Functional Inhibition in Direction-Selective Retinal Ganglion Cells: Spatiotemporal Extent and Intralaminar Interactions

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Stasheff, Steven F. and Richard H. Masland. Functional inhibition in direction-selective retinal ganglion cells: spatiotemporal extent and intralaminar interactions. J Neurophysiol 88: 1026–1039, 2002; 10.1152/jn.01033.2001. We recorded from on-off direction-selective ganglion cells (DS cells) in the rabbit retina to investigate in detail the inhibition that contributes to direction selectivity in these cells. Using paired stimuli moving sequentially across the cells’ receptive fields in the preferred direction, we directly confirmed the prediction of Wyatt and Daw (1975) that a wave of inhibition accompanies any moving excitatory stimulus on its null side, at a fixed spatial offset. Varying the interstimulus distance, stimulus size, luminance, and speed yielded a spatiotemporal map of the strength of inhibition within this region. This “null” inhibition was maximal at an intermediate distance behind a moving stimulus; \( \frac{1}{3} \) to \( \frac{1}{2} \) times the width of the receptive field. The strength of inhibition depended more on the distance behind the stimulus than on stimulus speed, and the inhibition often lasted 1–2 s. These spatial and temporal parameters appear to account for the known spatial frequency and velocity tuning of on-off DS cells to drifting contrast gratings. Stimuli that elicit distinct on and off responses to leading and trailing edges revealed that an excitatory stimulus on its null side, at a fixed spatial offset. The neural circuit underlying this null inhibition remains unknown. Neither the orientation tuning of a DS cell, the shape of its dendritic field, or any offset between on and off sublayers or between the dendritic field and the receptive field systematically reflect the cell’s preferred direction (Amthor et al. 1984, 1989; He et al. 1998; Yang and Masland 1992, 1994). Since GABA\(_A\) receptor blockade eliminates direction selectivity (Amthor and Daw 1982; Caldwell et al. 1978; Kittila and Massey 1995, 1997; Linn and Massey 1992), GABAergic amacrine cells likely play a key role. The cholinergic starburst amacrine cell, which co-releases GABA, has been a popular candidate, in part because its dendritic arbors co-stratify with those of the DS cell (Brandstatter et al. 1995; Famiglietti 1983b; Masland et al. 1984; Tauchi and Masland 1984). Laser ablation of a subset of starburst cells in the rabbit did not eliminate direction selectivity (He and Masland 1997), but immunotoxin-mediated ablation of the whole population in the mouse did (Yoshida et al. 2001). Mathematical modeling has highlighted the need to incorporate both temporal delay and anatomic offset between excitatory and inhibitory inputs to the ganglion cell (Koch et al. 1983, 1986; Torre and Poggio 1978). Recently, controversy has arisen as to whether direction selectivity is primarily subserved by presynaptic or postsynaptic mechanisms (Borg-Graham 2001; Taylor et al. 2000). In the more commonly encountered on-off class in the rabbit retina, Wyatt and Daw (1975) demonstrated that a small spot stimulus moving within a discrete region of the cell’s receptive field could inhibit the cell’s response to a localized stimulus (a constantly drifting contrast grating; Fig. 1A). This experiment defined the universe of all points from which the cell could be inhibited when excited by the drifting grating (shaded area). As the grating was placed in different locations about the receptive field, the region thus defined moved with it, remaining spatially offset by a constant amount. This spatial relationship implies that an asymmetric moving wave of inhibition accompanies any excitatory stimulus moving through the receptive field (shaded area in Fig. 1B), and always remains on the null side of the stimulus (thus is termed “null” inhibition). One of our goals was to test this implication directly, with moving stimuli.

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INTRODUCTION

Two classes, on and on-off, of direction-selective ganglion cells (DS cells) in vertebrate retinas respond selectively to stimuli moving in a particular direction (Amthor and Grzywacz 1993a; Barlow et al. 1964; Vaney et al. 2001). At least one type is felt to provide an essential input to drive smooth pursuit eye movements such as those in optokinetic nystagmus (Oyster et al. 1972; Yoshida et al. 2001).

The mechanism by which DS cells discriminate the direction of stimulus movement remains controversial (Borg-Graham 2001; Taylor et al. 2000; Vaney et al. 2001). In the more commonly encountered on-off class in the rabbit retina, Wyatt and Daw (1975) demonstrated that a small spot stimulus moving within a discrete region of the cell’s receptive field could inhibit the cell’s response to a localized stimulus (a constantly drifting contrast grating; Fig. 1A). This experiment defined the universe of all points from which the cell could be inhibited when excited by the drifting grating (shaded area). As the grating was placed in different locations about the receptive field, the region thus defined moved with it, remaining spatially offset by a constant amount. This spatial relationship implies that an asymmetric moving wave of inhibition accompanies any excitatory stimulus moving through the receptive field (shaded area in Fig. 1B), and always remains on the null side of the stimulus (thus is termed “null” inhibition). One of our goals was to test this implication directly, with moving stimuli.

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curves constructed from the responses of DS cells to drifting contrast gratings, the cell’s response drops rapidly at frequencies beyond which more than one cycle of the grating falls within the cell’s receptive field (He and Levičk 2000). We reasoned that the response of the DS cell to higher spatial frequencies might be suppressed because each bright band of the grating falls within the inhibitory wave elicited by the preceding bright band. If so, then in a pair of stimulus bars moving at a fixed distance from each other through the receptive field in the preferred direction, the cell’s response to the second stimulus would provide an estimate of the degree of inhibition conferred at that distance behind the first stimulus (Fig. 1C), from which we could construct a spatiotemporal map of the inhibition.

**METHODS**

**Tissue preparation, stimulation, and recording**

Whole-mounted pieces of rabbit retina were prepared and maintained in vitro for recording as previously described in detail (Peters and Masland 1996; Yang and Masland 1994). Briefly, New Zealand white rabbits of either sex (3–5 kg) were anesthetized with intramuscular xylazine (3–5 mg/kg) and ketamine (15–25 mg/kg). Each eye was topically anesthetized with proparacaine hydrochloride 0.5%, 1–10 μg of 4,6-diamidino-2-phenylindole (DAPI) was injected intraocularly to label the ganglion cells. The animal was allowed to recover, and 1–3 days later again was anesthetized with xylazine (5–10 mg/kg) and ketamine (30–100 mg/kg) to the point that the corneal reflex was abolished. The animal was enucleated, the globe hemisected, and the vitreous removed. The animal was killed with an overdose of ketamine, according to a protocol approved by the Subcommittee on Research Animal Care of the Massachusetts General Hospital. The eyecup was transferred to oxygenated (95% O2 -5% CO2) Ames’ medium at room temperature and kept in a recording chamber attached to a microscope stage and superfused at 2.5–3.5 ml/min with oxygenated Ames’ medium at 33–37°C.

Stimuli were generated on a monochrome monitor (Tektronix 608) using a Picasso image synthesizer (Innissfree, Cambridgeshire, England) driven by a 486 PC computer with user-written software. Images were reflected by a mirror and focused by a 20× objective (NA 0.4; Olympus Optical, Tokyo, Japan) on the photoreceptor layer of the retina. Illuminance values were calibrated using a photodiode and photometer (LS-100; Minolta, Tokyo, Japan), and typically fell between 9.4–12.2 cd/m². Extracellular recordings were made using tungsten-in-glass electrodes (Levičk 1972) placed close to the somata of ON-OFF DS ganglion cells identified visually under very brief fluorescence illumination (He and Masland 1997; Yang and Masland 1994). A Schmidt trigger circuit identified action potentials, whose time of occurrence relative to the stimulus generation (within 1 ms) was recorded by the computer for later off-line analysis. Following an experiment, the recorded cell was usually injected with Lucifer yellow CH (4%; Sigma Chemical Co., St. Louis, MO) and photographed immediately, before the tissue was fixed in 4% formaldehyde/0.1 M phosphate buffer and mounted in Vectashield (H-100; Vector Laboratories, Burlingame, CA) for subsequent further photomicroscopy.

**Experimental protocol**

DS ganglion cells were initially identified by direction-selective responses to small spots of light maneuvered manually. Each cell’s receptive field then was mapped using small (approximately 100 μm) flashing spots. Next the length and speed of a 100-μm wide moving bar was optimized for maximal cell response, and the preferred direction determined using this stimulus bar. The cell’s baseline response to this bar moving in the preferred direction was obtained, and a series of trials was conducted in randomized order. In each trial, a pair of such stimulus bars, separated by one of at least five different distances, was presented outside the receptive field (each stimulus starting from the same position) and passed through and beyond it. Each group of randomized trials was repeated 10 times. In various experiments, blocks of such trials were conducted in which the length, width, luminance, or speed of motion was varied, as detailed in RESULTS.
Data analysis

Spike times recorded during the experiments were converted off-line to peristimulus time histograms (PSTHs) and cumulative spike rates using user-written software in QuickC and/or Matlab (MathWorks, Inc., Cambridge, MA). For estimating the degree of inhibition, the start of the cell’s response to a given stimulus was determined as the first bin of the first four nonzero bins in a row in the PSTH, and the end of the response as the last nonzero bin preceding the first four empty bins after that. (In some cases the criterion number of bins was adjusted to avoid obvious stray spikes or brief gaps in the responses.)

The degree of inhibition was then calculated as the ratio of the total number of spikes within this region of the PSTH to the total number of spikes in the same region of the PSTH for the trial with a single stimulus bar. Where responses to two stimuli overlapped, we used only the region of the PSTH without overlap (estimated from the time course of the response to the isolated single stimulus) for this calculation.

RESULTS

Inhibition of the response to a moving stimulus by one preceding it

Data presented are from 37 ON-off direction-selective ganglion cells (Fig. 2), each from a separate retina preparation and rabbit. The cells typically were studied for 5–8 h (range, 2–10 h).

The first panel of Fig. 3A is a PSTH showing the response of a representative ON-off DS ganglion cell (DS cell) to a single stimulus bar passing through its receptive field in the preferred direction. The subsequent panels demonstrate the response to a pair of such stimuli separated by increasing distances. In the second panel, the two bars exactly overlap, creating one brighter stimulus; this demonstrates that the cell was capable of increasing its response beyond that evoked by the single stimulus—i.e., the stimuli are within the cell’s dynamic range. In subsequent panels, the response to the first stimulus is seen again. If no inhibition were present, the second stimulus should evoke a similar and distinct response (red trace). However, this response is markedly suppressed. Note that the maximal suppression of the second stimulus response does not occur at the

FIG. 2. ON-off DS ganglion cell injected with Lucifer yellow at the end of a recording session, showing characteristic bistratified morphology (Anthor et al. 1984; Yang and Masland 1992). Scale bar, 200 μm.

FIG. 3. Responses of an ON-off DS cell to pairs of stimuli moving in the preferred direction. A: peristimulus histograms of the response to a single stimulus bar (top panel) or a pair of bars at various separations (subsequent panels), represented in diagrams to the right of each histogram. Gray (first stimulus) and black (second stimulus) lines below each histogram indicate time of stimulus presentation, from onset outside the receptive field through offset on the opposite side of the receptive field. Thin red traces show the expected response to the 2nd bar, reproduced from the top panel. Bin size, 10 ms. B: cumulative plot of the data, showing the cumulative number of spikes fired. Colors match the color of patches next to the same trials in A.
smallest inter-bar separation, but at an intermediate distance (fourth panel). These data are replotted in Fig. 3B as the cumulative spike number. Here the response to the second stimulus would be seen as a second plateau in the curves, but is seen clearly only in the magenta curve, for the greatest bar separation. In 32 such experiments, the maximal inhibition of the DS cell’s response to the second bar occurred at an inter-bar separation of approximately 50–150% \([80.2 \pm 25.3\% \text{ (SD)}, \, n = 25]\) of the receptive field width (approximately 170–670 \(\mu\text{m}\)). At this separation, the response was suppressed by 89.00 ± 10.27% \((n = 24)\) from the response to a single isolated stimulus.

**Dependence of inhibition on stimulus size and luminance**

In five experiments, the length of the stimulus bars was systematically varied from 100 \(\mu\text{m}\) to as much as twice the receptive field width, with qualitatively similar results. We also found similar results in five experiments in which the luminance of both bars was varied, with the exception of low luminance \((3.1–7.6 \text{ cd/m}^2)\) stimuli that elicited only small responses individually. In the case of such low luminance bars, the response to the first stimulus was facilitated when another such stimulus followed it, but the response to the second stimulus still was markedly suppressed (Fig. 4). This finding is also in agreement with prior studies (Grzywacz and Amthor 1993). Our measurements are robust across a variety of stimulus sizes and luminance values.

**Spatial and temporal extent of the inhibition**

The variation in the strength of null inhibition with inter-stimulus distance indicates the extent of the inhibitory wave in both time and space. To sort out whether the strength of inhibition is more dependent on the spatial distance or temporal delay between stimuli, we conducted trials at different speeds in the same cell. In each block, stimulus bars were paired at a different separation in each of five trials. All trials within the block were presented at the same speed, and one block was conducted at each of at least three speeds within the range to which the cell responded vigorously. In three experiments, the spatial separations between stimuli were identical at the various speeds (thus temporal delay varied with stimulus speed), and in two experiments the temporal delays between stimuli were identical (so that spatial separation varied with stimulus speed). Figure 5 illustrates a case in which spatial separation was held constant. The beginning and end of the trials in each block are aligned, so that distance along the \(x\) axis always translates to the distance the stimulus travels along its path through the receptive field. Within each block of trials, the response to the second stimulus bar is most suppressed in the trial where the bars are separated by an intermediate distance (fourth panel of each block). The relative degree of inhibition for each of the inter-stimulus separations is similar at each speed. Thus the strength of inhibition depends primarily on the distance between the pair of stimuli. However, comparison of any trace in the slowest and fastest blocks shows clearly that at a given inter-stimulus distance the response to the second bar is greater at slow speeds. Presumably, the inhibition fades more during the longer time interval between the stimuli at a slower speed.

This may be seen more directly in surface plots that summarize the degree of inhibition (percent change in expected response) in such experiments, and serve as spatiotemporal maps of null inhibition (Fig. 6, A and B). The strength of inhibition varies more with the distance between stimulus bars than with the temporal delay between them or with stimulus speed. Figure 6A again illustrates that the maximum inhibition occurs at an intermediate distance between the stimuli (cf. Fig. 3). Figure 6C demonstrates that null inhibition lasts a remark-

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FIG. 4. Facilitation of response to 1st stimulus at low luminance. Peristimulus histograms (A) and cumulative spike number (B) show increased response to the 1st stimulus at most interstimulus distances (compare with red trace reproduced from the top panel), but the response to the 2nd stimulus is suppressed. Display format as in Fig. 3.
ably long time, generally on the order of 1 s. We searched for and found 20–62% inhibition as long as 2 s after the first stimulus bar passed through the receptive field (median/H11005 38%, n/H11005 6 cells).

We also confirmed the long time course of null inhibition using a stationary test stimulus, since null inhibition evoked by

FIG. 5. Inhibition as a function of stimulus speed. Peristimulus histograms for a series of trials as in Figs. 3 and 4, conducted at (A) 0.909, (B) 0.610, and (C) 0.365 mm/s. Time scale is different for each speed, and histograms are aligned according to the beginning and end of trials. The degree of inhibition is similar at each interstimulus distance across various speeds.

FIG. 6. Plots of the distribution of null inhibition in space and time. A: percent change in response of a DS cell to the 2nd stimulus bar as a function of the distance between the stimulus bars and of stimulus velocity. Note that inhibition varies more with stimulus distance than with velocity (thus more than with time delay between stimuli). B: change in response of another cell as a function of the time delay between stimuli and of stimulus velocity. C: change in response as a function of the delay between stimuli, demonstrating the prolonged time course of inhibition. Mean ± SD of 3 cells (2 cells at 2,000 ms and 1 cell at 2,250 ms).
a moving stimulus may be expected to suppress this response as well. Thus in some experiments a stationary flashing spot was presented in the receptive field center, following the moving bar at various delays. Suppression of the response to this spot followed a time course similar to that measured with two moving stimuli (see RESULTS).

**Anticipatory inhibition of the first stimulus**

In addition to the second stimulus response being markedly suppressed, in many cases the response to the first stimulus also was decreased relative to when it was presented alone. There was both a decrease in the total number of spikes elicited and an increase in the latency of this response (Fig. 7). Of note, in some trials, the first stimulus was suppressed even when the second stimulus evoked very little response itself (fourth panel). This “anticipatory” inhibition on the preferred side of the stimulus was most clearly seen under conditions of the highest luminance and the extremes of bar length and was not seen in trials with low luminance. It always was much weaker than inhibition on the null side, and may reflect the portion of the null inhibition wave that extends a short distance to the preferred side of the stimulus (Amthor and Grzywacz 1993b; Wyatt and Daw 1975). Alternatively, it may be explained by the fact that in most trials the trailing stimulus was in the cell’s classic inhibitory surround as the leading bar entered the receptive field. The present experiments do not allow us to distinguish between these possibilities.

**Interactions between ON and OFF responses**

In this same preparation, Kittila and Massey (1995) blocked ON pathway responses with 2-amino-phosphonobutyric acid (APB), showing that direction selectivity remains intact in a pharmacologically isolated OFF pathway. This finding has been taken to suggest that each of the distinct ON and OFF sublaminae of the dendritic fields of DS cells is capable of mediating direction selectivity independently. We tested whether inhibitory interactions between these sublayers occur in the pharmacologically intact retina, by using elongated stimulus bars to separate leading edge from trailing edge responses (Fig. 8, top panel). When two such bars pass through the receptive field sequentially, the leading edge of the second bar directly follows the trailing edge of the first. In eight cells, after initially mapping null inhibition with a pair of thin stimulus bars, we repeated each of these trials with elongated bars, setting the same separation between their edges as between the thin bars (Fig. 8, subsequent panels). The trailing edge (OFF) response to the first stimulus suppressed the leading edge (ON) response to the second, to a degree that varied according to the same spatial profile as seen with the thin bars. (The leading edge of the first stimulus may also have made a minor contribution to this inhibition, but by comparison with the thin bar experiments from the same cell, its spatial profile suggests that the major contribution is made by the trailing edge.)

What about other combinations of ON and OFF edges? For example, Kittila and Massey’s results imply that in our experiments, an OFF edge response should inhibit a subsequent OFF edge response as well. To test all possible combinations, we conducted similar trials in which the background illumination was set to an intermediate level, from which the stimulus bar was made either darker or brighter. For example, a dark stimulus followed by a bright stimulus created two sequential ON-going edges in the middle of the trial. Results from all four possible sequences of ON and OFF edges are illustrated in Fig. 9 for a representative cell, at an intermediate edge separation where inhibitory effects were prominent. The trailing edge
FIG. 8. Inhibitory interactions between ON and OFF dendritic sublayers. *Top panel:* elongated bar was presented to elicit separate leading edge (ON) and trailing edge (OFF) responses. *Subsequent panels:* trials in which 2 elongated bars crossed the receptive field sequentially, so that the leading edge of the 2nd bar followed the trailing edge of the 1st by an increasing distance (see schematics). The trailing edge response to the 1st bar suppressed the leading edge response to the 2nd bar (compare with traces).
response of the first stimulus inhibited the leading edge response of the second, regardless of the polarity of either edge. That is, null inhibition acts both within each of the ON and OFF pathways, and between them. For all four conditions, the degree of inhibition varied with the distance between the edges, as seen in the prior experiments. In some cases it was stronger when conferred by one pathway than by the other (data not shown).

If these results reflect a general principle governing interactions between the ON and OFF dendritic layers of DS cells, then null inhibition evoked by a moving trailing edge also may be expected to suppress the response to a stationary flashing spot. Thus in five cells, we flashed a stationary spot in the center of the receptive field on and off at various intervals after the trailing edge of a long bar passed through the receptive field center in the preferred direction (Fig. 10). As expected, the trailing edge response inhibited the response to the flashing spot, and this inhibition faded with a time course similar to the previous experiments in the same cells.

**Equivalence of total inhibition conferred by preferred and null direction movement**

Wyatt and Daw’s model predicts that the same wave of inhibition follows a preferred-moving stimulus as leads a null-moving stimulus across the receptive field (Fig. 1B). If this is true, the total amount of inhibition conferred on the DS cell during the course of the stimulus’ passage through the receptive field will be the same for either stimulus. We tested this hypothesis, again using the stationary flashing spot as a probe for the amount of inhibition. (Our 2-bar protocol would not be useful in the null direction, since a null-moving stimulus would not evoke a response.) Typical suppression of the spot response following stimulus movement in the preferred direction lasts for more than 1 s (Fig. 11A). In the null direction, the wave of null inhibition precedes the moving stimulus and lasts more than 1 s, so that suppression of the spot response can be seen both before and after the stimulus moves through the receptive field center (Fig. 11B). The maximum suppression of the spot response and the time course of this inhibition are similar in both cases. We obtained similar results in two additional cells.

**Discussion**

**Direct mapping of null inhibition**

Our estimation of the spatial extent of null inhibition corresponds well with that expected from Wyatt and Daw’s study (Wyatt and Daw 1975). These experiments defined a boundary from within which a moving spot reduced the cell’s response below an arbitrary firing rate, corresponding to about 25% inhibition. It formed a roughly “cardioid” shape with a lateral extent (along the preferred-null axis) of 30–50% of the receptive field width. Apparent motion experiments using briefly flashed slits yielded similar parameters. From our experiments using paired thin bars of light, we estimate that the maximal degree of inhibition is seen at a distance from the leading bar that corresponds to approximately 50–150% of the receptive

![Figure 9](http://jn.physiology.org/)

**FIG. 9.** Inhibition acts both within and across dendritic sublayers. Schematic diagrams represent the use of stimuli brighter or darker than background illumination to test each of the 4 possible combinations of sequential ON and OFF edges. In each case, the top two peristimulus histograms illustrate response to each of the stimulus bars presented alone, and the bottom histogram shows response when both stimuli are presented together. Shaded area highlights inhibition of response to the leading edge of the 2nd bar (compare with trial of 2nd bar alone) in all 4 cases.
field width. In the cases tested, we found 20–62% inhibition at distances as far as three times the receptive field width (median = 45%, n = 5). One previous study used stationary flashing stimuli and light “steps” to derive a similar estimation of these parameters, finding that the degree of inhibition was greatest at the smallest inter-stimulus distances, but remained significant (approximately 70%) at distances equivalent to one-half of the receptive field width (Amthor and Grzywacz 1993b).

The spatiotemporal parameters of null inhibition also help to explain the spatial frequency and velocity tuning of these cells. Maximal inhibition was seen when the stimulus bars were separated by a mean of 313 ± 116.4 μm (n = 25), corresponding to a spatial frequency of ~0.61 ± 0.167 cycles/°. The response of DS cells to square wave gratings peaks at about 0.8 cycles/° (4.62 cycles/mm) and falls rapidly at higher spatial frequencies (He and Levick 2000). This sharp roll-off reflects the inability of the cell to respond to closely spaced bars moving in the preferred direction; unless it moves extremely slowly, a second bar that follows closely on the first encounters the powerful and long-lasting wave of null inhibition. Thus the earlier statement that the cells have a “high grating resolution” (Koch et al. 1982) is incorrect. The paradox is that the cells are able to detect localized movement at a high spatial resolution: a DS cell with a 540-μm receptive field can respond to and discriminate the direction of movement of a 50-μm spot displaced for 50 μm (Barlow and Levick 1965).

Furthermore, the long duration of null inhibition offers an explanation for the DS cell’s responsiveness to slow stimulus speeds. In our experiments and in previous studies (Amthor and Grzywacz 1993b; Wyatt and Daw 1975), significant inhibition remains 1–2 s after passage of the wave through a given location. Hence, a second bar following the first closely enough in space to encounter null inhibition still will see significant inhibition, whether it follows rapidly or slowly. It follows that the velocity tuning curve of DS cells is relatively broad, as shown previously (Barlow et al. 1964; He and Levick 2000; Oyster 1968; Oyster et al. 1972). Thus the same physiologic mechanism (null inhibition) appears to serve two functions: conferring direction selectivity and adjusting the spatiotemporal dynamic range of the cell.

The spatial and temporal parameters of this inhibition also set important limitations on potential mechanisms of direction selectivity (e.g., anatomic extent of amacrine cell dendritic

![FIG. 10. ON-OFF interactions demonstrated using a stationary test probe. Peristimulus histograms show ON and OFF responses to a moving elongated bar (top panel) and to a stationary flashing spot (2nd panel), and the suppressed response to a stationary spot when preceded by the moving bar (compare with traces). Display format as in Fig. 3.](image-url)
FIG. 11. Total amount of inhibition is equivalent whether stimulus moves in the preferred or null direction. A: response to a stationary spot (top panel) is suppressed when it is preceded by a bar moving in the preferred direction (subsequent panels, various intervals; compare with trace). B: test spot is also inhibited to a similar degree when it is presented before or after the bar moving in the null direction. Display format as in Fig. 3.
fields or networks, time course of neurochemical signaling). Such long-lasting inhibition is not expected from the known pharmacology of direction selectivity since this is mediated by GABA<sub>A</sub> receptors (Ariel and Daw 1982; Caldwell et al. 1978; Kittila and Massey 1995, 1997; Linn and Massey 1992; Massey et al. 1997; Zhou and Fain 1995), which typically produce briefer postsynaptic potentials (PSPs) such as those recorded in recent patch-clamp studies of rabbit DS cells (Taylor et al. 2000). There may be more prolonged inhibitory shunting effects that are not directly reflected in these PSPs. Alternatively, PSPs of up to several seconds' duration may be mediated by GABA<sub>B</sub>, GABA<sub>C</sub>, or metabotropic glutamate receptors, all of which are found in mammalian retinal cells (Bowery 1989; Cai and Pourcho 1999; Friedman and Redburn 1990; Johnston 1996; Kouten et al. 1998; Lukasiewicz 1996; Neal and Cunningham 1995; Slaughter 1995; Thoreson and Witkovsky 1999; Zhang et al. 1998). Another possibility is that null inhibition is mediated by a combination of short-duration postsynaptic PSPs and longer-lasting presynaptic inhibition of glutamate release from bipolar cells.

Interactions between on and off dendritic fields

A question of particular interest has been whether the on and off pathways, anatomically segregated in distinct dendritic sublaminae of the inner plexiform layer (IPL) (Famiglietti 1983a; Nelson et al. 1978), each sustain functionally independent direction-selective mechanisms in the corresponding dendritic sublayers of the on/off DS cell (Amthor et al. 1984, 1989; Oyster et al. 1993; Yang and Masland 1994). Grzywacz and Amthor (Grzywacz and Amthor 1993; Amthor and Grzywacz 1993b) used prolonged light flashes to demonstrate that interactions between transitions of like sign (on-on or off-off) were usually inhibitory and of somewhat greater amplitude than those of crossed sign (on-off or off-on), leading the authors to conclude that direction selectivity is computed independently in each pathway. However, significant inhibitory interactions were seen among crossed sign interactions as well, particularly in cases where the sequence of light flashes mimicked apparent motion in the preferred direction.

Kittila et al. investigated such interactions using a more direct approach with moving stimuli and pharmacologic blockade of the on pathway with APB, demonstrating that direction selectivity remains intact in the isolated off pathway (Kittila and Massey 1995). This also suggested that the two pathways might implement mutually independent direction selective mechanisms. Strictly speaking, this finding indicates that the

![](https://example.com/fig12.png)

**FIG. 12.** Anatomically based models of the inhibitory events that occur in 1 arbor of a DS cell during a traverse of the retina by a small moving spot. Relative sizes are appropriate for a ganglion cell in the mid-periphery of the rabbit retina and for CD-15 bipolar cells, shown to co-stratify with the off arbor of the starburst/DS dendritic plexus (Brown and Masland 1999). A: presynaptic model, in which a stimulus (orange bar) moving in the preferred direction "outruns" the wave of null inhibition (fading green patch), but one moving in the null direction evokes less transmitter release (orange trace below DS cell) from bipolar cells, because the wave of inhibitory feedback on bipolar cell axon terminals precedes stimulus. Integration of inhibitory and excitatory inputs is performed by the bipolar cell. B: postsynaptic model, in which, during movement in the preferred direction, the spatial offset between postsynaptic excitatory (orange trace below DS cell) and inhibitory input (green trace below DS cell) creates a local region where excitation predominates. In the null direction, excitation and inhibition at the leading edge coincide, suppressing cell response. Integration of inhibitory and excitatory inputs is predicted to occur in local regions of the DS cell dendrites. Total amount of inhibition within the receptive field is the same in null and preferred directions in both models.
off pathway is capable of maintaining functional direction selectivity in the absence of on pathway signals, but does not demonstrate whether the opposite is true, nor whether there is any interaction between the two in a pharmacologically intact retina. It does make clear that on-off interactions are not required to generate direction selectivity.

Our experiments with separated leading edge and trailing edge responses demonstrate that when all pharmacologic pathways in the retina remain intact, inhibition is transmitted not only within either the on or off pathway, but also between the two (Figs. 8 and 9). Both within-pathway and cross-pathway inhibition follows a spatial distribution and time course similar to that seen in the experiments with two thin bars, and thus likely also reflects the null inhibition that contributes to direction selectivity in these cells. The difference between our results and those of Grzywacz and Amthor may depend on the use of moving rather than static stimuli, but are not at odds with their findings of mixed-sign interactions in some cases (Amthor and Grzywacz 1993b; Grzywacz and Amthor 1993). Our findings also are consistent with Kittila and Massey’s finding that direction selectivity may be maintained by the isolated off pathway (Kittila and Massey 1995) and complete the picture for other cases, including one (an isolated on pathway) that cannot be directly tested with currently available pharmacologic tools. One might also ask whether similar actions between on and off sublayers could be seen for stimuli moving in the null direction; however, this cannot be tested directly with our paradigm, since null-moving edges do not evoke a baseline response.

In the larger picture, our results fit well into the emerging theme that vertical interactions between the on and off sublayers of the IPL play an important role in shaping the responses of retinal ganglion cells (Roska and Werblin 2001; Uchiyama et al. 2001; Werblin et al. 2001) and the neurons to which they project (Ibbotson and Clifford 2001). How might this interaction between the two input pathways of DS cells be mediated? One possibility is that a large inhibitory shunting current delivered to a local region of the cell’s off dendritic arbor spreads rapidly to reach the on arbor, or vice versa. However, branches connecting the two arbors are relatively few in rabbit on-off DS cells (Amthor et al. 1984, 1989; Oyster et al. 1993) and are distributed more sparsely than the scale of the small (approximately 50 μm) functional subunits of the receptive field that are capable of direction discrimination (Barlow and Levick 1965).

An alternative hypothesis to explain cross-pathway inhibition is that multistratified amacrine cells transmit it. Until recently, the on-off division of the IPL has been thought to keep the on and off pathways strongly segregated. Although a few examples of amacrine cells whose dendritic fields cross the on-off border have been known since Cajal’s first descriptions, more recent studies have shown that more than one-half of all amacrine cell types possess this feature (MacNeil and Masland 1998; MacNeil et al. 1999). Any of these might transfer signals from the excitatory inputs of one DS cell arbor directly to the other arbor, or to a GABAergic monostratified cell (such as a starburst cell) in the opposite layer. Such a scheme also might explain why direction selectivity was preserved after laser ablation of some starburst cells in only the on layer (He and Masland 1997), while this mechanism would not be preserved after immunotoxin-mediated ablation of virtually all starburst cells (Yoshida et al. 2001).

Alternatively, this cross-pathway null inhibition may be mediated earlier in the on and off pathways—e.g., via networks of multiple amacrine cells or amacrine cells transmitting signals between on and off bipolar cell axon terminals (Marc and Liu 2000; Watt et al. 2001). This would require that the inhibition be presynaptic to the ganglion cell (Borg-Graham 2001).

The DS cell as a nonlinear integrator of synaptic inputs

Wyatt and Daw’s model predicts that the wave of null inhibition maintains a fixed special relationship to the stimulus. Thus it follows a preferred-moving stimulus and leads a null-moving one, but the total amount of inhibition conferred on the DS cell over the whole stimulus path is the same for movement in either direction (Fig. 1B) (Wyatt and Daw 1975). Our estimates of null inhibition are similar for stimuli moving in either direction (Fig. 11), consistent with this hypothesis.

Extracellular recordings measure the net functional inhibition that surely results from complex interactions between excitatory and inhibitory currents impinging on the DS cell. They do not, for example, allow us to distinguish between potential pre- and postsynaptic interactions between these currents. The key excitatory and inhibitory inputs to the DS cell are shown schematically in Fig. 12 for stimuli moving in preferred and null directions. The receptive field of the DS cell is represented in transverse view, with a small moving stimulus depicted as an orange bar at an instant during its traverse of the receptive field, and the zone of inhibition created by this stimulus shown as a green bar, fading to illustrate the decay of inhibition following the stimulus. As Wyatt and Daw’s model predicts and our estimates confirm, in a postsynaptic model (Fig. 12B), the total amount of excitation and of inhibition is equivalent from the perspective of the soma, in either case. Thus in a postsynaptic model the DS cell cannot operate in a classic “integrate and fire” manner. It has no means of differentiating the direction of stimulus movement based on simply summing at the soma all excitatory and inhibitory inputs.

The directional discrimination could be accomplished in any of several ways. In a presynaptic model (Fig. 12A), the bipolar cell could directly receive and integrate the total excitatory and inhibitory input, and pass on a weaker excitatory input to the DS cell for movements in the null direction. Such a scheme predicts directional responses in bipolar cells, the existence of which is controversial (Devoe et al. 1985; Marchiafava 1979; Werblin 1970). Alternatively, Koch et al. (1986) proposed a postsynaptic model of “on path” inhibition, but updated estimates of the key biophysical parameters now make this unlikely (Segev and Burke 1998). A first-level analysis searching for a systematic anatomic relationship between the locations of putative excitatory and inhibitory synapses in the on layer of a single DS ganglion cell (Jeon et al. 2002) did not discover any such systematic pattern, suggesting that not only the spatial relationship, but also the timing between excitatory and inhibitory inputs is essential to the computation of direction selectivity. A third potential mechanism is a local dendritic nonlinear interaction involving dendritic action potentials (Chen et al. 1997; Golding and Spruston 1998; Torre and Poggio 1978; Velte and Masland 1999), calcium currents (Euler et al. 2001),

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or modification of signals at branch points. Such models predict locally direction selective responses within the distal dendritic arbor of the DS cells.

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