Physiological Properties of Neurons in the Optic Layer of the Rat’s Superior Colliculus

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Lo, Fu-Sun, R. John Cork, and R. Ranney Mize. Physiological properties of neurons in the optic layer of the rat’s superior colliculus. J. Neurophysiol. 80: 331–343, 1998. We made intracellular recordings from 74 neurons in the optic layer of the rat superior colliculus (SC). Resting membrane potentials were $−62.3 \pm 6.2$ (SD) mV, and input resistances were $37.9 \pm 10.1$ MΩ. Optic layer neurons had large sodium spikes ($74.2 \pm 12.3$ mV) with an overshoot of 12 mV and a half-amplitude duration of $0.75 \pm 0.2$ ms. Each sodium spike was followed by two afterhyperpolarizations (AHPs), one of short duration and one of longer duration, which were mediated by tetraethylammonium (TEA)-sensitive ($I_{Ca}$) or apamin-sensitive ($I_{Ca}$) calcium-activated potassium currents, respectively. Sodium spikes were also followed by an afterdepolarization (ADP), which was only revealed when the AHPs were blocked by TEA or apamin. In response to hyperpolarizing current pulses, optic layer neurons showed an inward rectification mediated by H channels. At the break of the current pulse, there was a rebound low-threshold spike (LTS) with a short duration of $<25$ ms. The LTS usually induced two sodium spikes (doublet). Most optic layer neurons (84%) behaved as intrinsically bursting cells. They responded to suprathreshold depolarization with an initial burst (or doublet) followed by a train of regular single spikes. The remaining 16% of cells acted as chattering cells with high-frequency gamma (20–80 Hz) rhythmic burst firing within a narrow range of depolarized potentials. The interburst frequency was voltage dependent and also time dependent, i.e., showed frequency adaptation. Unmasking the ADP with either TEA or apamin converted all of the tested intrinsically bursting cells into chattering cells, indicating that the ADP played a crucial role in the generation of rhythmic burst firing. Optic layer neurons receive direct retinal excitation mediated by both N-methyl-D-aspartate (NMDA) and non-NMDA receptors. Optic tract (OT) stimulation also led to γ-aminobutyric acid-A (GABA$_A$) receptor–mediated inhibition, the main effect of which was to curtail the excitatory response to retinal inputs by shunting the excitatory postsynaptic current. Intracellular staining with biocytin showed that the optic layer neurons that we recorded from were mostly either wide-field vertical neurons or other cells with predominate superficially projecting dendrites. These cells were similar to calbindin immunoreactive cells seen in the optic layer. The characteristics of these optic layer neurons, such as prominent AHPs, strong shunting of inhibition, and short-lasting LTS, suggest that they respond transiently to retinal inputs. This is consistent with a function for these cells as the first relay station in the extrageniculate visual pathway.

INTRODUCTION

In addition to the primary visual pathway from the retina, via the dorsal lateral geniculate nucleus (LGN) to the visual cortex, there is an extrageniculate visual pathway that projects to the cortex after relays in the superficial layers of the superior colliculus (SC) and the lateral posterior nucleus-pulvinar complex (LP). Several lines of evidence, including positron-emission tomography (PET) scanning, lesion studies, and single-unit recordings, suggest that this second pathway is involved in visual orientation and spatial attention (Bender and Butter 1987; Corbetta et al. 1991; Grafton et al. 1992; LaBerge and Buchsbaum 1990; Petersen et al. 1987; Rafal and Posner 1987; Robinson and Petersen 1992). A computer modeling study (Olshausen et al. 1993) predicts that the functional role of this ascending pathway is to provide an attentional ‘‘window’’ that is essential for the primary visual cortex to select and route important visual information to the higher cortical areas. The extrageniculate visual pathway may also be involved in other aspects of visual processing such as motion perception (Casanova and Savard 1996; Chalupa 1991; McIlwain 1978b; Rauschecker 1988). A study of the physiological properties of those neurons in the optic layer of the rat SC that receive retinal input, and presumably project to the LP, should help us understand the functions of this relay station in the extrageniculate visual pathway.

We and others (Cork et al. 1995, 1998; Lane et al. 1993, 1997) have noted that a high percentage of the neurons in the optic layer of the rat SC are immunoreactive for calbindin$_{28K}$ (CB). Although the exact physiological role of this calcium-binding protein (CaBP) has not yet been determined, it has been useful as a marker for functional groups of neurons that are assumed to have some unifying physiological properties. In the rat SC, CB and another CaBP, parvalbumin (PV), are expressed in alternating bands of cells, possibly representing different components of the parallel visual pathways (Cork et al. 1995, 1998). There is a distinctive band of CB neurons within the dorsal two-thirds of the optic layer, most of which are medium-sized cells with dendrites projecting dorsally into the superficial layers (Lane et al. 1993; Cork et al., 1998). Lane et al. (1993) showed that most of these cells project to the LP.

The presence of CB in these neurons is of particular interest because several lines of evidence suggest that expression of one or the other of these CaBP is related to the firing patterns of neurons. PV is often found in “fast spiking” cells with nonadapting trains of calcium spikes, whereas CB is often found in cells with low-threshold calcium spikes and adapting trains of calcium spikes (Kawaguchi and Kubota 1993). Exogenous CB converts phasic supraoptic nucleus neurons to a continuous firing pattern, whereas inhibiting CB function with antibodies converts continuous firing...
into phasic firing (Li et al. 1995). It remains controversial, however, whether CB is associated with any specific neuronal firing pattern. Although Kawaguchi and Kubota (1993) found that nonpyramidal cells in layer V of rat frontal cortex all had low-threshold calcium spikes (LTSSs), a more recent report from Cala et al. (1997) showed that nonpyramidal cells exhibiting LTSS do not have a unique pattern of CaBP mRNA expression.

The goal of the present study was to determine the electrophysiological characteristics of neurons in the optic layer of the rat SC, to see whether any of them might correlate with CB expression. By means of intracellular recording from optic layer neurons, we investigated their passive membrane properties, active ion conductances, and synaptic inputs from the optic tract (OT). Our results show that these neurons have a short-lasting low-threshold spike and prominent spike afterhyperpolarizations (AHPs). The γ-aminobutyric acid-A (GABA_A) receptor-mediated inhibitory postsynaptic potential (IPSP) of these cells dramatically curtails the excitatory response induced by retinal inputs. All of these properties make the optic layer neurons fire transiently in response to stimulation of the OT. Another striking characteristic of these cells is the tendency to display high-frequency gamma (20–80 Hz) rhythmic burst (or doublet) firing, especially when the AHPs are blocked. These properties are completely different from those of cells in the LGN, indicating different roles played by the two visual pathways. Preliminary data have been published in abstract form (Lo et al. 1995, 1997).

METHODS

Sprague-Dawley rats of both sexes weighing 40–200 g were deeply anesthetized with Fluothane (Halothane) and killed by decapitation. The brain was removed quickly and immersed in ice-cold (4°C) sucrose artificial cerebrospinal fluid (ACSF) that was oxygenated with 95% O_2-5% CO_2 and contained (in mM) 234 to 254 NaCl, 1.25 NaH_2PO_4, 10 MgSO_4, 24 NaHCO_3, and 11 dextrose (Fukuda and Prince 1992). Parasagittal slices of the SC (400–500 μm thick) were cut in sucrose ACSF with a vibratome (Electron Microscopy Sciences) and incubated at room temperature in normal ACSF that contained (in mM) 124 NaCl, 2.5 KCl, 1.25 NaH_2PO_4, 10 MgSO_4, 26 NaHCO_3, 10 dextrose, and 2 CaCl_2; pH 7.4 after oxygenation. A slice was placed in an interface-style recording chamber (Fine Science Tools) and allowed to recover at 33°C for ≥1 h before recording.

Neurons in the optic layer were impaled with micropipettes filled with 4 M potassium acetate (KAc, 70–90 MΩ) or 2% biocytin in 2 M KAc (100–130 MΩ). Intracellular recording was performed in Bridge (current clamp) mode or DCC (discontinuous current clamp) mode with an Axoclamp-2B amplifier. For DCC mode, we set the sample rate to 3–4 kHz and adjusted the ‘‘capacitance neutralization’’ to an optimum level by monitoring the output of the headstage (Monitor). Discontinuous single electrode voltage clamp (DSEVC) was also performed to study the kinetics of the H current. Data were acquired and analyzed with Pulse 8.05 software (HEKA) running on a Macintosh Power PC 9500. For electrical stimulation of the slice, a pair of stimulating electrodes was placed on the optic tract fibers at the level of the caudal thalamus. Negative electrical pulses (0.1–2 ms, <250 μA) were applied to evoke postsynaptic responses from neurons in the optic layer.

Different antagonists or blockers were focally applied close to the cell from which recordings were being made. A small volume (0.5 μl) of such drugs was applied onto the surface of the slice through a Hamilton syringe. Control experiments showed that the effects of focally applied solutions were similar to the effects of bath-applied solutions of 1/10 the concentration. We therefore estimated the effective concentration of any focally applied drugs to be 1/10 of the concentration in the drop of solution. Solutions for focal application included the following: 10 or 20 mM tetraethylammonium chloride (TEA, Sigma), 2 μM apamin (RBI), 10 mM CsCl (Sigma), 10 mM NiCl_2 (Sigma), 1 mM D-2-amino-5-phosphonovaleric acid (D-APV; RBI), 250 μM 6,7-dinitroquinoxaline-2,3-dione (DNQX, RBI), 100 μM (-)-bicuculline methiodide (RBI), and 1 mM 2-hydroxyaclofen (RBI).

For intracellular biocytin injection, alternate ±1 nA current pulses (60 ms for each cycle) were passed via a biocytin-filled micropipette for 100–200 cycles. One hour after current injection, slices were fixed with 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) for 48 h and then transferred into phosphate-buffered saline (PBS) at 4°C. Slices were reacted in 10% methanol + 3% H_2O_2 overnight; after thorough rinsing in PBS, they were incubated overnight at 4°C with avidin-biotin complex [1:100 Elite kit (Vectastain) in PB with 1.8% NaCl and 0.5% Triton X-100]. After washing in PBS and 0.1 M acetate buffer (pH 6.0), slices were stained with nickel/glucose oxidase–intensified 3,3′-diaminobenzidine, rinsed in acetate buffer and PBS, dehydrated and stored in methysalicylate. Slices were wet mounted on microscope slides in methylsalicylate and examined with a light microscope to identify the location of labeled cells. A drawing tube attached to a Zeiss upright microscope was used to make drawings of the slice and filled cells. The location of the cell in the slice was mapped onto a drawing of the slice and filled cells. The location of the cell in the slice was mapped onto a drawing of the slice and filled cells. The location of the cell in the slice was mapped onto a drawing of the slice and filled cells.

RESULTS

Passive membrane properties of optic layer neurons

We made intracellular recordings from 74 neurons in the optic layer of the rat SC. Neurons were characterized, when they were impaled, by large overshooting sodium spikes. They had stable resting membrane potentials of −62.3 ± 6.2 (SD) mV and input resistances at rest of 37.9 ± 10.1 MΩ.

Responses to membrane depolarization

In response to depolarizing current pulses (0.2–0.4 nA, 300 ms), optic layer neurons gave a train of single spikes with a fast rising phase and a slower decay (Fig. 1A and B) and a half-amplitude duration of 0.75 ± 0.2 ms. The threshold for sodium spikes was about −45 mV, and they had a mean amplitude of 74.2 ± 12.3 mV, with an average overshoot of 12 mV. In contrast to neurons in the intermediate gray layer (Lopez-Barneo and Llinas 1988), sodium spikes in the optic layer neurons did not have a clear ‘‘shoulder,’’ suggesting that large potassium currents were preventing a calcium spike. This was confirmed when blocking potassium channels with TEA at an estimated concentration of 2 mM (Rudy 1988) induced a long-lasting shoulder, or plateau, in the falling phase of the sodium spikes (n = 3; Fig. 1C). Trains of spikes showed spike-
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FIG. 1. Sodium spikes in the optic layer neurons of the rat superior colliculus (SC). A: waveform of single sodium spikes. Threshold for the spikes is −45 mV. B: superimposed sodium spikes show a fast rising and a slower falling phase. Note no shoulder in the falling phase of the sodium spike. C: high concentration of tetroethylammonium (TEA; 2 mM) induces a shoulder or plateau in the falling phase of the sodium spike (trace 1, before; trace 2, after drug application; same for following figures). D: depolarization from a hyperpolarized potential (−70 mV) evokes an initial burst of spikes followed by single spikes. Each sodium spike is followed by two AHPs, denoted by f AHP and mAHP, respectively.

Frequency adaptation; i.e., a constant depolarizing current pulse induced repetitive firing with increasing interspike intervals (Fig. 1D).

Each sodium spike was followed by two AHPs (Figs. 1D and 2 A and E). There was a fast AHP (fAHP) followed by a longer duration one (mAHP) (Lorenzon and Foehring 1992; Sah 1996). A low concentration of TEA (around 1 mM) (Rudy 1988) completely abolished the fAHP (n = 7, Fig. 2B), suggesting that it was most likely mediated by the TEA-sensitive calcium-activated-potassium current, IC. Blocking fAHP widened the sodium spike (Fig. 2C), revealed an afterdepolarization (ADP; Fig. 2B), and shortened the interspike interval slightly (Fig. 2D). Thus the main effect of fAHP in optic layer neurons is to contribute to sodium spike repolarization (Lorenzon and Foehring 1992; Sah 1996).

The mAHP was not sensitive to TEA (Fig. 2B), but could be blocked by 200 nM apamin (n = 6, Fig. 2F), indicating that it was probably mediated by the apamin-sensitive calcium-activated-potassium current, IAHP (Lorenzon and Foehring 1992; Rudy 1988; Sah 1996). When mAHP was blocked, the ADP following each sodium spike was clearly seen (Fig. 2F). In contrast to TEA, apamin did not change the duration of the sodium spikes (Fig. 2G), but it did dramatically increase the frequency of spikes (Fig. 2H) and make the interspike interval constant, abolishing spike-frequency adaptation (Fig. 2, F and H). Thus the effect of mAHP in optic layer neurons is mainly to limit the firing frequency.

As mentioned above, sodium spikes were followed by an ADP that was usually masked by AHPs. The exact mechanism underlying the ADP has not been determined in the present study; however, the following results will show that the ADP is involved in the generation of high-frequency rhythmic burst firing.

Inward rectification

In most of the optic layer neurons (68/74), hyperpolarizing current pulses (−0.5−1.0 nA, 300 ms) induced a depolarizing “sag” on the passive electrotonic potential (arrow in Fig. 3A). This is inward or anomalous rectification and was blocked by 1 mM CsCl (n = 3, Fig. 3B). Both the pharmacology and the voltage dependency of this depolarizing sag suggested that it was most likely mediated by H channels (McCormick and Pape 1990). Voltage-clamp data showed that an inward (negative) current developed during hyperpolarizing current pulses (Fig. 3C). As the H current activated slowly, we could measure both the current at the beginning of the hyperpolarizing pulse (li) and at steady state (Iss). Plots of li and Iss against membrane potential (Vm; Fig. 3D) show that an inward current (the difference between Iss and li) was activated at membrane potentials negative to −65 mV.

Low-threshold calcium spike

In 71 of 74 optic layer neurons, at the break (cessation) of a hyperpolarizing current pulse 300 ms long, there was a rebound low-threshold calcium spike (LTS) usually with two sodium spikes (a doublet) riding on it (Fig. 4A). This LTS was reversibly blocked by NiCl2 (n = 3, 1 mM) as shown in Fig. 4, B and D. The magnitude and duration of the Ni-sensitive potential was estimated by superimposing records taken before and after NiCl2 application (Fig. 4C). In optic layer neurons the LTS was rather short in duration,
FIG. 2. AHPs in the optic layer neurons of the rat SC. A and B: low concentration of TEA (1 mM) specifically blocks the fAHP and discloses an afterdepolarization (ADP). C: TEA (1 mM) increases the duration of the spike. D: TEA slightly shortens interspike intervals at the same depolarized membrane potential. E and F: apamin (200 nM) specifically blocks the mAHP. G: apamin does not change the duration of the spike. H: apamin shortens interspike intervals dramatically at the same depolarized potential.

usually lasting for <25 ms. This is quite different from the long-lasting LTS with three to six sodium spikes riding on it that is seen in LGN relay cells (Crunelli et al. 1987; Lo et al. 1991; Lo and Sherman 1990; McCormick and Feeser 1990; Scharfman et al. 1990). One possible reason for the short duration of the LTS is that different potassium currents shorten it. Because neither TEA (n = 7) nor apamin (n = 6) increased the LTS duration or abolished the hyperpolarization following it, we suggest that potassium currents other than $I_C$ and $I_AHP$ may be responsible (Fig. 4, E and F).

**Chattering cells: high-frequency gamma rhythmic burst firing of neurons in the optic layer**

The threshold potential for LTS production was about −60 mV. When depolarized from a hyperpolarized membrane potential to about −60 mV, optic layer neurons produced only an LTS (Fig. 4A); however, in response to larger depolarizations (Fig. 1D), a train of single sodium spikes followed a single initial LTS. On the whole, the firing pattern of most optic layer cells (62/74, 84%) was the same as “intrinsically bursting cells” described in the cerebral cortex (Connors and Gutnick 1990), although a small proportion (12/74, 16%) of the neurons had high-frequency gamma rhythmic burst firing, just like “chattering cells” in the visual cortex (Gray and McCormick 1996) and somatosensorimotor cortex (Calvin and Sypert 1975). Current-clamp recordings, in DCC mode, showed that such repetitive bursting occurred only within a narrow range of membrane potentials from −47 to −53 mV (Fig. 5, B–F). Beyond this range, the neurons behaved like intrinsically bursting cells; namely, they gave either a LTS alone (Fig. 5A) or a LTS followed by single spikes (Fig. 5G). Within the range of rhythmic burst firing, the in-
terburst frequency was voltage dependent. The initial interburst frequency was 20 Hz at a membrane potential of −53 mV and 90 Hz at −47.5 mV (Fig. 5H). This range of burst frequencies corresponds approximately to the range of gamma frequencies (Gray and McCormick 1996; Pedroarena and Llinás 1997). Interburst intervals were not, however, constant at any given membrane potential; the rhythmic bursting firing, just like the trains of single spikes, showed frequency adaptation making the interburst frequency time dependent. Figure 5H shows the time-dependent burst frequencies plotted against the burst number at different membrane potentials. The initial burst frequency, between the first pair of bursts, was always the highest, and then burst frequency declined during later bursts. Each burst (doublet) usually contained two sodium spikes riding on a prominent ADP (Fig. 5I), suggesting that the ADP plays an important role in the generation of rhythmic burst firing.

Blockade of AHPs converts intrinsically bursting cells into chattering cells

As mentioned above, application of TEA or apamin blocked AHPs and unmasked the ADP. We therefore hypothesized that blocking the AHPs might also induce rhythmic burst firing. We tested this assumption on 10 optic layer neurons that behaved as intrinsically bursting cells. Before drug application, these cells produced a lone LTS after the hyperpolarizing pulse had ceased (Fig. 6, A and B). After application of 1 mM TEA (n = 5) or 200 nM apamin (n = 5), the neurons responded to the cessation of a similar hyperpolarizing pulse by firing rhythmic bursts (Fig. 6C and D). Fast sweep records (Fig. 6, E and F) showed that, after blocking the AHPs, the second spike in a burst was elicited by the ADP. In all 10 tested cells, blocking fAHP or mAHP converted the intrinsically bursting cells into chattering cells. Note that several undeveloped spikes (denoted by asterisk) were present together with fully developed bursts (Fig. 6C). There were also some undeveloped spikes (denoted by asterisk) on the ADP (Fig. 6F). These undeveloped spikes or bursts suggest that the rhythmic burst firing originates from dendrites (Pedroarena and Llinás 1997).

If AHPs were blocked, rhythmic burst firing could be induced, not only by changing membrane potential from hyperpolarization to depolarization, but also by stimulation of the OT. This was first demonstrated in extracellular recordings (Fig. 6, G–I) and then confirmed with intracellular recordings (Fig. 6J). Before blocking the AHPs, a single electrical shock applied to the OT merely evoked a short train of spikes (Fig. 6G). After the application of 1 mM TEA, the evoked response was followed by a silent pause and a series of rhythmic bursts (n = 4, Fig. 6H). After the TEA was washed out, the rhythmic bursts disappeared (data not shown), but they were restored by an application of 200 nM apamin (n = 3, Fig. 6J). Intracellular recordings also showed that rhythmic bursts could be evoked by OT stimulation after blocking the AHPs with TEA (Fig. 6J) or apamin (not shown). Again, undeveloped spikes or bursts were seen.
FIG. 4. Low-threshold calcium spike (LTS) in the optic layer neurons of the rat SC. A: LTS with 2 sodium spikes riding on it, occurs at the end of a hyperpolarizing pulse. B: LTS is blocked by NiCl₂ (1 mM). C: traces from A and B are superimposed to show the size and duration of the LTS. D: recovery of the LTS after wash out of NiCl₂. E and F: duration of the LTS is not changed after the blockade of fAHP by TEA (1 mM). G and H: duration of the LTS is not changed after the blockade of mAHP by apamin (200 nM).

together with fully developed bursts (Fig. 6 J). Because there was a silent pause between the synaptic excitation and the rhythmic bursts, it could be that rhythmic burst firing was induced at the end of the hyperpolarization by an IPSP. However, as described in the following section, IPSPs barely hyperpolarized the cell soma, so the silent pause before rhythmic bursting probably results from hyperpolarization due to the AHPs.

**Synaptic inputs to neurons in the optic layer**

All of the 39 optic layer neurons tested responded to stimulation of the OT with an excitatory postsynaptic potential (EPSP). When we changed the intensity of OT stimulation, the amplitude of the EPSP increased in an incremental way (Fig. 7 A), indicating that the neurons received multiple inputs from the OT. The latencies of different EPSPs were all about the same (Fig. 7 A), suggesting that they are mediated by a monosynaptic pathway. At the resting membrane potential, the EPSP produced a short train of sodium spikes (Fig. 7 B). At hyperpolarized potentials, the duration of the EPSP shortened, although its amplitude increased (Fig. 7 B). This voltage dependency indicated that the EPSP evoked by OT stimulation contained both NMDA (late) and non-NMDA (early) components. When the membrane was hyperpolarized, the former decreased and the latter increased in amplitude. Pharmacological study further supported this conclusion. Application of 100 μM d-APV, an NMDA receptor antagonist, shortened the duration of the EPSP, blocked the later spikes, but spared the first one (n = 6, Fig. 7, C and D). Additional application of 25 μM DNQX, a non-NMDA receptor antagonist, usually abolished the remaining EPSP.
FIG. 5. High-frequency gamma rhythmic burst firing in the optic layer neurons of the rat SC. A: depolarization to −54 mV from a hyperpolarized potential (−80 mV) induces an LTS with 2 sodium spikes riding on it. B–F: depolarization to between −53 and −47.5 mV from the same hyperpolarization (−80 mV) induces high-frequency rhythmic burst firing. G: greater depolarization, from −80 mV to −45 mV, induces a burst followed by single spikes. H: within the range −53 to −47.5 mV, the interburst frequency is voltage dependent. At each potential, the interburst frequency decreases. I: fast sweep record shows that the rhythmic burst firing is riding on rhythmic ADPs.

(n = 3, Fig. 7E). Therefore neurons in the optic layer receive direct retinal excitation that is mediated by both NMDA and non-NMDA receptors.

All EPSPs were followed by GABA_A receptor–mediated IPSPs. These IPSPs were very difficult to detect in optic layer neurons, because at the resting membrane potential they hardly hyperpolarized the membrane negative to the baseline (Fig. 7F, arrow), and at more hyperpolarized potentials, there was no obvious second peak due to a reversed IPSP (e.g., Fig. 7F, bottom trace). If the EPSP triggered a sodium spike, however, a real hyperpolarization did appear, and this was diminished but not reversed in polarity at −75 mV (Fig. 7G). If the EPSP triggered two spikes (Fig. 7H, top trace), the hyperpolarization was longer than that after just one spike (Fig. 7H, other traces). This hyperpolarization was not reversed even at −105 mV. Therefore the hyperpolarization that followed an EPSP with sodium spikes was the mAHP rather than the IPSP. The only way to identify the IPSP was to apply bicuculline to block GABA_A receptors. As shown in Fig. 7, I and J, application of bicuculline prolonged the evoked response markedly (n = 4). Thus the GABA_A receptor–mediated IPSP was characterized by its ability to curtail the late NMDA component of the EPSP rather than by its ability to hyperpolarize the membrane. By looking at the duration of the EPSP after OT stimulation, we could demonstrate the existence of the GABA_A receptor–mediated IPSP. We did not record any GABA_B receptor–mediated IPSPs from optic layer neurons, and 100 µM saclofen, a GABA_B receptor antagonist, failed to change the response evoked by OT stimulation (n = 4).

**Morphology of neurons recorded in the optic layer**

We injected biocytin into 16 neurons in 11 slices, and recovered 12 cells after fixation and labeling. Of these 12 cells, 9 were filled sufficiently to enable their morphology to be categorized based on previously published descriptions (e.g., Labriola and Laemle 1977; Langer and Lund...
FIG. 6. High-frequency rhythmic burst firing after blockade of AHPs in the optic layer neurons of the rat SC. A and C: apamin (200 nM) induces rhythmic burst firing. Note undeveloped spikes or bursts denoted by asterisk. B and D: TEA (1 mM) induces rhythmic burst firing. E and F: fast sweep records show that the burst (doublet) is induced by ADPs after application of either apamin or TEA. G–I: extracellular recordings show that the blockade of AHPs by apamin or TEA induces rhythmic burst firing after stimulation of the optic tract (OT). Note that a silent pause is intercalated between the evoked response and the rhythmic burst firing. J: intracellular recording confirms the effect of TEA on generation of rhythmic burst firing. Note premature bursts or spikes denoted by asterisk.

1974). Examination of these nine neurons revealed that they could be divided into two broad cell groups. Both groups had medium-sized cell bodies that were located at depths between 200 and 500 μm from the surface of the SC and had dendritic fields with vertically oriented dendrites that projected into the superficial gray layer. The two groups differed, however, in the breadth of their dendritic spread.

Narrow-field neurons had three to five primary dendrites that extended from a round or elliptical cell body. Although some of the primary dendrites were of large caliber (Fig. 8, arrows), secondary dendrites were generally thin with occasional thornlike extensions from the main dendrite (Fig. 8, arrowheads). Although short dendritic thorns were common, few obvious ball-on-stalk spines were found. Regardless of the initial orientation of the primary dendrites, the overall orientation of their dendritic fields was usually vertical, with the longest dendrites extending toward the pial surface. A subset of the narrow-field neurons had more elaborate, tortuous dendritic fields (Fig. 8, cell B). The as-
FIG. 7. Synaptic responses of SC optic layer neurons after OT stimulation. 

A: excitatory postsynaptic potentials (EPSPs) evoked by OT stimulation at different intensities. The relatively fixed latency of the EPSPs suggests that the EPSPs are mediated by a monosynaptic pathway. 

B: the voltage dependency of the EPSPs suggests that they are mediated by both N-methyl-D-aspartate (NMDA) and non-NMDA receptors. 

C-E: pharmacology of the EPSP. The late component of the EPSP is blocked by 100 μM D-2-amino-5-phosphonovaleric acid (D-APV), whereas the early component is blocked by the additional application of 6,7-dinitroquinoxaline-2,3-dione (DNQX; 10 μM). 

F: OT stimulation evokes an inhibitory postsynaptic potential (IPSP; ↓) that at resting potentials hardly hyperpolarizes the soma membrane. At more hyperpolarized potentials the reversal of the IPSP is obscured by the relatively larger EPSP. 

G and H: when the EPSP triggers sodium spikes, the hyperpolarization after OT stimulation results from AHPs that are not reversed even at −105 mV. 

I and J: bicuculline (100 μM) prolongs the response from OT stimulation dramatically, suggesting a strong shunting effect of the GABA<sub>A</sub> receptor–mediated IPSP.

Discussion

Our results reveal a fairly striking uniformity in both the physiological and some morphological properties of the neurons within the optic layer of the rat SC. In the vast majority of neurons, sodium spikes were followed by two AHPs mediated by different calcium-activated potassium currents; they had inward rectification mediated by Ih and low-threshold calcium spikes on the rebound from hyperpolarizing current pulses; they had bursting responses to depolarization with a subclass acting as chattering cells; all cells became chattering cells if the AHPs were blocked to reveal an ADP; the cells were monosynaptically activated by retinal input that had both NMDA and non-NMDA components; they also had a small GABA<sub>A</sub> receptor–mediated IPSP that could shunt the EPSP evoked after optic tract stimulation; and finally their morphologies had several common features, most notably medium-sized cell bodies with most dendrites projecting up into the superficial layers of the SC.

Gamma rhythmic burst firing in the optic layer

High-frequency (20–80 Hz) gamma rhythmic burst firing has been observed in layer V cells of the somatosensormotor cortex (Calvin and Sypert 1975) and layer II and III cells.
FIG. 8. Examples of narrow-field neuron morphologies. Drawings were made from biocytin-filled cells in 400-μm-thick slices. Cells are shown as they were oriented in the slice, i.e., the dorsal SC surface is toward the top of the page. All scale bars are 25 μm. A–D: examples of cells with narrower dendritic fields and most dendrites projecting into the superficial SC layers.

of the visual cortex (Gray and McCormick 1996; also see Gray and Singer 1989; Livingston 1996). Rhythmic bursting activity was found to be triggered by ADPs (Calvin and Sypert 1975; Gray and McCormick 1996; Li and Hatton 1996). In this study we report another example of gamma rhythmic burst firing in the optic layer of the rat SC. Our results also show that bursts of spikes are triggered by ADPs, although, in the optic layer, only a small proportion of neurons normally display this firing pattern. Most of the optic layer neurons are intrinsically bursting cells; they do not show the rhythmic burst firing because their ADPs are masked by AHPs. If ADPs are unmasked by blocking the AHPs, these intrinsically bursting cells are converted into chattering cells with high-frequency gamma rhythmic burst firing. Thus the ADP plays a crucial role in the generation of rhythmic bursting. Why only a small proportion of optic layer neurons normally show rhythmic burst firing is unknown, but it could be related to variability in the size of the AHP. In fact, we often observed rhythmic burst firing from neurons that were poorly impaled or damaged. In such neurons, I_c and I_{AHP} were not detectable. To prevent such impalement artifacts from influencing our data, we only included data from cells that were judged to be healthy on the basis of their resting membrane potential, input resistance, and spike size. Nevertheless, even when all data from damaged cells was discarded, the AHPs of the chattering cells were indeed smaller than those of the other neurons. Thus the chattering neurons in the optic layer may belong to a subgroup of neurons with intrinsically smaller AHPs. An alternative explanation for the chattering firing pattern is that such cells may have a larger ADP. The exact ion mechanism underlying the ADP in the optic layer remains unclear. In the supraoptic nucleus, the activation of ADP depends on an increase of intracellular Ca^{2+} concentration, and CB suppresses the ADP by buffering cytosolic Ca^{2+} (Li et al. 1995). If this is also the case in the optic layer, the chattering neurons may represent a minority of neurons in the optic layer that are CB negative and thus have larger ADPs. This
assumption will be tested by double labeling experiments using biocytin to fill the cells and CB immunoreactivity to determine the CB content and morphology of chattering neurons.

Is there a common electrophysiological signature for CB-immunoreactive cells?

In the optic layer of the rat SC, ~80% of neurons are CB-immunoreactive cells (Lane et al. 1993), and >90% of the CB-immunoreactive neurons project to the LP (Lane et al. 1997). In the present study, we recorded from a physiologically homogeneous population of neurons located in the optic layer, suggesting that most of the neurons we recorded from are likely to be the CB-containing neurons that project to the LP. Also, the morphologies of those cells we filled with biocytin are quite similar to those CB-positive LP projecting neurons described by Lane et al. (1993). CB has been reported to play an important role in determining intrinsic firing patterns of cortical and supraoptic neurons (SON) (Kawaguchi and Kubota 1993; Li et al. 1995). However, the effect of CB seems not to be the same for the cell types in these two regions. For instance, in SON neurons, CB suppresses the Ca^{2+}-dependent ADP and converts firing pattern from phasic to continuous, whereas in frontal cortical neurons, CB is found in cells with an LTS followed by a burst of spikes with spike-frequency adaptation. Whether or not there is a unique firing pattern for all CB-containing neurons throughout the brain is thus still an open question.

An LTS is certainly not an exclusive feature of CB-positive neurons. It is present in the Y- and X-relay cells of the cat LGN (Lo et al. 1991; Lo and Sherman 1990), which are thought to be CB negative (Mize et al. 1992), and parvalbumin-positive, CB-negative perigeniculate neurons (Deemeulemeester et al. 1991) have a long LTS (Huguenard and Prince 1992). On the other hand, it may be that LTS duration is correlated with CB content in the SC. In the LGN relay cells and other thalamocortical projection cells, the LTS is >40–60 ms long with three to six spikes riding on it (Crunelli et al. 1987; Jahnsen and Llinás 1984a,b; Lo et al. 1991; Lo and Sherman 1990; McCormick and Feeser 1990; Scharfman et al. 1990). Most of these relay cells except a small subgroup of X-cells in the cat LGN are CB negative (Deemeulemeester et al. 1991). In CB-positive frontal cortical neurons the LTS is also ~50 ms long (Kawaguchi and Kubota 1993). In the colliculus, however, the LTS of the presumed CB-positive optic layer neurons is rather short (<25 ms), and we have observed that CB-negative neurons in the intermediate gray layer have long-lasting LTSs (unpublished data). Thus a short LTS might be an index of CB-positive neurons in the SC.

Functional significance of the electrophysiological properties of neurons in the optic layer

The SC is a laminated structure. The superficial layers, especially the superficial gray layer and optic layer, serve as relay stations in two of the visual pathways. In the cat,
the upper superficial gray layer receives inputs from W-retinal ganglion cells (Berson 1988; Berson et al. 1990; McIlwain 1978a; Mize 1996), and many of its cells project to the LGN (Hartner et al. 1991; Huerta and Harting 1984; Mize 1996). The deep superficial gray layer and upper optic layer receive inputs from Y-retinal ganglion cells, and many of its cells project to the LP (Berson and McIlwain 1982; Caldwell and Mize 1981; Huerta and Harting 1984; Mize 1996). In the rat SC, LP-projecting neurons are located in the optic layer (Donnelly et al. 1983; Lane et al. 1993, 1997; Mason and Groos 1981; Miguel-Hidalgo et al. 1989; Mooney et al. 1988). Although we do not know the receptive-field properties of optic layer neurons in the rat SC, by analogy with the cat and hamster, the optic layer neurons should have Y-type receptive-field properties. Specifically, they should have a transient (phasic) response to visual stimulation and be sensitive to motion. The prominent mAHPs after each sodium spike in the optic layer neurons may slow down repetitive discharges or even stop the firing through spike-frequency adaptation. In addition, the GABA_A receptor–mediated IPSP may dramatically curtail the excitatory response evoked by OT inputs. All of these properties should contribute to a transient response with a high temporal resolution. It remains to be determined in an in vivo preparation whether these properties correlate with Y-type receptive-field projections.

**Synaptic organization in the optic layer**

Because the EPSPs evoked by OT stimulation in optic layer neurons have a relatively fixed latency, they are most likely mediated by monosynaptic retinal inputs. The source of the IPSPs, which follow the EPSPs, is less certain. In our brain slice preparation, most connections to the optic layer are severed, so we believe that the IPSPs must be mediated by an intrinsic local circuit rather than by some long-looped inhibitory circuit such as the projection from the substantia nigra (Ficalora and Mize 1989). Based on our physiological data, we do not know whether the IPSP evoked by OT stimulation is a feed-forward or a recurrent (feed-backward) IPSP. Nevertheless, the effects of the IPSP provide some clues to the synaptic organization underlying it. Because the IPSP does not markedly hyperpolarize the cell soma where we were recording, the inhibitory presynaptic terminals are most likely located on distal dendrites. As reviewed by Ferster and Jagadeesh (1992), postsynaptic inhibition can operate by two distinct mechanisms: 1) hyperpolarizing inhibition, by which IPSPs interact linearly with EPSPs and 2) shunting inhibition, by which IPSPs attenuate EPSPs in a nonlinear fashion. In the optic layer neurons, the IPSP appears to operate mainly by a shunting mechanism, because blocking the IPSP dramatically prolongs the EPSP. In a study of rat LGN, Xia and Lo (1996) suggested that a feed-forward IPSP has a powerful shunting effect, whereas a recurrent IPSP would have little such effect. The shunting effect is thought to be mediated by synaptic triads in which synapses from a retinal axon to a relay neuron and an inhibitory interneuron are in close proximity to a third synapse from the inhibitory interneuron to the relay neuron. Thus excitatory retinal input stimulates both the interneuron and the relay neuron, but the inhibitory feed-forward from the interneuron to the relay neuron shuts out the EPSP produced in the relay neuron. It is reasonable to speculate that the IPSPs we recorded in the optic layer are mediated by such a feed-forward circuit with triadic synapses on distal dendrites. Consistent with this, Mize (1992) has reported a similar GABA-immunoreactive circuit that could produce feed-forward inhibition in the superficial layers of the cat, rabbit, and monkey SC. Lund (1969) reported similar synapses in the rat.

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