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Non-monotonic contrast behavior in directionally selective ganglion cells and evidence for its dependence on their GABAergic input

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Abstract

We serendipitously discovered that the preferred-direction responses of ON–OFF directionally selective (DS) ganglion cells in the rabbit retina fall as a function of contrast when the contrast of a moving bar exceeds about 100%. Null-direction responses did not fall for contrasts up to 400%. Because the non-monotonic (rise-then-fall) behavior as a function of contrast occurred only for preferred-direction responses, it must depend on the mechanism of directional selectivity. It became thus of interest to investigate how this non-monotonicity depends on the major synapses involved in directional selectivity. Blockades of nicotinic acetylcholine (ACh) and NMDA glutamate receptors reduced responses without eliminating preferred-response non-monotonicity. Blocking GABAergic inhibition, however, did eliminate non-monotonicity. These results pose a difficult puzzle, since in the accompanying paper (Grzywacz et al., 1998), we showed that residual responses under combined nicotinic and NMDA blockades are not statistically significantly directionally selective. How is it possible that null-direction GABAergic inhibition affects non-nicotinic-non-NMDA residual responses without generating directional selectivity? This may happen if there exists an asymmetric GABAergic input to distal dendrites of the DS cell while the excitatory, non-nicotinic-non-NMDA input is to proximal dendrites. In support of this hypothesis, bath-applied GABA reduces responses to exogenous ACh under synaptic block, providing for the first time in the rabbit's retina, direct evidence of GABA receptors on DS cells.

Keywords: Retinal ganglion cells, Directional selectivity, Contrast, Saturation, GABA

Introduction

Previously, the contrast dependence of both the flashed-spot (Merwine et al., 1995) and apparent-motion (Amthor & Grzywacz, 1991) responses of ON–OFF DS ganglion cells of the rabbit's retina have been modelled with modified Michaelis-Menten equations. The Michaelis-Menten function accounted for the rapid rise and gradual plateauing of responses with increasing contrasts. However, most previous experiments used contrasts lower than 100%. We have found that the preferred-direction responses to bar motions do not simply saturate at contrasts larger than 100%, but begin to fall (see Fig. 2 of the accompanying paper—Grzywacz et al., 1998). Here we report on the phenomenology of this non-monotonic contrast behavior and relate it to the known pharmacology of retinal directional selectivity. Thus, we have utilized ACh, GABA, curare (nicotinic antagonist), AP7 (NMDA antagonist), and picrotoxin (GABA antagonist), to study the pharmacological substrate of this unusual non-monotonicity. A preliminary report of our findings has been presented in abstract form (Merwine et al., 1997).

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Methods

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

Physiological preparation

ON–OFF DS ganglion cells were recorded extracellularly in an everted, perfused eyecup preparation. In brief, adult Dutch belted pigmented rabbits were initially anesthetized with urethane (2 g/kg), followed by pentobarbital sodium given to effect. Following dark adaptation, an eye was removed, hemisected, and mounted in a recording chamber under dim red light. The eyecup was superfused with a transparent medium similar to that described by Ames and Nesbett (1981). All cells were recorded just below the visual

streak. A total of 55 cells from 25 retinas were recorded. Of these, 18 were tested for the effects of AP7; ten of which being further tested for the effects of combining curare with AP7. Six cells were tested with picrotoxin. Nine cells (plus an ON DS) were tested for GABA's effects on exogenous ACh application while under synaptic block.

Pharmacology

Following the collection of control responses, superfusion was switched to drug-containing media. The saturating concentrations of curare and picrotoxin for rabbit ON–OFF DS cells were previously shown to be 13 ± 11 μM and 28 ± 18 μM, respectively (Grzywacz et al., 1997). We used 60 μM curare and 30 or 60 μM (three cells each) picrotoxin. No dose–response function for AP7 has been determined, and we chose to examine both 250 μM (five cells, AP7 only condition) and 500 μM concentrations (remaining 13 cells; see accompanying paper for a justification of these concentrations—Grzywacz et al., 1998). To test for the presence of GABA receptors on the DS cell, synaptic block was first obtained by the addition of 0.75 or 1 mM $CoCl_2$ to the medium (Grzywacz et al., 1997). Block occurred within approximately 1 min as judged by the disappearance of responses to a high-contrast sweeping bar. ACh (50 μM) plus 30 μM physostigmine to boost the ACh response (Masland & Ames, 1976) was perfused after 4 min and the spike response recorded. Following return to normal medium, the same procedure was performed but with the inclusion of 5 mM GABA in the ACh superfusate. Responses were collected and the cell returned to control superfusion. Data from each experimental type were used only if recovery of directionally selective responses was obtained following return to normal medium.

Visual displays and responses

The visual stimulus was a light bar (600 μm wide and 2000 μm long) on a gray background moving perpendicular to the bar's long side (1000 μm/s) across the receptive field every 3 s. To reduce surround-inhibitory effects, the stimulus was limited by a mask (outer diameter = 1500 μm) to a square with sides of 200 or 300 μm at the center of the cell's receptive field. As a control, 11 cells were run in both masked and unmasked conditions. Motions tested were in the preferred or null direction. In each experiment, the contrasts displayed were a subset of 15, 25, 30, 40, 50, 60, 75, 80, 90, 100, 120, 150, 200, 300, and 400%, where contrast is defined as $C_{bar} = 100\% \ (F - B)/B$, with F and B representing foreground and background illumination, respectively. In all experiments, B = 23 lx. Each combination of contrast and tested direction was presented 20 times in pseudorandom order. The response measure was the mean number of spikes per trial collected over the entire motion.

The strength of directional selectivity was quantified at each contrast by calculating the directional selectivity index (*DSI*—Grzywacz & Koch, 1987), where $DSI = (p - n)/(p + n)$, with p and n representing the responses to preferred- and null-direction motions, respectively.

Results

Contrast dependence of responses

Fig. 1 shows the response histograms of an ON–OFF DS cell (e652c3) to motions in the preferred and null directions for contrasts that ranged from 25–200%. The preferred-direction response increased with contrast at low contrasts, but decreased as contrast exceeded 100%. The null-direction response continued to grow over the same range of contrasts. Not surprisingly, response onset began earlier for both preferred- and null-direction responses as contrast increased. However, for preferred- but not null-direction motions, the late portions of the response for both the leading and trailing edges progressively weakened (see arrows—Fig. 1 and Discussion). Fig. 2 presents the response-*versus*-contrast functions for this cell. Of 55 cells tested, 51 showed preferred-direction response decrements with a mean maximum decrease for all cells of 7.1 ± 1.0 spikes/trial [mean ± standard error (S.E.)]. This is equivalent to a mean percent reduction of 25 ± 3%. Of the 51 cells whose null-direction responses were measured, 46 showed continued growth, with a mean increase past the 100%-contrast point of 3.2 ± 0.5 spikes/trial, or 53 ± 9%. Due to the decrease in preferred-direction and increase in null-direction responses, the mean *DSI* fell for these cells from 0.54 ± 0.03 at 100% contrast to 0.29 ± 0.03.

Many authors, though not probing contrast dependence, studied ON–OFF DS cells with contrasts well above 100% (Barlow & Levick, 1965: 1900%; Wyatt & Daw, 1975: 2400%; Cohen & Miller, 1995: 900–1500%). It was curious, then, that our data showed directional selectivity falling far below normal for contrasts well below 1000%, yet these authors recorded strong directionally asymmetric responses. We show here that a protocol difference may help explain this discrepancy. To elicit significant responses from our cells while they were under excitatory blockade, we masked the surround. This limited stimulation to the excitatory center. Thus, surround inhibition (Merwine et al., 1995) as well as extra-receptive-field facilitation (Amthor et al., 1996) (for motions in the preferred direction) and inhibition (Amthor & Grzywacz, 1993) (for motions in the null direction) were removed. To study the effect of this removal, we recorded from ten cells with and without surround masking for comparison. Fig. 3 presents data from a cell (e692c4) under both conditions. When unmasked, cells had reduced responses to both preferred- and null-direction motions, presumably because of surround inhibition. Additionally, null responses were often reduced more than preferred ones, indicative of the asymmetric, extra-receptive-field inhibition (Amthor & Grzywacz, 1993).* As a result, for the cell of Fig. 3, the masked directional selectivity index (*DSI*—see Methods) at 400% contrast was only 0.12, while unmasked it was 0.65. For the ten cells, null-response reductions due to unmasking improved *DSI* at 400% contrast from a mean of 0.18 ± 0.04 (mean ± S.E.) to 0.40 ± 0.06. Thus, extra-receptive field processes seem necessary for effective DS cell function at high contrasts.

Although seven of the ten cells retained their preferred-response non-monotonicity when unmasked, it should be noted that three cells no longer showed the response fall-off, and there was a reduction in the magnitude of the fall for the majority of the remaining cells. Thus, surround masking, though not responsible for the phenomenon, did enhance the non-monotonic contrast dependence of the preferred-motion responses.

Because the non-monotonic (rise-then-fall) behavior as a function of contrast occurs only for preferred-direction responses, it must depend on the mechanism of directional selectivity. It be-

*The slopes of the linear, rising portions of both the preferred and null response-*versus*-contrast functions were also reduced when unmasked, consistent with the division-like nature of surround (Merwine et al., 1995) and null-direction (Amthor & Grzywacz, 1991) inhibition.

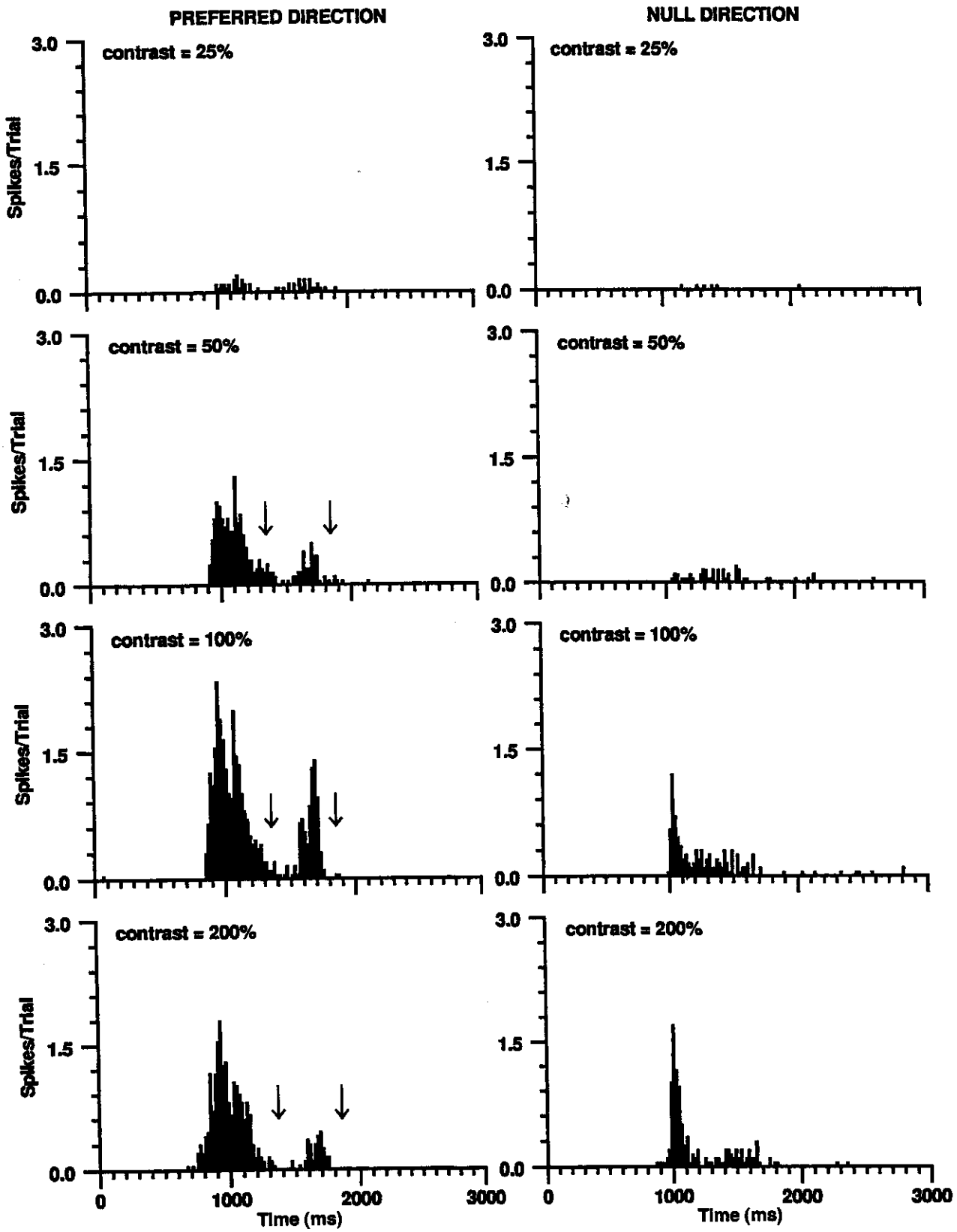

Fig. 1. Influence of contrast on poststimulus histograms of a cell (e652c3) responding to a bar moving in either the preferred (left column) or null (right column) direction. The rows correspond to different contrasts, which were, from top to bottom, 25%, 50%, 100%, and 200%. Time zero in the histograms corresponds to the onset of motion and the histogram's binwidths were 20 ms. For preferred- but not null-direction motions, both the leading- and trailing-edge responses fall as contrast exceeds 100%. Arrows indicate the loss of the late portions of the responses.

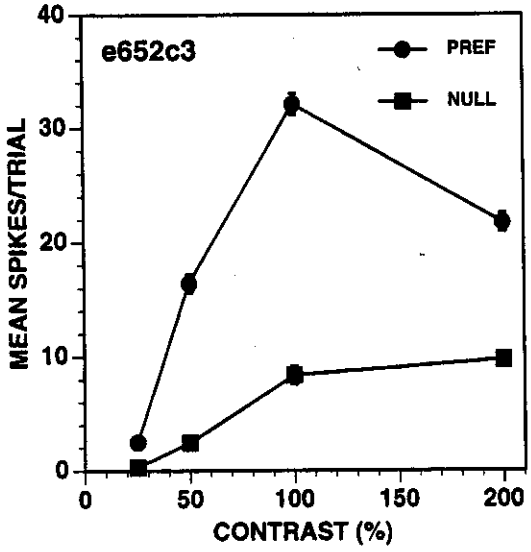

Fig. 2. The integrals of the preferred and null responses of the cell in Fig. 1 as a function of contrast. The circles represent response to motion in the preferred direction and the squares the response to motion in the null direction. The error bars stand for standard error of the response. Preferred but not null responses fall as contrast exceeds 100%.

comes thus of interest to investigate how this non-monotonicity depends on the major synapses involved in directional selectivity. In particular, we wondered whether blocking one or both of the primary excitatory pathways to the DS cells would prevent desensitization (Katz & Thesleff, 1957; Magleby & Pallotta, 1981) or depression (Takeuchi, 1958; Rosenthal, 1969) and thus eliminate the non-monotonicity.

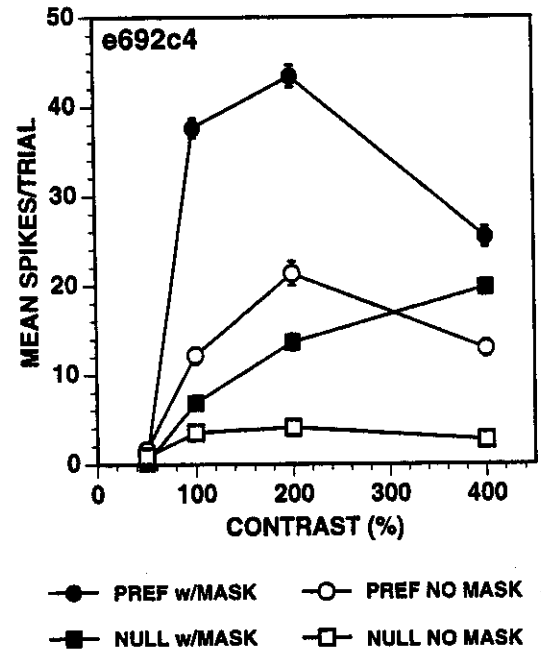

Fig. 3. Effect of surround masking on DS cell responses. Filled symbols show masked results and open symbols show results with no mask. Remaining conventions are as in Fig. 2. Removal of the mask causes reductions in both preferred and null responses, yet preferred-response non-monotonicity remains. Additionally, reduction of the null response by unmasking substantially improves directional selectivity at high contrasts.

Drug effects on non-monotonicity

The effect of 500 μM AP7 on the responses of the cell in Figs. 1 and 2 is shown in the top plot of Fig. 4. Both the preferred and null responses fell at all contrasts tested, and this result was typical and expected (Cohen & Miller, 1995; Kittila & Massey, 1997; Grzywacz et al., 1998). Of interest is that the non-monotonic contrast dependence of the preferred response was unaffected by AP7. AP7 was applied to 18 cells. One cell's responses fell to 0 for all contrasts in both directions. Fifteen of the remaining 17 cells retained the decrement in preferred response at high contrasts. A simple binomial test shows a chance probability for decrements in 15 of 17 cells of less than 0.0012. The mean preferred-response decrement over the 17 cells was 4.8 ± 1.0 spikes/trial or 28 ± 17%. (The large S.E. value is primarily due to one cell, which gave an outlier value.) Over the same contrast ranges, null responses grew 1.1 ± 0.3 spikes/trial or 76 ± 17% past the 100%-contrast value.

Curare was applied in conjunction with AP7 for ten cells. An example of curare's effect is shown in the middle plot of Fig. 4. Again, excitatory blockade reduced responses without affecting the non-monotonic contrast dependence of preferred-direction responses. One cell of the ten had no responses to any contrast tested under the drug combination (the same cell whose responses were eliminated by AP7 alone). Eight of the remaining nine cells retained the high-contrast response fall-off with a mean decrease over the nine cells of 1.1 ± 0.5 spikes/trial or 18 ± 19%. A simple binomial test shows a chance probability for decrements in eight of nine cells of less than 2%. Five of nine null-response functions showed growth past the 100%-contrast point, with a mean increase of 0.1 ± 0.4 spikes/trial or 11 ± 12%. Thus, because both AP7 and curare reduced responses without eliminating the rise-then-fall behavior, desensitization or depression of excitatory synapses cannot be the cause of non-monotonicity.

Six cells were tested with contrasts higher than 100% during picrotoxin administration. The bottom plot of Fig. 4 presents an example of picrotoxin's effects. Expectedly, preferred and null responses were greatly enhanced (Caldwell et al., 1978; Ariel & Daw, 1982; Grzywacz et al., 1997), but directional selectivity remained as previously shown for 47% of ON–OFF DS cells in the rabbit under full GABA blockade by Grzywacz et al. (1997). What was not expected is that GABAergic blockade eliminated the non-monotonic contrast dependence of the preferred-direction response for this cell. Non-monotonicity of the preferred response was eliminated by picrotoxin in five of six cells tested. The mean preferred response for contrasts larger than 100% showed an increase of 9.1 ± 5.6 spikes/trial or 34 ± 61%. Null responses continued to grow for all six cells with a mean increase of 11.2 ± 5.6 spikes/trial or 47 ± 29% past the 100%-contrast value. The AP7, curare, and picrotoxin results pose a difficult puzzle, since in the accompanying paper (Grzywacz et al., 1998), we showed that residual responses under combined nicotinic and NMDA blockades are not directionally selective. How is it possible that GABAergic inhibition causes the non-nicotinic-non-NMDA residual responses to be non-monotonic for preferred-direction but not null-direction motions, without making the responses directionally selective? In the Discussion, we will explain that this may happen if GABAergic input is to distal dendrites of the DS cell, while the excitatory, non-nicotinic-non-NMDA input is to proximal dendrites. An im-

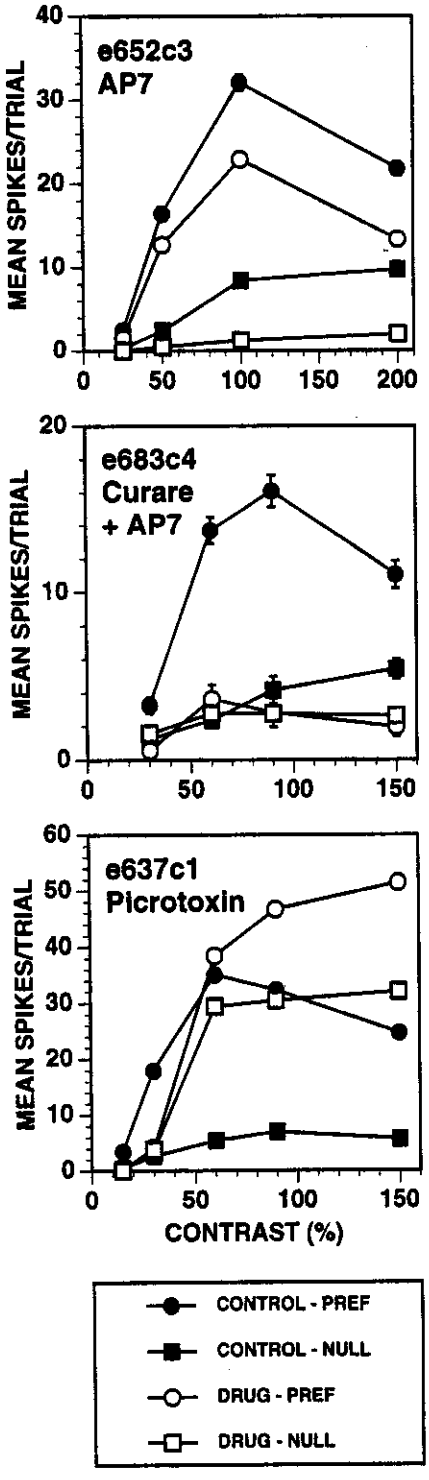

Fig. 4. Examples of the effects of various drugs on the contrast dependence of the integral of the preferred and null responses. Filled symbols represent control results and open symbols represent drug results. Remaining conventions are as in Fig. 2. AP7, curare, and picrotoxin concentrations were 500 μM, 60 μM, and 30 μM, respectively. Only picrotoxin eliminates the non-monotonic contrast dependence found during control data collection.

portant missing link in this hypothesis is a direct demonstration of GABA receptors on the DS cell. Such a demonstration exists for turtle (Ariel & Adolph, 1985), but not as yet for rabbit. Next, we report on an experiment in rabbit similar to that performed by Ariel and Adolph (1985) in turtle.

GABA receptors on the DS cell

To determine whether GABA receptors are present on ON–OFF DS ganglion cells, we studied the effect of bath-applied GABA (5 mM) on the responses to exogenous ACh (50 μM) during synaptic blockade. As shown in the turtle (Ariel & Adolph, 1985), GABA drastically reduces the response to applied ACh. Fig. 5 shows the results for one cell (e644c3). During synaptic block, exogenous ACh (plus 30 μM physostigmine) caused a brief burst of 159 spikes. The brevity of the burst was most likely due to the cell entering a depolarization block as observed previously (Masland & Ames, 1976; Grzywacz et al., 1997). With GABA present in the superfusion, the response to ACh was only 5 spikes. Similar results were obtained in nine of nine cells, with a mean number-of-spike reduction of 81 ± 6%. An ON DS cell (e645c1) was also recorded with similar results (ACh spikes = 318, ACh + GABA spikes = 1). These data demonstrate the existence of GABA receptors on rabbit DS ganglion cells.

Discussion

Phenomenology

While pharmacologically testing whether nicotinic and NMDA synapses have complementary roles for directional selectivity in ON–OFF DS cells of the rabbit retina (Grzywacz et al., 1998), we discovered an unusual contrast dependence for responses to preferred-direction motions of a sweeping bar. Because combined application of curare and AP7 dramatically reduced responses, we thought to compensate for the loss by increasing contrast. To our surprise, as contrast increased beyond 100%, preferred-direction responses did not just saturate, they fell. In contrast, null-direction responses did not fall over the same range of contrasts. This non-monotonic contrast dependence of the preferred-direction response was not previously described. In some ways, this is not surprising as sine-wave studies, for example, could not present contrasts larger than 100%.† Moreover, our prior studies tailored the examined contrast range to collect data over the rising portion of the response-*versus*-contrast function, generally stopping at contrasts less than 100%.

What is the importance of this non-monotonic contrast behavior? Because reflectances of natural objects are in a narrow range (Krinov, 1953), it has been suggested that natural contrasts are low (Shapley & Enroth-Cugell, 1984). Consistently, measurements of natural contrasts obtained with a photometer equipped with a detector whose field of view was about 1 deg found that natural contrasts are typically below 50% (Vu et al., 1997). However, measurements made with calibrated CCD technology at much higher spatial resolution (≈ 0.1 deg of visual angle) showed that natural RMS contrasts (100% times the standard deviation over the mean of pixel intensities) are much higher (van der Schaaf & van Hateren, 1996). In a sample of 276 images, these contrasts ranged from about 20% to about 230%, having a mean of 92 ± 44%

†Recall that the contrast of a grating is typically defined as $C_{grating}$ = 100% $[(L_{max} - L_{min})/2]/[(L_{max} + L_{min})/2]$, where L_{max} and L_{min} are the maximum and minimum luminances of the grating, respectively. Thus, as L_{min} approaches zero, $C_{grating}$ approaches its maximum of 100%. Strictly speaking, this definition of $C_{grating}$ is different from the definition of C_{bar} given in Methods. However, they become practically the same thing when one remembers that the mean intensity is $(L_{max} + L_{min})/2$ for gratings and to a close approximation B for thin bars.

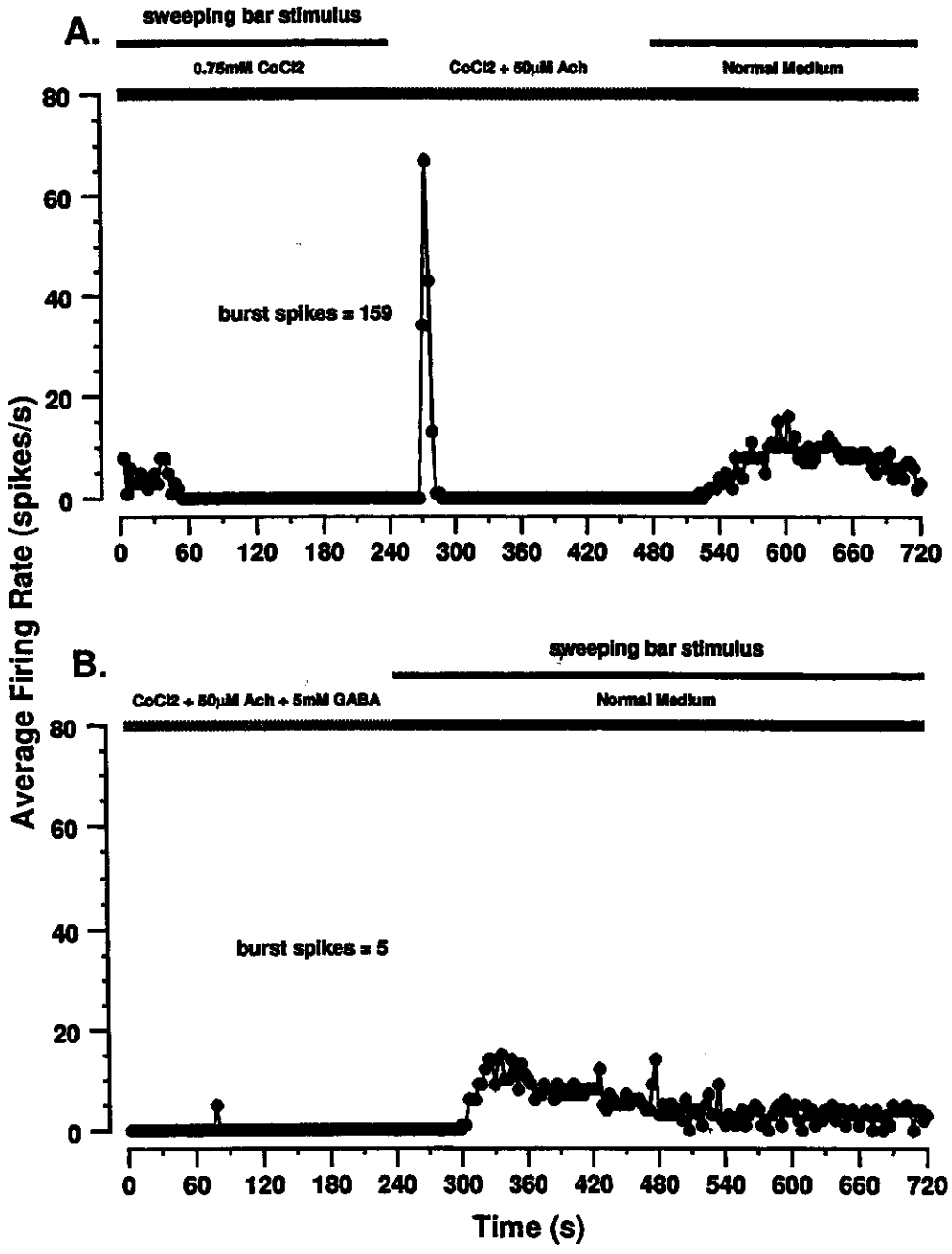

Fig. 5. Effect of 5 mM GABA on the response to exogenous ACh during synaptic block. (A) During sweeping bar stimulation, superfusion was switched to a mixture containing 0.75 mM $CoCl_2$, causing synaptic block in about 1 min. After 4 min, the stimulus was removed and 50 μM ACh (plus 30 μM physostigmine) was added to the superfusate causing a brief burst of 159 spikes. The cell was then allowed to recover. (B) After repeating synaptic block, ACh (plus physostigmine) was again delivered, this time with 5 mM GABA in addition. GABA reduced the burst to 5 spikes, a reduction of 97%. Recovery of light responses confirm that the cell was not lost during drug treatment.

(standard deviation). These high spatial resolution contrasts are more relevant for the rabbit than the low-resolution ones. Based on measurements of rabbit photoreceptor density (Young & Vaney, 1991), Grzywacz et al. (1994) estimated that the acceptance angle of rabbit photoreceptors can be as narrow as 0.013 deg (1.9 μm). Consequently, it is conceivable that rabbit retinas have to deal with contrasts higher than 100%, possibly making the non-monotonic contrast dependence behaviorally relevant for the animal. It is unclear, though, whether this dependence has any function for the rabbit. A possible function stems from the reduction of response at high contrasts occurring primarily at its late portion (Fig. 1). Hence, the preferred response becomes more transient (faster) and possibly endows higher temporal resolution to the cells. In theory, this change in temporal resolution can occur at high contrasts, because these contrasts evoke high signal-to-noise ratios without requiring much temporal integration.

Possible mechanisms for the non-monotonicity

Besides having a possible behavioral significance, the non-monotonic contrast behavior puts strong constraints on mechanisms of directional selectivity. A first explanation for the non-monotonic behavior is that some voltage-dependent channel in the DS cell activates or inactivates as it depolarizes with contrast. This would cause something equivalent to a depolarization block or a refractory process. However, such processes cannot provide the answer, since reduction of depolarization with curare and AP7 does not affect non-monotonicity. Another simple alternative for the non-monotonic contrast dependence would be postsynaptic receptor desensitization (Katz & Thesleff, 1957; Magleby & Pallotta, 1981). Such an inactivation would occur as the concentration of neurotransmitter in the synaptic cleft rises with contrast. However, again, our pharmacological data do not support such receptor inactivation as the explanation for non-monotonicity. Blockades of the major excitatory pathways, the nicotinic and NMDA receptors (Ariel & Daw, 1982; Cohen & Miller, 1995), result in decreased responses without eliminating preferred-response non-monotonicity. It is also unlikely that inactivation of one of the minor excitatory pathways involved (for instance, muscarinic or non-NMDA glutamatergic—Kittila & Massey, 1997) mediate the non-monotonic behavior. The problem with this alternative is that it does not explain the large fall of response with contrast (≈25%) in control conditions.

Rather, the data indicate that an active GABAergic inhibition is necessary for the non-monotonicity. Furthermore, this inhibition is probably spatially asymmetric, since the non-monotonic behavior occurs only for responses to preferred-direction motions. And this inhibitory process should act on the nicotinic, NMDA, and non-nicotinic-non-NMDA systems, or their outputs. This is both because directional selectivity remains despite blockade of either the nicotinic or NMDA pathways, and because the percental fall in response with contrast at high contrasts is similar regardless of the excitatory systems present. These arguments for a role of asymmetric inhibition in directional selectivity speak against any models in which the only asymmetry is in an excitatory pathway (Vaney, 1990; Borg-Graham & Grzywacz, 1992). They are consistent with models based solely on asymmetric inhibition (Barlow & Levick, 1965; Wyatt & Daw, 1975) or models based on both asymmetric inhibition and asymmetric excitation (Grzywacz et al., 1997).

Parsimony requires that the GABAergic inhibitory system acts on all these excitatory systems in a single cell, most likely the DS ganglion cell itself. We now have evidence for GABA receptors on the DS cell (Fig. 5). Here we examine this evidence further, and in particular, the assumption of synaptic blockade. Synaptic blockade eliminates conventional synaptic inputs to the DS cell. Thus, exogenous GABA should not have its inhibitory effect on the DS cell through synaptic contacts from other cells.‡ However, there may be gap junctions between DS cells and starburst amacrine cells (Xin & Bloomfield, 1997), which could allow network effects during blockade. It seems highly doubtful, though, that these gap junctions would permit the response decrement in the presence of GABA, as this would require that the ACh-induced current be shunted twice, from the DS cell to the starburst cells, and then from the starburst cells to the extracellular space. Evidence against the first form of shunting is that receptive fields of DS cells correlate tightly with their dendritic trees (Amthor et al., 1984; Yang & Masland, 1992). Hence, starburst-ganglion gap junctions do not appear to be sufficiently strong to enlarge the DS cells' receptive fields. Therefore, because this gap-junction explanation is not plausible, we conclude that there probably are GABA receptors on the DS cell. This does not mean that DS cells obligatorily receive GABAergic inhibition—directional or otherwise. It is possible that directionally asymmetric GABA could be released onto both bipolar and starburst amacrine inputs, and that the GABA receptors on the DS cell are involved in some other function, such as center-surround inhibition (or had a developmental role and are no longer functional). However, their presence is highly suggestive of a functionally asymmetric, direct, GABAergic inhibitory input to DS ganglion cells.

But if this is the case, why is the residual response under simultaneous curare and AP7 application not statistically significantly directionally selective (accompanying paper—Grzywacz et al., 1998)? In the accompanying paper (Grzywacz et al., 1998), we proposed that the non-nicotinic-non-NMDA excitatory input to the DS cell is into proximal dendrites, whereas the GABAergic input is into distal ones. In this case, because null-direction inhibition in rabbit DS cells appears to be of the shunting-type (Amthor & Grzywacz, 1991), it would be poorly positioned to transform this excitatory input into a directionally selective output (Koch et al., 1982). A distal GABAergic input and a proximal glutamatergic excitatory input have previously been proposed to explain the finding that physostigmine could saturate the directionally selective mechanism without saturating the DS cell itself (Grzywacz et al., 1998). Thus, we propose that residual responses under nicotinic and NMDA blockades are nondirectionally selective because the asymmetric GABAergic inhibition is not placed appropriately to affect significantly the residual, proximally dominated excitation.

We propose that the non-monotonic behavior emerges from a shortening of the delay of GABAergic inhibition in the preferred direction as contrast increases. At low contrasts, inhibition would be too delayed to have an impact on preferred responses. But at high contrasts, inhibition might reach a sufficiently high value at times early enough to inhibit at least part of the excitation. There is support for this notion in Fig. 1, which shows a progressive loss of the late portions of the response for both the leading and trailing edges as contrast increases. In comparison, for null-direction motions, shortening of delay with contrast would have no impact, since, in this case, inhibition is thought to begin before excitation takes off.

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‡The only known unconventional synapse in the retina releases GABA itself through transporters (Schwartz, 1982; O'Malley et al., 1992; Johnson et al., 1996). But the exogenous GABA could only inhibit DS cells through this synapse, if the DS cell had GABA receptors, which is the point that we are making anyway.

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