Interactions of Inhibition and Excitation in the Light-Evoked Currents of X Type Retinal Ganglion Cells

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Cohen, Ethan D. Interactions of inhibition and excitation in the light-evoked currents of X type retinal ganglion cells. J. Neurophysiol. 80: 2975–2990, 1998. The excitatory and inhibitory conductances driving the light-evoked currents (LECs) of cat and ferret ON- and OFF-center X ganglion cells were examined in sliced and isolated retina preparations using center spot stimulation in tetrodotoxin (TTX)-containing Ringer. ON-center X ganglion cells showed an increase in an excitatory conductance reversed positive to +20 mV during the spot stimulus. At spot offset, a transient inhibitory conductance was activated on many cells that reversed near \( E_{Cl} \). OFF-center X ganglion cells showed increases in a sustained inhibitory conductance that reversed near \( E_{Cl} \) during spot stimulation. At spot offset, an excitatory conductance was activated that reversed positive to +20 mV. The light-evoked current kinetics of ON- and OFF-center X cells to spot stimulation did not significantly differ in form from their Y cell counterparts in TTX Ringer. When inhibition was blocked, current-voltage relations of the light-evoked excitatory postsynaptic currents (EPSCs) of both ON- and OFF-X cells were L-shaped and reversed near 0 mV. The EPSCs averaged between 300 and 500 pA at -80 mV. The metabotropic glutamate receptor agonist 2-amino-4-phosphonobutyric acid (APB), was used to block ON-center bipolar cell function. The LECs of ON-X ganglion cells were totally blocked in APB at all holding potentials. APB caused prominent reductions in the dark holding current and synaptic noise of ON-X cells. In contrast, the LECs of OFF-X ganglion cells remained in APB. An increase in the dark holding current was observed. The excitatory amino acid receptor antagonist combination of d-amino-5-phosphono-pentanoic acid (d-AP5) and 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo-(F)-quinoxalinedione (NBQX) was used to block ionotropic glutamate receptor retinal neurotransmission. The LECs of all ON-X ganglion cells were totally blocked, and their holding currents were reduced similar to the actions of APB. For OFF-X ganglion cells, the antagonist combination always blocked the excitatory current at light-OFF; however, in many cells, the inhibitory current at light-ON remained. ON-center X ganglion cells receive active excitation during center illumination, and a transient inhibition at light-OFF. In contrast OFF-center X ganglion cells experience a sustained active inhibition during center illumination, and a shorter increase in excitation at light-offset. Cone bipolar cells provide a resting level of glutamate release on X ganglion cells on which their light-evoked currents are superimposed.

INTRODUCTION

The retina of mammals contain two common physiological types of ganglion cells termed ON- and OFF-center ‘‘X’’ cells, known to correspond to the anatomic classification termed ‘‘β’’ (Boycott and Wassele 1974; Henderson 1985; Vitek et al. 1985; Wassele and Boycott 1991). The receptive-field physiology of X cells are associated with ‘‘sustained’’ light responses, linear spatial summation, and narrow receptive-field centers. X ganglion cell types receive extensive amounts of cone bipolar and amacrine cell synaptic input (Cohen and Sterling 1991, 1992; Freed and Sterling 1988; McGuire et al. 1986). Thus X cells make good candidate neurons to study the interactions of these inputs in the formation of the classical ganglion cell receptive-field center response.

Ganglion cells receive both excitatory and inhibitory synaptic inputs. Inputs from bipolar cells are thought to release the excitatory amino acid glutamate (Tachibana and Okada 1991), and anatomic studies reveal most bipolar cells to be glutamate immunoreactive (Marc et al. 1990). Inputs from amacrine cells are thought to contain inhibitory neurotransmitters such as γ-aminobutyric acid (GABA) or glycine (Marc 1986; Pourcho 1980). The light-evoked excitatory synaptic currents to ganglion cells have been studied in a series of reports in tiger salamander (Diamond and Copenhagen 1993; Mittman et al. 1990), whereas less is known about the currents generating light-evoked inhibition. In addition, inhibition can operate both directly at the ganglion cell and also presynaptically at the bipolar cell level (Famiglietti and Kolb 1975; McGuire et al. 1984, 1986). How excitation and inhibition interact to generate the light-evoked currents of ON- and OFF-center ganglion cells in mammals has never been examined.

Several different models of ganglion cell function have been proposed. Early studies suggested that ganglion cell excitation largely follows that of the presynaptic bipolar cell inputs (Famiglietti et al. 1977; Frumkes et al. 1979; Wunk and Werblin 1979). More recent studies have shown that inhibitory in addition to excitatory processes shape the light-evoked responses of ON- and OFF-center ganglion cells (Belgum et al. 1982, 1987). The cellular identities of these inhibitory processes are unknown at present, with some authors proposing a bipolar cell origin (Sterling 1983), and others suggesting amacrine cells may be involved (Belgum et al. 1987). Anatomic evidence for both amacrine and bipolar cells containing the inhibitory neurotransmitters GABA and glycine have been presented (Chun and Wassele 1989; Jojich and Pourcho 1996; Pourcho 1980; Vardi 1995; Yang and Yazulla 1994). Recent physiological studies have shown that a heterogeneous set of processes exists, particularly on OFF-center ganglion cells (Arkin and Miller 1987; Belgum et al. 1987). However, none of these studies have examined light-evoked conductances on identified ganglion cell types.

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whose synaptic circuitry is known, such as those found in mammalian systems.

The results of this study indicate that cat and ferret X ganglion cells receive a resting release of glutamate by bipolar cells on top of which the bipolar light-evoked glutamate release is superimposed. This resting release accounts for the high spontaneous discharge rates of X cells. In addition, ON-center X ganglion cells receive active excitation during spot illumination, and a transient inhibition at light-OFF. In contrast most OFF-center X ganglion cells experience a sustained active inhibition during center spot stimulation, and excitation at spot-offset. For ON-center cells, both light-evoked excitation and inhibition are dependant on ON-center cone bipolar input, while the mechanism for OFF-cells is more complex.

METHODS

The eyes of cats and ferrets were obtained using university approved protocols. Adult and young cats were used for these experiments. Sixteen adult cats (age >1 yr) were used in the experiments using pentobarbital sodium anesthesia (25 mg/kg). The remaining 70% of the feline experiments used kittens or young cats (averaging 74 days of age) whose eyes were typically obtained from a series of physiology experiments using halothane anesthesia. The development of the receptive-field surrounds of cat ganglion cells is mature by 4–5 wk of age (Rusoff and Dubin 1977), thus cats younger than 35 days were avoided. Ferret eyes were obtained from animals 2–4 mo of age, anesthetized with intraperitoneal drugs were purchased from Sigma (St. Louis, MO). All other drugs were purchased from P-L Biochemicals (La Jolla, CA), or Tocris Cookson (St. Louis, MO). ATP was purchased from P-L Biochemicals (Milwaukee, WI). All other drugs were purchased from Sigma (St. Louis, MO).

Recording

Patch electrodes were drawn from borosilicate glass (1.5 mm OD, 0.86 mm ID) on a Brown Flaming P-87 electrode puller (Sutter Instruments, San Rafael, CA). Pipettes typically had resistances in the bath of 3±6 GΩ. Cells were voltage clamped using a DAGON 3900A amplifier and held at a holding potential of −80 mV unless noted otherwise (Minneapolis, MN) and PCLAMP6 software (Axon Instruments, Foster City, CA). Recordings were Bessel filtered at 2 or 10 kHz (voltage, current clamp, respectively) and were stored digitally on VCR tape at 18.5 kHz using an Instrutech VR10b data recorder (Great Neck, NY). Data analysis was performed off-line using ORIGIN software (Microcal, Northampton, MA). The seal resistance before rupture was typically around 10 GΩ. On formation of a gigohm seal with the patch electrode, the light-evoked response of the ganglion cell was examined and characterized as ON- or OFF-center from the extracelluar spike currents observed during seal formation. On rupture of the patch, voltage-clamp charging currents to a 5-mA calibration pulse were compensated using the DAGON 3911A whole cell expander controls. The settings for series resistance compensation, and cell capacitance were noted for each cell, and series resistance compensation was used for all whole cell recordings (typically 40–60%, fast setting). Estimated series resistances were 8–20 MΩ. Thus for the largest light-evoked currents (LEC) at −80 mV, a 1-nA current would generate an uncompensated holding potential error of at most 12 mV. Cat and ferret ganglion cells typically displayed a large delayed rectifier current activated at holding potentials
LIGHT-EVOKED CURRENTS OF X GANGLION CELLS

TABLE 1. Composition of internal ringers

<table>
<thead>
<tr>
<th>Ringer</th>
<th>A</th>
<th>B</th>
<th>C</th>
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</thead>
<tbody>
<tr>
<td>KCH₃SO₄</td>
<td>120</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CsCH₃SO₄</td>
<td>0</td>
<td>120</td>
<td>0</td>
</tr>
<tr>
<td>KCl</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>MgCl₂</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>HEPES</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>EGTA</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>CaCl₂</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>TEA-Cl</td>
<td>0</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>TBA-Cl</td>
<td>0</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>4-AP</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

Values are in mM. All above Ringers contained 1 mM glutathione, 1 mM ATP, and 0.5 mM GTP, and 0.05% Lucifer yellow (Li⁺ or methoxycarbonyl salt), pH 7.2. HEPES, N/2-hydroxypiperazine-N’-2-ethanesulfonic acid; EGTA, ethylene glycol-bis(β-aminooxyethyl ether)-N,N,N’,N’’-tetraacetic acid; TEA-Cl, tetraethylammonium chloride; TBA-Cl, tert-butyrammonium chloride; 4-AP, 4-aminopyridine.

above −20 mV, making our early measurements of reversal potentials difficult using simple Cs⁺-tetraethylammonium (TEA)–based electrodes. A combination of the potent quaternary amine, tert-butyrammonium chloride (TBA) (French and Shoukimas 1981) and Cs⁺ proved effective at reducing current in this case. The standard recording pipette (internal) solutions were methanesulfonate based and contained low concentrations of Cl⁻ to distinguish inhibitory postsynaptic currents (IPSCs) from excitatory postsynaptic currents (EPSCs). The calculated EC₅₀ of the internal solutions was −67 to −70 mV (Table 1). The reference electrode was a AgCl wire or agar bridge connected to the bath located out of the light path. Holding potentials were corrected for liquid junction potentials, −10 mV (Barry 1994; Lide 1997; Neher 1992). After recording, the Lucifer yellow–filled cell was examined with epifluorescence illumination, and the position and branching pattern of the stained cell’s dendritic arborization was observed with transmitted illumination in the slice to determine where the dendrites were positioned relative to the borders of the inner plexiform layer (IPL) (see Cohen et al. 1994). This information was used to classify the ganglion cells as either ON- or OFF-center and a or β morphology (Nelson et al. 1978) (see Fig. 1, C and D). For cells in the isolated retina, the response polarities were verified in some cases by application of L-APB to the bath (see text) and on recovery of the neurobiotin-stained cell. The stained cells were then photographed at x130 using a Zeiss 35mm camera adapter and Olympus SC35 SLR camera (Olympus Instruments, Lake Success, NY).

Light-evoked current-voltage (I-V) relations were examined in a series of 20-mV steps, between −100 and +40 mV and were leak subtracted. I-V relations were initially performed in the presence of tetrodotoxin-containing Ringer (TTX Ringer) to separate ON- and OFF-center inward current mechanisms. For isolation of the excitatory currents, picrotoxin (120 μM, 40–80 μM in a few early experiments) and strychnine (1–2 μM) were added to the bath Ringer (PST Ringer). The average holding current during the 100 ms before onset/offset of the stimulus was subtracted from the light-evoked response components, depending on the polarity of the ganglion cell receptive-field center. For displaying cumulative data on a sample of ganglion cells, normalized I-V curves were employed (see Mittman et al. 1990 for details). The light-evoked conductance of an individual cell was estimated by linearly fitting the I-V curve slope at all holding potentials ±0 mV. This conductance value was then used for normalizing the individual I-V curves of single cells before averaging. The magnitude of the current at each holding potential reflects the average value of the conductance estimate for all cells in the sample. The addition of picrotoxin and strychnine to the Ringer (PST) distorted the LECs of some OFF-center ganglion cells in the slice and isolated retina preparations. On these cells, the EPSC at spot-off could in some cases be delayed by ≈200 ms.

I-V relations of the LECs of normally responding Y ganglion cells showed a large static leak component reversing near their resting potential. LECs at holding potentials significantly away from the resting potential were not used due to poor space clamp. This may be partially due to Y cell coupling. When recorded in the isolated retina, Y cells often showed neurobiotin staining of their neighbors (n = 6 cells) (see also Mastronarde 1983; Vaney 1991).

Membrane properties of X cells

Using K⁺-based electrodes (internal solution A, Table 1), a series of charging curves were performed on ganglion cells in slices or the isolated retina at −70 or −80 mV in current clamp. Series resistance steps and electrode capacitative charging transients (typically <1 ms) were subtracted out using the DAGAN bridge balance circuitry. Curves were fit to a single exponential. Curves were tested in HEPES Ringer containing the following synaptic blockers: 5 μM NBQX, 100 μM D-amino-5-phosphono-pentanoic acid (d-APV), 120 μM picrotoxin, 1 μM strychnine, and 200 mM TTX. The average input resistance of peripheral X cells was low (299 ± 165 MΩ, mean ± SD), similar to values commonly reported in the literature for many CNS spiking cells (see Jonas et al. 1993; Spruston et al. 1994, for examples). The membrane time constant of a sample of seven X ganglion cells in retinal slices in HEPES buffer averaged 7.8 ± 1.6 ms. These values were also observed in ganglion cells where a normal light-evoked response was evident, using current- or voltage-clamp recording.

Light stimuli

The retina was viewed for whole cell recording using an upright Zeiss Axioskop microscope (Thornwood, NY) equipped with a ×40 fluor water immersion lens, and a high numerical aperture condenser whose iris was fixed in position. Retinal slices and isolated retina were manipulated and viewed under infrared illumination or dim red light (Corning long pass Filter No. 2030; 1% transmission at 650 nm or equivalent), as cat cones λmax peak at mid-spectral and short wavelengths (Daw and Pearlman 1970). In addition, the exposure duration was limited by a foot pedal–controlled shutter. On recording from a ganglion cell, white light spots, optimally enlarged and positioned to fit the receptive-field center, were used to stimulate the ganglion cell. A 1-s duration spot was used as the test stimulus (0.7 s in a few early experiments), which was repeated every 10 s. The light stimuli were controlled by precision timing generators (WPI, Mod 302-1) driving electromagnetic shutters (models VS-25 or VS-6, Vincent Associates, Rochester, NY). Experiments used either a shutter placed in the optical path of the microscope illuminator to stimulate the slice or a Maxwellian view optical system imaged on the slice through the microscope condenser. The light was attenuated by neutral density filters placed in the optical path of the microscope. The unattenuated illumination at the slice was ~150 lux. The light stimuli used in these experiments were of high intensity and were designed to activate both rod and cone bipolar pathways to ganglion cells. Cat rods are unable to follow flickering stimuli >9 Hz (Nelson 1985). Using retinal slices, three ganglion cells were tested with near saturating spot stimuli (1 = 0, or −1) square wave modulated at a rate of 10 Hz. All three cells showed large current components able to follow the flickering stimulus, indicating that cone pathways were active in the slice preparation. In most experiments in the isolated retina, a dim background light was added in the optical path of the microscope or projected on the preparation.
FIG. 1. Examples of light-evoked response properties and morphology of ganglion cells recorded in the isolated retina and slice preparation. **A**: light-evoked spiking of a whole cell recorded cat ON-X cell from the isolated retina. **Top trace**: capacitative spiking currents of the ganglion cell recorded in the cell-attached clamp mode before patch rupture at −80 mV. Spot stimulation causes a sustained increase in the firing rate of this cell. **Middle trace**: whole cell recording of the ganglion cell on patch rupture. Resting potential: −59 mV. **Bottom trace**: light stimulus (Internal A, spot size 1,000 μm, I = −1.0). **B**: voltage-gated currents of an ON-X cat ganglion cell recorded in the slice preparation. Stepping the cell to more positive holding potentials elicited a fast inward Na⁺ current, and a sustained outward delayed rectifier K⁺ current was activated. Holding potential: −70 mV. **Inset**: 20-mV command potential protocol. Hyperpolarizing pulses to −90 mV activate no large voltage-gated conductances (Internal solution A). **C**: neurobiotin-filled OFF-X (β) ganglion cell in tangential view recorded in the isolated retina. Note the characteristic bushy dendritic tree and medium sized cell body. **D**: examples of ON (left) and OFF (right) ferret β ganglion cells recorded in the retinal slice preparation at the same magnification. IPL, inner plexiform layer; INL, inner nuclear layer; GCL, ganglion cell layer.

(∼0.01 lux) as noted in RESULTS. However, no differences in the pharmacology of the responses were noted under these conditions.

**RESULTS**

**Physiology-anatomic correlations**

Figure 1 shows examples of the firing patterns and morphologies of cat and ferret X ganglion cells whole cell recorded in the isolated retina and slice preparations. Figure 1A, **top panel**, shows the light-evoked firing patterns of a cat ON-X cell in the cell-attached mode in voltage clamp. Most cat X cells showed prominent spontaneous firing rates. Upon rupture of the patch, and going to the whole cell configuration in current clamp, a similar firing pattern was observed (bottom panel). The light-evoked depolarizations of cat ganglion cells were often severely limited by voltage-gated currents (see also Diamond and Copenhagen 1995). Center spot stimulation elicited a transient increase in action potentials at spot onset, followed by a more sustained firing pattern that lasted for the duration of the spot stimulus. At light-offset a prominent transient hyperpolarization was commonly observed on ON-X cells. With potassium electrodes, corrected resting potentials for ON- and OFF-X cells recorded in current clamp mode averaged −60 ± 9.0 mV (n = 9 cells), and −61 ± 4 mV (n = 5 cells), respectively. An example of the voltage-gated currents of a cat ON-X cell recorded in the retinal slice preparation is shown in Fig. 1B. In voltage-clamp mode, cat and ferret X cells showed a fast inward current due to sodium activated at voltage steps above −50 mV. This inward current was blocked in TTX-containing Ringer. At holding potentials positive to −20 mV, a series of sustained outward potassium currents was activated (see also Kaneda and Kaneko 1991; Skaliora et al. 1993).

In the isolated retina preparation, a series of 79 cat and 5 ferret ganglion cells were recorded of both X and Y types. After recording, the morphology of the cell was examined
with epifluorescence microscopy and stained using avidin-HRP histochemistry (see METHODS). Figure 1C shows an example of a stained cat X ganglion cell recorded with a neurobiotin-filled patch electrode. The narrow bushy dendritic morphology termed “β” corresponding to the X-type physiology can be seen in a neurobiotin-filled cell in tangential view from the isolated cat retina. In addition Y/α ganglion cells could also be whole cell recorded and stained (compare with Cohen et al. 1994).

In the retinal slice preparation, the light-evoked responses of 99 X and Y ganglion cells were whole cell recorded, and their morphologies were examined with the use of epifluorescence microscopy. Most of the slice recordings (86%) were from cat ganglion cells; however, a limited number of ferret cells (12) were also recorded. Examples of ferret X cells recorded in retinal slices are shown in Fig. 1D. The morphology of ferret X ganglion cells did not noticeably differ from their X cell counterparts, with the exception of their soma sizes being somewhat smaller (Henderson 1985; Stryer and Zahs 1983; Vitek et al. 1985; cf. Fig. 1 of Cohen et al. 1994).

**Light-evoked current kinetics: effects of stimulus intensity**

To correlate the ganglion cell’s receptive-field center response polarity with the LECs recorded in the whole cell configuration, the light-evoked firing patterns of cat ganglion cells were examined in the cell-attached mode. An example of the spiking pattern and corresponding LEC of a cat ON-X cell is shown in Fig. 2A. At spot onset in the cell-attached configuration, this cell showed a rapid increase in spiking, followed by a lower sustained spiking rate for the duration of the spot stimulus. Upon rupture of the patch and going whole cell, the kinetics of the corresponding synaptic current showed a rapid inward transient and a smaller sustained component. Figure 2B shows intensity-response currents for an ON-X ganglion cell over 2 log units of stimulus intensity. For ON-center cells in Ames Ringer, the inward currents to center spot stimulation were associated with small graded sustained currents near stimulus threshold that increased in amplitude with stimulus intensity. At higher stimulus intensities near response saturation, a prominent initial inward transient current appeared ~10–20 ms in width. For ON-X cells, the transient currents could also be seen in TTX Ringer and also in PST Ringer, where inhibition was removed. At high spot intensities, the sustained inward current components were always smaller than the initial transient component, and decayed with time constants of 160–210 ms for a 1-s spot stimulus. By spot offset, the LECs of ON-X cells to bright spot stimulation declined to only 16.6 ± 10.4% of the peak value (n = 9, ON-X cells). Thus the LECs generating sustained spiking in ON-X ganglion cells are composed of inward currents with large transient and smaller sustained components.

The LEC kinetics of cat OFF-center X cells showed a different pattern to center spot stimulation as shown in Fig. 2, C and D. Upon rupture of the patch and going whole cell, the LEC at spot-on was a small outward current, whereas at spot offset a large inward current was observed. In current-clamp mode, this LEC at spot-on was reflected as a hyperpolarization of the OFF-X ganglion cell (data not shown). In voltage clamp, the synaptic currents evoked at light-on and light-off often had different polarities as shown in the intensity-response current relations of Fig. 2D. Higher intensity stimuli evoked a larger sustained outward current during the spot stimulus, and a larger rapidly inactivating inward current at spot offset. This seemed to suggest that the two synaptic currents were generated by largely different ionic conductances.

Because it was found that the light-evoked center current mechanisms were virtually identical between the slice preparation and the isolated retina, results in the slice and isolated retina have been pooled throughout this paper. The magnitudes of these currents have been compared between the two cat preparations in Table 2. For most ON- and OFF-ganglion cell types examined, their LECs were similar in magnitude. Ferret X cells were also similar in size to their cat counterparts (Table 2).

**Comparison X and Y ganglion cell light-evoked currents**

Figure 3 shows examples of the LECs of cat ON- and OFF-X (sustained) cells and their Y (transient) ganglion cell counterparts recorded in the isolated retina in the presence of TTX Ringer. Several different response mechanisms have been proposed to confer a transient response to light in Y ganglion cells. These have included transient presynaptic inputs, sustained inhibition, and voltage-dependent conductances among others (Kaneda and Kaneko 1991; Maguire et al. 1989; Mobbs et al. 1992). Although transiently responding Y ganglion cells might be expected to display a transient excitatory postsynaptic potential to a spot stimulus, the light-evoked EPSCs of ON-Y (n = 9 cells) and ON-X cells were similar in form in TTX Ringer, with the minor exception that the current noise seemed larger on the X ganglion cell types. The lower current noise observed in these largest of cat ganglion cells reflects their lower membrane resistances observed when compared with X cells (see METHODS) (Mastronarde 1983; Vaney 1991). Note the LECs of both Y (phasic) and X (sustained) ganglion cell classes were both composed of an initial transient inward current, followed by more sustained components (Fig. 3, A and C). OFF-Y ganglion cells (n = 8 cells) showed a similar correspondence in form to their OFF-X counterparts (Fig. 3, B and D). These results may reflect the commonality of many ON- and OFF-center cone bipolar inputs to these two different ganglion cell types (see Cohen and Sterling 1992; Freed and Sterling 1988; Kolb and Nelson 1993; McGuire et al. 1986). Examples of the LECs of ferret ON- and OFF-X ganglion cells are shown in the bottom insets of the figures.

**Light-evoked inhibitory currents in OFF-X ganglion cells**

In the presence of TTX Ringer, a series of I-V relations were performed on the light-evoked center currents to help determine the reversal potentials of the synaptic currents driving the receptive-field centers of ON- and OFF-center X ganglion cells (Fig. 4). Substantial light-evoked inhibitory currents were often revealed particularly for OFF-center X cells (Fig. 4B). During spot stimulation, using internal solutions containing low [Cl−] (Table 1), most OFF-center X cells dis-
FIG. 2. Comparison of the light-evoked spiking and synaptic currents of ON- and OFF-center X cat ganglion cells recorded from the isolated retina in Ames Ringer to center spot stimulation (bar). Holding potential, -80 mV. Aa: light-evoked spiking of an ON-X ganglion cell in the cell-attached mode. Ab: light-evoked currents (LECs) of the same ON-X cell, whole cell recorded on patch rupture. An inward current is observed during the spot stimulus (spot diam. 2.000 μm, I = -1, Internal A). B: intensity response series of LECs of an ON-X cell to spots of increasing intensity in the light-adapted condition I = -2, -1.5, -1.0, -0.5, -0.0 log units attenuation. Note at low-stimulus intensities, the response was sustained in nature, with many spikelike current transients (arrow), whereas high-intensity stimuli evoke a prominent initial transient current (arrow) (spot diam. 1.100 μm, Internal B). Ca: light-evoked spiking of an OFF-X ganglion cell in the cell-attached mode. Cb: LECs of the same OFF-X cell, whole cell recorded on patch rupture. The corresponding synaptic current shows a small outward current activated during spot stimulation (note reduced current noise), whereas at spot-offset a large rapid inward current is evoked that decays to the baseline (spot diam. 900 μm, I = -0.5, Internal B). D: intensity-response series of LECs to spot stimulation of another OFF-X cell, whole cell recorded. Note during spot stimulation a sustained outward current appears on this cell, whereas at spot offset, a more transient inward current was observed. Increasing spot intensities evoke larger outward currents at light-ON, and larger inward currents at light-OFF (spot diam. 700 μm, I = -1, 0.5, 0.0 Intensities, Internal A).

played an increase in a current reversing at hyperpolarized holding potentials. This active synaptic inhibition at light-ON was present in nearly all (85%) of the OFF-X cells examined (n = 11/13 cells). The inhibitory currents (IPSC) on OFF-center ganglion cells could be seen in either the slice or the isolated retina preparation. In TTX Ringer, the inhibitory conductance was sustained in nature and lasted for the duration of the spot stimulus. When compared at the midpoint of spot

TABLE 2. Light-evoked current magnitudes of cat and ferret X and Y ganglion cells in the isolated and sliced retinal preparations, at a holding potential of -80 mV.

<table>
<thead>
<tr>
<th>Preparation, Ringer</th>
<th>ON-X</th>
<th>ON-Y</th>
<th>OFF-X</th>
<th>OFF-Y</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cat isolated, TTX</td>
<td>789 ± 584 (17)</td>
<td>392 ± 197 (5)</td>
<td>244 ± 144 (10)</td>
<td>362 ± 177 (6)</td>
</tr>
<tr>
<td>Cat slice, TTX</td>
<td>274 ± 229 (8)*</td>
<td>352 ± 160 (5)</td>
<td>257 ± 227 (7)</td>
<td>450 ± 239 (5)</td>
</tr>
<tr>
<td>Cat slice, PST</td>
<td>486 ± 382 (12)</td>
<td>394 ± 562 (9)</td>
<td>296 ± 218 (8)†</td>
<td>334 ± 339 (4)†</td>
</tr>
<tr>
<td>Ferret (All), TTX</td>
<td>561 ± 512 (7)</td>
<td></td>
<td></td>
<td>349 ± 450 (4)</td>
</tr>
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</table>

Values are the average currents as means ± SD expressed in pA. TTX, tetrodotoxin; PST, picrotoxin-strychnine tetrodotoxin. * Some measurements determined in Ames Ringer alone. † Current measurements for this cell type were slightly distorted in PST Ringer.
stimulation to the dark current, the reversal potential of the inhibitory conductance for OFF-X cells averaged $-68.2 \pm 13.7$ mV ($n = 7$ cells) near the calculated $E_{Cl}$ of the internal solutions ($K^+$ or Cs$^+$ based; Fig. 4B). At light-offset a second more positive reversing conductance was observed, which decayed to the dark baseline with time. Occasionally, one could find ganglion cells where one component of the response was lost, lending support to the notion that the two components were from different cellular sources. At holding potentials near the resting potential, OFF-Y cells also showed an outward component at light-ON, and an inward component at light-OFF; however, due to the low membrane resistances of these cells (see METHODS), reversal of the inhibition was not observed. The inhibition during spot onset could be removed from the OFF-X cells by the addition of the GABA receptor antagonist picrotoxin and the glycine receptor antagonist strychnine to the Ringer (see DISCUSSION, Fig. 7). This result suggests that the active inhibition of OFF-X cells at light-ON may be mediated by amacrine cell(s).

Light-evoked inhibitory currents in ON-X ganglion cells

The LECs of ON-X cells showed a different pattern to spot stimulation. At light-ON, all ON-X cells showed an increase in an inward current that reversed positive to $-20$ mV (see also Freed and Nelson 1994). In contrast at light-OFF, many ON-center X ganglion cells showed activation of an inhibitory current (Fig. 4A). In TTX Ringer, this transient-like inhibition at spot offset decayed within 200–300 ms of the spot stimulus. $I-V$ relations revealed that the inhibition at spot-OFF in ON-X cells appeared to reverse at an average of $-72.3 \pm 12.0$ mV ($n = 7$ cells), similar to the inhibition during light-ON for OFF-X cells. However, the inhibition at light-OFF was seen in only 50% of the ON-X cells whose light-evoked $I-V$ relations were examined in TTX Ringer ($n = 8/16$ cells), and it would often run down. Although all ON-center X ganglion cells showed rapid truncation of the inward current activated during the light at light-offset, not all cells showed activation of an inhibitory current at
light-offset (e.g., Fig. 5B). This suggests that there may also be presynaptically located inhibitory mechanisms truncating the synaptic release by ON-center cone bipolar cells at light-OFF.

Tests of inhibitory processes in X cells: blockade of ON-bipolar input reveals no inhibitory bipolar input at light-OFF in ON-X cells

Several anatomic and physiological studies have suggested that some bipolar cells may release an inhibitory neurotransmitter. Evidence for these in cat retina include the localization of glycine or GABA (either by radioactive uptake or the presence of their synthetic enzymes) to cone bipolar cells (Chun and Wassle 1989; Pourcho 1980; Pourcho and Goebel 1987; Sterling 1983; Vardi and Auerbach 1995). In addition, physiological evidence has found evidence for ON-hyperpolarizing cone and rod bipolars in the ON-sublamina of the IPL contacting ON-center amacrine and ganglion cells (Nelson and Kolb 1983). Although this point has been contested in other species (i.e., Dacheux and Raviola 1986), and APB has been shown to modulate cat ganglion cell firing using extracellular recording (Bolz et al. 1985; Chen and Linsenmier 1989), it has never been directly tested on the LECs of ON- and OFF-center cat X ganglion cells.

To isolate synaptic processes dependent on OFF-center bipolar input, 50 ± 100 μM APB was applied to ON- and OFF-center X ganglion cells in TTX Ringer (n = 26 cells). APB is a type III metabotropic glutamate receptor agonist known to block the light responses of ON-center bipolar cells by mimicking the dark resting release of glutamate (Massey et al. 1983; Nawy and Jahr 1990; Slaughter and Miller 1981; Tian and Slaughter 1995). Most experiments used the L-form of APB. In the presence of APB, only the OFF type of bipolar cell should function in the retina.

The effects of APB were examined on ON-X ganglion cells at holding potentials of either −60 or −80 mV (n = 7 and 11, cells respectively). Figure 5A shows the effects of L-APB on the LECs of an ON-X cell recorded in TTX Ringer at a holding potential of −60 mV. In the control condition, the ganglion cell showed a prominent transient-sustained inward current with transient and sustained components that reverses positive to −20 mV. At spot offset, a 2nd more transient series of currents are evoked (arrowhead) that reverse near $E_C$. Slice preparation (spot diam: 400 μm, I = −0, Internal C). B: LECs of an OFF-X cell held at a series of different holding potentials in the presence of TTX. During the spot stimulus, a sustained inhibitory current (arrowhead) is activated that reverses near $E_C$. At spot offset, an inward current is evoked that reverses positive to −20 mV. Isolated retina (spot diam. 1,000 μm, I = −1, Internal solution A).

FIG. 4. LECs of cat ganglion cells are composed of excitatory and inhibitory conductances arranged in an opposing type of mechanism. A: LECs of an ON-center X cell in TTX Ringer held at a series of different holding potentials (indicated at left). During the spot stimulus (bar), light activates an inward current with transient and sustained components that reverses positive to −20 mV. At spot offset, a 2nd more transient series of currents are evoked (arrowhead) that reverse near $E_C$. Slice preparation (spot diam: 400 μm, I = −0, Internal C). B: LECs of an OFF-X cell held at a series of different holding potentials in the presence of TTX. During the spot stimulus, a sustained inhibitory current (arrowhead) is activated that reverses near $E_C$. At spot offset, an inward current is evoked that reverses positive to −20 mV. Isolated retina (spot diam. 1,000 μm, I = −1, Internal solution A).
result implies that a significant current component reduced in APB was an excitatory current, because reductions in an inhibitory current at −80 mV would appear as an increase in holding current at −60 mV. Furthermore, when synaptic inhibition was blocked in PST Ringer, APB-induced reductions in the dark holding current could also be observed (n =
4 ON-X cells, −80 mV). These results suggest that APB reduced a resting synaptic current due to glutamate release in the dark by ON-center cone bipolar cells.

The LECs of off-center X ganglion cells persisted in the presence of 50–100 μM L-APB in TTX Ringer (n = 8 cells). An example of the effects of APB on the LECs of an OFF-X cell can be seen in Fig. 5C. In contrast to ON-cells, the inward current at light-off was often potentiated in APB. L-APB (50–100 μM) caused the LECs of OFF-X cells to increase an average of 44 ± 41% (n = 6 cells). However, changes were also observed to the inhibitory currents activated at light-on as well. On this cell, the inhibitory current at light-on became more outward (Fig. 5C). I-V relations of three OFF-X cells in APB showed a loss of the inhibitory current activated at light-on (data not shown). In contrast to ON-X cells, the resting holding currents of OFF-X cells were slightly increased in APB, averaging +33 ± 29 pA (n = 5 cells). Thus in OFF-X ganglion cells, the inward current at light-off remained in L-APB.

Blockade of ionotropic glutamate receptor input to OFF-X cells revealed a persistent light-evoked inhibitory current at light-on in some cells

Because OFF-center ganglion cells showed a prominent activation of an inhibitory conductance at spot-on, we examined the effects of blockade of ionotropic excitatory amino acid (EAA) receptor input to ON- and OFF-center ganglion cells in a mixture of the N-methyl-d-aspartate (NMDA) receptor antagonist D-AP5 (100 μM) and the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)/kainate (KA) receptor antagonist NBQX (5 μM). Direct synaptic inhibition of OFF-X ganglion cell dendrites, such as from the inhibitory synapses of the glycinergic AII amacrine cell could be revealed by this experiment (Famiglietti and Kolb 1975) (see Fig. 8).

Excitation of OFF-X ganglion cells occurs when cone photoreceptor release glutamate at light-off, exciting AMPA/KA receptors on OFF-cone bipolar cells (Sasaki and Kaneko 1996; Slaughter and Miller 1983). OFF-Cone bipolar cells in turn release glutamate on NMDA and AMPA/KA EAA receptors on OFF-X ganglion cells, causing excitation (Cohen et al. 1994). Application of the NBQX/D-AP5 mixture should block the OFF-cone bipolar pathway to OFF-X ganglion cells; however, metabotropic receptor–mediated OFF-cone bipolar cell pathways would continue to function. In addition, ON-center AII amacrine cell inhibition would also function. Although ON-center rod bipolar synaptic excitation to AII amacrine cells would be blocked [it uses AMPA/KA receptors (Boos et al. 1993)], depolarization of the AII amacrine by gap junctional coupling with ON-cone bipolar cells would remain operational (Fig. 8) (Famiglietti and Kolb 1975). Thus in the NBQX/D-AP5 mixture, light-on inhibition of OFF-X ganglion cells by AII amacrine cells could persist. Results of this experiment are shown in Fig. 6.

For control purposes, the D-AP5/NBQX antagonist mixture was applied to ON-X ganglion cells. This resulted in their LECs being blocked in all cases (n = 8/8 cells; Fig. 6A). At a holding potential of −80 mV, only 3 ± 3% of the light response remained when the D-AP5/NBQX mixture was added to the TTX Ringer (n = 8 cells). Similar to the actions of L-APB, the resting current in the presence of the D-AP5/NBQX mixture was reduced on all ON-X cells by an average of 235 ± 247 pA (n = 6 cells).

The D-AP5/NBQX antagonist mixture was then applied to OFF-X ganglion cells. As expected, the antagonist mixture blocked the excitatory inward current at light-off on all OFF-X cells examined (Fig. 6B). In the antagonist mixture, the light-evoked inward current at light-off averaged only 4 ± 4% of control values (n = 8 cells). The resting current of OFF-X center ganglion cells was also reduced in the NBQX/D-AP5 mixture, although less significantly than for ON-X cells and averaged −69 ± 105 pA.

If OFF-center X ganglion cells, dendrites received a prominent inhibitory synaptic current through the AII amacrine cell synapse, the sustained inhibition at light-on would not be blocked by the D-AP5/NBQX antagonist mixture. Results with this experiment were mixed. In four OFF-X cells this inhibitory current at light-on was largely blocked by the NBQX/D-AP5 antagonist mixture (Fig. 6B). In contrast in another four X cells, the light-evoked inhibition at light-on clearly remained in the antagonist mixture, whereas the light-evoked excitation at light-off was blocked (arrow, Fig. 6C). Further studies will be needed to determine the mechanisms involved in modulating the light-evoked inhibition at light-on in OFF-X cells (see DISCUSSION).

Isolation of excitatory components of the light-evoked EPSC

To isolate the light-evoked EPSCs of X cells, picrotoxin and strychnine were added to the Ringer to block interference from GABAergic and glycinergic inhibitory synaptic currents. These chloride-mediated currents become larger at positive holding potentials due to the asymmetric distribution of chloride across the ganglion cell (see Cohen et al. 1994, Fig. 6). Addition of PST to the Ringer enhanced the LECs of individual ON-center X cells, and in the isolated retina preparation, this increase averaged 68 ± 103% to an average conductance of 6.9 ± 4.6 nS (n = 13 cells). However, PST Ringer caused little change in the shape of the waveform of the LECs for most ON-X cells.

Cat ganglion cells show direct conductances to exogenously applied NMDA and AMPA/KA on their dendrites (Cohen et al. 1994). The LECs of X ganglion cells reversed near 0 mV, typical for an EAA receptor–mediated synapse (Fig. 7). Figure 7A shows the raw current records of an ON-X ganglion cell recorded at a series of different holding potentials in PST Ringer. At positive holding potentials, the light-evoked EPSC was considerably larger in amplitude than the corresponding EPSC at negative potentials, indicative of an L-shaped I-V relation. Both the initial peak and later portions of the LECs showed this L shape, suggestive of a strong NMDA receptor contribution (Ascher and Nowak 1988; Diamond and Copenhagen 1995; Mittman et al. 1990; Nowak et al. 1988). Figure 7B shows the normalized average I-V curves of the initial peak current for eight ON-X cells. At holding potentials of −80 to −100 mV, the mean I-V relation did not decline to zero, suggesting that an AMPA/KA receptor contribution to the light-evoked EPSC exists as well (Diamond and Copenhagen 1995; Mittman et al. 1990). The EAA receptor pharmacology of these LECs...
FIG. 6. Blockade of ionotropic glutamate neurotransmission to X ganglion cells reveals evidence of an inhibitory synaptic current on some off-center cells at light-on. A: ON-center X ganglion cell. Application of the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)/kainate (KA) antagonist 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo-(F)-quinoxalinedione (NBQX) and the N-methyl-D-aspartate (NMDA) antagonist D-amino-5-phosphono-pentanoic acid (D-AP5) blocked all LECs on this ganglion cell. Recovery was observed (right panel). Holding potential −80 mV (spot stimulus: 550 μm diam, I = −0.7, Internal A). B: OFF-X cell. Application of the same antagonists blocked all LECs on some OFF-X cells. When held at holding potentials above and below E_Cl, both the inhibitory current at spot-on and the excitatory current at spot-off on this cell were largely reduced. Spot stimulus: 550 μm diam, I = 1, Internal B). C: in contrast, the light-evoked inhibitory currents in other OFF-X cells prominently remained in D-AP5 (100 μM) and NBQX (5 μM). This may be due to inhibition from AII amacrine cells. Left panel: control OFF-X ganglion cell LEC at holding potentials above and below E_Cl. An inhibitory current is activated at light-on, whereas at light-off an inward excitatory current is seen reversing positive to +40 mV (arrow). Right panel: LECs in the D-AP5/NBQX mixture. The inhibitory current at light-on remains in D-AP5/NBQX, whereas in contrast, the excitatory current at light-off is totally blocked (spot stimulus: I = −1, 700 μm diam, Internal A).

have been studied in a previous report and paper (Cohen 1994; Cohen et al. 1994).

The light-evoked EPSCs of off-center X ganglion cells also reversed near 0 mV in PST Ringer. While the normal light-evoked inhibitory current activated during spot illumination was blocked in PST Ringer, the inward currents at light-off were distorted slightly as shown in the raw EPSCs of an OFF-X cell in Fig. 7C. Note that, in contrast to the ON-X cell, the synchronization of the inward current to light-off was more variable (Fig. 7C, dotted line). Similar to the ON-X cells, the I-V relations of OFF-X cells showed an L shape, suggestive of a prominent NMDA receptor contribution. Like the ON-X cells, their I-V relations did not decline to zero at more negative holding potentials. During both the early peak and later phases of the light-evoked EPSC, the I-V relation continued to be L shaped. Similar I-V relations were seen on all of the light-evoked EPSCs of seven OFF-X cells examined in PST Ringer (Fig. 7D).

DISCUSSION

The main finding of this study is that the LECs of OFF- and ON-center cat X ganglion cells use excitatory and inhibitory conductance mechanisms that tend to operate in opposition.
FIG. 7. I-V relations of the light-evoked excitatory postsynaptic currents (EPSCs) of cat X cells reveal an L-shaped I-V relation in picrotoxin-strychnine Ringer. A: light-evoked I-V relations of an ON-X cell from a retinal slice preparation. Holding potentials are indicated at right. Note in the absence of inhibition, the LECs of the ON-X cell show a combination of a fast transient and slower sustained component. Dotted lines indicate 50 ms averaging region (spot diam 2,000 μm, I = −2.0, Internal B). B: average normalized I-V relations of 8 cat ON-X cells in PST Ringer. A 50-ms average of the peak current at light-on was used (indicated region Fig. 7A). Note that the light-evoked EPSCs are larger at positive potentials, reverse around 0 mV, and display a negative inflection at −20 to −40 mV. These features are suggestive of NMDA receptor function. At more negative holding potentials, the EPSC does not decline to the baseline. C: OFF-X cell, retinal slice preparation. LECs of an OFF-X ganglion cell shows a similar transient-sustained EPSC in PST Ringer. Dotted lines indicate 50-ms averaging region (spot diam. 1,600 μm, I = −1.5, Internal B). D: average normalized I-V relations for 7 OFF-X cells (6 cat, 1 ferret). A prominent L-shaped NMDA-mediated component to the EPSC is also seen. Error bars indicate standard deviation of the mean conductance (see METHODS for details).

Virtually all OFF-center X ganglion cells showed activation of a sustained inhibitory current to receptive-field center stimulation at light-on that appeared to reverse at negative holding potentials. The average reversal potential of the inhibitory current activated at light-on was near the calculated ECl of the internal solution, suggesting that it may be chloride mediated. OFF-X ganglion cells display direct GABA_A and glycine ligand-gated chloride conductances on their dendrites (Cohen et al. 1994). Addition of the GABA and glycine receptor antagonists picrotoxin and strychnine blocked these inhibitory currents at light-on on OFF-X ganglion cells. At light offset, an excitatory current predominated that appeared to reverse near 0 mV, the estimated reversal potential for ionotropic type glutamate receptor-gated channels.

In contrast, ON-X center ganglion cells showed a large transient-sustained inward excitatory current at light-on that reversed near 0 mV, whereas a only a transient inhibition was observed at light-off, which appeared to reverse near −70 mV. Yet roughly one-half of the ON-X cells examined lacked any evidence of strong light-evoked inhibition at light-off in TTX Ringer. The LECs of these ON-X cells were also rapidly terminated at light-offset (see Figs. 2A and 5B). This result may suggest that presynaptic inhibition of glutamate release, operating at the bipolar cell level, may also be important for modulating the light-evoked LECs of ON-X ganglion cells.

Previous physiological studies of sustained ganglion cell LECs in amphibians have proposed several different models of how light-evoked inhibition and excitation interact to generate the light response. Early studies showed that the light-evoked depolarizations of ON- and OFF-center sustained ganglion cells basically followed their presynaptic bipolar excitatory inputs, and that inhibition was largely transient in nature (Frumkes et al. 1981; Wunk and Werblin 1979). Later studies revealed that, in addition to the excitatory bipolar input on the ganglion cell, inhibition coexisted and operated simultaneously in a cooperative manner generating a “push-pull” like mechanism (Belgum et al. 1982, 1987). For ON-center ganglion cells, light would activate excitatory synaptic inputs, while synaptic inhibition was concurrently withdrawn. Darkness activated inhibitory synaptic inputs, while synaptic excitation was concurrently withdrawn. A similar set of mechanisms would operate on OFF-center ganglion cells. Darkness would cause excitation with concurrent removal of inhibition, while light would activate inhibition with concurrent removal of excitation. Anatomic studies have provided evidence of inhibitory neurotransmitters in some bipolar cells, in addition to numerous amacrine cell...
types (Chun and Wassele 1989; Pourcho 1980; Yang and Yazulla 1994) that could be the source(s) of the inhibition.

The current study, based on the LECs of identified X ganglion cell types in mammals shows a more heterogeneous picture of the interaction of excitation and inhibition, as shown in Fig. 8. ON-Center X ganglion cells receive an excitatory current from ON-center cone bipolar input at light-ON. However, at light-OFF, a transient inhibitory current could be activated in some cells. However, application of L-APB in TTX Ringer completely eliminated all LECs to center spot stimulation for all ON-center ganglion cells, and little inhibitory current remained at light-OFF. Thus for the ON-X ganglion cell, both excitatory and inhibitory currents seem dependant on ON-center cone bipolar input. We did not find any evidence of inhibitory currents activated at light-OFF in APB, such as might be generated by the OFF-center CB6 cone bipolar reported by Nelson and Kolb (1983), which is anatomically known to contact ON-X ganglion cells in sublaminas b of the IPL (Cohen and Sterling 1992; McGuire et al. 1986; Nelson and Kolb 1983). This result is similar to previous physiological studies with APB using extracellular recording in mammals (Chen and Linsenmier 1989; Cohen and Miller 1994; Massey et al. 1983; Muller et al. 1988), but in anurans, responses to light-OFF often appear on ON-center ganglion cells in APB (Arkin and Miller 1987). How inhibition at light-OFF is transmitted to ON-X ganglion cells is unclear, but could involve OFF-center spiking amacrine cells.

Experiments using APB suggest that ON-X ganglion cells receive a substantial resting release of glutamate in the dark. Application of APB reduced the resting current noise normally found in ON-X cells at holding potentials above $E_C$ (e.g., Figs. 4B and 5B), where chloride-mediated current noise would be expected to increase. This suggests that APB must also reduce some resting inhibitory conductances as well. The presence of a normal resting release of glutamate onto ganglion cells, particularly the ON-X ganglion cell, has important implications on glutamate receptor desensitization (Lukasiewicz et al. 1995) and models of ganglion cell excitation and inhibition will be unaffected by the NBQX/D-AP5 antagonist mixture (see also Cook et al. 1998). Its function may be impaired in TTX-containing Ringer.

The interaction of excitation and inhibition in OFF-center ganglion cells shows a more complicated picture. Excitation at light-OFF in OFF-X cells is primarily due to input from excitatory OFF-center cone bipolar cells. Anatomic evidence suggests that two cone bipolar types contact OFF-X ganglion cell dendrites (Kolb 1979; McGuire et al. 1986; Kolb and Nelson 1993). Addition of the NBQX/D-AP5 EAA receptor antagonist mixture to block ionotropic receptor input to OFF-X ganglion cells in all cases, blocked the light-evoked excitatory currents at light-OFF. In addition, the inhibitory current at light-ON was also blocked in four cases. This would fit with a model similar to the ON-X ganglion cell, where both amacrine-mediated inhibition and excitation are dependent on glutamate release by the bipolar cell. However, in the remaining four cases, OFF-center X ganglion cell synaptic inhibition at light-ON was not blocked in the NBQX/D-AP5 antagonist mixture (see Figs. 4B and 6C). This result could be due to direct inhibition of ganglion cell dendrites by OFF-center AII amacrine cells. The lobular appendages of the glycinergic AII amacrine are known to contact OFF-center ganglion cell dendrites (Famiglietti and Kolb 1975; McGuire et al. 1986; Kolb and Nelson 1993). Because the AII amacrine is coupled to ON-center cone bipolars, this light-evoked inhibition will be unaffected by the NBQX/D-AP5 antago-
nist mixture. In addition, the influence of this pathway could depend on the adaptation level of the retina, because all amacrine cell function is poor under light-adapted conditions (Bloomfield et al. 1997; Dacheux and Raviola 1986; Nelson 1982). This could potentially explain the lack of residual inhibition observed in the d-AP5 and NBQX antagonist blockade on some OFF-X ganglion cells. However, the AII amacrine provides only about one-sixth of the amacrine input to OFF-X ganglion cells (Kolb and Nelson 1993). It is likely that several amacrine cells also provide inhibitory input to OFF-X ganglion cells, particularly under light-adapted conditions. Further study will be needed to determine whether the inhibition of OFF-X ganglion cells at light-on is due solely to amacrine-mediated mechanisms or the existence of additional inhibitory cone bipolar inputs in the OFF-sublamina of the IPL (Pourcho 1980; Vardi and Auerbach 1995; but see Euler et al. 1996).

Comparison of the LEC waveforms of sustained firing X ganglion cells and their transiently firing Y counterparts were similar in form in TTX Ringer. Both X and Y cells can show transient and sustained LEC components. These components probably reflect the commonality of many cone bipolar inputs to ON- and OFF-X and Y ganglion cells (Freed and Sterling 1988; McGuire et al. 1986; Nelson and Kolb 1993; Saito 1981, 1983). In addition, glutamate receptor desensitization could potentially play a role in transient generation. Although intense stimuli tend to enhance the transient in the LEC of X cells, dimmer stimuli elicit more sustained LECs (e.g., Fig. 2B). The form of the light response for ON-X cells, with transient and sustained components resembles that seen by Boos et al. (1993) in rat AII amacrine cells and a few neonatal ganglion cells whose responses did not terminate with the light stimulus (Rorig and Grantyn 1993). How the LEC of Y cells generate their transient spiking responses to light is unclear. While voltage-dependent K+ channels might play a role in generating these response properties (Mobbs et al. 1992), cellular coupling is likely to play a role by reducing their effective membrane resistance (Mastronarde 1983; Vaney 1991), thereby enhancing the transient LEC component’s firing contributions.

The magnitudes of the LECs of X and Y ganglion cells are surprisingly large compared with their ON- and OFF ganglion cell counterparts in anurans (i.e., Diamond and Copenhagen 1995; Mittman et al. 1991). Assuming a reversal potential of 0 mV, these conductance values ranged from 3 to 5 nS for most cell types in the isolated and sliced retina preparations, whereas ON-X cells recorded in the isolated retina in TTX Ringer gave a higher value, averaging 9.8 nS (Table 2). Given that the number of synapses to a ganglion cell increases with retinal eccentricity (Kier et al. 1995), this may account for some of the variability in size of conductances observed. Previous sharp electrode-based estimates by Freed and Nelson (1994) of the light-evoked conductance of ON-X cells pooled from eccentricities of 1–21°, have given a conductance value similar to that found here for ON-X cells in the slice preparation. However, they also found no differences in the mean conductance of the transient and sustained portions of the light response. Further studies will need to examine how the size of the LECs of X ganglion cells scale with retinal eccentricity, and their relationship with voltage-gated currents.

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