tein matrix of the chromosomes, an indication that the protein associations of satellite DNA differ from those of main-band DNA. Yasmineh and Yunis (*16*) found that most of the DNA extracted from isolated heterochromatin of the mouse liver and brain cells was satellite DNA. The euchromatin prepared in these same experiments contained only DNA of main-band density.

Our results clearly demonstrate that heterochromatin is not a single category. It has been recognized for some time that certain chromosome regions maintain their heterochromatic character at all times. This type of heterochromatin has been termed constitutive and is usually considered genetically inert. Other chromosome regions or entire chromosomes, such as the mammalian X (*17*), become heterochromatic only in some particular stages or tissues. The second type of heterochromatin has been termed facultative or functional and is often associated with a turning-off of the genes on the chromosomes involved. The regions adjacent to the centromere are usually thought of as constitutive heterochromatin. This heterochromatin contains a specific type of DNA which is not present in the facultative heterochromatin of the sex chromosomes. It has also been reported that mouse chromosomes have constitutive heterochromatin at the telomeres (*18*). If these regions do contain satellite DNA sequences, these sequences are too few to be detected on our slides.

Although we have localized mouse satellite DNA in the centromeric heterochromatin, this localization does not establish a function for either satellite DNA or heterochromatin. It seems that this function is one which is necessary to the chromosome since the proportion of satellite DNA is maintained in established mouse cell lines even though the chromosomes have undergone other morphological change. We have found the same pattern of satellite hybridization in all of the mouse cell lines which we have studied. In this respect it becomes important to establish whether the apparent lack of hybridization at the Y centromere indicates a quantitative or qualitative difference from the other chromosomes. It is possible that centromeric heterochromatin might bind the proteins of the spindle microtubules during mitosis. We have investigated the binding of purified microtubular subunits to mouse satellite DNA in vitro (*19*) but our preliminary experiments showed no binding under the conditions used in our assay (*20*).

Centromeric heterochromatin has been described in the chromosomes of many animals and plants. It seems possible, therefore, that the centromeres are regularly adjacent to regions of repetitive DNA. Experiments in this laboratory have shown that the fly *Rhynchosciara hollaenderi* has a satellite of repetitive DNA. This satellite, like that of the mouse, has been localized in the centromeric heterochromatin by cytological hybridization (*21*). Cytological hybridization has also shown the presence of repetitive DNA at the centromeres in *Triturus viridescens* (*22*) and *Drosophila melanogaster* (*23*). It now seems important to determine whether noncentromeric heterochromatin consists of repetitive DNA with nucleotide sequences different from those of centromeric heterochromatin (*24*).

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Motoneuron Morphology and Synaptic Contacts: Determination by Intracellular Dye Injection

Abstract. *The structure and dendritic connections of an identified crustacean motoneuron were analyzed by intracellular injection of dye. Some processes of the neuron end in the ganglionic neuropil, but most terminate on axons which pass through the ganglion in specific, identifiable tracts. The former processes are ipsilateral to the soma, while the latter, as well as their connections, display bilateral symmetry. Structural and functional evidence suggests that the demonstrated contacts are synaptic junctions, and that the approach can therefore be used to study patterns of synaptic organization in complex neural networks.*

One goal of neurophysiologists is to associate the functional properties of specific nerve networks with the underlying and presumably causal structural "circuitry." Toward this end Stretton and Kravitz (*1*) recently developed a technique for determining the geometry and positions of single neurons and their dendritic fields. A single nerve cell is injected with the fluorescent dye Procion Yellow, which becomes distributed throughout the cell and remains confined within it during subsequent histological procedures. When viewed with a fluorescence microscope, the dye-filled branches of the neuron appear brilliant yellow against a deep green background of uninjected tissue. This technique has been used, for example, in the study of the morphology and spatial relationships of the giant fibers and fast flexor motoneurons in the crayfish abdomen (*2*).

I have applied the dye injection technique to motoneurons supplying relatively complex locomotory appendages, the abdominal swimmerets of the lobster *Homarus americanus*. The mo-

tor output patterns which control the rhythmic swimmeret movements have been analyzed in some detail (*3–5*), so that a satisfactory physiological basis exists for correlating neuronal function in this system with the structural data described here. The more general significance of my study is that it demonstrates the usefulness of the dye injection technique for determining the pattern of synaptic connections in a relatively complex network.

The method of Stretton and Kravitz (*1*) was used except that the dye was injected into the cell by pressure rather than by iontophoresis (*5a*). Neurons were functionally identified as described elsewhere (*5*). Fourteen motoneurons were injected and processed, of which 12 were power-stroke excitatory neurons located in the third abdominal ganglion [figure 2B in (*5*)]. In the eight injections which yielded useful data, dye was injected at a rate sufficient to expand the cell body by 50 percent in 5 seconds, and this procedure was repeated several times over a period of ½ to 1 hour. The dye was then allowed to become distributed for 72 hours at 4°C. It was usually possible to predict the outcome of an experiment within a few seconds of the initial injection. The best preparations were immediately made evident by the appearance of yellow dye in the neurite, sometimes for a distance of several hundred micrometers.

The neuron selected for detailed analysis was a power-stroke excitor (Fig. 1). The remaining neurons which were injected successfully appeared similar in gross morphology. For example, all were monopolar and most, including a single return-stroke excitor, sent branches to the opposite side of the ganglion through the commissural tracts (*6, 7*). A similar bilateral distribution of dendrites characterizes all other crustacean motoneurons which have been studied by intracellular dye injection (*1, 2*).

The major dendritic branches of the neuron described above gave off numerous thin processes, many of which were less than 5 μm in diameter and more than 100 μm long. These processes ended abruptly in tight couplings with the cylindrical walls of axons which pass through the ganglion in characteristic, identifiable tracts (Figs. 2 and 3).

The presence of similar contacts between giant fibers and the dendrites of the abdominal flexor motoneurons in crayfish is invariably accompanied by synaptic transmission from the former to the latter (*2*). By analogy, the contacts seen here are presumably synaptic junctions. The alternative explanations, namely, that the contacts are nonfunctional *en passant* associations or that the fine processes result from the undesired leakage of dye from the injected neuron, seem extremely unlikely, for the connections are made symmetrically with homologous groups of axons on both sides of the ganglion (Fig. 3). Such a highly specific pattern of contact would not be expected to result from random processes such as *en passant* association or dye leakage. Moreover, examination of several injections of power-stroke motoneurons revealed connections to homologous axons in different experiments.

The axons contacted by the injected motoneuron lie in the same position in the cross section of the cord as axons which carry information to the swimmeret motoneurons (Fig. 3), adding functional support to the proposal that the contacts seen here are synapses, and suggesting further that the direction of transmission across them is from cord axons to the fine dendritic processes of the motoneurons. Synapses between presynaptic axon cylinders and postsynaptic dendrites also link the crayfish giant fibers to the abdominal flexor motoneurons (*2, 9*). The similar arrangement found in the swimmeret system supports the suggestion (*9*) that in the crustacean nervous system synaptic contacts are established in large part by ramification of the postsynaptic neuron. The same type of synaptic relationship is found in the vertebrate

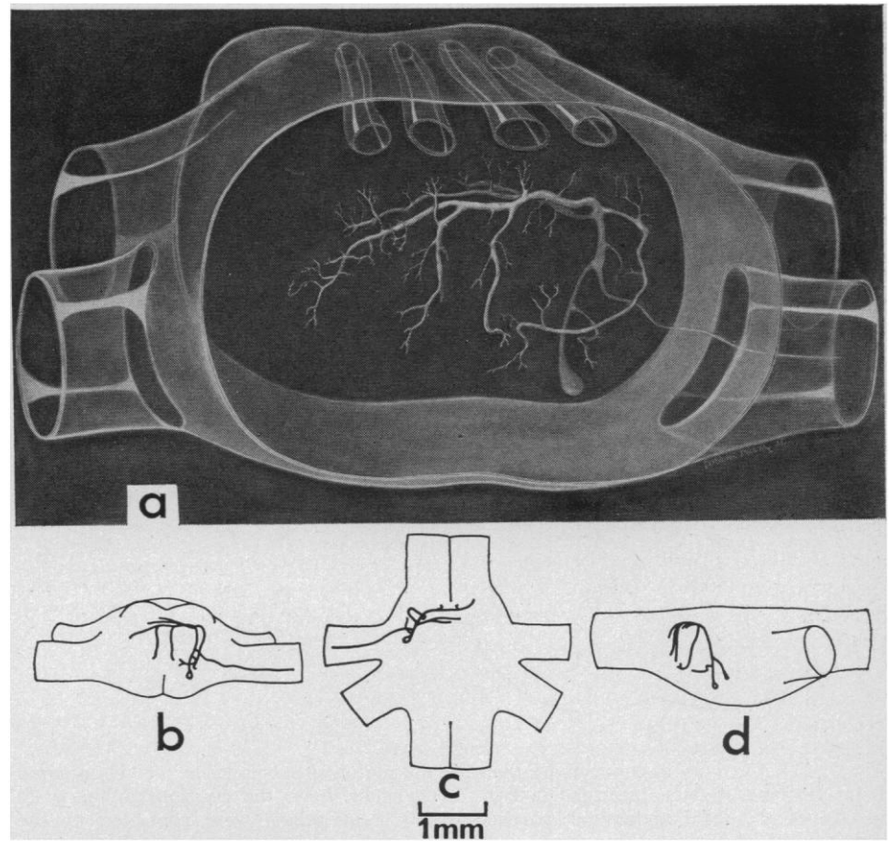

Fig. 1. Reconstructions of the third abdominal ganglion of the lobster and a single motoneuron within it. Before its injection with the marker dye Procion Yellow (see text), the neuron was functionally identified as one of the six excitatory motoneurons innervating the main power-stroke muscle of the swimmeret (muscle 4-8; *4*). (a) A three-dimensional model of the ganglion and the injected neuron, seen from the front. The model was constructed from tracings of color photomicrographs of 10-μm transverse sections through the ganglion. The salient features of each section were traced onto transparent sheets of plastic, which were then stacked up to form the model. The four giant fibers on the top of the illustration identify the dorsal side. The two major branches nearest the main axon terminated in the ipsilateral neuropil; the remaining dendritic processes course through the commissural tracts (*8*) and terminate on other axons (see text). The drawing is accurate down to the finest branches shown. (b–d) Planar projections of the major branches of the neuron. The ganglion is viewed from the front (b), top (c), and side (d). Anterior is toward the top in c, and toward the left in d. The size calibration applies to b–d.

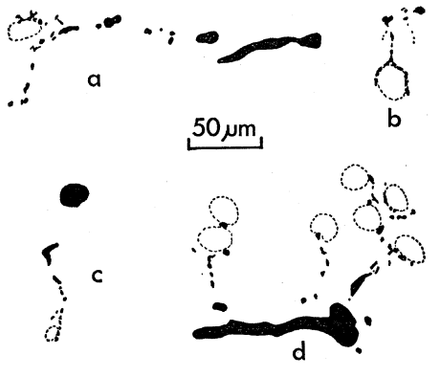

Fig. 2. Tracings made from high-magnification color photomicrographs of 10-μm transverse sections through the ganglion and neuron shown in Fig. 1. The black areas identify processes of the injected neuron, as determined by the presence of Procion Yellow; the dotted circles represent the cross sections of axons which pass through the ganglion. In the text evidence is discussed that the connections which are illustrated here are synaptic junctions. The connections shown in (a) and (b) were on the opposite side of the ganglion from the cell body, while those in (c) and (d) were on the same side.

cerebellar cortex, between granule and Purkinje neurons (*10*).

The best-known crustacean synapse in which the presynaptic element is the cylindrical wall of an axon is electrical (*11*). By analogy, the contacts mapped in my study may be electrical synapses. If confirmed by electron microscopy, this observation implies that electrical transmission is more widespread in the crustacean central nervous system than currently believed. My results also suggest that a great deal of nervous integration in the swimmeret system occurs at the motoneurons. Moreover, since only two processes of the motoneuron studied terminate in that region which is classically regarded as neuropil (Fig. 1), this integration apparently occurs outside of the neuropil to a greater extent than previously recognized (*7*). The neuropil may nevertheless exert a powerful influence on the motor output, for those dendritic processes which terminate in the neuropil lie in close proximity to the region in which the main axon branches off, and, by analogy with other crustacean motoneurons, this region is probably a spike-initiating zone (*7*). Whatever the functional role of the neuropil, my results show that in the swimmeret system, at least, it will not be necessary to untangle this complex region in order to gain at least a partial picture of the patterns of synaptic connection.

In summary, the synaptic contacts between identified neurons in a complex network can be mapped with good structural resolution by intracellular injection of dye. The evidence suggests that, for a given neuron, the population of presynaptic axons represents a unique, stable, and highly characteristic feature. It should be possible to identify this presynaptic population for specific motoneurons in the adult lobster before and after the animal is subjected to various conditioning paradigms (*12*) and also in various developmental stages. Thus the approach used in this study should lead to detailed correlations between nervous structure and function within a network; extensions of the approach may help to answer questions about the disposition of synaptic contacts during learning and development.

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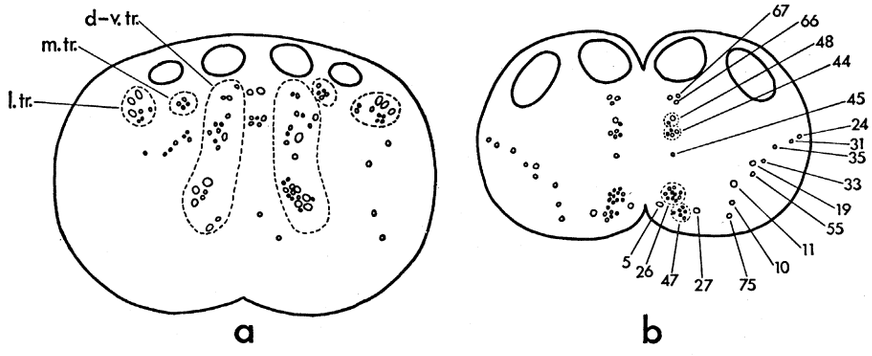

Fig. 3. (a) A structural connectivity map for the motoneuron shown in Fig. 1, prepared from the type of data illustrated in Fig. 2. The map shows the positions in the cross section of the third abdominal ganglion of all axons which were contacted by the dendrites of the injected motoneuron. The major groups include axons whose cross-sectional profiles lie in distinct, bilaterally symmetrical medial tracts (*m. tr.*), lateral tracts (*l. tr.*), and dorso-ventral tracts (*d-v. tr.*). Axons in the latter tracts were contacted by the two prominent ventral branches of the motoneuron (Fig. 1). The four dorsal giant fibers are shown only for purposes of orientation; they were not contacted by the injected neuron. (b) An "input" map which shows the positions of identified primary sensory neurons and interneurons in the cross section of the crayfish ventral nerve cord. The map, prepared from the data of Wiersma and Hughes (*8*) using their identification numbers, shows only those neurons which are known independently to mediate inputs to the swimmeret motoneurons (*4*). The relative positions of axons in the cord are preserved in the region of the ganglia (*6*), allowing the input map to be directly compared with the connectivity map in (a). This comparison reveals that certain populations of axons in the input map occupy the same positions as presumably homologous axons in the connectivity map. This topographical correspondence supports the interpretation that the connections identified in this study are synaptic junctions (see text).

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