Functional circuitry for peripheral suppression in mammalian Y-type retinal ganglion cells.

Kareem A. Zaghloul\textsuperscript{1}, Michael B. Manookin\textsuperscript{3}, Bart G. Borghuis\textsuperscript{1}, Kwabena Boahen\textsuperscript{2} and Jonathan B. Demb\textsuperscript{1,3,4,5}

Departments of Neuroscience\textsuperscript{1} and Bioengineering\textsuperscript{2}
University of Pennsylvania, Philadelphia, Pennsylvania 19104

Neuroscience Program\textsuperscript{3}, Depts. of Ophthalmology & Visual Sciences\textsuperscript{4} and Molecular, Cellular & Developmental Biology\textsuperscript{5}
University of Michigan, Ann Arbor, Michigan, 48105

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Contact information:
Jonathan Demb, Ph.D.
University of Michigan
Kellogg Eye Center
1000 Wall Street
Ann Arbor, Michigan 48105
734-647-7469 (tel)
734-936-7231 (fax)
jdemb@umich.edu
Abstract

A retinal ganglion cell receptive field comprises an excitatory center and an inhibitory surround. The surround has two components: one driven by horizontal cells at the first synaptic layer, and one driven by amacrine cells at the second synaptic layer. Here, we characterized how amacrine cells inhibit the center response of ON- and OFF-center Y-type ganglion cells in the in vitro guinea pig retina. A high spatial frequency grating (4-5 cyc mm\(^{-1}\)), beyond the spatial resolution of horizontal cells, drifted in the ganglion cell receptive field periphery to stimulate amacrine cells. The peripheral grating suppressed the ganglion cell spiking response to a central spot. Suppression of spiking was strongest and observed most consistently in OFF cells. In intracellular recordings, the grating suppressed the subthreshold membrane potential in two ways: a reduced slope (gain) of the stimulus-response curve by \(\sim 20-30\%\) and, in OFF cells, a tonic \(\sim 1\) mV hyperpolarization. In voltage-clamp, the grating increased an inhibitory conductance in all cells and simultaneously decreased an excitatory conductance in OFF cells. To determine if center response inhibition was presynaptic or postsynaptic (shunting), we measured center response gain under voltage-clamp and current-clamp conditions. Under both conditions, the peripheral grating reduced center response gain similarly. This result suggests that reduced gain in the ganglion cell subthreshold center response reflects inhibition of presynaptic bipolar terminals. Thus, amacrine cells suppressed ganglion cell center response gain primarily by inhibiting bipolar cell glutamate release.
Introduction

The retina represents a model system for understanding how neural circuitry
generates the receptive field properties of sensory neurons. Ganglion cells are the output
neuron of the retina and have been well characterized at the level of extracellular
recording. A receptive field typically features a center region, which can be excited by
an effective stimulus (Kuffler, 1953; Enroth-Cugell and Robson, 1966). For an ON-
center cell, the effective stimulus is a brightening over the center, whereas for an OFF-
center cell, the effective stimulus is a dimming over the center. Under cone-driven
conditions, the receptive field center primarily corresponds to a feed-forward pathway
from cones to bipolar cells to the ganglion cell (Figure 1) (Masland, 2003; Sterling and
Demb, 2004; Wässle, 2004). The ganglion cell center shows an approximately Gaussian
spatial profile, which arises from the dome-like distribution of bipolar synapses onto the
ganglion cell dendritic tree (Kier et al., 1995).

The receptive field center response is suppressed by stimulation of the surround
(Kuffler, 1953) (Figure 1). In the classical description, the surround represents a broad
region extending across and beyond the center (Rodieck, 1965; Enroth-Cugell and
Robson, 1966). This classical surround exhibits poor spatial resolution and therefore
senses low spatial frequencies, including broad-field stimuli and low spatial-frequency
gratings. In addition to the classical surround, the ganglion cell center can be suppressed
by high spatial frequency contrast patterns in the peripheral receptive field. This
‘peripheral suppression’ can be distinguished from the classical surround based on spatial
resolution. For example, the spiking response to a central spot can be suppressed by
peripheral contrast, such as high spatial-frequency drifting gratings (~1 cyc deg⁻¹ or ~4-5
cyc mm⁻¹ on the retina), that would not stimulate the classical surround (Caldwell and
Daw, 1978a, 1978b; Shapley and Victor, 1979; Enroth-Cugell and Jakiela, 1980;
Lankheet et al., 1992; Solomon et al., 2006). Peripheral suppression is not equally strong
in all cell types but is particularly prominent in the ‘brisk-transient’ cell types, which
include the parasol/Magnocellular-pathway cells in primates and alpha/Y-type cells in
other mammals (Caldwell and Daw, 1978a, 1978b; Shapley and Victor, 1979; Enroth-
Cugell and Jakiela, 1980; Solomon et al., 2006).
Surround mechanisms are created at both synaptic layers in the retina (Figure 1). At the outer plexiform layer, horizontal cells inhibit cones and bipolar cells (Figure 1) (Lankheet et al., 1992; Mangel, 1991; McMahon et al., 2004; Duebel et al., 2006). Horizontal cells are electrically coupled to one another in a syncytium (Baldrige et al., 1998). Thus, the horizontal cell network has requisite properties to convey the classical surround mechanism, sensitive to low spatial frequency stimuli. At the inner plexiform layer, amacrine cells inhibit bipolar cell synaptic terminals and ganglion cell dendrites (Figure 1). Amacrine cells are driven by bipolar cells, which themselves have relatively narrow receptive fields that would yield sensitivity to high spatial frequencies (Dacey et al., 2000). Furthermore, a nonlinearity at the bipolar cell synapse creates a ‘subunit’ structure in postsynaptic cells and extends sensitivity to high spatial frequencies (Hochstein and Shapley, 1976; Demb et al., 2001). Thus an amacrine cell mechanism for surround inhibition would be sensitive to both low and high spatial frequencies (Cook et al., 1998; Cook and McReynolds; Demb et al., 1999; Taylor, 1999; Flores-Herr et al., 2001).

Here, we asked how amacrine cell inhibition acts to suppress the center response of a ganglion cell. To this end, we presented high spatial frequencies in the ganglion cell’s receptive field periphery to selectively stimulate amacrine pathways. Direct measurements showed that such frequencies only minimally stimulate horizontal cells. Therefore, the suppressive effects measured in the ganglion cell must be driven by amacrine circuitry. An amacrine cell’s inhibitory signal can reach the ganglion cell either directly, through a synapse on the dendrite, or indirectly, through a synapse onto a presynaptic bipolar terminal (Cook and McReynolds, 1998; Cook et al., 1998; Taylor, 1999; Flores-Herr et al., 2001; Shields and Lukasiewicz, 2003). Here, we tested the relative contributions of these two synaptic sites and determined their individual roles in the suppression of the excitatory center response of the ganglion cell.
Materials and Methods.

Tissue preparation

For each experimental session, a retinal whole-mount (flattened eye cup) was prepared from a Hartley guinea pig (200-800 g). Procedures were in accordance with University of Pennsylvania, University of Michigan and NIH guidelines. In some cases, an animal was anesthetized with ketamine (100 mg kg\(^{-1}\)), xylazine (20 mg kg\(^{-1}\)) and pentobarbital (150 mg kg\(^{-1}\)), and both eyes were removed following which the animal was killed by anesthetic overdose. In other cases, an animal was anesthetized with ketamine (40 mg kg\(^{-1}\)) and xylazine (4 mg kg\(^{-1}\)) and decapitated, and then both eyes were removed. Each eye was hemisected and the vitreous was peeled off in one piece. Five slits were cut, so that the eye-cup would lay flat. The flattened eye-cup, which included the neural retina attached to the pigment epithelium, choroid, and sclera, was mounted on a piece of circular filter paper and then mounted flat in a chamber on a microscope stage. The filter paper was held in place by a teflon ring that fit tightly in the chamber walls. The tissue was superfused (~4-6 ml/min) with oxygenated (95% O\(_2\), 5% CO\(_2\)) Ames medium (Sigma, St. Louis, MO) at 32-36\(^\circ\) C (Demb et al., 1999). In some experiments, glucose was added to the medium (0.8-3.6 g/L); this increased osmolarity by ~2-6%, and did not have noticeable effects on the recordings.

Electrophysiology

For intracellular recordings of ganglion cells, Acridine orange (0.001%; Molecular Probes, Eugene, OR) was added to the superfusate, allowing ganglion cell somas to be identified by fluorescence during brief exposure to UV light. A soma in the visual streak was penetrated with a glass electrode (tip resistance 80-200 M\(\Omega\)), filled with 1% pyranine (Molecular Probes) in 2M potassium acetate. Voltage was recorded with an intracellular amplifier (NeuroData, IR-283, NeuroData Instruments Corp., Delaware Water Gap, PA) and digitized at 5 kHz using AxoScope software (Axon Instruments, Foster City, CA). For horizontal cells, recordings were obtained with glass electrodes (impedance 80-200 M\(\Omega\)) back-filled with Alexa 488 or 568 and neurobiotin (5%) in 1.5 M potassium acetate. Membrane potential was amplified (NeuroData, IR-283), sampled at 2 kHz and digitized by a computer for online analysis and storage (Apple Macintosh;
10-bit AD board; custom built software). Resting potential was recorded upon cell penetration and continuously monitored during the recording. Following the recording, horizontal cells were injected with the dye and fixed for confocal microscopy (see below).

For extracellular (loose-patch) and whole-cell recordings from ganglion cells, we identified large somas visualized with a cooled CCD camera (Retiga 1300C, QCapture software; Qimaging Corporation, Burnaby, British Columbia) (Hu et al., 2000). Positive pressure was applied through a patch electrode (3-6 MΩ), filled with Ames medium, and this electrode was used to ‘burrow’ through the inner limiting membrane and clean the area surrounding the targeted cell (Roska and Werblin, 2001). The same electrode was used to form a loose seal onto the targeted cell (~30-100 MΩ) and record spikes under voltage clamp (Vhold = 0 mV). This electrode was then withdrawn, and a second electrode (3-6 MΩ) filled with intracellular solution was used to obtain a >1 GΩ seal. The patch was ruptured and recordings were made in the whole-cell configuration.

Intracellular solution for current-clamp recordings contained (in mM): 140 K methylsulfate, 8 NaCl, 10 HEPES, 0.1 EGTA, 2 ATP-Mg, 0.3 GTP-Na₂, adjusted to pH 7.3. For voltage-clamp recordings, QX-314-Br was added (5 mM), to block sodium channels and improve space clamp, and NaCl was reduced to 3 mM. The calculated reversal for inhibitory responses (E_{GABA/glycine}) would be approximately -73 mV for the first solution. For the second solution, containing Br⁻, E_{GABA/glycine} should be more depolarized given that GABA/glycine channels are more permeable to Br⁻ than Cl⁻ by ~20% (Bormann et al., 1987; Robertson, 1989). In calculations below, we assumed that with Br⁻ in the pipette, E_{GABA/glycine} was approximately -68 mV. Junction potential (-9 mV) was corrected in all cases.

For voltage-clamp recordings, the holding potential was compensated for the voltage drop across the electrode tip, based on the following equation: V_{h,corr} = V_{h} - (I_{leak} R_s (1 - R_{s,correct})), where V_{h} is the apparent holding potential before the stimulus (in mV), I_{leak} is the leak current (in nA), R_s is the series resistance (typically 12-30 MΩ), and R_{s,correct} is the series resistance compensation (typically between 0.25 and 0.50). Holding potentials were typically restricted to a range negative to -30 mV; positive to -30, the cells showed a large outward current, which resembled a delayed-rectifier potassium
current. Signals were recorded with a MultiClamp 700A amplifier and digitized at 10 kHz using pClamp 9.0 software (Axon Instruments).

Programs were written in Matlab (Mathworks, Natick, MA) to analyze responses (down-sampled to 1 kHz) separately in the spike rate and subthreshold membrane potential, as described previously (Demb et al., 2001a; Zaghloul et al., 2003, 2005). To exclude the spikes from the voltage response, we down-sampled the raw data (recorded at 5 or 10 kHz) by taking the median value of every 5 or 10 sample points; this had the effect of removing spikes but preserving the subthreshold waveform. In some cells, we instead used a linear interpolation method, where we ‘clipped out’ each spike with a line from 1 msec before to 1-2 msec after each spike (Zaghloul et al., 2003).

Data are reported as mean ± sem. Statistical significance was assessed using one-tailed t-tests.

**Horizontal cell morphology**

After recording horizontal cells, the retina was fixed in 4% paraformaldehyde for 20 minutes. Cells were visualized either based on the Alexa dye staining, or by reacting for Neurobiotin by the following procedure: incubate with blocking buffer (10% normal goat serum, 5% Triton-X in 5% sodium phosphate buffer, 1 hour); react with streptavidin-fluorescein (8 mg/ml) or streptavidin-Cy5 (40 mg/ml) for 3 hours; wash with 5% sodium phosphate buffer (3 x 10 min); mount on a slide using Vectashield (Vector Laboratories, Burlingame, CA). Cells were imaged using a confocal microscope (Leica, Nussloch, Germany; 40X objective, n.a. 1.25).

**Visual stimulus**

The stimulus was displayed on a miniature monochrome computer monitor (Lucivid MR1-103, Microbrightfield, Colchester, VT) projected through a microscope port and through a 2.5X or 4X objective focused on the photoreceptors. The monitor had a vertical refresh of 60 Hz and a spatial resolution of 640 x 480. The stimulus was confined to the central 480 x 480 pixels which, when projected onto the retina, subtended 3 x 3 or 3.7 x 3.7 mm. The mean luminance was \( \sim 10^3 - 10^4 \) isomerizations per middle-wavelength sensitive cone or rod per second, which is within the mesopic range (Yin et al.,
2006); in two cases, a lower mean luminance was used (~$10^2$ isomerizations per cone or rod per second). The relationship between gun voltage and monitor intensity was linearized in software with a lookup table. Stimuli were programmed in Matlab as described previously (Brainard, 1997; Pelli, 1997; Demb et al., 1999).

All ganglion cell experiments used a dynamic modulation of a low contrast spot (diameter, 0.5 mm) centered on the cell body and therefore approximately centered on both the dendritic tree and the ~0.5 – 0.7 mm diameter receptive field center (Demb et al., 2001a, 2001b; Dhingra et al., 2003). The spot will necessarily stimulate both the classical center and the surround, since these overlap in space (Enroth-Cugell and Robson, 1966). However, the purpose of the spot was to stimulate the bipolar cells that synapse onto the ganglion cell dendritic tree, and for this purpose the spot, even if centering was off by ~0.1 mm, would be adequate to stimulate most of these bipolar cells.

The spot intensity was updated at 60 Hz with values drawn randomly from a Gaussian distribution. This stimulus approximates “white-noise” (Marmarelis and Marmarelis, 1978; Sakai and Naka, 1987; Figure 2). The stimulus distribution is presented in contrast units, where the intensity has been normalized by subtracting the mean luminance and then dividing by the mean luminance. Thus, the stimulus had a mean of zero and a range of -1 to +1. Stimulus contrast is defined by the Gaussian SD, which was always 0.10. The stimulus lasted 240 seconds and included 12 20-second periods: 10 seconds of the spot (spot alone) alternating with 10 seconds of the spot plus a drifting grating (spot + grating). The grating was presented in the periphery, excluded from a 1-mm patch centered on the cell body. The grating had a square-wave profile with a spatial frequency of 4.3 or 5.0 cycles mm$^{-1}$ (bar width = 116 or 100 µm), a temporal frequency of 2 Hz, and a contrast of 1.0. The analysis was performed on data collected during the last 8 seconds of each ten-second half-period. Thus, for each contrast, there was 8 x 12 = 96 seconds of data. This relatively short stimulus increased the probability of highly stable intracellular recordings and provided data with sufficient signal-to-noise for the analysis. Due to the alternating contrast half-periods, any instabilities during the recording should be distributed equally between the two half-
periods. Horizontal cell recordings used drifting sine-wave gratings (2 Hz, 0.7 contrast, 0.05 – 6 cyc mm\(^{-1}\)) presented over a field of 2.4 x 3.2 mm.

**Analysis: linear-nonlinear model**

We analyzed both subthreshold and spiking responses using a linear-nonlinear (LN) cascade analysis (Chichilnisky, 2001; Carandini et al., 2005). In this analysis, a linear filter represents the impulse response function of a cell, or the theoretical response to a brief light flash (Figure 2). This filter, plotted in reverse, represents the cells ‘weighting function.’ The linear prediction of the response (i.e., the linear model) is calculated at a given point in time by multiplying the stimulus by the weighting function and summing the result (i.e., convolution; Carandini et al., 2005). To compute the L filter, \( f(t) \), we cross-correlated the stimulus and the response (Wiener, 1958; Lee and Schetzen, 1965; Sakai et al., 1995; Chichilnisky, 2001). To generate the L model of response \( r_L(t) \), we convolved (*) the stimulus \( s(t) \) and the L filter:

\[
r_L(t) = f(t) * s(t).
\]

Finally, the L model is passed through a nonlinear input-output function to generate a LN model of the response (Figure 2):

\[
r_{LN}(t) = N(r_L(t)).
\]

The N function accounts for rectification (e.g., the spike threshold) and saturation in the response (Victor, 1987; Sakai et al., 1995; Chichilnisky, 2001; Kim and Rieke, 2001; Baccus and Meister, 2002; Zaghloul et al., 2003).

To generate the N function, we plotted the L model versus the actual response (analyzed after down-sampling to 1 kHz) and binned the data (~1000 samples/bin). These binned points represent the average response output (in spikes sec\(^{-1}\), mV or pA) at each level of the L model value (in arbitrary ‘input’ units). For the simultaneous fitting procedure described below, we required a descriptive function fitted through the points of the N function. A Gaussian cumulative distribution function (cdf) provides a good fit to the spike N function (Chander and Chichilnisky, 2001; Zaghloul et al., 2003, 2005):

\[
f(x) = \alpha C(\beta x + \gamma),
\]

where \( C() \) is the cumulative normal density, \( x \) is the input value, and the parameters roughly correspond to the maximum response (\( \alpha \)), the response gain (\( \beta \)) and the spike
threshold (\(\gamma\)) (Chichilnisky, 2001; Chander and Chichilnisky, 2001). The fit was performed using standard routines in Matlab that minimize the mean-squared error (MSE) between the data and the fitted line (Figure 2). For the membrane N points, we fit the data with the function:

\[ f(x) = \alpha C(\beta x + \gamma) + \delta, \]

where the additional parameter \(\delta\) allows for a vertical offset (because the membrane N function goes negative). As we found previously, the Gaussian cdf provided a good fit for the membrane potential N function for ON cells but not for OFF cells (Zaghloul et al., 2003). Thus, for OFF cells we fit the positive and negative sides of the membrane N function separately (Zaghloul et al., 2003, 2005).

Analysis: simultaneous fit of spike responses to the spot alone and spot + grating conditions.

There is a free scale-factor in the LN model: the y-axis of the filter and the x-axis of the nonlinear function can be scaled by the same amount without changing the output of the model (Chander and Chichilnisky, 2001; Kim and Rieke, 2001; Zaghloul et al., 2005). Therefore, to compare between the two conditions, we initially multiplied each filter by a factor, so that the variance of the two linear models was equal (Baccus and Meister, 2002). We then scaled the nonlinearities by the same factors (i.e., a stretch along the x-axis) so that each LN model output remained unchanged. After normalizing in this way, the two filters showed only slight differences in kinetics, but there was a large difference between the N functions: the peripheral grating typically reduced the spike rate, which resulted in an apparent rightward shift of the N function.

We considered three models to explain the effect of the peripheral grating on the N stage of the model. First, we considered a ‘gain change model’ in which the two curves were fit with the same Gaussian cdf, except for a scaling along the x-axis. Specifically, both curves were fit with the same \(\alpha\) and \(\gamma\) parameters, but unique \(\beta\) parameters (i.e., total of four parameters). This model was used previously to account for the reduced gain evoked by an increase in the contrast of the spot itself (Chander and Chichilnisky, 2001; Kim and Rieke, 2001; Zaghloul et al., 2005; Beaudoin et al., 2007). However, based on a preliminary analysis of OFF cells, it was apparent that this model
failed in a systematic way: an underestimation of the small amplitude responses to the spot alone and an overestimation to the small amplitude responses in the presence of the grating (see below). Therefore, we considered a ‘threshold change model’ in which the two curves were fit with the same Gaussian cdf except for a horizontal shift along the x-axis. Specifically, both curves were fit with the same $\alpha$ and $\beta$ parameters, but unique $\gamma$ parameters (i.e., total of four parameters). Finally, we considered a ‘combined model’ that allowed for both a gain change and a threshold change. Specifically, both curves were fit with the same $\alpha$ parameters, but unique and $\beta$ and $\gamma$ parameters (i.e., total of five parameters).

The gain change and threshold change models have the same form and equal number of parameters so we could directly compare the mean-squared error (MSE) of these models to determine which provided a better fit to the data. The combined model has an additional parameter and so we expect it to yield a lower MSE. However, the combined model allowed us to test whether the gain change or threshold change dominated in explaining the difference between the two contrast curves when both gain and threshold were allowed to vary.

Analysis: simultaneous fit of subthreshold responses to the spot alone and spot + grating conditions.

For membrane potential or current responses, we fit both low and high contrast N functions with the Gaussian cdf plus offset, mentioned above. The low and high contrast functions were fit with the same $\alpha$ and $\gamma$ parameters but unique $\beta$ and $\delta$ parameters. Thus, the fitted N functions were similar to those for the spike N functions, except that membrane potential N functions were allowed to have unique offsets (i.e., unique y-intercepts because of the unique $\delta$’s). The unique offsets were necessary, because in some cases the y-intercept of the membrane N function differed between contrasts by several mV or tens of pA (see below; Baccus and Meister, 2002). Furthermore, the gain change between conditions (change in $\beta$) was independent of the change in the y-intercept. For ON cells, we performed the simultaneous fit with the cdf to the full nonlinear function, whereas for OFF cells, we fit just the excitatory (depolarizing or inward current) side of the nonlinear function (see above; Zaghloul et al., 2003; 2005).
For the subthreshold response, the above gain change model showed good fits, without systematic errors of any sort, and so we did not consider alternative models as we did for the spike response.

**Evaluation of the LN model.**

The LN model is useful to the extent that it provides an accurate representation of a cell’s response. We evaluated model accuracy by building the model based on data collected with one stimulus and then testing the model’s predictive power (explained variance: $r^2$) on the averaged response to a second stimulus. The model-building stimulus was a modulated spot, as described above: 12 20-sec periods (10 sec of spot alone and 10 sec of spot + grating). The model-testing stimulus was a two-sec segment of spot modulation, repeated ten times in a 20-sec period (10 sec of spot alone and 10 sec of spot + grating). The model-testing period (MT) was interspersed six times within the model building periods (MB): 3 MT; 2 MB; 3 MT; 2 MB; 3 MT; 2 MB; 3 MT. In all cases, we only analyzed the last eight seconds of data from each 10-sec half-period. Thus, the LN model was built from 96-sec of data for both spot alone and spot + grating conditions. The response to the two-sec model-testing segment was averaged over 24 repeats for both spot alone and spot + grating conditions. We then tested how well the LN model predicted the averaged response to the two-sec model-testing segment (Figure 2). The model was assessed in this way for 14 cells (11 OFF cells, 3 ON cells) and for both spiking and subthreshold measurements. For spiking responses, we evaluated the model in cases where the firing rate was at least one spike sec$^{-1}$ for both conditions. We evaluated model fits for the threshold change model, which generally yielded better fits than the gain change model (see below).

For spiking responses, the $r^2$ between the LN model and the data was 0.80 ± 0.03 for the spot alone condition and 0.65 ± 0.03 for the spot + grating condition (n = 13). For membrane potential responses, the $r^2$ between the LN model and the data was 0.94 ± 0.01 for the spot alone condition and 0.90 ± 0.01 for the spot + grating condition (n = 11). Thus, the LN model generally gave a better fit for the spot alone condition than for the spot + grating condition, although this difference was most marked for spiking responses; membrane potential responses were very well captured by the LN model in either
condition. The relatively low $r^2$ value for spiking response to the spot + grating condition was likely caused by the lower spike rates in this condition.

Results.

Basic cellular properties.

We targeted Y-type ($\alpha$) ganglion cells, because these cells show significant ‘peripheral suppression’ in vivo, and because we could identify them routinely by their large cell bodies in the visual streak (Caldwell and Daw, 1978a; Enroth-Cugell and Jakiela, 1980; Peichl et al., 1987; Demb et al., 1999; 2001a). We presented a series of stimuli to confirm that the cell had the characteristic properties: a “brisk-transient” center response to a spot (0.5 mm diameter), an antagonistic surround response to an annulus (inner diameter, 0.74; outer diameter, 2.0 mm) and a nonlinear (frequency-doubled) response to a contrast-reversing grating (spatial frequency = 4.3 or 5.0 cycles mm$^{-1}$) (Enroth-Cugell and Robson, 1966; Cleland and Levick, 1974; Hochstein and Shapley, 1976; Demb et al., 2001a; 2001b). In whole-cell recordings, cells had an input resistance of $32 \pm 3 \text{ M\Omega}$ (mean $\pm$ sem; $n = 17$), similar to our previous measurements and similar to Y-types cells in the cat retina (Cohen, 2001; O’Brien et al., 2002; Zaghoul et al., 2003; Manookin and Demb, 2006; Beaudoin et al., 2007).

Here we report on 65 ganglion cells (48 OFF-center, 17 ON-center). As in previous studies, we had a bias towards successful recordings from OFF-center cells (Zaghoul et al., 2003; 2005), which outnumber their ON-center counterparts by about two-fold (B. Borghuis, unpublished observations). We recorded extracellularly from 36 cells (26 OFF-center, 10 ON-center), which, when viewing a gray screen at mean luminance, fired spontaneously at $10 \pm 3$ spikes s$^{-1}$ (range = 0-57). We recorded intracellularly from 32 cells (25 OFF-center, 7 ON-center), which fired spontaneously at $5 \pm 2$ spikes s$^{-1}$ (range = 0-49) and rested at -64.1 $\pm$ 0.9 mV. Ten cells were recorded with ‘sharp’ intracellular electrodes and 22 were recorded with whole-cell patch electrodes. We made additional whole-cell recordings with QX-314 (5 mM) added to the pipette solution to block spiking ($n = 13$ cells). Some cells were recorded by both extracellular and intracellular methods ($n = 23$ cells). Our main criteria for stable
intracellular recordings were a low resting potential and a well-modulated light response to the flickering spot stimulus described below.

Spatial tuning of horizontal cells in the guinea pig retina.

We recorded horizontal cells to assess their spatial sensitivity in the guinea pig retina. Here we report on cells that were successfully filled with a fluorescent dye to identify the type (A-type or B-type; Peichl and Gonzalez-Soriano, 1993). At mean luminance, horizontal cells rested at -47 ± 5 mV (mean ± sem; n = 30). Responses were recorded to sine-wave gratings at a range of spatial frequencies; the response was quantified as the amplitude of the best fitting sine-wave at the 2-Hz drift frequency (F1 amplitude). Maximal response amplitudes were 3.8 ± 0.5 mV (A-type; n = 24) and 3.2 ± 1.1 mV (B-type; n = 6). Both types of horizontal cell showed low-pass spatial sensitivity with half-maximal sensitivities near 0.4 - 0.5 cyc mm⁻¹ (Figure 3D). At 5 cyc mm⁻¹, the response declined to ~4% (HA cells, n = 24) or ~7% (HB cells, n = 6) of the peak response (Figure 3D). In the following experiments, we used spatial frequencies of 4.3 or 5.0 cycles mm⁻¹. At these frequencies, the bar width (~100 microns) should match the putative width of a bipolar cell receptive field, which drive amacrine cells, but is too fine to strongly stimulate horizontal cells (Figure 3) (Werblin, 1972; Lankheet, 1992; Demb 1999, 2001a, b; Dacey et al., 2000; Passaglia et al., 2001).

Peripheral contrast suppresses the ganglion cell spiking response to a central stimulus.

We first quantified the effect of the peripheral grating on the ganglion cell receptive field center as measured in the spike response. To stimulate the cell, a 0.5-mm diameter spot flickered over the center; on each frame of the monitor (16.7 ms) an intensity value was drawn randomly from a Gaussian distribution (see Materials and Methods; Figure 4A). This stimulus approximates ‘white noise,’ with approximately equal stimulus energy over the range of temporal frequencies to which the cell is most sensitive (Zaghloul et al., 2005). In alternate 10-second half-cycles, the spot was presented either alone or in the presence of a grating in the peripheral receptive field (Figure 4A). During the 10-second presentation, the grating drifted at 2 Hz, and thus
maintained a constant contrast signal at each point in the periphery (Enroth-Cugell and Jakiela, 1980).

The central spot, presented alone, caused a series of spike bursts in the ganglion cell, and this spiking was suppressed by the addition of the peripheral grating. Similar results were observed in spiking responses recorded by extracellular and intracellular methods, and so results on spiking, here and below, were combined across recording conditions. We analyzed the effect of the grating on the spike rate over the last 8 seconds of each 10-second half-cycle. For OFF cells, the spike rate reduced from $12.2 \pm 1.3$ to $5.6 \pm 0.8$ spikes sec$^{-1}$, a drop of $6.7 \pm 0.6$ (n = 44 cells, 26 extracellular; p < 0.001). For ON cells, the spike rate reduced from $32 \pm 4$ to $30 \pm 4$ spikes sec$^{-1}$, a drop of $1.8 \pm 1.9$ (n = 16 cells, 10 extracellular; n.s.) (Figure 3C). We further quantified the effect of the grating by testing for a significant reduction in spiking within individual cells. For each cell, we compared the spike rate between the two conditions across the 12 cycles and performed a t-test (two-tailed). For every OFF cell, the grating significantly reduced the firing rate (p < 0.01 for 41 cells; p < 0.05 for 3 cells) (Figure 4B, D). For ON cells, the grating significantly reduced the rate in five cells (p < 0.05), had no significant effect in four (p > 0.05) and significantly increased the rate in seven (p < 0.05; Figure 4B, D); the increased rate in some ON cells could result from inadvertent stimulation of the edge of the receptive field center (see below). Thus, the grating consistently reduced the spike rate in OFF cells but had a more variable effect in ON cells.

**Peripheral contrast causes the linear filter to become more biphasic in OFF-center cells.**

To further quantify the impact of the peripheral grating on the center response, we analyzed the spike train with a linear-nonlinear (LN) analysis (see Materials and Methods; Figure 2). The goal of the LN analysis is to separate changes in the temporal sensitivity of the cell from changes in contrast sensitivity (Chichilnisky, 2001; Carandini et al., 2005). The linear filter indicates the temporal sensitivity of the cell. The nonlinear function shows the relationship between the filtered contrast (i.e., the linear model of the response) and the output of the cell; the slope of this function indicates the contrast sensitivity (see Materials and Methods). For the spike response, we restricted our analysis to those cells that fired at a rate of at least one spike sec$^{-1}$ in both conditions, and cells
that showed a significant reduction in firing rate during the spot + grating condition (n = 40 OFF cells, 5 ON cells).

For OFF cells, the grating caused the linear filter to become more biphasic (Figure 5C). To quantify the change in the shape of the filter, we compared the amplitude of the first phase of the response (i.e., positive response for ON cells; negative response for OFF cells), with the second phase of the response (i.e., negative response for ON cells; positive response for OFF cells). We calculated a biphasic index, which was the second phase amplitude (s2 or sg2, for the spot alone or spot + grating) divided by the first phase amplitude (s1 or sg1). If there were no second phase of the response, the index would be zero, whereas if the second phase amplitude equaled the first phase amplitude, the index would be -1. Most cells, with or without the peripheral grating present, had an index between -0.2 and -1.0 (Figure 5D).

For OFF cells (n = 40 cells), the biphasic index was -0.55 ± 0.02 for the spot alone condition, compared with -0.73 ± 0.03 for the spot + grating condition, a difference of -0.17 ± 0.02 (p < 0.001). For ON cells (n = 5 cells), the biphasic index was -0.91 ± 0.07 for the spot alone condition, compared with -0.86 ± 0.09 for the spot + grating condition, a difference of -0.05 ± 0.04 (n.s.). Thus, the peripheral grating caused the linear filter to become more biphasic in OFF cells but had no significant effect in ON cells. The increased biphasic quality of the OFF cell filter, caused by the peripheral grating, should correspond to a relatively decreased sensitivity to low temporal frequencies.

**Peripheral suppression in OFF cells is best explained by an increased spike threshold.**

We next compared the effect of the grating on the spiking nonlinear function. We considered two models, with an equal number of parameters (four), to describe the suppression of spiking caused by the grating. In the first model, the grating causes a ‘gain change.’ The reduced gain corresponds to a reduced slope of the nonlinear function. We also considered a second model, in which the grating causes an increased threshold for spiking (‘threshold change model’). This increased threshold corresponds to a rightward shift (on a linear axis) in the nonlinear function in the spot + grating condition (see Materials and Methods). For an OFF cell, the *threshold change* model
provided a more satisfactory fit (Figure 5A). We also considered a ‘combined model,’ with an additional parameter (five), in which the two nonlinear functions differed by both their gain (slope) and their threshold (horizontal position along the x-axis).

To quantify the difference between the gain change model and the threshold change model, we fit both models for each cell and compared the difference in mean-squared error (MSE) between each model and the data. For OFF cells (n = 40 cells), the threshold change model yielded lower MSE (7.2 ± 0.8 spikes/sec) compared to the gain change model (11.1 ± 1.4 spikes/sec); the MSE was relatively lower for the threshold change model by 27 ± 4% (p < 0.001). The combined model fit yielded a lower MSE (6.8 ± 0.7 spikes/sec) compared to the models above, as expected based on the additional parameter added in the fit. However, the MSE for the combined model was only slightly lower than that for the threshold change model above. Furthermore, in the combined model fit, the relative gain in the presence of the grating was 0.98 ± 0.02 (i.e., reduction of ~2%), and thus a gain change did not play a major role in describing the suppressed spiking. In the combined model, the threshold change (i.e., rightward shift) was 23.8 ± 1.4 arbitrary (linear model) units, similar to that found using the threshold change model (23.3 ± 1.5 units). Thus, for OFF cells the effect of the grating could be described concisely as an increased spike threshold.

For ON cells (n = 5), the MSE was not significantly lower for the threshold change model (31 ± 8 spikes/sec) compared to the gain change model (57 ± 27 spikes/sec); the threshold change model was lower by 19 ± 14% (n.s.). For the combined model (MSE = 28 ± 7), the peripheral grating caused both a significant gain reduction (0.85 ± 0.03; i.e., reduction of ~15%) and an increased threshold (rightward shift, 19 ± 6 units). Thus, for this subset of ON cells (which showed significantly reduced firing during the grating) the effect of the grating could be described as a combination of an increased spike threshold and a reduced gain.

In the above experiments, the grating was always presented at full contrast (1.0). In six OFF cells, we further tested the effect of the grating at a lower contrast (0.25). Data were analyzed using the threshold change model. For these cells, the low contrast grating caused a rightward shift of the nonlinear function of 8 +/- 2 linear model units at low contrast and 20 +/- 3 units at high contrast. Thus, there was a significant effect of the
grating at low contrast with a significantly greater effect at high contrast (difference of 12 +/- 2 units; p < 0.01). Therefore, at the lower contrast level, the effect of the grating was not saturated.

**Peripheral contrast causes tonic membrane hyperpolarization in OFF cells.**

To understand the mechanism underlying the above effects on spiking, we analyzed the effect of the peripheral grating on the subthreshold membrane potential. In OFF cells, the grating evoked a tonic membrane hyperpolarization (Figure 5). The hyperpolarization was largest at the onset of the peripheral stimulus and slowly declined over the 10-second half-cycle. During the period of analysis (two to 10 seconds after grating onset or offset), the grating hyperpolarized the membrane potential from -64.6 +/- 0.9 mV to -65.7 +/- 0.8 mV, a difference of 1.2 +/- 0.2 mV (n = 25 cells; p < 0.001). At the offset of the peripheral stimulus, the membrane initially depolarized strongly and then this depolarization declined during the half-cycle (Figure 6C).

Individual ON cells showed either a tonic depolarization or hyperpolarization, so that the average effect of the grating on membrane potential was negligible. The membrane potential was -59.7 +/- 2.6 mV during the spot alone and -59.4 +/- 2.5 mV in the presence of the grating (n = 7). ON cells that showed a depolarization during the grating corresponded to those that showed an increased spike rate (see above and Figure 4B); whereas cells that showed a hyperpolarization corresponded to those that showed a decreased spike rate (n = 5; Figure 6C). As discussed below, the depolarization in some ON cells probably arises from unintended stimulation of excitatory bipolar cell synapses at the edge of the ganglion cell’s receptive field center.

**Peripheral contrast reduces the response gain of subthreshold responses.**

In addition to tonic hyperpolarization of the membrane potential, the peripheral grating suppressed the amplitude of response fluctuations to the central spot (Figure 6A). To quantify the nature of this suppression, we performed an LN analysis of the subthreshold response, similar to the analysis described above for the spike rate. Two LN models were fit that allowed, between conditions, a tonic membrane polarization (difference in the y-intercept of the nonlinearity) plus a gain change (difference in the
slope of the nonlinearity; see Materials and Methods). The example OFF cell shows the typical effect: the grating caused both hyperpolarization and a reduced gain (Figure 7A; see also Figure 2). The accompanying spike response is shown with the threshold change model fitted to the data (Figure 7A; see also Figure 2). For ON cells, there were two patterns. In the first pattern, the grating caused a hyperpolarization and reduced gain, similar to the OFF cell (Figure 7B). In the second pattern, the grating caused a depolarization and reduced gain (Figure 7C). The accompanying spike responses for the two ON cells are shown with the combined model fitted to the data (Figure 7B, C). For both patterns, the grating reduced the slope of the spike nonlinearity, but the rightward shift of the spike nonlinearity was only present in the case where the grating evoked hyperpolarization (Figure 7B).

Next, we compared quantitatively the reduced gain during the spot + grating conditions across cells. For every cell recorded intracellularly (n = 25 OFF cells, 7 ON cells), the grating reduced the gain of the subthreshold response. For OFF cells, the gain was reduced significantly below 1.0 to 0.68 ± 0.02 (p < 0.001; i.e., reduction of ~32%). For ON cells, the gain was reduced to 0.78 ± 0.06 (p < 0.001; i.e., reduction of ~22%).

The change in the y-intercept is a measure of membrane polarization, independent of the nonlinearity. The y-intercept showed a hyperpolarization for OFF cells (-1.0 ± 0.2 mV; p < 0.001), similar to the direct analysis of membrane potential above, but no significant change for ON cells (+0.3 ± 0.4 mV). Thus, the reduced gain in the membrane potential response, observed in all cells, was not always accompanied by membrane hyperpolarization. The apparent independence of these two inhibitory effects suggests distinct underlying cellular mechanisms.

To further analyze the relationship between the suppressive effects on spiking and subthreshold responses, we measured three correlations. This analysis was performed on OFF cells, where the effect of the grating on spiking could be described concisely as an increased threshold (see above); the analysis was further restricted to those OFF cells that fired at a rate of at least one spike sec⁻¹ for both conditions (n = 21 cells). There was not a significant correlation between the reduced gain in the subthreshold response and the hyperpolarization (i.e., the change in the y-intercept of the nonlinear function) (Figure 8A). Thus, these two effects on the subthreshold response could arise from different...
cellular mechanisms; we further support this conclusion with analyses below. The rightward shift of the spike response was correlated with both the hyperpolarization ($r = -0.57; p < 0.05$) and the reduced gain in the subthreshold response ($r = -0.79; p < 0.01$) (Figure 8B, C). Thus, the suppression of the spiking response was related to both suppressive effects expressed in the subthreshold potential.

**Peripheral contrast increases ganglion cell membrane conductance.**

We tested whether the hyperpolarization in OFF cells, caused by the peripheral grating, could be explained by a direct inhibitory synapse onto the ganglion cell dendrite. To test this, we made intracellular recordings with QX-314 in the pipette to block spiking and improve the space clamp. We then measured the response to the peripheral grating in voltage clamp at several holding potentials. Every cell showed an increased conductance (i.e., positive slope on the I-V plot) during both the transient and sustained periods of the grating (Figure 9). For OFF cells ($n = 6$), the conductance increased by $8 \pm 2$ nS for the transient response and $1.3 \pm 0.4$ nS for the sustained response. The transient response reversed at $-94 \pm 4$ mV, and the sustained response (2-10 sec after grating onset) reversed at $-97 \pm 18$ mV.

The above reversal potential measurements suggest that, for OFF cells, the grating simultaneously increased an inhibitory conductance ($\Delta g_{GABA/glycine}$) and decreased an excitatory conductance ($\Delta g_{cation}$), which would move the reversal potential for the summed conductance ($\Delta g_{total}$) negative to $E_{GABA/glycine}$. To determine the relative contributions of the two underlying conductances (where the conductances represent changes [$\Delta$] from resting conductances), we used the following formula:

$$a = \frac{(E_{GABA/glycine} - E_{total})}{(E_{total} - E_{cation})},$$

where $a$ is the ratio between the conductances ($\Delta g_{cation}/\Delta g_{GABA/glycine}$), $E_{GABA/glycine} = -68$ mV, $E_{cation} = 0$ mV and $E_{total}$ was the measured reversal potential for the leak-subtracted response to the grating. The above equation follows from Ohm’s law, given that, during the sustained period of the grating, $\Delta i_{cation} = -\Delta i_{GABA/glycine}$. From this equation, we could divide the total conductance into the two underlying conductances (after establishing their relative contributions). This procedure is depicted graphically in Figure 9C. For OFF cells ($n = 6$), the grating evoked an inhibitory conductance of $1.7 \pm 0.4$ nS ($p < 0.05$;
two-tailed t-test) in parallel with a decreased excitatory conductance of 0.43 ± 0.14 nS (p < 0.05; Figure 9D).

For ON cells (n = 4), the conductance increased by 5 ± 3 nS for the transient response and 2.2 ± 0.6 nS for the sustained response. The transient response reversed at -29 ± 1 mV, and the sustained response reversed at -55 ± 6 mV. For the sustained response, the grating evoked an inhibitory conductance of 1.8 ± 0.5 nS (p < 0.05) in parallel with an increased excitatory conductance of 0.41 ± 0.22 nS (n.s.; Figure 9D). Therefore, in all cells the grating evoked an increased inhibitory conductance, consistent with a direct inhibitory synapse on the ganglion cell dendrite. This inhibitory conductance was accompanied either by a decreased excitatory conductance (OFF cells) or a trend towards an increased excitatory conductance (ON cells) that depended on cell type.

Evidence that peripheral contrast reduces the gain of subthreshold ganglion cell center responses by inhibiting presynaptic bipolar cells.

We next tested whether the reduced gain of the subthreshold response could be explained by postsynaptic ‘shunting’ inhibition or rather by presynaptic inhibition of bipolar terminals. To test this, we made intracellular recordings with QX-314 in the pipette, to block spiking and improve space clamp, and then compared recordings of membrane current (I_m; with the holding potential, V_hold, near the resting potential) to recordings of membrane voltage (V_m). Consider first the voltage-clamp condition, where conductances in parallel would add. We considered the total membrane conductance as a sum of three conductances: synaptic conductance driven by the central spot (g_center), synaptic conductance driven by the peripheral grating (g_grating), and a leak term (g_leak), each with an associated reversal potential:

\[ I_m = g_{\text{center}}(V_{\text{hold}} - E_{\text{center}}) + g_{\text{grating}}(V_{\text{hold}} - E_{\text{grating}}) + g_{\text{leak}}(V_{\text{hold}} - E_{\text{leak}}). \]

If the peripheral grating acts purely by post-synaptic inhibition, then its effect would be exclusively on g_grating. During the analysis period (two to 10 seconds after grating onset or offset), we expect g_grating and g_leak to be relatively steady (Figure 9), and we assume all reversal potentials to be constant. Thus an effect on g_grating would evoke a tonic offset in current between the two conditions, with no additional effect on the gain of the center
response, as the modulation amplitude of $g_{\text{center}}$ would remain the same. If instead the peripheral grating acts through inhibition of the presynaptic bipolar terminal, then, in the equation above, the modulation amplitude of $g_{\text{center}}$ itself would change, which should evoke a reduced gain under voltage-clamp similar to that observed under current-clamp. More generally, postsynaptic shunting inhibition, caused by tonic inhibitory synapses, should only be present under current-clamp.

We used the LN model above to compare the effects of the grating on $I_m$ and $V_m$. (Figure 10B, C). Across 13 cells ($n = 9$ OFF cells, 4 ON cells), the reduction in gain was the same in both conditions: the grating reduced the center gain to $0.73 \pm 0.04$ (i.e., ~27% reduction) for $I_m$ and to $0.72 \pm 0.04$ (i.e., 28% reduction) for $V_m$ (Figure 10). The effect was similar for OFF cells ($V_m$, 0.73 ± 0.03; $I_m$ 0.74 ± 0.03) and ON cells ($V_m$, 0.70 ± 0.10; $I_m$ 0.69 ± 0.10). These gain reductions in $V_m$ are consistent with those recorded with standard pipette solution above (~22-32%). The reduced gain was accompanied in OFF cells by a slight hyperpolarization as reflected by a change in the y-intercept of the nonlinearity of $-0.6 \pm 0.5$ mV ($n = 9$). This hyperpolarization was somewhat small and inconsistent across cells, compared to recordings above, perhaps caused by the more depolarized value for $E_{\text{GABA}}/\text{glycine}$ (i.e., with Br$^-$ in the pipette solution) and the accompanying decreased driving force on inhibition (see Materials and Methods). Under voltage-clamp OFF cells showed a small outward current ($16 \pm 13$ pA). For ON cells, there was little change in the y-intercept under either condition (current-clamp: $-0.2 \pm 0.7$ mV; voltage-clamp: 7 ± 28 pA). In general, our main conclusion from the comparison of current-clamp and voltage-clamp recordings is based on the similar reduction in gain across the two conditions. The reduced gain of the ganglion cell center $V_m$ response must be primarily caused by amacrine cell inhibition of presynaptic bipolar cell terminals.

**Discussion.**

Here, we measured the effect of peripheral suppression on the center response of mammalian retinal ganglion cells using intracellular recording. We used a peripheral grating with a spatial frequency that was beyond the resolution of the horizontal cell network in order to selectively stimulate amacrine cells (Figure 3). Our main finding was that the peripheral grating caused two suppressive effects on the ganglion cell
subthreshold membrane potential: a reduced gain of the center response, evident in all cells (Figures 7, 8), and a tonic membrane hyperpolarization, most consistently observed in OFF cells (Figures 5-8). The tonic membrane polarization was consistent with an increased inhibitory conductance at the ganglion cell dendrite in parallel with a decreased (OFF cells) or increased (ON cells) excitatory conductance (Figure 9). For both cell types, the reduced gain of the center response was similar under current-clamp and voltage-clamp conditions, which suggests that this reduced gain reflects an inhibition of presynaptic bipolar terminals (Figure 10).

Circuitry of amacrine-mediated peripheral suppression.

A model explaining the main results is shown in Figure 11. The drifting grating in the periphery stimulates bipolar cells, which each have a nonlinearity at their synaptic output (Enroth-Cugell and Freeman, 1987; Demb et al., 2001a). Each bipolar cell then acts as a ‘nonlinear subunit’ which can increase its release more than it can decrease its release (i.e., rectification). The drifting bars stimulate the subunits asynchronously, which leads to a steady increase in the summed excitatory drive onto the amacrine cell (Enroth-Cugell and Robson, 1966; Hochstein and Shapley, 1976; Demb et al., 2001a; Olveczky et al., 2003). Tonic stimulation of the amacrine cell drives tonic inhibition of the recorded ganglion cell and its presynaptic bipolar cells. The long-range inhibitory signal is carried by a spiking amacrine cell, which fires conventional sodium spikes, blocked by tetrodotoxin (TTX) (Demb et al., 1999, 2001a; Taylor, 1999; Flores-Herr et al., 2001; Olveczky et al., 2003; Roska and Werblin, 2003). These amacrine cells are presumably those that extend axons millimeters across the retina (Vaney et al., 1988; Dacey, 1989; Famiglietti, 1992; Stafford and Dacey, 1997). These ‘long-range’ amacrine cells represent a class of cell that includes several types (Volgyi et al., 2001; Lin and Masland, 2006). The specific types that synapse onto Y-type cells and their presynaptic bipolar terminals are unknown.

The reduced gain of the center response in the subthreshold membrane potential is apparently driven by amacrine cell inhibition of the central bipolar terminals (i.e., those conveying the spot response; Figure 11). The hyperpolarization, shown most consistently in OFF cells, is driven by a combination of direct inhibition of the ganglion cell and
inhibition of tonic glutamate release from presynaptic bipolar terminals (Figures 9, 11). For ON cells, the grating sometimes caused a slight depolarization that could be explained if the edge of the grating sometimes inadvertently caused direct stimulation of bipolar cells at the edge of the receptive field center (Figures 4, 7, 11). This may have occurred more frequently in ON cells because they are relatively larger than OFF cells in guinea pig retina, as found in primate and human parasol cells (Dacey and Petersen, 1992; Chichilnisky and Kalmar, 2002; J. Demb, unpublished observations). This inadvertent center stimulation may have also occurred in a few OFF cells and explain why the grating did not cause a tonic membrane hyperpolarization in all cases (Figure 8B). Despite this variability in tonic membrane polarization, the grating reduced the gain of the center response in the subthreshold membrane potential for every ON and OFF cell studied. This suggests that the peripheral grating always evoked an inhibitory effect on those central-most bipolar terminals that conveyed the spot response.

Our data support two sites of synaptic inhibition driven by long-range amacrine cells: bipolar terminals and ganglion cells. This conclusion is consistent with previous studies in mammalian retina (Taylor, 1999; Flores-Herr et al., 2001; Roska and Werblin, 2003). However, we have added to these previous efforts in four regards. First, we have used a high spatial frequency surround stimulus and show that it does not stimulate horizontal cells (Figure 3). Thus, surround effects studied here can be ascribed specifically to amacrine cells. Second, we have used the LN model to investigate effects of amacrine cell stimulation on the temporal tuning of the center response. Under our conditions, effects on the temporal tuning were minimal. The grating caused a slightly more biphasic filter in OFF cells, which should attenuate low temporal frequencies (Figure 5). Third, we used the LN model to quantify gain changes separately from static nonlinear (i.e., rectifying) influences on membrane voltage or current. Fourth, by directly comparing voltage-clamp and current-clamp recordings in the same cell, we could determine whether shunting of the ganglion cell leads to reduced gain of the center response. The shunting effect was shown to be negligible (Figure 10).

Comparing the circuit for peripheral suppression between mammals and lower vertebrates.
The circuit revealed here in a mammalian retina shows both similarities and
differences to a circuit proposed in lower vertebrate retina (Werblin, 1972; Werblin and
Copenhagen, 1974; Thibos and Werblin, 1978). In mudpuppy, a spinning windmill
stimulus, like the drifting grating stimulus in the present study, was shown to stimulate
amacrine cells but not horizontal cells. The windmill stimulus, presented in the receptive
field periphery, suppressed the spiking response of ganglion cells by causing a membrane
hyperpolarization. However, in several studies the windmill apparently did not suppress
bipolar cells (Werblin, 1972; Werblin and Copenhagen, 1974; Thibos and Werblin,
1978). Thus, in mudpuppy, the amacrine cells appear to act mostly at the ganglion cell
dendrite, whereas we demonstrated here in a mammal an important role for suppression
of the presynaptic bipolar terminals.

The amacrine cells that mediate the windmill-evoked suppression rely on sodium
action potentials to convey signals laterally over long distances (Cook and Werblin,
1994; Cook et al., 1998). Bath-applied TTX blocked suppression in ganglion cells but
not bipolar cells in one study (Cook and McReynolds, 1998; Cook et al., 1998).
However, another study showed that inhibition at salamander bipolar terminals is TTX-
sensitive (Shields and Lukasiewicz, 2003). Thus, there may be a role for presynaptic
inhibition in the salamander circuit for peripheral suppression similar to the presynaptic
mechanism shown here. In ON-OFF ganglion cells, the OFF pathway can be selectively
suppressed during transient shifts of a grating in the surround, consistent with amacrine-
mediated suppression of presynaptic OFF bipolar terminals (Geffen et al., 2007).

Asymmetry between ON and OFF pathways.

Asymmetries exist between the parallel ON and OFF ganglion cell pathways, for
example, in receptive field size and the pattern of excitatory and inhibitory synaptic input
(Chichilnisky and Kalmar, 2002; Pang et al., 2003; Zaghloul et al., 2003; Murphy and
Rieke, 2006; Sagdullaev et al., 2006). Here, we found that, in the presence of the grating,
OFF cells showed a more consistent suppression of their spike rates and a more
consistent membrane hyperpolarization. However, these asymmetries must be
interpreted with some caution. For example, we did not systematically explore multiple
spatial and temporal frequency conditions for the surround grating, and doing so might
reveal conditions that evoke larger suppressive effects in ON cells. Indeed, there were clear cases where ON cells showed suppressive effects, including suppressed spike rates, reduced gain of the subthreshold response and membrane hyperpolarizations (Figures 4-7). We conclude that different amacrine cell pathways interact with ON and OFF Y cells (i.e., there is not a single ON-OFF amacrine pathway that inhibits both ganglion cell circuits). Consistent with this, in the in vivo rabbit retina, picrotoxin (GABA<sub>AC</sub> receptor antagonist) blocked the suppressive effect of a peripheral windmill stimulus for OFF-center Y cells but not ON-center Y cells, which also predicts the involvement of two distinct amacrine cell mechanisms (Caldwell and Daw, 1978b).

**Peripheral suppression throughout the visual system**

Mechanisms for peripheral suppression appear first in the retina but are repeated at several stages of the visual pathway. For example, cells in the lateral geniculate nucleus (LGN) of the thalamus show peripheral suppression (Solomon et al., 2002; Bonin et al., 2005; Webb et al., 2005a). Some of the suppression at the LGN can presumably be explained by a retinal mechanism, but other central mechanisms may also play a role. Peripheral suppression also exists in the primary visual cortex, where its orientation sensitivity, binocular nature and time course suggest an intra-cortical mechanism (DeAngelis et al., 1994; Bair et al., 2003; Webb et al., 2005b; Smith et al., 2006). Further surround effects appear in extrastriate areas (see Allman et al., 1985; Albright and Stoner, 2002). At all stages, the apparent role of peripheral suppression is to create a context in which to interpret the strength of a central stimulus. It will be interesting to learn whether the circuitry for peripheral suppression described here is repeated at other stages.
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References.


Network, 12, 199-213.

Chichilnisky EJ, Kalmar RS (2002). Functional asymmetries in ON and OFF ganglion 

Cleland BG, Levick WR (1974). Brisk and sluggish concentrically organized ganglion 

Cohen ED (2001). Synaptic mechanisms shaping the light-response in retinal ganglion 

Cook PB, Werblin FS (1994). Spike initiation and propagation in wide field transient 

Cook PB, Lukasiewicz PD, McReynolds JS (1998). Action potentials are required for the 
lateral transmission of glycinergic transient inhibition in the amphibian retina. J 
Neurosci, 18, 2301-2308.

Cook PB, McReynolds JS (1998). Lateral inhibition in the inner retina is important for 

Neurol 284:275-293.

Dacey D, Packer OS, Diller L, Brainard D, Peterson B, Lee B (2000). Center surround 

Dacey DM, Petersen MR (1992). Dendritic field size and morphology of midget and 


nonlinear spatial summation in the brisk-transient (Y) ganglion cell in mammalian 


Figure legends

**Figure 1. Circuitry for receptive field center and surround mechanisms.**
The excitatory receptive field (RF) center response of the ganglion cell (g) arises from cone photoreceptors (c) releasing glutamate onto bipolar cells (b), which in turn release glutamate onto the ganglion cell (g). A split into ON and OFF pathways arises at the cone synapse, where postsynaptic bipolar cells express either ionotropic glutamate receptors (OFF pathway), which preserve the sign of the cone response, or metabotropic receptors (ON pathway), which invert the sign of the cone response (Sterling and Demb, 2004). The surround arises partly from cone-driven horizontal cell (h) feedback. Horizontal cells excite neighboring cells via gap junctions and ultimately inhibit the cones and bipolar cells that make up the center pathway. The surround also arises from bipolar cell-driven amacrine cell (a) feedback. The amacrine cell-mediated inhibition can act directly onto the ganglion cell and onto the presynaptic bipolar terminal. Shown is the circuit for an OFF-center ganglion cell; the ON-center cell would have a similar circuit except that cone synapses are hyperpolarizing.

**Figure 2. The linear-nonlinear (LN) model of spiking and subthreshold membrane potential responses.**
The stimulus (in contrast units; see Materials and Methods) is convolved with a linear filter to generate the linear model of response. The linear model (in ‘input units’) is passed through an instantaneous (static) nonlinearity to generate the LN model of the response (in spikes sec\(^{-1}\) or mV). The model (green lines), built from one data set, can be compared to the average response to a brief test stimulus (black or red lines), from a separate data set (averaged over 24 repeats to reduce noise). The model corresponds closely to the data (see Materials and Methods). Shown are models for spiking (top row) and subthreshold membrane potential responses (bottom row) to the spot alone and spot + grating conditions. The \(r^2\) between model and data was: 0.88 (spikes, spot alone); 0.67 (spikes, spot + grating); 0.95 (membrane potential, spot alone); 0.91 (membrane potential, spot + grating).
Figure 3. Spatial bandwidth of horizontal cells.

A. An A-type horizontal cell in the visual streak of the guinea pig retina. The fluorescence image was converted to grayscale, and the contrast was inverted.

B. Intracellular responses of the cell in A. to drifting sine-wave gratings at various spatial frequencies (0.7 contrast). Each trace shows the average of two repetitions. Resting potential was -47 mV.

C. The cell's spatial transfer function is low-pass with a half-maximal response at ~0.2 cyc mm⁻¹ (same cell as in A and B). Responses are F1 amplitudes at each spatial frequency. Smooth gray line shows a sigmoidal fit (Matlab; least-squares method). Dashed line shows the F1 amplitude to a blank screen (spontaneous).

D. Average spatial transfer functions for a population of A-type and B-type horizontal cells. Responses have been normalized by subtracting the spontaneous response and then dividing by the maximal response. At 5 cyc mm⁻¹, the responses are attenuated to less than 10% of the peak response (see text).
**Figure 4. Peripheral contrast suppresses spiking responses to a central spot.**

**A.** Ganglion cell extracellular response to one cycle of the stimulus protocol (bottom trace). Each cycle consisted of a 10-second period of a flickering spot (0.5 mm diameter, centered on ganglion cell body) followed by a 10-second period of the spot plus a drifting grating in the periphery; spot and grating time courses are indicated above the trace. The grating was excluded from a central patch (1-mm diameter; see image at top). Grating was full contrast with 100-μm wide bars and a 2-Hz drift rate. The grating suppressed the spiking response to the spot.

**B.** Effect of the grating on firing rate in individual cells. Plotted are the firing rates during the last eight seconds of each half-cycle. The grating reduced the firing rate in all OFF cells and in some ON cells (points below the dashed identity line). Error bars indicate ±1 SEM across the 12 cycles.

**C.** Firing rate across the 20-second stimulus cycle, averaged across 12 cycles (same cell as part A). Error bars indicate ±1 SD across cycles. Line above data, here and in D, indicates grating time course (as in A).

**D.** Normalized spiking response across the 20-second stimulus cycle. The normalized response is the firing rate at each point in the stimulus cycle minus the average rate over the entire cycle. Figure at left shows the average for 44 OFF cells, all of which showed a significant decrease in firing rate during the grating (see Results). Figure at right shows the average of five ON cells that showed a decreased spike rate (gray symbols) and seven ON cells that showed an increased spike rate during the grating (white symbols; see Results). Error bars indicate ± 1 SEM across cells.
Figure 5. Peripheral contrast changes response kinetics and increases the threshold for spiking consistently in OFF cells.

A1. Linear filter for the spot alone and spot + grating conditions. The peripheral grating caused the filter to become more biphasic. To quantify the biphasic nature of the filter, we measured the amplitude of the first phase of the filter (negative response for OFF cells, positive for ON cells) for both the spot alone (s₁) and spot + grating condition (sg₁), and compared this to the amplitude of the second phase of the filter (positive response for OFF cells, negative for ON cells) for the spot alone (s₂) and spot + grating condition (sg₂). Filters are from the OFF cell in Figure 4A.

A2. Nonlinearity for the spot alone and spot + grating conditions. Data points show the binned spike rate as a function of the linear model values (see Materials and Methods). The data are fit (smooth lines) with Gaussian cumulative distribution functions (cdfs) that are identical except for a scale factor that ‘stretches’ the curves along the x-axis (gain change model) or shifts the curves laterally along the x-axis (threshold change model; see Materials and Methods). The threshold change model fit the data with a lower mean squared error (MSE, 4.7 spikes/s) than the gain change model (10.7 spikes/s). The gain change model underestimated the response to small responses in the spot alone condition and overestimated the response to small responses in the spot + grating condition (gray brackets).

A3. Residuals (difference between data and fit) from A2.

B1. Same format as A1 for an ON cell. The grating did not evoke a change in the shape of the filter.

B2. Same format as A2 for an ON cell. Data were better described by the threshold change model than the gain change model in this cell.

C. Population analysis of the effect of the peripheral grating on response kinetics. The grating caused OFF cells to become more biphasic (points below the identity line). ON cells were relatively unaffected. The pattern of results (here and in D.) was consistent for spikes measured with extracellular (extra) and intracellular (intra) recordings. The analysis here and in D was restricted to those cells that fired at a rate of at least one spike sec⁻¹ for both conditions and for which the grating caused a significant suppression of the spike rate (n = 40 OFF cells, 5 ON cells).
D. The *threshold change model* best explains the suppression of spiking in the presence of the peripheral grating for OFF cells. Plot shows the MSE for the *gain change* and *threshold change models*. Most OFF cell points fall below the identity line, indicating that the *threshold change model* yields lower MSE. ON cells were about equally well fit by the two models (see Results).

**Figure 6. Peripheral contrast hyperpolarizes the membrane potential in OFF cells**

A. OFF ganglion cell intracellular response to one cycle of the stimulus protocol; shown are the first second, the middle two seconds and the last second of the 20-second cycle.

B. Average subthreshold membrane potential across the 20-second stimulus cycle (same cell as A). The grating caused a hyperpolarization that partially recovered during the 10-second half-cycle. Error bars indicate ±1 SD across cycles.

C. Normalized membrane potential across the 20-second stimulus cycle. The normalized response is the membrane potential at each point in the stimulus cycle minus the average membrane potential over the entire cycle. Figure at left shows the average for 25 OFF cells. Figure at right shows the average of 2 ON cells that showed a hyperpolarization (gray symbols) and 3 ON cells that showed a depolarization during the grating (white symbols; see Results). Error bars indicate ±1 SEM across cells.
**Figure 7. Peripheral contrast reduces the gain of subthreshold responses.**

**A1.** Linear filter and static nonlinearity for the membrane potential response to the spot alone and spot + grating conditions. Inset shows the linear filters for the two conditions (normalized to have equal variance; scale bar indicates 100 ms; y-axis is in arbitrary filter units). Points show the two static nonlinearities. The depolarizing (rightward) sides of the nonlinearities were fit with Gaussian cdfs that differed by a gain change (i.e., scaling along the x-axis) and a tonic hyperpolarization (i.e., vertical shift, reflected by a drop in the y-intercept). The grating reduced the gain by 36% and hyperpolarized the membrane by 2.6 mV. Data is a whole-cell recording of the OFF cell in Figure 6A.

**A2.** Analysis of the spiking response for the cell in A1. The threshold change model was fit to the data (rightward shift of 32 input units).

**B1.** Same as A1 for an ON cell. The grating reduced gain by 23% and hyperpolarized the membrane by 1.0 mV.

**B2.** Analysis of the spiking response for the cell B1. The combined model was fit to the data (reduced gain by 21%; rightward shift of 12 input units).

**C1.** Same as B1 for a second ON cell. The grating reduced gain by 21% and depolarized the membrane by 0.4 mV.

**C2.** Analysis of the spiking response for the cell in C1. The combined model was fit to the data (reduced gain by 27%, leftward shift of 1 input unit).
Figure 8. Peripheral contrast effects on subthreshold and spiking responses are correlated.

A. Relationship between the effect of the grating on the average membrane potential and the reduced gain of the membrane potential (n = 21 OFF cells with firing rates of at least one spike sec\(^{-1}\) in both conditions). The change in the average membrane potential was measured as the change in the y-intercept of the nonlinear function (negative values indicate that the peripheral grating caused hyperpolarization). The gain change shows the gain in the spot + grating condition (\(g_{s+g}\)) relative to the gain in the spot alone condition (\(g_s\)). The correlation was not significant.

B. Relationship between the effect on the average membrane potential and the horizontal shift in the spike nonlinearity (positive values indicate rightward shift in the presence of the grating). The correlation was significant (p < 0.05).

C. Relationship between the reduced gain in the membrane potential and the horizontal shift in the spike nonlinearity. The correlation was significant (p < 0.01).
Figure 9. Peripheral contrast causes an increased membrane conductance.

A1. OFF cell response to the peripheral grating was measured in voltage clamp, $V_{\text{hold}} = -58$ mV or -74 mV. Traces are leak-subtracted and show the response to a single presentation of the grating.

A2. I-V plots show the amplitude of the transient current, right after grating onset (dark symbol), and the sustained current during the main period of analysis, 2-10 sec after grating onset (white symbols). Lines show regression fits; the x-intercept of the fit is the apparent reversal potential. This reversal was -95 mV (transient response) or -90 mV (sustained response).

B1. Same format as A1. for an ON cell, $V_{\text{hold}} = -37$ mV or -63 mV.

B2. Same format as B2. for the ON cell in B1. The reversal was -32 mV (transient response) or -71 mV (sustained response).

C. The sustained response from A2. is shown with the calculated underlying excitatory (dashed gray line) and inhibitory conductances (solid gray line; see Results). The two underlying conductances, when summed equal the total conductance (i.e., the regression fit to the data; solid black line).

D. The average excitatory conductance ($g_{\text{ex}}$) and inhibitory conductance ($g_{\text{in}}$) during the sustained response to the grating for OFF cells ($n = 6$) and ON cells ($n = 4$). Error bars indicate $\pm 1$ SEM.
Figure 10. **Peripheral contrast reduces the gain of the subthreshold response similarly in membrane voltages and currents.**

A. Time course of the effect of the peripheral grating for an OFF cell measured in current-clamp or voltage-clamp ($V_{hold} = -73$ mV); same format as Figure 6A. The recording solution included QX-314 to block sodium channels.

B. Linear-nonlinear model for the OFF cell in A., measured in current-clamp or voltage-clamp. The grating reduced the gain to a similar degree under current clamp (49%) and voltage clamp (46%). Fits to the nonlinear functions are based on depolarizing responses (current clamp) or inward currents (voltage clamp). Note that for the voltage-clamp recording here and in C., the y-axis of the linear filter and both axes of the nonlinearity have been plotted in reverse (i.e., negative values are going upward or rightward) to facilitate comparison with the LN model for the current-clamp recording.

C. Same as B. for an ON cell. The grating reduced the gain to a similar degree under current clamp (13%) and voltage clamp (18%; $V_{hold} = -47$ mV).

D. Across cells ($n = 9$ OFF cells, 4 ON cells), the reduced gains measured under voltage-clamp and current-clamp conditions were similar. The gain change was measured in the spot + grating condition ($g_{s+g}$) relative to the gain in the spot alone condition ($g_s$). Points lie near the identity line.

Figure 11. **Circuit model to explain the influence of long-range amacrine signaling on the ganglion cell center response.**

For OFF cells, amacrine cell inhibition acts at two points: a synapse onto the ganglion cell dendrite and a synapse onto the presynaptic bipolar cell terminal. Both synapses result in tonic hyperpolarization of the ganglion cell. The synapse on the bipolar terminal further causes a reduced gain of the center response to the spot. For ON cells, the circuit would be similar except that, under the present conditions, the grating sometimes caused a tonic depolarization that could be explained by inadvertent stimulation of bipolar cells at the edge of the ganglion cell dendritic tree (arrow pointing from bipolar terminal to extended ganglion cell dendrite, in dashed lines).
Figure 1. Circuitry for receptive field center and surround mechanisms. The excitatory receptive field (RF) center response of the ganglion cell (g) arises from cone photoreceptors (c) releasing glutamate onto bipolar cells (b), which in turn release glutamate onto the ganglion cell (g). A split into ON and OFF pathways arises at the cone synapse, where postsynaptic bipolar cells express either ionotropic glutamate receptors (OFF pathway), which preserve the sign of the cone response, or metabotropic receptors (ON pathway), which invert the sign of the cone response (Sterling and Demb, 2004). The surround arises partly from cone-driven horizontal cell (h) feedback. Horizontal cells excite neighboring cells via gap junctions and ultimately inhibit the cones and bipolar cells that make up the center pathway. The surround also arises from bipolar cell-driven amacrine cell (a) feedback. The amacrine cell-mediated inhibition can act directly onto the ganglion cell and onto the presynaptic bipolar terminal. Shown is the circuit for an OFF-center ganglion cell; the ON-center cell would have a similar circuit except that cone synapses are hyperpolarizing.
Figure 2. The linear-nonlinear (LN) model of spiking and subthreshold membrane potential responses. The stimulus (in contrast units; see Materials and Methods) is convolved with a linear filter to generate the linear model of response. The linear model (in 'input units') is passed through an instantaneous (static) nonlinearity to generate the LN model of the response (in spikes sec⁻¹ or mV). The model (green lines), built from one data set, can be compared to the average response to a brief test stimulus (black or red lines), from a separate data set (averaged over 24 repeats to reduce noise). The model corresponds closely to the data (see Materials and Methods). Shown are models for spiking (top row) and subthreshold membrane potential responses (bottom row) to the spot alone and spot + grating conditions. The r² between model and data was: 0.88 (spikes, spot alone); 0.67 (spikes, spot + grating); 0.95 (membrane potential, spot alone); 0.91 (membrane potential, spot + grating).
Figure 3. Spatial bandwidth of horizontal cells. A. An A-type horizontal cell in the visual streak of the guinea pig retina. The fluorescence image was converted to grayscale, and the contrast was inverted. B. Intracellular responses of the cell in A. to drifting sine-wave gratings at various spatial frequencies (0.7 contrast). Each trace shows the average of two repetitions. Resting potential was -47 mV. C. The cell’s spatial transfer function is low-pass with a half-maximal response at ~0.2 cyc mm⁻¹ (same cell as in A and B). Responses are F1 amplitudes at each spatial frequency. Smooth gray line shows a sigmoidal fit (Matlab; least-squares method). Dashed line shows the F1 amplitude to a blank screen (spontaneous). D. Average spatial transfer functions for a population of A-type and B-type horizontal cells. Responses have been normalized by subtracting the spontaneous response and then dividing by the maximal response. At 5 cyc mm⁻¹, the responses are attenuated to less than 10% of the peak response (see text).
Figure 4. Peripheral contrast suppresses spiking responses to a central spot. A. Ganglion cell extracellular response to one cycle of the stimulus protocol (bottom trace). Each cycle consisted of a 10-second period of a flickering spot (0.5 mm diameter, centered on ganglion cell body) followed by a 10-second period of the spot plus a drifting grating in the periphery; spot and grating time courses are indicated above the trace. The grating was excluded from a central patch (1-mm diameter; see image at top). Grating was full contrast with 100-?m wide bars and a 2-Hz drift rate. The grating suppressed the spiking response to the spot. B. Effect of the grating on firing rate in individual cells. Plotted are the firing rates during the last eight seconds of each half-cycle. The grating reduced the firing rate in all OFF cells and in some ON cells (points below the dashed identity line). Error bars indicate ±1 SEM across the 12 cycles. C. Firing rate across the 20-second stimulus cycle, averaged across 12 cycles (same cell as part A). Error bars indicate ±1 SD across cycles. Line above data, here and in D, indicates grating time course (as in A). D. Normalized spiking response across the 20-second stimulus cycle. The normalized response is the firing rate at each point in the stimulus cycle minus the average rate over the entire cycle. Figure at left shows the average for 44 OFF cells, all of which showed a significant decrease in firing rate during the grating (see Results). Figure at right shows the average of five ON cells that showed a decreased spike rate (gray symbols) and seven ON cells that showed an increased spike rate during the grating (white symbols; see Results). Error bars indicate ± 1 SEM across cells.
Figure 5. Peripheral contrast changes response kinetics and increases the threshold for spiking consistently in OFF cells. A1. Linear filter for the spot alone and spot + grating conditions. The peripheral grating caused the filter to become more biphasic. To quantify the biphasic nature of the filter, we measured the amplitude of the first phase of the filter (negative response for OFF cells, positive for ON cells) for both the spot alone (s1) and spot + grating condition (sg1), and compared this to the amplitude of the second phase of the filter (positive response for OFF cells, negative for ON cells) for the spot alone (s2) and spot + grating condition (sg2). Filters are from the OFF cell in Figure 4A. A2. Nonlinearity for the spot alone and spot + grating conditions. Data points show the binned spike rate as a function of the linear model values (see Materials and Methods). The data are fit (smooth lines) with Gaussian cumulative distribution functions (cdfs) that are identical except for a scale factor that 'stretches' the curves along the x-axis.
(gain change model) or shifts the curves laterally along the x-axis (threshold change model; see Materials and Methods). The threshold change model fit the data with a lower mean squared error (MSE, 4.7 spikes/s) than the gain change model (10.7 spikes/s). The gain change model underestimated the response to small responses in the spot alone condition and overestimated the response to small responses in the spot + grating condition (gray brackets). A3. Residuals (difference between data and fit) from A2. B1. Same format as A1, for an ON cell. The grating did not evoke a change in the shape of the filter. B2. Same format as A2, for an ON cell. Data were better described by the threshold change model than the gain change model in this cell. C. Population analysis of the effect of the peripheral grating on response kinetics. The grating caused OFF cells to become more biphasic (points below the identity line). ON cells were relatively unaffected. The pattern of results (here and in D.) was consistent for spikes measured with extracellular (extra) and intracellular (intra) recordings. The analysis here and in D was restricted to those cells that fired at a rate of at least one spike sec-1 for both conditions and for which the grating caused a significant suppression of the spike rate (n = 40 OFF cells, 5 ON cells). D. The threshold change model best explains the suppression of spiking in the presence of the peripheral grating for OFF cells. Plot shows the MSE for the gain change and threshold change models. Most OFF cell points fall below the identity line, indicating that the threshold change model yields lower MSE. ON cells were about equally well fit by the two models (see Results).
Figure 6. Peripheral contrast hyperpolarizes the membrane potential in OFF cells. A. OFF ganglion cell intracellular response to one cycle of the stimulus protocol; shown are the first second, the middle two seconds and the last second of the 20-second cycle. B. Average subthreshold membrane potential across the 20-second stimulus cycle (same cell as A). The grating caused a hyperpolarization that partially recovered during the 10-second half-cycle. Error bars indicate ±1 SD across cycles. C. Normalized membrane potential across the 20-second stimulus cycle. The normalized response is the membrane potential at each point in the stimulus cycle minus the average membrane potential over the entire cycle. Figure at left shows the average for 25 OFF cells. Figure at right shows the average of 2 ON cells that showed a hyperpolarization (gray symbols) and 3 ON cells that showed a depolarization during the grating (white symbols; see Results). Error bars indicate ±1 SEM across cells.
Figure 7. Peripheral contrast reduces the gain of subthreshold responses. A1. Linear filter and static nonlinearity for the membrane potential response to the spot alone and spot + grating conditions. Inset shows the linear filters for the two conditions (normalized to have equal variance; scale bar indicates 100 ms; y-axis is in arbitrary filter units). Points show the two static nonlinearities. The depolarizing (rightward) sides of the nonlinearities were fit with Gaussian cdfs that differed by a gain change (i.e., scaling along the x-axis) and a tonic hyperpolarization (i.e., vertical shift, reflected by a drop in the y-intercept). The grating reduced the gain by 36% and hyperpolarized the membrane by 2.6 mV. Data is a whole-cell recording of the OFF cell in Figure 6A. A2. Analysis of the spiking response for the cell in A1. The threshold change model was fit to the data (rightward shift of 32 input units). B1. Same as A1 for an ON cell. The grating reduced gain by 23% and hyperpolarized the membrane by 1.0 mV. B2. Analysis of the spiking
response for the cell B1. The combined model was fit to the data (reduced gain by 21%; rightward shift of 12 input units). C1. Same as B1 for a second ON cell. The grating reduced gain by 21% and depolarized the membrane by 0.4 mV. C2. Analysis of the spiking response for the cell in C1. The combined model was fit to the data (reduced gain by 27%, leftward shift of 1 input unit).
Figure 8. Peripheral contrast effects on subthreshold and spiking responses are correlated. A. Relationship between the effect of the grating on the average membrane potential and the reduced gain of the membrane potential ($n = 21$ OFF cells with firing rates of at least one spike sec$^{-1}$ in both conditions). The change in the average membrane potential was measured as the change in the y-intercept of the nonlinear function (negative values indicate that the peripheral grating caused hyperpolarization). The gain change shows the gain in the spot + grating condition ($g_{s+g}$) relative to the gain in the spot alone condition ($g_s$). The correlation was not significant. B. Relationship between the effect on the average membrane potential and the horizontal shift in the spike nonlinearity (positive values indicate rightward shift in the presence of the grating). The correlation was significant ($p < 0.05$). C. Relationship between the reduced gain in the membrane potential and the horizontal shift in the spike nonlinearity. The correlation was significant ($p < 0.01$).
Figure 9. Peripheral contrast causes an increased membrane conductance. A1. OFF cell response to the peripheral grating was measured in voltage clamp, Vhold = -58 mV or -74 mV. Traces are leak-subtracted and show the response to a single presentation of the grating. A2. I-V plots show the amplitude of the transient current, right after grating onset (dark symbol), and the sustained current during the main period of analysis, 2-10 sec after grating onset (white symbols). Lines show regression fits; the x-intercept of the fit is the apparent reversal potential. This reversal was -95 mV (transient response) or -90 mV (sustained response). B1. Same format as A1. for an ON cell, Vhold = -37 mV or -63 mV. B2. Same format as B2. for the ON cell in B1. The reversal was -32 mV (transient response) or -71 mV (sustained response). C. The sustained response from A2. is shown with the calculated underlying excitatory (dashed gray line) and inhibitory conductances (solid gray line; see Results). The two underlying conductances, when summed equal the total conductance (i.e., the regression fit to the data; solid black line). D. The average excitatory conductance (gex) and inhibitory conductance (gin) during the sustained response to the grating for OFF cells (n = 6) and ON cells (n = 4). Error bars indicate ±1 SEM.
Figure 10. Peripheral contrast reduces the gain of the subthreshold response similarly in membrane voltages and currents. A. Time course of the effect of the peripheral grating for an OFF cell measured in current-clamp or voltage-clamp (Vhold = -73 mV); same format as Figure 6A. The recording solution included QX-314 to block sodium channels. B. Linear-nonlinear model for the OFF cell in A., measured in current-clamp or voltage-clamp. The grating reduced the gain to a similar degree under current clamp (49%) and voltage clamp (46%). Fits to the nonlinear functions are based on depolarizing responses (current clamp) or inward currents (voltage clamp). Note that for the voltage-clamp recording here and in C., the y-axis of the linear filter and both axes of the nonlinearity have been plotted in reverse (i.e., negative values are going upward or rightward) to facilitate comparison with the LN model for the current-clamp recording. C. Same as B. for an ON cell. The grating reduced the gain to a similar degree under current clamp (13%) and voltage clamp (18%; Vhold = -47 mV). D. Across cells (n = 9 OFF cells, 4 ON cells), the reduced gains measured under voltage-clamp and current-clamp conditions were similar. The gain change was measured in the spot + grating condition (gs+g) relative to the gain in the spot alone condition (gs). Points lie near the identity line.
Figure 11. Circuit model to explain the influence of long-range amacrine signaling on the ganglion cell center response. For OFF cells, amacrine cell inhibition acts at two points: a synapse onto the ganglion cell dendrite and a synapse onto the presynaptic bipolar cell terminal. Both synapses result in tonic hyperpolarization of the ganglion cell. The synapse on the bipolar terminal further causes a reduced gain of the center response to the spot. For ON cells, the circuit would be similar except that, under the present conditions, the grating sometimes caused a tonic depolarization that could be explained by inadvertent stimulation of bipolar cells at the edge of the ganglion cell dendritic tree (arrow pointing from bipolar terminal to extended ganglion cell dendrite, in dashed lines).