

ROLES FOR ELECTRICAL COUPLING IN NEURAL CIRCUITS AS REVEALED BY SELECTIVE NEURONAL DELETIONS

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SUMMARY

Understanding fully the operation of a neural circuit requires both a description of the individual neurones within the circuit as well as the characterization of their synaptic interactions. These aims are often particularly difficult to achieve in neural circuits containing electrically-coupled neurones. In recent years two new methods (photoinactivation after Lucifer Yellow injection and intracellular injection of pronase) have been employed to delete selectively single neurones or small groups of neurones from neural circuits. These techniques have been successfully used in the analysis of circuits containing electrically-coupled neurones. In several systems new roles for electrical synapses in the integrative function of neural circuits have been proposed.

In the nervous systems of both the leech and lobster it is now thought that synaptic interactions previously thought to be direct are mediated through an interposed, electrically-coupled neurone. In the pyloric system of the stomatogastric ganglion of the lobster, *Panulirus interruptus*, the Lucifer Yellow photoinactivation technique has permitted a separate analysis of the properties of several electrically-coupled neurones previously thought quite similar. We now know that the Anterior Burster (AB) interneurone and the Pyloric Dilator (PD) motor neurones, which together act as the pacemaker ensemble for the pyloric network, differ in many regards including (a) their intrinsic ability to generate bursting pacemaker potentials, (b) their neurotransmitters, (c) their sensitivity to some neurotransmitters and hormones, (d) the neural inputs they receive and (e) their pattern of synaptic connectivity.

These results will be discussed in the context of the role of electrical coupling in neuronal integration.

INTRODUCTION

To understand how the nervous system processes information it is necessary to know the pattern of synaptic connections among its constituent neurones. In a similar fashion, to determine the function of a specific neurone in a neural circuit, it is useful to be able to remove that neurone from the neural circuit, and study the operation of the circuit without it. Until very recently, although it was possible to perform large-scale ablations of whole regions of nervous systems, or to cut nerves containing axons

of many neurones, it was not possible to delete selectively a single neurone, or small groups of neurones from a neural circuit. Recently two different methods have become available that allow the experimenter to remove single neurones irreversibly from neuronal circuits. These methods have been used to solve the patterns of synaptic connectivity in neuronal circuits, to assess the function of specific neurones, and to characterize the properties of individual neurones.

The first of these methods is the intracellular injection of protease (Parnas & Bowling, 1977; Bowling, Nicholls & Parnas, 1978). The second method is photoinactivation of neurones previously injected with the dye, Lucifer Yellow (Miller & Selverston, 1979). In this paper, I will briefly discuss these methods, and some of the results which have been obtained with them. The major emphasis of this review however, will be a discussion of how these methods have provided an invaluable tool for the analysis of circuits containing electrically-coupled neurones, and have led to new insights into the role of electrical coupling in integration.

CELL DELETION BY PROTEASE INJECTION

Parnas & Bowling (1977) pioneered the use of protease or pronase injection into individual leech neurones for the purpose of selectively killing them. In this method, pronase is injected intracellularly from a microelectrode, and within 12–24 h following the injection the injected neurone and its associated processes die. Pronase does not cross electrical junctions or interfere with other neurones in the ganglion (Bowling *et al.* 1978; Muller & Scott, 1981). This allows the selective deletion of any neurone into which sufficient enzyme can be injected (in any animal or ganglion that can survive long enough following the injection for useful study). This technique has been used extensively for studies of regeneration in the leech nervous system, because the animals can be injected, and then allowed to recover before analysis weeks or months later (Parnas, 1981; Muller & Nicholls, 1981; Elliott & Muller, 1982, 1983; Muller & Scott, 1979). Additionally, this technique has recently been used to produce selective partial denervations of a crustacean muscle (Parnas, Dudel & Grossman, 1982), a procedure which is difficult to achieve merely by cutting the nerve innervating the muscle.

This technique is of limited usefulness in experimental preparations in which it is not possible to wait for many hours following the injection to allow the injected neurone to die. Obviously, it is necessary to be able to penetrate the neurone in order to inject it.

CELL DELETION BY LUCIFER YELLOW PHOTOINACTIVATION

The Lucifer Yellow photoinactivation technique was first used by Miller & Selverston (1979) to kill neurones of the stomatogastric ganglion of the lobster, *Panulirus interruptus*. In this technique the dye, Lucifer Yellow (Stewart, 1978), is injected into a neurone and allowed to diffuse for only several minutes. The ganglion is then irradiated with an intense blue light, which results in the rapid death of the dye-filled neurone. The advantage of this technique is that the whole procedure takes less than 1 h (even allowing time for the ganglion to be washed with physiological saline, to remove any neurotransmitter or ions released during the photoinactivation process).

disadvantage is that it requires additional, expensive, optics in the experimental set-up. The potential applicability of this technique to different nervous systems also varies. It has thus far been most useful in non-pigmented Arthropod ganglia that can be easily illuminated, although it has also been successfully used in the leech (Peterson, 1983; Granzow & Kristan, 1983). In ganglia in which Lucifer Yellow (or other small molecular weight dyes) do not cross electrical junctions (Miller & Selverston, 1982*a,b*; Eisen & Marder, 1982; Audesirk, Audesirk & Bowsher, 1982), this technique allows the selective deletion of single neurones. In preparations in which Lucifer Yellow crosses from one electrically-coupled neurone to others (Stewart, 1978), this technique will not always allow the selective removal of single neurones. If the Lucifer Yellow technique is coupled with focused illumination of small regions of the injected neurones, it can be used for the ablation of portions of single neurones (Miller & Selverston, 1982*b*; Miller & Jacobs, 1984, this volume).

THE USE OF CELL DELETIONS TO DETERMINE THE FUNCTION OF SPECIFIC NEURONES

One of the most important uses of cell deletion experiments is to determine the function of a specific neurone in a neural circuit. Often, hyperpolarization of the neurone in question with a microelectrode is either not feasible or is insufficient to enable the investigator to determine whether the neurone in question is required for a given function. An example of this use of cell deletions is the work of Camhi (1983) and Comer (1983) on the giant fibre system of the cockroach. These investigators were interested in determining the behavioural function of the giant fibres in the cockroach. They therefore injected these fibres with pronase, allowed the animals to recover and the fibres to die, and then tested the animals' behavioural responses to stimuli.

Peterson (1983) used the Lucifer Yellow photoinactivation technique in his analysis of the neural control of the leech heartbeat, to ask whether a specific synaptic interaction was the only one mediating a given function. To determine whether the reciprocal connection between the HN(3) cells is the only link across the midline, Peterson inactivated one HN(3) cell, and discovered that activity became uncoupled across the midline, indicating that the connection in question was sufficient for this purpose.

The most extensive use of the Lucifer Yellow photoinactivation technique is that of Selverston and his colleagues on the stomatogastric ganglion. Miller & Selverston first analysed the roles of specific neurones in the pyloric system in the lobster stomatogastric ganglion (Selverston & Miller, 1980; Miller & Selverston, 1982*a,b*). More recently Selverston & Wadepuhl have analysed the gastric system (Selverston, Miller & Wadepuhl, 1984).

PROBLEMS POSED BY CIRCUITS CONTAINING ELECTRICALLY-COUPLED NEURONES

Electrical coupling among neurones is now known to occur in a wide variety of regions in both invertebrate and vertebrate nervous systems (Bennett, 1972, 1973, 1974). We have only begun to appreciate the full range of integrative functions that electrical coupling can confer on neural circuits. Part of the problem arises from the

difficulty in demonstrating that neurones are electrically coupled in preparations that are not easily amenable to recordings from more than one neurone simultaneously. Another problem arises from the fact that it is often impossible to study separately the actions of electrically-coupled neurones. In this paper, I will describe several systems in which cell deletion experiments were used to demonstrate the presence of electrically-coupled neurones in a network in which they were not previously known to occur. I will then discuss work in which cell deletion experiments were used to study separately the actions of neurones already known to be electrically coupled. The results of these studies show that electrical coupling is an integral part of the function of many neuronal circuits, and that electrical coupling may serve a number of roles in nervous systems.

THE PROBLEM OF MONOSYNAPTICITY IN CIRCUITS CONTAINING ELECTRICALLY-COUPLED NEURONES

It can be surprisingly difficult to establish the pattern of synaptic connectivity in nervous systems that contain electrically-coupled neurones (see also Roberts *et al.* 1982).

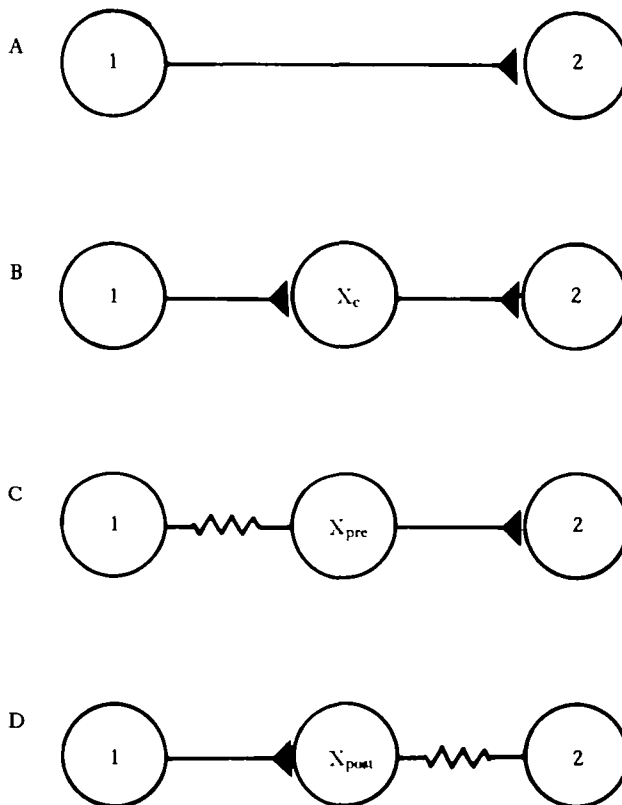


Fig. 1. The problem of interposed electrically-coupled neurones. Triangles denote excitatory chemical synapses; resistor symbols denote electrical synapses. (A) Direct monosynaptic connection between neurones 1 and 2. (B) Polysynaptic pathway involving a chemical synapse. Presence of an interposed interneurone, X_c , in the pathway between neurones 1 and 2. (C) Neurone 1 is electrically coupled to neurone X_{pre} which synapses onto neurone 2. (D) Neurone 1 synapses onto neurone X_{post} that is electrically coupled to neurone 2.

A great deal of attention has been paid to the problem of how to demonstrate with electrophysiological techniques that a unitary chemical synaptic potential is monosynaptic (Kandel, 1976) (as drawn in Fig. 1A), without the intervention of an unidentified interneurone. A number of strategies have been proposed to demonstrate (a) that an excitatory postsynaptic potential (EPSP) is chemical rather than electrical and (b) that the connection is monosynaptic, without an intervening chemical synapse (Fig. 1B). These are described by Kandel (1976). They include the use of low Ca^{2+} -containing salines to block chemical synaptic transmission, of high Ca^{2+} -containing salines to raise the threshold of an intervening neurone, the use of TEA injections into the presynaptic neurones (Kehoe, 1972), and the measurement of synaptic latencies. However, far less attention has been paid to determining whether there are intervening electrically coupled neurones, either on the presynaptic (Fig. 1C) or the postsynaptic sides (Fig. 1D). The behaviour of neurones connected *via* electrically-coupled neurones (as in Fig. 1C, D) may thus appear indistinguishable from monosynaptically connected ones (as in Fig. 1A) in terms of synaptic latencies, manipulations of divalent cation concentrations, and the other tests of monosynapticity suggested by Kandel (1976).

There are several reports of previously supposed direct synaptic connections that are now known to involve an intervening electrically-coupled neurone. In most of these, the ability to delete neurones selectively from the circuit was necessary for the demonstration.

Muller & Scott (1981) showed that an electrical connection in the leech, previously thought to be direct, is mediated *via* another, electrically-coupled interneurone. The touch cells (T cells) in the leech respond to touch of the animal's skin. These cells are electrically coupled to the S cells, and the electrical synapse between the S and T cells is unaffected by bathing the ganglion in high Mg^{2+} solutions, arguing that there is

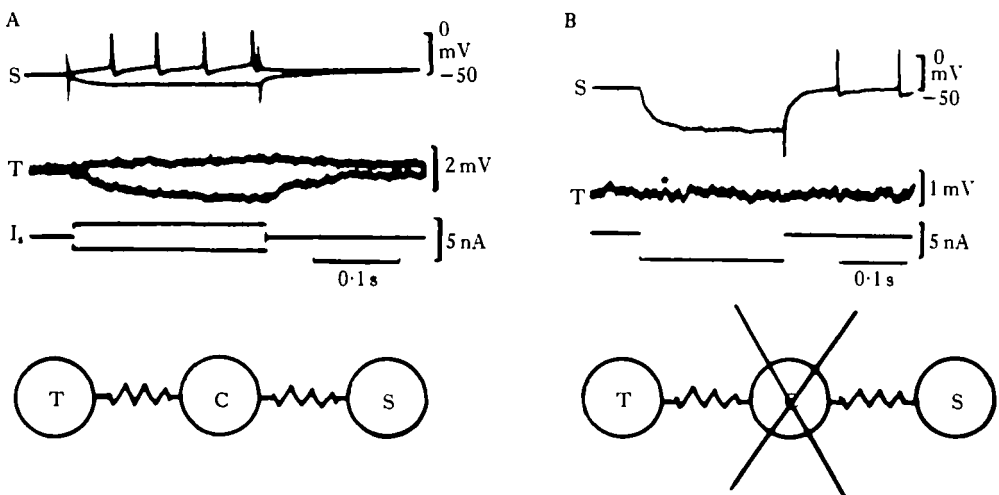


Fig. 2. Demonstration that the T cell and the S cell in the leech are coupled *via* the interposed electrically-coupled C interneurone. (A) Passing current into the S cell produces an electrotonic potential in the T cell. (B) After deletion of the C cell by pronase injection, hyperpolarization of the S cell no longer produces hyperpolarization of the T cell. Figure modified from Figs 2B and 7C of Muller & Scott (1981).

no intervening chemical synapse. When Muller & Scott injected the S cells and \square cells, in the same ganglion, with horseradish peroxidase (HRP), they discovered that these cells did not come into contact with each other. However, when the S cells were injected with Lucifer Yellow, the dye spread into two small neurones, called the

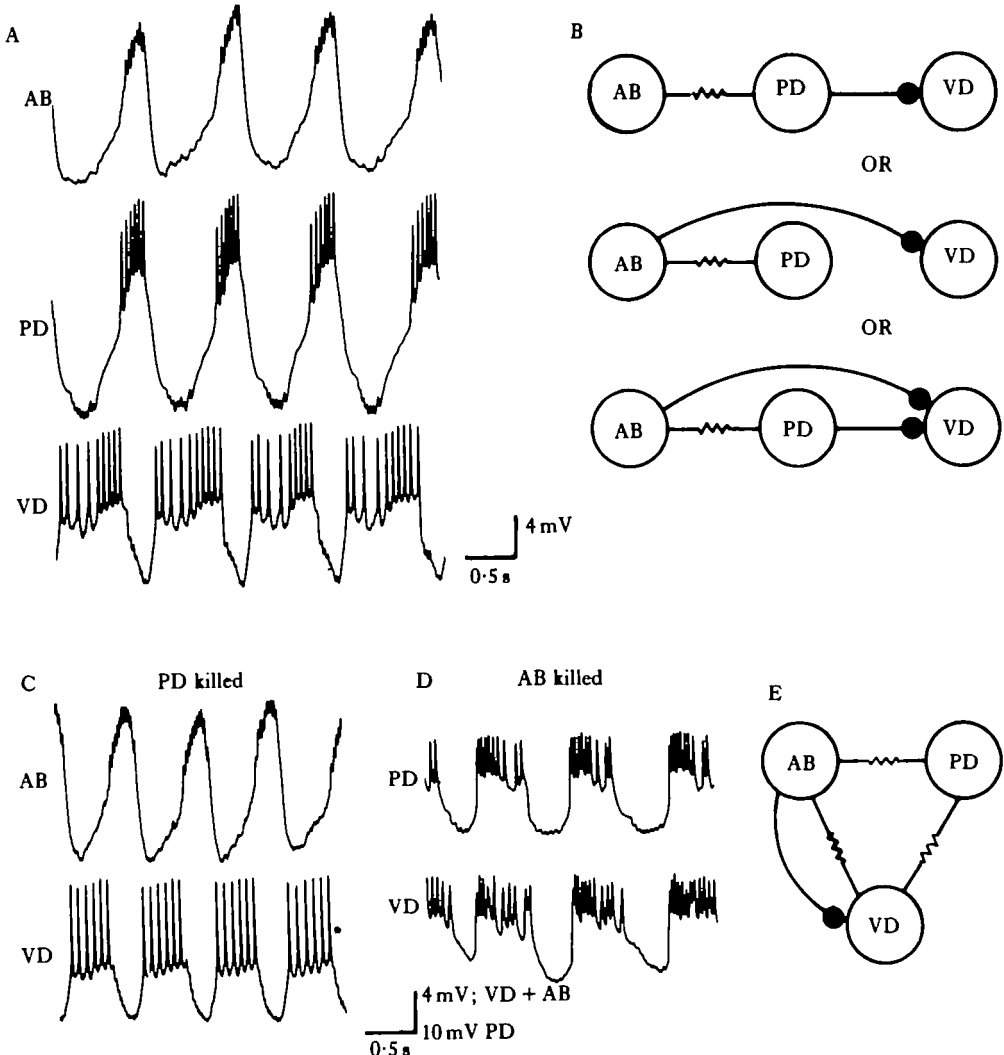


Fig. 3. Use of Lucifer Yellow photoinactivation technique to determine which of two electrically coupled neurones is presynaptic to another neurone. (A) Simultaneous intracellular recordings from the AB, PD and VD neurones of the lobster stomatogastric ganglion. Associated with the synchronous depolarization in the AB and PD neurones is a compound IPSP in the VD neurone. (B) Possible circuits that could explain the inhibition from the electrically-coupled AB and PD neurones to the VD neurone. Resistors denote electrical synapses, filled circles denote chemical inhibitory synapses. (C) Simultaneous intracellular recordings from AB and VD neurones after photoinactivation of the PD neurones. Note that depolarization of the AB neurone still evokes the compound IPSP in the VD neurone. (D) Simultaneous intracellular recording from a PD neurone and the VD neurone after photoinactivation of the AB neurone. Depolarization of the PD neurone no longer evokes an inhibition in the VD neurone. In fact the VD neurone now fires in phase with the PD neurone, due to the electrical junction between the VD neurone and the PD neurones. (E) Synaptic connectivity among the AB, PD and VD neurones. The AB but not the PD neurones inhibits the VD neurone. (Modified from Eisen & Marder, 1982.)

Dupling neurones (C neurones) which are electrically coupled to both the T and the S cells. The electrical connection from the T to the S cells appears to be mediated entirely by coupling through the C cells, because the electrical coupling from the S to T cells was abolished after killing the C cells by protease injection (Fig. 2). These results corroborate their anatomical findings, and are an example of the circuit drawn in Fig. 1B.

Eisen & Marder (1982) used the Lucifer Yellow photoinactivation technique to demonstrate that two synapses (long thought to exist in the stomatogastric ganglion, STG, of the lobster *Panulirus interruptus* from electrophysiological recordings) are mediated *via* electrical connections to other neurones. In one, the supposed presynaptic neurone is electrically coupled to another neurone that actually liberates the neurotransmitter (e.g. Fig. 1C). In the second, it is the supposed postsynaptic neurone that is electrically coupled to another neurone which receives the synaptic potential (e.g. Fig. 1D).

The STG contains two pyloric dilator (PD) motor neurones that are electrically coupled to the single anterior burster (AB) interneurone. These neurones depolarize synchronously, and in so doing inhibit the other neurones in the pyloric network, among them the ventricular dilator (VD) motor neurone (Fig. 3A). On the basis of recordings such as those in Fig. 3A, it was presumed that the PD neurones inhibited the VD neurone. However, Fig. 3B shows that there are at least three different patterns of synaptic connectivity that could account for the recordings in Fig. 3A. It was possible to decide among these by selectively deleting either the AB or the PD neurones. After the AB neurone was photoinactivated, depolarization of the PD neurones failed to evoke inhibitory postsynaptic potentials (IPSPs) in the VD neurone (Fig. 3C). On the other hand, after photoinactivation of the PD neurones, depolarizations of the AB neurone evoked IPSPs in the VD neurone (Fig. 3D). Thus, the IPSP recorded in the VD neurone, when the PD and AB neurones depolarize simultaneously, is due to neurotransmitter released only by the AB neurone (Fig. 3E is the actual pattern of synaptic connectivity among the PD, AB and VD neurones, Eisen & Marder, 1982), which is important since the AB and PD neurones release different neurotransmitters.

Granzow & Kristan (1983) described a similar result in the leech. Depolarization of the ventral inhibitor motor neurone inhibits the dorsal excitor motor neurone. However, after photoinactivation of the dorsal inhibitor motor neurone, which is electrically coupled to the ventral inhibitor, depolarization of the ventral inhibitor no longer inhibits the dorsal excitor. From these data, Granzow & Kristan (1983) concluded that the inhibition from ventral inhibitor to dorsal excitor is mediated *via* the electrical connection between the inhibitors (as drawn in Fig. 1C).

The converse situation occurs in the synaptic connections between the lateral pyloric (LP) neurone and the PD and AB neurones, again in the lobster stomatogastric ganglion. Recordings from the AB, PD and LP neurones show IPSPs in both the AB and PD neurones associated with LP neurone action potentials (Fig. 4A). Although these IPSPs are slightly smaller and slower when recorded in the AB neurone than in the PD neurones, they are distinct, and it was always assumed that the IPSP recorded in the AB neurone was direct. However, Fig. 4B shows that the IPSP evoked by the LP neurone in the PD and AB neurones can be explained by three

different patterns of connectivity. After photoinactivation of the AB neuron, depolarization of the LP neurone still evokes an IPSP in the PD ones (Fig. 4C). After photoinactivation of the PD neurones, depolarization of the LP neurone no longer evokes an IPSP in the AB neurone (Fig. 4D). Thus, it was concluded that the IPSP recorded in the AB neurone is mediated *via* the electrical connection to the PD neurones (as in Fig. 1D; see Eisen & Marder, 1982 for additional evidence). The implications of this result bear further study: the AB neurone is largely responsible

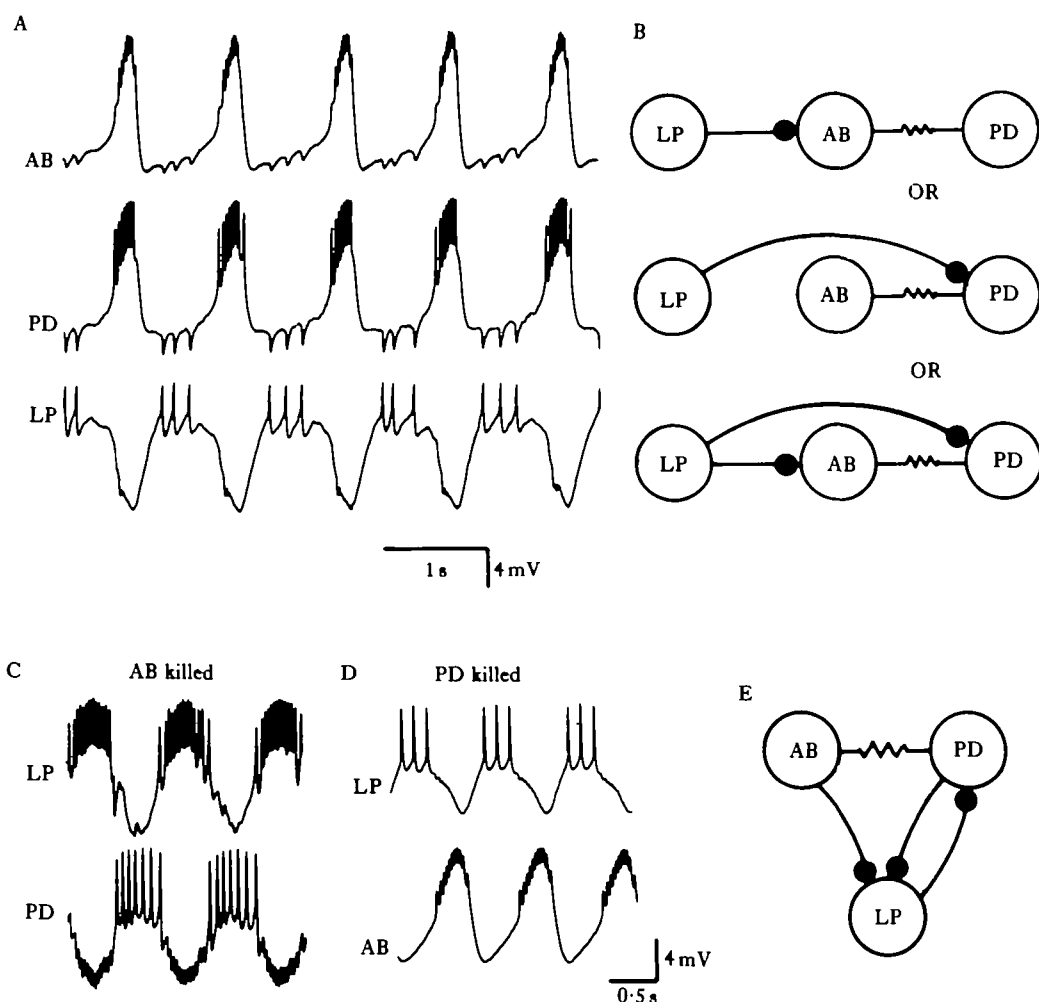


Fig. 4. Use of the Lucifer Yellow photoinactivation technique to determine which of two electrically coupled neurones is postsynaptic to another neurone. (A) Simultaneous intracellular recordings from the AB, PD and LP neurones in the lobster stomatogastric ganglion. Note that each action potential in the LP neurone is associated with an IPSP in both the PD and AB neurones. (B) Possible circuits to explain the pattern of connectivity responsible for the recordings shown in A. (C) Simultaneous intracellular recordings from the LP and PD neurones after photoinactivation of the AB neurone. Note that each LP neurone action potential still evokes an IPSP in the PD neurone. (D) Simultaneous intracellular recordings from the AB neurone and LP neurone after photoinactivation of the PD neurones. Action potentials in the LP neurone no longer evoke IPSPs in the AB neurone. (E) Synaptic connectivity among the AB, PD and LP neurones. The LP neurone directly inhibits the PD neurones but not the AB neurone. (Modified from Eisen & Marder, 1982.)

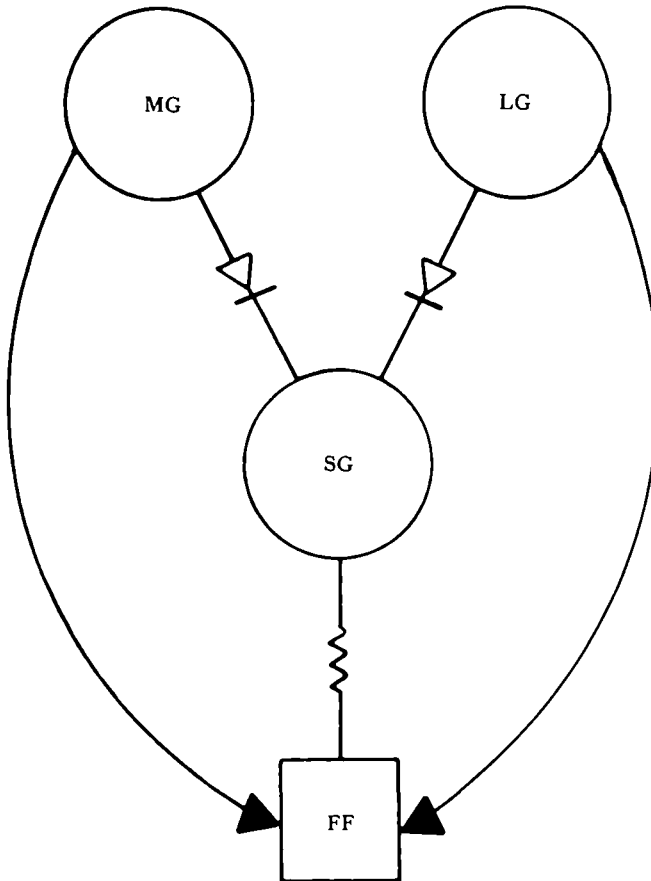


Fig. 5. Parallel pathways in the crayfish escape system. The LG (lateral giant) and MG (medial giant) neurones are connected to the fast flexor motor neurones (FF) by two pathways, one direct, the other *via* the (SG) segmental giant interneurones. (Modified from Roberts *et al.* 1982.)

for setting the frequency of the pyloric rhythm, but all feedback to the AB neurone from other neurones in the central pattern generator is through its electrical connections with the PD and VD neurones.

THE PROBLEM OF PARALLEL PATHWAYS

One of the most difficult problems in the analysis of neural circuits is assessing the functional importance of parallel pathways, that is, two or more pathways that connect the same pre- and postsynaptic neurones. This is illustrated dramatically in the escape system of the crayfish (Wine & Krasne, 1982). The lateral and medial giant axons in the crayfish make monosynaptic connections with the fast flexor motor neurones. Additionally, the giants are electrically coupled to an intraganglionic interneurone, the segmental giant (SG) that also excites the fast flexor motor neurones (Kramer, Krasne & Wine, 1981) (see Fig. 5).

Roberts *et al.* (1982) determined, by a variety of physiological techniques (not including cell deletions), that the direct connection from the giant fibres to the fast flexor

motor neurones is functionally weak, and that the pathway involving the SG interneurones is responsible for virtually all of the normal activation of the fast flexor motor neurones. However, in other preparations, the assessment of the relative importance of two parallel pathways may be much easier if it is possible to delete a neurone selectively in at least one of the pathways (for example, the SG interneurone, Fig. 5).

It is interesting that in another escape pathway, in *Drosophila*, there is also an intervening electrically coupled interneurone (Tanouye & Wyman, 1980). In this animal it may be possible to perform deletions of specific cells or pathways with mutations (Thomas & Wyman, 1982).

THE USE OF CELL DELETIONS TO STUDY SEPARATELY THE PROPERTIES OF ELECTRICALLY-COUPLED NEURONES

Recently the cell killing technique has demonstrated that electrically coupled neurones, which normally act synchronously, can have very different cellular properties. This observation was first made by Miller & Selverston (1982a) on the PD and

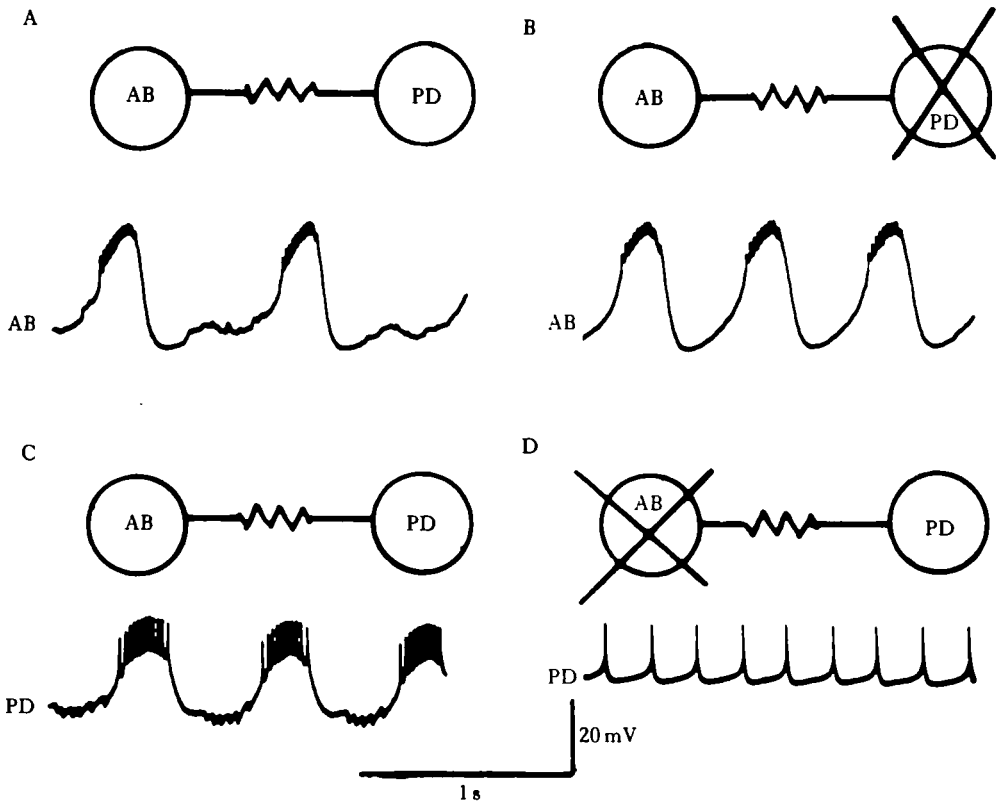


Fig. 6. Electrically-coupled neurones differ in their intrinsic capacity to generate bursts. (A) Intracellular recording from an AB neurone in an intact ganglion. (B) Intracellular recording from an AB neurone after photoinactivation of the PD neurones. (C) Intracellular recording from a PD neurone in an intact ganglion. (D) Intracellular recording from a PD neurone after photoinactivation of the AB neurone. Note that the isolated AB neurone continues to generate bursting pacemaker potentials, but the isolated PD neurone fires tonically. (Modified from Miller & Selverston, 1982a.)

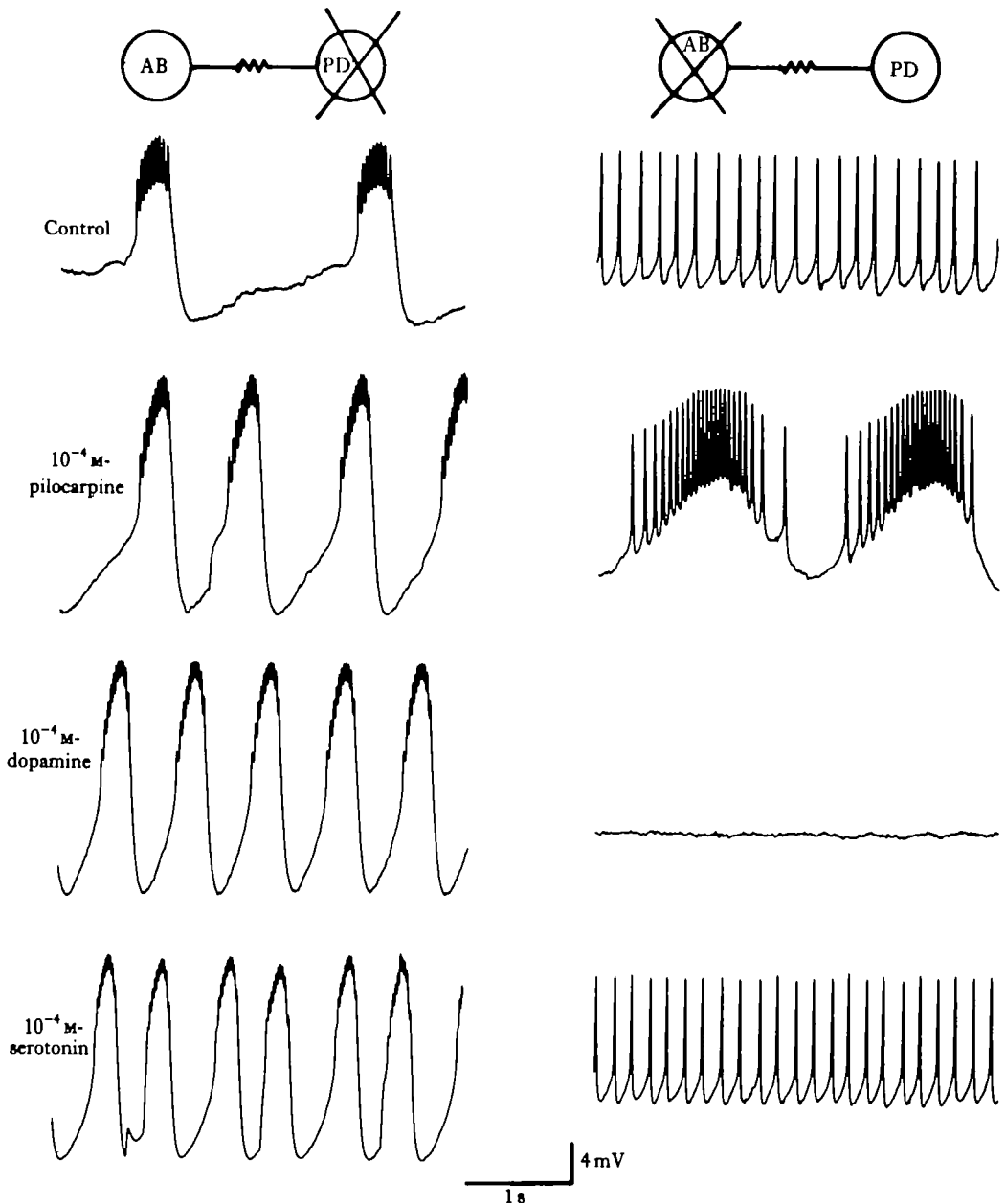


Fig. 7. Electrically-coupled neurones differ in their sensitivity to neurotransmitters. Recordings from isolated AB and PD neurones of the lobster stomatogastric ganglion. Left-hand column: recordings from an AB neurone after photoinactivation of the PD neurones and the VD neurone. Right-hand column: recordings from a PD neurone after photoinactivation of the AB neurone. Top row: control saline. The AB neurone continues to produce bursts, but the PD neurone fires tonically. Second row: in the presence of 10^{-4} M-pilocarpine. The amplitude and frequency of the AB neurone bursts are increased, and the PD neurone now produces long, slow bursts. Third row: in the presence of 10^{-4} M-dopamine. The amplitude and frequency of the bursts in the AB neurone are increased, but the PD neurone is hyperpolarized and inhibited. Bottom row: in the presence of 10^{-4} M-serotonin. The amplitude and frequency of the AB neurone bursts are increased but the PD neurone is unaffected. (Modified from records in Marder & Eisen, 1984b.)

AB neurones of the pyloric system of the stomatogastric ganglion. The PD and AB neurones are synchronously active in the intact ganglion, and together act as the pacemaker for the pyloric system. However, these neurones differ in their intrinsic ability to generate bursts in the absence of extrinsic inputs to the ganglion (Fig. 6, taken from Miller & Selverston, 1982a). In this experiment, Miller & Selverston photoinactivated the two PD neurones in the ganglion, and showed that the AB neurone retained its intrinsic ability to generate bursts (Fig. 6A, B). However, after the AB neurones were photoinactivated, the PD neurones fired tonically, and not in bursts (Fig. 6C, D).

We subsequently found other differences in the cellular properties of the AB and PD neurones (Marder & Eisen, 1984b) (Fig. 7). We studied the responses of isolated PD and AB neurones to bath application of dopamine, serotonin and pilocarpine (the muscarinic agonist). All three increased the amplitude and frequency of the AB neurone bursts after deletion of the PD neurones (Fig. 7, left). After deletion of the AB neurone, the PD ones hyperpolarized in response to dopamine, were unaffected by serotonin, but produced long slow bursts with pilocarpine.

The PD and AB neurones release different neurotransmitters, and evoke IPSPs of different time courses in follower neurones (Eisen & Marder, 1982; Marder & Eisen, 1984a) (Fig. 8). The AB neurone releases glutamate and the PD neurones release ACh (Marder & Eisen, 1984a). The AB neurone evokes a rapid IPSP, as can be seen after photoinactivation of the PD neurones, while the PD neurones evoke a slow IPSP, as can be seen after photoinactivation of the AB neurones (Fig. 8). During normal physiological activity, the AB and PD neurones depolarize synchronously, and the evoked IPSPs contain two components (mediated by different neurotransmitters), an early, AB-derived, and a late, PD-derived component.

ROLES OF ELECTRICAL COUPLING IN INTEGRATION

Electrical coupling in the nervous system was first thought to be a relatively rare feature. Initially two main functions for electrical coupling were proposed: speed and synchrony (Bennett, 1972, 1973, 1974).

Speed

It was reasoned that since the synaptic delay at electrical synapses was usually less than at chemical synapses, the propagation of an action potential would be faster through a polyn neuronal pathway that employed electrical synapses (Bennett, 1972). Frequently cited as evidence for this argument is the presence of electrical junctions in the escape pathways of a number of animals, where speed is presumably of the essence (Furshpan & Potter, 1959; Bennett, 1972; Wine & Krasne, 1982; Tanouye & Wyman, 1980). It is certainly true that a pathway in which the giant fibre interneurones are connected by electrical synapses to motor neurones conducts action potentials rapidly, and allows an animal to respond rapidly in a stereotyped escape response. However, in the best described system, that of the crayfish, the giant fibre to motor neurone system is paralleled by other pathways, with slower conduction velocities (Wine & Krasne, 1982). Most interestingly, Reichert & Wine (1983) have

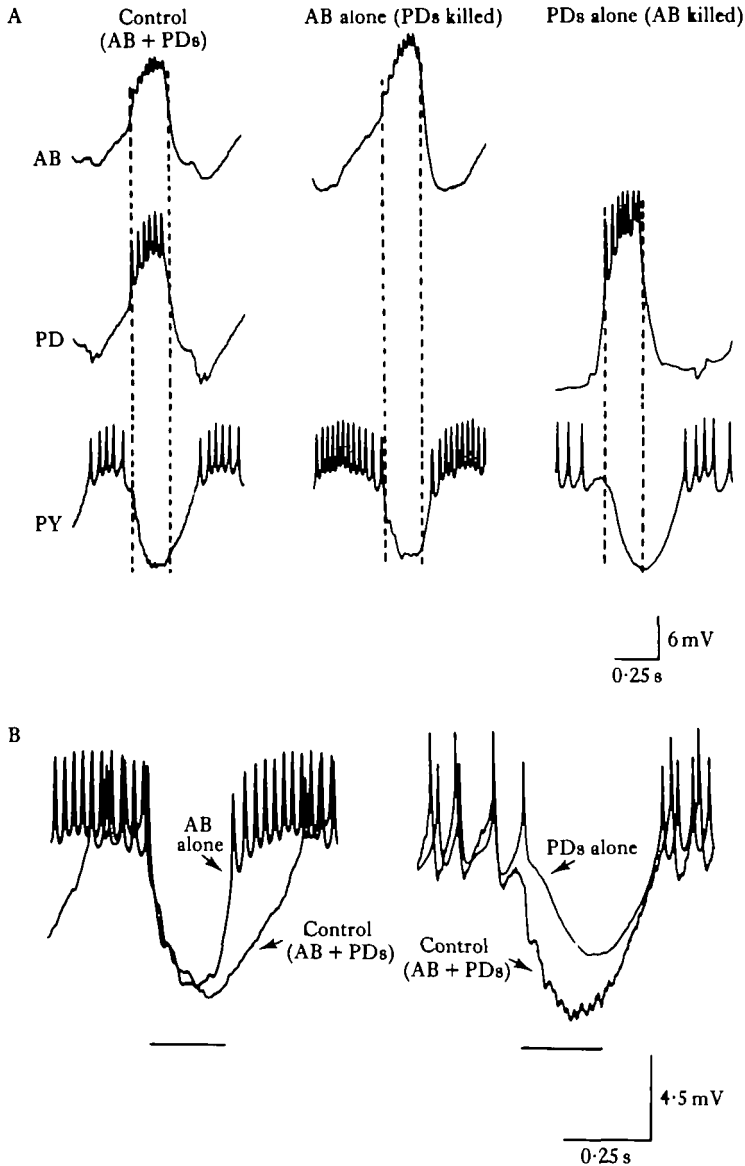


Fig. 8. Electrically-coupled neurones evoke IPSPs of different time course. Recordings from the AB, PD and PY neurones of the lobster stomatogastric ganglion. (A) Left: simultaneous intracellular recordings from the AB, PD and PY neurones. The IPSP recorded in the PY neurone starts soon after the onset of depolarization of the AB and PD neurones, and persists long after the repolarization of the AB and PD neurones. Middle: recording from AB and PY neurones after photoinactivation of the PD neurones. The IPSP in the PY neurone has a rapid onset, and terminates soon after repolarization of the AB neurone. Right: recordings from a PD neurone and a PY neurone after photoinactivation of the AB neurone. The IPSP in the PY neurone has slow onset, and persists long after the repolarization of the PD neurone. (B) Photographic superposition of the recordings from the PY neurones before and after photoinactivation of one of the presynaptic neurones. Time of depolarization of the presynaptic neurones indicated by bars. Left: PD neurones photoinactivated, the AB neurone-evoked IPSP is early and rapid. Right: AB neurone photoinactivated, PD neurone-evoked IPSP is slow and late. (Modified from Marder & Eisen, 1984a.)

shown that the difference in conduction velocity between a pathway containing giant fibres and electrical synapses and one with chemical synapses is used by the animal to order in time a series of behaviour activities.

Synchrony and asynchrony

It is almost a truism that the coupling of neurones (or cells of any type) by electrical junctions, that allow current to flow between them, will tend to synchronize their activity. Certainly, there are many examples in the literature in which electrical junctions are found between neurones that synchronously depolarize and fire action potentials (Gettings, 1974; Kaneko, Merikel & Kater, 1978; Egelhaaf & Benjamin, 1983). Electrically-coupled neurones can also fire out of phase with each other (Bennett, 1974; Mulloney & Selverston, 1974; Maynard & Selverston, 1975; Korn & Bennett, 1975; Mulloney, Perkel & Budelli, 1981; Egelhaaf & Benjamin, 1983). Some groups of electrically-coupled neurones fire in phase, under some conditions, but out of phase in others. A number of mechanisms have been proposed to explain how this might happen.

One way is to uncouple the neurones reversibly. Spira & Bennett (1972) showed that synaptic input to electrically-coupled neurones in *Navanax* functionally uncouples them, by increasing the conductance of the extrajunctional membrane. Certain pharmacological agents and neurotransmitters appear to uncouple neurones in the retina, by what appears to be a direct action on the junctional membrane (Neyton, Piccolino & Gerschenfeld, 1982; Piccolino, Neyton, Witkovsky & Gerschenfeld, 1982).

The ocular motor neurones of teleost fish fire synchronously during the fast phase of nystagmus, but generate smoothly-graded compensatory movements at other times. Korn & Bennett (1975) showed that when the cell bodies of these neurones are depolarized by synaptic inputs, the neuronal activity is synchronized. However, impulses that are initiated in the dendrites do not result in synchronized firing. Therefore, it is the spatial distribution of the synaptic inputs relative to the sites of electrical coupling that governs the physiological response of the neurones. The coupling interneurone in the leech (Muller & Scott, 1981) appears to serve a similar function.

Another very simple circuit allows neurones to fire synchronously under some conditions but out of phase under others. It is one in which one of a pair of electrically coupled neurones inhibits the other (Mulloney & Selverston, 1974). Mulloney *et al.* (1981) modelled the output of a circuit containing this feature as its central element (Fig. 9). They showed that when both the electrical and chemical inhibitory synapses were weak, the relative firing phase of the neurones drifted (Fig. 9A). When the electrical junction was strong, but the chemical synapse weak, the electrically-coupled neurones tended to fire synchronously (Fig. 9B). However, when the inhibitory chemical synapse was strong, and the electrical connection weak, the neurones tended to fire out of phase with each other (Fig. 9C).

The firing time of the VD neurone in the stomatogastric ganglion is an example of this behaviour. Its firing varies from in phase to in antiphase with the PD/AB neurone group (J. L. Ayers & A. I. Selverston, personal communication). The circuit diagram in Fig. 3 indicates that if the strength of the inhibitory synapse from the AB to the VD neurone were increased, or the strength of the coupling between the VD and the AB

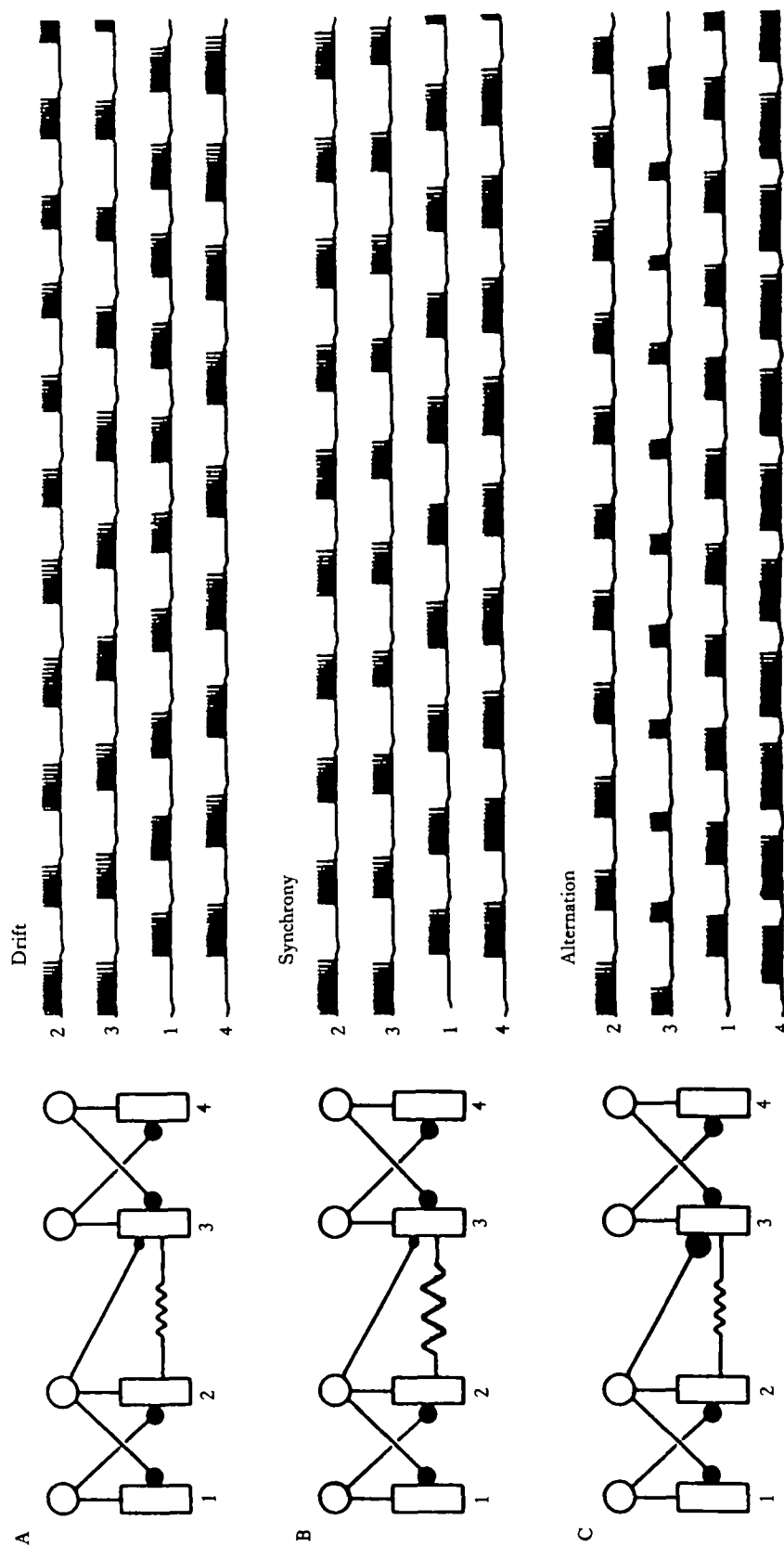


Fig. 9. Shift from synchronous firing to antiphasic firing according to the strength of electrical and chemical inhibitory synapses. Results of a computer modelling study of the circuits shown to the left of the traces. In the circuit diagrams, resistor symbols denote electrical coupling and filled circles chemical inhibitory synaptic connections. The size of the symbol indicates the strength of the interaction. (A) Neurons 2 and 3 are weakly electrically connected, and neuron 2 weakly inhibits neuron 3. As a result the firing times of neurons 2 and 3 drift past each other. (B) Neurons 2 and 3 are strongly electrically coupled, and neuron 2 weakly inhibits neuron 3. As a result, neurons 2 and 3 fire synchronously. (C) Neurons 2 and 3 are weakly electrically coupled, but neuron 2 strongly inhibits neuron 3, and therefore neurons 2 and 3 fire alternately. (Modified from Figs 1 and 2 of Mulloney, Perkel & Budek, 1981.)

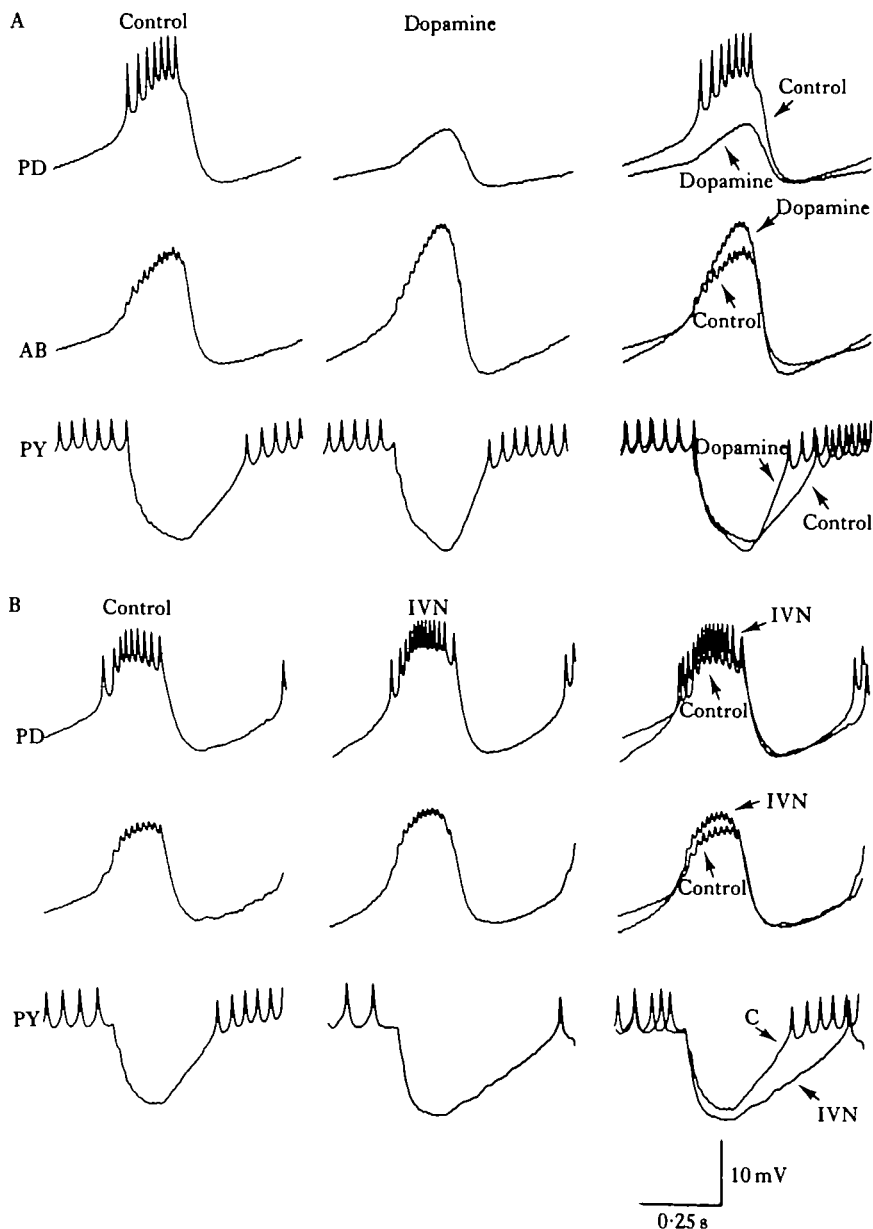


Fig. 10. Modulation of the amount of transmitter released by the PD neurones results in variable duration IPSP. (A) Simultaneous intracellular recordings from PD, AB and PY neurones in control conditions (left), and in the presence of 10^{-4} M-dopamine (middle). The right-hand panel is a photographic superposition of the dopamine and control traces. In the presence of dopamine the amplitude of the slow depolarization in the AB neurone increased, while the amplitude of the slow depolarization in the PD neurone decreased. The duration of the IPSP recorded in the PY neurone associated with the depolarization of the PD/AB neurones is decreased, and the PY neurone resumes firing earlier. (B) Simultaneous intracellular recordings of the PD, AB and PY neurones in control conditions (left) and directly after high frequency stimulation of the inferior ventricular nerve (IVN) (middle). The right-hand panel is a photographic superposition of the left and middle panels. After IVN stimulation the amplitude of the slow depolarization in the PD neurone is increased, the duration of the IPSP recorded in the PY neurone is longer and the PY neurone resumes firing later. (Modified from Eisen & Marder, 1984.)

And PD neurones decreased, then the VD would tend to fire out of phase with the AB and PD neurones. However, if the strength of the coupling were increased, or that of the inhibitory synapse decreased, then VD would tend to fire with the AB and PD neurones. The recordings in Fig. 3 show that the VD neurone shifts its phase of firing after the inhibitory connection from the AB neurone is removed, exactly as predicted. In the next section I will show that it is possible to influence the amount of transmitter released by the PD neurones with inputs that selectively influence the PD neurones. Therefore, it is likely, but not proved, that the phase of the VD neurone firing time may be controlled by inputs to the AB neurone.

Phase modulation

Circuits involving electrical junctions are capable of modulation of function in still other ways. One example is the modulation of firing phase of the pyloric (PY) neurones of the stomatogastric ganglion of *Panulirus interruptus* (Eisen & Marder, 1984). As discussed above, the PD and AB neurones are electrically coupled and release different neurotransmitters that evoke IPSPs in PY neurones with different time courses (Fig. 8). Since the PD neurones are responsible for the late component of the compound IPSP, excitation of them causing the release of more transmitter should prolong the time course of the compound IPSP. On the other hand, inhibition of the PD neurones should decrease the time course of the IPSP. To test this idea, we sought experimental conditions that would enable us to change the excitability of the PD neurones without directly affecting PY neurones. Stimulation of the inferior ventricular nerve (IVN) produced a slow depolarization of the PD neurones (Marder & Eisen, 1984b) but no effect on the PY neurones directly. Dopamine applications hyperpolarized and inhibited the PD neurones (Fig. 7) but had no direct effect on PY neurones (Eisen & Marder, 1984). Therefore, we predicted that the IPSP recorded in PY neurones due to depolarization of the AB/PD neurones would be increased in duration subsequent to stimulation of the IVN, but decreased in duration in the presence of dopamine. Fig. 10 shows that in the presence of dopamine, the IPSP is shorter in duration, and the PY neurones start firing earlier after the activity of the AB and PD neurones (Fig. 10A). The converse is true after IVN stimulation (Fig. 10B). Here, the IPSP is prolonged, and the firing time of the PY neurones is delayed. Fig. 11 shows that when we measured the mean firing phase of PY neurones relative to the AB/PD neurones, we found a significant phase shift of PY firing time (Eisen & Marder, 1984). Thus, since the IPSP evoked by the electrically-coupled AB and PD neurones has two components, each evoked by the action of different transmitters, with different time courses, it is possible to modulate the firing time of the follower neurones in a simple, reversible fashion (Fig. 11).

Switching the sign of a synaptic potential

In Fig. 12 I have drawn some hypothetical circuits that show that if two electrically-coupled neurones release different transmitters, one excitatory, the other inhibitory, the compound synaptic potential could actually switch from being predominantly excitatory to predominantly inhibitory. It is possible that such an arrangement exists in complex nervous systems. Without an appreciation of this possibility, such a synapse might by extremely difficult to understand.

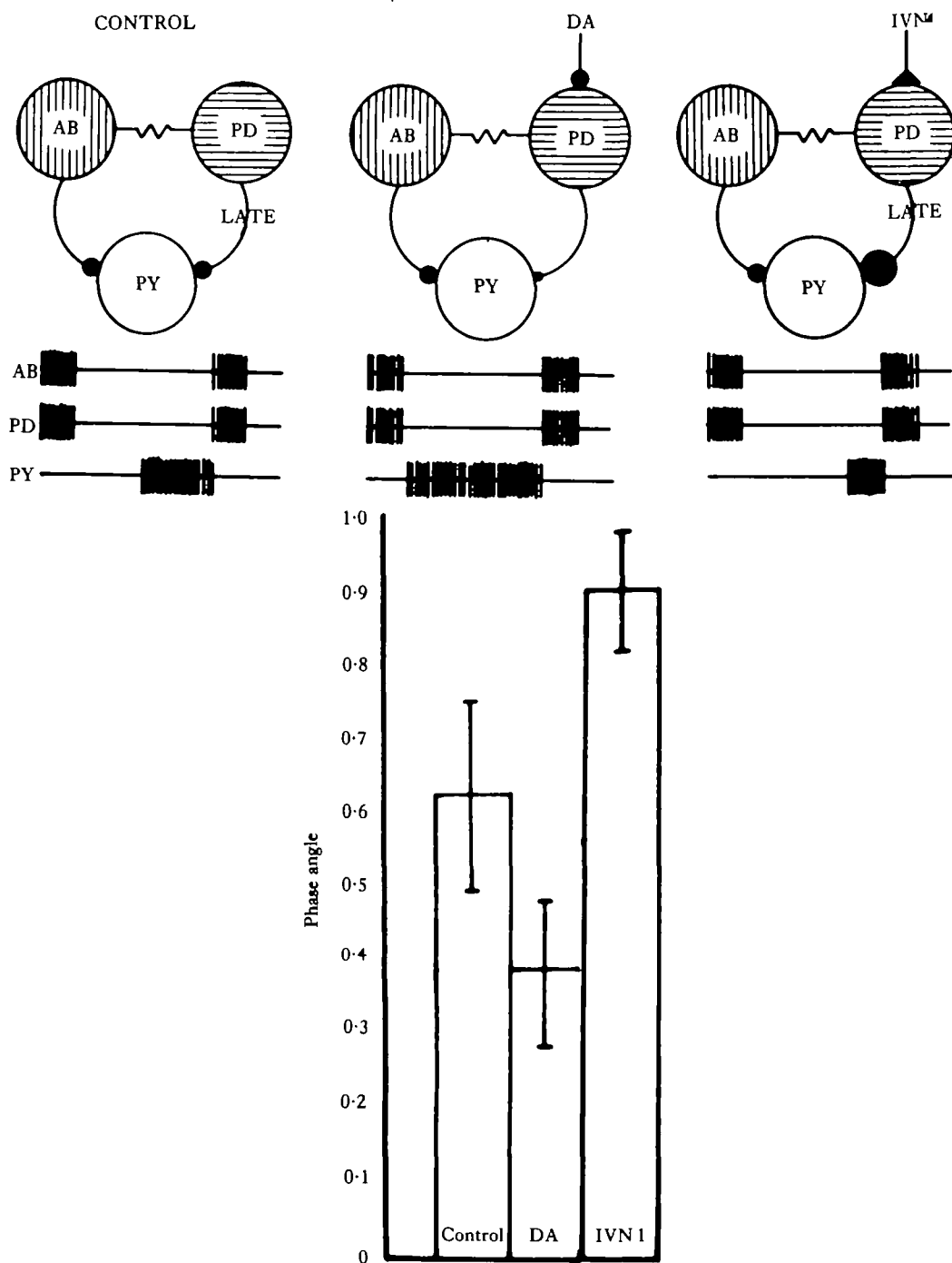


Fig. 11. Histograms show the firing phase of PY neurones in control saline, in the presence of dopamine (DA), and after stimulation of the inferior ventricular nerve (IVN). Cartoon model of the control of firing phase of the PY neurones. Filled circles indicate chemical inhibition, resistor symbols, electrical connections, and filled triangle, excitation. Size of symbol denotes strength of the interaction. Hand-drawn extracellular records point out that the time of resumption of PY neurone firing is controlled by the PD neurones. (Modified from Eisen & Marder, 1984.)

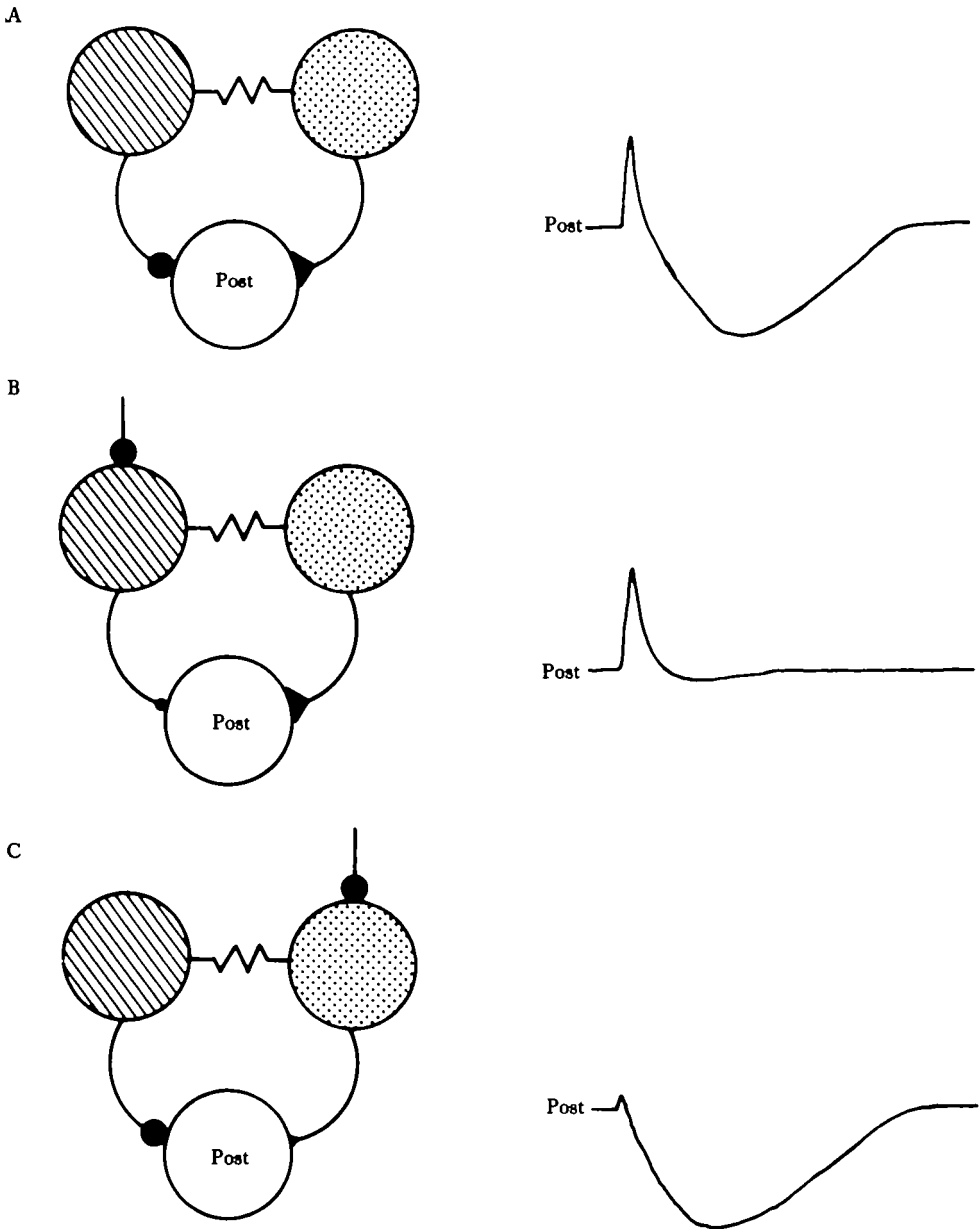


Fig. 12. Electrically-coupled neurones that release different neurotransmitters with opposite synaptic actions can act as a synapse that 'switches' sign. Filled circles indicate chemical inhibitory connections. Filled triangles indicate chemical excitatory connections. Resistor symbols indicate electrical junction. Size of symbol denotes strength of the connection. (A) Electrically-coupled neurones synchronously depolarize and release neurotransmitter, resulting in a biphasic synaptic potential. (B) The neurone that releases the excitatory transmitter is inhibited, the resulting synaptic potential is predominantly an IPSP. (C) The neurone that releases the inhibitory neurotransmitter is inhibited, the resulting synaptic potential is predominantly an EPSP. Post, postsynaptic neurone. Right-hand panel shows hypothetical recordings from postsynaptic neurones in the circuits shown on the left. (Modified from Eisen & Marder, 1984.)

CONCLUSIONS

The use of the Lucifer Yellow photoinactivation technique and pronase injection method for deletions of single neurones has simplified analyses of the patterns of synaptic connectivity in circuits containing electrically-coupled neurones. These techniques have allowed us to study separately the properties of electrically-coupled neurones in a way not possible previously. Electrical synapses serve functions other than just providing a through pathway for rapid impulse propagation, or a way of ensuring synchrony. Rather, it is becoming clear that electrical synapses are an important site for modulation of the function of neural circuits.

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