# Arthropod Preparation for Behavioral, Electrophysiological, and Biochemical Studies

Abstract. *Electrodes and cannulea can be permanently implanted in the horseshoe crab,* Limulus polyphemus. *Recordings of electrical activity can be obtained from the optic nerve, heart, and abdominal ganglia and the cannulae are effective routes for introducing isotopes into the nervous system. The animals survive for at least 13 days and are not behaviorally impaired.*

In studying the relations between neurophysiological and behavioral events, the anatomical and behavioral characteristics of many arthropods offer advantages over higher organisms. Behavioral patterns in arthropods are typically stereotyped. The central nervous systems are anatomically and functionally well differentiated, and the coordinating mechanisms for many response sequences exist in distinct ganglionic masses.

We have been interested in developing an arthropod preparatioin useful for investigating the neural substrates of learning and memory. Our requirements were that the animal be capable of learning, that its anatomy permit the permanent fixation of electrodes in relevant places without seriously limiting the animal's behavior, and that its circulatory system be accessible for cannulation so that radioisotopes and other agents could be injected at any time during learning.

The horseshoe crab (*Limulus polyphemus*) meets these requirements and offers other advantages as well. Large specimens (15 to 25 cm in diameter) may be obtained at relatively low cost (*1*) and kept for several months in an artificial sea-water medium (*2*). A conditioned tail reflex can be established in response to a light stimulus presented to the lateral eye (*3*). The proportionately large size of the nervous system enables ganglia and fiber bundles to be readily located and makes available amounts of nerve tissue sufficient for biochemical analyses. Once implanted, electrodes and cannulae can be permanently attached to the rigid dorsal carapace.

Our goals were to obtain recordings of the electrical activity in the optic nerve of the lateral eye and, at that area of the ventral cord which sends fibers to the flexors and extensors controlling tail movements, the postbranchial ganglia (*4*). To detect any changes in other aspects of the functioning of the nervous system, recordings of the neurogenic heart rhythm were also desired. Activity had to be recorded from electrodes in each position for 6 to 8 days, the minimum time needed to establish a conditioned tail reflex in response to light. Permanent cannulation was necessary so that injections could be made by the same routes in different animals. In addition, prior implantation of a cannula would minimize any stress that might be caused by direct insertion of a syringe.

Electrodes and cannulae were implanted in eight animals ranging from 12 to 25 cm in diameter. The time each animal survived the various combinations of implanted electrodes is indicated in Table 1. All surgery was accomplished with the animals packed in ice, a procedure which kept the animals immobilized and kept bleeding at a minimum.

Electrodes were made from 26-gauge stainless steel wire. The tips were formed electrolytically and insulation was achieved by the methods of Grundfest *et al.* (*5*); tip diameters ranged from 10 to 20 $\mu$. Recordings were monopolar, amplified with Tektronix 122 a-c amplifiers, and monitored on an oscilloscope. Permanent records were obtained by means of a Polaroid camera attachment.

To implant an electrode in the optic nerve, a 2-cm hole was drilled just anterior to the lateral eye. Three stainless steel screws were then inserted into the carapace around the rim of the hole, forming a triangle. The surrounding carapace was scraped and the screws covered with prosthetic cement (Nu-Weld). This procedure provided an anchor securely fixed to the animal. A Teflon strip, hooked at one end, was used to grasp the nerve (see Fig. 1*A*). Its upper portion was cemented to the anchoring ring, thus fixing the distance between the fiber bundle and carapace. The electrode was lowered into the nerve with the aid of a micromanipulator, and the upper part of its shaft

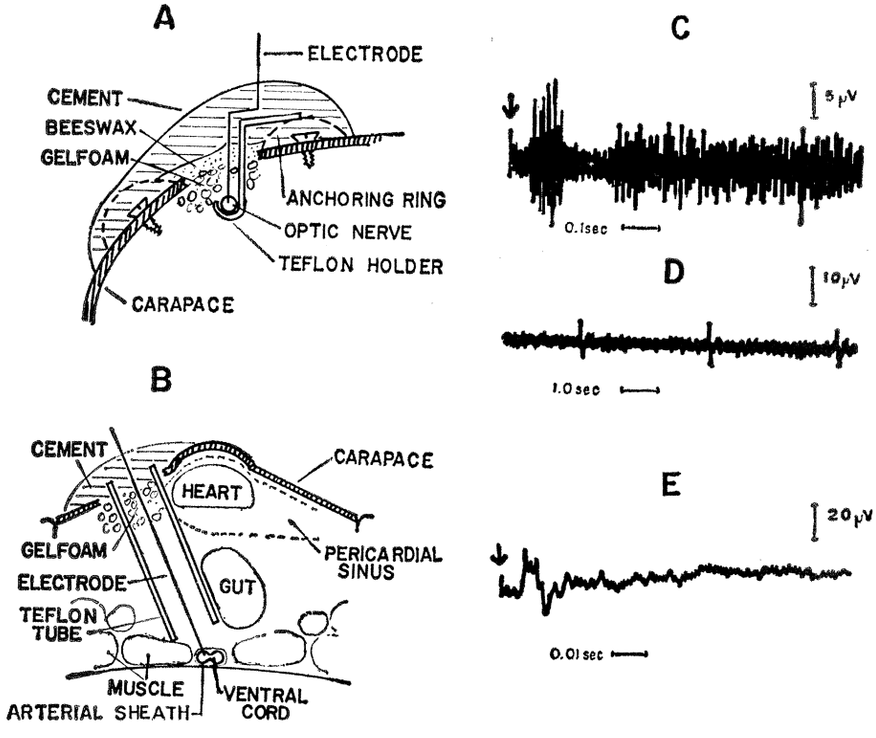


Fig. 1. (*A*) Method of placing electrode in optic nerve. (*B*) Method of placing electrode in the ventral cord. (*C*) Response of optic nerve to constant light source (arrow indicates light onset). (*D*) Spontaneous heart activity. (*E*) Response of postbranchial ganglia to a 20-volt shock, 20 $\mu$sec in duration, delivered across the posterior carapace. Recordings taken 12 days after surgery. Upward deflection indicates a negative response.

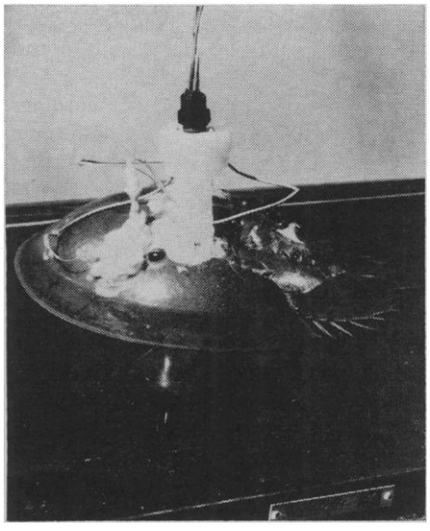

Fig. 2. A *Limulus* with permanently implanted electrodes.

cemented to the anchoring ring. The wound was filled with Gelfoam (Upjohn), sealed with beeswax, and covered with a layer of cement. The beeswax protected the nerve, which is close to the carapace, from the cement.

To obtain recordings of heart rhythms, a 1-mm hole was drilled in the medial ridge of the carapace of the cephalothorax, 2.5 cm from its posterior edge. The electrode was lowered 1 cm below the surface of the carapace and secured by pouring cement around the point of entry of the electrode.

Placement of electrodes in the postbranchial ganglia was the most difficult, mainly because these ganglia are located ventrally. The approach and methods of ensuring electrode stability are depicted in Fig. 1*B*. A hole was cut in the posterior carapace, extending laterally from the midline to the chitinous infoldings, and longitudinally from the third to the fifth pair of entapophyses [see (*4*)]. The pericardial sinus and gut were moved centrally and a teflon tube (0.7 cm in diameter) placed over the cord as shown in Fig. 1*B*. The electrode was then lowered into the cord, piercing the enveloping arterial sheath. The tube prevented the gut and other internal structures from displacing the electrode.

The large dorsal pericardial sinus was the most accessible vessel for cannulation. A hole, slightly smaller than the threaded part of the cannula (*6*), was drilled 1 cm off the midline of the posterior carapace opposite the third pair of entapophyses. The cannula was inserted through the hole and screwed tightly against the carapace, its shaft extending into the sinus.

The reference electrode was placed in the egg masses of the cephalothorax. All electrodes were connected with shielded cable to a seven-pin miniature tube socket. The socket was contained in a teflon bridge attached to the carapace as shown in Fig. 2.

The surgery did not appear to produce any serious changes in behavior, and we found that animals with permanently implanted electrodes could be conditioned. Most animals were kept in a free-living state in an aquarium between recording sessions, and during these periods feeding, mating, and egg-laying were observed.

During recording sessions the animals were placed in a small aquarium containing 3 cm of sea-water, or, if they were unusually active, they were secured in a manner similar to that used by Smith and Baker (*3*). Examples of electrical potentials obtained 12 days after surgery are shown in Fig. 1, *C* through *E*.

To determine whether the cannula was an adequate route for getting an isotope into central nervous-system structures, 50 $\mu$c of $P^{32}$ were injected into each of four cannulated animals. In one fraction consisting of ribonucleic acid nucleotides, counts of at least 90 per minute over background have been found per nucleotide. Further details will be published elsewhere.

These findings thus demonstrate the feasibility of permanently implanting electrodes and cannulae in *Limulus* while leaving the animal in a behaviorally unrestricted state.

Table 1. Placements of electrodes and cannulae in eight *Limulus polyphemus*, and the time each survived after surgery. An "X" in any column indicates that the animal received the implantation listed at the top of the column.

| Electrodes | | | Cannula | |
|---|---|---|---|---|
| Optic nerve | Ventral cord | Heart | Dorsal pericardial sinus | Survival (days) |
| X | X | X | | 13 |
| | | X | X | 18 |
| | | X | | 23 |
| | | X | X | 36 |
| X | X | X | X | 14 |
| | | | X | 78 |
| X | | | | 14 |
| X | | X | X | 57 |

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**References and Notes**

1. Specimens may be obtained from W. Taylor, RD 2, Cortez, Fla., or from the Marine Biological Laboratory, Woods Hole, Mass.
2. Artificial sea water was made with Rila marine mix and kept at a *p*H of 7.5 to 8.2.
3. J. C. Smith and H. D. Baker, *J. Comp. Physiol. Psychol.* **53**, 279 (1960).
4. W. Patten and W. A. Redenbaugh, *J. Morphol.* **16**, 1 (1900).
5. H. Grundfest, R. W. Sengstaken, W. H. Oettinger, R. W. Gurry, *Rev. Sci. Instrum.* **21**, 360 (1950).
6. Collison intraventricular cannulae, C. F. Palmer Ltd., London.
7. Supported by NASA grant NSG 475.

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## Selective Attentiveness and Cortical Evoked Responses to Visual and Auditory Stimuli

Abstract. *Cortical evoked responses to flashes and clicks were recorded from human subjects performing visual or auditory tasks under three conditions of selective attentiveness. The subjects were required to attend to the flashes and to ignore alternating clicks, or vice versa. Responses to flashes recorded from the occipital area were larger when attention was directed toward visual stimuli, and responses to click stimuli recorded from the temporal area were larger when attention was directed toward auditory stimuli.*

In a previous study (*1*) we demonstrated that the magnitude of visually evoked responses was correlated with fluctuations in attentiveness during a prolonged visual vigilance task. In contrast, the study reported here was concerned with short-term attentiveness to either click or flash stimuli presented alternately. Average evoked potentials were recorded from visual and auditory areas while subjects attended to stimuli within one of the two sense modalities and ignored those in the other. Three methods of inducing and maintaining attentive sets were compared.

Thirteen subjects performed under all three experimental conditions: vigilance, key-pressing, and counting. Un-