tozoa were dispersed by ultrasonic treatment in water distilled in glass. Minute drops of the suspension were placed on Formvar-coated grids and allowed to dry in air. The grids were shadow-cast with palladium. The vesicles of the other males were fixed in 1 percent osmic acid buffered with Palade's solution (*6*), dehydrated in ethyl alcohol, and embedded in Epon according to Luft (*7*). Sections were stained with an aqueous solution of uranyl acetate to intensify contrast. Micrographs were obtained by means of a Siemens Elmiskop I (*8*).

Two basic types of spermatozoa are shown in Fig. 1. The gyres in some are directed dextrally (Fig. 1*A*); in others they are sinistral (Fig. 1*B*). Differences in the directions of the gyres occur only in the head, however; the gyre of the tail piece is always dextral (Fig. 1*C*). The central axial filament complex of the tail piece (see Fig. 2), which is of the usual type, is crossed at intervals of approximately 8.9 (one interval being the ratio of the diameter of the axial filament to the distance between cross-over points) by the two mitochondrial bands coiled dextrally around it. In spermatozoa with dextrally coiled heads the gyre continues uninterrupted from the tip of the head to the end of the tail piece, whereas in spermatozoa with sinistrally coiled heads the gyre is reversed in the tail piece.

There is some evidence that production of the two types of spermatozoa is related to the sex ratio of the offspring. When the proportion of the two types of spermatozoa in the female spermatheca is compared with the sex ratio of the progeny produced by the females, a much lower proportion of spermatozoa with dextrally coiled heads always occurs in the sex-ratio strain which has few females than in wild stock which has many females. In a series of tests with females of strains producing low proportions of females, 5 percent of the offspring being female, the proportion of dextral to sinistral spermatozoa in their spermathecae was 387 to 627, or 38 percent dextral; with females from wild stock, where 90 percent of the offspring were female, the proportion was 998 to 421, or 70 percent dextral.

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Electrical Connections between Visual Cells in the Ommatidium of Limulus

Abstract. *When microelectrodes are inserted in two cells of the same ommatidium in* Limulus, *current applied through the membrane of one cell produces a change in potential across the membrane of both cells. The large spikes recorded from one cell and the small spikes recorded from the other always appear synchronously. However, the small spikes are abolished selectively during the hyperpolarizing response. The discrete waves recorded from eyes adapted to darkness occur simultaneously in cells of the same ommatidium.*

The lateral eye of *Limulus* consists of several hundred ommatidia, each of which contains about 12 retinular cells and one or occasionally two eccentric cells. An elaborate structure of microvilli formed by the membrane of retinular cells, the rhabdom, is thought to contain a photosensitive substance and to initiate the process leading to vision. Eccentric cells possess a fairly large axon, from which impulses can be recorded following illumination (*1*). Smaller axons in the optic nerve are supposed to originate from retinular cells, but no one has succeeded in recording activity from them (*2*). When a microelectrode is inserted in an ommatidium, a negative potential is frequently recorded, suggesting penetration of an ommatidial cell. In darkness, the potential difference between the intracellular electrode and the outside is around −50 mv. This potential is decreased (and in some cells it can even be transiently reversed) during illumination.

In the majority of penetrated cells, small spikes can be recorded superimposed on the slow potential. In a smaller number of cells, the spikes are considerably larger. MacNichol (*3*) and Tomita *et al.* (*4*) have suggested that units of the first type are retinular cells and the others are eccentric cells (*3*, *4*).

In agreement with the observation that no activity can be recorded from the small optic nerve fibers, it has been suggested that the small spikes recorded with microelectrodes are not due to impulses originating in the retinular cells themselves but are reflections of the impulses of the adjacent eccentric cell. This interpretation implies that the two types of cells are electrically connected, as suggested by Tomita *et al.* (*4*).

In our work, two electrodes were inserted in the same ommatidium as done previously by Tomita and Miller (*5*) and by Behrens and Wulff (*6*). By means of a carefully balanced Wheatstone bridge similar to that described by Frank and Fuortes (*7*), it was possible to pass currents through either electrode while recording potentials simultaneously from both.

In most experiments, one electrode was placed in a cell producing large spikes (eccentric) and the other in a cell giving only small spikes (retinular), as shown in Fig. 1*d*. The currents applied through one electrode produced a change in potential in both cells. Similar results were obtained when the two microelectrodes were placed in two retinular cells in the same ommatidium. However, no electrical interaction could be detected when the two electrodes were placed in different ommatidia.

Figure 1*a* shows the result obtained when a bright flash of light was applied to the eye. Electrode 1 recorded a small slow potential and large spikes while electrode 2 recorded a larger slow potential but only barely detectable spikes. Large and small spikes were always synchronous. Similar results were obtained in all other pairs of cells studied. In the experiment illustrated in Fig. 1*b*, a current applied through electrode 2 evoked hyperpolarization of both cells. Reversal of the current depolarized both cells and evoked firing as illustrated in Fig. 1*c*; large spikes were recorded by electrode 1 and synchronous small spikes by electrode 2.

In many instances, the hyperpolarization evoked by applied currents developed in two steps, the first at the onset of the current and the second after some delay. The delayed change in potential is similar to that observed in other cells and is referred to as the hyperpolarizing response. It is due

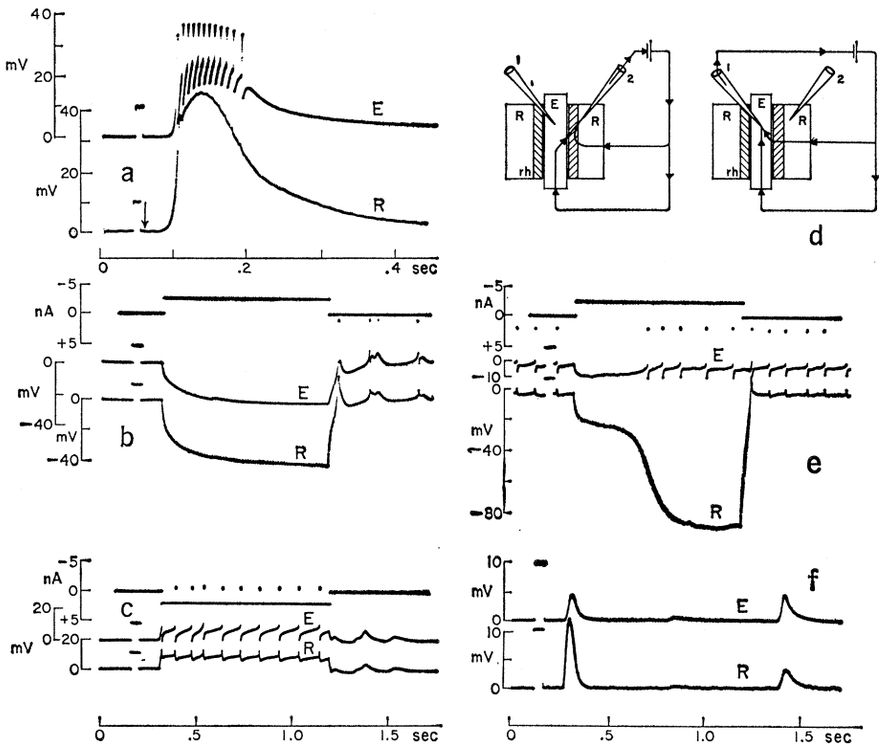

Fig. 1. Potential changes evoked by light or current in two cells of the same ommatidium. (*a*) Response of an eccentric cell *E* and a retinular cell *R* to a bright flash of light of 10 msec delivered at arrow. Square pulse at left in these and other records is a 10-mv calibration. (*b*) Potential changes induced by hyperpolarizing constant current through electrode impaling the retinular cell. Current pulse shown in uppermost trace. Current calibration indicated at left (1 na = 10^{-9} amp). (*c*) Potential changes induced by depolarizing constant current through retinular cell. Note synchronous spikes in the two cells. (*d*) Diagram of longitudinal section through ommatidium, showing impaling electrodes and presumed distribution of currents. About 12 retinular cells are arranged radially around the eccentric cell in each ommatidium (*rh*, rhabdom). Currents were passed either through electrode 2 (left) or through electrode 1. In either case potential changes were always recorded from both cells. (*e*) Hyperpolarizing response of retinular cell evoked during illumination of the ommatidium. Constant current through retinular cell evokes a delayed increase of hyperpolarization in *R* while hyperpolarization is reduced and firing is restored in *E*. Illumination was used to evoke steady firing. Hyperpolarizing responses can be obtained also in darkness. (*f*) Discrete waves recorded during dim illumination. These randomly appearing waves were always synchronous in the two cells. Observe large size of the first wave in *R* and of the second wave in *E*.

to an increase of resistance of the cell membrane, critically evoked by currents exceeding a certain intensity (*8*).

A hyperpolarizing response is shown in Fig. 1*e*. In this experiment the eye was illuminated by a weak constant light in order to evoke repetitive firing. At first, the current evoked hyperpolarization of both cells. The initial drops in potential were smaller than those evoked by the same current in darkness (Fig. 1*b*), indicating that membrane resistances were reduced by the background light (*9*). During this phase, firing was abolished in both cells. With the onset of the hyperpolarizing response, the drop in potential recorded by electrode 1 decreased and the large spikes reappeared while no coincident small spikes could be seen in the other cell. No hyperpolarizing response was evoked by currents passed through electrode 1 and the potential drops recorded by electrode 2 remained approximately proportional to those recorded by electrode 1 for all current intensities used.

The observed electrical interactions between cells can be interpreted qualitatively on the basis of the simplified diagrams of Fig. 1*d*. When the current is passed through one of two impaling electrodes, a fraction of the current flows from one cell to the other. During the hyperpolarizing response, the resistance between eccentric and retinular cells increases so that the coupling between the two cells is reduced. Reappearance of the large but not of the small spikes during the hyperpolarizing response would be expected if spikes were generated only by the eccentric cell. However, the results of this experiment do not exclude the possibility that retinular cells, when not hyperpolarized, may generate spikes in synchrony with the firing of the eccentric cell.

Results obtained with dim illumination were in general agreement with the views derived from the previous results and may furnish additional information on the processes initiated by light. It has been shown that, with dim illumination, discrete potential waves of variable amplitude and frequency may be recorded from visual cells of *Limulus* (*10*). When recorded simultaneously from two cells, these waves appeared synchronously in the two records. As shown in Fig. 1*f*, the size and shape of the waves was not identical in the two traces. Some waves were larger in the record taken from one cell, while some waves were larger in the other record. In general, the smaller wave reached its peak later than the corresponding larger wave.

Presumably, individual waves can originate in different sites in the ommatidium and spread electrically from one cell to the others. If a wave originates in or near the impaled retinular cell it will be larger there than in the eccentric cell. Conversely, a wave will be larger in the eccentric cell if it originates in the eccentric cell itself or in a retinular cell closer to the eccentric cell than to the impaled retinula.

It is not possible at this time to establish whether the slow potentials (Fig. 1*a*) and the discrete waves (Fig. 1*f*) are actively produced by retinular cells only or by both retinular and eccentric cells, but more information may be obtained from a quantitative analysis of the results.

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Amino Acids Released from the Cerebral Cortex in Relation to Its State of Activation

Abstract. *The rate of release of γ-aminobutyric acid from the perforated pial surface of the cerebral cortex in the cat showed systematic variations with the state of activation or "arousal" of the electrocorticogram. In animals subjected to midbrain coagulation and showing constant spindle patterns characteristic of sleep, the rate of release was three times greater (about 2 micrograms per hour per square centimeter) than in animals showing a largely "aroused" electrocorticogram, which had sections of the upper cervical cord or were neuraxially intact but had been given local anesthesia. The rate of release of glutamic acid was lower in the "sleeping" animals than in "aroused" animals, while no such differences were found for glutamine and aspartic acid. Such studies may lead to a better understanding of chemical mechanisms involved in the control of states of consciousness by the brain stem.*

During recent years it has become apparent that "activation" of the cortex in the "arousal" reaction is manifested by both excitatory and inhibitory effects upon individual cortical neurones and that the synchronization of the electroencephalogram in states of impaired consciousness may be due in part to active inhibitory processes of brain stem origin (*1*). It may be presumed that inhibitory effects are mediated by some chemical substances liberated from synaptic terminals of a different set of cortical afferent fibers from those responsible for the excitatory effects, believed to be mediated by acetylcholine (*2*).

As a preliminary test of this hypothesis we have measured the release of γ-aminobutyric acid (γ-ABA), a candidate for the role of an inhibitory transmitter substance in the brain (*3*), and of glutamic acid, glutamine, and aspartic acid from the cerebral cortex of cats. Measurements were made both before and after the brain stem was transected; such transection is known to cause changes in the electrocorticogram (ECG) similar to those which occur during (synchronized) sleep or barbiturate narcosis. Glutamic acid, glutamine, aspartic acid, and γ-ABA are closely related metabolically. Glutamic acid is known to have an excitatory action on cortical neurones (*4*), while aspartic acid and glutamine serve as controls for (or indicators of) indirect effects such as changes in cerebral circulation.

A plastic chamber (*5*) was sealed into the skull. The pia-arachnoid membrane over the 1 cm^2 of the midsuprasylvian gyrus covered by the chamber was punctured in six to eight avascular points. The trachea was cannulated for artificial respiration and, when called for, transection or coagulation of the brain stem was carried out with suitable instruments under stereotaxic control. The fifth nerve nucleus and occipital nerves, and all points subjected to pressure by the stereotaxic frame, were injected with Zylocaine. The animal was then paralyzed with Flaxedril and allowed to recover from the surgical procedures and the general anesthesia for at least an hour. (Further injections of the local anesthetic were made from time to time during the experiment. A tendency to natural sleep was taken as assurance that the animal was free from pain.) The ECG was monitored by silver-ball electrodes resting on the cortex within the chamber.

Cats were prepared in five different ways: (i) neuraxially intact with desynchronized "aroused" ECG throughout most of the collection period (sometimes maintained so by mild sensory stimulation); (ii) "encephale isolé," upper cervical cord transections, which also showed aroused ECG patterns; (iii) "cerveau isolé," upper midbrain transections, with ECG showing constant spindle pattern characteristic of sleep; (iv) hemisection of upper midbrain with an "aroused" pattern in the ECG from the side opposite to the section in response to mild sensory stimuli and a constant sleep pattern over the ipsilateral cortex; and (v) hemicoagulation of the mesial brain stem at the mesodiencephalic junction, with ECG patterns the same as in (iv).

The chambers were irrigated with warm saline solution until the perfusing fluid was clear. Then each chamber was filled with 1 ml of warm saline solution which was allowed to remain for 15 minutes with gentle stirring and was then withdrawn by suction and frozen. Similar samples were often taken simultaneously from chambers on the two hemispheres, and in some experiments two chambers were placed over each hemisphere, making four in all, to increase the cortical area from which samples were taken.

The fluids obtained from the chambers were lyophilized and the residues were extracted with a mixture of acetone and HCl. Each extract was evaporated to dryness at reduced pressure and the residue taken up in water. The solution was passed through a column of Dowex 50 (H^+ form) and the amino acids were eluted with ammonia solution. The eluate was evaporated to

Table 1. Release of amino acids from the surface of the cerebral cortex. (γ-ABA, γ-aminobutyric acid; *P* indicates significance of difference of the mean from the mean for neuraxially intact animals; ns, difference not significant.)

Preparation	ECG pattern	No. of samples	Amino acid released ($\mu g\ hr^{-1}\ cm^{-2}$)			
			Glutamic	γ-ABA	Glutamine	Aspartic
Neuraxially intact	Aroused	8	9.2 ± 0.8*	0.7 ± 0.2	1.0 ± 0.6	2.1 ± 1.0
Cervical section	Aroused	6	8.6 ± 0.2	0.7 ± 0.25	1.1 ± 0.15	1.8 ± 0.4
Midbrain section	Sleep	13	5.7 ± 1.0	2.0 ± 0.7	1.1 ± 0.3	1.7 ± 0.4
			$P < 0.01$	$P < 0.01$	ns	ns
Left midcollicular hemisection						
Right hemisphere	Aroused	2	7.2, 7.0†	1.1, 0.8	2.0, 2.0	2.5, 2.0
Left hemisphere	Sleep	2	5.0, 4.5	2.6	2.2, 2.2	2.5, 2.0

* Mean plus or minus standard deviation. † Individual results for the two preparations.