respectively.

The high values of the PSD and the CC indicate that spontaneous depolarization of the pairs of SGI neurons is well synchronized. To clarify whether spontaneous depolarization of 2 neurons was induced simultaneously, we compared the onsets of depolarization between 2 neurons. Figure 1C shows a recording obtained from another pair of SGI neurons (cells 3 and 4). The faster sweep records clearly illustrate that in an event (underlined 2 in Fig. 1C1), the onset of depolarization of cell 3 preceded that of cell 4 (Fig. 1C2), whereas in another event (underlined 3 in Fig. 1C1), the onset of depolarization of cell 4 preceded that of cell 3 (Fig. 1C3). Figure 1D shows a histogram of the distribution of the time difference in onset of depolarization between the 2 neurons. The distribution of the time difference was not biased in either a positive or a negative direction from the center (zero ms).

In all pairs (n = 11) of SGI neurons, the onset of depolarization of one neuron could both precede and follow that of the other neuron. Figure 1E shows a histogram illustrating the
distribution of the time difference constructed from data obtained from 11 pairs of SGI neurons (176 events of depolarization for a total of 38 min). Although some events were induced almost simultaneously (20 events occurring within ±5 ms), most events were induced with some time difference. Figure 1F shows a histogram illustrating the distribution of the average time difference of depolarization for each neuron pair. The range of the average time difference was −19.3 to 38.9 ms. Only 2 pairs of neurons exhibited an average time difference within ±5 ms (1.9 and 3.0 ms). These results indicate that spontaneous depolarizations induced in the presence of Bic plus low Mg²⁺ are propagated among SGI neurons, and that the propagation of spontaneous depolarization can be bidirectional.

Bic has been shown to block the apamine-sensitive calcium-activated potassium channels (SK-type channel; Debarbieux et al. 1998; Johnson and Seutin 1997; Khawaled et al. 1999). Because the SK-type channels are responsible for spike afterhyperpolarizations, blockade of the channels may enhance the activities of individual neurons, resulting in an enhancement of activities of neuronal population. Therefore it is possible that synchronous depolarization might not have been attributable to its effect on the GABA_A receptors, but rather to its effect on the calcium-activated potassium channels. To test this possibility, we used another GABA_A receptor antagonist, SR95531, which does not affect the SK-type channels (Seutin et al. 1997). Application of 10 μM SR95531 plus low Mg²⁺ induced synchronous depolarizations similar to those induced by Bic plus low Mg²⁺ (data not shown). The mean PSD and CC of 8 adjacent SGI neuron pairs were 61.1 ± 9.3 and 0.85 ± 0.07, respectively. There was no significant difference in the PSD (P = 0.33, unpaired t-test) and the CC (P = 0.80) between neurons exposed to Bic plus low Mg²⁺ (n = 11) and neurons exposed to SR95531 plus low Mg²⁺ (n = 8).

Several kinds of voltage-dependent channels, such as a low-threshold (T-type) Ca²⁺ channel and an inward rectifying channel, were expressed in SGI neurons (Saito and Isa 1999). The T-type Ca²⁺ channel is activated below the threshold of action potential generation, and activation of this channel gives rise to low-threshold spikes and bursting firings, which has been reported in several central neurons such as the thalamic neurons (Jahnsen and Llinás 1984). Because this channel is inactivated when the membrane potential is depolarized, if this channel is involved in the generation of spontaneous depolarizations of SGI pairs, the rise in the membrane potential should abolish spontaneous depolarizations. Another is an inward rectifier potassium channel. It has been reported that this channel is involved in forming the down-state of bistable spontaneous fluctuations in the medium spinystellate cells in the striatum (Wilson and Kawaguchi 1996). Thus if this channel is involved in synchronous depolarization, hyperpolarization of the membrane potential should change the synchronous depolarization. Therefore to investigate whether synchronous depolarization arises from intrinsic voltage-dependent mechanisms, we held the membrane potentials of both SGI neurons at 3 different levels, control (−70 to −55 mV), hyperpolarized (< −75 mV), and depolarized (> −50 mV) by injection of steady-state current, and recorded spontaneous membrane potentials by application of Bic plus low Mg²⁺. Figure 2 shows a recording obtained from a pair of SGI neurons (cells 5 and 6, distance = 29.1 μm). Synchronous depolarization was also observed when the neurons were hyperpolarized (Fig. 2B1) and depolarized (Fig. 2C1). Phase plots of membrane potentials were mostly similar in the 3 conditions (Fig. 2, A2, B2, and C2). Figure 2, D and E show comparisons of the PSD (D) and the correlation coefficient (E) of spontaneous depolarization induced at the 3 different holding potentials (n = 5 pairs).
High spontaneous depolarizations in SGI neurons were also observed in the presence of low Mg$^{2+}$ (0.1 mM) in the solution containing Bic alone (1.66 ± 0.88) was significantly different from that in Bic plus low Mg$^{2+}$ (6.82 ± 4.12, P < 0.01, paired t-test), indicating that spontaneous depolarization is less frequently induced in the solution containing Bic alone. These results suggest that Bic is necessary to induce synchronous depolarization, but reduction in extracellular Mg$^{2+}$ is needed for induction of more frequent depolarization.

Spontaneous depolarization in the superficial layer neurons

To examine whether synchronous depolarization occurs in neurons in the superficial layers by application of Bic plus low Mg$^{2+}$, we recorded spontaneous membrane potentials from pairs of adjacent neurons in the SGS. Figure 5A shows a recording obtained from a pair of SGS neurons (cells 11 and 12, distance = 66.9 μm) in the presence of Bic plus low Mg$^{2+}$. In this pair of neurons, spontaneous depolarization was observed, but clusters of depolarizations that were often observed in SGI pairs were rarely observed in the SGS. The phase plots of normalized membrane potentials revealed that the number of plots in area II were small (Fig. 5A2, PSD = 16.6, CC = 0.15), indicating that synchronous depolarization occurs infrequently. Although in many SGS pairs synchronous depolarization did not occur frequently, a small number of SGS pairs exhibited highly synchronous depolarization. Figure 5C shows a recording obtained from another pair of SGS neurons (cells 13 and 14, distance = 51.7 μm). In this pair, clusters of depolarization were observed (Fig. 5C1) and the depolarization was synchronous (Fig. 5C2, PSD = 57.7, CC = 0.72). The time difference

(PSD = 15.7, CC = 0.17) in contrast to those in the low Mg$^{2+}$ solution containing Bic (Fig. 3B2, PSD = 51.3, CC = 0.83). Figure 3, C and D show comparisons of the PSD and the CC between the low Mg$^{2+}$ solution with and without Bic (n = 8 pairs of adjacent SGI neurons). The PSD and the CC were low in the low Mg$^{2+}$ solution (PSD = 7.9 ± 9.8, CC = 0.10 ± 0.22), whereas they became significantly higher in the solution containing Bic plus low Mg$^{2+}$ (PSD = 48.0 ± 9.8, CC = 0.76 ± 0.14, P < 0.01, paired t-test). These results indicate that reduction in extracellular Mg$^{2+}$ can induce spontaneous depolarization, but the addition of Bic is needed for induction of synchronous depolarization.

Next, to examine the effect of release from GABA$\text{A}$ inhibition on the induction of synchronous depolarization, we investigated spontaneous membrane potentials in the presence of Bic alone. Figure 4, A and B show recordings obtained from a pair of SGI neurons (cells 9 and 10, distance = 70.8 μm). Application of Bic also induced spontaneous depolarization and the depolarization appeared to be synchronous (Fig. 4A1). However, the frequency of depolarization appeared to be low. After washing out Bic by superfusion with the control solution for more than 5 min, application of Bic plus low Mg$^{2+}$ induced repetitions of spontaneous depolarization (Fig. 4B1). The phase plots of the normalized membrane potentials were almost similar in both conditions (Fig. 4, A2 and B2). Figure 4, C and D show comparisons of the PSD and the CC in the presence of Bic alone or Bic plus low Mg$^{2+}$ (n = 8 pairs of adjacent SGI neurons). There was no significant difference in the PSD (P = 0.50, paired t-test) and the CC (P = 0.29) between recordings obtained in the solution containing Bic alone (PSD = 48.3 ± 11.9, CC = 0.81 ± 0.07) and in the solution containing Bic plus low Mg$^{2+}$ (PSD = 51.4 ± 14.9, CC = 0.84 ± 0.08). However, the normalized duration of synchronous depolarization during the recording time (Fig. 4E) in the solution containing Bic alone (1.66 ± 0.88) was significantly different from that in Bic plus low Mg$^{2+}$ (6.82 ± 4.12, P < 0.01, paired t-test), indicating that spontaneous depolarization is less frequently induced in the solution containing Bic alone. These results suggest that Bic is necessary to induce synchronous depolarization, but reduction in extracellular Mg$^{2+}$ is needed for induction of more frequent depolarization.
in the onset of depolarization was analyzed from 10 SGS pairs (72 events of depolarization for a total of 27 min). The range of the average time difference was −43.3 ms to 51.3 ms (Fig. 5F).

Previous anatomical and physiological studies have shown that neurons in the superficial layer express GABA\(_B\) (Binns and Salt 1997; Bowery et al. 1987; Chu et al. 1990; Endo and Isa 2002) and GABA\(_C\) receptors (Pasternack et al. 1999; Schmidt et al. 2001) as well as GABA\(_A\) receptors. Therefore it may be possible that the low frequency of synchronous depolarization in many SGS pairs is attributable to activation of GABA\(_B\) and GABA\(_C\) receptors, even when GABA\(_A\) receptors are blocked by Bic. Addition of GABA\(_B\) receptor antagonist CGP 55845A (2 \(\mu M\)) and GABA\(_C\) receptor antagonist TPMPA (50 \(\mu M\)) to the solution containing 10 \(\mu M\) Bic plus low Mg\(^{2+}\), however, appeared not to enhance the frequency of synchronous depolarization (Fig. 5B). Figure 5, D and E show comparison of the PSD and the CC between recordings obtained before (PSD = 14.2 ± 20.5, CC = 0.29 ± 0.26, \(n = 10\) pairs of adjacent SGS neurons) and after the addition of CGP 55845A and TPMPA (PSD = 15.3 ± 18.7, CC = 0.32 ± 0.29). There was no significant difference in the PSD (\(P = 0.74\), paired \(t\)-test) and the CC (\(P = 0.71\)). These results suggest that infrequent synchronous depolarization observed in SGS pairs of neurons is not attributable to the activation of GABA\(_B\) and GABA\(_C\) receptors.

In the deep SGS and the SO as well as the upper SGI, there is a particular group of neurons called wide-field vertical (WFV) cells that have characteristic morphological and electrophysiological properties (Isa et al. 1998; Langer and Lund 1974; Lo et al. 1998; Saito and Isa 1999). Morphologically, WFV cells exhibit large soma and extend dendrites widely into the dorsal superficial layer (Fig. 6, A and B). Electrophysiologically, they exhibit voltage sag (Fig. 6C, arrow) caused by a hyperpolarization-activated current in response to hyperpolarizing current pulses. Figure 6D shows an example of spontaneous membrane potentials obtained from the pair of WFV cells in SO (cells 15 and 16, distance = 86.7 \(\mu m\)) following application of Bic plus low Mg\(^{2+}\). In this pair of neurons, clusters of depolarization were observed. The PSD and the CC values were high (PSD = 54.1, CC = 0.78), although some plots were observed in areas I and III (Fig. 6E). The time difference in the onset of depolarization was analyzed from 14 SO pairs (166 events of depolarization for a total of 49 min). The range of the average time difference was −52.2 to 58.6 ms (Fig. 6F).

**FIG. 5.** Spontaneous membrane potentials from pairs of SGS neurons. A and B: spontaneous membrane potentials from a pair of stratum griseum superficiale (SGS) neurons (cells 11 and 12), and phase plots of normalized membrane potentials of cell 11 against those of cell 12 in the presence of low Mg\(^{2+}\) (0.1 mM) plus 10 \(\mu M\) Bic (A1, A2) and low Mg\(^{2+}\) plus GABA antagonist cocktail [10 \(\mu M\) Bic + 2 \(\mu M\) CGP 55845A + 50 \(\mu M\) (1,2,5,6-tetrahydropyridine-4-yl) methylphosphinic acid (TPMPA)] (B1, B2). Both plots were obtained from individual recordings for 2 min. C1: spontaneous membrane potentials from another pair of SGS neurons (cells 13 and 14) in the presence of low Mg\(^{2+}\) (0.1 mM) plus 10 \(\mu M\) Bic. C2: phase plots of normalized membrane potentials of cell 13 against those of cell 14 (obtained from a recording of 1-min duration). D and E: comparison of the PSD (D) and the CC (E) between recordings obtained in the presence of low Mg\(^{2+}\) plus Bic, and low Mg\(^{2+}\) plus GABA antagonist cocktail. F: histogram illustrating the distribution of the average time difference for each SGS pair.

Interlaminar comparison of synchronous depolarization between adjacent cells

A comparison of the PSD and the CC was performed among 19 SGI pairs (\(n = 11\): Bic; \(n = 8\): SR95531), 21 SGS pairs (\(n = 16\): Bic; \(n = 5\): SR95531), and 18 WFV cell pairs (\(n = 14\): Bic; \(n = 4\): SR95531). The distance between neuron pairs was <100 \(\mu m\), and not significantly different among SGI (46.3 ± 20.3 \(\mu m\)), SGS (48.0 ± 24.2 \(\mu m\)), and WFV pairs (48.1 ± 23.0 \(\mu m\), Fig. 7A; \(P > 0.9\) in all cases, ANOVA post hoc test). Figure 7, B and C show plots of the PSD and the CC in SGI, SGS, and WFV pairs. The PSD in most SGI pairs was high, and only a few pairs exhibited low values (<50). In the latter pairs, the decay time course of depolarization was considerably different between the 2 recorded neurons, which probably accounted for the low values. On the other hand, the PSD values of SGS and WFV pairs were distributed over a wide range. A few SGS pairs exhibited high values comparable to those in the SGI pairs, but the values were zero or near zero in some SGS pairs. In many SGS pairs exhibiting low values, either of the recorded neurons frequently did not show spontaneous depolarization. Although many pairs of WFV cells exhibited high values of PSD, comparable to those seen in SGI pairs, a few pairs had low values, comparable to those in SGS pairs. The pairs of WFV cells exhibiting low values of PSD did not frequently show clusters of depolarization, although spontaneous depolarization was frequently induced. Statistical analysis revealed that the PSD of SGS pairs (20.6 ± 21.0) was significantly smaller than the PSD of SGI (57.9 ± 11.8) and WFV neuron pairs (46.1 ± 19.8, \(P < 0.01\), ANOVA post hoc
test). Similarly, the CC of SGS pairs (0.39 ± 0.27) was significantly smaller than the CC of SGI and WFV pairs (SGI = 0.84 ± 0.07, WFV = 0.83 ± 0.10, \( P < 0.01 \)). The PSD and the CC were not significantly different between SGI and WFV pairs (\( P = 0.15 \) and \( P = 0.99 \)).

**Interlaminar comparison of intensity of the lateral excitatory connection**

The results described above suggest that the excitatory connections composed of adjacent SGI and WFV neurons are extensive in contrast to those composed of SGS neurons. To clarify how the lateral excitatory connections are organized in the SC, we investigated the relationship between the synchronicity of spontaneous depolarization and the distance between 2 recorded neurons. Figure 8A shows a recording obtained from a pair of SGI neurons separated by a distance of about 500 \( \mu \)m. The phase plots presented on the right are extremely diffuse (PSD = 24.7, CC = 0.55), in contrast to the phase plots obtained from pairs of adjacent SGI neurons (Fig. 1B1). This suggests that the increase in time difference in the induction of depolarization, as well as nonsynchronous depolarization, occurs in the SGI pairs. The synchronicity of depolarization was reduced when the recordings were obtained from a more remote SGI pair of neurons (Fig. 8B, PSD = 7.7, CC = 0.18, by comparison with Fig. 1B1). In a pair of WFV cells separated by a distance of about 400 \( \mu \)m, synchronous depolarization occurred less frequently (Fig. 8C, PSD = 19.3, CC = 0.58), by comparison with a pair of adjacent WFV neurons (Fig. 6, D and E). In SGS pairs that were separated by a distance of about 300 \( \mu \)m, depolarization between 2 neurons was much less synchronous (PSD = 9.8, CC = 0.34) than that in SGI and WFV pairs of neurons. Figure 8, E and F show plots of the PSD and the CC against the distance between 2 recorded pairs of neurons in SGI (top panel), WFV (middle panel), and SGS (bottom panel), respectively. The PSD of SGI pairs decreased gradually in parallel with the increase in distance, whereas that of SGS pairs was low regardless of the distance. The PSD of WFV pairs appeared to exhibit an intermediate distribution between that of SGI and SGS neuronal pairs. In particular, the PSD was widely distributed when neurons were separated by a distance of \(< 500 \mu \)m. The CC also decreased as the distance between neuron pairs increased, but the decrease was gradual in contrast to the decrease in the PSD in the 3 kinds of neuron pairs.

**DISCUSSION**

In the present study, we demonstrated the putative intralaminar distribution of lateral excitatory connections in the SC from an analysis of synchronicity in spontaneous depolarization between pairs of neurons recorded simultaneously. The present findings indicate the distribution of a neuronal population participating in synchronous depolarization that may be essential for burst activities mediated by SC neurons.
Properties of synchronous depolarization

Application of 10 μM Bic and reduction of extracellular Mg$^{2+}$ concentration (0.1 mM) induced synchronous depolarization in adjacent neurons in the SC, which was prominent in the SGI. We have found that spontaneous depolarization shows the following properties: 1) propagation of spontaneous depolarization in the SGI can be bidirectional; 2) induction of spontaneous depolarization is not caused by activation of intrinsic voltage-dependent conductances; 3) reduction in extracellular Mg$^{2+}$ itself can induce spontaneous depolarization, but the depolarization is less synchronous; and 4) application of Bic alone induces synchronous depolarization, but the depolarization occurs less frequently.

Measurements of the onset of depolarization in SGI neuronal pairs revealed that the onset of depolarization of a neuron can both precede and follow that of another neuron. This finding indicates that spontaneous depolarization induced in the presence of Bic plus low Mg$^{2+}$ is propagated among a neuronal population in the SC, and propagation of spontaneous depolarization can be bidirectional. This bidirectional propagation of neuronal activity was observed even in a pair of SGI neurons that exhibited unidirectional synaptic connectivity (Isa et al., 2003). Although not described in the present study, in our preliminary study, we only rarely could find direct synaptic connections between adjacent cells. We suppose that the connections between individual SGI neuron pairs are rather sparse or the synaptic strength between the pair is not strong. Instead, individual neurons may communicate with many neighboring neurons. The synchronous depolarization would arise from common inputs from a vast majority of neighboring neurons, and with noisy connections among such a large number of neurons may result in a long time delay such as 50 ms. Spontaneous depolarizations often occurred in clusters that were sustained for several seconds. This suggests that the neuronal activities reverberate in the excitatory circuits of the SC.

The synchronous depolarization was induced even when SGI neurons were held at depolarized and hyperpolarized membrane potentials, suggesting that voltage-dependent ionic conductances are not involved in the synchronicity of spontaneous depolarization. This finding further supports network-based generation of synchronous depolarization in the SC.

The present study has revealed that application of low (0.1 mM) Mg$^{2+}$ alone, or 10 μM Bic alone, induce different features of spontaneous depolarization in SGI neuronal pairs.
Although application of low Mg\(^{2+}\) alone induced spontaneous depolarization, clusters of depolarizations were rarely observed, and depolarizations between 2 recorded neurons were less synchronous. On the other hand, by application of Bic alone, clusters of depolarizations were observed and the depolarizations were synchronous, comparable to those in Bic plus low Mg\(^{2+}\). However, the frequency of spontaneous depolarization was low. These results suggest that disinhibition from GABAergic inhibition may enhance synchronicity of depolarization in SGI neurons to activate the excitatory circuits such as reverberating circuits in SC, whereas activation of NMDA receptors may control the threshold for generation of spontaneous depolarization.

Recent studies have demonstrated that the inhibitory interneurons exhibiting highly synchronous activity are extensively interconnected by electrical synapses (Deans et al. 2001; Galarreta and Hestrin 1999; Perez Velazquez and Carlen 2000). Therefore the highly synchronous depolarization observed in adjacent SGI neurons may imply the presence of electrical coupling among SGI neurons. However, depolarizing current pulses applied to one of the recorded neurons never caused depolarizing response in the other cell recorded in the present study (data not shown). This suggests that SGI neurons exhibiting synchronous depolarization are unlikely to communicate with each other through gap junctions.

**Interlaminar comparison of properties of lateral excitatory connection**

Comparison of the synchronicity of spontaneous depolarization of adjacent neuron pairs in different layers of the SC has revealed that spontaneous depolarization of SGI and WFV neurons is highly synchronous in contrast to that of SGS neurons. Even in slice preparations that were 350–400 \(\mu\)m thick, most SGI pairs exhibited synchronous depolarization, suggesting that SGI neurons form intensive local excitatory connections. On the other hand, many SGS pairs rarely exhibited synchronous depolarization, but depolarization of a few pairs were highly synchronous, suggesting that excitatory connections in SGS are sparse, or particular types of SGS neurons form intensive local excitatory connections. In SGS, several types of neurons exhibiting different morphological characteristics have been reported (Langer and Lund 1974; Lee and Hall 1995; Mooney et al. 1988; Özen et al. 2000). Although we did not perform a systematic morphological analysis of the recorded SGS neurons, whether there is a relationship between cell morphology and synchronous depolarization is an interesting problem that should be addressed. The PSD and the CC in adjacent WFV pairs was not significantly different from those in adjacent SGI pairs, but some WFV pairs exhibiting low synchronicity could be observed. This suggests that although most of WFV neurons form intensive excitatory connections, a few are isolated from these connections.

The interlaminar difference in synchronicity of spontaneous depolarization was closely related to the distance between the 2 recorded neurons. The PSD in SGI pairs decreased gradually with increasing distance, whereas that in SGS pairs decreased abruptly at a distance of about 100 \(\mu\)m. These results suggest that the SGI neurons form extensive excitatory connections, whereas SGS neurons form local excitatory connections that may be sparse, or limited to subsets of neurons. The WFV neurons may form connections that are intermediate between those in SGI and in SGS. Because the recordings of neuronal pairs were performed in frontal slices through the SC, distributions of neuronal pairs are limited to the mediolateral direction. Previous morphological studies in cat SC demonstrated that the extensive organization of intrinsic axonal connections is present in both mediolateral and rostrocaudal directions, although these studies could not distinguish between excitatory and inhibitory connections (Behan and Appell 1992; Behan and Kime 1996). This finding implies that the rostrocaudal distribution of lateral excitatory connections is almost similar to the mediolateral distribution. In the superficial layer, small groups of neurons exhibit intralaminar projections up to several millimeters as well as interlaminar projections (Behan and Appell 1992). Our results, however, do not support extensive excitatory connections in SGS. The long-range projections of SGS neurons may be inhibitory ones (Behan and Appell 1992; Rizzolatti et al. 1974). On the other hand, in the intermediate layers, the majority of terminals of intralaminar projections are observed at 1–2 mm from the injection site, but terminals are also observed \(\leq 5\) mm (Behan and Kime 1996), indicating extensive intrinsic axonal connections in the deeper layers.

From focal electrical stimulation study, it was estimated that \(\geq 30\%\) of neurons in the intermediate gray layer of cat SC (output neurons that were \(\leq 2\)–3 mm from the stimulus site) were excited (McIlwain 1982, 1991), although the study did not take into account the possibility that the passing fibers originated outside of the SC were also stimulated. The present findings that synchronous depolarizations occur in SGI pairs, even in reduced preparations, suggest that the extensive excitatory connections are intrinsic to the SC. In the rat SC used in the present study, the mediolateral extent of the colliculus is about 3 mm at the widest point. Thus our finding that synchronous depolarizations could be observed between SGI pairs separated at a distance of \(>1\) mm, although the synchronicity is low, suggests that a large number of SGI neurons can participate in synchronous depolarization through lateral excitatory connections. The deeper layers of the SC may use a population code to determine the amplitude and vector of saccades, and several models for saccade accuracy produced by a neuronal population have been proposed (Lee et al. 1988; McIlwain 1991; Sparks and Gandhi 2003; Sparks et al. 1990; Van Gisbergen et al. 1987). In any model of saccade generation, the extensive lateral excitatory connections in SGI may be essential for the population coding that generates saccades with appropriate metrics.

Differences in the structure of excitatory connections between the SGS and the SGI may reflect differences in signal processing: fine processing in the SGS and distributed, coarse processing in the SGI. One of the examples supporting the laminar differences in signal processing is that receptive fields in the SGS are small and well defined, whereas receptive and movement fields in the SGI are large (Cynader and Berman 1972; Goldberg and Wurtz 1972; Schiller and Koerner 1971). Although inhibitory circuits may form and modify the spatiotemporal signal processing in the SGS and the SGI, the different excitatory structure may contribute to a layer-specific signal processing. The intermediate feature of the excitatory connections of WFV cells suggests that WFV cells have a unique relationship with the SGI as well as the SGS.
Previous anatomical studies have demonstrated that the SGI exhibits a discontinuous patchy structure, which corresponds to the different density of output neurons as well as the terminals of afferents from other brain regions (Graybiel 1978; Graybiel and Illing 1994; Huerta and Harting 1984; Wiener 1986). The diameter of patches is 150–250 μm in rats. Although we did not establish whether recorded neurons were inside or outside such patches, the relationship between synchronicity of depolarization and distance did not change abruptly at particular distances, as shown in Fig. 8. This suggests that the distribution of lateral excitatory connections is not related to the patchy structure of the SC. However, more detailed analysis is needed to draw conclusions about the relationship between lateral excitatory connections and the patchy representation of the SC.

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