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RAPID PUBLICATION

Artificial Electrical Synapses in Oscillatory Networks

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SUMMARY AND CONCLUSIONS

1. We use an electronic circuit to artificially electrically couple neurons.

2. Strengthening the coupling between an oscillating neuron and a hyperpolarized, passive neuron can either increase or decrease the frequency of the oscillator depending on the properties of the oscillator.

3. The result of electrically coupling two neuronal oscillators depends on the membrane potentials, intrinsic properties of the neurons, and the coupling strength.

4. The interplay between chemical inhibitory synapses and electrical synapses can be studied by creating both chemical and electrical synapses between two cultured neurons and by artificially strengthening the electrical synapse between the ventricular dilator and one pyloric dilator neuron of the stomatogastric ganglion.

INTRODUCTION

Network behavior results from a complex interplay between the intrinsic membrane properties of the constituent neurons and their synaptic interactions. Electrical coupling often plays an important role in shaping network function. However, it is difficult to determine the impact of electrical connections on network output because it is frequently impossible to manipulate the strength of electrical synapses without also modifying other synaptic interactions and intrinsic properties of the neurons (Carrow and Levitan 1989; Neyton and Trautmann 1986; Piccolino et al. 1984). Several studies using small networks of neurons grown in culture have allowed the examination of some properties of networks containing coupled oscillatory elements (Kleinfeld et al. 1990a,b; Syed et al. 1990). However, these studies depend on the serendipitous formation of connections and do not allow the investigator to manipulate selectively the strength of electrical synapses once they are formed. To eliminate these problems, we use an electronic circuit to electrically couple neurons in dissociated cell culture and in a small rhythmic neural network. This method allows us to assess the interplay between electrical coupling and intrinsic membrane properties in emergent network behavior.

METHODS

Cell culture

Stomatogastric ganglia (STGs) from *Cancer borealis* were removed and desheathed in sterile physiological saline. They were placed into calcium- and magnesium-free saline containing 2% each collagenase (Sigma) and dispase (Boehringer Mannheim) for 2 h. The solution was then changed to the culture medium [50% Leibovitz's L-15 Medium (GIBCO) diluted to physiological concentration and containing 50 μg/ml gentamicin (GIBCO)]. Cells were then removed from the ganglion with gentle suction applied through a fire-polished micropipette. The isolated cells were placed into a Nunclon (Thomas) plastic culture dish containing culture medium; the dish was covered and left undisturbed for 12 h. The cells were allowed to grow for 2–10 days before experimentation.

Recordings

The cultures were perfused with normal physiological saline (in mM: 440 NaCl, 11 KCl, 13 $CaCl_2$, 26 $MgCl_2$, pH 7.45) for $\geq$30 min before recording. Recordings were made with the use of single intracellular electrodes filled with 0.6 M K_2SO_4 and 20 mM KCl. The recording amplifiers (Axoclamp-2A) were used in discontinuous current clamp mode with a sampling rate of 5 kHz.

Electrical synapse

The artificial electrical synapse is created by an analog circuit that compares the membrane potential of the neurons to be coupled and injects a current into each cell proportional to the magnitude of the difference in their membrane potentials (Fig. 1*A*). Because the injected current is based on the voltage difference, the circuit functions as an artificial conductance. This conductance is functionally equivalent to a nonrectifying gap junction between the somata of the coupled neurons. The effective coupling conductance is controlled by adjusting the gain on the output of the circuit (Fig. 1*A*). The output of our circuit provides a direct measure of coupling currents.

Chemical synapse

The artificial chemical synapse was formed by triggering a brief iontophoretic pulse of γ-aminobutyric acid (GABA) onto the postsynaptic cell from the rising phase of the presynaptic cell's oscillation.

RC-cell

The RC-cell we used in Fig. 2*A* is the clamp-1 model cell (Axon Instruments). It simulates a neuron with a 50-MΩ input resistance, a 25-ms time constant, and a 50-MΩ electrode resistance.

RESULTS

We first studied electrical coupling between cultured neurons dissociated from the STG of the crab, *Cancer borealis* (Golowasch et al. 1990; Graf and Cooke 1990; Krenz et al. 1990). After several days in primary tissue culture, these neurons retain much of their ability to respond to transmitters and modulators (Golowasch et al. 1990) and display a range of intrinsic membrane properties including tonic fir-

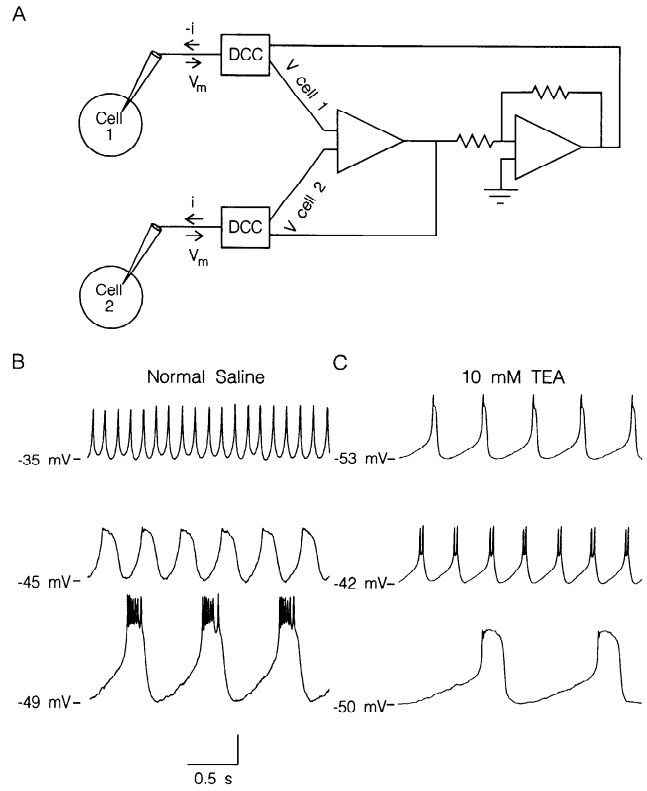

FIG. 1. Experimental components. *A*: schematic of recording configuration. *B*: examples of oscillatory behavior in 3 different nonidentified stomatogastric ganglia (STG) neurons in culture at the membrane potentials indicated. *C*: effect of 10 mM tetraethylammonium on the same 3 neurons as in *B*. All neurons in both parts have been depolarized with a constant 0.1 nA current. Vertical calibration bar is 10 mV in *B*; 20 mV in *C*.

ing and bursting (Fig. 1*B*). The frequency and wave form of the oscillations in these neurons can be modified by continuous current injection or by standard pharmacological techniques (Fig. 1*C*). This gives us the ability to study the effects of electrical coupling on many different types of oscillators (Fig. 1, *B* and *C*).

Figure 2*A* shows the effect of artificially electrically coupling an oscillating neuron in culture to an electronic cell that consists solely of a resistance and capacitance. As the coupling strength was increased, the frequency of the oscillator first increased and then decreased (Fig. 2*B*). This surprising result was anticipated in a theoretical study (Kepler et al. 1990). Other oscillatory neurons showed a steady decrease in frequency as the coupling strength was increased (data not shown). The type of response depended on the wave form of the oscillator as previously predicted (Kepler et al. 1990). Although Fig. 2*A* shows an electronic cell, similar results were obtained when the passive cell was a neuron with no active properties in the relevant voltage range.

The behavior of two cell networks can exhibit interesting emergent properties when they are constructed from neurons both of which have dynamic properties in the relevant voltage range. Figure 2*C* shows the effect of electrically coupling an oscillating neuron to another neuron that was capable of oscillating when depolarized. The initial state of the second neuron was more hyperpolarized than its threshold for oscillation. When the oscillator was weakly coupled to the silent cell (0.8 nS), the oscillator frequency decreased. As the coupling was increased (1.8 nS), the second cell was brought over its threshold and started to display oscillations as the result of the activation of its own regenerative properties. These oscillations were weak and unstable and caused a further decrease in the frequency of the first cell's oscillations. The first neuron's oscillations also became partially dependent on the oscillations of the second cell. As we increased the coupling strength still further (2.3 nS), both the frequency and stability of the two oscillators increased. These changes reflect strengthening of the regenerative properties of the second cell by the coupling.

Figure 2, *D* and *E*, shows that when two oscillating neurons are electrically coupled, the network output results from an interplay between the behaviors of the isolated neurons. In Fig. 2*D*, low coupling (1.7 nS) caused an unstable phase delay between the two oscillators. Higher coupling (2.3 nS) caused the oscillations to become synchronous. Figure 2*E* shows that when one of the oscillators was altered pharmacologically, the coupled cells phase-locked at a lower coupling strength (2 nS), but a slight delay remained between them (Fig. 2*E*, *middle*).

Figure 2*E* (*right*) shows the results of adding an artificial chemical synapse to the artificially coupled oscillatory neurons. The chemical synapse was a unidirectional inhibitory synapse from cell 1 onto cell 2 and was created by triggering a brief iontophoretic pulse of GABA during the rising phase of cell 1. The chemical synapse caused a decrease in the oscillation frequency that was proportional to the duration of the inhibitory stimulus whereas the electrical synapse maintained a close synchrony between the two oscillators. Under these conditions cell 2 acts as the network pacemaker, because the network period depends on cell 2's release from chemical inhibition and its ability to initiate its own oscillation.

An early theoretical study (Mulloney et al. 1981) showed that when two oscillators are coupled by both electrical and inhibitory chemical synapses, that changes in the relative strengths of the electrical and chemical connections modify the phase relationship between the two oscillators. We have used the artificial electrical synapse to investigate the behavior of such a network within an intact ganglion. Figure 3 shows the results of adding further electrical coupling between the ventricular dilator (VD) and one of the pyloric dilator (PD) neurons of the intact stomatogastric ganglion of the crab.

Under normal conditions, the anterior burster (AB) and two PD neurons comprise the pacemaker unit of the pyloric rhythm. The VD neuron is weakly electrically coupled to the pacemaker but normally fires out of phase with the pacemaker because it is also inhibited by the AB neuron (Eisen and Marder 1982; Marder and Eisen 1984). The VD synchronizes with the pacemaker when the inhibitory synapse is blocked by picrotoxin (Bidaut 1980; Eisen and Marder 1982; Marder and Eisen 1984) because in the absence of inhibition the electrical synapse now dominates. The artificial electrical synapse allows us to effectively modify the strength of the VD's electrical coupling to the pacemaker.

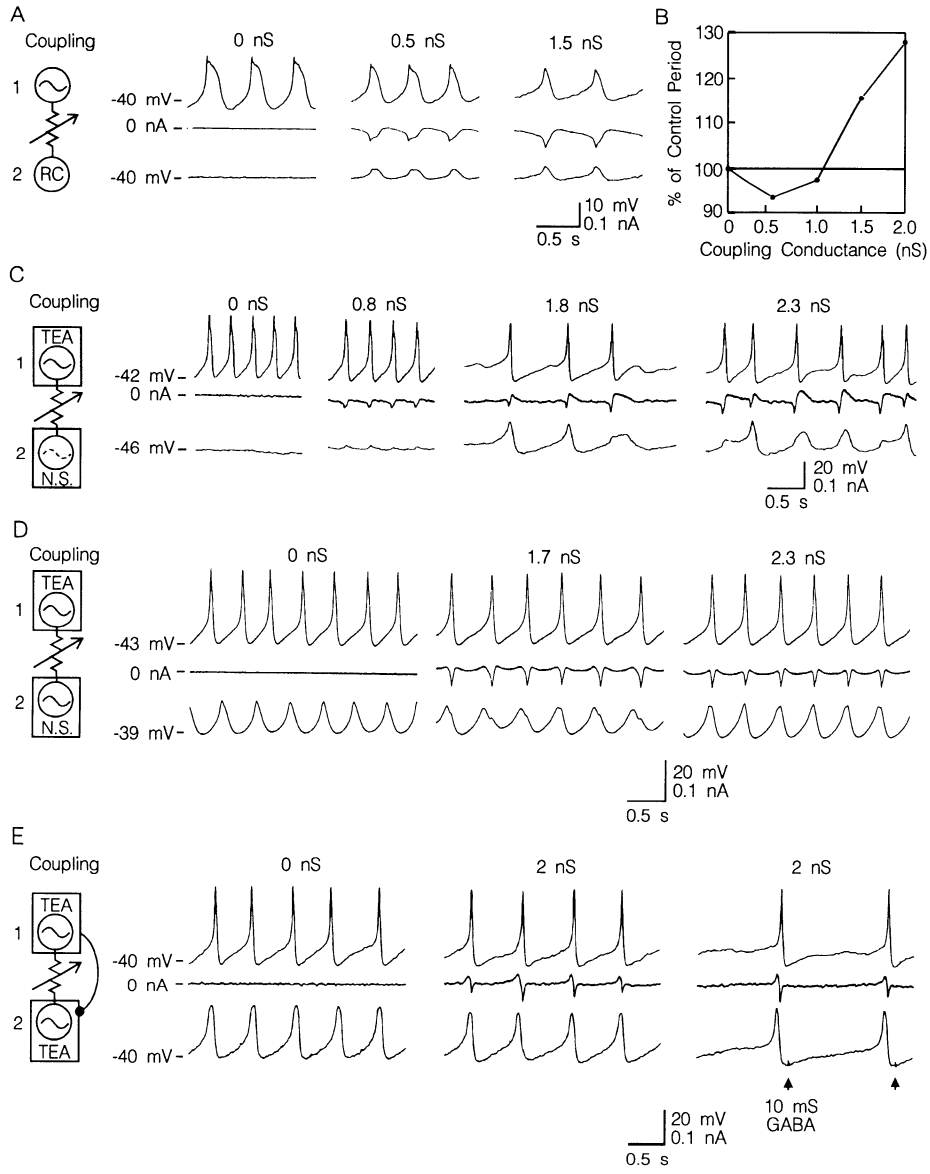

FIG. 2. Effect of coupling conductance on isolated neuronal oscillators. *A*: neuron 1 is a nonidentified STG neuron in primary cell culture. It has been depolarized with constant current such that the ensuing oscillation is dominated by the hyperpolarizing phase. Neuron 2 is a resistance and capacitance (RC) circuit. The *top* and *bottom rows* of recordings are the membrane potentials of neurons 1 and 2, respectively. The *center row* is the current injected into neuron 1 as a result of the coupling conductance (shown above each column). *B*: effect of coupling conductance on oscillation period from cells in *A*. *C*: neurons 1 and 2 are nonidentified stomatogastric ganglia neurons in separate recording chambers. Neuron 1 has been depolarized in 10 mM tetraethylammonium (TEA) so that it maintains a stable oscillation. Neuron 2 is in normal saline and was hyperpolarized relative to its threshold for oscillation. *D*: neurons 1 and 2 are the same neurons as in *C* and were perfused with the same solutions. Both neurons were depolarized so they maintained stable oscillations. *E*: neurons 1 and 2 are the same as in *C*, but they are both in 10 mM TEA. The amount of constant current injected into the two cells is the same as in *D*. In the *right* column an artificial chemical synapse was added from neuron 1 onto neuron 2. The artificial chemical synapse was formed by a 10-ms iontophoretic pulse of γ-aminobutyric acid with a 100-ms delay from the trigger point. The iontophoretic pulse caused a 4-mV hyperpolarization that lasted for ~0.5 s on neuron 2 at rest (−40 mV).

Figure 3 shows that when an electrical synapse was added between the somata of the VD neuron and a PD neuron, as the electrical coupling strength was increased, a continuous variation of the phase of VD was seen until the VD and PD neurons synchronized. This demonstrates that the phase of the VD neuron depends on the strength of both electrical and chemical synapses and suggests that neuromodulation of the strength of either synapse would modify the phase of firing of the VD neuron.

DISCUSSION

The results presented here demonstrate the utility of an artificial electrical synapse in revealing the role of electrical coupling in shaping network function. We have shown that it is possible to determine how the wave form and frequency of an oscillator changes with coupling strength and how the emergent properties of the network are determined by the interaction of coupling strength and the intrinsic properties of the neurons. The electronic nature of this synapse allows us to couple neurons in separate dishes, permitting the separate pharmacological modification of individual cultured neurons. We have demonstrated that electrical synapses can be created or amplified not only in culture but in intact ganglia. The addition of artificial synapses to small networks grown in culture should add to the insights already gained with this approach (Kleinfeld et al. 1990a,b; Syed et al. 1990).

One of the dilemmas of neural modeling is how to retain the essential features of the component neurons while permitting systematic study of the effects of parameters on network function. Our electronic methods of coupling real biological neurons provide a technique for varying parameters at will as in simulations of similar small circuits (Abbott et al. 1991; Mulloney et al. 1981; Wang and Rinzel 1992; Sherman and Rinzel, 1992). However, we are not forced to make ad hoc assumptions or simplifications in the represen-

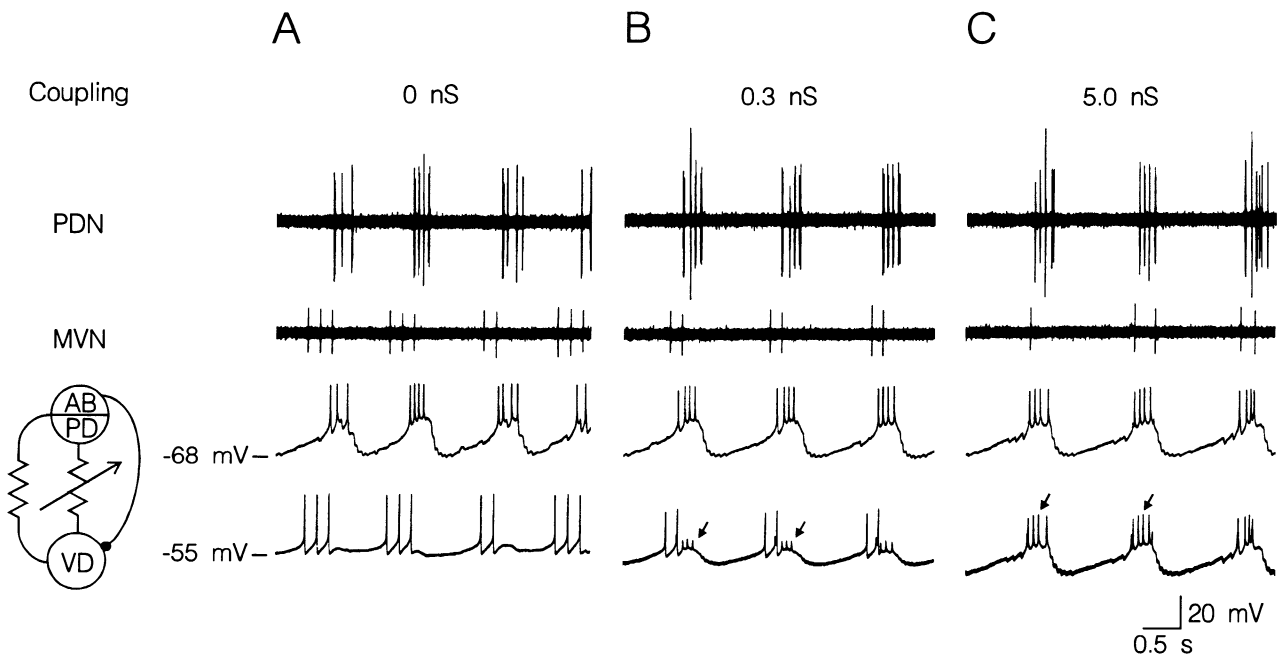

FIG. 3. Effect of artificial electrical coupling between the somata of the ventricular dilator (VD) and a pyloric dilator (PD) in the intact stomatogastric ganglia. The *top* traces are extracellular pin recordings from the pyloric dilator nerve (PDN) that show action potentials from both PD neurons. The second set are extracellular recordings of the medial ventriculator nerve (MVN) that show VD neuron action potentials. The third and fourth set of traces are intracellular recordings from the PD and VD, respectively. *A*: control. *B* and *C*: increased electrical coupling. Notice that the rapid coupling potentials (arrows) recorded in the VD can be differentiated from real action potentials by comparisons to the MVN recordings.

tation of our model because we are coupling real neurons. These methods should provide an important new tool for understanding neural networks.

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NOTE ADDED IN PROOF

Subsequent to our submission of this paper we learned that a similar method has been employed to couple cardiac cells. (R. W. Joyner, H. Sugiwara, and R. C. Tau. Unidirectional block between isolated rabbit ventricular cells coupled by a variable resistance. *Biophys J.* 60: 1038–1045, 1991)

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