Proc. Natl. Acad. Sci. USA
Vol. 78, No. 10, pp. 6540–6542, October 1981
Neurobiology

Linear information processing in the retina: A study of horizontal cell responses

(vision/intracellular recording/sinusoidal gratings/spatial summation)

DANIEL TRANCHINA*, JAMES GORDON*†, ROBERT SHAPLEY*, AND JUN-ICHI TOYODA‡

*Laboratory of Biophysics, The Rockefeller University, New York, New York 10021; †Department of Psychology, Hunter College of the City University of New York, New York, New York 10021; and ‡Department of Physiology, St. Marianna University School of Medicine, Kawasaki, Japan, 213

Communicated by Floyd Ratliff, July 1, 1981

ABSTRACT **A basic question about visual perception is whether the retina produces a faithful or a distorted neural representation of the visual image. It is now well known that in some retinal pathways there are significant nonlinear transductions which distort the neural image. The next natural question is, What are the locations of the nonlinear stages within the retinal network? We report here on an investigation of linearity and nonlinearity of responses of horizontal cells in the turtle retina as an assay of the degree of nonlinearity in the outer plexiform layer of the retina. The visual stimuli were sinusoidal gratings; these patterns were modulated by contrast reversal with a sinusoidal time course. The conclusion from our experiments is that the turtle's horizontal cell responses show evidence only of linear spatial summation even at moderately high contrasts on moderately high background levels. Our work thus indicates that there is no significant distortion of the visual image by the photoreceptors or by the neural summation of photoreceptor signals by horizontal cells under normal physiological conditions. These results are consistent with the view that the major nonlinearities of the retina are proximal to the outer plexiform layer.**

Horizontal cells in several fish retinas have been reported to be nearly linear in their response to diffuse illumination (1–3). A model based on the concept that the network of electrically coupled horizontal cells behaves as a single flat cell with passive spread of current injected at the synapses works well for horizontal cells of catfish (4, 5) and turtles (6). When the membrane conductance changes caused by the light stimulus are not too large, this model implies linear spatial summation. Horizontal cells in the cat respond to sinusoidal modulation of the luminance of spots with significant harmonic distortion only when relatively high modulation depths are used at frequencies below 2–3 Hz (7).

We report here that luminosity horizontal cells (which hyperpolarize to all wavelengths of light) in the turtle and their presynaptic receptors act as linear tranducers in response to diffuse illumination and also in response to spatial patterns which test properties of spatial summation.

METHODS

Intracellular recordings were obtained from horizontal cells in isolated eye-cup preparations of *Pseudemys scripta elegans* (red-eared turtle) and *Chelydra serpentina* (common snapping turtle) which gave similar results. The eye-cups were maintained in a moist oxygenated chamber at 16–20°C. Fine-tipped microelectrodes filled with 4 M potassium acetate (resistance, 100–300 MΩ) gave satisfactory results. The visual stimulus was a raster type display produced on a cathode ray tube oscilloscope screen (Tektronix 5103N, P31 phosphor, which has its spectral peak at 525 nm) by a special-purpose microcomputer (8) which also signal-averaged the response. A monochromator was used to test the spectral sensitivity of the neurons. The stimuli in these experiments were like those used in the past for studying the receptive field characteristics of retinal ganglion cells of several vertebrates (8–11). The typical stimulus was temporal modulation of the contrast of a sinusoidal grating pattern superimposed on a steady background illumination. In particular, we used sinusoidal contrast reversal. A special case was sinusoidal modulation of the spatially uniform illuminance. The mathematical form of the stimulus is given below, and the range of each variable used in this study is given in parenthesis following each definition:

$$s(x,t) = L_0[1 + \sin(2\pi ft)m \sin(2\pi kx + \theta)] \qquad [1]$$

in which L_0 is the background illuminance (2 lumens/m^2), f is the temporal frequency (0.125–32 Hz), m is the contrast[§] (0.1–0.8), k is the spatial frequency (0–5 cycles/mm), x is the position along one rectangular coordinate axis on the retina, and θ is the spatial phase (position) of the grating (0–2 π radians). For the sake of comparing our light stimulus with those used in other studies, we note that a 0.5-sec incremental step of illuminance with contrast 0.3 produced the same response as a 0.5-sec step of 640-nm light with a flux of 6.18×10^{10} quanta sec^{-1} cm^{-2} (or 1.92×10^{-8} W cm^{-2}). The background illuminance was well above threshold and produced a steady hyperpolarization in horizontal cells of about 15 mV (25–30% of maximum hyperpolarization). Fourier analysis of the signal-averaged responses was performed to determine the component at the input frequency and also the higher-order harmonics caused by nonlinear distortion. It has already been established (8–11) that, if a neuron responds to this stimulus at the input frequency with an amplitude that depends sinusoidally on the spatial phase (position) of the grating, it must receive inputs that are linear locally and that are summed linearly.

RESULTS

Under these conditions, luminosity horizontal cells responded almost exclusively at the modulation frequency (Fig. 1). Sinusoidal modulation of the full-field illuminance gave a sinusoidal response with very little harmonic distortion (Fig. 1 *Left*). When the contrast was 0.3, the amplitude of the fundamental response was 8.5 mV (peak to trough), and the amplitude of the second harmonic was less than 5% of the fundamental (Fig. 1 *Center*). The amplitude of the response to contrast reversal of a sinusoidal

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

§ The term "contrast" is defined implicitly by Eq. 1 and can also be defined as equal to $(L_{max} - L_{min})/(L_{max} + L_{min})$ in which L is the retinal illuminance.

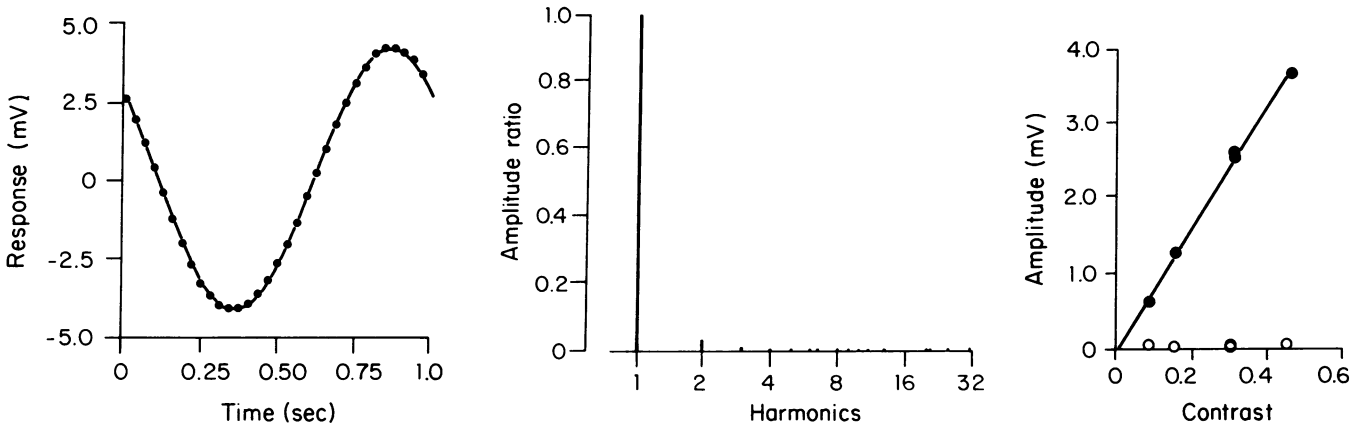

FIG. 1. (*Left*) Horizontal cell response to sinusoidal modulation (1 Hz) of the spatially uniform illuminance of the retina. The response was sampled at 64 discrete points per cycle and averaged over 16 cycles. Every other point is plotted for sake of clarity. The data deviate little from the continuous curve which is the best-fit sinusoid with frequency 1 Hz. (*Center*) Fourier analysis of the response. The height of each line represents the ratio of the amplitude of the n^{th} harmonic frequency component of the response to the fundamental (input frequency) component. For $n > 1$, these are the distortion coefficients, all of which are small. (*Right*) ●, Amplitude (peak to trough) of the fundamental component of the response to sinusoidal modulation (1 Hz) of the contrast of a sinusoidal grating pattern (0.5 cycle/mm) as a function of contrast; the response amplitude was proportional to contrast. ○, Corresponding amplitudes of the second harmonic frequency components.

grating was proportional to contrast (Fig. 1 *Right*), and the temporal phase of the response remained constant. Over the range of temporal frequencies used in this study, when the contrast was 0.3, the amplitude of the second harmonic was rarely more than 10% of the fundamental. The harmonic distortion increased with contrast. In one experiment (not shown here), sinusoidal modulation of the full-field illuminance with a contrast of 0.8 gave a peak-to-trough response of 16 mV, and the amplitude of the second harmonic was 15% that of the fundamental.

Fig. 2 demonstrates the linearity spatial summation. In this set of experiments the stimulus was sinusoidal contrast reversal of a grating which was placed in various positions with respect to the receptive field. The amplitude of the response was a sinusoidal function of the spatial phase (position) of the grating (Fig. 2 *Upper*). The responses when the grating was positioned for maximal response (peak position) and for minimal response (null position) are shown in Fig. 2 *Lower*. These two positions were separated by 90° in spatial phase. On either side of the null position the temporal phase of the response flipped by 180°, as expected.

Luminosity horizontal cells behaved in a similar fashion over the entire range of spatial and temporal frequencies explored. The degree of linearity depended on contrast as well as spatial and temporal frequency. The extent of linearity decreased somewhat for temporal frequencies below 1 Hz and increased for frequencies above 1 Hz. Because the second harmonic component of the response was the only significant higher-order harmonic under conditions of low to intermediate stimulus contrast, the ratio of the second harmonic to the fundamental component of the response serves as a good measure of the extent of nonlinearity. The ratio of the second harmonic to the fundamental component of the response to modulation of full-field retinal illuminance with contrast 0.3 was measured for 29 luminosity horizontal cells at 0.125, 1, and 4 Hz. The mean (± SD) ratio was 8.3 ± 2.2% at 0.125 Hz, 6.7 ± 2.1% at 1 Hz, and 4.0 ± 1.8% at 4 Hz. The most obvious departure from linearity was under the simultaneous conditions of high contrast (which undoubtedly produced significant harmonic distortion in the receptors) and very high spatial frequencies (which, as a consequence of spatial summation, produced essentially no response at the modulation frequency). However, under these conditions, the overall response magnitude was relatively small—i.e., 0.5 mV for a spatial frequency of 2 cycles/mm and a contrast of 0.6.

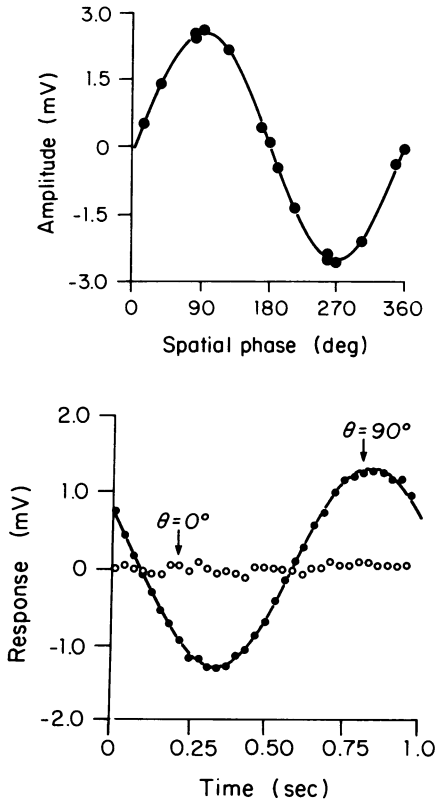

FIG. 2. (*Upper*) Amplitude (peak to trough) of the response to sinusoidal modulation (1 Hz) of the contrast (0.3) of a sinusoidal grating as a function of spatial phase (position) of the grating with respect to the receptive field. The actual data (●) deviate little from the continuous curve which is the best-fit sinusoid. This indicates that spatial summation is linear. (*Lower*) Responses at the positions which gave maximal (●) and minimal (○) amplitudes. These two positions were separated by 90° in spatial phase. The continuous curve is a best-fit sinusoid.

DISCUSSION

We conclude from the data above that, in response to stimuli whose contrast covers a large part of the physiological range, red-sensitive cones behave (to a good approximation) as linear transducers and that a linear transduction takes place at the synapses between these cones and the luminosity horizontal cells. Linear inputs from each local region of the receptive field are simply added together to produce the overall response. This conclusion is based on the fact that luminosity horizontal cells in the turtle retina receive their major input from red-sensitive (630 nm) cones (12–16). We verified this fact during our experiments. Because the inputs to luminosity horizontal cells from green- (13, 14, 16) and blue- (13) sensitive cones and rods (15) are relatively small, we cannot yet make a compelling argument for similar behavior of these receptors and their respective synapses.

In general, turtle cones respond nonlinearly when the response magnitude exceeds roughly 10% of the overall dynamic range, for several reasons which have been discussed in detail by Baylor *et al.* (17). It has also been shown by Normann and Perlman (18) that signal transmission from red-sensitive cones to horizontal cells is, in general, nonlinear. The relevant finding of this study is that, although the magnitude of the horizontal cell responses elicited by our stimuli were relatively small compared to the maximal response magnitude, the stimuli that elicited nearly linear responses had contrasts covering a large part of the physiological range. Therefore, we may conclude that, although cones respond nonlinearly over the major portion of their dynamic range, most of this range is rarely used in response to stimuli in the natural environment. Furthermore, the synapse between red-sensitive cones and horizontal cells must behave linearly for stimuli consisting of moderate perturbations about a mean level of illumination. An abrupt change in mean level of illumination, as occurs when moving from shade to direct sunlight, can be expected to elicit nonlinear responses in the outer plexiform layer initially but, after the retina has adapted to the new mean level, perturbations around this mean will again elicit primarily linear responses. (This conclusion is justified provided that the phenomenon of linearity is not peculiar to the particular level of background illumination used in our experiments.)

As in other vertebrates, there are neurons that respond nonlinearly in the turtle retina. Others have recorded responses of neurons that produced a burst of impulses both at the onset and offset of an illuminated spot (19–21). In the course of this study we encountered spiking neurons that responded with predominant second harmonic distortion (frequency doubling) to the same stimuli used to test the linearity of horizontal cells (22). This nonlinearity cannot be traced back to the level of the luminosity horizontal cells. The findings presented here are consistent with the hypothesis that receptors, horizontal cells, and bipolar cells act as linear spatiotemporal filters and that the major retinal nonlinearities occur more proximally in the retina (23). This view is supported by recent experiments on neurons in the catfish retina (24).

A linear model has recently been proposed for the spatiotemporal properties of catfish horizontal cells (25). We conclude that a linear model will be effective for turtle horizontal cells as well, and we expect that the techniques of linear systems analysis (26) will be applicable to the task of modeling the outer plexiform layer in general.

This work was supported by National Institutes of Health National Research Service Award 7524 from the National Institute of General Medical Science and Grants EY 188, EY 1428, and EY 1472 from the National Eye Institute. R.S. was supported by a Career Development Award from the National Eye Institute. Some special equipment was provided by Grant RR 07065 from the National Institutes of Health. Computer time was provided in part by the City University of New York–University Computer Center System.

1. Spekreijse, H. & Norton, A. L. (1970) *J. Gen. Physiol.* **56,** 1–15.
2. Toyoda. J. I. (1974) *J. Gen. Physiol.* **63,** 214–234.
3. Marmarelis, P. Z. & Naka, K. I. (1973) *J. Neurophysiol.* **36,** 619–633.
4. Naka, K. I. & Rushton, W. A. H. (1967) *J. Physiol. (London)* **192,** 437–461.
5. Marmarelis, P. Z. & Naka, K. I. (1973) *Biophys. J.* **12,** 1515–1532.
6. Lamb, T. D. (1976) *J. Physiol. (London)* **263,** 239–255.
7. Foerster, M. H., van de Grind, W. A. & Grusser, O.-J. (1977) *Exp. Brain Res.* **29,** 367–385.
8. Shapley, R. M. & Gordon, J. (1978) *J. Gen. Physiol.* **71,** 139–155.
9. Enroth-Cugell, C. & Robson, J. G. (1966) *J. Physiol. (London)* **187,** 517–552.
10. Hochstein, S. & Shapley, R. M. (1976) *J. Physiol. (London)* **262,** 237–264.
11. Gordon J. & Shapley, R. M. (1978) in *Visual Psychophysics and Physiology,* eds. Armington, J. C., Krauskopf, J. & Wooten, B. R. (Academic, New York), pp. 315–329.
12. Simon, E. J. (1973) *J. Physiol. (London)* **230,** 199–211.
13. Fuortes, M. G. F. & Simon, E. J. (1974) *J. Physiol. (London)* **240,** 177–198.
14. Yazulla, S. (1976) *Vision Res.* **16,** 727–735.
15. Perlman, I. & Normann, R. A. (1979) *Vision Res.* **19,** 903–906.
16. Fuortes, M. G. F., Schwartz, E. A. & Simon, E. J. (1973) *J. Physiol. (London)* **234,** 199–216.
17. Baylor, D. A., Hodgkin, A. L. & Lamb, T. D. (1974) *J. Physiol. (London)* **242,** 685–727.
18. Normann, R. A. & Perlman, I. (1979) *J. Physiol. (London)* **286,** 509–524.
19. Schwartz, E. A. (1973) *J. Physiol. (London)* **230,** 1–14.
20. Baylor, D. A. & Fettiplace, R. (1979) *J. Physiol. (London)* **288,** 107–127.
21. Bowling, D. B. (1980) *J. Physiol. (London)* **299,** 173–196.
22. Tranchina, D. (1981) Dissertation (Rockefeller Univ. New York).
23. Victor, J. D. & Shapley, R. M. (1979) *J. Gen. Physiol.* **74,** 671–689.
24. Davis, G. W. & Naka, K. I. (1980) *J. Neurophysiol.* **43,** 807–831.
25. Krausz, H. I. & Naka, K. I. (1980) *Biophys. J.* **29,** 13–36.
26. Brodie, S. E., Knight, B. W. & Ratliff, F. (1978) *J. Gen. Physiol.* **72,** 167–202.