interaction of breaks induced by the two agents. Merz *et al.* (*10*) reported that in the combination treatment the interaction of breaks produced by x-rays with those produced by the chemicals, or vice versa, increased two-hit aberrations to an extent approximately twice the sum of aberrations induced by the two agents in separate treatments. In the present study, the number of bridges in the combination treatment was 2.4 times the sum of the separate treatments. This increase might well be attributed to the interaction of breaks if the bridges are assumed to result from interchanges. But the similar increase in acentric fragments arising from the combination treatment could not be explained on the very same basis because the interaction between breaks would actually reduce the yield instead of increasing it. Therefore, it is highly doubtful that the interaction of breaks was the real or major cause for the increase in the number of bridges. Additional evidence (unpublished) in favor of the synergism interpretation comes from a limited observation on aberrations at metaphase, particularly the chromatid breaks which showed about a nine-fold increase in the combination treatment over the sum of the two separate treatments (*11*).

F. K. S. Koo

Agricultural Bio-Sciences Division,
*Puerto Rico Nuclear Center,**
Mayagüez

References and Notes

1. S. Greer and S. Zamenhof, *Am. Chem. Soc. Abstr. 131st Meeting*, (1957) p. 3C; S. Greer, *J. Gen Microbiol.* **22**, 618 (1960).
2. B. Djordjevic and W. Szybalski, *J. Exptl. Med.* **112**, 509 (1960); R. L. Erickson and W. Szybalski, *Biochem. Biophys. Res. Commun.* **4**, 258 (1961); G. Ragni and W. Szybalski, *J. Mol. Biol.* **4**, 338 (1962).
3. H. S. Kaplan, K. C. Smith, P. Tomlin, *Radiation Res.* **16**, 98 (1962).
4. Reviews by W. Szybalski, in *The Molecular Basis of Neoplasia* (Univ. of Texas Press, Austin, 1962).
5. W. Sauerbier, *Virology* **15**, 465 (1961); P. Howard-Flanders, R. P. Boyce, L. Theriot, *Nature* **195**, 51 (1962).
6. W. Szybalski, in *The Molecular Basis of Neoplasia* (Univ. of Texas Press, Austin, 1962), p. 147.
7. T. C. Hsu and C. E. Somers, *Proc. Natl. Acad. Sci. U.S.* **47**, 396 (1961); C. E. Somers and T. C. Hsu, *ibid.* **48**, 937 (1962).
8. J. H. Taylor, W. F. Haut, J. Tung, *ibid.*, p. 190.
9. B. A. Kihlman, *Exptl. Cell Res.* **27**, 604 (1962).
10. T. Merz, C. P. Swanson, N. S. Cohn, *Science* **133**, 703 (1961); T. Merz, C. P. Swanson, C. N. Hemalatha, *Brookhaven Symp. Biol.* **14**, 53 (1961).
11. I thank Edith R. de Irizarry for technical assistance.
* Operated by the University of Puerto Rico for the U.S. Atomic Energy Commission.

14 May 1963

Electrotonic Junctions between Teleost Spinal Neurons: Electrophysiology and Ultrastructure

Abstract. *Electrotonic transmission between spinal neurons is correlated with distinctive apposition of cell processes involving membrane fusion. In the same neurons, postsynaptic potentials appear to arise at typical synaptic knobs where there is an intercellular space.*

Although it is now generally believed that nervous transmission is for the most part chemically mediated (*1*) electrical transmission has been described at several vertebrate and invertebrate junctions (*2*). The two types of transmission occur at junctions that appear morphologically different. In typical vertebrate synapses where transmission is known to be chemical, the pre- and postsynaptic neurons are separated by a space of about 200 Å (*3*). Thickenings of the apposed membranes and clusters of presynaptic vesicles and mitochondria further characterize these junctions. Close apposition of membranes to actual fusion occurs at several invertebrate junctions where transmission is electrical (*4*). In smooth and cardiac muscle, fusions of apposing membranes have been considered to be the sites of electrotonic transmission between muscle fibers (*5*).

Our studies reveal two modes of indirect excitation of spinal electromotor neurons in Mormyrid electric fish (*Gnathonemus* sp., *Marcusenius* sp.). Typical postsynaptic potentials result from stimulation of descending fibers. Furthermore, a spike directly evoked in one cell spreads to all the others. These results are illustrated in Fig. 1, where responses of pairs of neurons were recorded simultaneously. The recordings

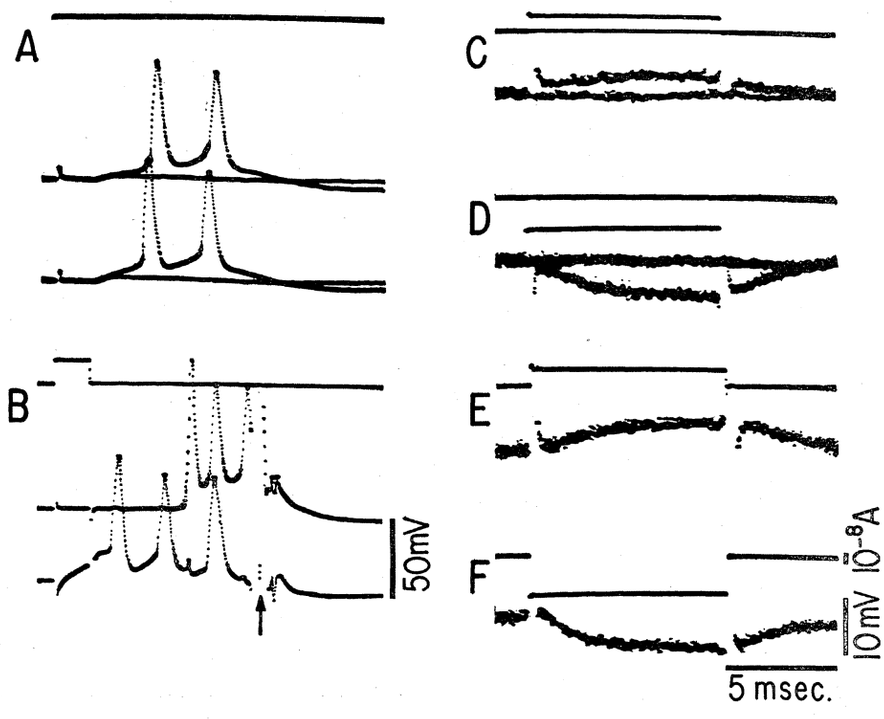

Fig. 1. Indirect excitation of electromotor neurons and electrotonic spread between them. *A*, *C–F*, from a pair of cells 0.3 mm apart; *B*, from a pair of cells 3 mm apart in a different fish. Upper trace: current applied through one recording electrode in a bridge circuit, depolarization indicated by an upward deflection. Lower traces: intracellularly recorded potentials. *A*, *B*, see text. *C*, *D*, de- and hyperpolarization of the more rostral cell of the same pair as in *A* resulted in spread of polarization to the more caudal cell (superimposed traces show the baseline). The recording from the rostral cell was omitted because of excessive bridge imbalance. *E*, *F*, when polarizing current was applied in the more caudal cell, electrotonic spread was recorded in the rostral cell. The recording from the caudal cell was omitted. The electrotonic spread was not an artifact, since the potentials were greatly reduced or absent when one or both electrodes were just extracellular.

in *A* were taken from two neurons a few cell diameters apart; they show superimposed traces of sub- and suprathreshold postsynaptic potentials in response to spinal stimulation rostral to the electromotor nucleus. The stronger stimulus elicited two spikes in each cell. In *B*, from cells at opposite ends of the electromotor nucleus, the more caudal cell (lower trace) was excited by current passed through the recording microelectrode and the resulting activity propagated to the more rostral cell. Each cell generated three spikes which were followed by a small asynchronous electric organ discharge (arrow). The generation of multiple-spike responses to brief stimuli (*B*) appears to be intrinsic to this neuronal membrane and related, like the spread of excitation between cells, to synchronization of the electric organ discharge (*6*). The transmission between electromotor neurons is electrotonic as shown in *C–F* (Fig. 1) from the same pair of cells as in *A*. Depolarizing current applied in either cell depolarized the nearby cell (*C, E*). Hyperpolarization was also transmitted in both directions (*D, F*).

The two modes of indirect excitation can be ascribed to morphologically distinct types of junction. Material was prepared for electron microscopy by a modification of the method of Palay *et al.* for perfusion with OsO_4 (*7*). Numerous areas were observed showing close apposition between dendrite-like processes of the electromotor neurons. Figure 2 shows such a region of close apposition 8 μ long, in which there are several areas of membrane fusion (*A*). No vesicular elements or mitochondria are immediately related to these areas, but a fine fibrillar material dispersed in the cytoplasm is occasionally organized with respect to them. Such membrane fusions are considered to be the morphological substrate for electrotonic transmission between the electromotor neurons. Typical synaptic knobs (arrows, and *B*) are observed on other areas of the neurons and provide the morphological basis for the postsynaptic potentials.

In conclusion, distinctive appositions of neuronal processes have been found which involve membrane fusion and appear to mediate electrotonic transmission between neurons. These data are of particular interest because the cells are in the vertebrate central nervous system and evolved from spinal motoneurons. Further work on other

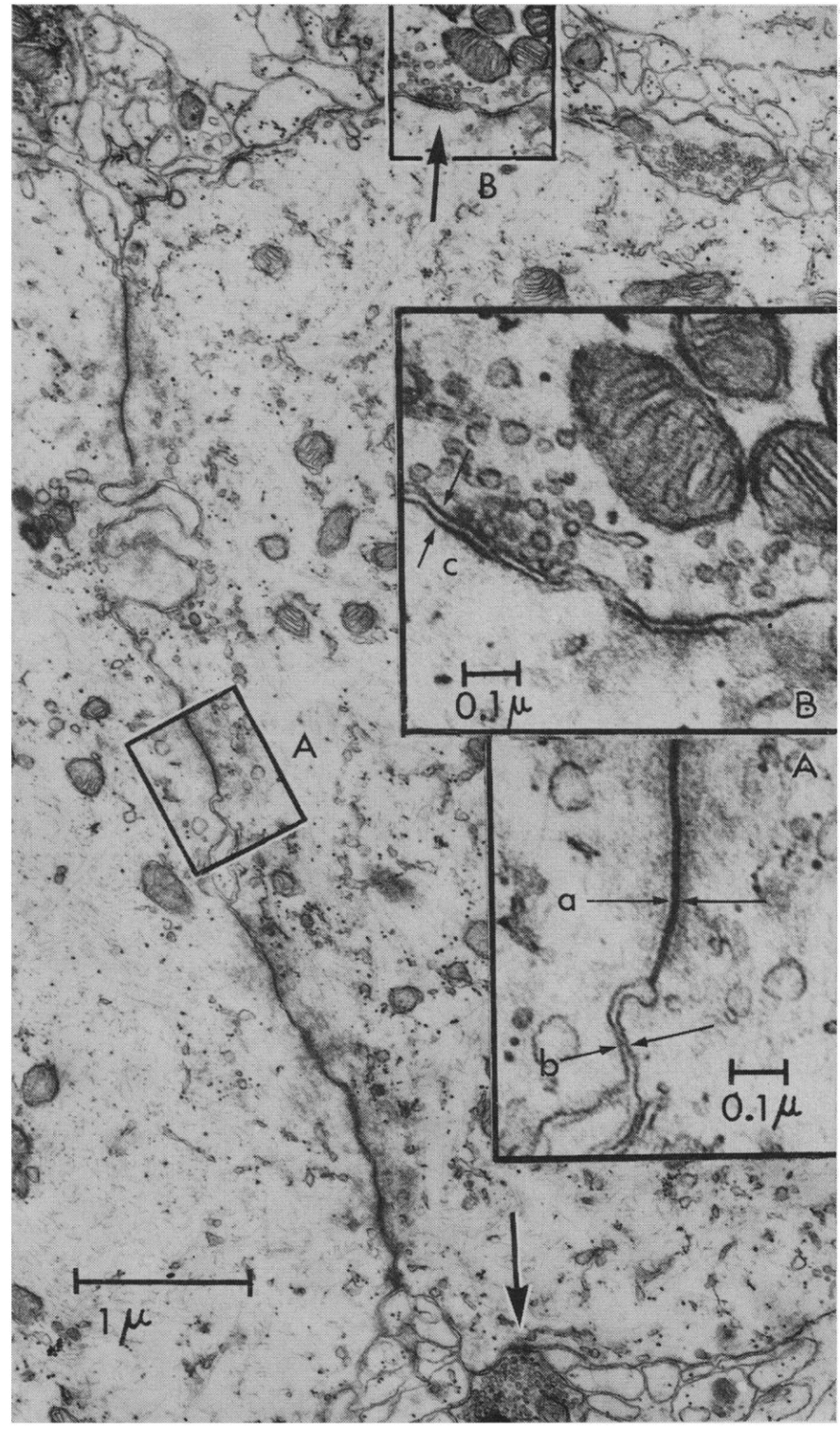

Fig. 2. Electron micrograph of a section (OsO_4 perfused, Epon embedded) through a region of apposition between two dendrite-like processes of electromotor neurons. No intercellular space is apparent in certain areas of close apposition. Typical synaptic knobs (arrows) are found at the top and bottom of the micrograph (× 25,000). Inset *A:* enlargement of area *A*. At *a* the overall width measures about 140 Å. At *b*, where an intercellular space is apparent, the overall width is about 200 Å (× 78,000). Inset *B:* enlargement of the synaptic knob of area *B*. The overall width at *c* measures about 270 Å (× 78,000).

tissues may verify the correlation between anatomy and mode of transmission, and allow either electrical or chemical transmission to be inferred from the morphology of the junction. This type of inference would be of particular importance for such structures as apical dendrites where direct electrophysiological evidence is difficult to obtain (*8*).

M. V. L. BENNETT*, E. ALJURE†
Y. NAKAJIMA, G. D. PAPPAS
Departments of Neurology and *Anatomy, College of Physicians and Surgeons, Columbia University, New York*

References and Notes

1. J. del Castillo and B. Katz, *Progr. Biophys. Biophys. Chem.* **6**, 121 (1956); J. C. Eccles, *Physiology of the Nerve Cell* (Johns Hopkins Univ. Press, Baltimore, 1957), p. 270; H. Grundfest, in *Handbook of Physiology, Neurophysiology I*, J. Field, Ed. (Am. Physiol. Soc., Washington, D.C., 1959), p. 147.
2. M. V. L. Bennett, *Federation Proc.* **19**, 282 (1960); R. Eckert, *J. Gen. Physiol.* **46**, 573 (1963); E. J. Furshpan and D. D. Potter, *J. Physiol.* **145**, 289 (1959); S. Hagiwara and H. Morita, *J. Neurophysiol.* **25**, 721 (1962); C-Y. Kao and H. Grundfest, *ibid.* **20**, 558 (1957); A. R. Martin and G. Pilar, *Federation Proc.* **21**, 355 (1962); A. Watanabe and T. H. Bullock, *J. Gen. Physiol.* **43**, 1031 (1960); A. Watanabe and H. Grundfest, *ibid.* **45**, 267 (1961).
3. S. L. Palay, *Exptl. Cell. Res. Suppl. 5* (1958), p. 275; D. P. de Robertis and H. S. Bennett, *J. Biophys. Biochem. Cytol.* **1**, 47 (1955).
4. K. Hama, *ibid.* **6**, 61 (1959); *Anat. Record* **141**, 275 (1961); J. D. Robertson, *Ann. N.Y. Acad. Sci.* **94**, 339 (1961).
5. M. M. Dewey and L. Barr, *Science* **137**, 670 (1962); *Anat. Record* **145**, 222 (1963).
6. M. V. L. Bennett and E. Aljure, *Federation Proc.* **22**, 220 (1963); paper in preparation.
7. S. L. Palay, S. M. McGee-Russell, S. Gordon, Jr., M. A. Grillo, *J. Biophys. Biochem. Cytol.* **12**, 385 (1962).
8. Supported in part by grants from Muscular Dystrophy Associations of America, National Institute of Neurological Diseases and Blindness (B-3728, NB-03270-2; 2B-5328 R1) and (B-2314 and B-3448); National Science Foundation (G-19969), and United Cerebral Palsy Research and Educational Foundation.

* Research career development fellow, National Institute of Neurological Diseases and Blindness.
† Rockefeller Foundation fellow.

11 April 1963

Tsetse Fly Puparia: A New Collecting Technique

A glossinologist often needs to collect tsetse fly puparia for experimental use. The conventional method is to scratch tsetse breeding soil and collect the puparia brought to view. This method is time-consuming, uneconomical, and not very productive, particularly in an area with a low incidence of the fly. The searchers are unable to detect all puparia present in the soil (*1*). In a second method for separating tsetse puparia from the soil, sieves are used. This method becomes inefficient because of lumping of the soil, which blocks the sieves if the soil is slightly moist.

We tried these techniques when seeking puparia of *Glossina palpalis* (R-D) on the river Lofa, Western Province, Liberia, reported to have a low fly population (*2*), but failed to collect enough puparia to colonize this species. We describe, in this report, a new technique of tsetse puparia collection which enabled us to obtain over 5000 puparia from the same locality.

A 4-gal water barrel, a few sheets of filter paper, and a 2-inch glass vial are supplied as puparia-colecting kits to two collectors who work together. The barrel is filled with river water and carried to the possible breeding sites. Depending on the depth of breeding soil, about 0.5 to 1.5 inches of topsoil are gathered from such sites and examined for puparia by pouring the soil into water. The movement of soil and puparia in reverse directions in the water allows the puparia contained to be separated out on the surface of the water. If the soil is poured slowly almost all the puparia are set free to float, but if the process is carried out rapidly it becomes necessary to disturb the soil gently by hand in order to release the puparia trapped by the mud. The floating puparia are fished out, dried on filter paper, and stored in the glass vial containing a little sand.

The short exposure of puparia to water during collection has no bad effect on the viability of puparia, for 88.5 percent of puparia collected by this method produced live flies, a 4-percent higher emergence than that observed in the case of puparia obtained by hand-picking in the same area.

This method of puparia collection detects all the puparia in the soil examined and can be used as a method of control against this insect.

Z. H. ABEDI
MAX J. MILLER
Liberian Institute of the American Foundation for Tropical Medicine, Harbel, Liberia

References

1. G. R. Jewell, "Quantitative studies of the breeding places of *G. morsitans*," East African Trypanosomiasis Organization Report, 1956–57, pp. 58–59 (1958).
2. K. R. S. Morris, *J. Trop. Med. Hyg.* **65**, 12 (1962).

4 June 1963

Transport of Neurohormones from the Corpora Cardiaca in Insects

Abstract. *Evidence from electron microscope studies of aphid and cockroach nerves, and from the bioassay of extracts of the aortal nerves of cockroaches indicates that some neurohormones are distributed from the corpora cardiaca along nerve axons to their target organs.*

The corpora cardiaca of insects are small ganglia which are situated immediately behind the brain and connected to it by nerves. From the corpora cardiaca, nerves have been traced in various insects to the alimentary canal, salivary glands, aorta, prothoracic gland, and various muscles (*1*, *2*).

Substances produced by neurosecretory cells in the brain have been shown to pass along nerve axons to the corpora cardiaca from which they are thought to be released into the blood (*3*). In this respect the protocerebrum-corpus cardiacum system of insects is considered to be analogous to the hypothalamus-neurohypophysis system of vertebrates and the X organ-sinus gland system of crustacea.

Recent work has suggested that at least some materials may be distributed from the corpora cardiaca along nerve axons. In the fly, secretory material was observed in a nerve passing from the corpora cardiaca to the esophagus (*4*); in the cockroach it has been shown by ligaturing experiments that material passes along nerves from the corpora cardiaca to the subesophageal ganglion (*5*); and, in aphids, material staining with paraldehyde fuchsin and believed to be secretory material was traced from the corpora cardiaca along the aortal nerves and along nerves passing to muscles (*2*).

We have therefore studied the nerves leaving the corpora cardiaca in several groups of insects using paraldehyde fuchsin stain for neurosecretory material. Under the compound microscope no material was found in any of these nerves, although large amounts were sometimes present in the brain and corpora cardiaca.

Extracts of the corpora cardiaca of the cockroach can cause increased rate of heartbeat. One active factor is a peptide which is thought to stimulate the pericardial cells to release an indolalkylamine which, in turn, acts on the