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16. Osmolality was kept constant by reducing the Na^+ concentration by the same amount.
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Extracellular Potassium Ions Mediate Specific Neuronal Interaction

Abstract. *The giant interneurons from the nerve system of the cockroach* Periplaneta americana *exhibit a peculiar reciprocal synaptic interaction. The synaptic potentials are not blocked by addition of 5 millimolar cobalt chloride and have an extrapolated reversal potential close to 0 millivolt. Hyperpolarizing current injected into one cell does not spread to the other. Intracellular injection of tetraethylammonium ions into one giant interneuron increases the duration of the action potential of the injected cell to 30 milliseconds and reduces the rise time and amplitude of the postsynaptic response recorded in the other giant interneuron. These results indicate that the interaction between the interneurons is not mediated by conventional chemical or electrotonic synapses. All evidence points to generation of the potentials by localized increases in extracellular potassium concentrations as a consequence of firing of one neuron.*

Chemical and electrotonic synapses are the bases for specific connections between excitable cells. The specificity of connections mediated by chemical synapses is a consequence of the specific chemical interaction between neurotransmitters released from the presynaptic terminal and receptors located on postsynaptic cells. In electrotonically coupled neurons, specific connections are provided by relatively large hydrophilic channels that directly connect cells (*1*). In the present report we describe a specific connection between identifiable interneurons which is probably mediated by potassium ions. The interaction is made possible by close membrane proximity in restricted and defined regions.

We described previously (*2*) a monosynaptic connection between two adjacent giant interneurons in the metathoracic ganglion (T_3) of the cockroach *Periplaneta americana*. This connection exhibits unusual characteristics (see Fig. 1). Intracellular injection of current into one such neuron, *a* (see Fig. 1A), generates an action potential (Fig. 1A, upper trace) that is followed by a synaptic potential recorded from an adjacent giant interneuron, *b*. Likewise, stimulation of *b* generates a similar synaptic potential in *a* (Fig. 1B). Thus, the interaction between these giant interneurons is reciprocal (giant-to-giant interneuron synaptic potential or GGSP). The GGSP appears after a delay of 1 msec and has a rise time of 1 to 1.5 msec and a long decay time (60 to 100 msec). The GGSP rise time is faster than the rise time of chemically mediated synaptic potentials (2.8 to 3 msec) recorded at the same place and generated in the same area. For example, see the arrow in Fig. 1B, where a spontaneous synaptic potential in interneuron *a* is recorded (*3*). To characterize the properties of the GGSP we studied the dependence of GGSP amplitude on transmembrane potential of the postsynaptic axon. In Fig. 1D, the first potential (arrow) is a GGSP evoked by intracellular stimulation of an adjacent giant interneuron, whereas the second potential, the result of a chemical synapse, is generated by extracellular stimulation of the contralateral thoracic connective. Although the GGSP amplitude is only slightly increased by hyperpolarization of the membrane, the chemically mediated postsynaptic potential is increased by more than twofold when the giant interneuron membrane is hyperpolarized by 20 mV (Fig. 1C). On the basis of this type of experiment we estimate that the reversal potential of the GGSP is between −10 and 0 mV, whereas postsynaptic potentials from other sources reverse between −65 to −57 mV (*4*). The GGSP also differs from other postsynaptic potentials evoked at T_3 by its insensitivity to the addition of 5 m*M* $CoCl_2$ to the physiological solution. All other evoked and spontaneous potentials are blocked by the addition of cobalt ions, but the GGSP is not. The only observed effect of 5 m*M* $CoCl_2$ on the GGSP is a reduction in its decay time (*5*).

Several features of the GGSP, namely, the relatively short latency and the insensitivity to cobalt ions in the bathing solution and to transmembrane potential, points strongly to the presence of electrotonic synapses (*1*). This possibility, however, was ruled out by direct experiments. Hyperpolarizing or depolarizing rectangle current pulses sufficient to produce a −40 mV to +20 mV shift in the membrane potential of the injected giant interneuron did not produce any change in the membrane potential of the adjacent interneuron. Since these results are not consistent with conventional chemically or electrotonically mediated transmission, we investigated the possibility that the GGSP is mediated by an increase in the extracellular potassium concentration following the genera-

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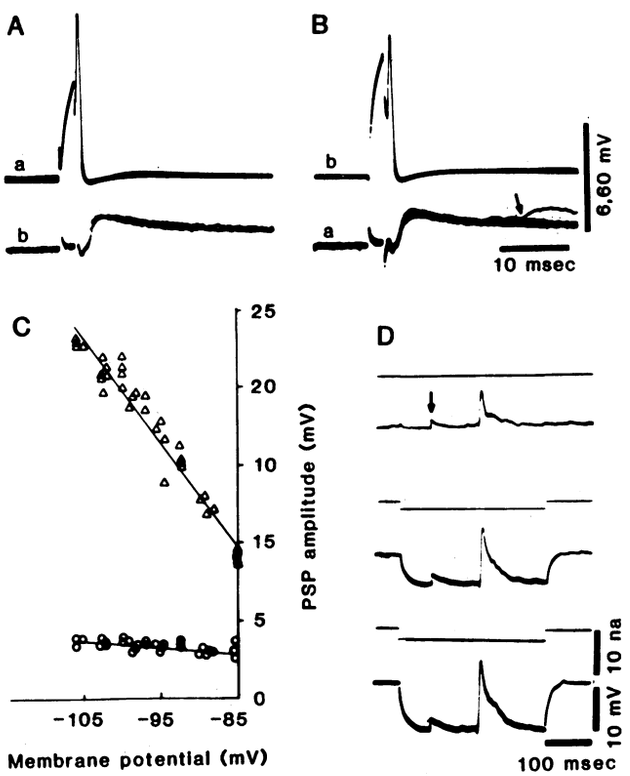
tion of an action potential in a giant interneuron. It is of interest that the expected apparent reversal potential for depolarization produced by K^+ accumulation was the same as that found in these experiments (*4*).

Intracellular injection of tetraethylammonium ions (TEA) decreases the outflow of K^+ during an action potential, thereby increasing the spike duration (*6*). If the GGSP's were mediated by either a chemical or electrotonic synapse, an increase in the duration of the presynaptic action potential would be expected to produce an increase in GGSP amplitude and duration (*7*). In contrast, a decrease in GGSP amplitude would be expected if this potential is produced by accumulation of extruded K^+ ions in the extracellular cleft following an action potential in the presynaptic fiber. Figure 2, A to D, shows simultaneous recordings from two giant interneurons, at the base of T_3 and between ganglia A_1 and A_2 (electrode arrangement in Fig. 2G). Extracellular stimulation of the abdominal connective generates an action potential in both fibers (Fig. 2A). An intracellular stimulus delivered to one interneuron (A_1–A_2 electrode) generates a GGSP in the other (T_3 electrode) (Fig. 2B, upper and lower trace illustrate the same response on two different time scales). Figure 2, C and D, shows the responses to the same stimuli as in Fig. 2, A and B, 15 minutes after the TEA injection (*8*). Although the injected fiber generates a prolonged action potential (up to 30 msec) (Fig. 2C), both the rate of rise and the amplitude of the GGSP are significantly reduced (Fig. 2D). These results argue strongly against the possibility of either chemical or electrotonic synapses and are consistent with the assumption that the GGSP is mediated by the accumulation of K^+ in the extracellular space.

The plasma membranes of two giant interneurons must come into close proximity for such K^+-mediated interaction to occur after a single action potential (*9*). The giant interneurons in the connectives and within the ganglia are densely wrapped by multiple layers of glial processes (*10*). This glial envelope



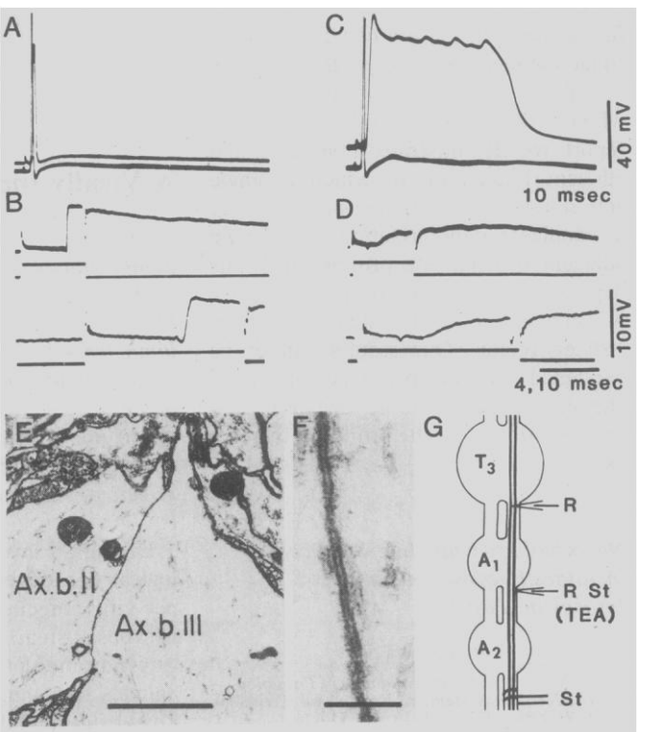


Fig. 1 (left). Synaptic potentials produced by giant interneurons of *P. americana*. (A and B) Characterization of the reciprocal interaction (GGSP) between giant interneurons in the metathoracic ganglion (T_3). Two adjacent interneurons were impaled by microelectrodes, one at the caudal base of T_3 (*a*) and the other at the anterior edge of the ganglion (*b*). (A) Action potential initiated by intracellular stimulation of *a* produces a fast-rising (1.4 msec) depolarizing potential in *b*. (B) Firing of *b* produces a fast-rising response in *a*. Note that the rise time of a spontaneous chemically mediated synaptic potential [arrow in (B)] is slower than the rise time of the GGSP. (C and D) Comparison of the dependence of GGSP amplitude and a chemically mediated postsynaptic response (PSP) on the transmembrane potential of the giant interneuron. (D) The GGSP (arrow) and a chemically mediated PSP (initiated by stimulation of the contralateral thoracic connective) are superimposed on hyperpolarizing pulses. Although the PSP amplitude is increased when the giant interneuron membrane is hyperpolarized [triangles in (C)], the GGSP amplitude is almost unaffected [circles in (C)]. Fig. 2 (right). The GGSP's are produced by K^+ accumulation (A to D). The effect of intracellular injection of TEA on the action potential of the injected giant interneuron and the GGSP. Two adjacent giant interneurons were impaled by microelectrodes, one for voltage recording at the caudal base of the metathoracic ganglion [lower traces in (A) and (C)] and the other for stimulation and injection of TEA placed in a giant interneuron 2 mm caudally to T_3 [between the first and second abdominal ganglia; for electrode arrangement see (G)]. (A) Extracellular stimulation of the abdominal connectives evoked an action potential in both interneurons. (B) Intracellular stimulation by the electrode placed between ganglia A_1 and A_2 evoked a GGSP in the other interneuron, shown on two time bases. After 15 minutes of TEA injection, a single extracellular stimulus to the abdominal connective evokes a long-lasting potential in the TEA-injected interneuron [upper trace in (C)]. The action potential in the other interneuron is normal. Under these conditions, intracellular stimulation of the TEA-injected interneuron evokes a GGSP in the other fiber (D). However, its amplitude is greatly reduced [compare (D) to (B)]. These results rule out the possibility that the GGSP is mediated by either a chemical or an electrotonic synapse. (E and F) The putative morphological structure for specific and restricted potassium-mediated interaction. (E) Low-power electron micrograph (2 μm) showing two identified branches of giant interneurons II and III [nomenclature after Harris and Smyth (*12*)]. (F) Magnification (0.2 μm) of the region in which the two neurites come into close proximity reveals that the space between the interneurons in this region is only 7 to 10 nm.

prevents communication between the fibers (*11*). However, we found that at the metathoracic ganglion the axon gives off several neurites which ramify in the neuropil. In several series (three preparations) of serial sections through the metathoracic ganglion, we found that the branches from two different giant interneurons come into close proximity (Fig. 2E) (*12*). In this area the distance between the giant interneurons' neurite membranes is only 7 to 10 nm (Fig. 2F). We have observed up to three such regions between two branches (*9*).

That K^+ has an important role in neuronal interaction has been demonstrated before (*13*). In most of these studies the increase in extracellular K^+ concentration was either due to stimulation of a single neuron at high frequency or the combined action of a group of neurons; furthermore, a fraction of adjacent neuronal elements in the vicinity of the stimulated pathway were affected, whereas others were not. In the present report we demonstrate a specific and efficient interaction in which a single action potential is capable of generating a significant depolarization in a single adjacent neuron. We propose that this depolarization is due to an increase in K^+ concentration at a defined and restricted region. Our results cannot be explained by the presence of either chemical or electrotonic synapses and are consistent with the proposed hypothesis.

Y. YAROM
M. E. SPIRA

Neurobiology Unit, Life Sciences Institute, Hebrew University, Jerusalem, Israel

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3. For these experiments we used late nymphal stages of *P. americana*. At these stages chemically mediated synaptic inputs with a rise time of 2.8 msec and a reversal potential range between −65 and −57 mV can be activated by stimulation of seven different nerves connected to the metathoracic ganglion (M. E. Spira and Y. Yarom, unpublished observations). The nerve cord was isolated as described (*2*). The isolated nerve cord was continuously perfused by physiological solution containing 214 m*M* NaCl, 3.1 m*M* KCl, 7 m*M* $CaCl_2$, and 1 m*M* tris. The *p*H was adjusted to 7.2 to 7.4.
4. The relation between GGSP amplitude and membrane potential (Fig. 1C) extrapolates to an apparent reversal potential ranging from −10 to 0 mV. This result is expected if the GGSP arises from an increase in the extracellular K^+ concentration [P. Rudomin, E. Stefani, R. Werman, *J. Neurophysiol.* **42**, 912 (1979)].
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9. Assuming that a single action potential in a giant interneuron produces an efflux of 4×10^{-12} mole of potassium ions per square centimeter [A. L. Hodgkin, *The Conduction of the Nervous Impulse* (Thomas, Springfield, Ill., 1964), pp. 42–44] and taking into account the extracellular space between the closely apposed interneuron branches and the membrane properties, we calculate that a single action potential in one giant interneuron would evoke a 1-mV depolarizing potential in the other, provided the radius of the closely apposed membranes is 10 μm, or 16 junctions with radii of 2.5 μm form between the interneurons. In our ultrastructural study we have observed up to three regions of close membrane apposition between two branches. Each of the giant interneurons studied emits up to seven branches (Y. Yarom and M. E. Spira, in preparation). Thus, if each branch makes up two to three such junctions the total area of membrane in close apposition will be about that estimated by our calculations.
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A Virally Induced Obesity Syndrome in Mice

Abstract. *An obesity syndrome was found in a number of mice infected as young adults with canine distemper virus, a morbillivirus antigenically related to measles. Body weights of obese animals 16 to 20 weeks after infection were comparable to those reported for genetically obese mice and for mice rendered obese by hypothalamic lesions. The total number of adipocytes in specific fat deposits was greater in obese animals than in their lean littermates. This hyperplasia was accompanied by moderate cell enlargement. Pancreatic islet tissue was also hypercellular in the obese mice. Brain tissue from the obese mice showed no overt pathology, and immunofluorescence staining for viral antigens was negative. There may be a selective, virus-induced disruption of critical brain catecholamine pathways.*

During an investigation of the pathological consequences of canine distemper virus infection of the mouse central nervous system, we found that an obesity syndrome developed in a number of animals surviving the infection. To our knowledge, an infectious process has not been considered a possible etiological factor in the pathogenesis of obesity. Experimental models of obesity have fallen into three classes: hypothalamic obesity, produced by electrolytic, chemical, or knife injury to the hypothalamus or to fibers leading to and from the hypothalamus; dietary obesity; and genetically transmitted obesity. Strain-dependent differences in the phenotypic expression of obesity have been noted, for example, at the level of adipose tissue morphology (*1*). Thus, in certain

Table 1. Outcome of canine distemper virus infection in NCS/R mice. Abbreviations: CNS, central nervous system; CDV, canine distemper virus.

Inoculum	*N*	Acute encephalitis*	CNS disease 4 to 24 weeks after infection	Obesity 6 to 20 weeks after infection	No CNS disease, no obesity
CDV (10^3 PFU, intracerebrally)	120	52	14	18	36
CDV (5×10^4 PFU, intraperitoneally)	12			2	10
Normal suckling mouse brain suspension (intracerebrally)	35				35
HBSS (intracerebrally)	45				45

*Animals dead or dying 2 weeks after inoculation.

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