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Demonstration of Anatomy of the Giant Fiber System of the Squid by Microinjection.* (21254)

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There are a number of morphological, physiological and pharmacological technics available for the study of the gross and microscopical features of the nervous system. In this paper is described still another technic, that of microinjection which has been adapted to demonstrate the finer structures at the cellular and intercellular levels. With this technic, single neurons can be colored and their exact courses, branches and connection traced.

Materials and methods. The squid (*Loligo pealii*) was used, the gross aspects of the giant fiber system having been demonstrated by Young(1). If only the mantle fibers and the stellate ganglion were needed, the animal was decapitated; if the central nervous system was needed intact, the animals were narcotized with chloral hydrate (1 g/liter filtered sea water). The mantle was opened by a mid-ventral incision, flattened against a glass plate, and firmly anchored (Fig. 1). The specimen was kept wet with sea water during its use. It was not necessary to remove the fibers from the mantle because of the latter's translucence. However, when the second order fiber from the stellate ganglion into the central nervous system was to be injected, it had to be dissected free from the other fibers making up the bundle. A binocular dissecting microscope was adequate. The specimen on a glass plate was lighted from below. A simple right-angle coarse-geared micromanipulator was used. Micropipettes with tips 5-10 μ were hand drawn from 0.85 mm bore hard glass tubing, according to the method of Barber as modified and extensively used by Chambers(2,3). Pipettes were filled just before use with 0.1-0.5% Trypan Blue or 1% Trypan Red(4). Under the dissecting microscope the microtip

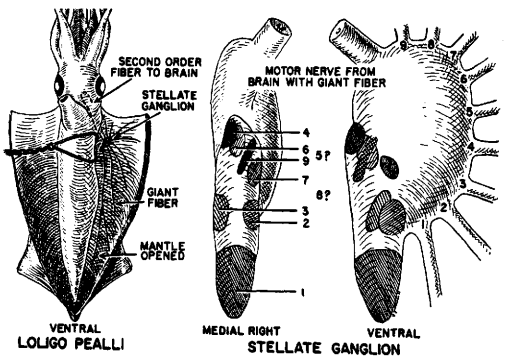

FIG. 1. The giant fibers of the mantle decrease in size from posterior to anterior so that fiber 1 is huge compared with 8 or 9. Nucleus 1—Several injections. This is the largest cell mass because it forms the largest fiber. It occupies the entire posterior of the ganglion's cell mass. Nucleus 2—Several injections. This mass is somewhat smaller, since its fiber is smaller. It lies immediately anterior to 1, but occupies only the ventral portion of the total mass. Nucleus 3—Three injections. A smaller elongated group of cells lying dorsal to 2. Nucleus 4—One injection. A small cell group at the antero-dorsal tip of the extraganglionic cell mass. Nucleus 5—No satisfactory injection. One attempt. Nucleus 6—One injection. About the same size as 4 and lying just medial to it. Nucleus 7—One injection. A small group just anterior to 2 on ventral side of the whole cell mass. Nucleus 8—No satisfactory injection. Three attempts. Nucleus 9—One injection. A very small elongated group of cells lying ventral to 6.

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was pushed at 30-40° angle directly through the mantle muscle to penetrate the giant fiber at some point in its course. Dye was injected by slight pressure through a closed water column attached to a 5 cc syringe. Injection was continued until the dye reached the nuclear mass in the stellate ganglion or, in the case of the second order fiber into the brain, until approximately 10 mm^3(2) of dye was injected. Injected specimens were fixed in 15% formalin, dehydrated in alcohol, cleared in xylene, and imbedded in paraffin to be sectioned later.

Results. 1) By injecting each of the giant fibers of the mantle, it was possible to outline the cells of origin of each in the stellate ganglion. The diagrams show a ventral view and side view of the squid's right-side stellate ganglion, and map out each of the cellular masses except the fifth and eighth, for which no satisfactory results were obtained. When the same fiber was injected in different specimens the nuclear mass was always constant in location.

2) The nature of the giant synapse in the stellate ganglion, although already known, is easily demonstrated by the intracellular injection method. By injecting one of the mantle axons with 0.5% Trypan Blue to its nuclear origin and then exerting slightly more pressure, the interaxonal plasma membranes can be ruptured and all the giant axons filled with bright blue dye. Then 1% Trypan Red is injected into the second order fiber, whose end feet form the giant synapse. When viewed through the low power dissecting microscope, the giant synapse is shown in brilliant red and blue. There is no admixture of dye, showing that the axon end feet are separated from the giant fiber axons by some form of membrane.

3) The cells of origin of the second order fiber in the brain, previously unknown, were easily located by gross inspection; however, finer localization must await microscopic sections. The second order fiber leaves the stellate ganglion and penetrates the muscular body wall, traveling in a nerve bundle along the dorsal side of the body cavity to pierce the cartilaginous brain case almost at the midline, just dorsal to the esophagus. Within the brain the fiber bends sharply laterally and ventrally and ends after completing a hemicircle in a small ventrolateral lobe behind the large optic lobe.

Summary. 1. A new intracellular staining method is proposed as a way of obtaining definitive knowledge as to the location of single fiber nerve tracts, their connections, branches and cells or origin. 2. The method is applied to the giant fibers of the squid with definitive results.

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Development of a Strain of Spontaneously Hypertensive Rabbits.* (21255)

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Essential hypertension develops in man without demonstrable renal, neural, or vascular involvement and becomes more severe in older age groups. Hamilton, *et al.* believe that essential hypertension is not a disease entity but merely a condition seen in that section of the population presenting arterial pressures higher than an arbitrarily selected value(1). Without a definite disease to which they can attribute the elevated pressure, in-

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