

C. N. SHUSTER, JR., PH.D.

FUNCTIONAL AUTONOMY IN THE LATERAL EYE OF THE HORSESHOE CRAB, *LIMULUS POLYPHEMUS*¹

M. E. BEHRENS and V. J. WULFF

Masonic Medical Research Laboratory, Utica, N.Y. 13501
2150 Bleeker Street

(Received 27 July 1966)

INTRODUCTION

THE multifaceted lateral eye of the horseshoe crab, *Limulus polyphemus*, consists of several hundred ommatidia, the majority of which contain 10-15 retinula cells and one eccentric cell (DEMOLL, 1914). Light-initiated electrical activity recorded intracellularly (HARTLINE *et al.*, 1952; MACNICHOL, 1956; TOMITA, 1956; YEANDLE, 1958; FUORTES, 1958) is characterized by a slow potential change on which a train of spike potentials may be superimposed. The correlation of intracellular responses with the cell types (TOMITA and MILLER, 1962; KIKUCHI and UEKI, 1965; BEHRENS and WULFF, 1965) has demonstrated that both slow and spike potentials can be recorded from retinula as well as from eccentric cells and the following observations were made: (1) the magnitude of the light-initiated slow potential change of retinula cells is, on the average, larger than that of the eccentric cell; (2) superimposed spike potentials are absent from retinula cell responses more frequently than from eccentric cell responses; (3) the magnitude of spike potentials recorded from eccentric cells are, on the average, of larger amplitude than those recorded from retinula cells; and (4) spike potentials recorded simultaneously from two cells in the same ommatidium are synchronous. The observed synchrony of the intraommatidial spike discharge suggests a single source of spike potentials and considerable evidence (HARTLINE *et al.*, 1952; WATERMAN and WIERSMA, 1954; TOMITA *et al.*, 1960; KIKUCHI and UEKI, 1965) points to the eccentric cell as the source of the spike potentials. The spreading of the spike potential discharge to the visual cells within an ommatidium indicates electrical coupling among these cells, which has been suggested by TOMITA *et al.* (1960) and demonstrated by SMITH *et al.* (1965) and STIEVE (1965).

Although there is little doubt concerning the source of the spike potential discharge within an ommatidium, there is considerable uncertainty regarding the origin of the light-initiated slow potentials which have been recorded from retinula and eccentric cells. It has been suggested that both spike potentials and slow potential changes originate in eccentric cells (MACNICHOL, 1956, 1958), but the results of subsequent investigations suggest that retinula cells probably generate a slow potential change in response to illumination. This impression was enhanced by the observation (BEHRENS and WULFF, 1965) of a

¹ This investigation was aided by grant NB 03721 from the National Institute of Neurological Diseases and Blindness, National Institutes of Health, Public Health Service, Department of Health, Education and Welfare.

retinula cell response which maintained its magnitude and wave form while the simultaneously recorded response of the eccentric cell in the same ommatidium became markedly abnormal, indicative of deterioration of the cell. This observation, together with the finding that the passage of current through a cell coincident with microelectrophoretic injection of dyes for identification purposes routinely severely altered or abolished the responses to light, suggested the use of controlled doses of current to selectively destroy one of two cells monitored simultaneously within the same ommatidium. The results of these experiments are presented below.

METHODS

The basic techniques employed in these experiments were described in detail in a previous paper (BEHRENS and WULFF, 1965).

Two micropipette electrodes containing different colored dyes were introduced into visual cells, usually within the same ommatidium of an isolated lateral eye of the horseshoe crab, *Limulus polyphemus*. Light-initiated responses were recorded before and after current from an external source was passed through one of the two impaled cells. Small quantities of current (0.6×10^{-6} – 9.6×10^{-6} coulomb; in most experiments 1.2×10^{-6} C, micropipette negative) were used to abolish or markedly alter the responses recorded from one of two impaled sense cells (Fig. 1). Frequently, the administration of current had to be repeated to produce the desired result. At the termination of each experiment the dyes in the micropipettes were injected into the impaled cells preparatory to histological identification, as previously described.

RESULTS

Data were obtained from four types of cell combinations: two retinula cells in the same ommatidium (5 exps.); an eccentric cell and a retinula cell in the same ommatidium (6 exps.); cells in adjacent ommatidia (1 exp.); and one experiment in which both electrodes

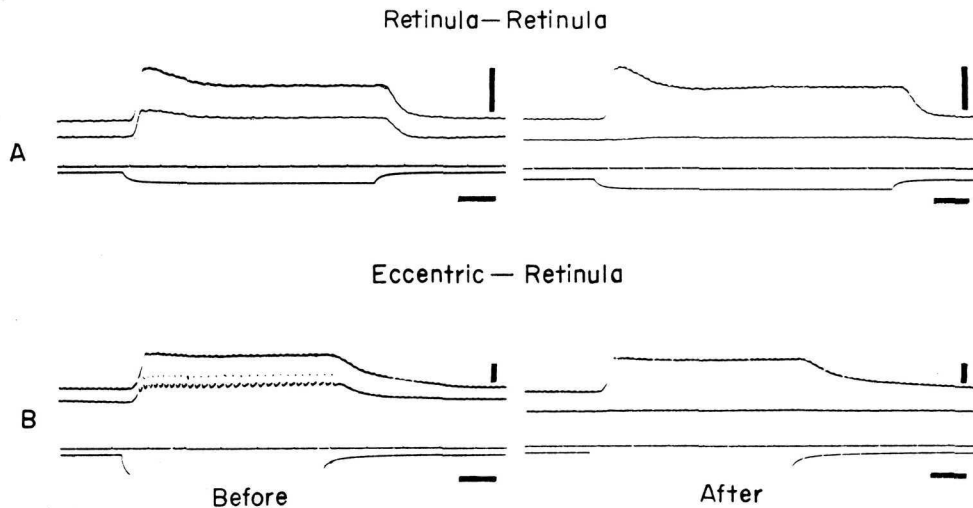


FIG. 1. Light-initiated intracellular responses (photographically recorded) from two retinula cells (A) and a retinula-eccentric cell combination (B, lower response from eccentric cell) before and after application of current to one retinula cell (A) and to the eccentric cell (B). In each experiment, the cells were located within the same ommatidium. In all records, the upper two traces define the light-initiated responses, the third trace indicates elapsed time and the lowest trace the onset and duration of the light stimulus. The horizontal bars indicate 0.1 sec elapsed time and the vertical bars indicate the deflection produced by a 20 mV test signal. For additional details please refer to the text.

had penetrated the same eccentric cell. The quantity of current required to alter or abolish the recorded response of a cell varied. Satisfactory results were most frequently obtained when 1.2×10^{-6} C was applied and the current application repeated as required. Following treatment, a response to the first of a series of light flashes could often be recorded from the treated cell, even though responses to subsequent light flashes were apparently absent.

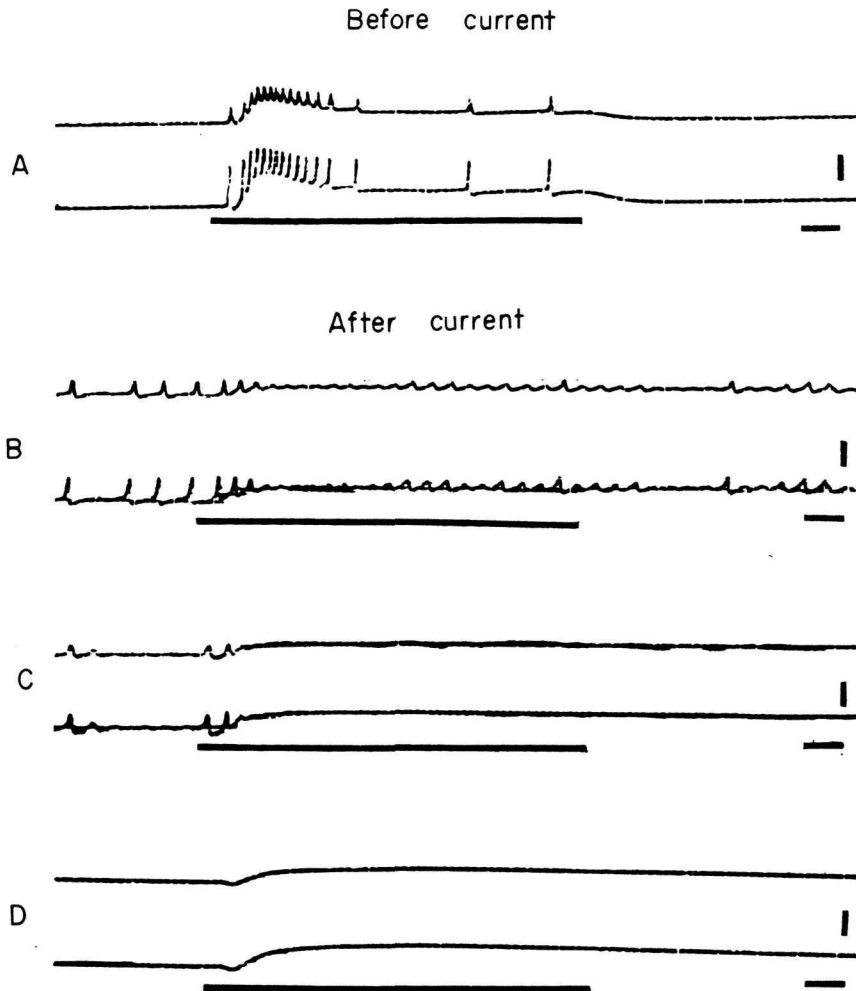


FIG. 2. Dynagraph (two channel) records of light-initiated responses detected by two micropipettes located within the body of one eccentric cell before (A) and after one application of current (B, C and D). The light stimulus was not recorded and is approximated by the line beneath each set of records. The vertical bars indicate the deflection produced by a 10 mV test signal and the horizontal bars at the right represent 0.1 sec elapsed time. For further details please refer to the text.

Light-initiated responses recorded before and after application of current are shown in Fig. 1. It was possible to abolish or markedly alter the response recorded from one retinula cell without significantly affecting the response recorded from a second retinula cell located in the same ommatidium (in 3 out of 5 cases) even when the impaired retinula

cells were found to be adjacent (in 2 of the 3 cases; Fig. 1A). It was also possible to abolish or markedly alter the response recorded from an eccentric cell without significantly affecting the response recorded from a retinula cell located in the same ommatidium (in 4 of 6 cases; Fig. 1B). In two cases in which the retinula cell response changed, the changes in the eccentric cell response preceded those seen in the retinula cell response. In the experiment in which both electrodes had impaled the same eccentric cell, the responses recorded by both electrodes were similarly altered by current passed through one of the micropipettes (Fig. 2). In this case, the colored markers were both located within the body of the eccentric cell, but it was not possible to estimate the distance between the tips of the micropipettes.

The ease with which the spike potentials were abolished from the recorded responses by the imposed current varied with the identity of the cell. Spike potentials were always abolished from the responses recorded from both impaled cells when the eccentric cell was exposed to current, provided both cells were located within the same ommatidium; in one experiment in which spike potentials persisted in the responses recorded from a retinula cell after passing current through the eccentric cell, it was found that the cells were in adjacent ommatidia. Passage of current through retinula cells did not abolish the spike potentials in all cases, even after the light-initiated slow potentials were abolished or had become diphasic or of reversed polarity.

The effect of the imposed current on the response of an impaled cell persisted for the duration of each experiment and may well be irreversible; in one experiment the altered response persisted unchanged for 30 min after the final application of current.

DISCUSSION

The results presented above indicate that the light-initiated responses of the retinula cells may persist unaltered in the face of deliberate alteration or abolition of the response of the eccentric cell or another retinula cell in the same ommatidium. These observations indicate that the retinula cell response occurs independently of the response of the eccentric cell and, perhaps, independently of the response of other retinula cells. However, the validity of this inference hinges on the assumption that the abnormal response detected by the microelectrodes is typical of the entire cell. It is possible that regions of the cell remote from the sensing electrode continue to function under these conditions, or that the electrode has moved to a less favorable position in the cell. The results of two experiments argue against these possibilities. In one experiment, in which both electrodes were located in the same eccentric cell, current applied through one electrode produced similar changes in the responses recorded by each of the two microelectrodes (Fig. 2). Further, in a number of experiments, repeated current applications resulted in stepwise "deterioration" of the recorded responses (Fig. 3); it is considered unlikely that these changes were caused by electrode movement. If it is valid to assume that the imposed current affects the response of the entire cell, then the observed constancy of retinula cell responses when an adjacent retinula cell or the eccentric cell is "destroyed", indicates that electrical interaction of the type demonstrated by SMITH *et al.* (1965) and STIEVE (1965) may play only a minor role, if any, in the function of the sense cells within an ommatidium.

These experiments provide additional evidence for the contention that there is a single source for the spike potentials within an ommatidium, presumably the eccentric cell. In our experiments, current applications always resulted in similar changes in the frequency and amplitude of the spike potentials recorded from two cells, provided they were located

in the same ommatidium. In some of the experiments in which the retinula cell was exposed to current, currents sufficient to abolish or markedly alter the recorded slow potential change failed to abolish the spike potentials. In every case in which currents were applied

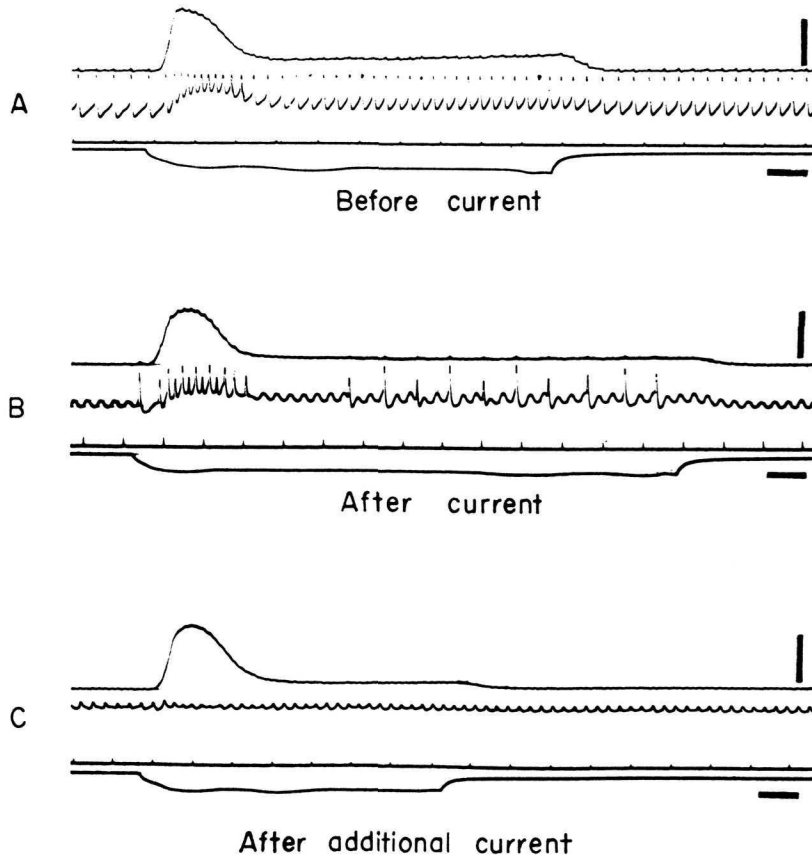


FIG. 3. Light-initiated intracellular responses (photographically recorded) from a retinula-eccentric cell combination within the same ommatidium before (A) and after two successive applications of current to the eccentric cell (B and C). Identity of traces and calibrations are described in Fig. 1. For additional details please refer to the text.

to the eccentric cell the spikes were abolished even though, in some cases, the slow potential change could still be recorded.

REFERENCES

- BEHRENS, M. E. and WULFF, V. J. (1965). Light-initiated responses of retinula and eccentric cells in the *Limulus* lateral eye. *J. Gen. Physiol.* **48**, 1081-1093.
- DEMOLL, R. (1914). Die Augen von *Limulus*. *Zool. Jb. Anatomie und Ontogenie der Tiere*, **38**, 443-464.
- FUORTES, M. G. F. (1958). Electrical activity of cells in the eye of *Limulus*. *Am. J. Ophthalm.* **46**, 210-223.
- HARTLINE, H. K., WAGNER, H. G. and MACNICHOL, E. F., Jr. (1952). The peripheral origin of nervous activity in the visual system. *Cold Spring Harb. Symp. quant. Biol.* **17**, 125-141.
- KIKUCHI, R. and UEKI, K. (1965). Double-discharges recorded from single ommatidia of horseshoe crab, *Tachypleus tridentatus*. *Naturwissenschaften* **52**, 458-459.
- MACNICHOL, E. F., Jr. (1956). Visual receptors as biological transducers. In: *Molecular Structure and Functional Activity of Nerve Cells*. American Institute of Biological Sciences, Washington, D.C.

- MACNICHOL, E. F., Jr. (1958). Subthreshold excitatory processes in the eye of *Limulus*. *Expl. Cell Res. Suppl.* **5**, 411-425.
- SMITH, T. G., BAUMANN, F. and FUORTES, M. G. F. (1965). Electrical connections between visual cells in the ommatidium of *Limulus*. *Science, N.Y.* **147**, 1446-1447.
- STIEVE, H. (1965). Interpretation of the generator potential in terms of ionic processes. *Cold Spring Harb. Symp. quant. Biol.* **30**, 451-456.
- TOMITA, T. (1956). The nature of action potentials in the lateral eye of the horseshoe crab as revealed by simultaneous intra- and extracellular recording. *Jap. J. Physiol.* **6**, 327-340.
- TOMITA, T., KIKUCHI, R. and TANAKA, I. (1960). Excitation and inhibition in lateral eye of horseshoe crab. In: *Electrical Activity of Single Cells*. Igakushoin, Hongo, Tokyo.
- TOMITA, T. and MILLER, W. H. (1962). Personal communication.
- WATERMAN, T. H. and WIERSMA, C. A. G. (1954). The functional relation between retinula cells and optic nerve in *Limulus*. *J. exp. Zool.* **126**, 59-85.
- YEANDLE, S. (1958). Electrophysiology of the visual system, discussion. *Am. J. Ophthal.* **46**, 82-87.

Abstract—The interaction of light-initiated responses of sense cells within individual ommatidia were investigated by recording simultaneously from two cells (subsequently identified) before and after the deliberate "destruction" of one cell by the passage of current. The slow response of an eccentric or retinula cell could be altered or abolished without affecting the response of the second cell (retinula), suggesting a measure of autonomy for the retinula cell slow response. The spike discharge was always suppressed when eccentric cells were treated but not when retinula cells were treated with current.

Résumé—On étudie l'interaction des réponses à la lumière des cellules sensorielles dans une ommatidie individuelle en enregistrant simultanément les réponses de deux cellules (identifiées ultérieurement) avant et après "destruction" délibérée d'une des cellules par passage d'un courant. La réponse lente d'une cellule excentrique ou de la rétine est altérée ou abolie sans affecter la réponse de la seconde cellule (de la rétine), ce qui suggère une mesure de l'autonomie de la réponse lente de ces cellules. Les décharges sont toujours supprimées quand on soumet au courant les cellules excentriques, mais pas les cellules de la rétine.

Zusammenfassung—Die Wechselwirkung lichtinduzierter Reaktionen zwischen Sinneszellen innerhalb einzelner Ommatidien wurde durch gleichzeitige Registrierung von zwei Zellen (die anschließend identifiziert wurden), vor und nach der absichtlichen Zerstörung einer Zelle durch Stromfluß, untersucht. Die langsame Reaktion einer exzentrischen- oder Retinulazelle konnte verändert oder aufgehoben werden, ohne die Reaktion der anderen Zelle zu beeinflussen, was auf ein gewisses Maß von Unabhängigkeit für die langsame Reaktion der Retinulazelle hindeutet. Die Spikeentladung wurde immer unterdrückt, wenn exzentrische Zellen, aber nicht wenn Retinulazellen mit Strom behandelt wurden.

Резюме — Было исследовано взаимодействие вызываемых светом реакций чувствительных клеток внутри отдельного омматидия, путем одновременной записи от двух клеток (затем идентифицированных), до и после преднамеренного «повреждения» одной клетки пропусканием через нее электрического тока. Медленная реакция эксцентрической или ретинулярной клетки может быть изменена или же совсем подавлена без изменения реакции другой клетки (ретинула), что заставляет думать об известной степени автономности медленных реакций ретинулярной клетки. Быстрый разряд всегда подавлялся, если ток пропускался через эксцентрические клетки, но не подавлялся, если ток пропускался через ретинулярные клетки.