Direction Tuning of Inhibitory Inputs to the Turtle Accessory Optic System

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Ariel, Michael and Naoki Kogo. Direction tuning of inhibitory inputs to the turtle accessory optic system. J Neurophysiol 86: 2919–2930, 2001. Neurons in turtle accessory optic system (basal optic nucleus, BON) were studied to compare excitatory and inhibitory visual inputs. Using a reduced in vitro brain stem preparation with the eyes attached, previous studies only showed a monosynaptic retinal input to the BON from direction-sensitive retinal ganglion cells that share a common preferred direction. Now using an intact brain stem preparation, not only did BON neurons display inhibitory postsynaptic potentials [IPSP(C)s] spontaneously, but IPSP(C)s were also evoked by visual pattern motion, they had their polarity reversed near the chloride equilibrium potential ($E_{Cl}$ -) and they were blocked by the GABA$_A$ antagonist bicuculline. Because excitatory postsynaptic currents had reversal potentials >0 mV, BON cells were recorded using patch electrodes filled with QX-314 or Cs$^+$ to measure the cell’s direction tuning also at that higher reversal potential. For most of the BON neurons studied, their visual excitation and inhibition had a very similar preferred direction, indicating that both synaptic inputs were maximally active onto the same cell under the same stimulus conditions. These competing inputs may result from connections between the pretectum and accessory optic nuclei. Such synaptic interactions may serve a functional role in the visual processing necessary to create retinal slip signals for oculomotor control.

INTRODUCTION

Sensory information converges on single sensory neurons as either excitatory or inhibitory inputs. In some cases, one sensory stimulus evokes multiple synaptic responses via different paths and different neurotransmitters, resulting in nonlinear interactions of the different membrane currents. Here, we describe experiments that demonstrate that “retinal slip,” the sensory code of global visual pattern motion, results from both excitatory and inhibitory inputs to the same cell in the vertebrate brain stem. This convergence occurs in the retinal target neurons of the accessory optic system (called the basal optic nucleus, BON, in turtle brain stem). These neurons integrate excitatory synaptic currents from many retinal ganglion cells from the contralateral eye to form the retinal slip signal (Kogo and Ariel 1997). The individual retinal inputs to a given BON cell have been shown to be from direction-sensitive (DS) ganglion cells that have a common preferred direction, thus creating a larger receptive field that encodes global image motion (Kogo et al. 1998). Global image motion is also encoded in the pretectum [mammalian nucleus of the optic tract (marsupial, Ibbotson et al. 1994; primate, Mustari and Fuchs 1990; opossum, Volchan et al. 1989); nucleus lentiformis mesencephali (Katte and Hoffmann 1980; amphibians, Manteuffel 1985; pigeon, Winterson and Brauth 1985)].

Visually evoked inhibition was not initially detected in extracellular studies of BON cell receptive field properties because the recordings used an in vitro brain stem preparation whose neurons have little or no spontaneous spike activity (but see inhibition during elevated spike activity in Fig. 3 of Rosenberg and Ariel 1990). Visually evoked inhibition was also not observed in the initial intracellular recordings from BON that used a reduced brain stem preparation (Kogo and Ariel 1997) in which the dorsal midbrain (including the pretectum) was surgically removed (see Fig. 4 of Rosenberg and Ariel 1991). The absence of inhibition suggests that it is mediated by a pathway through the dorsal midbrain.

In the experiments described in this paper, whole cell recordings from BON neurons in an intact turtle brain stem preparation with the eyes attached consistently showed inhibition evoked by pattern motion presented to the contralateral eye. The nonlinear combination of that inhibition with the direct convergence of excitatory retinal inputs to BON cells (Kogo et al. 1998) is interesting because these two sets of synapses encode very similar information about visual motion yet pass current across the cell’s membrane in opposite directions. The impact of such an arrangement on the visual processing or retinal slip and its control of oculomotor stabilization is discussed.

METHODS

The animal care and experimental preparation were described in detail elsewhere (Kogo and Ariel 1997; Rosenberg and Ariel 1990). Turtles, Pseudemys Scripta Elegans, were maintained in a room-temperature aquarium prior to the >1 h of cryanesthesia in ice water. The entire brain was removed with the two eyes attached. The telencephalon was removed within 15 min of decapitation, preventing conscious sensations before the tissue equilibrated to room temperature. Then the eyes were hemisected so that visual stimuli could be focused onto each retina. The brain stem was placed ventral side up into the superfusion chamber. The superfusate [containing (in mM) 130 Na, 2.0 K, 3.0 Ca, 2.0 Mg, and 97 Cl$^-$] was bubbled with 95% O$_2$–5% CO$_2$ gas so that solution’s pH was ~7.6 ± 0.05 and its osmolarity was ~274 ± 2 mOsmol (means ± SD). On occasion, 50–200 µM bicuculline was added to this superfusate.

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The tips of glass patch pipettes (5–9 MΩ) were filled with a standard pipette solution [containing (in mM) 124 KMeSO₄, 2.3 CaCl₂, 1.2 MgCl₂, 10.0 HEPES, 5.0 EGTA, and 2.0 ATP; pH = 7.3–7.4; osmolarity = 264 mOsm]. Assuming complete replacement of the cytoplasm with this pipette solution following rupture of the membrane patch, the computed equilibrium potentials in millivolts based on the Nerst equation are: \( E_{K} = 90.4 \), \( E_{Ca} = -107.2 \), \( E_{Na} = -68.3 \), \( E_{Cl} = 6.9 \), \( E_{La} = 13.3 \). In some cases (\( n = 47 \)), this pipette solution was modified by replacing 10 mM of KMeSO₄ by same concentration of a lidocaine derivative, QX-314, which blocked Na⁺ action potentials. Although QX-314 may affect more than just spike responses (Perkins and Wong 1995), synaptic responses appear unaffected by this drug. In other cases (\( n = 23 \)), the K⁺ of this pipette solution was replaced with Cs⁺ to reduce the K⁺-mediated spike repolarization. During Cs⁺ substitution, the voltage-dependent Na⁺ currents are rapidly inactivated by depolarizing the cell >10 mV, although the spike waveform measured near rest was not otherwise affected.

The reversal potentials for visually evoked excitatory postsynaptic currents (\( E_{EPSC} \)) and inhibitory postsynaptic currents (\( E_{IPSC} \)) were estimated as follows. Current pulses (<500 µA, 100 µs) presented via a bipolar electrode placed in the eye’s optic disk produced biphasic responses. From voltage-clamp recordings, the slope and amplitude of the early and late components were measured at holding potentials between −120 and +40 mV, and a linear regression of these data were performed to estimate \( E_{EPSC} \) and \( E_{IPSC} \), respectively.

**Visual stimulation**

Details of visual stimulation can be found elsewhere (Amamoto and Ariel 1993). In a darkened room, a full-field stimulus was generated on a computer monitor and focused through a lens to cover the whole retinal eyecup contralateral to the recording. Checkerboard stimuli were moved in 12 different directions, each for 4 s following a 1-s stationary period. Following each set of 12 responses, another trace was recorded during a full 5-s stationary period. All 13 responses were usually averaged over three stimulus sets. From the geometry of the 640 × 480 pixel monochrome monitor positioned above this retina, a video pixel equaled 11 µm on the retina or \( \approx 0.13^\circ \) (8.25') of visual angle (Northmore and Granda 1991) resulting in checks of 2.6'.

BON cells in vitro are known to respond best to the motion of large complex visual patterns (Rosenberg and Ariel 1990). In vivo recordings from other accessory optic systems suggest that these cells may differentiate between full-field stimuli that simulate body translation and those that simulate body rotation (nucleus of the basal optic root of pigeon) (Wylie and Frost 1999). To test for this property, in some cases (\( n = 47 \)), synaptic responses appear unaffected by TTX. In other cases (\( n = 23 \)), the K⁺ of this pipette solution was replaced with Cs⁺ to reduce the K⁺-mediated spike repolarization. During Cs⁺ substitution, the voltage-dependent Na⁺ currents are rapidly inactivated by depolarizing the cell >10 mV, although the spike waveform measured near rest was not otherwise affected.

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**Data analyses**

Preferred directions were analyzed from >1,000 direction-tuning curves from 57 BON cells, 21 of which were also studied with bicusculine. Response strength was measured for both EPSP(C)s and IPSP(C)s by computing the area for excitatory and inhibitory membrane deflections that exceeded the baseline measured just before the motion of the visual pattern (an average of 100–1,000 ms). For current-clamp recordings, positive areas of voltage indicated excitation and negative areas of voltage indicated inhibition. Alternatively, during voltage-clamp recordings, areas of outward current indicated inhibition and areas of inward current indicated excitation.

The direction-tuning curves were fit to the three parameters of the wrapped normal equation (see Rosenberg and Ariel 1998) to estimate objectively each cell’s preferred direction. These estimates were rejected from further analysis if their correlation coefficients fell <0.6 [the criterion value of direction sensitivity, twice as much response in the preferred direction than in the null direction (Rosenberg and Ariel 1991)]. Preferred directions are shown on the polar plots of direction-tuning as a line emanating from the plot origin (—: excitatory or inward current; -- : inhibitory or outward current). Small arrows (Figs. 3D and 7A) denoted preferred direction estimates measured from the area opposite the dominant current (i.e., outward or inward current when the cell was set near \( E_{EPSC} \) and \( E_{IPSC} \), respectively; see DISCUSSION).

**RESULTS**

To measure excitation and inhibition inputs to a given neuron, we chose a physiological approach of recording the excitatory responses at the reversal potential for the inhibitory response, and vice versa. This approach requires that the inward and outward currents can be measured and that the reversal potentials for each are far from one another. Because excitatory monosynaptic retinal ganglion cell input to the BON is blocked by 6-cyano-7-nitroquinoxalene-2,3-dione (CNQX) (unpublished observations), it appears that AMPA receptors mediate retinal input on BON cell dendrites. AMPA receptors have been shown to control a nonselective cation channel that reverses >0–10 mV (Spruston et al. 1995). Moreover, an inhibitory input to BON has been found to originate in the pretectum, perhaps from the nucleus Lentiformis Mesencephali, which is known to encode the direction of full field visual motion (Fan et al. 1995). This inhibitory input is mediated through GABA\(_A\) receptors via a Cl⁻ conductance (Kaila 1994) that reverses near −70 mV (actually −68.3 mV for BON cells calculated from the extracellular and pipette solutions using the Nerst equation).

Injecting current through a ruptured patch in a voltage-clamp mode easily held the BON cell’s membrane potential to −70 mV (\( E_{EPSC} \)) because it was close to the resting potential near −60 mV. Visual responses under these conditions show an isolated excitatory response. On the other hand, recording at the AMPA reversal potential is problematic due the presence of voltage-gated Na⁺ channels that generate action potentials. The addition of TTX into the bath would block this channel but also block the visual responses traveling to the BON.

Therefore the patch pipette filling solutions were modified with either QX-314 or Cs⁺ ions to inactivate the spiking mechanism of the recorded cell without affecting the neural processing of the brain stem. We then tested the direction...
tuning properties of BON cells to see if they are affected by dialysis of the modified pipette solution into its cytoplasm. Extracellular spike activity was recorded prior to rupturing the patch, followed by intracellular membrane recordings (Fig. 1). The direction-tuning curves were similar when measured before rupture, just after rupture when the cell is not yet dialyzed with Cs\textsuperscript{+} pipette solution, and many hours later, long after the Cs\textsuperscript{+} solution has had its effect on the cell. This shows that Cs\textsuperscript{+} pipette solution did not affect a cell’s ability to spike nor its preferred direction of motion. Presumably the synaptic responses that underlie the visual response are also unaffected. On the other hand, Cs\textsuperscript{+} substitution of the K\textsuperscript{+} ions in the patch pipette permitted the long-term depolarization of the BON cell well above spike threshold, presumably by blocking a delayed K\textsuperscript{+} current that prevents the voltage-gated Na\textsuperscript{+} channel from inactivating.

**Estimates of reversal for excitatory (E_{EPSC}) and inhibitory currents (E_{IPSC})**

As an initial approximation of $E_{EPSC}$ and $E_{IPSC}$ of visual responses, we measured the excitatory and inhibitory response components to optic nerve stimulation and assumed that visual responses reversed at similar holding potentials. Short-latency graded potentials were evoked by current pulses to the optic nerve of as low as 5 $\mu$A. At low stimulus current levels, only a monophasic excitatory response was observed (Fig. 2A) that was similar to those observed in a brain stem preparation in which dorsal midbrain was removed (onset latency of 3.9 ms ± 1.3) (Kogo and Ariel 1997). However, at membrane potentials between −70 and 0 mV (our first guess of $E_{GABA}$ and $E_{AMPA}$, respectively), a biphasic response was evoked: an excitatory wave of inward synaptic current followed by an inhibitory wave of outward synaptic current (observed in 67 cells; onset latency of 14 ± 3.2 ms). Figure 2 shows the response in a BON cell for which these two components were clearly separated in time. Unlike the monophasic excitatory responses of BON cells in preparation without the dorsal midbrain, the occurrence of the biphasic response suggests that the excitatory response represents direct retinal afferent input to the BON and that the delayed, less-sensitive inhibitory response is due to direct or indirect retinal excitation of an inhibitory path perhaps via the pretectum (Fan et al. 1995).

Each component of this biphasic response to optic nerve shock was also shown to reverse polarity (Fig. 2). Somewhere more than +20 mV, the first response component became an outward current (presumably at the EPSC reversal potential). Similarly, when less than −70 mV, the second component of the biphasic response became an inward current (presumably at the IPSC reversal potential). Figure 2B shows examples both

![Image](http://jn.physiology.org/)

**FIG. 1.** Long-term ruptured whole cell voltage recordings from a basal optic nucleus (BON) cell using a patch pipette in which K\textsuperscript{+} ions have been substituted with Cs\textsuperscript{+} ions. Time is given in hours:min. A: autoscaled direction-tuning plots of a BON cell to the same full-field pattern moving on the contralateral retina. The polar plots were fit with a wrapped normal function to estimate the cell’s preferred direction under each condition. A1: the tuning curve of extracellular spike activity after the gigaohm sealing had been established by the patch pipette but prior to rupturing the membrane patch. A2: the tuning curve of the depolarization area of a current-clamp recording made immediately after the patch was ruptured. Insets: voltage traces during motion in the preferred and null directions. A3 is like A2 and A4 is like A1 except measured hours after the Cs\textsuperscript{+} pipette solution diffused into the BON cell. Preferred directions of this cell are also shown as M12 in Fig. 6. B: 2 superimposed traces of spontaneous spikes, expanded and aligned from the traces of A, 2 and 3. During the Cs\textsuperscript{+} effect (thick line), the spike waveform was similar to spikes recorded just after the patch was ruptured (thin trace).
From the sample of control biphasic optic nerve responses studied in 35 BON cells, \( E_{\text{EPSC}} \) and \( E_{\text{IPSC}} \) were estimated to be \( 45.9 \pm 17.5 \) mV \((n = 35)\) and \( -49.2 \pm 18.2 \) mV \((n = 20)\). These uncorrected values do not correspond well to those for a cation AMPA channel and a Cl\(^-\)-mediated GABA\(_A\) conductances, respectively (but see DISCUSSION). However, application of bicuculline (only to the brain chamber) did block the second component of the biphasic response (unpublished data) (see also Fig. 4), indicating that it was indeed mediated by a GABA\(_A\) receptor. In fact, during bicuculline, the estimated \( E_{\text{EPSC}} \) increased from its control value of \( 49.6 \pm 17.5 \) mV to \( 70.4 \pm 21.8 \) mV \((n = 8)\). Bicuculline not only decreased the amplitude of the second component of the biphasic optic nerve response, but also decreased the first component (observed in 10 of 11 cells for which the cell’s impedance at rest was unchanged by bicuculline or even increased slightly). The latter finding suggests that GABA may also be involved in the early optic nerve response.

Visual response properties of EPSPs and IPSPs during full-field retinal stimulation

The major excitatory input to the BON comes from retinal ganglion cells. We have previously reported that one can evaluate the direction tuning of the excitatory synaptic input by counting individual EPSPs (Kogo et al. 1998). EPSCs are infrequent and brief enough in BON cells so that very few are obscured by another near-coincident EPSC event (see expanded trace in Fig. 3A, inset). However, measuring EPSCs in this way does not account for different numbers of events that were of large or small amplitude or short or long duration.

To quantify excitatory and inhibitory inputs in the same cell, one could simply count the two sets of synaptic events. However, ESPCs and IPSCs have very different amplitudes and durations and so have different effects on a BON cell. Therefore instead of counting synaptic events, measurements were made of the area of inward and outward current deflections during visual motion relative to a baseline measurement made during the stationary visual pattern. Figure 3 shows a comparison of the direction-tuning using this area measurement of inward current versus that of counting EPSCs. In this example, EPSCs were not contaminated by IPSCs because the EPSCs were recorded near the reversal potential for the IPSC and because the brain was bathed with bicuculline. Figure 3A, left and right traces, shows the membrane currents measured in the voltage-clamp mode, during pattern motion on the contralateral retina in the preferred and null direction, respectively. Using 12 directions of stimulus motion, EPSCs were counted, and area of inward current was measured from the same traces. The results of that analysis shown in Fig. 3B show that the direction tuning of the area measurement was very similar to that of the EPSC counts.

Next, the area of IPSC deflections was measured just like that of EPSCs so the two types of events can be compared independent of their different range of amplitudes and durations. Unlike the EPSCs of the same cell (Fig. 3A), IPSCs were prolonged so that they overlap onto each other (see expanded trace). These IPSCs also were visually responsive and direction sensitive. Note that the preferred direction of the outward current for the cell shown was quite similar to that of the inward current (Fig. 3D, - - - compared with ——).
EFFECT OF HOLDING POTENTIALS ON DIRECTION-TUNING ESTIMATES.

During the recordings, a given BON cell was studied at holding potentials estimated by eye to be the reversal potentials for each of the two components of the optic nerve response. For example, the EPSC data for the BON cell used in Fig. 3 were collected at the holding potential of −70 mV (the estimated \( E_{\text{IPSC}} \) during the experiment), but the later analysis revealed a computed \( E_{\text{IPSC}} \) estimate of −50 mV. In this case, we know that our estimate of the excitatory preferred direction is correct even at −70 mV by comparing the preferred directions with and without bicuculline.

However, errors caused by poor estimates of \( E_{\text{IPSC}} \) may not be detected if the preferred directions of the EPSCs and IPSCs are similar. Also, holding potentials used during an experiment may have been exactly at the reversal potential for some synaptic currents but slightly higher or lower for other synapses that were less well “voltage-clamped.” To understand the impact of choosing holding potentials that differed from the \( E_{\text{IPSC}} \) and \( E_{\text{EPSC}} \) of each synapse, we analyzed a BON cell for which the preferred directions of its excitatory and inhibitory visual inputs were different (inferior- and superior-temporal, respectively). This cell’s direction tuning at a holding potential near zero was not similar to either that of the excitatory or inhibitory response. However, Fig. 4B, top, shows that preferred directions for visual responses at holding potentials of −70 and −24 mV are very similar even though those potentials are far from −33 mV, the estimate of \( E_{\text{IPSC}} \) based on optic nerve responses. Similarly, Fig. 4B, bottom, is based on area measurements of outward current. Their preferred directions are also similar even though the holding potentials of +10 and +45 mV are quite different from +70 mV, the estimate of \( E_{\text{EPSC}} \).

Therefore inaccuracies in choosing a holding potential to isolate an excitatory or inhibitory visual inputs should not have a dramatic effect on the estimate of the preferred direction of that input.

The analysis of the averaged optic nerve responses at different holding potentials in Fig. 4A also shows that inaccuracies in choosing holding potentials do not have a dramatic effect on the isolation of a response. When the optic nerve responses during bicuculline were subtracted from the control responses, an isolated inhibitory current can be viewed which has a long latency and slow time course (Fig. 4A, bottom). These inhibitory traces had similar shapes even when the holding potentials (0 to +40 mV) were near the computed \( E_{\text{EPSC}} \) of +70 mV. On the other hand, pure excitatory traces (Fig. 4A, middle) did not appear to have the same amplitude or shape when the holding potentials were less than −10 mV because of the occasional spikes that occurred during these voltage-clamp recordings.

To verify that these visually evoked outward currents were in fact due to GABA inhibition, similar recordings were made in the presence of bicuculline for another cell with different excitatory and inhibitory preferred directions. Figure 5 shows that bicuculline blocked the outward current (measured at +43 mV) but did not affect the inward current’s response amplitude or direction tuning when recorded with a holding potential at −70 near the \( E_{\text{IPSC}} \). Spontaneous IPSP(C)s were also observed in most BON cells recorded in the intact brain stem preparation (112 of 177 cells). Bicuculline did block these spontaneous IPSP(C)s as well as the IPSP(C)s evoked by electrical stimulation directed to the pretectum (unpublished data). The addition of bicuculline verifies that excitatory and inhibitory inputs to the BON cells can be separated in voltage-clamp recordings using different holding potentials.

ANALYSIS OF ENTIRE BON CELL SAMPLE. Although all BON cells receive DS excitation (a criterion for their identification) (Kogo and Ariel 1997), we recorded from 57 BON cells that also displayed DS IPSP(C)s (27 with normal pipette solution, 15 with QX-314 added, and 15 with Cs+ substitution of the K+ ions). Following the onset of pattern motion, many BON cells exhibited a brief visual response that was greater than the
steady-state response (see large deflection followed by a plateau, Fig. 5). Therefore initial analyses were performed separately on an initial response period (usually from 0 to 600 ms that includes time prior to the response onset) and a steady-state response (usually 600–3,000 ms). The initial response values were noisier because their durations were more than four times shorter than the steady-state response measurement. Nevertheless, comparing the preferred directions of the transient and sustained response components of all the BON cells studied revealed that 91% of the preferred directions were within 40° of visual angle to each other. Given that the average half-width of tuning curves of BON cells is 141° (Rosenberg and Ariel 1991), those differences in direction tuning of transient and steady-state responses were not considered further.

A final analysis was performed of full-field visual responses of the 20 cells for which preferred directions could be estimated at both $E_{\text{IPSC}}$ and $E_{\text{EPSC}}$. Each direction-tuning curve was fit to a wrapped normal, as described by Rosenberg and Ariel (Fig. 6, correlation coefficients for each fit) (Rosenberg and Ariel 1998). Like extracellular spike responses, the preferred directions of the excitatory and inhibitory inputs are variable within the BON. However, for most of the cells, the relative alignments of the two inputs are similar, even considering that representing the direction tuning by a single vector can be quite inaccurate due to the broad tuning curves of their inputs (Fan et al. 1995; Rosenberg and Ariel 1991). On the other hand, Fig. 6 does show a couple of examples of BON cells where the excitatory and inhibitory preferred directions are quite different (>1 quadrant).

**Visual response properties of EPSPs and IPSPs during local retinal stimulation**

Six of the 20 cells studied with full-field retinal stimulation were also recorded during local retinal stimulation. In all those cases, a mask was placed on the computer monitor that was imaged onto the retina, to find the approximate location of the receptive field center. With this mask in place, recordings were repeated during 12 directions of visual motion and compared with one stationary condition. An example is shown in Fig. 7. In this case, the mask reduced the stimulus size from stimulation of $64 \times 85^\circ$ of visual angle to only region of 40° square. Although the response was slightly reduced, it is clear that the preferred direction of the excitatory and inhibitory inputs to the BON cell are approximately the same (Fig. 7A, compare top and middle rows). This was true of every cell studied with local retinal stimulation.
The direction tuning of the near periphery of the BON cells was also investigated. Often when the receptive field center was simply blocked to expose the periphery, excitatory and inhibitory responses were either ineffective or weak. However, when responses occurred, the preferred direction of the excitatory and inhibitory inputs to the BON cell were similar to that of the center responses (Fig. 7A, bottom).

Responses to smaller parts of the receptive field were also measured using computer-generated focal stimuli (see Methods). Figure 7B shows a few such responses, indicating that stimulation of the receptive field evoked the greatest response and that the preferred directions of both the visual excitation and inhibition to the cell remain the same even for these small stimulus fields. Similar data were also generated using moving patterns for the entire 4 × 4 grid of retinal stimulus positions in other cells with similar findings.

** Discussion **

Using whole cell patch recording techniques in an intact turtle brain stem in vitro, neurons of the accessory optic system were held at either the reversal potential of the excitatory synaptic input or the inhibitory synaptic input. In this way, visually evoked synaptic inputs were separated into inhibitory and excitatory membrane currents, respectively. Each of these two membrane responses was direction sensitive and have receptive fields that overlap within the visual world.

The average resting membrane potential of a BON cell is \(-59.6 \pm 3.7\) mV, which is close to its average spike threshold \(-43.0 \pm 5.5\) mV (Kogo and Ariel 1997). Because the BON cell’s resting membrane potential is near both the potential where inhibitory synaptic currents are smallest (\(E_{\text{IPSC}}\)) and the threshold potential for cell spiking, a linear response to the excitatory input should dominate the cell’s spike activity. However, GABA\(_A\) receptor-mediated shunting inhibition near \(E_{\text{Cl}^-}\) may still modulate the strength of the excitatory response in a nonlinear manner. In 18 of 20 cells studied, the preferred directions for excitatory and inhibitory responses were within the same quadrant. Therefore in those cells, its inhibitory input may affect the response strength rather than the preferred direction of visual pattern motion.
We have previously reported that retinal slip signals can be created in the BON by a simple spatial summation of similar DS ganglion cells from across the retina’s surface (Kogo et al. 1998) to be relayed to oculomotor nuclei, vestibular system, and cerebellum to modulate motor reflexes. The results of these experiments suggest that, even at the first site of the generation of a retinal slip signal by the brain, there may be complex processing that occurs by an interaction between excitatory and inhibitory visual inputs.

**Measurement of visually evoked EPSCs and IPSCs recorded in BON cells**

Our previous study reported that the direction-tuning of EPSP inputs to BON cells were well matched to its spike output (Kogo et al. 1998) to be relayed to oculomotor nuclei, vestibular system, and cerebellum to modulate motor reflexes. The results of these experiments suggest that, even at the first site of the generation of a retinal slip signal by the brain, there may be complex processing that occurs by an interaction between excitatory and inhibitory visual inputs.

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**FIG. 6.** Estimates of the preferred directions of all BON cells measured at $E_{\text{IPSC}}$ (——) and $E_{\text{EPSC}}$ (-----) using full-field pattern stimuli. The plots are arranged in chronological order with reference names that identify cells found in other figures. Listed under each name are the correlation coefficients of the fits to the excitatory and inhibitory inputs. All but 2 cells had the preferred directions of their visually evoked excitation and inhibition within the 90° quadrant of visual angle.
inhibitory components of the cell's response to visual motion. Because EPSCs and IPSCs have different amplitudes and shapes, counting individual synaptic events, as in our previous report, would not describe how they influence the spike output.

Objections can be raised on theoretical grounds about our use of an area measurement to characterize opposing signals from a single waveform. Specifically, values of positive and negative area from a waveform are not independent measures. An increase in one value always results in a decrease in the other. This criticism can be described for our polar plot data as follows. Plotting spontaneous synaptic activity alone (a fixed area of positive and negative current independent of stimulus direction) would be displayed as one “positive area” polar plot and one “negative area” polar plots; each a circle centered on the origin. If moving the visual pattern in one direction causes a positive deflection in its trace, the positive area polar plot would appear DS in that direction and the negative area polar plot would appear DS in the opposite direction. Thus a single response in one direction may cause a measurement artifact of preferred directions for inward and outward currents in a BON cell that are equal and opposite.

However, such measurement artifacts were not observed. In fact, the direction tuning of inward and outward currents of a BON cell were almost never equal and opposite. Any such measurement artifact appears to have been overwhelmed by the size of the opposing physiological responses. One aspect of the data analysis methods that may have reduced this artifact was using a baseline threshold. Baseline area values computed during stationary stimuli were subtracted from the area values measured during pattern motion (see METHODS), thus creating a threshold that a response must exceed to be measured.

Another requirement for measuring the area under synaptic responses is the accurate determination of the trace's baseline. For example, if the selected baseline value is lower than the true baseline, the inward current area will be smaller in the preferred direction and the outward current area will be larger in the null direction than the actual biological responses. Those data would indicate a net outward current exists that is DS in a direction that is exactly opposite the preferred direction of the actual inward current response. A baseline mismeasurement may have occurred in our data because there was a short 1-s interval between stimulus presentations that alternate in direction, leaving a small residual effect of one stimulus on the next epoch's measurement of the prestimulus baseline.

In Figs. 3D and 7A, there were small yet statistically significant DS responses of visual inputs near their own reversal potential (small arrows that point opposite to the larger preferred direction measured using the opposite area). However,
these data are derived from cells (Y62 and M09) for which the excitatory and inhibitory visual inputs had very similar preferred directions. It is therefore unlikely that such a DS synaptic input exists that would be evident near its own reversal potential and have a preferred direction different from the preferred direction measured at other holding potentials. Such small analysis artifacts were even observed for outward currents at $E_{\text{IPSC}}$ during bicuculline.

**Synaptic interaction within BON cells**

Visually evoked inhibition can play a role in BON cell output, even near the resting potential. Although IPSP amplitude is small when a BON cell is at rest (spontaneous IPSPs were typically 1- to 2-mV hyperpolarizations), their long duration leads to temporal summation when evoked by visual stimuli (see Fig. 3C). Because $E_{\text{Cl}}$ is also close to the resting potential, a large Cl$^-$ current may shunt the membrane, reduce the EPSP amplitude and thereby reduce the spike response. Fan et al. suggested in 1995 that the pretectal nucleus lentiformis mesencephali (nLM) might be a source of this inhibition to the BON. A push-pull interaction between these nuclei (Fig. 8B) is suggested by the finding that the abundance of nLM cells preferring nasal motion was complementary to a paucity of BON cells preferring that direction. On the other hand, BON cells that receive an inhibitory nLM input with the same direction tuning as the retinal excitation (Fig. 8C) would require selective synaptic wiring between the nLM and BON, even though the populations of nLM and BON cells appear complementary with regard to their direction-tuning (Fan et al. 1995).

In other vertebrates, there is also evidence for push-pull interactions between the pretectum and accessory optic system. Stimulation of the pretectum inhibited the extracellular activity of certain cell types in the pigeon’s nucleus of the basal optic root (Nogueira and Britto 1991). Similarly, lesions of the rat pretectal nucleus of the optic tract reduced certain responses in accessory optic system neurons homologous to the BON (Natal and Britto 1987). The exact circuitry of such effects is complicated by the possibility of commissural connections or re-
ciprocable connections between the pretectum and the accessory optic system (pigeon, Baldo and Britto 1990; frog, Lazar et al. 1989; rat, Schmidt et al. 1998).

In turtle, evidence for a role for nLM as a relay of visual inhibition is limited. Electrical microstimulation of nLM evoked IPSPs in BON cells that reversed near $E_{Cl}$ and was blocked by bicuculline. Spontaneous IPSPs in BON were seldom seen in a brain with its dorsal midbrain removed (Kogo and Ariel 1997), yet spontaneous IPSPs were observed in intact brains even after lidocaine was injected into both eyes, indicating a central origin (Ariel, in preparation). Finally, in situ hybridization of mRNA for glutamic acid decarboxylase, the synthetic enzyme for GABA, labels cells in the turtle pretectum as well as the dorsal BON (J. Martin and M. Ariel, unpublished data).

Difficulties in estimating reversal potentials for inward and outward currents

To isolate excitatory from inhibitory currents, estimates of $E_{EPSC}$ and $E_{IPSC}$ for BON cells were made using the biphasic optic nerve response of a given BON cell. This requires a reliable space clamp of the cell. However, like most brain stem neurons, BON cells have a complex morphology (Martin and Ariel, unpublished results). Estimates of optic nerve responses may represent a mixture of synapses at different positions on the dendrites. There may also be gradients of the pipette solution within the BON cells. In cases when Cs$^+$ pipette solution enters the BON cell soma after the patch is ruptured, the absence of K$^+$ ions may create an abnormal K$^+$ gradient along the dendrite due to the presence of a K$^+$/Cl$^-$ co-transporter along the dendrites (Jarolimek et al. 1999). Gradients may also result if the ions in the pipette solutions were not well matched with that in the BON cell.

Another source for error in our estimates of reversal potential was an inability to correct for voltage offsets of the micropipette. The recording baseline was set to 0 mV once the electrode tip was advanced into the chamber’s superfusate. However, this zero value may be affected by tip potentials as the electrode contacts brain tissue when the pressure is reversed on the internal pipette solution or when filling solution dialyzes into the cytoplasm. We estimated the liquid junction potential present between the patch pipette filling solution and the chamber’s superfusate based on a theoretical calculation (JPCalc) (Barry 1994). That software indicated that the recorded membrane potential in current clamp or holding potential was an inability to correct for voltage offsets of the micropipette.

Preferred directions of excitatory and inhibitory inputs to a single BON cell may be similar

Excitatory synaptic responses in BON cells are due to release of excitatory transmitter released by spike responses of DS retinal ganglion cell axons (Kogo and Ariel 1997). The convergence of these direct retinal inputs generated the dominant direction-tuning of each BON cell (Kogo et al. 1998). These findings have now been extended by finding a DS inhibitory input onto the BON cells. If the preferred direction of an inhibitory input was nearly opposite that of excitatory synaptic input, these two visual inputs would form a push-pull system that strengthens direction tuning of neurons in the accessory optic system. For example, when visual pattern motion directly excites a BON cell (downward on the retina in Fig. 8B), inhibition of the same cell via the pretectum is weakest. Similarly, when visually evoked inhibition is strongest (upward on the retina in Fig. 8B), the direct excitation is weakest. Shunting of AMPA excitation by GABA$_A$ inhibition would seldom occur because the synaptic events would rarely be temporally coincident.

Our finding that some BON cells have very different preferred directions for excitatory and inhibitory inputs is consistent with the extracellular recordings in other vertebrate accessory optic systems where noncollinear excitatory and inhibitory inputs to neurons have been reported (chickens, Burns and Wallman 1981; cats, Grasse and Cynader 1982; Soodak and Simpson 1988; pigeons, Wylie and Frost 1990). These reports relied on extracellular recordings of increases and decreases in spike activity during visual pattern motion but without control of the cell’s membrane potential needed to separate the excitatory and inhibitory inputs. Computer fits showed that the preferred direction of the excitation (increase above the spontaneous rate) was not exactly in the opposite direction as the preferred direction of the inhibition (decrease below the spontaneous rate) (Soodak and Simpson 1988). However, like our analysis to measure positive and negative area from the same current trace, it may be difficult to separate two preferred directions from the single data set to conclude opposite direction tuning of excitatory and inhibitory inputs.

The hypothesis of separate excitatory and inhibitory inputs to neurons in the rabbit accessory optic system is supported by the finding that the receptive fields of the two inputs were spatially segregated (Fig. 8A) (see Simpson et al. 1988). In our in vitro turtle experiments, local retinal stimulation has not revealed any separate regions within the receptive field, although it is possible that the in vitro preparation is not normal or that optical stimulation of the retinal eyecup presents a distorted image to the receptive field. Moreover, spatially segregated responses may require a full 360° optic flow pattern stimulating the full visual fields of both eyes in a three-dimensional visual environment.

Although the rabbit cells had nearly opposite preferred directions for their excitatory and inhibitory measures, most of the turtle BON cells neurons had similar preferred directions for its two antagonistic inputs. It is possible that we are describing an inhibitory path to the accessory optic system in turtle that also exists other vertebrates but could not be measured from the extracellular spike recordings in which membrane potential remains very close to the $E_{IPSC}$, the reversal potential for the inhibitory input.

Role for concurrent, conflicting synaptic inputs

It is interesting to consider a possible function for conflicting sensory signals to the same neuron in the brain stem. In many BON cells, release of excitatory and inhibitory synaptic input occurred simultaneously during preferred direction motion (Fig. 8C), potentially leading to the nonlinear shunting effects.
on the membrane potential. A similar finding has been observed in DS retinal cells (Borg-Graham 2001). The accessory optic system may compute the direction and strength of the retinal slip signal based on a nonlinear interaction of two competing inputs. One potential role for competing BON inputs is that, as the stimulus strength increases, the concomitant depolarization serves as a brake that increases the visually evoked hyperpolarization and limits the spike frequency of the retinal slip signal. Alternatively, other inputs to the accessory system neurons may exist that depolarize the cells’ membrane potential, thereby controlling the strength of the retinal slip signal to the oculomotor system and its reflex gain. Networks of balanced excitatory and inhibitory activity can result in neuronal responses that react more rapidly to visual stimuli (van Vreeswijk and Sompolinsky 1996). This modulation would affect the accuracy of reflexes of retinal image stabilization while preventing instabilities that are inherent in negative feedback control systems. Similar control mechanisms may operate in other reflex arcs in the brain stem.

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