

Complementary roles of two excitatory pathways in retinal directional selectivity

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Abstract

The two major excitatory synapses onto ON–OFF directionally selective (DS) ganglion cells of the rabbit retina appear to be nicotinic cholinergic and NMDA glutamatergic. Blockade of either of these synapses with antagonists does not eliminate directional selectivity. This suggests that these synapses may have complementary roles in the computation of the direction of motion. To test this hypothesis, quantitative features of the DS cell excitatory pathways were determined by collecting responses, under nicotinic and/or NMDA blockade, to a sweeping bar, hyperacute apparent motions, or a drifting sinusoidal grating. Sweeping bar responses were reduced, but directional selectivity not eliminated, by blockade of either excitatory path, as previously shown (Cohen & Miller, 1995; Kittila & Massey, 1997). However, residual responses under combined blockades were not statistically significantly DS. NMDA blockade reduced responses more than nicotinic blockade for each protocol, and shifted hyperacute motion thresholds to higher values. This supported the notion that glutamate provides the main excitatory drive to DS cells, that is, the one responsible for contrast sensitivity. In turn, nicotinic, but not NMDA blockade eliminated directional selectivity to a drifting low spatial-frequency sinusoidal grating in these cells. This suggested that acetylcholine (ACh) is the main excitatory input with regards to directional selectivity for some textured stimuli, that is, those with multiple peaks in their spatial luminance profile. Moreover, nicotinic blockade raised the low temporal-frequency cutoff of the grating responses, consistent with the proposal that preferred-direction facilitation, which is temporally sustained, is dependent on the cholinergic input. These different properties of the NMDA and nicotinic pathways are consistent with a recently proposed two-asymmetric-pathways model of directional selectivity.

Keywords: Directional selectivity, Retinal ganglion cells, Acetylcholine, Glutamate, GABA

Introduction

Excitatory receptive and dendritic fields of ON–OFF directionally selective (DS) ganglion cells of the rabbit retina correlate tightly (Amthor et al., 1984; Yang & Masland, 1992). This suggests that glutamatergic, bipolar synapses, which have narrow receptive fields (Dacheux & Raviola, 1986), are the dominant excitatory inputs to DS cells. (Excitatory inputs with broad receptive fields would, of course, yield an excitatory receptive field larger than the DS-cell dendritic tree.) If glutamate provides the dominant excitatory input, it may mediate the DS cell response's high contrast sensitivity (Grzywacz et al., 1990) and directional hyperacuity (Grzywacz et al., 1994). We formulate this hypothesis, because it seems unlikely that the ACh input, which is too weak to detect when mapping the excitatory receptive field, could support these functions. Surprisingly, N-methyl-D-aspartic acid (NMDA) receptors were found to mediate most of the glutamatergic excitation in ON–OFF DS cells (Cohen & Miller, 1995), with α -amino-3-hydroxy-5-

methyl-4-isoxazolepropionic acid (AMPA)/kainate (KA) receptors responsible for only a weak portion of the glutamatergic input (Kittila & Massey, 1997).

In contrast to the receptive-field/dendritic-tree coincidence, preferred-direction facilitation extends outside the excitatory receptive field (Amthor et al., 1996). [Preferred-direction facilitation is the enhancement of the response to a test spot, when it is the second spot of a two-spot apparent motion in the preferred direction (Grzywacz & Amthor, 1993).] Hence, it is likely that an amacrine cell mediates facilitation. Due to their unique positioning (Famiglietti, 1983; Amthor et al., 1984), pharmacology (Ariel & Daw, 1982; Famiglietti, 1983), and size (Tsuchi & Masland, 1984; Grzywacz & Amthor, 1993; Amthor et al., 1996), the cholinergic, starburst amacrine cells are the most likely candidate for this role. The cholinergic input to ON–OFF DS cells has been shown to be carried primarily by nicotinic receptors (Kittila & Massey, 1997). (Although a weak, muscarinic input was suggested based on responses to bath-applied carbachol, synaptic block was not performed, so the muscarinic effect may have been transsynaptic.) Intracellular and whole-cell patch recordings from starburst cells (Bloomfield, 1992; Peters & Masland, 1996) and the time course

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of facilitation (Grzywacz & Amthor, 1993) imply that the nicotinic input is probably temporally sustained. This contrasts with the temporally transient responses of DS cells to steps of light (Barlow & Levick, 1965). As we will see in Results, that the ACh input is temporally sustained may be crucial for the support of directional selectivity at low temporal frequencies or speeds.

Blockade of either nicotinic or NMDA synapses does not eliminate directional selectivity (Kittila & Massey, 1997). This suggests that these synapses may have complementary roles in the computation of motion direction. In this study, then, we have utilized nicotinic and NMDA blockades to test this hypothesis. Curare, a competitive nicotinic antagonist, whose critical concentration in our setup has previously been determined (Grzywacz et al., 1997b), was used to block nicotinic ACh excitation. We used AP7, which acts as a competitive NMDA antagonist (Cohen & Miller, 1995), to limit NMDA glutamatergic excitation. A preliminary report of these findings has been presented in abstract form (Grzywacz et al., 1997a).

Methods

Anesthesia, surgery, recording, and stimulation methods were essentially the same as previously published by Amthor et al. (1989) and Grzywacz et al. (1997b).

Physiological preparation

Adult Dutch belt-pigmented rabbits were used for all experiments. The animals were initially anesthetized with urethane (2 g/kg), followed by pentobarbital sodium given to effect. Following dark adaptation, an eye was enucleated, hemisected, divitreated, everted, and mounted in a recording chamber under dim red light. The eyecup was superfused (3–5 ml/min) with a simulated cerebrospinal fluid (Ames & Nesbett, 1981) saturated with 95% O₂–5% CO₂. Extracellular recordings were obtained from the eyecup with carbon fiber in glass microelectrodes. All cells were recorded from just below the visual streak. A total of 30 eyes were used, from which we successfully obtained pharmacological data on 54 cells. Cells which did not recover directionally selective responses following drug wash-out were discarded from analysis.

Pharmacology

For each experiment, superfusion was switched to drug-containing media following the collection of control responses. The saturating concentration for curare in our setup has previously been determined as $13 \pm 11 \mu\text{M}$ (Grzywacz et al., 1997b), and a concentration of $60 \mu\text{M}$ was used here. AP7 was applied alone, or in combination with $60 \mu\text{M}$ curare, at either 250 or $500 \mu\text{M}$ concentration, well beyond the $100 \mu\text{M}$ level described as fully blocking responses to exogenously applied NMDA in the rabbit (Kittila & Massey, 1997). There is reason to suspect, however, that the critical concentration for AP7 may be higher than $100 \mu\text{M}$, and this issue will be discussed later.

Visual displays and responses

Three separate visual tests were applied in this study: a sweeping bar, hyperacute shifts of an extended edge, and a drifting sinusoidal grating. For each test, a mask was centered over the cell's excitatory receptive field to limit surround inhibition. The mask

had a circular outer edge with a diameter of $1500 \mu\text{m}$ and a square inner opening with sides measuring either 200 or $300 \mu\text{m}$.

The sweeping bar stimulus was a light bar ($2000 \mu\text{m}$ long and $600 \mu\text{m}$ wide) of varying contrast on a gray background, moving perpendicular to the bar's long side ($1000 \mu\text{m/s}$)* across the receptive field every 3 s. Motions tested were in either preferred direction only, preferred and null, or preferred, null and both orthogonal. Each combination of direction and contrast was presented 20 times in pseudorandom order. The contrasts displayed were a subset of 15, 25, 30, 40, 50, 60, 75, 80, 90, 100, 120, 150, 200, 300, and 400%, with contrast defined as $C_{\text{bar}} = 100\% (F - B)/B$, with F and B representing the foreground and background illuminations, respectively. For these experiments, $B = 23 \text{ lx}$. The measured response was the mean number of spikes per trial collected over the full motion.

The hyperacute edge-motion stimulus has been previously described (Grzywacz et al., 1994). In brief, responses were recorded to the appearance of an extended (light or dark) edge followed by a preferred- or null-direction apparent motion. No-motion cases, caused by the edge appearance alone, were also obtained to remove the transient responses through base subtraction (Amthor & Grzywacz, 1993; Grzywacz & Amthor, 1993). Apparent-motion displacement magnitudes ranged from 3.4 to $163 \mu\text{m}$. The background illumination was 60 lx , and the edge contrast was 99.2%, with contrast defined as above. The order of presentation of combinations of displacement magnitudes and directions of motion were randomized within a set and presented 30 times each.

The final stimulus protocol utilized a sine-wave grating ($240 \mu\text{m}/\text{cycle}$) drifting at rates ranging between 0.125 and 32 Hz , in both the preferred and null directions, for either 8 s (five trials), 12 s, or 16 s (three trials) each. The grating contrast was 99.2% with contrast defined as $C_{\text{grating}} = 100\% (L_{\text{max}} - L_{\text{min}})/(L_{\text{max}} + L_{\text{min}})$, where L_{max} and L_{min} were the maximal and minimal luminances, respectively. Mean luminance was 60 lx . The low-velocity cutoff was defined as the lowest velocity whose response was not less than half of the peak response. Thus, the cutoff was determined against normalized responses.

Results

Sweeping bar responses under cholinergic blockade

We began by verifying previous reports that nicotinic blockade will reduce DS cell preferred responses to a sweeping bar without eliminating directional selectivity (Ariel & Daw, 1982; Cohen & Miller, 1995; Grzywacz et al., 1997b; Kittila & Massey, 1997). Curare (nicotinic cholinergic antagonist) was applied to seven cells during the collection of response-versus-contrast functions and preferred responses were reduced, regardless of contrast, for each cell. The mean preferred response decrement at 50% contrast was $39 \pm 7\%$ (mean \pm S.E.).

Sweeping bar responses under NMDA blockade

We have also verified the reduction in response to a sweeping bar stimulus without loss of directional selectivity for NMDA block-

*This speed is slightly lower than that required for maximum responses to slits (Wyatt & Daw, 1975) and roughly optimal for square-wave gratings (Amthor & Grzywacz, 1998). Importantly, measurements of the strength of directional selectivity show an insensitivity to speed for speeds spanning over two orders of magnitude (Amthor & Grzywacz, 1998).

ade (Fig. 1—Massey & Miller, 1990; Cohen & Miller, 1995; Kittila & Massey, 1997). Fig. 2 shows the response-versus-contrast functions for an ON-OFF DS cell (the same one as in Fig. 1) before, during, and after application of 250 μM AP7 (NMDA glutamatergic antagonist). Regardless of contrast, both preferred- and null-direction responses were reduced. Similar results were found in all 17 cells tested with AP7. For five cells, 250 μM AP7 caused preferred-response reductions at 50% contrast of $48 \pm 7\%$ and at 100% contrast of $45 \pm 5\%$. These values were consistent with the 42% reduction ($n = 7$) reported by Cohen and Miller (1995) using 200–250 μM AP7. The remaining 12 cells were tested with 500 μM AP7 and had reductions of $71 \pm 7\%$ and $67 \pm 7\%$ for 50 and 100% contrasts, respectively. [One cell (e683c1) gave no significant responses under 500 μM AP7 for any stimulus—maximum tested contrast = 300%.] The difference between the

effects of AP7 at 500 μM and at 200–250 μM was statistically significant ($t = 2.69$ and $P < 0.01$ for 50% contrast, and $t = 2.38$ and $P < 0.025$ for 100% contrast, one-sided t -test, 15 degrees of freedom). Furthermore, the reduction for 500 μM AP7 was statistically significantly larger than that found by Kittila and Massey (1997), $31 \pm 7\%$, utilizing (\pm)-3-2-carboxypiperazin-4-yl phosphonic acid (CPP) at twice the concentration they describe as being sufficient to block fully a saturating dose of exogenously applied NMDA. The reduction was also considerably larger than the aforementioned 42% reduction ($n = 7$) of Cohen and Miller (1995), and the 34% reduction ($n = 1$) utilizing 100 μM AP7 [the critical level for AP7 as reported by Kittila and Massey (1997)] reported by Massey and Miller (1990). Fig. 3 plots the percentage of response reduction due to AP7 (as well as equivalent CPP) as a function of concentration for all four studies. This plot strongly

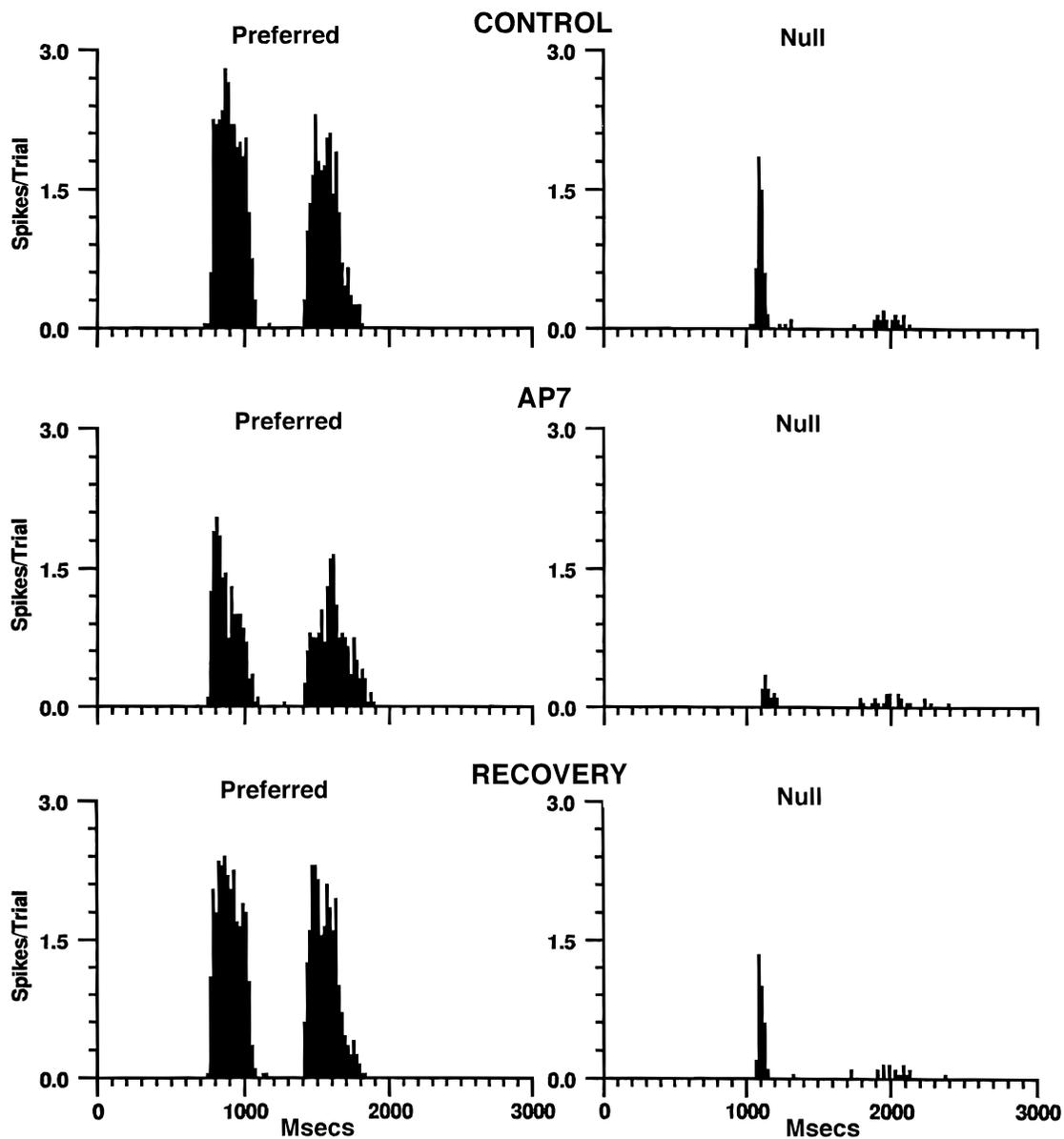


Fig. 1. Influence of 250 μM AP7 on poststimulus histograms of a DS cell (e651c3) responding to a bar sweeping along its preferred-null axis. Top, middle, and bottom rows are control, AP7, and recovery, respectively; preferred responses are to the left, null responses to the right. Responses decrease without loss of directional selectivity.

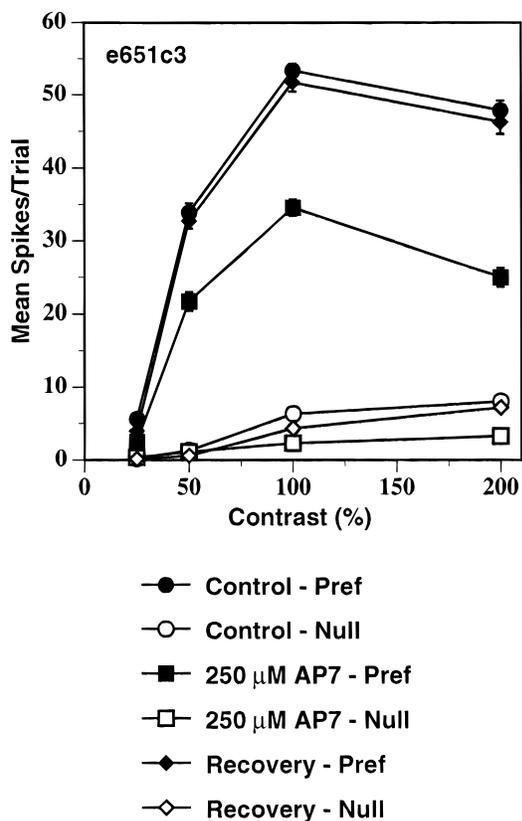


Fig. 2. Response-versus-contrast functions for the cell of Fig. 1. Solid and empty symbols are for preferred and null responses, respectively. Error bars stand for the standard error of the response. AP7 reduces responses at all contrasts but directional selectivity remains. The fall in the preferred response functions at high contrasts is typical and is the subject of the accompanying paper (Merwine et al., 1998). Although not all cells recovered as fully as here, directional selectivity was recovered or the cell's data were not used in the paper (see Methods). For clarity, recovery is not shown in the remaining figures.

suggests that, at this range of concentration, the effect of AP7 increases with concentration. However, that the effect of AP7 does not seem to saturate at 100 μ M as suggested by Kittila and Massey (1997) may also be related to protocol differences and will be considered further in the Discussion.

Sweeping bar responses under combined NMDA and cholinergic blockades

When 60 μ M curare was added to the 500 μ M AP7 superfusate, responses were generally further reduced, consistent with Kittila and Massey's (1997) results with hexamethonium bromide (plus atropine) and CPP. We have found this reduction for some contrast in eight of nine cells (recall that e683c1's responses were eliminated by AP7 alone). The mean additional reduction from the AP7-alone response was $12 \pm 3\%$ at 100% contrast, but $0 \pm 3\%$ at 50% contrast. This difference in additional reduction, for 50 and 100% contrasts, was perhaps due to the varying contrast dependencies of the two excitatory mechanisms. Recall that the reduction due to curare alone, at 50% contrast, was $39 \pm 7\%$ ($n = 7$). This large difference in curare's effect when presented alone, and following AP7 administration, supported the claim that the excit-

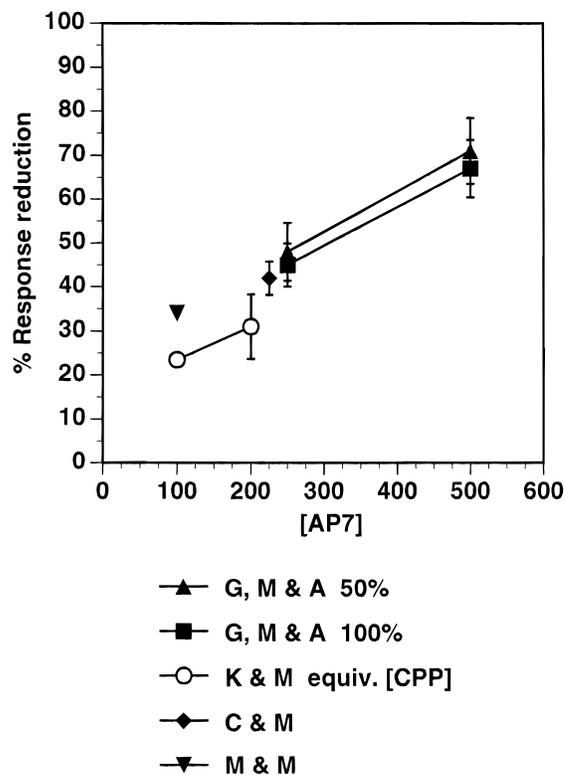
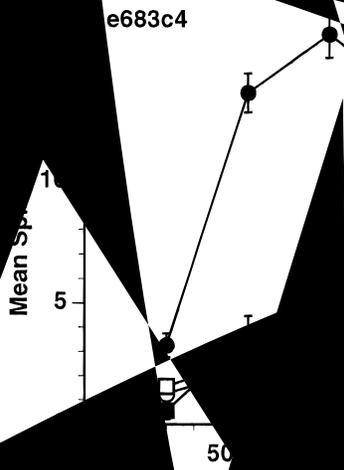


Fig. 3. Mean % DS cell response reduction as a function of AP7 (or CPP) concentration. The concentration of CPP was translated to an equivalent AP7 concentration by equating them when they block a saturating dose of exogenous NMDA (Kittila & Massey, 1997) and scaling them proportionally. G, M, and A refers to this paper, K and M refers to the Kittila and Massey paper, C and M refers to Cohen and Miller (1995), and M and M refers to Massey and Miller (1990). The data suggest that the critical concentration for NMDA receptor blockade may be higher than previously believed (100 μ M for AP7 as reported by Kittila & Massey, 1997). However, protocol differences make comparisons across studies difficult (see Discussion).

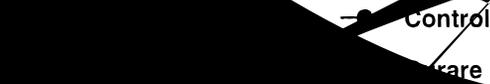
atory inputs to DS cells are not fixed in their contribution to the final response (Kittila & Massey, 1997). The residual responses with both antagonists applied were reduced from control $84 \pm 5\%$ and $88 \pm 5\%$, for 50 and 100% contrasts, respectively (see Fig. 4 for an example), neither of which is significantly different from the value of $92 \pm 2\%$ found by Kittila and Massey (1997) ($t = 1.36$ and 0.72 for 50 and 100% contrasts, two-sided t -test, 13 degrees of freedom) during blockade with hexamethonium bromide (plus atropine) and CPP. However, in contrast to Kittila and Massey's (1997) result, directional selectivity remained for only one cell under combined blockade. The remaining cells showed either no difference between preferred and null responses (four cells—Fig. 4), or preferred-direction reversal (three cells).

Directional hyperacuity

Previously, we demonstrated that DS cells can respond in a directional manner to edge motions spanning as little as 1.1 μ m, whereas the minimal displacement for DS responses to a drifting sine-wave grating is about 125 μ m (Grzywacz et al., 1994). Here we tested whether this directional hyperacuity still existed under either curare or AP7. A representative example of their effects on a DS



for a DS cell responses (e683c4) under control, curare, and AP7 conditions. Conditions are the same as above and directions of motion are the same. Directional selectivity is lost.



(e670c3) responses as a function of distance from the cell. The motion direction is the same as above. The motion speed is 10 $\mu\text{m/s}$. The motion distance is $< 3 \mu\text{m}$ under control to $13 \mu\text{m}$ under curare to $13 \mu\text{m}$ under AP7.

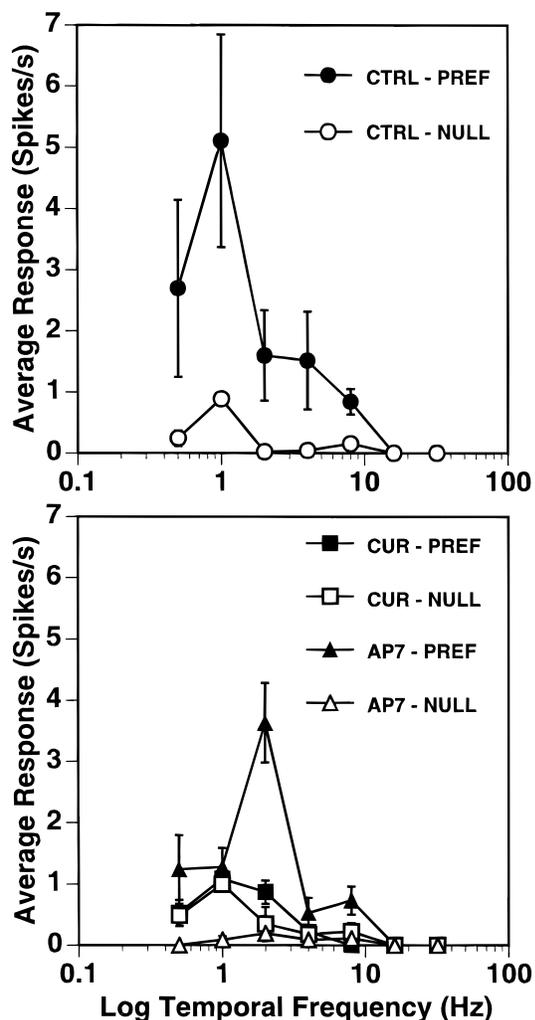


Fig. 7. Effects of curare and AP7 on the average response of a DS cell (e673c2) to a drifting sinusoidal grating. The top panel displays control responses while the bottom panel shows responses under drug conditions. Conventions are as in Fig. 2. Curare and AP7 both lowered responses, but only the former eliminated directional selectivity.

happen with the moving bar (for more details, see Grzywacz et al., 1998). In contrast, as seen in Fig. 7, for those cells for which AP7 did not eliminate the response (an example of such an elimination appears in Fig. 8), AP7 did not seem to eliminate grating-elicited directional selectivity.† In four of five cells, directional selectivity elicited by the grating remained intact under AP7. In curare's case, grating-elicited directional selectivity was eliminated in 11 of 12

†Some definitions of directional selectivity would say that if no spike responses remain after drug application, then the cell is no longer DS. However, this ignores the possibility that directional, subthreshold responses exist. Many standard measures of the strength of directional selectivity (for example, p/n , or $(p - n)/(p + n)$, where p and n are the number of spikes elicited by preferred- and null-direction motions, respectively) will be undefined if no responses remain under pharmacological blockade. Thus, we prefer to limit our consideration to those cells whose responses were not eliminated by drug application.

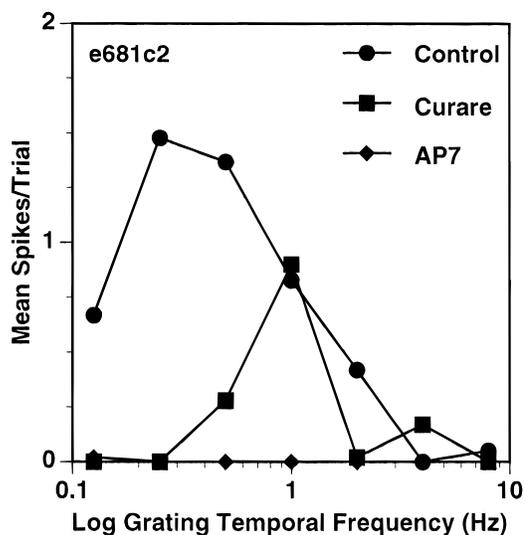


Fig. 8. Preferred-direction responses of a DS cell (e681c2) to a drifting $240 \mu\text{m}/\text{cycle}$ sinusoidal grating as a function of temporal frequency under control, curare, or AP7 conditions. Curare raised the low temporal-frequency cutoff from 0.25 to 1 Hz. AP7 eliminated all grating responses for this and 13 of the remaining 18 cells.

cells. Therefore, cholinergic action through nicotinic receptors, but not NMDA action, seems to be the main excitatory process supporting directional selectivity for a moving, rugged visual surface, at least when it has low spatial frequency, like in our stimulus.

We also examined the changes in the sinusoidal temporal-frequency tuning curves caused by the blockade of nicotinic or NMDA inputs. In particular, our interest lied on the low temporal-frequency cutoff, since nicotinic inputs are probably temporally sustained. It thus seemed reasonable to hypothesize that nicotinic inputs contribute directly to low temporal-frequency responses. Fig. 8 shows the responses of a cell (e681c2) to a $240 \mu\text{m}/\text{cycle}$ grating drifting at temporal frequencies ranging from 0.125 to 8 Hz. The low and high temporal-frequency cutoffs, under control condition, were 0.25 and 1 Hz, respectively. Curare application reduced response strength at most temporal frequencies, and raised the low temporal-frequency cutoff to 1 Hz. (The high cutoff was unchanged.) For five cells, responses under curare were not significantly nonzero, and four others were not tested at temporal frequencies low enough to determine a difference between curare and control. For the remaining ten cells, however, there was an increase in the low temporal-frequency cutoff as shown in Fig. 8. Fig. 9 presents the low temporal-frequency cutoffs under control and curare for these ten cells, and Fig. 10 quantifies the amount of the shift on a cell-by-cell basis. These data support the notion that the sustained, nicotinic input to the DS cell is required for responses at low temporal frequencies. Unfortunately, AP7 application reduced grating responses to insignificance for the cell in Fig. 8. This, in contrast to the nicotinic-blockade experiments, was the case in 14 of the 19 cells. Thus, we can say little about NMDA's involvement in determining the temporal-frequency bandwidth of these cells. The data were suggestive of a higher low temporal-frequency cutoff with AP7, but this result was not statistically significant.

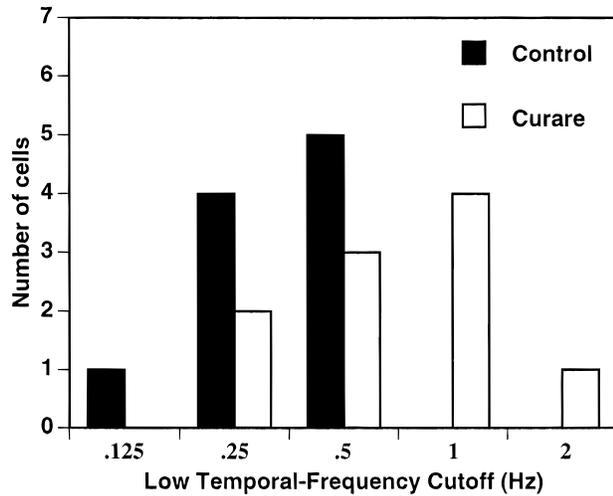


Fig. 9. Population distributions showing curare's effect on the low temporal-frequency cutoff. Curare raises the low temporal-frequency cutoff for the population.

Discussion

Under either nicotinic or NMDA receptor blockade, quantitative data were collected to a sweeping bar, hyperacute apparent motions, or a drifting grating. Each data set is considered separately below. Interpretation of the nicotinic-blockade data is somewhat straightforward, as the only cells that release ACh in the rabbit retina are the starburst amacrine cells (Famiglietti, 1983), and their connections are predominantly to ganglion cells (Famiglietti, 1991). The NMDA-blockade data, however, must be approached with caution. The responses of some amacrine cells depend on NMDA inputs, and thus AP7 may have both direct and network effects on DS cells. Cohen and Miller (1995) and Kittila and Massey (1997)

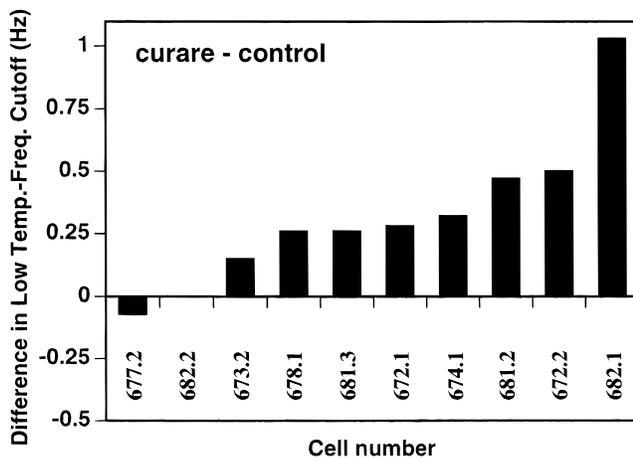


Fig. 10. Difference between curare and control low temporal-frequency cutoffs on a cell-by-cell basis. For the majority of cells, curare raises the low temporal-frequency cutoff. Cells are the same as in Fig. 9.

have shown, though, that AMPA/KA glutamatergic receptors control the amacrine cell release of GABA and ACh onto rabbit DS cells. Thus, the primary amacrine inputs to DS cells will not be strongly affected by NMDA blockade. Nevertheless, other trans-synaptic effects of NMDA blockade could occur through, for example, the blocking of amacrine release of glycine.‡ Glycine has been shown to increase the late component of the DS-cell response (Caldwell et al., 1978). As our response measure is spikes per trial and not maximal firing rate, a loss of the late portion of the DS-cell response would lead to a response reduction, and perhaps an over-estimation of the effect of NMDA blockade.

Before discussing the sweeping-bar, hyperacuity, and drifting-grating data, we should briefly consider the possible roles of non-nicotinic ACh and non-NMDA glutamate receptors. Although the pharmacological analyses of Kittila and Massey (1997) demonstrated that nicotinic and NMDA receptors carry the majority of the direct excitation to DS cells, minor inputs through muscarinic and AMPA/KA receptors were shown as well. Nevertheless, two experimental issues necessitate careful consideration of muscarinic and AMPA/KA receptors. First, NMDA receptor responses depend on both glutamate and membrane voltage. This is because Mg^{2+} blocks NMDA channels, unless removed by depolarization (Nowak et al., 1984). Excitatory inputs from non-nicotinic-non-NMDA receptors could, therefore, exert substantial effects by depolarizing the DS cell, removing the Mg^{2+} block, and thereby augmenting NMDA-receptor responses. Evidence for such an enhancement by removal of Mg^{2+} is now available for DS cells (Tjepkes & Amthor, 1998). Second, as discussed above, AMPA/KA receptors control the release of GABA and ACh onto rabbit DS cells. Elimination of both of these (putatively) asymmetric pathways (Grzywacz et al., 1997a) would cause the DS cell to lose its directional selectivity. Cohen and Miller (1995) have shown that AMPA/KA antagonists eliminate directional selectivity in the rabbit retina. Yet responses to stationary light stimuli are only modestly affected, as they depend primarily on NMDA receptors.

Sweeping bar

Because the DS cell's excitatory receptive and dendritic fields tightly correlate (Amthor et al., 1984; Yang & Masland, 1992), it seems that the DS cell's dominant excitatory input is from (glutamatergic) bipolar cells, since they have narrow receptive fields. Kittila and Massey (1997) have shown that NMDA and nicotinic receptors mediate most of the direct glutamatergic and cholinergic excitation onto DS cells, respectively. Hence, NMDA excitation is probably the dominant input onto these cells, and thus, NMDA blockade would be expected to reduce responses more than nicotinic blockade. Such is the case with the data here, with 500 μM AP7 reducing responses on average $71 \pm 7\%$ ($n = 12$) at 50% contrast, and 60 μM curare reducing responses $39 \pm 7\%$ ($n = 7$). (As discussed above, some of the response decrement due to curare might be due to an effect on NMDA receptors through removal of Mg^{2+} block.) This dominance of the response by NMDA inputs is the reverse of that reported by Cohen and Miller (1995) and Kittila and Massey (1997). We feel there are at least two possibilities for this difference. First, the critical concentration for full NMDA

‡Transsynaptic effects through bipolar inputs to the DS cells should be minor, because NMDA blockade practically eliminates these inputs anyway (Kittila & Massey, 1997).

blockade is in doubt,§ as suggested by Fig. 3. We explain the reasons for this doubt as follows: Kittila and Massey (1997) reported that 100 μM AP7 completely blocks the response to a saturating concentration of exogenous NMDA. However, it is possible that the transient levels of light-evoked glutamate are higher than the saturating dose, thus requiring higher doses of AP7 for blockade. Moreover, it is possible that the affinity for NMDA is lower in DS cells than the affinity for the endogenous transmitter. And it is possible that although 100 μM AP7 eliminated all extracellular responses to NMDA in the Kittila and Massey experiment, subthreshold responses lingered. Second, to enhance the DS cell response under excitatory blockade, we used a surround mask. This mask would prevent extra-receptive-field facilitation, which is likely to arise from cholinergic amacrine cells (Amthor et al., 1996—see Introduction for more details). Thus, our stimulus protocol itself tips the excitatory balance in favor of the NMDA, bipolar input.

Combined blockades of nicotinic and NMDA receptors did not fully eliminate DS cell responses to the sweeping bar stimulus. Small DS-cell responses to muscarine and AMPA have been described by Kittila and Massey (1997), so this result is not a surprise. However, unlike the Kittila and Massey (1997) report, we found directional selectivity becoming statistically insignificant for the DS cell's residual response. We propose that this may occur due to differential locations of excitatory non-NMDA-non-nicotinic and inhibitory GABAergic inputs in the dendritic tree of the DS cell. Based on independent data, the glutamatergic inputs have been hypothesized to occur on both proximal and distal dendrites, whereas the GABAergic input was hypothesized as distal only (Grzywacz et al., 1997b). We now extend that hypothesis to propose that the minor, non-NMDA-non-nicotinic component of the excitatory input is relatively proximal. As null-direction inhibition appears to be of the shunting type (Amthor & Grzywacz, 1991), it is necessary that inhibition and excitation be electrotonically close for the inhibition to be effective (Koch et al., 1982). Thus, we feel that the loss of directional selectivity in residual responses may simply be due to the inhibition being placed too far from the proximal, non-NMDA-non-nicotinic excitatory input (for more evidence of this, see accompanying paper; Merwine et al., 1998). A possible explanation for the lingering directional selectivity in Kittila and Massey's data is that they failed to block the distal excitatory NMDA receptors completely (see previous paragraph).

Directional hyperacuity

Rabbit DS cells have been shown to respond in a directional manner to apparent motions as short as 1.1 μm (Grzywacz et al., 1994). This distance is about two orders of magnitude shorter than the rabbit's acuity to sine-wave gratings, and thus qualifies as a hyperacuity. If glutamate, through NMDA receptors, provides the dominant excitatory input to these cells with the nicotinic input primarily facilitatory, one would expect NMDA blockade to affect hyperacute motion sensitivity more strongly than nicotinic blockade. For seven of nine cells tested such was the case, and, on average, NMDA blockade increased the hyperacute motion threshold by $53 \pm 21 \mu\text{m}$ more than nicotinic blockade. Thus, NMDA input is integral to the DS cells' responses to positional changes in the hyperacuity range. Because retinal hyperacuity (though not its

directionality) is tightly related to contrast sensitivity (Shapley & Victor, 1986), we also infer that NMDA synapses are the main ones, endowing DS cells with their exquisite contrast sensitivity (Grzywacz et al., 1990).

Sine-wave grating

As mentioned previously, the cholinergic amacrine input to the DS cell appears to carry extra- (and most likely intra-) receptive-field facilitation. This facilitation is temporally sustained, and therefore may be utilized for low temporal-frequency responses. We tested this temporal-frequency prediction using a sine-wave grating drifting at various rates. Nicotinic blockade raised the low temporal-frequency cutoff for DS cells. This agreed with the prediction above and suggested that, although not the primary excitatory drive in DS cells, ACh, nonetheless, may have a crucial role in extending the low-velocity end of the DS cell temporal-frequency profile. That curare eliminated directional selectivity for a grating was significant, since the same drug fails to do so for a sweeping bar. In a separate paper, we point out that the glutamatergic system would be inefficient in dealing with moving textures, that is, images with multiple luminance peaks or elements, postulating that under these conditions the cholinergic system would be necessary for directional selectivity (Grzywacz et al., 1998). We would say that exactly as the glutamatergic system has a role in contrast sensitivity, the cholinergic system has its own roles, for instance, dealing with textures. In this case, one should view these systems as complementary. Their contribution to responses overlap over some range of stimulus parameters, explaining why nicotinic blockade fails to eliminate directional selectivity to a sweeping bar and blockade of NMDA fails to eliminate directional selectivity to a moving grating. This dual system is consistent with recent models of directional selectivity (Grzywacz et al., 1997b; Kittila & Massey, 1997), as is the differential distribution of transmitter types discussed in Sweeping bar.

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§One cannot be certain from our experiments that the effects of 500 μM AP7 are specific to glutamatergic receptors. However, by the same token, one cannot be certain that AP7 fully blocks them (Fig. 3).

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