K^+ affects the hyperpolarized cell.

Increases in K^+ concentration sufficient to reduce the electrogenic hyperpolarization occur physiologically as the result of neuronal firing. A reliable estimate can be made of the buildup in K^+ concentration in the immediate vicinity of the neuronal membrane, the undershoot of the action potential being used as an indicator (*4*). With this technique it can be shown that when a single cell is stimulated at 45 stimuli per second for a few seconds the K^+ concentration around it may increase by 3 mmole/liter. When a whole nerve root is stimulated, the average concentration within the extracellular spaces can rise still further, to more than 10 mmole/liter, as estimated from the change in the membrane potential of the surrounding glial cell (*5*). Under such conditions, hyperpolarized neurons will be much more depolarized by K^+ than if their membrane potential had been at the normal resting level.

A speculation that can be made on the basis of these results is that K^+ liberated by active neurons might differentially affect adjacent cells depending on whether they had previously been active and were therefore hyperpolarized. Thus a cell which had conducted impulses would be more depolarized by a given increment of external K^+ produced by its neighbors than one which had been quiescent. If such a mechanism did operate, the glial compartmentation of neurons might influence the patterns of interaction around synaptic regions. For example, a common compartment might enclose one group of neurons, allowing them to interact by means of K^+ while reducing the effect of K^+ liberated from other cells.

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Lateral Giant Fibers of Crayfish: Location of Somata by Dye Injection

Abstract. *Using a new technique for injecting fluorescent dye, we have analyzed the intraganglionic architecture of the lateral giant interneuron in crayfish. Each lateral giant interneuron comprises a giant axon, an ipsilateral dendritic arborization of fairly constant form, and a contralateral soma. Antidromic axon spikes produce only small, electronic potentials in the soma; direct depolarization there fails to produce electrically excitable membrane responses and cannot discharge the main axon.*

The lateral giant fibers (LG) of crayfish are among the best-known of the giant cells in invertebrate nervous systems. Extensive electrophysiological (*1*) and morphological (*2*) studies have shown the LG to be separate segmental interneurons connected end-to-end by unpolarized electrical septa. Each cell also (i) makes synaptic contacts in the neuropil of its own ganglion (*3*); (ii) forms a rectifying electrical junction with a "motor giant" neuron (*4*); and (iii) joins with its contralateral partner in a nonrectifying commissural connection (*1*). Although the principal electrical junctions have been studied thoroughly, the major branches have not been mapped, nor have the somata been found. This information is needed for three purposes. First, the morphology of ganglionic branches will be helpful in interpreting the motor output produced by single-cell stimulation (*5*, *6*). Second, some invertebrate giant fibers are formed from the processes of many cells. Until the LG soma is found, its status as a single element is in doubt. Third, the relationship between the electrical activity of an interneuron soma and its connection with the axon could explain the puzzling fact that microelectrode records from somata in crustacean abdominal ganglia have revealed no interneurons (*6*). We here report the application of a new intracellular injec-

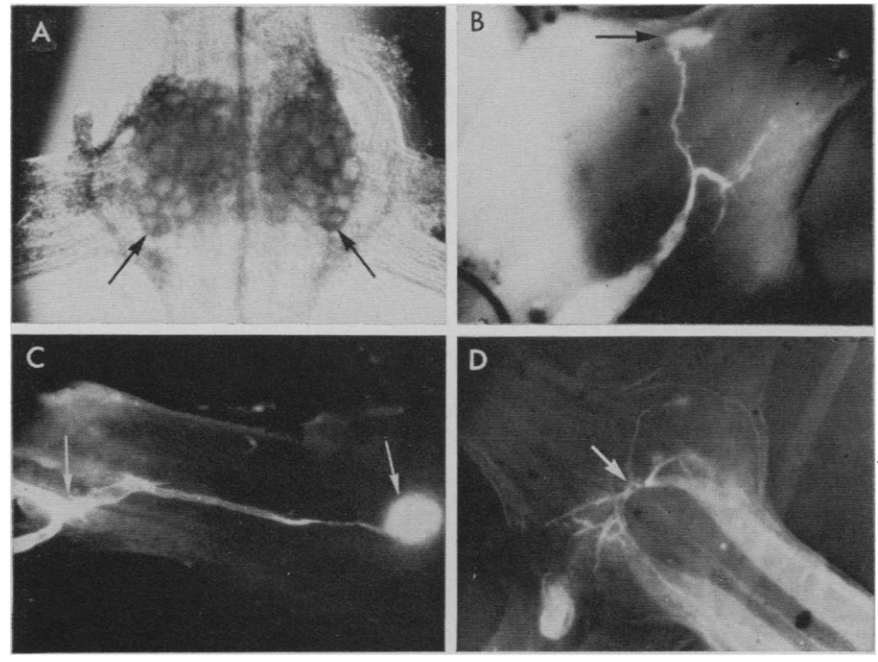

Fig. 1. (A) Third abdominal ganglion as it appears when viewed in the dissecting microscope. Arrows indicate the position of the two LG somata. (B) Whole mount of a cleared ganglion photographed with fluorescence microscopy after injection of the LG axon with Procion Yellow. The cell body is visible on the contralateral side (arrow); the dendritic branches are in the ipsilateral neuropil. (C) Electrical junction (left arrow) between fast flexor motoneuron and LG axon. The fluorescent dye crossed this junction, but on the other side remained confined to the motor giant cell whose soma (right arrow) is filled. (D) Double injection of LG axons to illustrate the commissural synapse (arrow). The lower cell body was broken off during the mounting procedure. For all figures, the width of the ganglion (1.0 mm) provides a size reference.

tion technique, which employs a dye first used by Kravitz *et al.* (*7*), to locate the position of the LG somata and to map their branches. The electrical properties of the somata so found have also been related to the morphology of the cells.

Crayfish (*Procambarus clarkii*) were pinned ventral side up in a paraffin-filled lucite dish and covered with cold van Harreveld's (*8*) solution. After exposing the abdominal nerve cord, we removed the sheath around ganglion 3 and around the connective between ganglion 2 and ganglion 3, stripped away the fibers overlying the LG axon, and blew out the ganglionic connective tissue with a fine jet of saline. To inject the LG we lifted the anterior portion of the connective with a metal hook and inserted a micropipette from the rostral end nearly parallel with the axon, so that the tip could be advanced longitudinally toward the third ganglion. The micropipette was filled with a saturated (about 6 percent) solution of aqueous Procion Yellow (M4RS); a connected micrometer syringe was used to inject a small amount of the dye. The cord was then ligated near the point of injection to prevent leakage of the dye, and the preparation was stored at 4°C to permit diffusion of the dye into the ganglionic branches of the cell. These filled after 2 to 96 hours, and could easily be seen in a dissecting microscope.

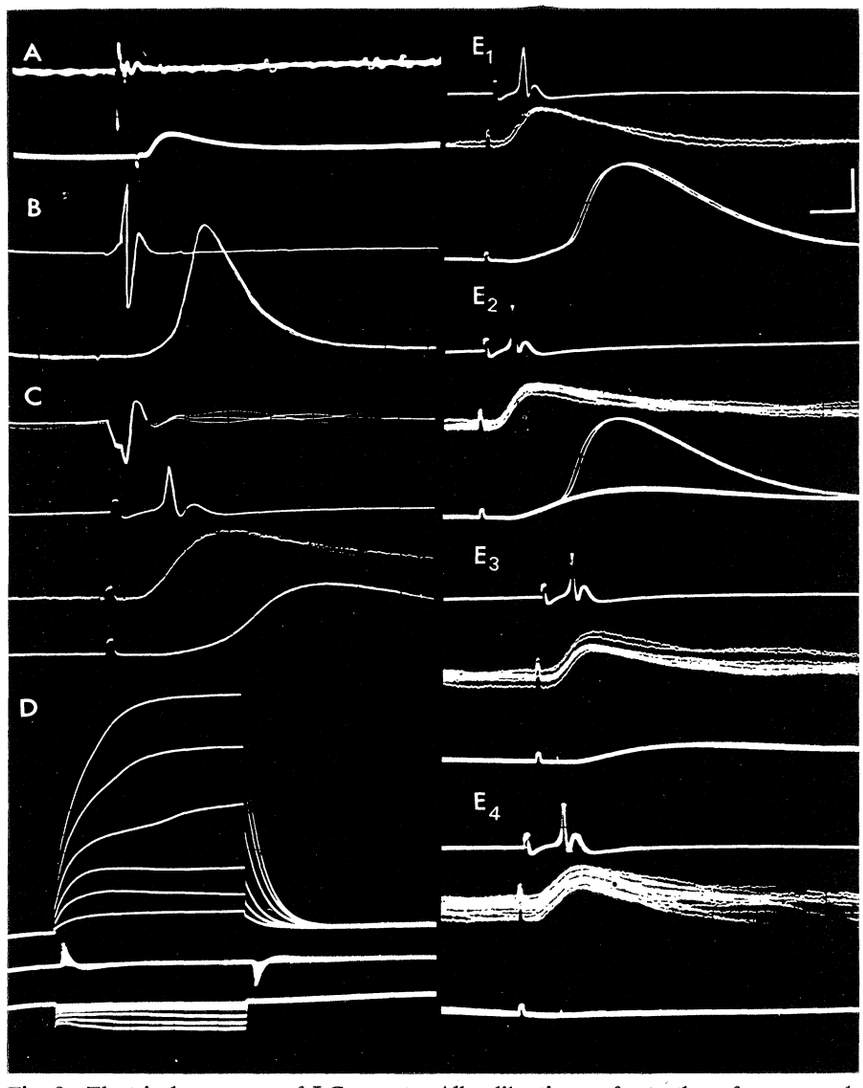

Fig. 2. Electrical responses of LG somata. All calibrations refer to the reference marks in E_1. (A and B) Typical antidromic soma potentials (lower traces), obtained by stimulation of LG axon (the extracellular record in upper traces). Calibrations: time, 2 msec; 0.5 mv in A and 5 mv in B. (C) Soma responses from LG (trace 3, 1 mv) and a nearby flexor motoneuron (trace 4, 2.5 mv) activated by stimulation of LG axon (trace 1). The motoneuron soma tires after a synaptic delay. Trace 2 is of the heterolateral LG axon. Time calibration, 2 msec. (D) Purely passive responses of the LG soma to direct depolarization through a second microelectrode. Upper trace, soma response; middle trace, extracellular record from contralateral LG axon, to show absence of an evoked discharge. Calibrations: time, 10 msec; current (lower trace), 2×10^{-8} amp; voltage, 20 mv. (E_1–E_4) Illustrates failure of motoneuron (lower trace, 5 mv) to follow LG axon spikes at high frequencies. Upper traces, contralateral LG axon; middle trace (1 mv), LG soma. Stimulus frequencies: 10, 20, 40, and 80 per second from E_1 to E_4, respectively. Time calibration, 2 msec.

When one views a desheathed ganglion with dark-field illumination, the larger cell bodies are seen to form a characteristic array on the ventral surface (*9*). Three moderately large cell bodies are aligned longitudinally next to the exit of the second root (Fig. 1A); the most caudal one of these always stained when the contralateral LG axon in the rostrally adjacent connective was injected. To determine the course of ganglionic processes, we fixed the tissue in Bouin's solution, dehydrated it in alcohols, cleared it with xylene, and made whole mounts of the ganglia in Fluoromount for observation with a Zeiss fluorescence microscope with exciter filter II and barrier filters 50 and 44 (Fig. 1, B–D). The dendritic field is ipsilateral to the LG axon; the neurite crosses as a thin branch to the contralateral side, and gives off finer processes as it does so (Fig. 1B). Although the dye does not normally cross the known electrical junctions made by the LG, excessive pressure will sometimes cause it to pass these membranes selectively. Figure 1C shows an instance in which dye injected into the LG axon crossed to the motor giant cell without spreading to extracellular sites or to other nearby cells. In the experiment shown in Fig. 1D, both LG axons were injected. The commissural junction between them can be seen at the point where their processes cross in the midline.

In other experiments, we recorded from the soma while stimulating its axon and monitoring the axon spike in an adjacent segment. Such intracellular recordings showed a small, electronically decaying depolarization that was associated at fixed latency with spikes in the main LG axon (Fig. 2, A and B). These depolarizations differed in two ways from the responses of motoneuron somata driven postsynaptically by the LG. First, they were always much smaller, ranging from 50 μv to 5 mv instead of greater than 10 mv; second, they followed repetitive LG stimulation at frequencies up to 100 per second, instead of failing at 10 to 15 per second (Fig. 2E). Direct depolarization of the LG soma (by as

much as 80 mv for 50 msec, as recorded with a second intracellular electrode) never caused a spike in the main LG axon, nor was there evidence for electrically excitable activity in the soma membrane (Fig. 2D). This result indicates that the electrotonic spread of soma depolarization is insufficient to activate axonal spike-initiating sites, and would be predicted from the length and small diameter of the neurite.

After several experiments, injection of dye into the axon was used to identify the recorded soma as that of LG. Cells showing large soma potentials tended to fill more rapidly than those showing small ones, suggesting that the length and diameter of the neurite is variable. Longer, thinner neurites presumably provide an extra impediment to the flow of dye, and also impose more electrotonic decrement.

In the thinness of its neurite and the heterolateral position of its soma the LG resembles the nonseptate median giants, whose architecture in the brain has recently been elucidated with conventional anatomical techniques (see *10*).

The detailed morphology of an identified interneuron has thus been worked out with a new and useful anatomical technique. The results show the LG to be a conventional neuron with a single soma which is electrically and spatially isolated from the conductile process, and whose membrane is electrically inexcitable. Previous microelectrode explorations of crustacean segmental ganglia may have failed to reveal soma responses of other interneurons because they, too, are more isolated from conductile and integrative sites than are the somata of motoneurons.

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5 July 1968

Insulin Secretion from Toadfish Islet Tissue Stimulated by Pyridine Nucleotides

Abstract. *The addition of glucose stimulated release of insulin from the isolated islet tissue of the toadfish incubated in vitro. Reduced nicotinamide-adenine dinucleotide also stimulated insulin release, whereas the oxidized form had no effect. Both oxidized and reduced nicotinamide-adenine dinucleatide phosphate stimulated insulin release, but the reduced form was significantly more effective.*

Athough the role of glucose in stimulating insulin secretion from the beta cells of the islets of Langerhans has been well studied with the isolated perfused pancreas (*1, 2*) and slices of pancreas (*3–6*), the mechanism controlling the insulin release remains unknown. Several theories have been proposed (*7–9*), and the one most widely held is that glucose acts by giving rise, during metabolism, to some substrate or cofactor which then triggers insulin release (*9*). This is supported by the finding that glucose has no effect on insulin secretion when its metabolism is blocked by 2-deoxyglucose (*10*), glucosamine (*11*), mannoheptulose (*3, 4*), 2,4-dinitrophenol (*2, 3, 5*), or anoxia (*3, 5, 12*).

We have examined the effect of a number of metabolic intermediates and cofactors whose concentration in the islets might be expected to change as a result of glucose metabolism. We used the toadfish as the experimental animal. In contrast to mammals, the islet tissue of the toadfish is separated from the acinar pancreas and segregated into one or more discrete bodies; thus difficulties encountered by the presence of acinar tissue are eliminated. In our studies, reduced pyridine nucleotides markedly stimulated insulin release from islet tissue.

Toadfish islets (1 to 6 mg each) were obtained as described (*13*), decapsulated, and incubated for 10 minutes in 0.140*M* NaCl buffered at *p*H

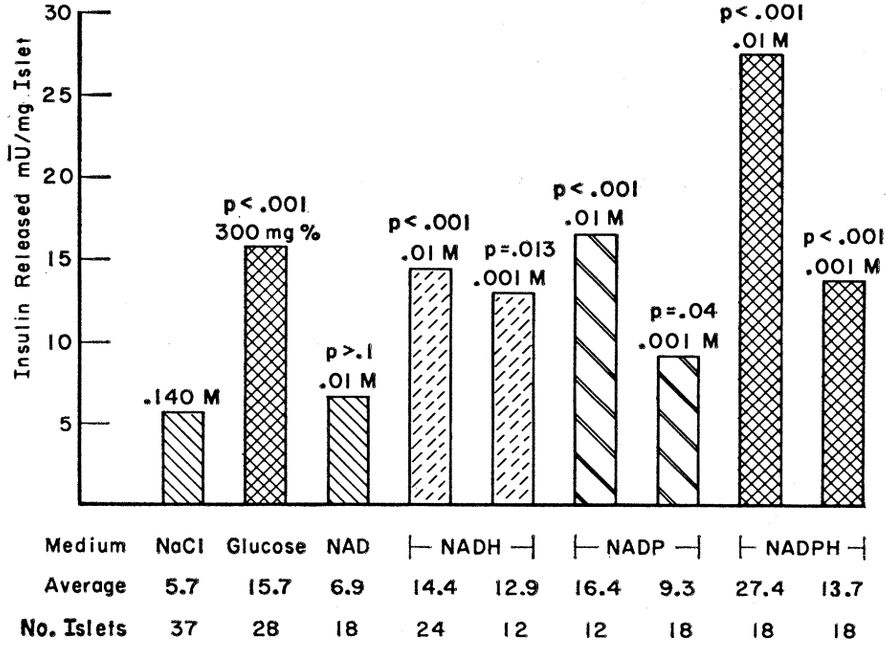

Fig. 1. Effect of pyridine nucleotides (NAD, NADH, NADP, NADPH) on insulin release from toadfish islets. The *P* values shown refer to the difference between the test substance indicated and the NaCl control.