At 10 hours, there was, in addition, light staining of nerve fibers in the inner plexiform layer. Thus, it appears likely that the amacrine cells are the major source of the AChE of the inner plexiform layer of the normal rabbit retina.

The appearance of AChE in the majority of the neurons of the ganglion-cell layer of the rabbit, in contrast to the pattern in the cat where extremely few ganglion cells contain detectable AChE (*2*), was unexpected, since the optic nerve in those species studied contains extremely low concentrations of AChE (*11*) and of choline acetylase (*12*).

The presence of AChE in the amacrine cells of the retina has an interesting analogy in the organ of Corti, where AChE is confined to the terminals of the olivo-cochlear bundle (*13*). The amacrine cells are involved in lateral interactions in the retina, which may, like the olivo-cochlear bundle (*14*), exert an inhibitory effect on the transmission of peripheral sensory impulses to the central nervous system (*15*). In spite of their AChE content, the olivo-cochlear fibers are apparently not cholinergic, since their action is not blocked by characteristic cholinergic blocking agents but is highly sensitive to blockade by strychnine (*16*). On the basis of the ability of strychnine to prevent the release of acetylcholine from cholinergic fibers, it has been proposed that acetylcholine is a mediator in the release of an unidentified, hyperpolarizing transmitter by the olivo-cochlear fibers (*17*). Similarly, the production by strychnine of rhythmic retinal discharges (*18*), and its blockade of post-excitatory inhibition in the retina (*19*) may be caused by interference with an acetylcholine-mediated release of an inhibitory transmitter by the amacrine cells.

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Crayfish Muscle Fiber: Ionic Requirements for Depolarizing Synaptic Electrogenesis

Abstract. *Presence of sodium in the bathing medium is not essential for the electrically excitable depolarizing electrogenesis of crayfish muscle fibers, production of action potentials being dependent on calcium. The depolarizing electrogenesis of the excitatory synaptic membrane component does require sodium, however, and this ion cannot be replaced by lithium as it can in spike electrogenesis of many cells. Ionophoretic applications of glutamate, which in the presence of sodium depolarize the cell by activating the excitatory synaptic membrane, are without effect in the absence of sodium. Not only is there no depolarization, but the membrane conductance also remains unchanged. Thus, in the absence of inward movement of sodium across the synaptic membrane there is also no outward movement of potassium. Accordingly, it seems that increased conductance for potassium is not an independent process in the synaptic membrane, whereas it is independent of sodium activation in spike electrogenesis. Chloride activation is independent, however; increase in conductance and the electrogenesis of the inhibitory synaptic component are not affected by the absence of sodium. Implications of these findings regarding the structure of differently excitable membrane components are discussed.*

Axons and muscle fibers can generate action potentials (spikes) after all the Na of the bathing medium has been replaced with Li (*1*). However, excitatory postsynaptic potentials (EPSP's) in cat superior cervical ganglion (*2*) and *Tenebrio* muscle fibers (*3*) are blocked when Li replaces all the Na. Furthermore, the generator potential evoked by light in photosensitive neurons of *Limulus* when Na is present is abolished by substitution of Li (*4*).

Generator potentials and EPSP's are both variants of depolarizing electrogenesis in electrically inexcitable membrane components (*5*). The above-mentioned findings suggested the possibility of further conclusions regarding the ionic channels that must be opened during the synaptic activity. Because of its theoretical importance, we investigated the matter in several parallel studies. Our work on crayfish neuromuscular junctions is the most extensive, but other findings will be mentioned.

Neuromuscular preparations of crayfish (*Procambarus* and *Orconectes*) respond to ionophoretic applications of glutamate with large depolarizations caused by activation of the excitatory synaptic membrane (*6*). They also exhibit inhibitory electrogenesis caused by a different ionic mechanism (*7*). Thus, the effects of Li could be tested on two bioelectric generators. In most experiments, recordings with intracellular microelectrodes were made from individual fibers of various muscles of the walking legs and the abdominal extensors. In some, however, single fibers were prepared as described by Girardier *et al.* (*8*). The results ob-

tained with the different genera, with different muscles, and with single fibers or whole preparations did not appear to differ in any significant manner.

The synaptic membranes were excited either by stimulation of the appropriate axons with brief electrical pulses or by application of glutamate to elicit the excitatory electrogenesis or of γ-aminobutyric acid (GABA) for the inhibitory. The glutamate was applied ionophoretically (*6*). γ-Aminobutyric acid was added to the medium in a dosage large enough to activate the inhibitory membrane fully. Intracellular polarizing currents were applied with a microelectrode.

The standard saline containing Na was that described by Van Harreveld (*9*). The NaCl was stoichiometrically substituted either with LiCl or tris(hydroxymethyl)aminomethane (Tris) chloride. In some experiments the concentration of Ca was increased isosmotically up to as much as 88.3 mmole/liter.

The usual neurally evoked response of a muscle fiber is shown in Fig. 1A. When the fiber was transferred from the standard Na medium to the Li saline, the amplitude of the EPSP decreased slowly (Fig. 1, B–D). The response disappeared after the fiber had been exposed for 2 hours and 40 minutes to the Li medium. Slow disappearance of the EPSP was characteristic for the neurally evoked response.

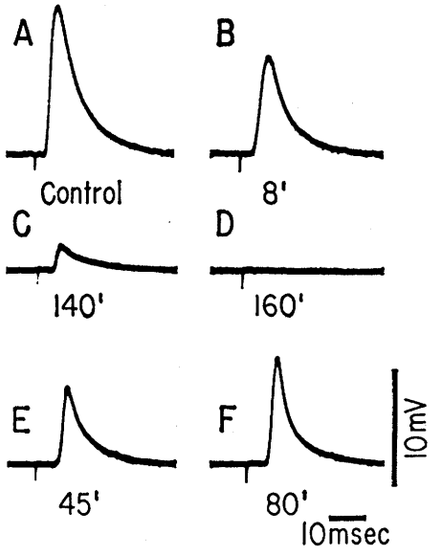

Fig. 1. Reversible elimination of the EPSP on substitution of Li for Na in the bathing medium. This and all other figures are of *Orconectes* neuromuscular preparations. Intracellular recording from a muscle fiber in the medial portion of the deep abdominal extensor muscle. The resting potential (—74 mv) was unchanged on substitution of Li for Na and on the subsequent replacement of Li by Na. The artifacts signal single stimuli to the exciter axon. (A) control; (B–D) the EPSP disappeared slowly after immersion of the preparation in the Li saline. Times after introduction of Li are shown on records. (E and F) Recovery of the EPSP at indicated times after restoration of Na.

The spikes of crayfish axons were unaffected by the substitution of Li for Na. Thus, Li must have produced some effect on the transmissional process. The site of the blockade was tested with ionophoretic applications of glutamate (Fig. 2). The depolarization elicited by a jet of glutamate in the presence of Na (Fig. 2A) was nearly abolished within 20 minutes after Na was replaced with Li (Fig. 2, B–D). The muscle fibers again responded with depolarization when Na was restored (Fig. 2E). The response to glutamate rapidly disappeared when a medium in which NaCl was replaced with TrisCl was applied (Fig. 2F); it returned when Na was restored (Fig. 2, G and H).

Crustacean muscle fibers can generate spikes in the absence of Na (*10*), and generally the depolarizing electrogenesis is effected by an increased permeability to Ca. The depolarizing electrogenesis of the EPSP, however, requires the presence of Na (Fig. 3). The depolarization by glutamate (Fig. 3A) was abolished (Fig. 3B) by substitution of TrisCl for NaCl in the bathing medium. The concentration of $CaCl_2$ was then increased from the normal amount in the saline (13.5 mmole/liter) to 88.3 mmole/liter with appropriate reduction of TrisCl. Only a small depolarization was observed when an ionophoretic jet of glutamate was applied (Fig. 3C), whereas when the muscle was exposed to a NaCl medium the depolarization elicited by glutamate was again large (Fig. 3D). Thus, while the electrochemical gradient for Ca may contribute somewhat to the depolarizing electrogenesis of the excitatory synaptic membrane, the electromotive force of the Na battery is by far the more important contributor.

The neurally evoked inhibitory electrogenesis of crayfish muscle fibers is usually small and is generally depolarizing in sign. Accordingly, a more sensitive index of activity of the inhibitory postsynaptic membrane component is provided by the increase in conductance produced when the inhibitory membrane is excited. We activated the membrane with GABA and tested the change in conductance by measuring the change in the slope of the current-voltage (I-E) relation when the muscle fiber was polarized by intracellulary applied currents.

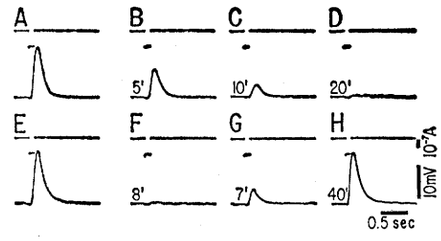

Fig. 2. The presence of Na is required to elicit depolarizations in response to ionophoretic applications of glutamate (abductor of carpopodite, resting potential —82 mv). Upper traces monitor the ionophoretic current; lower traces show the changes in membrane potential. (A) Control response; (B–D) diminution and virtual disappearance of the response with time, after substitution of Li for Na; (E) recovery of responsiveness 27 minutes after restoration of Na. The preparation was then placed in a sodium-free (Tris Cl) medium. (F) The response to glutamate disappeared within 8 minutes and (G and H) recovered gradually after reintroduction of Na.

A muscle was first bathed in a LiCl saline (Fig. 4, open circles) and then in the same saline supplemented with 10^{-5} mole of GABA per liter. The slope of the I-E relation changed markedly (triangles) indicating a considerable increase in conductance which denotes the activation of the inhibitory membrane. The electrogenesis was minimal, however, with a depolarization of only 2 mv. Thus, the inhibitory membrane remained responsive to its activator (GABA) even in the absence of Na. When the preparation was returned to the standard saline free of GABA, the effect of the agent was abolished, and the I-E relation (filled

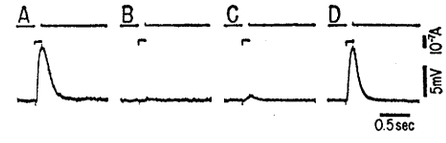

Fig. 3. Effects of Ca on depolarization induced by glutamate in a single muscle fiber, stretcher of meropodite (resting potential —77 mv). Registrations as in Fig. 1. (A) Control; (B) 7 minutes after transfer to a TrisCl saline; (C) 5 minutes after transfer to a medium containing 88.3 mmole of $CaCl_2$ per liter; and (D) 26 minutes after return to the standard medium.

circles) was the same as that for the LiCl medium in the absence of GABA. The resting potential also returned to its original value.

The excitatory postsynaptic electrogenesis is presumably due to an increase in conductance of both the Na and K batteries (*11*). In electrically excitable membranes the ionic channels for Na and K have pharmacologically distinctive properties and are independent (*12*). By substituting either Li or Tris (Fig. 5) for Na, we tested whether or not K activation was still produced in the synaptic membrane after the depolarizing electrogenesis had been eliminated in muscle fibers bathed in the sodium-free saline. An intracellularly applied current was used to change the membrane potential in either direction from the resting level. A jet of glutamate was applied during the polarization. If the muscle fiber had responded to glutamate with K activation, the increase in conductance should have caused a decrease in the polarization evoked by either direction of the applied current and would have resulted in a shift of the membrane potential toward the resting value. The application of glutamate, however, did not cause a change in the polarization. Thus, it seems that in the absence of an inward flux of Na, K activation of the synaptic membrane was also abolished.

Our data (Figs. 1 and 2) confirm findings on other varieties of electrically inexcitable membrane components that the depolarizing electrogenesis is effected by activation of Na channels and that Li cannot be substituted for Na in this electrogenesis. Some experiments on frog neuromuscular junctions (*13*) also showed that the substitution of Li for Na blocks the synaptic potential. However, these findings may not apply to other varieties of electrically inexcitable membranes that cause depolarizing electrogenesis. Thus, studies on the generator potential of crayfish stretch receptors (*14*) showed that the cells could still be depolarized on stretch when they were bathed in a LiCl saline.

The depolarization of the EPSP of crayfish muscle fibers is mainly due to movement of Na, and the ionic battery for Ca is minimally effective, if at all (Fig. 3).

The lack of effect of the substitution on the responsiveness of the inhibitory postsynaptic membrane (Fig. 4) is not unexpected. The inhibitory electrogenesis is due to Cl activation,

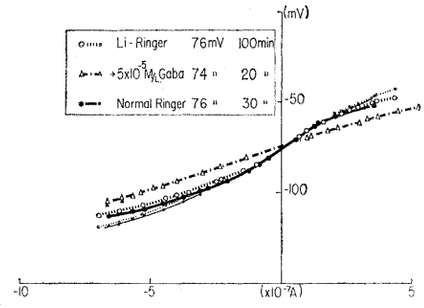

Fig. 4. Change in membrane conductance on application of GABA shows that activity of inhibitory synaptic membrane persists in the absence of Na (stretcher of meropodite, resting potential —76 mv). Abscissa, intracellularly applied current; Ordinate, membrane potential. Open circles show the current-voltage relation in a LiCl saline; the small symbols represent the initial potential, and the larger symbols denote the steady state of the hyperpolarizing Cl activation. Triangles show the change effected 20 minutes after the introduction of GABA (5×10^{-5} mole/liter) in a LiCl medium. Filled circles, 30 minutes after the preparation was replaced in the standard NaCl medium.

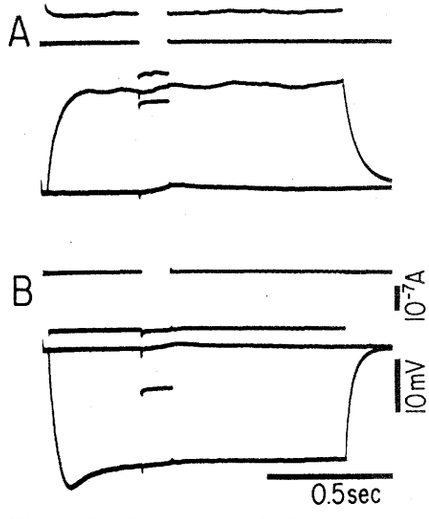

Fig. 5. In the absence of Na, the excitatory synaptic membrane component does not respond to glutamate with an increase in conductance. The abductor of carpopodite bathed in a TrisCl medium was used; resting potential of the impaled fiber was —82 mv. Two sweeps were photographed in each record, without and with an intracellularly applied current which was depolarizing in A and hyperpolarizing in B. The upper traces of each record monitor the depolarizing current as well as that producing an ionophoretic jet of glutamate. During application of current the trace was displaced upward in A and downward in B. The change in membrane potential (lower traces) produced by the applied current was not affected by the application of glutamate; this fact indicates that the glutamate had not increased the conductance of the membrane.

and the electrical conductance for this ionic battery was not affected by the absence of Na. The finding does, however, indicate that the block of the excitatory electrogenesis by Li was probably not due to some "toxic" effect of this ion on the membrane, but that it must have blocked specific channels to prevent inward movement of Na. A similar block of ionic movements is produced by application of Cs or Rb to eel electroplaques (*15*) and of Rb to frog muscle fibers (*16*). In both systems the K channels appear to be blocked, preventing flux of that ion during application of inward currents.

The absence of any increase in conductance when Na influx was eliminated (Fig. 5) implies that the presence of Li must have blocked K activation as well as Na activation. In electrically excitable membranes the activation processes for these two ions are quite independent (*12*). Various pharmacological agents also affect both ionic components differently (*11, 17*). These various data therefore raise the possibility that the movements of Na and K in the activated synaptic membrane do not take place in separate channels as they probably do in spike electrogenesis. However, the frog motor endplate can still respond to acetylcholine with a conductance increase when Na is fully substituted by K (*18*). This might suggest that the movements of Na and K in the synaptic membrane of frog muscle fibers are not as closely coupled as they appear to be in the crayfish synaptic membrane. However, the electrochemical and physiological conditions of the earlier study were sufficiently different from those of the present work, that results cannot be compared directly.

When Li was substituted for Na the neurally evoked EPSP's were eliminated much more slowly (Fig. 1) than was the depolarization produced by ionophoretic applications of glutamate (Fig. 2). Disappearance of the endplate potential of frog muscle fibers was also slow (*13*). There is good evidence that glutamate does in fact activate the synaptic membrane of the muscle fibers (*6, 19*). The difference between the two modes of synaptic excitation with respect to the time course of the block may, however, be due to nothing more than spatial factors. Synaptic activation by the presynaptic terminals takes place at many sites along the crayfish muscle fibers, and even in the relatively concentrated

junctions of the frog endplate the synaptic sites are small discrete patches (*20*). Removal of all Na from the deeper-lying synaptic clefts therefore may be quite slow. Application of glutamate was made at one spot along the crayfish muscle fiber, and, since the spots chosen for the measurements were the most strongly responsive (*6*), it is likely that these synaptic regions were relatively superficial, subject to rapid depletion of Na when this ion was eliminated from the bathing medium.

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Visual Reaction Times on a Circle about the Fovea

Abstract. *Reaction times to a dim photopic stimulus were measured on a circle about the fovea, 15° from the line of direct vision. Large variations in reaction time were found on various half meridians and were interpreted as reflecting the distribution of retinal receptors.*

Visual reaction time (RT) to a light stimulus is related to the subjective brightness of the flash. Bright flashes yield short RT's and dim flashes long RT's. Small changes in light intensity produce large changes in RT only for a low-intensity photopic stimulus. Thus RT provides a sensitive measure of suprathreshold visibility for dim visual stimuli.

Sensitivity of the retina has been mapped along the horizontal meridian (passing through the fovea and blind spot along the line from 0° to 180°, seen in Fig. 1) with both RT and thresholds (*1–3*). The profiles of the sensitivity curves for both RT and thresholds are the same, both measures reflect the distribution of rods and cones as counted and averaged 30° on either side of the horizontal meridian. There is histological evidence that the distribution of rods and cones along the horizontal meridian is not representative of their distribution along other meridians. In particular, the density of rods falls off most rapidly along the meridian from 45° to 225° (*4*). Also, a spot of increased sensitivity on a retina which compensates for the insensitivity of the blind spot of the other eye has been identified by measurements of both RT and threshold (*2, 5*).

Visual reaction time is faster on the nasal side than on the temporal side of the retina with the exception of the spot of increased sensitivity on the temporal retina corresponding to the blind spot of the other eye (*2, 3*). Further, the upper retina yields faster RT's than the lower retina (*6*).

The purpose of this study was to plot in detail a suprathreshold visibility curve measured by RT on a circle about the fovea. The stimulus was a light spot of a 15-minute visual angle produced by a gas-discharge lamp and passed through an American Optical LGM-5 152-cm fiber optic. The stimulus was viewed at 15° from the line of direct vision. This angle was chosen so that the image of the stimulus would

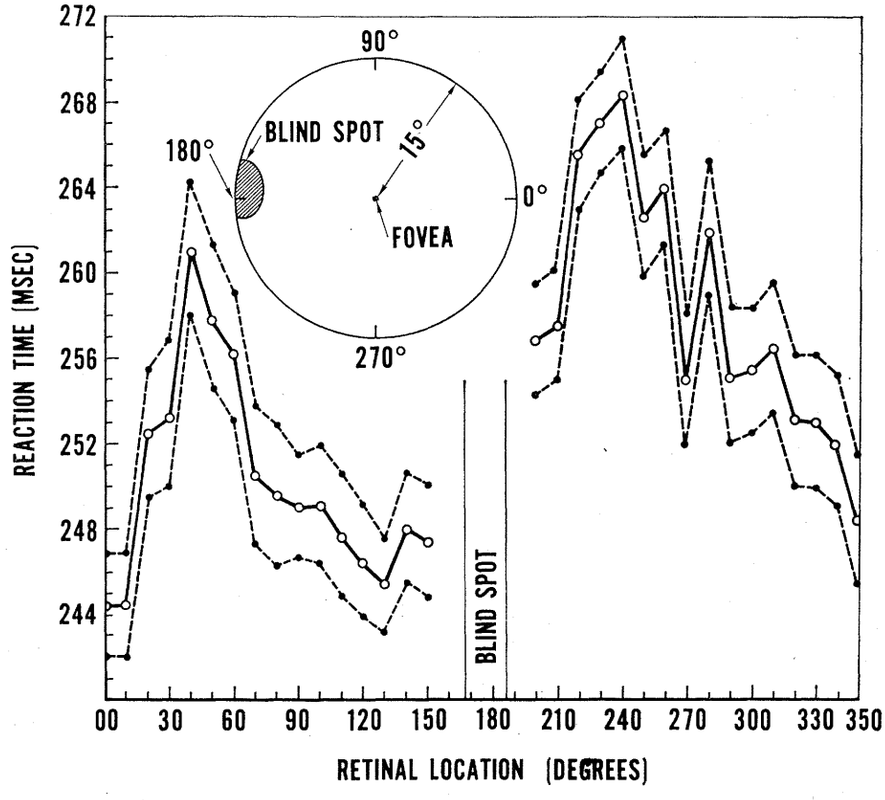

Fig. 1. Average reaction time to a dim visual stimulus plotted as a function of retinal location on circle 15° eccentric from the line of direct vision. The dashed lines define plus or minus two standard errors of the mean. Each average is based on 480 reaction times made on both eyes of two observers.