Synaptic Potentials Effect the Release of Transmitter from Locust Nonspiking Interneurons

Abstract. *An excitatory synaptic potential in a local nonspiking interneuron of a locust is able to effect the release of chemical transmitter. The consequence is that a discrete inhibitory synaptic potential is evoked in an identified postsynaptic motoneuron. These local interactions between interneurons and motoneurons are of behavioral significance in that they ensure the correct operation of a resistance reflex.*

Neurons within a central nervous system continually generate a wealth of synaptic potentials as a result of chemical and electrical signals from other neurons. In many neurons an obvious consequence of these inputs is that when they exceed a certain level, a spike is generated that can be carried to distant parts of the neuron. At the output synapses, the spike either transiently increases the rate of release of a chemical transmitter, or its current flows directly into the next neuron and produces a synaptic potential. This report provides direct evidence that synaptic potentials themselves can effect intercellular communication without the intervention of a spike, so that local interactions between neurons become possible. There is already strong circumstantial evidence that supports the concept of local neuronal circuits (*1*). Reconstruction of field potentials in the olfactory bulb of rabbits suggests that synaptic potentials in dendrites can influence the dendrites of other neurons (*2*). Studies using electron microscopy indicate that the appropriate serial and reciprocal synapses are present (*3*). Moreover, synapses of some neurons, such as receptor cells, can be activated by small voltage signals and thereby affect higher-order neurons (*4*). Neurons that normally spike can, when the spike is blocked, transmit in a graded fashion in response to voltage changes of a few millivolts (*5*). Local nonspiking interneurons in the segmental ganglia of insects (*6*) are able, by small changes in their membrane potentials, to effect graded chemical transmission onto their postsynaptic neurons (*7, 8*). Here it is shown by intracellular recording from a local interneuron and a postsynaptic motoneuron that a discrete (*9*) depolarizing synaptic potential of only 2 mV in the interneuron is sufficient to effect the release of transmitter. Each of these synaptic potentials in the interneuron evokes a discrete hyperpolarizing potential in the postsynaptic neuron so that patterned information is preserved. The synaptic potentials occur when the hind leg is moved, and are an essential element in a reflex.

The thorax of the locust was opened by dorsal dissection to expose the metathoracic ganglion (*10*). The femur of the hind leg was held rigidly, while the tibia could be moved by a mechanical activator driven by a waveform generator. Intracellular recordings were made simultaneously from neuropilar processes of an identified motoneuron innervating the flexor tibiae muscle of a hind leg and from a presynaptic interneuron (*11*). Intracellular staining with cobalt shows that the interneuron is a local one with all its processes within the metathoracic ganglion (Fig. 1). The interneuron does not produce spikes (*7, 8*). Injection of small amounts (< 5 nA) of depolarizing current into the interneuron evokes a hyperpolarization of the flexor motoneuron and spikes in the antagonistic slow extensor tibiae motoneuron (Fig. 2a). The hyperpolarization in the flexor motoneuron follows the onset of the stimulus with a delay of 1.5 msec. Intracellular recordings from the slow extensor motoneuron reveal a depolarization that follows with a similar latency. Connections between the interneuron and the two antagonistic motoneurons are therefore probably direct. The effects in the motoneurons are graded depending upon the amount of current injected into the interneuron and are mediated by the release of chemical transmitter. The evoked hyperpolarization is enhanced when the flexor motoneuron is depolarized by the direct injection of current (Fig. 2b), reaches an apparent reversal potential when the motoneuron is hyperpolarized (Fig. 2c), and finally reverses in polarity when more hyperpolarizing current is applied (Fig. 2d). Hyperpolarizing current injected into the interneuron produces no detectable voltage or resistance changes in the flexor motoneuron.

To evoke synaptic potentials in the interneuron, a resistance reflex was activated. Passive flexion of the tibia inhibits the flexor motoneuron and excites the slow extensor motoneuron, so that the imposed movement is resisted. Holding the tibia in a flexed position evokes a sequence of depolarizing potentials of 2 to 2.5 mV in amplitude in the interneuron (Fig. 2e). Each potential is followed by an inhibitory postsynaptic potential (IPSP) in the flexor motoneuron and sometimes by a spike in the extensor motoneuron (Fig. 2e). The depolarizing potentials in the interneuron are excitatory postsynaptic potentials (EPSP's); when the interneuron is hyperpolarized with injected current, they increase in ampli-

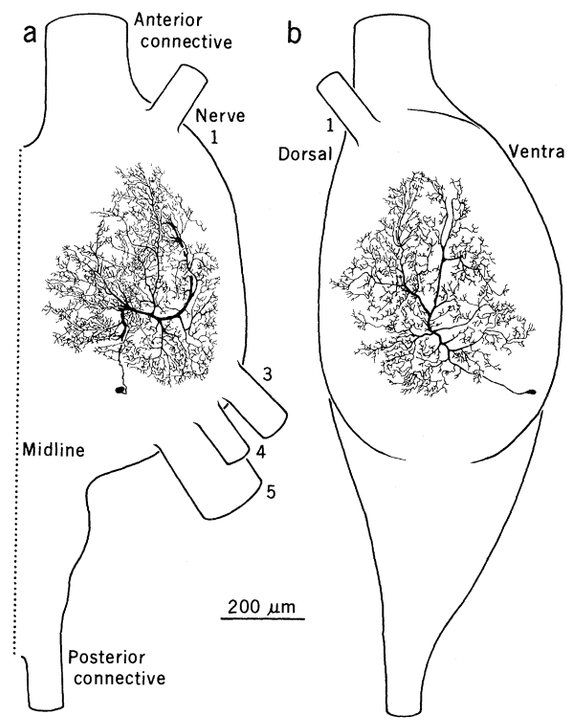

Fig. 1. Drawings of a nonspiking local interneuron that, when depolarized, evokes a hyperpolarization in a flexor tibiae motoneuron and spikes in the slow extensor tibiae motoneuron: (a) dorsal view and (b) side view. Drawings were made with a camera lucida from a whole mount of the metathoracic ganglion. The interneuron was identified physiologically and then stained by the intracellular injection of cobalt and the intensification of the cobalt sulfide precipitate with silver (*13*). The cell body of 15-μm diameter is in a ventral rind of cell bodies. A single process emerges from the cell body and gives rise to a profusion of branches, none more than 5 μm in diameter, within the central neuropil. Branches of the interneuron and the motoneurons are intermingled.

0036-8075/79/0406-0081$00.50/0

tude, and when it is depolarized they are diminished. The source of the EPSP's is thought to be spikes in an interneuron or a sensory neuron activated by the movement of the tibia (*12*). If the tibia is moved rhythmically, then upon each flexion there is a sequence of EPSP's in the interneuron, a hyperpolarization of the flexor motoneuron, and spikes in the extensor motoneuron (Fig. 2f). That the interneuron plays an essential role in mediating these changes in the motoneurons can be shown by tonically hyperpolarizing the interneuron and repeating the movement (Fig. 2g). There is then a reduction in the amplitude of the hyperpolarization in the flexor motoneuron and of the frequency of extensor spikes.

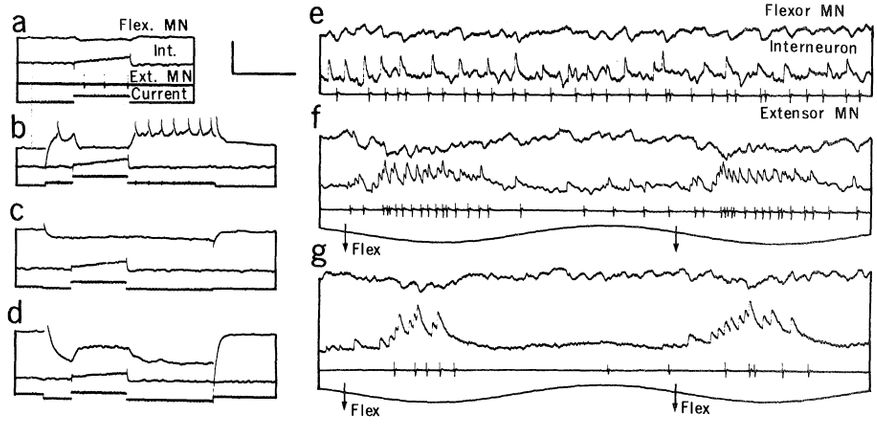

Fig. 2. Interactions of a nonspiking local interneuron with flexor and extensor tibiae motoneurons. (a) Depolarization of the interneuron (*Int.*) with current injected through the recording microelectrode by means of a bridge circuit evokes a hyperpolarization of the flexor motoneuron (*Flex. MN*) that persists for the duration of the current pulse. Spikes are also evoked in the slow extensor motoneuron (*Ext. MN*) [myogram on third trace, omitted in (b) to (d)]. The bottom trace in (a) to (d) monitors the current injected into both the interneuron and the motoneuron. (b) If the flexor motoneuron is depolarized by injected current to evoke spikes, and then the same pulse of current as in (a) is injected into the interneuron, the evoked hyperpolarization is accentuated. (c) If the flexor motoneuron is hyperpolarized, the effect from the interneuron reaches an apparent reversal potential. (d) If the flexor motoneuron is hyperpolarized still further, then the effect from the interneuron is reversed in polarity and is now depolarizing. (e) The tibia is fixed at an angle of 60° about the femur; EPSP's in the interneuron (middle trace) precede IPSP's in the flexor motoneuron (top trace). There is a tendency for spikes in the extensor motoneuron (bottom trace) to follow the EPSP's. (f) The tibia is forcibly flexed and extended in a sinusoidal way (bottom trace) about a femoral-tibial angle of 90° to 40°. Flexion evokes EPSP's in the interneuron, summed IPSP's in the flexor motoneuron, and an increased frequency of spikes in the extensor motoneuron. (g) The interneuron is hyperpolarized by 3.5 nA of current. Larger EPSP's occur in the interneuron with each flexion, but there is a reduction in the amplitude of the hyperpolarization in the flexor motoneuron, and of the number and frequency of spikes in the extensor motoneuron. Calibration. Vertical: voltage, flexor motoneuron, (a) to (d), 16 mV; (e) to (g), 4 mV; interneuron, (a) to (d), 40 mV; (e), 4 mV; (f) and (g), 8 mV; current, 26 nA; tibial movement, 80°. Horizontal: (a) to (d), 400 msec; (e) to (g), 200 msec.

To show more clearly the relationship between the EPSP's in the interneuron and the potentials in the motoneurons, a signal averager was triggered by an EPSP in the interneuron (Fig. 3). An IPSP in the flexor motoneuron follows an EPSP in the interneuron with a delay of 1.5 msec, as measured from the half-height of the rising phase of the EPSP to the onset of the IPSP (Fig. 3, a and b). A spike in the slow extensor motoneuron is more likely to follow the EPSP after a delay of 17.5 msec than with any other delay (Fig. 3c). These effects could be caused by common synaptic driving of the interneuron and the motoneurons, or the interneuron could be an essential link in the chain. To test whether the interneuron is necessary, it is held hyperpolarized by the steady injection of 3 nA of current. The absolute level of depolarization reached by the EPSP should be reduced so that it should now be less effective at releasing transmitter. When the hyperpolarizing current is applied, the amplitude of the IPSP in the flexor motoneuron is reduced by 75 percent, although the amplitude of the EPSP in the interneuron is increased by 24 percent and its duration, at half-height, by 25 percent (Fig. 3, d and e). The latency of the IPSP increases to 2.5 msec, which is consistent with the expectation that only the peak of the EPSP is now able to release transmitter. The reduction in the amplitude of the IPSP is graded and depends upon the amount of current injected into the interneuron. In addition, an extensor spike no longer occurs with a preferred relationship to the EPSP (Fig. 3f).

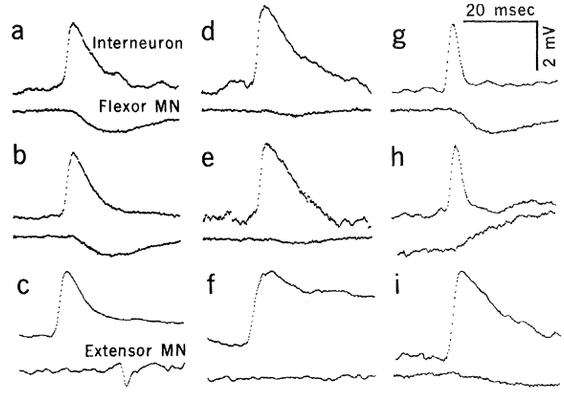

Fig. 3. EPSP's in an interneuron evoke IPSP's in a flexor tibiae motoneuron (*MN*). The EPSP's were first passed through a window discriminator, the output of which triggered a signal averager operating in a pretriggering mode. A simultaneous oscilloscope display of the EPSP's and the output of the window circuit ensured that only the EPSP's triggered the averager. The EPSP's and IPSP's were averaged simultaneously for 32 sweeps, the EPSP's and the extensor spikes, recorded at the muscle, for 256 sweeps. The EPSP's were evoked by holding the tibia flexed. (a) to (c) Three successive averages with the interneuron at its normal resting potential are shown. (a) and (b) An IPSP follows with a latency of 1.5 msec. (c) A spike in the extensor motoneuron occurs with a preferred latency. (d) to (f) Three successive averages with the interneuron held hyperpolarized by 3 nA of current are shown. (d) and (e) The EPSP is enlarged in amplitude and prolonged in duration, but the IPSP is substantially reduced in amplitude. (f) A spike in the extensor motoneuron no longer occurs with a preferred latency. (g) to (i) Averaged records from another locust are shown. (g) The interneuron is at its normal resting potential and the EPSP is followed by an IPSP in the flexor motoneuron. (h) The motoneuron is held hyperpolarized with the result that the IPSP is reversed in polarity. (i) The interneuron is held hyperpolarized by 3 nA of current, with the result that the IPSP in the motoneuron is reduced in amplitude.

These effects are consistent in the eight locusts so far examined. For example, in another locust the EPSP in the interneuron, although briefer, is followed by an IPSP with a latency of 1.5 msec in the same flexor motoneuron (Fig. 3g). As before, the IPSP can be reduced in amplitude by hyperpolarizing the interneuron (Fig. 3i). When hyperpolarizing current is applied directly to the motoneuron, the IPSP can be reversed in polarity (Fig. 3h). The reversal is brought about by the same amount of current as that needed to reverse the hyperpolarization evoked when depolarizing current is injected into the interneuron (Fig. 2, a to d). This indicates

that similar conductance changes underlie both potentials.

The evidence shows clearly that the local interneuron inhibits the flexor motoneuron by the graded release of transmitter and that such interactions between neurons are of behavioral significance. The possibility must be considered that there is widespread use of this method of information transfer among other neurons.

M. BURROWS

Department of Zoology,
University of Cambridge,
Cambridge CB2 3EJ, England

References and Notes

1. G. M. Shepherd, *The Synaptic Organization of the Brain* (Oxford Univ. Press, London, 1974); P. Rakic, *Local Circuit Neurons* (MIT Press, Cambridge, Mass., 1976); F. G. Worden, Ed., *The Neurosciences Fourth Study Program* (MIT Press, Cambridge, Mass., in press).
2. W. Ball and G. M. Shepherd, *J. Neurophysiol.* **31**, 884 (1968).
3. Examples of serial and reciprocal synapses seen in the electron microscope are numerous, for example, olfactory bulb: W. Rall, G. M. Shepherd, T. S. Reese, M. W. Brightman, *Exp. Neurol.* **15**, 44 (1966); superior colliculus: R. D. Lund, *J. Comp. Neurol.* **135**, 179 (1969); thalamus: H. J. Ralston III, *Nature (London)* **230**, 585 (1971), retina: J. E. Dowling and B. B. Boycott, *Proc. R. Soc. London Ser. B* **166**, 80 (1966); lobster ganglia: D. G. King, *J. Neurocytol.* **5**, 207 (1976); insect thoracic ganglia: D. G. King, *Neurosci. Abstr.* **3**, 558 (1977).
4. Small voltage changes in a variety of vertebrate and invertebrate receptor neurons are able to influence higher order neurons; for example, vertebrate rods and cones: J. F. Ashmore and G. Falk, *Nature (London)* **263**, 248 (1976); D. A. Baylor and R. Fettiplace, *Cold Spring Harbor Symp. Quant. Biol.* **40**, 429 (1976); G. L. Fain, A. M. Granda, J. H. Maxwell, *Nature (London)* **265**, 181 (1977); barnacle lateral eye: S. R. Shaw, *J. Physiol. (London)* **220**, 145 (1972); barnacle median ocellus: W. N. Ross and A. E. Stuart, *ibid.* **274**, 173 (1978); crab coxal receptors: B. M. H. Bush and A. J. Cannone, *ibid.* **232**, 95P (1973).
5. Graded synaptic transmission has, for example, been shown at spiking synapses of the following animals. Squid: B. Katz and R. Miledi, *J. Physiol. (London)* **192**, 407 (1967); K. Kusano, D. R. Livengood, R. Werman, *J. Gen. Physiol.* **50**, 2579 (1967); R. R. Llinas, in *Society for Neuroscience Symposia*, W. M. Cowan and J. A. Ferrendelli, Eds. (Society for Neuroscience, Bethesda, Md., 1977), vol. 2, p. 139; M. P. Charlton and H. L. Atwood, *Neurosci. Lett.* **5**, 165 (1977). Lamprey: A. R. Martin and G. L. Ringham, *J. Physiol. (London)* **251**, 409 (1975). Lobster: K. Graubard, *Neurosci. Abstr.* **7**, 652 (1975); ———, J. A. Raper, D. K. Hartline, *ibid.* **3**, 177 (1977).
6. K. G. Pearson and C. R. Fourtner, *J. Neurophysiol.* **38**, 33 (1975).
7. M. Burrows and M. V. S. Siegler, *Nature (London)* **262**, 222 (1976); (*8*). These papers present evidence that, in their normal functional state, these local interneurons do not spike. Small changes in their membrane potential effect the graded release of transmitter.
8. M. Burrows and M. V. S. Siegler, *J. Physiol. (London)* **285**, 231 (1978).
9. A "discrete" synaptic potential is defined as one that is separate and distinct from others at the particular recording site.
10. Full details of the methods are given by G. Hoyle and M. Burrows [*J. Neuobiol.* **4**, 3 (1973)] and in (*8*). Microelectrodes were filled with 2*M* potassium acetate and had d-c resistances of 50 to 80 megohms. All data were stored on magnetic tape. Locusts, *Schistocerca gregaria*, renamed *Schistocerca americana gregaria* by V. M. Dirsh [*Genus Schistocerca (Acridomorpha Insecta)* (Junk, The Hague, 1974)], were adults of either sex obtained from a crowded laboratory culture.
11. The tibia of a locust hind leg is moved by two antagonistic muscles, the flexor and the extensor. The flexor muscle is innervated by at least seven excitatory motoneurons and by two inhibitory neurons [M. Burrows and G. A. Horridge, *Philos. Trans. R. Soc. London Ser. B* **269**, 49 (1974)]. The extensor muscle receives, by contrast, only one slow and one fast (not used in walking) excitatory motoneuron, one inhibitory neuron, and one modulatory neuron. There are many local interneurons characterized physiologically (*7*, *8*) and anatomically [M. V. S. Siegler and M. Burrows, *J. Comp. Neurol.*, **183**, 121 (1979)] that affect these motoneurons. The interneuron discussed here has similar physiological and morphological characteristics in each locust. It is either the same neuron in each locust or a member of a limited class.
12. Experiments to date do not indicate which of the variety of possible sense organs in the hind leg are involved.
13. R. M. Pitman, C. D. Tweedle, M. J. Cohen, *Science* **176**, 412 (1972); J. P. Bacon and J. S. Altman, *Brain Res.* **138**, 359 (1977).
14. I thank my Cambridge colleagues for their helpful advice. Supported by a grant from the Science Research Council (United Kingdom); equipment provided by grants from the Royal Society of London and the Camilla Samuel Fund.

5 July 1978; revised 19 September 1978

Discrimination Learning Without Short-Term Memory: Dissociation of Memory Processes in Pigeons

Abstract. *Pigeons were trained to perform delayed matching to samples of food and no food when each sample concurrently served as the outcome of a discrimination learning trial which immediately preceded each matching trial. Ambient light presented during the delays after the samples of food or no food severely disrupted matching but had no effect on the rate of discrimination learning.*

A popular view of animal learning holds that things are learned about to the extent that their representations persist in a labile, short-term memory (STM) for some time after a learning episode. Learning is thus viewed as the transfer of associative information from STM to a long-term memory (LTM). The transfer processes have been variously referred to as "backward scanning" (*1*), "consolidation" (*2*), or "rehearsal" (*3*). The general idea is that rehearsal of information in STM promotes the eventual representation of that information in LTM. The rehearsal construct has also been used to interpret performances in tasks that appear to measure only maintenance of STM (*4*, *5*). However, rats (*6*) and humans (*7*) have been reported to show signs of LTM while showing little signs of STM, implying that maintenance of STM need not be prerequisite to formation of LTM. The findings reported here support such a dissociation between STM and LTM; discrimination learning was found to occur in the absence of measurable STM for outcomes of learning trials.

In the experiment described herein, STM was tested throughout associative learning. Pigeons were first trained to perform a version of delayed matching to sample in which reward for a choice between two comparison stimuli was conditional upon the prior occurrence of one of two sample stimuli (a presentation of food or no food). Then, discrimination learning trials were introduced in which the food and no-food samples were preceded by different stimuli. Thus, in the resulting procedure, shortly after each discrimination learning trial, a bird's memory for the outcome of that trial was tested. The measure of STM was the accuracy with which the birds could report occurrences (match to samples) of food and no food after a delay. The measure of association formation was the rapidity with which differential responding was established to the different signaling stimuli (discrimination learning). The question was whether a treatment, ambient light (*5*), that is known to reduce delayed matching performance (interfere with STM) would also reduce the rate of discrimination learning (interfere with the establishment of a new association).

Ten experimentally naive White King pigeons were maintained at about 80 percent of their free-feeding weights. The birds worked in a normally darkened operant conditioning chamber (*5*). The front panel of the chamber contained three pecking keys, a grain hopper mounted below the center key, and a houselight mounted above the center key. Stimuli were presented by in-line projectors mounted behind each key. The chamber was situated in a larger lightproof enclosure with ventilation and sound attenuation provided by an exhaust fan. Scheduling of experimental events and recording of data were accomplished with the aid of an Automated Data Systems 1800E computer located in an adjacent room.

Initially, the birds were trained to key-peck and then to perform delayed matching to samples of food and no food. After about 40 days of such training, each bird was performing the following task with accuracies in excess of 87.5 percent correct. After an intertrial interval of 20 seconds, each trial commenced with a white disk displayed on the center key. A single peck darkened the key and produced one of two samples: 2 seconds of access to grain from the illuminated food

0036-8075/79/0406-0083$00.50/0