Parallel Processing in Retinal Ganglion Cells: How Integration of Space-Time Patterns of Excitation and Inhibition Form the Spiking Output

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Roska, Botond, Alyosha Molnar, and Frank S. Werblin. Parallel processing in retinal ganglion cells: how integration of space-time patterns of excitation and inhibition form the spiking output. J Neurophysiol 95: 3810–3822, 2006; doi:10.1152/jn.00113.2006. Our goal was to understand how patterns of excitation and inhibition, interacting across arrays of ganglion cells in space and time, generate the spiking output pattern for each ganglion cell type. We presented the retina with a 1-s flashed square, 600 μm on a side, and measured patterns of excitation and inhibition over an 1,800-μm-wide region encompassing many ganglion cells. Excitatory patterns of ganglion cells resembled rectified versions of the voltage patterns of ON bipolar cells. Inhibitory patterns in ON ganglion cells resembled the rectified versions of voltage patterns of OFF bipolar cells. OFF ganglion cells received OFF excitation and ON inhibition. Many ganglion cells also received an additional wide field transient inhibition derived from the activity of both ON and OFF bipolar cells. Ganglion cell spiking was suppressed in those space-time regions dominated by inhibition. We classified each ganglion cell type by correlating its space-time patterns with its dendritic morphology. These studies suggest the bipolar and amacrine cell circuitry underlying the interplay between ON and OFF signals that generate spiking patterns in ganglion cells. They reveal a surprising synergistic interaction between excitation and inhibition in most ganglion cells.

INTRODUCTION

The inner plexiform layer of the mammalian retina is defined by an anatomic infrastructure consisting of ~10 different, well-defined strata (Boycott and Wassle 1999). These strata are formed by a superfamily of homologous transmembrane immunoglobulin molecules that mediate homophilic adhesion in vitro and direct laminar targeting in vivo (Yamagata et al. 2002). At each stratum, the terminals of specific different bipolar cell types, each with temporally different neural activity (Cohen and Sterling 1990a,b; DeVries et al. 2002; Euler and Wasse 1995, 1998; Euler et al. 1996; Famiglietti 1981; Ghosh et al. 2004; Jeon et al. 2002; Kolb et al. 1981; McGillem and Dacheux 2001; McGuire et al. 1984; Mills and Massey 1992; Pourcho and Goebel 1987; Vardi et al. 2000; Wasse and Boycott 1991) contact the dendritic trees of at least a dozen different ganglion cell types (Amthor et al. 1989a,b; Dacey et al. 2003; DeVries et al. 2002; Kock et al. 1989; Kolb et al. 1992; Magrum et al. 2002; O’Brien et al. 2002; Rao-Miroznik et al. 1995; Rockhill et al. 2002; Roska and Werblin 2001, 2003; Sun et al. 2002a,b; Vaney et al. 1981) with spatially diverse ramifications. Each stratum encompasses only a subset of bipolar and ganglion cell processes. Diverse amacrine cell subtypes (MacNeil and Masland 1998; MacNeil et al. 1999) carry information along and between these strata: the processes of some amacrine cell types ramify laterally within specific strata, whereas others send processes vertically that span several strata. Through these interactions, the retina computes a unique space-time neural representation of the visual scene at each stratum (Roska and Werblin 2001, 2003; Werblin et al. 2001). This set of about a dozen different neural representations is then carried by the ganglion cell axons to higher visual centers.

In an earlier study (Roska and Werblin 2001; Werblin et al. 2001), we began to investigate the space-time activity generated in these strata in response to an extended visual stimulus, specifically a 600-μm flashed square. That study showed a rich array of inhibitory and excitatory patterns representing the square stimulus, along with the spiking output, but the work did not include a full catalog of ganglion cell types or space-time patterns of cell activity. Here, we expand the description of cell types and show average space-time patterns for inhibition, excitation, and spiking derived from multiple measurements of each cell type. We associated the physiological space-time patterns with specific dendritic morphologies, confined to specific strata within the inner plexiform layer (IPL). From these studies, we infer both circuitry and underlying interactions between the ON and OFF signals that converge at ganglion cell dendrites.

METHODS

Electrophysiology

Using 94 ganglion cells, we recorded in the visual streak of isolated, light-adapted, whole-mount retinas of 2.5-kg New Zealand White rabbits using an Axopatch 200B amplifier (Axon Instruments). From each ganglion cell, we recorded with two electrodes. First, we recorded spiking with a loose cell-attached electrode (resistance: 3–4 MΩ) filled with Ames solution. Second, we recorded inhibitory and excitatory currents with a whole cell electrode (5–8 MΩ) filled with (in mM) 113 CsMeSO₄, 1 Mg SO₄, 7.8×10⁻³ CaCl₂, 0.1 bis-(oaminophenoxo)-N,N,N',N"-tetraacetic acid (BAPTA), 10 HEPES, 4 ATP-Na₂, 0.5 GTP-Na₃, 5 QX314-Br, and 7.5 Neurobiotin-Cl, pH 7.2. The retinas were continuously perfused at 8–10 ml/min with Ames (pH 7.4) solution at 36°C, equilibrated with 95% O₂-5% CO₂ containing 50 mg/l kanamycin. Under these conditions, the light response of the isolated rabbit retina is preserved for 6–7 h (we have not tested longer periods). Excitatory currents were measured by clamping cells to an E_Cl of ~−60 mV. Inhibitory currents were measured by clamping the membrane to 0 mV, the reversal potential for ionotropic glutamate receptors (Roska and Werblin 2001, 2003). The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
Under whole cell patch recording, QX314 was used to block sodium currents internally. The data-acquisition software, RED, was written by M. Wang, E. Nemeth, D. Handwerker, and T. Lan. Data were analyzed in Mathematica (Wolfram Research).

Confocal reconstruction

Confocal reconstruction of ganglion cells was done on a MRC 1024 (Bio-Rad Laboratories) or an LSM 510 confocal microscope (Zeiss) as described previously (Roska and Werblin 2001, 2003). Briefly, cells were loaded with neurobiotin, fixed, and incubated with a streptavidin-Alexa Fluor 488 conjugate to stain the measured cell and ToPro3 to stain all cell nuclei. The 488-nm laser line was used to image the filled cell, and the 630-nm laser line was used to image nuclei. We mounted all retinas with the Pro-Long antifade kit (Molecular Probes).

Light stimulus

An LCD panel, illuminated by a variable intensity (0–2000 W) spatially homogenous lamp, projected the stimulus onto the photoreceptor layer of the light-adapted, whole-mount rabbit retina (Roska and Werblin 2001, 2003). The time constant of the LCD panel was 12 ms. Illumination consisted of a 600-µm square flashed at a series of 30 separate 60-µm displacements in the region of the recorded cell. For reference, 1 degree of visual angle is 170 µm on the rabbit retina (Hughes 1971).

Slice preparation

Segments of the visual streak were mounted on Millipore paper and sliced with a razor into 250-µm thick slices. The slices were turned on their sides so that a cross-section was visible through the objective. Bipolar cells were identified as cell bodies lying near the outer edge of the inner nuclear layer (INL).

RESULTS

Representing ganglion cell activity as space-time patterns

We attempted to gain an intuitive sense for how the visual world is represented in space-time by different classes of ganglion cells. The results approximate recording from a linear array of identical ganglion cells spaced at 60-µm intervals responding to a 600 × 600-µm flash presented at the center of the array. The representation in space-time of that ganglion cell population could show us how extended patterns would be represented as neural activity across the retina. A similar analysis could be accomplished using an array of electrodes (Baccus and Meister 2002; Chichilnisky and Kalmar 2003; Meister 1996; Meister et al. 1991, 1994), but electrode arrays only record spiking activity. We were interested in how excitation and inhibition interact at the ganglion cell membrane to generate spiking patterns. Current technological limitations limit us to a single (or possibly 2) patch electrode at 60-µm spatial increments at 30 locations across an 1,800-µm region (Fig. 1). We chose 600 µm so that neural activity at one side of the square would not interfere with activity at the opposite side. We chose 1 s so that all transient activity at light ON would settle before the flash terminated, separating the ON and OFF responses. This separation allowed us to measure the temporal transients at light ON and OFF and the spatial transients on the light and dark sides of each boundary independently. We assembled the

30 responses to generate a “space-time map” (Fig. 2). This map approximates the retinal representation generated by a linear array of 30 identical ganglion cells, each discharged by 60 µm, in response to a single 600-µm flashed square.

These space-time responses can be analyzed by correlating the response activity of ON and OFF ganglion and bipolar cells, which provide excitatory input to ganglion cells (Fig. 2). Mammalian cones hyperpolarize at light ON, then “rebound” with a depolarization at light OFF. Bipolar cells follow cones and also show this rebound. The flash ON bipolar cells therefore depolarize at light ON and hyperpolarize at light OFF. Bipolar cells hyperpolarize at light ON and depolarize at light OFF (Fig. 2B).

The response polarities are reversed in regions adjacent to the flash due to antagonistic horizontal cell feedback. At light ON, ON bipolar cells are depolarized in the region beneath the flash (flash) and hyperpolarized in regions peripheral to the flash (Periph, Fig. 2B). At light OFF, these ON bipolar cells hyperpolarize in the region of the flash and depolarize in peripheral regions. A similar but sign-reversed set of responses is recorded on the OFF bipolar cells (Fig. 2B).

The bipolar signals are rectified at the bipolar-to-ganglion cell synapse (compare Fig. 2, B with C). The depolarizing phases of bipolar activity are conveyed to the ganglion cells as excitation, but the hyperpolarizing phases of bipolar activity are lost. The excitatory currents recorded from a typical ganglion cell appear are displayed in color-coded regions only where the bipolar cells were depolarized (Fig. 2D).

All ON ganglion cells showed an excitatory pattern that is derived from bipolar cells, manifest in regions 2, 4, and 6 (Fig. 2D). All OFF ganglion cells showed an excitatory pattern that is derived from OFF bipolar cells in regions 1, 3, and 5 (Fig. 2D).
To confirm that the ON bipolar cells are responsible for excitation in the ON regions in these ganglion cell patterns, we blocked ON bipolar activity with APB (Slaughter and Miller 1983) in the four ganglion cell types shown in Fig. 3. In all cases, 2-amino-4-phosphonobutyric acid (APB) blocked activity in regions 2, 4 and 6, which are elicited by the ON bipolar cell input.

ON parasol cell

The ON parasol cell has broadly extending dendrites spanning \( \approx 250 \mu m \), located near the midline of the IPL (Fig. 4). These cells lie at a depth within the IPL similar to the G2 cells (Rockhill et al. 2002), but their dendritic arbor seems more densely branched. If this, or any of the subsequent cell types, was to represent the stimulus faithfully, the pattern would resemble the panel labeled “full rep” in Fig. 4. Neural activity would begin when at the onset of the flash, end at the termination of the flash, and span the full 600 \( \mu m \) width of the stimulus. However, none of the cells recorded show this complete pattern. Instead, each response is truncated, either in space, time or both. The ON parasol cell appears to receive excitatory input mainly from the ON bipolars with activity primarily in region 2 and with some excitation at light OFF in regions 4 and 6, the typical ON bipolar cell pattern. Excitation at ON spans the entire 600-\( \mu m \) width of the stimulus. Although the broad extent of the dendrites could have “diffused” excitatory activity, excitation does not extend beyond the 600 \( \mu m \) boundaries of the stimulus. The inhibitory pattern is complex, showing activity in all six regions, suggesting that inhibition is derived from both the ON and the OFF bipolar cells. Transient inhibition extends laterally by \( \approx 600 \mu m \), suggesting that it is carried laterally by widely ramifying inhibitory processes. We infer that this inhibition is carried by ON-OFF transient amacrine cells, similar to polyaxonal cells (Volgyi et al. 2001). In addition, a delayed, sustained buts spatially narrow inhibition appears in regions 2, 4, and 6. This narrowly confined inhibition is probably derived from ON bipolar cells because it

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**Fig. 2.** Interactions underlying the space-time activity patterns. Top: ON activity; bottom: OFF activity. A: spatial location of the flashes. Flash: spatial region of the flash; Periph: spatial region in the periphery of the flash. B: bipolar cell responses at center and peripheral regions. The center bipolar response is from a patch recording made in a retinal slice in response to a 1-s diffuse flash, showing transient depolarizing peak at light ON and transient hyperpolarizing peak at light OFF. Surround responses were not recorded but are presented here as inverted versions of the center response. C: ganglion cell excitatory currents. These responses are rectified versions of the bipolar activity in the center and peripheral regions. Excitation exists only in regions where bipolar cells were depolarized. The hyperpolarizing phases of the bipolar response are lost. D: color coded space-time maps of ganglion cell spiking activity formed by input from bipolar cells beneath the flash at light ON and input from bipolar cells adjacent to the flash at light OFF. ON cell excitation exists in regions 2, 4, and 6, although the representation of excitation in regions 4 and 6 in this figure is rather faint. Bottom: complementary waveforms and responses for the OFF bipolar and ganglion cells formed by rectified versions of OFF bipolar cell input. Right: resulting space-time map of excitatory currents in an OFF ganglion cell with activity in regions 1, 3, and 5 that are complementary to those of the ON ganglion cell. In this and subsequent figures, red denotes highest activity; blue lowest activity. Excitatory activity in regions 2, 4, and 6 are derived from the ON bipolar cells.

**Fig. 3.** ON excitation is lost in the presence of APB, an ON bipolar cell activity blocker. Top: excitatory responses for 4 different ganglion cell types, showing activity reflecting the inputs from ON bipolar cells. ON bipolar input is indicated by activity in regions 2, 4, and 6. The ON OFF DS cell shows input from both ON and OFF systems. Bottom: responses from the same cells in the presence of APB. Activity in regions 2, 4, and 6, corresponding to ON bipolar input, are lost, confirming that the ON activity recorded in the ON ganglion cells is derived from the ON bipolar cells. Integration of inhibition and excitation in space and time in response to a 600-\( \mu m \) flashed square.
follows the ON bipolar cell pattern. The inhibition is contained within the boundaries of the stimulus and not diffused, suggesting that it is carried by narrowly ramifying amacrine cells. This ON inhibition appears to overlap and interfere with excitation, so the spiking response at light ON is truncated and briefer than the excitatory response.

**ON bistratified ganglion cell**

The ON bistratified ganglion cell shown in Fig. 5 has dendrites at both edges of the IPL with an elongated dendritic arbor spanning ~150 μm. These cells may correspond to G3 cells (Rockhill et al. 2002). Despite the dual dendritic arbor, this cell receives only ON-like excitation, appearing only in region 2. OFF excitation, if it existed, would appear in region 5. Excitation is brief, lasting only ~100 ms, but it is distributed uniformly across the 600-μm flashed square. Inhibition is purely OFF-derived, appearing only in regions 1, 3, and 5, and is more sustained than excitation. Inhibitory activity at light ON appears to spread rather broadly, suggesting that it is carried laterally by processes that have an extent of >400 μm. Spiking is confined to region 2 in the areas that receive excitation.

**ON delta cell**

The processes for ON delta cells lie at the 80% level of the IPL and spread widely to >500 μm (Fig. 6), similar to G10 cells (Rockhill et al. 2002). These cells are likely ON directionally selective cells (Amthor et al. 1989a,b; He and Masland 1998; Oyster et al. 1980). The ON delta cells receive excitatory input from the ON bipolars, with activity in region 2. The response is more sustained than the ON parasol and ON bistratified cells, lasting the entire 1-s stimulus. This excitation also

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**FIG. 4.** ON parasol cell responses and morphology. Top: time course of each of the 32 recordings made as the stimulus square was moved across the retina in 60-μm increments. Excitation was measured with the membrane held at ~60 mV, inhibition was measured with the membrane held at 0 mV, and spiking, measured with a loose patch electrode. Peak excitatory currents were 200 pA; peak inhibitory currents were 500 pA. Peak spike rate was 100 spikes/s. n = 11 indicates the number of cells of this type that were averaged in these measurements. Second row: color-coded response patterns where red represents the highest activity. The micrograph at the left shows the dendritic structure for this cell type. The scale bar in this and in subsequent figures represents 50 μm. Layers: the approximate depth of the dendrites within the inner plexiform layer (IPL) stained in green (see METHODS for staining). Dendritic depths within these regions are compared for all cell types in Fig. 16. Full Rep: this red square is the space-time pattern that would be a faithful representation of the stimulus. The response begins at the onset of the flash (left vertical white line), exists uniformly throughout the 1-s flash interval, and terminates abruptly at the offset of the flash (right vertical white line). The red square also uniformly spans the 600-μm region subtended by the flash (distance between the 2 horizontal white lines). None of the measured representations here or below are faithful to the stimulus. They are all more truncated versions of this representation in space and/or time, showing the filtering characteristics in space and time.

**FIG. 5.** ON bistratified cell responses and morphology. In this and subsequent figures, the top row shows the space-time patterns of excitation, inhibition and spiking. Bottom left: dendritic ramifications. Bottom right: approximate depth of dendrites within the IPL.
seems to “leak” into adjoining regions 1 and 3 as one might expect given the cell’s large dendritic spread that can diffuse activity in space. Inhibition appears to be slightly delayed and transient, derived from both the ON and OFF bipolar cells. This inhibition spreads less laterally than inhibition for the previous two ON cell types. In this case, the delayed, transient inhibition appears to generate a slight “pause” in spiking response in region 2, the region between the two vertical red responses.

Local edge detector

Local edge detector cells have diffuse processes throughout the OFF sublamina (Fig. 7) (Berson et al. 1998; Rockhill et al. 2002; Zeck et al. 2005), although they show a greater mono-stratified dendritic tree than we observed here. The narrow responses at the edges of the stimulus probably results from their narrow dendritic arbor, which span <100 μm.

The local edge detector is one of the few cells that receives both excitation and inhibition at both ON and OFF, consistent with the location of its processes that span the ON and OFF sublamina, being diffuse throughout the 25–65% levels of the IPL. OFF excitation is relatively sustained, appearing in regions 1 and 3 at light ON and at the outer boundaries of region 5 at light OFF, confined to regions immediately adjacent to the edges of the stimulus. ON excitation appears to be somewhat more transient in region 2 at light ON,
falling within the boundaries of the stimulus. A transient inhibition, spanning the width of the stimulus, appears at both light on and off in regions 2 and 5. The spiking response reflects the interaction between the excitatory and inhibitory inputs. Early transient inhibition blocks early spiking in region 2, allowing only the sustained spiking in regions 1 and 3. Transient inhibition at light off also precludes early responses at light off, leaving only delayed spiking in region 5. The off-driven excitation in regions 1 and 3 is sustained, most likely derived from the excitatory input from sustained off bipolar cells.

**ON OFF DS cells**

ON OFF DS cells have dendrites that reach both the on and off strata and extend broadly ≤300 μm in diameter (Fig. 8). These DS cells, with process levels near 20 and 80%, correspond to G7 ganglion cells (Rockhill et al. 2002) of the DS class (Taylor and Vaney 2003).

The excitatory patterns, measured at both on and off, manifest in regions 2 and 5, reflecting the on and off locations of the dendritic processes. Inhibition is also derived from both the on and off bipolar cell systems and appears to be carried by widely ranging processes. These cells probably receive inputs from several distinct amacrine cell types, operating on different temporal and spatial scales. The broad regions of inhibition appear to dramatically truncate excitatory activity, so the spiking is limited to brief durations in regions 2 and 5, immediately following light on and off. This simple stimulus paradigm belies the sophisticated signal processing the DS cells are capable of performing (Fried et al. 2002, 2005; Taylor and Vaney 2003).

**ON beta cells**

ON beta cell processes (Fig. 9) resemble those of previously reported ON beta cells in cat (Boycott and Wassle 1974). Their dendrites span ~300 μm and lie between the 40 and 60% levels. They are somewhat diffuse G4 cell types (Rockhill et al. 2002). The processes of these cells show a few thick primary dendrites with a profusion of thinner and shorter branches. OFF beta cells (following text) showed similar branching patterns.

ON beta cells show a brief excitation that is strongest in regions just inside the boundaries of the stimulus at light on. Inhibition, however, is primarily derived from sustained on bipolar cells. The role of inhibition here is curious because excitation itself is extremely brief, and inhibition does not appear to interact with excitation. Spiking reflects excitation, appearing most strongly at the inner boundaries of the stimulus, suggesting that this cell type can detect edges. This relatively narrow representation in the excitatory pattern is surprising, given the relatively large dendritic spread of the processes. It may reflect significant presynaptic interactions to which we have no access in these measurements.

**OFF beta cells**

The OFF beta cells in Fig. 10 correspond to the previously described OFF beta cells (Boycott and Wassle 1974). They lie between the 20 and 40% level and span a diameter of ~200 μm, similar to G4 cells (Rockhill et al. 2002). These cells represent the outside edges of the stimulus at light on and the inside edges at light off, a reflection of the excitatory input from exclusively off bipolar cells. Both patterns are relatively brief, unlike that of the local edge detector. The inhibitory response is broad in space, and likely derived (via wide field amacrine cells) from both on and off bipolar cells. Spiking occurs at off after a significant delay, a reflection of the brief off inhibition that appears immediately at off. There is also a more sustained, purely on inhibition apparent in region 2, probably indicating an amacrine cell input derived from on bipolar cells that is distinct from the on-off wide-field inhibition seen in regions 1, 3, 4, and 6.
OFF parasol cell

These cells ramify primarily at the 20% level, with a dendritic diameter of \( \sim 250 \, \mu m \) (Fig. 11) resembling G5 cells (Rockhill et al. 2002). They also may correspond to zeta cells (Berson et al. 1998). These cells show a conventional, transient OFF-derived excitation that is strong in region 5 and weak in regions 1 and 3. Inhibition is transient and primarily derived from ON bipolar cells, being strongest in regions 2, 4, and 6, as well as some broad transient ON-OFF inhibition. The spiking activity is primarily in region 5 at light OFF, reflecting the strong excitatory input unopposed by inhibition.

OFF coupled cell

The processes of the OFF coupled cells lie at the outer boundary of the IPL and are dramatically asymmetric (Fig. 12). Rockhill and coworkers do not show cell types of this mor-
Excitation with dendrites pushed up against the inner margin of the inner nuclear layer.

Excitation to the OFF coupled cell is derived from OFF bipolar cells, showing relatively sustained activity in regions 1, 3, and 5. Inhibition is sustained and appears to be primarily derived from ON bipolar cells because activity is located in regions 2, 4, and 6. However, the overall response seems to be truncated because sustained excitation results in only brief spiking at light ON. Curiously, there is no apparent direct inhibitory input in regions 1 and 3, measurable at the ganglion cell, to truncate this excitation.

**OFF delta**

The OFF delta cells have dendrites that ramify near the border of the INL (Fig. 13) and resemble G9 cells (Rockhill et al. 2002), which were identified as OFF delta types found in cats (Dacey 1989; Wassle et al. 1987). These cells derive excitatory input from a sustained OFF bipolar input. As with the ON delta cell, the relatively wide dendritic field of the OFF delta cell results in some diffusion of OFF activity in region 5 into ON regions 4 and 6. The inhibitory input is derived from sustained ON bipolar cells. The interaction between excitation and inhibition appears to “clean
up" the response, suppressing the leakage into ON regions and assuring that spiking is confined to region 5.

**ON alpha cells**

Due to their rarity and biasing in selection of cells while patch clamping, only a small number of ON alpha cells were recorded. No space-time maps were obtained, although a small number of variable spot-size records were measured with morphology (Fig. 14). Excitation showed both sustained and transient components at small spot sizes, but a larger and purely transient response to large spots. Inhibition showed a delayed, sustained response that decreased with increasing spot size. These cells stratified deeply and showed a large dendritic spread primarily made up of radial processes, as did G11 cells (Rockhill et al. 2002) and alpha cells (Cleland and Levick 1974a,b; Peichl et al. 1987). These cells showed a transient excitation and a sustained inhibition in response to spots, both decreasing and becoming more transient with larger spot diameter.
DISCUSSION

The preceding figures describe the great diversity of ganglion cell activity, measured using the space-time plots. These representations of ganglion cell activity provide intuitive graphical information from which many inferences about retinal circuitry can be derived. These studies were done under light-adapted conditions with relatively high contrast stimuli. There was little spontaneous activity under our experimental conditions. We have not studied how these patterns change under different experimental conditions. Figure 15 shows an overview of the 10 ganglion cells characterized in this study. The figure includes an additional column labeled “region of interaction,” showing regions of significant overlap between excitation and inhibition. For many ganglion cells, the spiking activity can be predicted from the excitatory patterns alone, because inhibition appears in regions complementary to excitation. For ON cells, excitation usually occurs in regions 2, 4, and 6, whereas inhibition falls in regions 1, 3, and 5. However, for some ganglion cells, excitation and inhibition overlap, creating significant regions of interaction. Such interactions are shown for the ON Beta cell, where inhibition appears to truncate excitation leading to transient spiking, and the ON Delta cell, where ON excitation intersects ON inhibition generating a transient pause in spiking. There is also considerable overlap the local edge detector, where both ON and OFF excitation and inhibition interact, eliminating spiking in regions 2 and 5. Finally, overlap in the ON OFF DS cell appears to truncate excitation, leading to transient spiking at both ON and OFF.

Correlating ganglion cell layering with earlier studies

The distinct responses correspond to specific morphologies, where dendrites are confined to a subset of strata in the IPL. The cells we studied correspond to the cell types previously described (Rockhill et al. 2002), except for the OFF-coupled cell that appears to have no counterpart. The remarkable correspondence between our studies and those of Rockhill and colleagues are shown in Fig. 16.

Possible circuitry for generating the response patterns shown in the preceding text are illustrated in Fig. 17. Inferences are based on four assumptions: 1) narrowly ramifying inhibitory signals are carried by glycinergic amacrine cells; widely ramifying inhibition is carried by GABAergic amacrine cells (Roska and Werblin 2003). 2) Transient activity is located near the ON OFF boundary of the IPL; sustained activity is located closer to the proximal and distal borders of the IPL (Roska and Werblin 2001). 3) Inhibitory patterns showing local activity are likely carried by narrowly spreading amacrine cells; patterns showing broad, global activity are likely carried by broadly ramifying amacrine cells. And 4) each ganglion cell is excited...
by bipolar cells with terminals that lie at or near the depth of its dendrites (Fig. 17).

The convergence of OFF inhibition with ON excitation, and of ON inhibition with OFF excitation (Fig. 17, A and B), may serve a variety of functions: We describe three examples of these functions that would apply to ON cells, but similar interactions probably apply to the OFF system as well.

First, excitation and inhibition act in a complementary, push-pull synergy: when excitation increases, inhibition decreases, such that excitatory and inhibitory currents combine and enhance, rather than offset each other.

Second, the rectification at the bipolar to ganglion cell synapse could represent a design flaw because the antagonistic surround, so carefully crafted by horizontal cell feedback, is lost when the bipolar signal crosses the bipolar-to-ganglion cell synapse. However, for ON cells at light ON, the antagonistic surround, lost through rectification at the synapse, is replaced with active inhibition, derived from the OFF system, and carried to the ON cells via amacrine cell inhibition. This active inhibition of ON ganglion cells, derived from the OFF system, may be more effective than the hyperpolarizing surround brought from the outer retina (Fig. 18).

Third, reintroducing an active, inhibitory surround antagonism in regions around the square at the ganglion cell level may spatially constrain the blurring of excitation across the ganglion cell dendrites, which can extend $\leq 400\ \mu m$. The inhibition in surrounding regions will "embrace" excitation, limiting its spread (Fig. 18). Inhibition with a spatial profile that resembles either ON or OFF bipolar activity, such as the OFF Delta and OFF Coupled cells, may be delivered via amacrine

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**FIG. 17.** Inferring circuitry from the space-time maps. A: generation of the ON bistratified space-time map. Excitation in the ON bistratified ganglion cell arrives from ON bipolar cells. The ON bistratified ganglion cell dendrites are located at the 80% depth, indicating that the bipolar cells that drive them have axon terminals near that depth. The inhibitory input is OFF phase, driven by amacrine cells that receive their input from bipolars on the OFF sublamina. The OFF inhibition is sustained, suggesting that it is derived from distal layers near the 0% level. The inhibitory patterns are narrow, suggesting they are derived from glycnergic amacrine cells (Roska and Werblin 2003) that carry OFF signals to the ON layers with narrow lateral spread. These characteristics are represented by the narrow, vertical amacrine cell symbol in A. B: generation of the OFF delta cell space-time map. The OFF delta cells receive OFF-type excitation derived from OFF bipolar cells stratifying near the processes at the 20% depth. Inhibition is the ON type and sustained, suggesting that it is generated by ON bipolar cells near the proximal border of the IPL. Inhibition is constrained to regions near the boundary of the stimulus, suggesting that the vertically oriented amacrine cells that carry inhibition are narrow and glycnergic (Roska and Werblin 2003). This is represented by the amacrine cell symbol in B. C: generation of the space-time maps for the ON parasol cell. Excitation is the ON type and transient, consistent with the layering of the ON parasol cell dendrites. One form of inhibition is the OFF type and very broad, suggesting that it is carried by widely ramifying ON OFF amacrine cells, such as the GABAergic polyaxonal amacrine cells (Roska and Werblin 2003).

**FIG. 18.** Hypothetical generation of patterns of excitation and inhibition for a population of ON ganglion cells at the peak of the response to a flashed square. Top: patterns of activity for the ON and OFF bipolar cells to a bright flashed square. Upward deflection indicates depolarization, downward indicates hyperpolarization Green: ON bipolar activity, Tan: OFF bipolar activity. The ON bipolar responds with depolarization in the region of the square but with hyperpolarization in regions surrounding the square. The OFF bipolars respond with hyperpolarization in the region of the square but with depolarization in regions surrounding the square. Middle: rectified versions of the bipolar response, illustrating the response component that provides excitation to the ganglion cell. Only the depolarizing components of the response generate excitation; the hyperpolarizing components of the responses have been lost. Bottom: surround response from the OFF system drives local amacrine cells. The response is inverted because amacrine cells are inhibitory, and this surround inhibition embraces the center depolarization, reconstructing an active antagonistic surround for the ON ganglion cell. A similar surround is formed for the OFF ganglion cell.
cells that vertically span the ON-OFF sublaminae and ramify narrowly so that inhibitory activity is not diffused in space. This OFF-bipolar-derived inhibition brought to the ON ganglion cells is formed by horizontal cell antagonism but conveyed to the ON system via the OFF bipolar cells.

Conclusion

This and earlier studies (Roska and Werblin 2001, 2003) suggest that a limited number of visual channels, probably about a dozen, generate different patterns of neural activity, each carried intact by a separate class of optic nerve fibers to higher visual centers. These different ganglion cell classes form the fundamental “phrases” of a natural language of vision. The visual brain must infer every aspect of vision through this very limited set of representations. It remains a major challenge to understand how these patterns are integrated by the brain to convey the vast richness of the visual world.

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