# VOLTAGE INDEPENDENCE OF AN ELECTROTONIC SYNAPSE

M. F. JOHNSTON AND F. RAMÓN
*Department of Physiology, Duke University Medical Center, Durham, North Carolina 27710*

Since the description of electrotonic coupling between lateral axons of the crayfish nerve cord (Watanabe and Grundfest, 1961), this synapse has shown no sign of being other than a linear current pathway. Electrotonic junctions from other systems studied over a period of many years also behave as linear pathways: pancreatic acinar cells (Iwatsuki and Petersen, 1978); neurons from the central nervous system of marine molluscs (Getting, 1974), leech (Hagiwara and Morita, 1962; Penn and Lowenstein, 1966), and goldfish medulla (Korn and Faber, 1975); giant axons from earthworm (Brink and Barr, 1977); embryonic cells in a meroblastic egg (Bennett and Trinkaus, 1970); isolated and reaggregated *Fundulus* blastomeres (Bennett et al., 1978); normal liver and hepatoma cells in culture (Borek et al., 1978; Sheridan et al., 1978); salivary gland cells (Rose and Loewenstein, 1971; Kater and Galvin, 1978).

Recent studies demonstrating a voltage-dependent junctional conductance between amphibian blastomeres (Spray et al., 1979, 1981) have, however, prompted us to reconsider the question of the voltage dependence of junctional synapses. It is conceivable that current injection experiments lacked the resolution necessary to reveal a changing junctional conductance. We have implemented a voltage clamp of the crayfish axons septal membrane to examine the electrical properties of this synapse in more detail.

Experiments were performed on internally perfused lateral axons from the abdominal nerve cord of crayfish (*Procambarus clarkii*). The technique for internal perfusion of the axons has been previously described (Johnston and Ramón, 1981). Briefly, perfusion was achieved by cutting the axons distal to the ganglion and advancing a cannula into the cut end to a point as close as possible to the septum (within 1 mm). Solution flow proceeded from the cannula tip, into the axon, and out through the cut where the cannula was introduced. All experiments were performed at ~17°C.

Internal cannulas were constructed from capillary tubing (50 μm o.d., 35 μm i.d.) cemented into a broken microelectrode shank. To improve current-passing characteristics, a floating bare platinum wire (18-μm diameter) was inserted into the cannula, yielding a high-frequency impedance of ~200 kΩ and a DC resistance of ~10 MΩ. For voltage-clamp experiments, both axons of a coupled pair were cannulated and perfused simultaneously and a glass microelectrode was inserted into each axon near the septum to monitor voltage (Fig. 1*A*).

Internal solutions had (in mM): KF, 100; K-citrate, 37; mannitol, 16; and HEPES, 1 at pH 7.5. External solution had (in mM): NaCl, 205; KCl, 5.4; CaCl2, 13.5; MgCl, 2.6; and HEPES, 5 at pH 7.5. To minimize conductance changes in unclamped regions of axon membrane, the internal solution contained TEA-Cl, 25 mM, and the external solution TTX, $10^{-7}$M.

Fig. 1*A* is a diagram of the lateral axons to show the placement of electrodes and cannulas. Fig. 1*B* is a simplified diagram of the voltage-clamp circuit. The voltage across the septum was recorded differentially by feeding the preamplifier outputs (*1, 2*) to a difference amplifier (*5*). The output of this amplifier ($V_s$) is the voltage that was "clamped" by the main control amplifier (*6*). The return current pathway was via one of the axial electrodes (a perfusion cannula), connected to the current-measuring amplifier (*10*).

As shown in Fig. 1*A*, the current measured ($I_m$) is the sum of transseptal current ($I_s$) plus current in a parallel pathway through the axon membranes ($I_2$). A double clamp was used to minimize $I_2$ and restrict the measurement to $I_s$. In this scheme the bath potential ($V_b$) was monitored by a second chlorided silver wire and recorded differentially with respect to $V_2$ (amplifiers *3* and *4*). The resting potential was nulled with the balance control on the $V_b$ amplifier (*3*). This voltage was applied to a second control amplifier (*9*) which drove the other bath electrode (*Stim 2*, Fig. 1*A*), so that its potential followed $V_2$. Under

Dr. Johnston's present address is the Department of Neurobiology, Harvard Medical School, Boston, MA 02115; and Dr. Ramon's present address (where reprint requests should be sent) is the Departmento de Fisiología, Centro de Investigación del Instituto Politecnico Nacional, Apartado Postal 14-740, México 14, D.F. México.

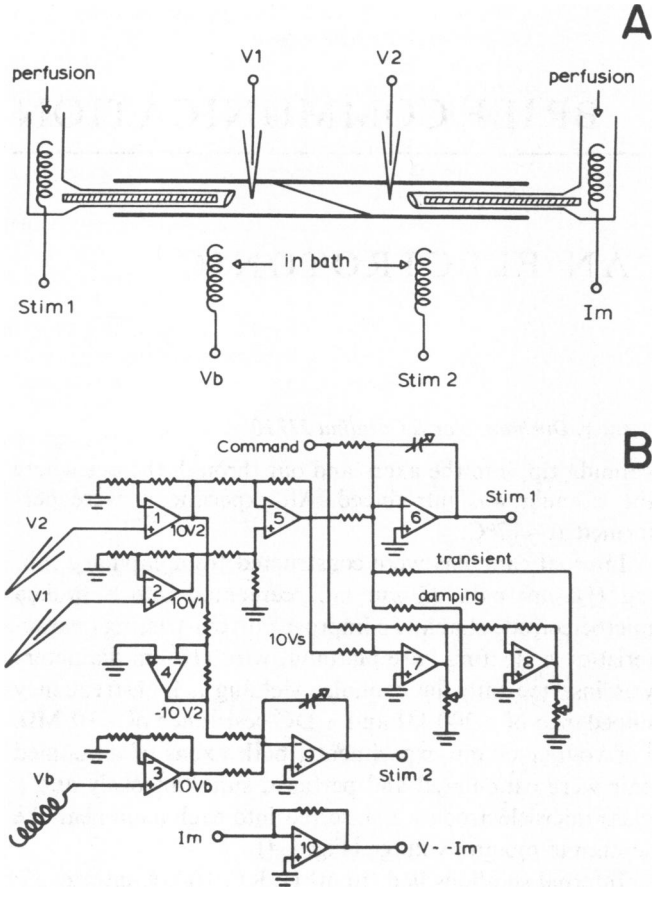


FIGURE 1*A* Diagram of the experimental setup used for voltage-clamp experiments. The two axial electrodes are shown along with internal microelectrodes. The two electrodes in the bath ($V_b$ and *Stim 2*) are Ag-AgCl electrodes. *B*, diagram of the voltage-clamp electronic circuit. Amplifier functions are: *1, 2, 3*, voltage preamplifiers; *4*, inverter for 10 $V_2$ signal; *5*, difference amplifier to generate transseptal voltage; *6*, control amplifier for main clamp; *7, 8*, damping and transient control systems; *9*, control amplifier for second (bath) clamp; *10*, current measuring amplifier.

these conditions $I_2$ is independent of the transseptal voltage, effectively eliminating the parallel pathway.

Fig. 2*A* shows transseptal voltage-clamp pulses (*top*) and the resulting currents (*bottom*). Septal currents recorded in response to square voltage-clamp pulses across the junctional region are almost rectangular, except for small on and off transients. The slight droop in the current traces probably reflects either a residual contribution of the axonal membrane, slow resistance changes, or polarization in the current-passing and measuring electrodes.

As expected from the records in Fig. 2*A*, the septal current voltage relationship is linear (Fig. 2*B*). The straight lines that fit the data have almost identical slopes for positive (182 kΩ) and negative (166 kΩ) excursions over the range ±100 mV around a holding potential of −85 mV. Membrane resting potentials, when not held, were 85 ± 2 mV.

Septal resistance in crayfish lateral axons averages ~17 kΩ (Johnston and Ramon, 1981). This low junctional resistance has serious implications for voltage clamp of the septal membrane, because the axoplasm resistance (~65 kΩ/mm) results in a large series-resistance error. Although this error can be minimized by careful microelectrode placement, a problem remains because the cannulas cannot be advanced closer than ~0.5 mm from the septum without damaging the axons. Since the septum spans a 0.2–0.5 mm length of the axon, it cannot be isopotential.

Another problem of these voltage-clamp experiments is the inability to completely eliminate the current pathway through the axon membranes. Trying to space clamp the axon is impractical because of the proximity of the cut end, which must remain open for perfusion (when perfusion is not flowing the axons collapse over the cannulas). Furthermore, as current is injected into one axon via the synapse, the longitudinal voltage profile in the axon will change, and $I_2$ will not be exactly constant (the direction of the change is determined by electrode placement and axon geometry). In all cases, however, currents obtained using the double clamp were more regular and reproducible than without, suggesting that deviations from ideality were not severe.

In summary, it appears that the voltage and time dependence of the junctional conductance of crayfish septate axons is negligible. This conclusion is consistent with results obtained by means of injection of current pulses (Watanabe and Grundfest, 1961). These results point to the existence of two classes of electrotonic synapses, voltage-dependent junctions which have only been observed in amphibian systems (Spray et al., 1979), and linear junctions which seem to account for all other instances of electrotonic coupling. Hopefully future studies will reveal the nature of the differences between these types of junctions.

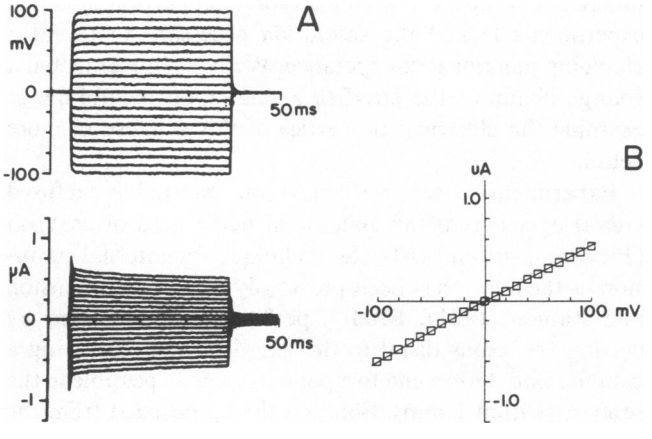


FIGURE 2*A* Family of voltage (*top*) and current (*bottom*) records from a double voltage-clamp sequence of rectangular pulses across the junctional region of segmented axons. External medium contained TTX and internal medium TEA. *B*, steady-state current voltage relationships derived from the family of current records shown in *A*. Data points were taken 25 ms after the beginning of each pulse.

## REFERENCES


Bennett, M. V. L., and J. P. Trinkaus. 1970. Electrical coupling between embryonic cells by way of extracellular space and specialized junctions. *J. Cell Biol.* 44:592–610.

Bennett, M. V. L., M. E. Spira, and D. C. Spray. 1978. Permeability of gap junctions between embryonic cells of *Fundulus:* a reevaluation. *Dev. Biol.* 65:114–125.

Borek, C., S. Higashino, and W. R. Loewenstein. 1969. Intercellular communication and tissue growth. IV. Conductance of membrane junctions of normal and cancerous cells in culture. *J. Membr. Biol.* 1:274–293.

Brink, P., and L. Barr. 1977. The resistance of the septum of the median giant axon of the earthworm. *J. Gen. Physiol.* 69:517–536.

Getting, P. A. 1974. Modification of neuron properties by electrotonic synapses. I. Input resistance, time constant, and integration. *J. Neurophysiol.* 37:846–857.

Hagiwara, J., and H. Morita. 1962. Electrotonic transmission between two nerve cells in leech ganglion. *J. Neurophysiol.* 25:721.

Iwatsuki, N., and O. H. Petersen. 1978. Electrical coupling and uncoupling of exocrine acinar glands. *J. Cell Biol.* 79:533–545.

Johnston, M. F., and F. Ramón. 1981. Electrotronic coupling in internally perfused crayfish segmented axons. *J. Physiol. (Lond.).* 317:509–518.

Kater, S. B., and N. J. Galvin. 1978. Physiological and morphological evidence for coupling in mouse salivary gland cells. *J. Cell Biol.* 79:20–26.

Korn, H., and D. S. Faber. 1975. An electrically mediated inhibition in goldfish medulla. *J. Neurophysiol.* 38:452–471.

Penn, R. D., and W. R. Loewenstein. 1966. Uncoupling of a nerve cell membrane junction by calcium-ion removal. Science *(Wash, D.C.).* 151:88–89.

Rose, B., and W. R. Loewenstein. 1971. Junctional membrane permeability. Depression by substitution of Li for extracellular Na, and by long-term lack of Ca and Mg: restoration by cell repolarization. *J. Membr. Biol.* 5:20–50.

Sheridan, J. D., M. Hammer-Wilson, D. Preus, and R. G. Johnson. 1978. Quantitative analysis of low-resistance junctions between cultured cells and correlation with gap-junctional areas. *J. Cell Biol.* 76:532–544.

Spray, D. C., A. L. Harris, and M. V. L. Bennett. 1979. Voltage dependence of junctional conductance in early amphibian embryos. *Science (Wash., D.C.).* 204:432–434.

Spray, D. C., A. L. Harris, and M. V. L. Bennett. 1981. Equilibrium properties of a voltage-dependent junctional conductance. *J. Gen. Physiol.* 77:77–93.

Watanabe, A., and H. Grundfest. 1961. Impulse propagation at the septal and commisural junctions of the crayfish lateral giant axons. *J. Gen. Physiol.* 45:267–308.