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RESEARCH NOTE

ADAPTATION POOLS AND EXCITATION RECEPTIVE FIELDS OF RAT RETINAL GANGLION CELLS

LILLIAN TONG and DANIEL G. GREEN
Vision Research Laboratory, University of Michigan, Ann Arbor, Michigan 48109, U.S.A.

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INTRODUCTION

Electrophysiological experiments on a variety of animals and psychophysical studies on man indicate that light adaptation spreads laterally across the retina. The evidence suggests that the magnitude of this spread is greater than can be attributed to scattered light (Rushton, 1965a, b; Easter, 1968; Cleland and Enroth-Cugell, 1968; Barlow and Andrews, 1967; Burkhardt and Berntson, 1972; Enroth-Cugell and Shapley, 1973; Green, Tong and Cicerone, 1977), implying that adaptive signals are neurally pooled. There is not, however, general agreement about whether the adaptive summation area is the same as or smaller than the size of the ordinary receptive field. The studies on goldfish (Easter, 1968) and frogs (Burkhardt and Berntson, 1972) show that the adaptation pool is narrower than the response receptive field. The work of Cleland and Enroth-Cugell (1968) and Enroth-Cugell and Shapley (1973) on the cat show that response summation areas and adaptation summation areas are the same size.

These conclusions are based on experiments using different methods. Easter (1968) placed a small suprathreshold flashing test in the receptive field of a goldfish ganglion cell. Another small spot was placed in various positions, and at each position its intensity was adjusted to reduce the response to the test to threshold. The intensity required at each position defined the adaptation receptive field. In the frog, Burkhardt and Berntson (1972) used similar methods to plot adaptation receptive fields for test spots centered in the receptive fields of retinal ganglion cells. The findings of Cleland and Enroth-Cugell (1968) and Enroth-Cugell and Shapley (1973) in the cat were based on the size of Ricco's law summation areas for response and adaptation.

Since different methods have been used on different animals, it is hard to know whether to ascribe various results to differences between methods or differences between the retinas of mammals and cold blooded vertebrates. A recent study of ours (Green, Tong and Cicerone, 1977) seemed to imply that rats have smaller adaptation pools than excitation receptive fields. In Green *et al.*, a small steady adaptation spot was placed within the rat ganglion cell receptive field, and its effect on the receptive field sensitivity profile was measured. The adaptation spot was found to produce a local decrease in sensitivity at and around the adapting spot. The experiments reported here were designed to determine the spatial extent of adaptive pooling by directly measuring the adaptation receptive field of rat retinal ganglion cells using the same methods that Easter (1968) and Burkhardt and Berntson (1972) had used on cold-blooded retinas.

METHODS

Surgery

Rats (Long–Evans hooded) raised in dim illumination were dark-adapted for 12 hr or more prior to the experiment. During the experiment only dim red illumination was used so that rats remained dark adapted. Rats were initially anesthetized with urethane (200 mg/100 g) injected intraperitoneally with subsequent small doses as necessary. The upper eyelid and conjunctiva of the left eye was removed and care was taken thereafter to keep the cornea moist. The rat was mounted in a standard stereotaxic apparatus and a rectangular hole (3 × 6 mm) was drilled in the skull to the right of midline and including bregma. The dura was reflected. The left eye was then stabilized with a semi-eye ring similar to that used by Enroth-Cugell and Shapley (1973). The half ring, designed to fit snugly around the eyeball, could be held rigidly by a long bar to the stereotaxic frame. Eastman 910 Adhesive was applied to the ring which was lowered into the upper half of the eyeball. Care was taken to keep the glue from spreading on to the cornea. The pupil was dilated with atropine sulphate 1%. A black contact lens, with a clear, plano pupil, 0.5 mm in dia, was placed on the left eye. The small pupil increased depth of focus. Calculations of diffraction effects using Fraunhofer diffraction show that the first minima falls about 2′ from the geometric edge of the stimulus. The smallest stimulus was a disk 1° in dia, so that the spread of light by diffraction is not a significant concern.

Stimulation

Small spots of light on a tangent screen were derived from two light sources: a xenon arc lamp for the adaptation stimulus and a tungsten filament lamp for the test stimulus. The wavelength composition of the tungsten and xenon sources differ slightly. These "color" differences are unlikely to be of any importance since we are probably stimulating just rods. Even when cones are excited, we have never seen any color-specific ganglion cell responses, though we have looked extensively for them. An electromagnetic shutter (Uniblitz) was used to produce a 0.5-sec flash of the test spot every 3 sec. The adaptation stimulus, when present, was a steady light. The test and adapting stimuli could be varied in size and position on the tangent screen placed approximately 40 cm from the animal's eye.

The intensity of the stimuli was controlled with neutral density filters.

Recording

Electrodes were glass pipettes filled with a low melting point metal (Cerrolow, 136) with a tip size ranging from 3 to 10 μm. The tip was plated with platinum. Only electrodes with a resistance less than 4 MΩ were used. Recording electrodes were selected by tip size: the larger tips (5–10 μm) were used early in the experiment to locate the optic tract; smaller tips (3–5 μm) were used to record single unit extracellular activity. The indifferent electrode, a chlorided silver wire, was inserted into the cheek of the rat. The difference signal, between the two electrodes, was amplified 10× with a FET preamplifier and displayed on a Tektronix RM565 dual beam oscilloscope. A trigger level could be adjusted so that only spikes from a single unit would initiate a 1-msec pulse to the *Z*-axis of a second oscilloscope, a storage oscilloscope (Tektronix 564), causing a bright dot on the screen. The sweep of this second scope was triggered by the onset of the test stimulus and was displaced downward with each stimulus presentation. Thus, with successive presentations of the stimulus, a dot pattern was formed on the storage screen (See Green, Tong and Cicerone, 1977). The response of the unit was evaluated by viewing these patterns formed on the storage scope and by listening to the spike discharge over a loudspeaker. The storage scope pattern usually served as a check on thresholds determined by auditory criteria.

Procedure

Half of a ping-pong ball was placed over the left eye of the rat and illuminated uniformly with the flashing test light. A microelectrode was positioned in the optic tract using coordinates from the stereotaxic atlas (de Groot, 1959) and landmark sutures on the skull. An electrode positioned 1.5 mm lateral and at bregma in the anterior–posterior plane would generally pass through the optic tract when lowered 8 mm.

Optic tract units fired synchronously with the light presentation and had the properties of fibers as opposed to cell bodies. Fibers generally have a faster rise time than cells and are monophasic rather than biphasic when recorded from a Cerrolow electrode. Action potentials from fibers can also be distinguished from cells when heard on the audio monitor by virtue of their shorter duration and greater high-frequency content. Histology on several animals confirmed that the electrode was in the optic tract. Once the optic tract was located, a smaller-tipped recording electrode was lowered at the same coordinates and single units isolated.

Once a unit was isolated, the receptive field was crudely located by removing the ping-pong ball and shining a hand-held light (ophthalmoscope) on a sheet of white paper in front of the eye. By rotating the animal, adjusting the height of the tangent screen, and moving the spot, the tungsten light stimulus which was used for threshold measurements could be placed in the position where a maximal response was obtained.

A dark-adapted excitation receptive field (ERF) profile was found for each unit by determining the amount of light necessary in a 1.5° flashing test spot to produce a criterion (threshold) response from the ganglion fiber. This determination was made for positions in 1.5° steps across the responsive region of the visual field. Thus, the ERF is a measure of the receptive field sensitivity of the unit.

Next, adaptation receptive fields (ARFs) were found for the unit. To determine ARFs, the test spot was flashed at an intensity ten times that necessary for a threshold response. A steady 1.5° adaptation spot was moved horizontally across the receptive field in 1.5° steps. At each position the intensity of the adaptation spot was adjusted to bring the response to the test flash from 10× threshold to threshold.

RESULTS

In this study, 15 rats were used. Excitation receptive fields and adaptation fields were measured in sufficient detail to allow comparison of 18 single "off" units. No surround mechanism with "on" response was seen for any of the dark-adapted off units. "On" units, which are found with about equal frequency, have not been studied. The tonic firing caused by adapting spots makes it nearly impossible to detect incremental responses using our auditory and visual display methods.

Excitation receptive fields (ERFs) were found by moving a 1.5° test in 1.5° steps across the responsive area of the visual field, adjusting the intensity of the test spot at each position until a criterion (threshold) response was obtained from the unit. Several determination of threshold were often obtained at a single position in the receptive field. Repeated measurements were usually within 0.1 or 0.2 log units of each other as long as the spike remained well-isolated. All ERFs were unimodal, and 78% were symmetrical in shape with a central region of equal sensitivity which we termed the plateau (see Fig. 1, open circles). This means that it was necessary to increase the intensity of the spot as it moved away from a central region in order to produce the criterion response. The plateau varied in size from unit to unit ranging from a single point to 6° visual angle, but averaging 3° visual angle. In the most sensitive region of the field, the intensity of the 1.5° spot necessary to give threshold response varied from 0.03 to 1.0 cd/m^2 with an average of 0.1 cd/m^2. Taking into account the pupil dia (0.5 mm), the color temperature of the source, and the smaller size of the rat's eye, one can calculate equivalent scotopic trolands. The average threshold intensity produced a retinal illumination equivalent to 0.01 scotopic td in man.

The excitation field sizes varied greatly. If we define excitation field size as the diameter of the field in degrees where the sensitivity is down 0.5 log units from peak sensitivity, they ranged from 3 to 10° of visual angle. Of the 21 ERFs measured, seven were from 3 to 6°, 12 from 6 to 9°, and two over 9°. No determination was made of the position of these fields in visual space.

Adaptation receptive fields (ARFs) were obtained for different positions of the test spot within the plateau of peak sensitivity. For each position, the test was set at a 10× threshold intensity and the adaptation spot moved in 1.5° steps across the field. The adaptation spot intensity was adjusted to bring the response to the test to threshold. Two basic types of results were obtained.

The most common result (15 out of 18 units, 83%) was that adaptation field profiles were narrower than excitation field profiles (see Figs. 1 and 2); i.e. as the adaptation spot moved away from the position of the test, the intensity of the adaptation spot had to be greater than could be expected from the sensitivity of its position as determined by the ERF. In all these units, the location of the narrower ARF depended on the position of the test spot. When the test was

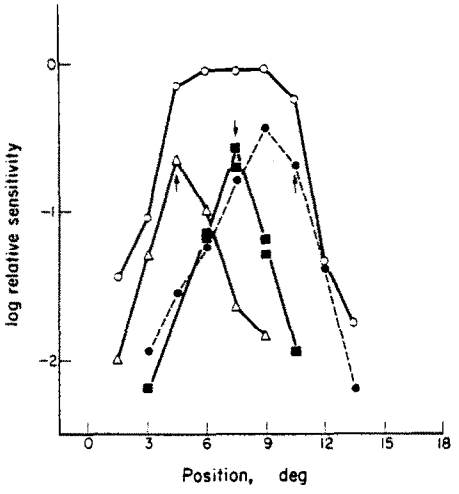

Fig. 1. Excitation receptive field (ERF) (○) and three adaptation receptive fields (ARFs) for a single optic tract fiber. For the ERF, the vertical axis gives for each position the log of one over the intensity of the test stimulus at threshold. With the ARFs the vertical axis plots at each position the log of one over the intensity of an adaptation spot which brought a ×10 threshold spot (at the position of the arrow) to threshold. ARFs are for tests positioned at 4.5° (△), 7.5° (■), and 10.5° (●) indicated by arrows. The adaptation spot is most effective at the site of the test for tests at 4.5 and 7.5°. The most effective position for adaptation is displaced from the 10.5° test toward the center of the field, however. Note that all ARFs are narrower than the ERF. In this and all figures, the abscissa refers to relative degrees in the receptive field of the unit.

in the center of the excitation field, the ARF peaked at the center of the field. However, for a test that was non-centered, but still on the plateau, the ARF center was displaced with respect to the ERF center, toward the site of the test. The ARF height at the position indicated with an arrow shows the adapting intensity required when test and adaptation are superimposed. The approximately equal heights of the

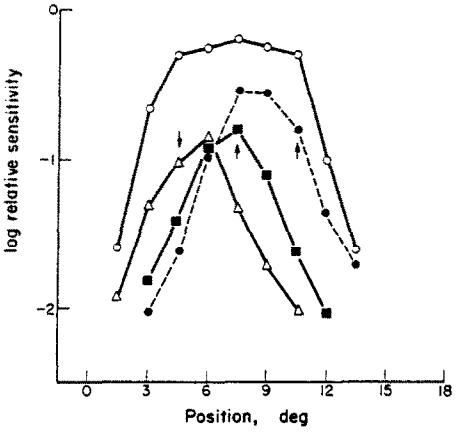

Fig. 2. ERF (○) and three ARFs for tests positioned at 4.5° (△), 7.5° (■), and 10.5° (●). For both ARFs with non-centered test positions, the most effective position for the adaptation spot is displaced from the position of the test toward the center of the receptive field. All ARFs are narrower than the ERF.

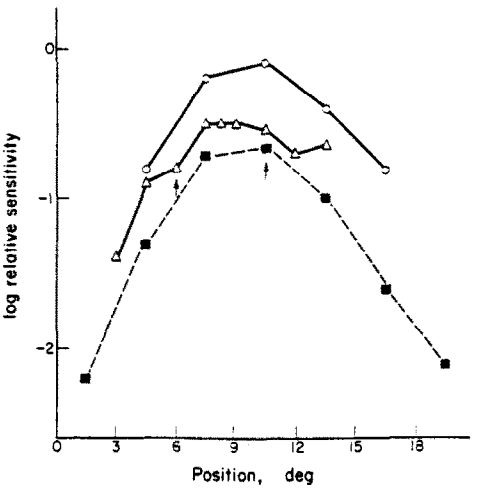

Fig. 3. ERF (○) and two ARFs for tests positioned at 6° (△) and 10.5° (■). ERF and ARFs have the same shape.

ARFs in Fig. 1, for example, indicate that within the ERF plateau the adaptability as estimated by coincident test and adaptation was also constant.

In two units the most effective adapting position for non-centered tests was the site of the test itself. That is, the effectiveness of the adaptation fell off as the spot moved away from the position of the test to either left or right, for example, in Fig. 1 when the test spot was on the left (△, 4.5°). In other cases for a non-centered test, the ARF did not peak at the site of the test but at a position displaced toward the center of the ERF plateau. The ARF was still narrower than the ERF, however. This result was found in seven units. In two units both types of results for non-centered tests were obtained for different test positions as in the unit in Fig. 1. This figure shows that the ARF for a test at 4.5° (△) peaks at the site of the test. However, the ARF for a test at 10.5° (●) peaks central to the test. The unit in Fig. 2 showed this latter result for two positions of the test. In the ARF for a test at 10.5°, displaced adapting spots at 7.5 and 9.0° are more effective by 0.3 log units than a superimposed adapting spot. This is a small effect and one might wonder if it is significant. The error in these determinations was of the order of ±0.1 log units. Thus the desensitizing effect with the adaptation displaced was at least as great as that found with it superimposed. The increased desensitizing effect of the displaced spot was greater than the ERF sensitivity increase. This was also the case for an ARF with a test at 4.5° (△) in the same unit. It is quite clear in all these units that the position of the test affects the shape of the adaptation profile, and that the ARF is narrower than the excitation field profile.

Three units (17%) displayed a second type of result. The adaptation field profiles were equal in size to the excitation field profiles, and position of test spot did not affect the shape (see Fig. 3). These three units all had excitation fields larger than 7.5°.

DISCUSSION

This study shows that when rat adaptation pools and excitation fields are measured with the methodo-

logy previously used in goldfish and frog, one comes to the conclusion that adaptation and excitation are pooled differently, the result found for goldfish and frog (Easter, 1968; Burkhardt and Berntson, 1973). Adaptation pools are in general found to be narrower than excitation fields. In addition, we find that the adaptation field is not an invariant property of the ganglion cell, but rather depends on where in the receptive field the test spot is positioned. This latter property suggests to us that light adaptation produces sensitivity changes distal to the point at which signals determining the receptive field are completely summed. For example if adaptation pooling took place in bipolars, a small adaptation spot would desensitize only a small subset of the bipolars converging on a ganglion cell. A small test exciting the same bipolars would therefore be more affected than a test elsewhere in the ganglion cell's field. Adaptation fields would be smaller than excitation fields and would vary with the position of the test spot. There is really nothing which necessarily implicates bipolar cells. The same argument would apply equally well to photoreceptors, or, for that matter, to horizontal or amacrine cells if these latter cells can locally modify the signals impinging on the ganglion cell. The neuron or neurons involved, however, must act prior to pooling of excitatory responses.

Our results also suggest that retinal sensitivity is controlled by pooling signals arising in spatially separated retinal areas. We have shown that the sensitivity of one area of the receptive field is altered by adapting some other spatially distinct area. This, however, is not sufficient ground for concluding that pooling occurs. Scattered light would produce such an effect even if adaptation occurred within individual photoreceptors with no neural spread. Our conclusion stems from the finding that in some instances the most adaptable position was not the position of the test spot. More adaptive light will be falling on to the receptors stimulated by the test when the adaptation is superimposed than when the adaptation spot is displaced. If only the steady light falling on to the receptors illuminated by the test could reduce sensitivity, then when one is on the receptive field plateau (as in Fig. 2), the most adaptable position should be the position of the test. This is not the case, so we infer that neural signal spreading across the retina must in part be responsible for the sensitivity changes. Other evidence that light adaptation spreads laterally comes from the three units in which spread of adaptation and spread of excitation were equal.

The question remains unanswered whether species differences may explain differences in the results of the spatial extent of adaptation and excitation pooling. Our results on rats show that it is not because of a fundamental difference between cold- and warm-blooded vertebrates. It seems possible that apparent differences between cat and rat retinas may be due to differences in method. Evidence in support of this comes from recent experiments of Harding (1977) on the cat, showing local adaptive effects, using a uniform and bipartite adapting field.

In conclusion, it seems likely that light adaptation in the rat occurs at a site or sites distal to the ganglion cell. Information about the external illumination which sets retinal sensitivity spreads across the retina. This spread seems to occur before the ganglion cells, possibly within the layer of photoreceptors, but more likely in the more proximal retina.

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