Electrotonic Coupling: Effective Sign Reversal by Inhibitory Neurons

Abstract. *Neurons in the buccal ganglia of* Navanax inermis *which control circumferential muscles of the pharynx showed typical electrotonic coupling when there was little synaptic activity in them. When there was much inhibitory activity, the effective sign of coupling was reversed; that is, hyperpolarization and depolarization of one cell caused depolarization and hyperpolarization of the others. A neural circuit explaining these results involves inhibitory neurons electrotonically coupled to and also inhibitory to the circumferential neurons that are themselves coupled. This circuit offers considerable flexibility for mediation of different activity patterns in this simple neuronal system.*

The occurrence of electrotonic synapses can in many instances be ascribed to a need for a high degree of synchronization of neuronal discharge (*1*). Junctional resistance at electrotonic synapses is not known to show any effect of the previous history of stimulation, which is consistent with a synchronizing function. This stability is in contrast to the variability of the postsynaptic potential (PSP) amplitude at most chemically transmitting synapses, where the amount of transmitter released is either increased or decreased by prior activity. It has been found that the synchronizing action at electrotonic synapses can be modulated by chemical synaptic inputs. The conductance increase associated with chemically mediated inhibition can shunt electrotonic spread between cells, apparently allowing the cells to fire more asynchronously (*2*). Also, synaptic inputs that increase cell resistance can increase electrotonic spread between coupled cells (*3*).

We report here further integrative capabilities in a population of electrotonically coupled cells that are made possible by chemical synapses. We have found neurons that under some conditions exhibit ordinary electrotonic coupling. Under different conditions, the same neurons exhibit coupling which is effectively negative; that is, depolarization of one cell hyperpolarizes the other, whereas hyperpolarization of the first cell depolarizes and may even excite the other.

The neurons showing this extraordinary change in sign of effective coupling are located in the buccal ganglia of the marine opisthobranch *Navanax inermis*. Stimulation of these cells causes circumferential muscles of overlapping regions of the pharynx to contract. For the experiments reported here, the buccal ganglia either were isolated with short lengths of the buccal nerves or remained connected to the pharynx. One or both ganglia were desheathed with fine forceps and pinned to Sylgard 184 encapsulating resin (Dow Corning) in the bottom of a dish. Except for the use of solutions high in Mg ions (*4*) to block chemical synapses, preparations were placed in filtered seawater at 18° to 22°C. For measurements of electrical coupling between cells, each of a pair of neurons was usually penetrated by two glass microelectrodes (one for recording and one for passing current), although a bridge circuit was sometimes used for simultaneous recording and current injection through a single electrode. Electrodes were filled with 3*M* potassium chloride or potassium acetate and had resistances of 5 to 15 megohms.

The phenomenon we discuss here is common among the five or so neurons in each ganglion that control the circumferential muscles. We refer to a pair of these neurons as cells A and B. When the baselines of cells A and B have little synaptic activity, these cells show ordinary electrotonic coupling. Hyperpolarization spreads from A to B (Fig. 1, b1, and Fig. 2, c1) and from B to A. Depolarization also spreads between cells (Fig. 2, c2), and impulses in one cell are followed by small depolarizations in the other (arrows in Fig 1a). Sometimes the cells fire quite synchronously, and firing of either cell can appear to trigger firing in the other (Fig. 1a).

Under other circumstances, particularly when the cells show considerable synaptic activity, the effects in cell B when cell A is polarized are remarkably different. Hyperpolarization in cell A results in depolarization in cell B (Fig. 1, b2 and b3, and Fig. 2a), while depolarization and firing of cell A hyperpolarize cell B (Fig. 2b). Thus, there is, in effect, a reversal in sign of coupling. (We have not systematically checked for symmetry of the ordinary or reversed coupling; however, both could be more pronounced in one direction.)

The cause of the sign reversal may be inferred from the experiment illustrated in Fig. 1b. After several hyperpolarizing pulses had been applied in cell A, it began to fire between pulses (Fig. 1, b1). An action potential in cell A was often followed by a large inhibitory postsynaptic potential (IPSP) in cell B (small arrows in Fig. 1, b1). These IPSP's were

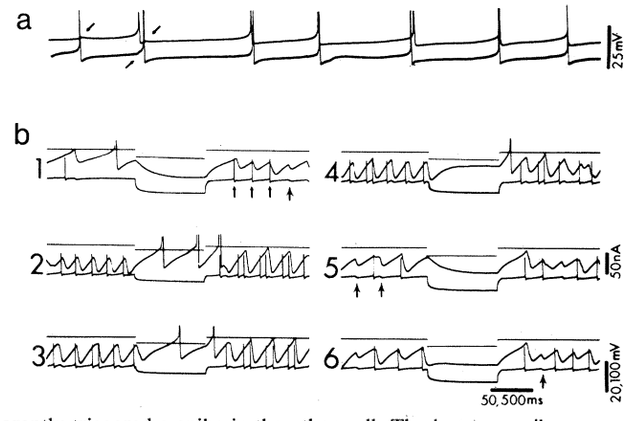

Fig. 1. Synchrony of firing and reversal of sign of effective coupling between two circumferential motoneurons. (a) The two cells were firing synchronously. Arrows mark apparent electrotonic spread of spikes from one cell to the other. These electrotonic PSP's can be seen to precede the second to fourth spikes in the cell of the lower trace. The fifth spike in the lower-trace cell apparently triggered a spike in the other cell. The last two spikes were nearly simultaneous in the two cells. (b) A different pair of cells. In the absence of IPSP's, the cells exhibited typical electrotonic coupling, but the cell of the lower trace began to fire (small arrows in b1) following the hyperpolarizing pulses (current on upper trace; the pulses were given every 2.5 seconds). Most spikes were followed by an IPSP in the middle-trace cell, although there was a large variability in the latency. Also, simultaneous IPSP's occurred in the two cells without firing of either cell (large arrows in b1, b5, and b6). Changes in amplitude of IPSP's in the middle-trace cell in this and subsequent records indicate the nonunitary nature of the IPSP. Hyperpolarizing the cell of the lower trace appears to have completely blocked IPSP's. When the hyperpolarizing current was weak, the cell of the upper trace was depolarized and began to fire, evidently because of disinhibition (b2 and b3). As the hyperpolarizing current was increased, the disinhibition was counteracted by electrotonically spread hyperpolarization, and the potential in the cell of the upper traces settled to an intermediate level (b4) or to a more hyperpolarized level (b5 and b6). The time course of the voltage record depended on the time of onset of the current pulse with respect to the spontaneously occurring IPSP's. The onset of the hyperpolarization had an ordinary time course when the pulse started at the end of an IPSP (b5); the onset could appear depolarizing when superimposed on the positive-going phase of an IPSP (b6). The middle trace of (b) was recorded at higher gain; a faster sweep was used in (a).

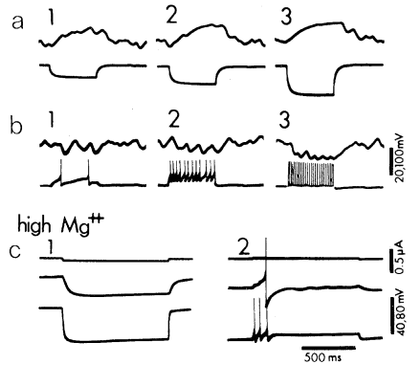

Fig. 2. Effect of high Mg^{2+} concentrations on interaction between two circumferential motoneurons. (a1 to a3) Hyperpolarization of the lower-trace cell induced depolarization of the upper-trace cell. This was associated with reduced PSP activity in the upper-trace cell. The depolarization was nearly maximal with the hyperpolarization in (a2), but the further hyperpolarization in (a3) may have reduced the PSP frequency. The saturation of the depolarizing effect and the absence of depolarizing transients at the termination of the hyperpolarizing pulses indicate that under these conditions the two cells were uncoupled (b1 to b3). Depolarization and firing of the lower-trace cell hyperpolarized the upper-trace cell. At low frequencies of firing there appeared to be IPSP's corresponding to each impulse (b1) but this correspondence was lost at high frequencies (b2 and b3). (c) After 45 minutes in a solution containing a high concentration of Mg^{2+} ions the cells were electrotonically coupled in a typical manner. Both hyperpolarization (c1) and depolarization (c2) spread from the lower-trace cell to the middle-trace cell. A small amount of PSP activity, presumably inhibitory, remained and was responsible for the oscillations in the middle-trace cell (c2, after the impulse). Current is shown on the upper trace in (c); lower gain was used for the lower-trace cell.

clearly not unitary, as may be seen by comparing the recording in cell B before and after the pulses in Fig. 1, b4 to b6. They were presumably generated by several unidentified neurons that we have termed the C group. The IPSP's could occur when A did not fire, and were then clearly visible in both cells, indicating that the C group inhibits both A and B (large arrows in Fig. 1b). Moreover, when a spike in A was closely followed by an IPSP in B, there was also an IPSP in A that could be seen as a small addition to the hyperpolarization after the spike. Neurons of the C group are likely to be coupled to each other, since IPSP components can occur relatively synchronously at very different latencies after an action potential in cell A (beginning of the record in Fig. 1, b3). In this experiment the smallest hyperpolarization that was applied in cell A appeared to block the entire C group, as indicated by the smooth time course of the voltages and absence of IPSP's in cells A and B during the current pulses in Fig. 1b. Block by hyperpolarization suggests that cell A was electronically coupled to the C group. The remarkable aspect of these observations is that a small hyperpolarization in cell A actually depolarized cell B and caused it to fire (Fig. 1, b2 and b3). The depolarization resulted from removal of the inhibition coming from the C group. Superimposed on this disinhibition are the electrotonic potentials, as can be seen from the small changes in the hyperpolarizing and depolarizing directions at the onset and termination of the pulses. As hyperpolarization in cell A was increased in amplitude, the electrotonic spread increased, while the disinhibition remained constant, apparently being virtually complete with even the smallest hyperpolarization (Fig. 1, b4 and b5). With large hyperpolarizations in cell A the appearance of the record in cell B depended on the timing of the pulse with respect to an IPSP from the C group. If the potential had recovered sufficiently after the last IPSP, the hyperpolarization was like that in the passive cell (Fig. 1, b5). If the IPSP's had just begun there was little change in its early time course, but further IPSP's were blocked so that the potential settled to an appropriate level hyperpolarized from the quiescent value (Fig. 1, b6).

In the experiment from which Fig. 1b is taken, the firing of cells A and B and of the C group began after hyperpolarizing pulses were started. This activity and that of B when the cell was disinhibited (Fig. 1, b2 and b3) seem likely to have resulted from post-hyperpolarization firing, as the records reveal no obvious EPSP's during or between the hyperpolarizing pulses.

The IPSP's did not always appear to consist of several unitary responses as in Fig. 1, and the inhibitory activity could be much more irregular (Fig. 2, a and b). Activity of inhibitory interneurons in causing effectively negative coupling is still indicated, however, for solutions with high Mg concentrations caused the baseline to become quiescent, and then the coupling between cells A and B was typically electrotonic (Fig. 2c).

A neural circuit that accounts for our findings is illustrated in Fig. 3. The two neurons A and B are electrotonically coupled. Each is also electrotonically coupled to the C group, here diagrammed as a single cell. [In Figs. 1 and 2 we only illustrate coupling from A to C; in many pairs of cells coupling to the C group from one of the cells (cell B) also occurred but was weaker.] The C group is inhibitory to both A and B. Other inputs regulate the excitability of the C group. When the C group is inactive and its excitability is low, A and B show ordinary electrotonic coupling and the cells can fire synchronously (Fig. 1a). The degree of coupling is rather low and they can also fire independently. At low levels of C group activity, the effects of electrotonic spread and alteration in level of inhibition can both be seen, as in Fig. 1b. Hyperpolarization of cell A turns off the C group, leading to depolarization of cell B, and at onset and termination of the hyperpolarizing pulses, electrotonic potentials are seen as short-latency shifts in hyperpolarizing and depolarizing directions. Furthermore, the steady potential in B during the hyperpolarizing pulse in A is more hyperpolarized the stronger the pulse in A.

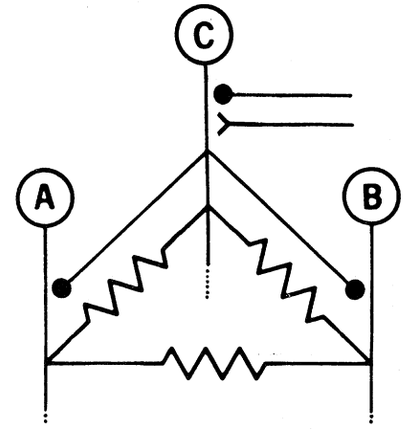

Fig. 3. A neuronal circuit that accounts for the data presented in this report. Cells A and B represent two circumferential motoneurons, and C represents a group of unidentified neurons that are inhibitory (filled circles) to A and B as well as electrotonically coupled to them. Independently of A and B, the C group receives inhibitory and excitatory (open ending) inputs that vary its excitability.

When the activity of the C group is high, as was apparently true for the experiment of Fig. 2, hyperpolarization in cell A can still decrease this activity and depolarization can increase it. However, in this case there may be no detectable electrotonic component in cell B; in particular, in Fig. 2a3 there was no immediate depolarizing shift in cell B when the hyperpolarization in cell A was terminated. A delay in onset of renewed C group activity and inhibition would be expected, but a positive-going electrotonic component should begin with little delay (Fig. 1, b3 and b4). Evidently, under these circumstances A and B were uncoupled by the inhibitory inputs to them, as described for other neurons in *Navanax* (*2*) and postulated elsewhere (*5*). (We are uncertain whether this uncoupling input was from the C group.)

Although a linear relation can be expected for the spread of subthreshold potentials between A and B when the C group is quiescent, the relation when the C group is active will be complex. The inhibition and disinhibition will both be somewhat delayed. Saturation will occur when the C group is completely blocked, as may have happened in the experiment of Fig. 1b, and nonlinearities could arise both in excitation of the C group and in its effects on cell B; a further factor in the input-output relation will be the effect of the C group back on cell A. Finally, synaptic inputs may modulate electrotonic spread between cells A and B.

Synaptic relations similar to those illustrated in Fig. 3 are found elsewhere in the *Navanax* buccal ganglia; subthreshold depolarizations of the expansion-controlling motoneurons excite neurons that inhibit them (*6*), but the effects are much weaker than in the circumferential neurons. Also, in the stomatogastric ganglion of the spiny lobster there are neurons connected both electrotonically and by inhibitory synapses (*7*). In one instance inhibition apparently causes the cells to fire out of phase when both are excited; however, during prolonged excitation the cells come to fire more or less synchronously, perhaps due to fatigue of the inhibition. Aside from this transition, no phenomena similar to the change in sign of effective coupling were noted. In addition, it was suggested that uncoupling produced by inhibition might be important in this preparation. The circuitry of Fig. 3 could account for the gating phenomenon described by Murray (*8*) in the cerebral ganglia of *Navanax*. In this case subthreshold polarizations in certain cells affect electrotonically mediated transmission of impulses from one set of cells to another set that have not been recorded from intracellularly. The cells that Murray calls gate cells could be electrotonically coupled to cells like the C group that inhibit the postsynaptic elements.

The flexibility of the circuitry described here may allow mediation and perhaps initiation of different patterns of movement involving the same neurons. When the C group neurons are inactive and not excited by the circumferential neurons, the latter cells are coupled typically and tend to fire synchronously. When activity of the C group is modulated by the circumferential neurons, the relation between circumferential cells is transformed into a different mode and the sign of effective coupling between them is reversed. Very large hyperpolarizations of circumferential neurons result from activity in expansion neurons, and post-hyperpolarization firing may operate in initiating circumferential contractions for swallowing. The hyperpolarizations in the circumferential neurons would also alter C group excitability. The pharynx sucks in food and swallows it by peristalsis or rejects it, depending on the circumstances (*9*). Because circumferential neurons control overlapping areas of the pharyngeal wall, synchronous and sequential activation will cause different kinds of movements. Understanding how the different patterns of movement are controlled seems feasible in this simple system.

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10. Supported in part by NIH grants HD-04248 and NS-07512 and NSF grant BNS75-19120 and an NIH postdoctoral fellowship to D.C.S. (1F32-NS05136).

1 June 1976; revised 27 July 1976

Interindividual Variation in Binding of Benzo[*a*]pyrene to DNA in Cultured Human Bronchi

Abstract. *The binding of benzo[*a*]pyrene to DNA in cultured human bronchus was measured in specimens from 37 patients. The binding values ranged from 2 to 151 picomoles of benzo[*a*]pyrene per milligram of DNA with an overall mean ± standard error of 34.2 ± 5.2. This 75-fold interindividual variation in the binding of benzo[*a*]-pyrene to DNA is similar in magnitude to that found in pharmacogenetic studies of drug metabolism. Aryl hydrocarbon hydroxylase is also inducible by benz[*a*]anthracene in the bronchial mucosa.*

Experimental systems are being developed to study the process of carcinogenesis in human tissues which are targets of environmental chemical carcinogens (*1*). The human bronchus, for example, is exposed to such carcinogens as polynuclear aromatic hydrocarbons (PAH) (*2*), which are present in the environment because of the incomplete combustion of fossil fuels and because of tobacco smoke (*3*). The carcinogenic PAH are procarcinogens requiring enzymatic activation into metabolites which bind covalently to cellular macromolecules (*4*). In some studies (*5*) a positive correlation has been found among binding of the PAH to DNA and their carcinogenicity. Whether this correlation will be found in studies of carcinogenesis in man is not known.

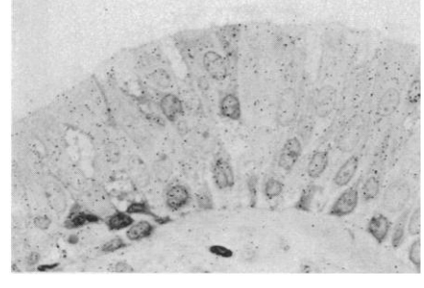

Fig. 1. Section of cultured human bronchus showing three major cell types (mucous, ciliated cells, and basal cells). The cells are well-preserved, and are overlaid with autoradiographic grains after incubation of the bronchi with tritiated BP (*7*).

Human bronchi maintained in explant culture have the capability to activate carcinogenic PAH into metabolites which bind to DNA (*6*). This binding is dependent on temperature of incubation, duration of exposure to PAH, and concentration of PAH (*7*). By means of an autoradiographic assay, it has been shown that in the bronchial mucosa the epithelial cells bind more PAH than the fibroblasts. 7,8-Benzoflavone (*7, 8*), an inhibitor of aryl hydrocarbon hydroxylase (benzopyrene hydroxylase; E.C. 1.14.14.2), decreased the binding of a PAH, benzo[*a*]pyrene (BP), to DNA.

Since the human population is genetically very heterogeneous and the metabolic activation of PAH may be under genetic control, we have studied the interindividual variation in the binding of BP to DNA in cultured human bronchial mucosa.

Human bronchial specimens were ob-