Adaptation to light fluctuations in the frog retina

Donner, K.

Elsevier
1991

Neuroscience Research Supplement. 1991. 15: S175-S184

http://hdl.handle.net/1975/945

Neuroscience Research, Suppl. 15 (1991) S175–S184

ADAPTATION TO LIGHT FLUCTUATIONS IN THE FROG RETINA

KRISTIAN DONNER, KAJ DJUPSUND, TOM REUTER and IRMA VÄISÄNEN
Department of Zoology, University of Helsinki, Arkadiankatu 7, SF-00100 Helsinki, Finland

INTRODUCTION

In this study, we characterize a retinal mechanism by which light fluctuations decrease the gain of ganglion cells on a time scale much longer than the integration time for photoresponses. The extended time scale (tens of seconds to minutes) makes it justified to speak about a true adaptation mechanism, distinct from the classical action of receptive field surrounds, where the gain control depends on events essentially simultaneous with the excitatory stimulus (1-6).

The experiments described investigate how repetitive stimulation or sinusoidal modulation of background lights desensitize ganglion cells in the frog retina under conditions where there are no significant changes in the adaptational state of the photoreceptors. The pharmacology of the underlying mechanism(s) is studied by addition of neurotransmitter agonists and antagonists to the retina. Our conclusion is that this kind of adaptation is not due to a buildup of "surround" neurotransmitters. In fact, it can be confined to subregions within the receptive field center, suggesting that the mechanism is a local gain change in a subset of excitatory synapses.

While the literature contains several observations indicating retinal desensitization by light fluctuations, its significance as a mechanism for light-adaptation may be much more general than has been appreciated. We propose that it could underlie much of the "proximal" adaptation to background light described in vertebrate retinas (5, 7-10). A main function would be to protect the dynamic range of ganglion cells from being congested by fluctuations which are neither spatially nor temporally coherent, e.g., photon noise and intrinsic noise in retinal neurons.

MATERIAL AND METHODS

Action potentials were extracellularly recorded from single ganglion cells in the eyecup of *Rana temporaria*. The main details of preparation, recording, optics, light calibrations and cell classification have been described elsewhere (11-17). The ganglion cells studied here were classified as class 1-2, class 3 or class 4, with the following distinguishing features of their responses to light:

Class 1-2 cells typically respond with long, low-frequency discharges to both the onset and the offset of small spots of light centered on the receptive field (RF), but not to large-field changes in illumination. They respond best to small light or dark spots moving across the RF, and continue to fire even for several seconds if the spot stops in the middle of the RF.

Class 3 cells are also on-off center cells but are distinguished from class 1-2 by responding

Presented at the 13th Taniguchi International Symposium on Visual Science, November 26–30, 1990

well to long moving edges and full-field changes in illumination. Their responses are typically brisk and short, especially with larger stimuli.

Class 4 cells are off-center cells responding with long discharges to any decrease in the illumination of the RF center, equally well to full-field stimuli as to spots matching the RF center. Unless the 432 nm "green" rods are stimulated (see below), they never give on-responses to center stimulation.

Two types of experiments were performed: 1) recording of the spike discharge patterns in response to repetitive square-wave stimulation with a small test spot of supra-threshold intensity in the photopic state; 2) recording of the mean discharge rate and flash thresholds under a dim full-field background, the intensity of which was either steady or sinusoidally modulated.

Experiment 1. The retina was continuously illuminated by a strong 558 nm background light. One "adaptation" sequence (duration 42.5 s) consisted of nine square-wave cycles of 2.5 s light pulses delivered at 2.5 s intervals superimposed on the background. Unless otherwise noted, this increment stimulus was a small (0.11 mm) circular spot of 615 nm light centered on the receptive field, of intensity 1 log unit above threshold. After the offset of the ninth light pulse, "recovery" was monitored by presenting one more pulse 10, 15, 20, 30 or 40 s later and a final one after 2-3 min. Thus, each run yielded one point (plus a final value) on the "recovery" function, and full characterization of one cell usually comprised 4 - 6 runs.

The combination of a strong 558 nm background plus a 615 nm stimulus was chosen to ensure that all responses were driven by a single type of photoreceptor, 575 nm cones. The frog retina has four types of receptors: 432 nm rods, 502 nm rods and (accessory members of double) cones, and 575 nm cones. All the ganglion cell classes 1-4 receive input from the 575 nm receptors (12). The 558 nm background saturates 502 nm rods, and the 615 nm stimulus pulses were sub-threshold for the 432 nm rods and 502 nm cones.

In the pharmacological experiments, the same protocol was repeated after addition of transmitter antagonists or agonists: GABA antagonist picrotoxin, glycine antagonist strychnine, dopamine agonist D-amphetamine sulfate, or dopamine antagonists haloperidol ("serenase"), chlorpromazine chloride, or pimozid (Janssen Pharmaceuticals, Holland). The drugs were applied by releasing (through a microsyringe) a 2 µl drop of drug Ringer into the ca. 0.2 mm layer of vitreous left in our eyecup preparations (15-17). The drug concentrations in the Ringer solutions were chosen to give estimated tissue concentrations of 50 - 500 µM (assuming homogeneous drug distribution in 15-20 mg tissue, which was the average weight of the retina + pigment epithelium + residual vitreous in our eyecups).

Experiment 2. Backgrounds which could be sinusoidally modulated at desired depth and frequency were provided by a 555 nm LED driven by a computer-controlled current source. The light was led through the normal "background" optical channel to produce a homogeneous field on the retina ("full-field", in fact ca. 1/3 of the retinal area). Test flashes (100 or 67 ms) fo threshold determination were delivered through the stimulus channel at 60 s intervals (0.11 mm spot of 513 nm light centered on the receptive field). Thresholds were determined, on one hand, during 15-20 min periods of steady background and, on the other hand, during 15-20 min periods of sinusoidal modulation (usually at 50 or 75% depth) around the same mean

intensity. During the modulation periods, 50 seconds of modulation alternated with 10 s intervals where the background was held steady at the mean level; the test flash was always given 2 s after the beginning of the steady interval. Thus, variation due to the phase of modulation and antagonism from the receptive field surround mechanisms were avoided and only persisting sensitivity changes were recorded. These experiments were recorded on tape for later analysis of the mean rate of discharge under the two types of backgrounds.

RESULTS

Repetitive stimulation in the photopic state

Time-course of adaptation and recovery. Fig. 1 A exemplifies how the full spike discharge patterns of one class 1-2 cell changed under the repetitive stimulation protocol (see Methods). Adaptation is evident as a decrease in the number of spikes and an increase in the latency of the responses to successive stimulus cycles. Both changes were virtually complete by the sixth cycle (after 25 s). The spike number was then only 18% of the original. When the cell was allowed a recovery period of 15 s between the last (ninth) light pulse and the recovery-monitoring pulse, the spike number returned to 65% of the original, and after ca. 2 minutes the response had attained its original strength. Fig. 1 B summarizes the adaptation and recovery of spike numbers for both on and off responses in a sample of class 1-2 cells. At the end of the

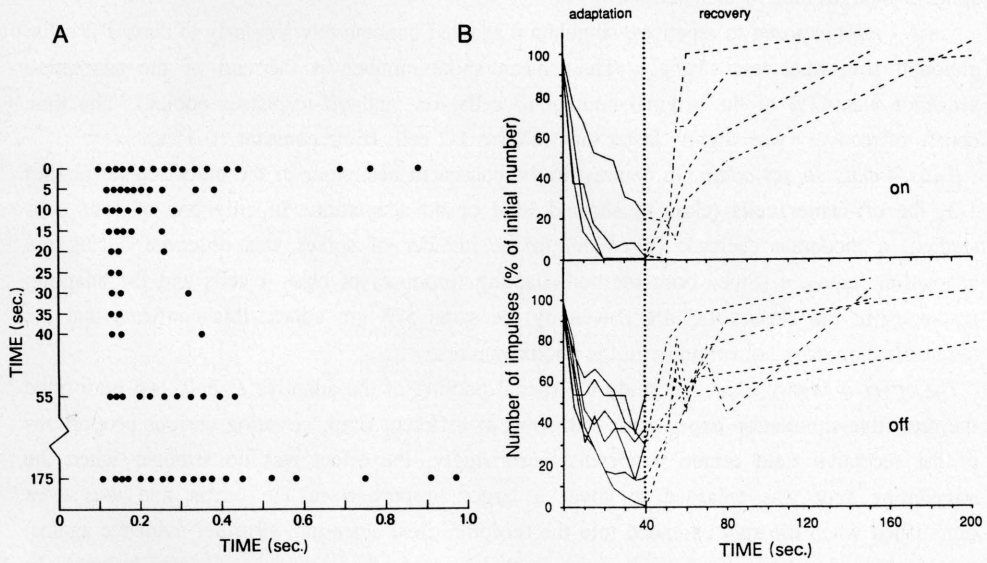

Fig. 1. (A) Adaptation of the off-discharges of a class 1-2 cell under one sequence of repetitive stimulation. The ordinate gives the moments of the falling edges of the successive 2.5 s light pulses. Each row of dots is the spike discharge in response to the falling edge (time 0 on the abscissa) in one stimulus cycle; each dot is one action potential. "Recovery" is monitored (in this run) by stimuli presented 15 and 135 s after the last adapting stimulus. (B) Time-course of adaptation and recovery of the number of spikes in class 1-2 cells: top panel, on-responses; bottom panel, off-responses. Each curve shows data from one cell; each point during the adaptation sequence is the mean of data from 4-6 runs, while the "recovery" points are usually single measurements.

adaptation sequence, the average spike number of the on-component had fallen to 15% (7 cells; only 4 shown in the figure to avoid clutter) and that of the off-component to 33% of the pre-adaptation average (9 cells of which 6 are shown). Both components adapted with the same "time constant" (time within which 63% of the total change occurred under these conditions) of 10-15 s (corresponding to an average of three stimulus cycles). The time constant of recovery was about 20 s.

In one class 1-2 cell, where the off-response was completely abolished after the eighth cycle, we compared this effect with the effect of a *steady* exposure of the adapting spot over the same time (42.5 s) as taken by one repetitive-stimulation sequence. When this steady light spot was turned off, the average response was 46% *stronger* than the unadapted off-response to a 2.5 s pulse, and the response to a 2.5 s pulse presented 5 s after the offset was almost as strong (92%) as the unadapted one. Thus, the effect of repetitive stimulation is not due to background adaptation in the photoreceptors, or to any standing potential in the retina that can be induced by a steady adapting light.

We also performed a quantitative analysis of changes in response latency, which reflects the size of the excitatory signal received by the ganglion cell more directly than does spike number. The theoretical basis for this is described in ref. (18). The adaptation process judged by latencies was essentially similar to that monitored by spike numbers (as can be qualitatively appreciated from Fig. 1 A). This fact indicates that the changes represent a real desensitization (gain decrease) and are not simply due to a lowered capacity of the ganglion cell to give long spike discharges (see further below).

Class 3 cells exposed to repetitive stimulation adapted qualitatively similarly to class 1-2 cells, although somewhat less strongly. Their mean spike number at the end of the adaptation sequence was 43% of the original number (7 cells, on- and off-responses pooled). The time course of recovery was slightly faster than in class 1-2 cells (time constant 10-15 s).

Class 4 cells do not adapt. In contrast to the consistent behaviour of the on-off center classes 1-3, the off-center cells (class 4) showed little or no adaptation. In only one of four cells studied, a moderate decrease (by 40%) in the number of spikes was observed during the adaptation sequence. Since both the non-adapting responses of class 4 cells and the adapting responses of the classes 1-3 are driven by the same 575 nm cones, this confirms that the desensitization does not originate in the photoreceptors.

The effect is highly local. To study the spatial pooling of the adaptive signals, we performed the repetitive-stimulation experiment with spots of different sizes, covering various proportions of the receptive field center. Somewhat surprisingly, the effect was no stronger when the stimulating spot was enlarged to cover a larger proportion of the center and was even diminished when the spot extended into the receptive field surround. (Further evidence against the involvement of surround mechanisms in this type of adaptation is considered below.). In fact, the very smallest spots could evoke the maximum possible desensitization within the area they covered.

The following experiment was performed to determine how local the effect really is (Fig. 2). First, two equisensitive sites in the receptive field of the cell under study were identified by

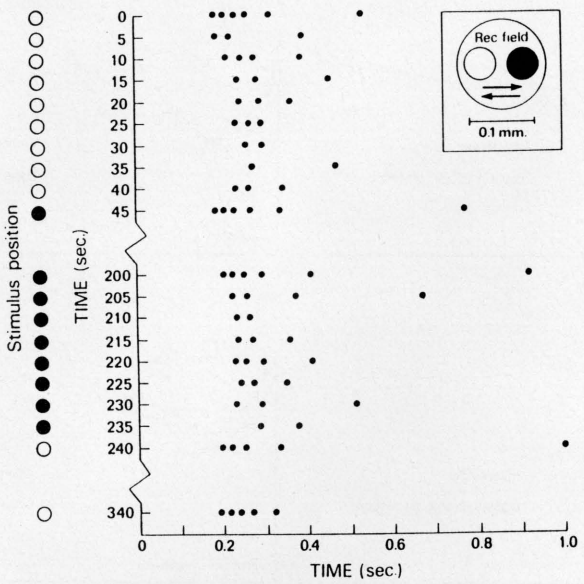

Fig. 2. Desensitization is confined to the subregion of the receptive field center which has been covered by the adapting spot. The inset (upper right) shows the receptive field and the two equisensitive sites (1 and 2) where a 50 μm diameter spot was presented. Stimulus protocol on the left: an open circle symbolizes a 2.5 s light pulse at site 1, a filled circle a pulse at site 2. Conventions otherwise as in Fig. 1 A.

mapping with a small spot (50 μm diameter). Site 1 was exposed to the usual adaptation sequence (nine cycles of stimulation). Then without break, a tenth 2.5 s pulse was presented at site 2. Instead of the highly enfeebled discharge which would have resulted, had the test stimulus been given at site 1, the cell now responded with a discharge of virtually the original strength. In other words, the desensitization due to the adapting sequence had not spread over the whole receptive field center. The result was similar when site 2 was adapted and site 1 tested thereafter (bottom part of Fig. 2, from 200 s onwards).

This experiment (done on 3 cells with similar results) yields two important conclusions: (1) the desensitization does not spread measurably beyond the subregion of the receptive field center which is covered by the adapting stimulus, and (2) it does not reside in the spike-generating mechanism of the ganglion cell.

Pharmacology of the mechanism. On stimulation of the receptive field center, "surround" antagonism is inevitably activated, since the center and surround mechanisms overlap spatially (17,19). Conceivably, the desensitization could be due to a gradual build-up of inhibitory or modulatory neurotransmitters. In pharmacological experiments, we focussed on the two main transmitters implicated in lateral inhibition, GABA and glycine (15-17,19-21), and particularly on dopamine, the transmitter of interplexiform and some amacrine cells and a major neuro-modulator in the retina (22,23). To interfere with the two former, we employed the commonly used antagonists picrotoxin and strychnine. To interfere with dopamine, we used the antagonists chlorpromazine, haloperidol and pimozid, as well as the agonist amphetamine.

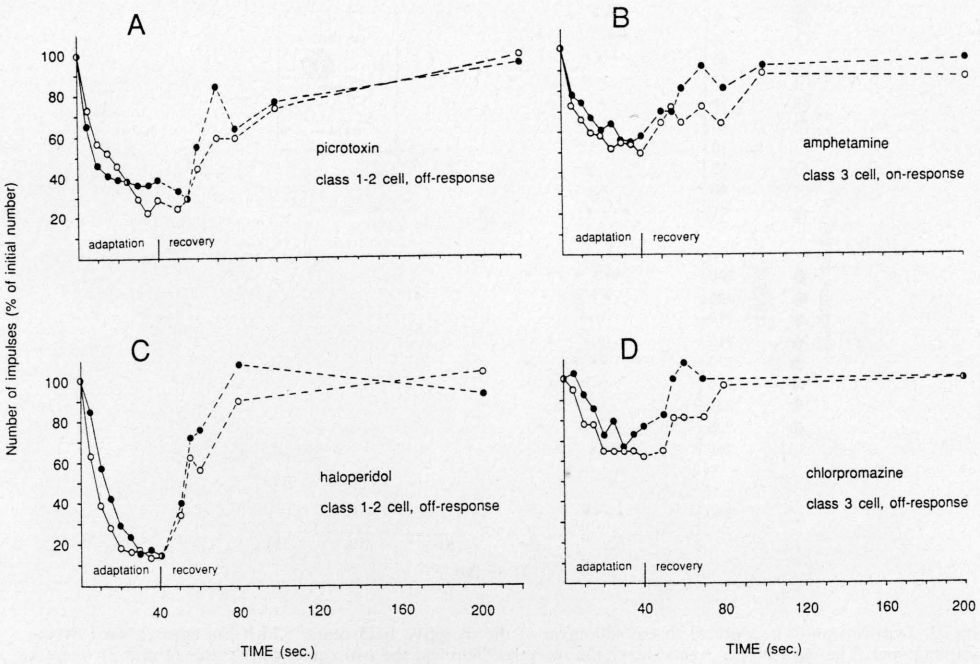

Fig. 3. Lack of changes in adaptation when (A) picrotoxin; (B) amphetamine; (C) haloperidol; (D) chlorpromazine were added to the retina. Tissue concentration 50 - 500 μM. Open circles before, filled circles after drug application.

All of these attempts to affect the adaptation to repetitive stimuli gave essentially negative results. The examples of Fig. 3 illustrate that, at least to a first approximation, the adaptation process remained unaffected. It should be noted that picrotoxin and strychnine applied by the same technique and in the same concentrations have been found to have strong effects on lateral inhibition (15-17).

Sine-wave modulated scotopic backgrounds

Adaptation to repetitive stimulation is not restricted to the photopic state. In the dark-adapted retina, this kind of adaptation is of special interest as, possibly, one expression of a mechanism for what we have termed "noise adaptation", i.e., the desensitization of ganglion cells in proportion to quantal noise from dim background lights (square-root or Rose-deVries law, see ref. 9). We have put forward the hypothesis that this type of desensitization could be driven by the fluctuations in the neural signal which result from the quantal fluctuations of the light (9). Here, we shall therefore consider an experiment designed to elucidate this possible connection, rather than simply reproducing, for the dark-adapted state, results of the repetitive-stimulation experiment. The idea was to add imposed fluctuations to the quantal fluctuations of the background light, and monitor effects on sensitivity and the maintained discharge.

Sensitivity. Cells (initially dark-adapted) were exposed to backgrounds delivering, in different experiments, from 0.03 to 3 photoisomerizations per rod per second (Rh^*s^{-1}). By comparing flash thresholds recorded under a certain background 1) when held steady, and 2) when modulated around the same mean intensity in sine-wave fashion, the effect of modulation as such could be assessed (see Methods). Modulation was always interrupted for 10 s starting 2 s before the presentation of flashes; thus the flash thresholds measured against modulated backgrounds reflected persisting gain changes and did not depend on phase relations or lateral antagonism.

The results qualitatively supported the idea that background fluctuations cause a longer-term desensitization of the ganglion cell. However, the sensitivity decrease evoked by the modulation was rather modest in these experiments with full-field backgrounds, typically 0.1 - 0.2 log units and never larger than 0.3 log units (11 on-off center cells). Fig. 4 shows the threshold rise in one cell as a function of modulation frequency. The greatest desensitization (at this temperature, 11 °C) was achieved with 0.2 - 0.5 Hz, while the effect fell off shallowly towards lower frequencies and more steeply towards higher frequencies.

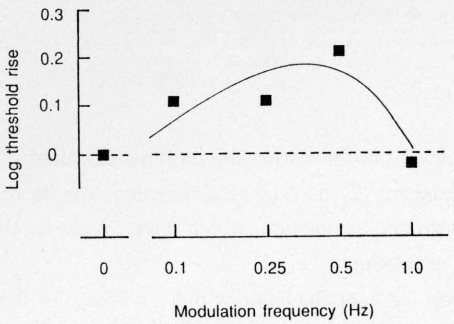

Fig. 4. Flash thresholds measured 2 s after the end of sine-wave modulation of a dim background (mean intensity 1.5 Rh^*s^{-1}, 75% depth), plotted as functions of the modulation frequency (Hz). Thresholds are given in relation to that measured against the same background when not modulated (log threshold rise = 0).

Maintained discharge. We have previously argued that the main "purpose" of noise adaptation in ganglion cells is to keep the maintained discharge low in the face of quantal fluctuations. After the onset of a dim steady background, the mean discharge rate first rises, but then falls back to approximately its dark level over some 5 - 10 min (9). It therefore appeared interesting to study whether the fluctuation-driven mechanism considered here could serve that purpose. Fig. 5 illustrates that it can. A dark-adapted cell with stable maintained discharge in darkness (panel A) was exposed to a dim background, which caused a transient rise in the rate of discharge followed by a gradual return to approximately the original level (panel B). When the steady background had been on for 12 minutes, sine-wave modulation around the mean was begun. The rate of discharge after the onset of modulation (panel C) was indeed found to rise and then relax with a time-course similar to that after the initial onset of the steady background.

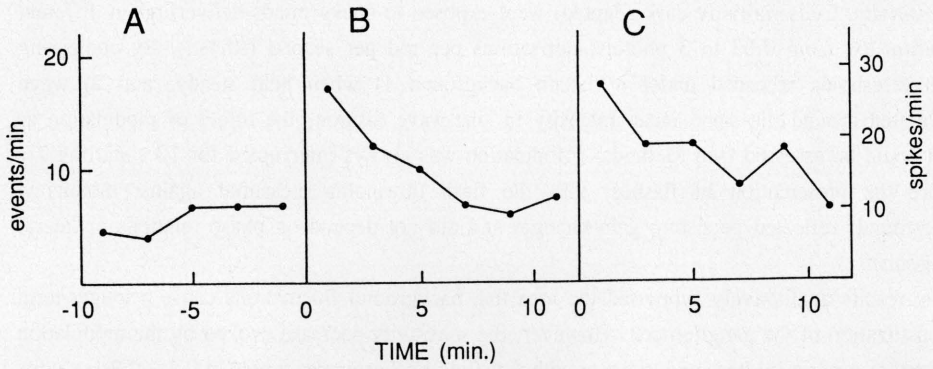

Fig. 5. The maintained discharge of a ganglion cell (A) in darkness during 10 minutes preceding background illumination, (B) during 12 minutes after a very dim steady background (0.03 Rh^*s^{-1}) has been turned on), (C) during 12 minutes after the beginning of sine-wave modulation of the same background (0.2 Hz, 75% depth). Each dot is the mean count over 2 minutes. In panels (A) and (B), the ordinate is our usual measure for maintained activity in frog, events/min (see refs. 9 and 14), but in panel (C) the dimension is spikes/min, since it is not meaningful to delimit events when they are paced by a periodic light. Note that both the background as such and the onset of modulation induced a transient rise in maintained activity which fell back with approximately the same time constant.

DISCUSSION

We shall consider three aspects of the fluctuation-driven desensitization of ganglion cells: (1) the site and physiological mechanism; (2) its biological function; (3) its relation to previously described components of background adaptation which take place in the proximal retina, independently of photoreceptor adaptation.

Mechanism. The desensitization by light fluctuations did not reside in the photoreceptors. In the dark-adapted state, modulation-driven adaptation was evident even with backgrounds giving each rod as little as one isomerization per 30 seconds on average. In the photopic state, it was found that the responses of class 4 cells did not generally adapt, although driven by the same 575 nm cones as the adapting responses of classes 1-3. The mechanism thus resides in the neural network between photoreceptors and the ganglion cell.

It is noteworthy that in cases where two mechanisms with different extents of spatial pooling of adapting signals have been implicated in the background adaptation of ganglion cells, (goldfish, rat and cat: refs. 24-26), photoreceptors have remained a likely site of the more local mechanism. Despite its very local nature, however, this is definitely not the case for the fluctuation-driven mechanism described here.

The desensitization showed no dependence on the action of some of the most obvious neurotransmitter candidates, i.e., GABA, glycine, or dopamine. Experiments with larger adapting spots indicated that stronger activation of the receptive field surround weakened rather than enhanced the desensitizing effect. Adaptation thus seems not to be based on surround-type antagonistic interactions. The mechanism appears to be a sharply localized, activity-dependent gain decrease in subsets of the excitatory synapses on the ganglion cell. Whether such a subset

corresponds to the input from any individual presynaptic cell (bipolar or amacrine) must await further study.

Function. The output (discharge rate) of ganglion cells has a narrow dynamic range and could easily be congested by maintained spiking (see discussion in ref. 9). What type of adaptational mechanism could be effective in preventing this? In principle, the threat of saturation of any neuron comes from precisely the types of stimuli to which it *responds* well. Signal transmission to ganglion cells commonly involves some degree of differentiation (see e.g. 27,28), so they would seem to need a mechanism to protect their output not against steady DC-signals, but specifically against *fluctuations* in the excitatory drive. Attenuation of responses to fluctuations that have little or no information content would help to protect the ganglion cell output and yet bring little biological disadvantage. Such fluctuations inevitably arise, e.g., from intrinsic noise in neurons in the receptive field, from photon noise in the incoming light, and from various types of flicker in the environment. Receptive field surround mechanisms serve admirably to attenuate spatially coherent (common-mode) fluctuations (1-6), but are not necessarily adequate to deal with spatio-temporally random variation. The fine-grained mechanism studied here could provide an appropriate complement to this end.

Relation to proximal background adaptation in the vertebrate retina. In the (nearly) dark-adapted retina, several types of proximal responses are desensitized by background lights which are too dim to affect rods. This has been reported at least for ganglion cell spike responses in cat (5,10), rat (35), toad (9) and skate (7), b-wave in rat (29) and skate (7) and the "proximal negative response" (PNR) in skate (8). On the other hand, desensitization due to repetitive stimulation has been described in ganglion cells of cat (30,31), frog (32,33, this study) and mudpuppy (34), b-wave in monkey (35), and PNR as well as b-wave in mudpuppy (34). Given this correlation, it is tempting to speculate that the two classes of observations might reflect the action of a common "gain box" sensitive to fluctuations in the excitatory drive.

ACKNOWLEDGEMENTS

This work was supported by the Academy of Finland (grant 01/455).

REFERENCES

1. Werblin FS (1974) J Gen Physiol 63:62-87
2. Werblin FS, Copenhagen DR (1974) J Gen Physiol 63:88-110
3. Copenhagen DR (1975) Vision Res 15:573-581
4. Shapley RM, Victor JD (1978) J Physiol (Lond) 285:275-298
5. Shapley RM, Enroth-Cugell C (1984) In: Osborne N, Chader G (eds) Progress in Retinal Research Vol 3. Pergamon, Oxford, pp 263-346
6. Donner K (1987) Vision Res 27:1211-1221
7. Green DG, Dowling JE, Siegel IM, Ripps H (1975) J Gen Physiol 65:483-508

8. Dowling JE, Ripps H (1977) J Gen Physiol 69:57-748.
9. Donner K, Copenhagen DR, Reuter T (1990) J Gen Physiol 95:733-753
10. Enroth-Cugell C & Shapley RM (1973) J Physiol (Lond) 233:311-326
11. Donner KO, Reuter T (1968) J Physiol (Lond) 199:59-87
12. Bäckström A-C, Reuter T (1975) J Physiol (Lond) 246:79-107
13. Bäckström A-C, Hemilä S, Reuter T (1978) Med Biol 56:72-83
14. Copenhagen DR, Donner K, Reuter T (1987) J Physiol (Lond) 393: 667-680
15. Bäckström A-C (1981) Med Biol 59:234-246
16. Grönholm M-L, Reuter T (1981) Vision Res 21:1649-1652
17. Donner K, Grönholm, M-L (1984) Vision Res 24:1807-1819
18. Donner K (1989) Visual Neurosci 3:39-51
19. Bäckström A-C (1982) PhD thesis, Univ Helsinki
20. Polishchuk NA, Zenkin GM (1981) Neurophysiol 13:395-400
21. Miller RF, Dacheux RF, Frumkes TE (1977) Science 198:748-750
22. Dowling JE (1986) Trends Neurosci 8:236-240
23. Douglas RH, Djamgoz MBA (1990) J Photochem B 4:425-431
24. Easter SS (1968) J Physiol 195:273-281
25. Green DG, Tong L, Cicerone CM (1977) Vision Res 17:479-486
26. Cleland BG, Freeman AW (1988) J Physiol 404:591-611
27. Baylor DA, Fettiplace R (1977) J Physiol (Lond) 271:425-448
28. Baylor DA, Fettiplace R (1979) J Physiol (Lond) 288:107-127
29. Green DG, Powers MK (1982) Vision Res 22:209-216
30. Enroth C (1952) Acta Physiol Scand 27 suppl 100:1-67
31. Enroth-Cugell C, Shapley RM (1973) J Physiol (Lond) 233:271-309
32. Morrison JD (1979) J Physiol (Lond) 290:45P
33. Grüsser-Cornehls U (1968) Pflügers Arch 303:1-13
34. Karwoski CJ, Proenza LM (1980) J Gen Physiol 76:479-497
35. Brown KT, Watanabe K (1965) Science 148:1113-1115