Shunting Inhibition in Accessory Optic System Neurons

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Ariel, Michael and Naoki Kogo. Shunting inhibition in accessory optic system neurons. J Neurophysiol 93: 1959–1969, 2005. First published November 24, 2004; doi:10.1152/jn.00214.2004. The interaction of excitatory and inhibitory inputs to the accessory optic system was studied with whole cell recordings in the turtle basal optic nucleus. Previous studies have shown that visual patterns, drifting in the same preferred direction, evoke excitatory and inhibitory postsynaptic events simultaneously. Analysis of the reversal potentials for these events and their pharmacological profile suggest that they are mediated by AMPA and GABA_A receptors, respectively. Here, neurons were recorded to study nonlinear interaction between excitatory and inhibitory responses evoked by electrical microstimulation of the retina and pretectum, respectively. The responses to coincident activation of excitatory and inhibitory inputs exhibited membrane shunting in that the excitatory response amplitude, adjusted for changes in driving force, was attenuated during the onset of the inhibitory response. This nonlinear interaction was seen in many but not all stimulus pairings. In some cases, attenuation was followed by an augmentation of the excitatory response. For comparison, the size of the excitatory response was evaluated during a hyperpolarizing current pulse that directly modulated voltage-sensitive channels of a slow rectifying I_h current. Injection of hyperpolarizing current did not cause the attenuation of the excitatory synaptic responses. We conclude that there is a nonlinear interaction between these excitatory and inhibitory synaptic currents that is not due to hyperpolarization itself, but probably is a result of their own synaptic conductance changes, i.e., shunting. Since these events are evoked by identical visual stimuli, this interaction may play a role in visual processing.

INTRODUCTION

Neurons may receive tens of thousands of synaptic inputs that are integrated throughout its membrane, often resulting in spike initiation at the axon hillock near the soma. This synaptic integration occurs during a temporal overlap of synaptic events that involve different synaptic conductances (e.g., excitatory and inhibitory responses; Qian and Sejnowski 1990). The experiments here investigate synaptic integration on a membrane level in a system for which it has been shown that natural stimuli evoke simultaneous excitatory and inhibitory inputs onto the same cell (Ariel and Kogo 2001). These cells in the accessory optic system relay retinal slip information to the cerebellum and numerous brain stem nuclei for compensatory head and eye movements (Breacha and Karten 1979; Simpson 1984).

The accessory optic system receives direct excitatory input from the retina (Kogo and Ariel 1997; Zhang and Eldred 1994) and indirect inhibitory input from the pretectum (Kogo et al. 2002). Both excitatory and inhibitory responses to pattern motion on the contralateral retina are direction-sensitive (Ariel and Kogo 2001). Surprisingly, these two inputs shared a similar preferred direction such that excitatory and inhibitory synaptic events reach the membrane simultaneously.

To study synaptic integration of these two inputs, microstimulation was employed to control exactly the relative timing of the excitatory and inhibitory events. A similar analysis was reported for pairs of unitary excitatory events stimulated directly from the retina (Kogo and Ariel 1999). In that case, the coincident arrival of pairs of excitatory inputs did not always sum linearly but exhibited response attenuation below the linear combination of their individual synaptic effects. The interval between stimulation of each input was incremented by 1–2 ms to quantify the time course of nonlinear summation. Response facilitation was never observed, but in about one-half of the pairs, the time course of attenuation was shorter than the time course of the decaying phase of the first excitatory postsynaptic potential (EPSP). This finding suggested that this attenuation involves nonlinear mechanisms due to the opening of excitatory synaptic channels.

The experiments described here perform a similar analysis of the interaction between excitatory and inhibitory inputs to the same cells in the accessory optic system of the turtle [basal optic nucleus (BON)]. The excitatory response from the retina is mediated entirely by AMPA receptors, while pretectal stimulation evokes an inhibitory response that is Cl− mediated and blocked by a GABA_A receptor antagonist (Kogo et al. 2002). The time course of nonlinear interactions of coincident excitation and inhibition were evaluated during whole cell patch recordings in the intact turtle brain stem. The neurons were characterized by their responses to natural visual stimuli presented to the contralateral retina prior to presenting electrical pulses to the retinal surface to evoke EPSPs and to the pretectum to evoke inhibitory postsynaptic potentials (IPSPs). A clear nonlinear interaction was observed that suggests that shunting occurs during coincident synaptic events. This shunting may play a role in the increased visual responses that are also observed in these neurons when their resting membrane potentials remain close to the Cl− equilibrium potential.

METHODS

Red-ear pond turtles, Pseudemys scripta elegans, were housed in an aquarium at room temperature with facilities to swim and bask on a 16/8-h light-dark cycle. In initial experiments, surgery was performed after >1 h of ice anesthesia, whereas in later experiments, turtles were injected with 12.5 mg thiopental. After the brain was removed with the eyes attached, the telencephalon was ablated (for details, see Kogo and Ariel 1997; Rosenberg and Ariel 1990). The eyes were hemisected, and the preparation was placed ventral side up in the
superfusion chamber. The ionic composition of the superfusate was (in mM) 130 Na, 2.0 K, 3.0 Ca, 2.0 Mg, and 97 Cl, bubbled with 95% O2-5% CO2 (pH 7.6). The eye contralateral to the recording was stimulated with a full-field checkerboard pattern drifting in 1 of 12 directions (Amamoto and Ariel 1993).

Whole cell ruptured patch recordings in BON were made with 5- to 9-MOhm pipettes filled with pipette solution (in mM: 114 KMeSO4, 2.3 CaCl2, 1.2 MgCl2, 10.0 HEPES, 10.0 QX314, 5.0 EGTA, and 2.0 ATP) (Ariel and Kogo 2001). This solution would diffuse into the neurons, and eventually QX314 would block action potentials so that responses could be studied above spike threshold. The membrane potentials of BON cells (approximately −60 mV at rest) were depolarized by continuous current injection to reveal hyperpolarizations evoked by pretectal stimuli. In cells for which the effect of the slow rectifying \( I_h \) current was studied, QX314 was not added to the pipette solution and the concentration of KMeSO4 was increased to 124 mM. Bipolar stimulating microelectrodes were positioned in the contralateral retina and in the pretectal area based on surface landmarks ipsilateral to the recordings to deliver current pulses (<500 \( \mu \)A, 100 \( \mu \)s).

The intervals between the pairs of stimulation were incremented by 1.0 or 2.0 ms to generate a set of 20 different intervals. This procedure was repeated 20–100 times, and the data were averaged. For comparison, retinal stimuli were also paired at 20-ms increments following the onset of a long 800-ms hyperpolarizing current injection through the recording electrode. In most of those experiments, a clear depolarizing sag was observed during the hyperpolarization, indicative of a strong \( I_h \) current (Kogo and Ariel 1997).

In total, 27 BON neurons were recorded using electrical stimulation; 18 of them were studied with pairs of retina + pretectum stimuli, and 9 neurons tested with pairs of retina stimuli + current injections were quantified for this study. Among the 18 neurons studied with pairs of retina + pretectum stimuli, 13 showed apparent nonlinear interactions between excitatory and inhibitory responses of their averaged data. These were quantified further using pairs of individual excitatory responses to retinal stimulation before and during the hyperpolarization. A paired t-test showed that 12/13 neurons had excitatory responses that were significant smaller during part of that hyperpolarization (\( P < 0.05 \)).

The excitatory response was measured at the time of its peak voltage, relative to the membrane voltage measured in the control response using the pretectal stimuli alone. Adjustments for driving force were based on the membrane potential just before the onset of the response to the retinal stimulation. For the retina + pretectum responses, the average value for the excitatory reversal potential of 46 mV was used for adjustments of driving force (Ariel and Kogo 2001). For the paired stimuli of retina + current injections, the driving force was adjusted using the two responses within 100 ms of the onset of the current injection.

The analysis of visual responses during biccuculline involved an additional 10 neurons studied without QX-314 or electrical stimulation. To measure the visual response amplitude, the baseline during the 3-s visual stimulation was first adjusted to zero by subtracting its minimum value. Then the area under each voltage traces was measured (mV · s). Using those values, polar plots were made to show the relative response in each direction, both before (control) and during drug (biccuculline). To compare the control and biccuculline data, the direction-tuning was fit to a wrapped normal curve (Rosenberg and Ariel 1998), aligned to the preferred direction and averaged across the sample.

**RESULTS**

Whole cell recordings were made of BON neurons in vitro from the brain stem’s ventral surface (Fig. 1). These cells are characterized by direction-sensitive synaptic responses to whole field visual pattern motion imaged on the contralateral retina (Fig. 1A). Excitatory and inhibitory responses were evoked in each cell by current stimulation of the retinal surface (RET) and the pretectum (PT), respectively (Fig. 1, B and C).

**Electrical responses**

Interactions between excitatory and inhibitory responses were examined in BON neurons by evoking both responses from the same neuron with an interstimulus interval that incremented by 1 or 2 ms. As seen in Fig. 2, A and B, the PT responses exhibited a prominent hyperpolarization of a longer duration than that of the RET responses. Therefore RET stimulation occurred after PT stimulation so that the responses would overlap in time.

During the overlap of PT and RET, the excitatory RET responses were often attenuated only during the initial phase of the inhibitory response so that the attenuation was short-lived (Fig. 2C). Because the RET response occurred during a membrane hyperpolarization, the phenomenon is best observed in a plot of the excitatory response amplitude (measured in reference to the unpaired PT response—see inset above the traces in Fig. 2C). Many of the neurons showed a similar short-lived attenuation of excitatory response during the beginning of the IPSP. During some pairs of RET-PT stimuli, BON neurons exhibited both attenuation and a delayed facilitation of the RET response (Fig. 3, see also Fig. 5). As with the example in Fig. 2, the attenuation of the RET response was short-lived, but it was also followed by responses that were greater than the control.

Because these RET responses occurred during membrane hyperpolarization, they were analyzed to determine whether they result from changes in ionic driving force. The synaptic pharmacology of the RET responses indicate that these responses are mediated by AMPA receptors, occasionally followed by a bicuculline-sensitive inhibition (Ariel and Kogo 2001). (Note that in both Figs. 2 and 3, the RET responses show small hyperpolarizations after their excitatory responses.) The reversal potential for the AMPA responses occur at positive membrane potentials, so the driving force is large for the RET responses. We therefore scaled down the measured excitatory amplitude if the response began during a membrane potential that was hyperpolarized relative to baseline and scaled it up if the response began at a membrane potential that was depolarized relative to baseline.

Because RET responses already involve a large driving force, the effect of compensating for the small baseline membrane changes evoked by PT were minimal. This is seen in the graphs of Figs. 2 and 3; triangles are used to plot the EPSP amplitude, whereas circles show amplitude values computed to compensate for changes in driving force. This adjustment did not reduce the clear attenuation of the RET response during the initial PT response. In fact, excitatory responses occurring during a hyperpolarization should have a greater driving force, hence the adjusted response showed a greater attenuation. Changes in driving force therefore do not cause the attenuation of RET responses.

Next, the effect of hyperpolarization was experimentally measured. BON neurons were injected with negative current pulses through the electrode (presumably at the soma) in lieu of
the PT IPSPs. This injected current may differ from synaptic current in dendritic membrane but often revealed a voltage-sensitive channel, an anomalous delayed rectifier current known as $I_h$ (Kogo and Ariel 1997), as is seen in Fig. 4. However, excitatory responses during the hyperpolarization evoked by current injection did not attenuate. In fact, as expected from an increased driving force, the excitation was immediately increased after onset of the hyperpolarization.

FIG. 1. Voltage responses of a neuron in the basal optic nucleus. A: responses to each of 12 stimulus directions are plotted each in the position related to its stimulus direction. B: 20 superimposed traces during retinal stimulation. C: 20 superimposed traces during pretectal stimulation. For B and C, a stimulus artifact in each trace indicates the time of stimulation. Membrane potential was adjusted to $-51$ mV to show a hyperpolarization during pretectal stimulation. Note that action potentials are blocked by QX314 in the patch pipette.
Although RET attenuation as shown in Figs. 2 and 3 was a short-lived phenomenon, the RET amplitude was also analyzed during a long-lasting hyperpolarizing current pulses that evoked the $I_h$ current (Kogo and Ariel 1997). $I_h$ current results from the opening of slow nonspecific cation channels hundreds of milliseconds after onset of hyperpolarization from the membrane voltage below $-60$ mV (Mayer and Westbrook 1983). We examined whether the delayed opening of these channels shunted the membrane and reduced the RET response. Figure 4, C–E, shows that the excitatory responses became smaller as the membrane repolarized during the current pulse. This is opposite the effect shown in Figs. 2 and 3, where the excitatory responses were more attenuated when the hyperpolarization began as compared with when it repolarized. However, the hyperpolarization necessary to evoke the $I_h$ current was large, and therefore its effect on the driving force was significant. Figure 4F shows a plot of the RET amplitude as a function of time in the current pulse (diamonds). Although the amplitude decreased as the $I_h$ current depolarized the membrane, this decrease appears to result for a change in driving force (Fig. 4F, squares). When the amplitudes were adjusted for the decrease in driving force, changes of the response amplitudes were not observed. This result was seen with all BON neurons studied (Fig. 4G). It appears that, unlike the synaptic currents evoked by PT stimulation, the channels of the BON’s $I_h$ current do not have significant nonlinear interactions with the RET excitatory synapses.

Assuming that the response attenuation during the coincident PT-RET stimulation is due to membrane shunting, why is
the RET response only briefly decreased, even as the PT hyperpolarization continues? Figure 5 shows another example of PT-RET stimulation for a BON neuron in which the time course of the inhibitory current was also measured using voltage-clamp recordings. When the membrane is depolarized (−30 mV) as with Figs. 2 and 3, the RET responses (upward deflections of Fig. 5A) were only attenuated during the initial phase of the IPSP (Fig. 5C, bottom trace). This brief attenuation was also observed when the resting membrane potential was near the reversal potential for the PT response (Fig. 5B). In that case, PT stimulation did not hyperpolarize the membrane, further indicating that a change in driving force is not responsible for RET attenuation. Below these PT-RET traces are two averaged PT response traces: an outward current evoked during a recording made in the voltage-clamp mode (IPSC, Fig. 5C) and a membrane hyperpolarization evoked in the current-clamp mode (IPSP, Fig. 5C). Figure 5D compares the data from Fig. 5, C and A, by superimposing the response graph adjusted for driving force (Fig. 5A, ○) on a vertically flipped IPSC trace (from Fig. 5C but outward current is displayed as downward). This comparison shows that the attenuation observed during PT-RET had a time course most similar the IPSC trace (Fig. 5D). Although the space-clamp condition of voltage-clamp recordings of neurons with complex dendritic trees is not expected to be perfect, the IPSC time course is still informative, especially when PSPs and PSCs are obtained from the same whole cell recording. The finding that the time course of RET attenuation is well matched to the IPSC is good evidence that synaptic responses at RET synapses are shunted by synapses activated by PT.

**Visual responses**

An analysis of the functional effects of shunting inhibition was made using visual response data from BON neurons previously recorded to analyze GABA inputs (Ariel and Kogo 2001). From those data, there were 10 neurons for which voltage traces were recorded between −60 and −70 mV and there were no visible IPSPs but strong direction-sensitive EPSPs. These neurons were recorded during full-field pattern motion in each of 12 directions across the contralateral retina. The amount of depolarization for each stimulus was measured

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**FIG. 3.** Averaged voltage responses exhibit initial attenuation of retinal response followed by responses greater than that of the control. A: amplitudes graphed for measured responses (○) and responses computed to compensate for the increased driving force (●) compared with the control (▲ and dashed line). B: responses to 50 retinal-pretectal stimuli, 20 pairs incremented by 1 ms. C: responses to stimulation of only retina (depolarization, upward deflection) and pretectum (hyperpolarization, downward deflection). Membrane potential was adjusted to −24 mV, permitting clear hyperpolarization during pretectal stimulation.
before and after the application of 100 μM bicuculline, an antagonist to the GABA_A receptor. That ionotropic receptor opens a chloride channel with a reversal potential of $-68.3 \text{ mV}$ (computed using the Nernst equation; Ariel and Kogo 2001), so that recordings near that value should not hyperpolarize the membrane but may reduce the excitatory synaptic drive to the neuron.

The average resting membrane potential was $-67.2 \text{ mV}$ ($n = 6$). Relative to the control traces, bicuculline did not cause any consistent change in the resting membrane potential ($0.37 \pm 1.21 \text{ (SD) mV, } n = 10$). Although the visual responses of two neurons were also unchanged during bicuculline, eight neurons showed obvious response increases (mean of 28% increase, ranging from 3 to 100%). Specifically, the response area (above the baseline) was 13.955 mV · s along the preferred direction prior to bicuculline, but was increased to 17.923 mV · s during bicuculline. There was an even larger effect of bicuculline during the transient response to motion (44% increase from 15.633 mV · s during control to 22.509 mV · s during bicuculline).

An example of the effect of bicuculline on the direction-tuning of BON neurons is shown in Fig. 6A. The voltage response to the onset of pattern motion on the contralateral retina was stronger to inferonasal motion, yet very little response occurred in the opposite direction. This direction tuning in the BON is mediated by GABA release by retinal amacrine cells because it is blocked by localized bicuculline application to the contralateral retina (Ariel 1992), causing equal responses to all directions of visual motion. Here, 100 μM bicuculline application only to the brain stem did not block direction sensitivity but increased the visual response, primarily in the preferred direction. The average responses of eight neurons (those aligned and normalized with the example shown in Fig. 6A are shown Fig. 6B). Therefore even when the membrane potentials of these neurons were near the GABA_A reversal potential, there was a GABA_A effect, perhaps attributable to the shunting phenomenon describe above.

DISCUSSION

Previous studies have shown that individual neurons may receive both excitatory and inhibitory synaptic drive during the same retinal image motion in its preferred direction (accessory optic system, Ariel and Kogo 2001; retina, Borg-Graham 2001; visual cortex, Monier et al. 2003). Here, coincident responses of excitation and inhibition were evoked in neurons of the accessory optic system by separate electrical stimulation of these two inputs to analyze any nonlinear interactions. It was found that, similar to the attenuation of coincident excitatory responses from retina (Kogo and Ariel 1999), excitatory responses during a coincident IPSP were attenuated. The time course of this attenuation indicates that it is due to membrane shunting that results from the opening of inhibitory synaptic channels following pretectum stimulation.

Nominally, shunting occurs without an apparent IPSP (at the reversal potential), when many synaptic channels open but there is no net current flow and thus no hyperpolarization. To exclude hyperpolarization during IPSPs as a cause for attenuation of the retinal responses, the retina was stimulated during hyperpolarizing pulses, yet no attenuation was observed. The interaction of these excitatory and inhibitory responses is consistent with the pharmacological identification of retinal inputs mediated by AMPA receptors and indirect visual input mediated by GABA_A receptors (Kogo et al. 2002). Because these two pathways are both active during the same direction of visual stimulus motion, this membrane shunting may be an important feature of neural processing in these brain stem neurons.

Voltage-sensitive interaction?

Attenuation of excitatory responses in many cells recovered sooner than the IPSP evoked by PT stimulation. This finding is inconsistent with a mechanism mediated by a change in driving force or another voltage-sensitive mechanism that might affect the RET response directly by depressing the AMPA receptor conductance. A voltage-sensitive mechanism for attenuation was also not supported by our experimental finding that membrane hyperpolarizations evoked by current pulses through the recording pipette did not cause any response attenuation. These current pulses did evoke an $I_h$ current, resulting in a membrane repolarization with a time course much slower than the attenuation observed during PT-RET stimulation. Although there was a reduction in RET responses during $I_h$ activation, those reductions seem to result from a decrease in the driving force created by the membrane repolarization.

An alternative explanation for RET attenuation is that PT stimulation evokes two inhibitory processes: membrane hyperpolarization of the BON cell and presynaptic inhibition of the excitatory retinal ganglion cell terminals in the BON. In this case, the correspondence of the attenuation time course with the IPSC would be simply fortuitous. A more parsimonious explanation for RET attenuation is that RET and PT interact by a shunting mechanism. This would predict that the time course of attenuation should relate to that of the conductance change, which is shorter than that of the IPSC or any voltage-sensitive mechanisms resulting from that hyperpolarization. In fact, the role for membrane shunting is supported by the clear correspondence in Fig. 5B of the attenuation time course with the IPSC in the absence of any change in baseline voltage.

In some cases, the response amplitude following the attenuation was actually greater than that of the control. This response increase may be delayed and thereby be a different cause for the short-lived nature of the attenuation. For example, the time course of attenuation shown in Fig. 5A during a membrane hyperpolarization is even shorter than the IPSC, suggesting that a mechanism exists that augments the RET

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**FIG. 4.** Retinal responses by a neuron in the basal optic nucleus during hyperpolarizing current pulses that evoked an $I_h$ current. A: 20 superimposed traces of retinal response. B: superimposed voltage traces showing individual responses to 8 steps of injected current. C: superimposed voltage traces showing membrane response to one 800-ms step of injected current and a set of individual retinal responses at different latencies incremented by 1 ms. D: 14 superimposed voltage traces of retinal responses to the 1st and last latency. E: average retinal responses at different latencies after onset of injected current pulse. F: graph of measured response amplitudes shown in E ( • ) and computed response amplitudes (○) adjusted for the increased driving force based on the control (▲). G: graph of measured and computed response amplitudes as in F based on the average of 6 neurons in the basal optic nucleus. Note that the adjusted response amplitudes do not change during the $I_h$ current.
FIG. 5. Average of responses of a neuron in the basal optic nucleus, studied in current- or voltage-clamp mode. A: as with the example in Fig. 3, retinal responses during current-clamp mode were attenuated during the initial pretectum (PT) response followed by responses greater than that of control. Membrane potential was adjusted to –30 mV to permit a clear hyperpolarization during pretectal stimulation. B: same as A except that the membrane potential was adjusted so that the same pretectal stimulation no longer evoked hyperpolarization. C: superimposed traces of averaged inhibitory postsynaptic currents (IPSC) and potentials (IPSP) recorded in current- or voltage-clamp modes, respectively (membrane potential = –30 mV). D: response component of traces in C except that the IPSC was inverted. Superimposed on the inverted IPSC are data points from B that plotted retinal surface (RET) response amplitudes adjusted for its driving force. Note that the time course of the graph is well matched to the IPSC.
FIG. 6. Direction-tuning of voltage responses, before and during bicuculline. A: pairs of visual responses to 4 of 12 stimulus directions are shown outside a polar plot in the direction of pattern motion imaged on the contralateral retina. Responses to one-third of a 2nd stimulation follow a 100-ms period of a stationary pattern. On the polar plot, the distance from the origin represents the response amplitude (control: gray ovals; bicuculline: black triangles); 100 μM bicuculline was applied to the brain stem and not to the retinal eyecups. Curved lines indicate a wrapped-normal fit to visual responses to all 12 directions and indicate the preferred direction (straight line from the origin). Note that action potentials are present because the patch pipette solution lacked QX314. B: mean direction-tuning curves of 8 neurons affected by bicuculline. These curves were the average of each neuron’s curve after they were normalized and aligned to the preferred direction of the neuron in A. Left polar plot is based on responses to the full 3-s stimulation, showing an increase in the preferred direction response during bicuculline. Right plot shows a greater responses increase during the initial 200-ms response after pattern motion onset. Note that bicuculline did not change the preferred direction of these neurons.
response while overlapping in time with the membrane shunting.

Interpretation of these experimental findings using simple cellular mechanisms is difficult in this intact complex tissue with mixed responses. Our previous demonstration of attenuation during coincident responses of two RET stimuli was also performed in the presence of bicuculline (Kogo and Ariel 1999), thus showing membrane shunting in the absence of mixed responses. In these experiments, however, electrically evoked responses may be a mixture of excitatory and inhibitory inputs. For example, Fig. 3C shows a small initial depolarization in the PT response and a small delayed hyperpolarization in the RET response. A biphasic nature to these responses has been described previously (Ariel and Kogo 2001) and shown by pharmacological means to be mediated by a combination of AMPA and GABA_A receptors (Kogo et al. 2002). In this study of PT and RET interactions, blockers of excitatory and/or inhibitory neurotransmission could not be present, which leaves open the possibility that the small component of these responses (excitation during PT stimulation or inhibition during RET stimulation) play some role in the observed attenuation.

The small mixed nature of the RET response may explain the late augmentation in the RET response. Perhaps RET alone activated a small amount of inhibitory inputs that are also activated by PT alone. When both stimuli are combined, the shared inhibitory inputs may still be in the refractory period, or GABA receptors may already be desensitized by a preceding PT stimulation. Then, when RET is stimulated later, RET activates less inhibitory components within its mixed response, thus evoking a larger response at those later intervals. While this mechanism may contribute to the late augmentation of the RET response, it is insufficient to account for the entire augmentation which can outlast the PT hyperpolarization. Isolation of these two phenomena, brief attenuation and late augmentation, is important for a better understanding of the basis for this nonlinear interaction.

**Location of synapses on BON membrane**

The interactions between PT and RET responses in the BON were variable. One interpretation is that the spatial arrangements of specific pairs of synapses evoked by specific stimuli on a certain neuron may vary, making the interactions more or less effective. This interpretation is based on the passive membrane properties of neurons in which the site of a synaptic input on a neuron’s dendrite plays a role in the strength of its response measured at the soma (cable model, Rall et al. 1967). In addition, synaptic interactions may be less effective if there are local changes in ion concentrations like Cl^- in a small structure like a dendritic spine (electrodiffusion model, Qian and Sejnowski 1990).

If synaptic interaction was equal for all pairs, it is possible that membrane shunting is due to PT-evoked large Cl^- conductance changes at or near the soma that affects all RET inputs. However, the variability of these experiments suggests that there are different positions of PT synapses along the BON dendrite, but not only on spines. Having some PT synapses onto spines could not account for a lack of RET attenuation because BON neurons lack spines (Martin et al. 2004). PT synapses are more likely on distal dendrites where some can interact with RET synapses. This locus is consistent with the observation that GAD-labeled presynaptic terminals in the BON are most commonly found on small postsynaptic profiles (J. Martin and M. Ariel, unpublished observations).

Unlike the PT-RET pairs, there was no membrane shunting of RET responses during activation of I_h. This current is due to opening of voltage-sensitive channels, which are more abundant in the distal dendritic membrane of mammalian pyramidal cells (Magee 1998; Stuart and Spruston 1998). Blockade of these channels increased the differences between the responses of proximal and distal synapses, suggesting that I_h compensates for a synapse’s location on the dendritic tree and thereby enables more linear summation along the dendrite (Magee 1999). The location of I_h currents in the membrane of BON neurons is unknown, but I_h often had an even larger effect on the membrane potential of BON cells than that of PT inhibition. Because opening these voltage-sensitive channels did not reduce the RET response, its locus in BON membrane may have minimal effect on the retinal inputs.

**Impact on visual processing**

A role for nonlinear interactions in visual processing has been examined in mammalian visual cortex, a site of complex excitatory/inhibitory interactions between many neuronal types (Borg-Graham et al. 1998; Douglas et al. 1988; Ferster and Jagadeesh 1992). Some of these reports did find a large increase in somatic conductance during flashed (Borg-Graham et al. 1998; Monier et al. 2003) or moving (Borg-Graham et al. 1998; Monier et al. 2003) visual stimuli, but a nonlinear reduction in excitatory responses was not observed during inhibitory light stimulation (Ferster and Jagadeesh 1992; but see Anderson et al. 2000).

The BON differs from visual cortex in many respects, being a relay structure with physically separate excitatory and inhibitory inputs that can be stimulated in the absence of much ongoing synaptic activity. These differences make BON neurons easier to study and more likely to exhibit nonlinear interactions. Shunting inhibition in BON is even more interesting because it may relate to actual excitatory/inhibitory interactions that occur during natural visual stimuli (Fig. 6; see Ariel and Kogo 2001). Recordings from neurons whose membrane potentials at rest were near the GABA_A reversal potential had their visual responses increased during bicuculline. Although this finding is consistent with shunting, a minor concern is that the visual stimulation itself raised the membrane potential above that GABA_A reversal potential so perhaps some of the increase was not due to shunting. The magnitude of the increase is also complicated by the presence of increased spike activity. Measurements made on the same voltage traces that were low-pass filtered to attenuate the spikes yielded comparable results, indicating that the contribution of spikes on response area (measured as mV \cdot s above the baseline) was minimal. Thus the visual response increase near the GABA_A reversal potential suggests a physiological role for shunting inhibition.

The turtle BON, like homologous structures in nuclei of the vertebrate accessory optic system, plays an essential role in mediating optokinetic head and eye movements (Ariel and Rosenberg 1991; Fite et al. 1979). BON neurons respond best to large patterns drifting slowly in a specific preferred direction...
Neurons in the pretectum give similar responses (Fan et al. 1995) yet have a different distribution of their preferred directions. Both nuclei appear to mediate reflexes that stabilize the retinal image (Simpson 1984). In fact, both anatomical and physiological evidence has been reported for interactions between these two nuclei in many species. 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). Electrical stimulation and lidocaine injections of the pigeon pretectum indicate mixed excitation and inhibition (Nogueira and Britto 1991; Wang et al. 2000). Commisural and/or reciprocal connections between the pretectum and the accessory optic system may mediate such effects (pigeon, Baldo and Britto 1990; frog, Lazar et al. 1989; rat, Schmidt et al. 1998). Similar connections between the turtle BON and PT may add a dynamic feedback component to the system that complicates the interpretation of the cellular responses shown above.

Because the connections between PT and BON are inhibitory, one might expect that this circuit would act as a push-pull system to enhance the detection of visual motion. On the contrary, the preferred directions of excitatory and inhibitory inputs to the BON were found to be similar (Ariel and Kogo 2001), indicating that excitatory and inhibitory synaptic currents were flowing during the same visual stimuli. The use of these coincident competing signals is not yet understood, but this study indicates that nonlinear interactions may occur between synaptic currents through excitatory AMPA and inhibitory GABA$_A$ receptors. The significance of this nonlinear process for the control of oculomotor reflexes remains to be investigated.

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