the tyrosine kinase region of *erb*B-2, was ligated to the 12.3 Sal I–Bcl I fragment of the LTR-EGFR containing the EGFR cDNA depleted of its tyrosine kinase region, to yield the LTR-EGFR/*erb*B-2^{TK} expression vector. The reciprocal recombination yielded the LTR–*erb*B-2/EGFR^{TK} expression vector; in the latter case, because of the presence of a second Bcl I site at position 3078 in the EGFR cDNA (18), an oligonucleotide was used to restore the 19 codons of EGFR encompassed between the two Bcl I sites.
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28. Antisera were prepared against synthetic peptides derived from the predicted amino acid sequences of EGFR and *erb*B-2 (*18*). Antipeptide antibodies M1, M6, and M7 were obtained by immunizing rabbits with synthetic peptides derived from the *erb*B-2 sequence (*18*), encompassing residues 866–880, 1218–1232, and 1240–1255, respectively (*18*). The antibodies E5 and E7 were obtained against synthetic peptides derived from the EGFR predicted sequence (*18*), encompassing residues 985–990 and 1172–1186, respectively (*18*). The αF antipeptide antibody, directed against the protein kinase C modulation domain of the EGFR, was a kind gift of J. Schlessinger.
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The Effect of Electrical Coupling on the Frequency of Model Neuronal Oscillators

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Neurons with oscillatory properties are a common feature of the nervous system, but little is known about how neural oscillators shape the behavior of neuronal networks or how network interactions influence the properties of neural oscillators. Mathematical models are used to examine the effect of electrically coupling an oscillatory neuron to a second neuron that is either silent or tonically firing. Models of oscillatory neurons with varying degrees of complexity show that this coupling can either increase or decrease the frequency of an oscillator, depending on its membrane potential wave form, the state of the neuron to which it is coupled, and the strength of the coupling. Thus, electrical coupling provides a flexible mechanism for modifying the behavior of an oscillatory neural network.

NEURONS THAT DISPLAY INTRINSIC oscillatory properties are important components of many biological neural networks (*1, 2*). In particular, networks that generate rhythmic motor patterns often use neurons with oscillatory membrane properties (*2*). Despite considerable advances in our understanding of rhythmic neural networks, we do not know precisely how the frequency of such networks is controlled. Recent studies of the pyloric network of the lobster stomatogastric ganglion (STG) showed that the frequency of the oscillator anterior burster (AB) neuron is influenced by electrical coupling to other neurons (*3*). Specifically, in the presence of the peptide proctolin the frequency of the pacemaker-driven network was about 1 Hz, whereas the frequency of the isolated AB neuron was about 2 Hz; in this study the investigators concluded that the electrically coupled neurons were providing a "load" on the pacemaker, slowing it down (*3*).

We are currently developing network models that contain oscillatory elements (*4*). While studying the effect of electrical coupling on an oscillating neuron within these models, we found that the situation is not nearly as simple as the "loading" picture would imply. Instead, electrical coupling provides a flexible way of modulating the frequency of an oscillator that depends critically on properties of the oscillator and of the coupled cell and on the coupling strength.

We begin by considering the effect of electrical coupling in a simple model of a bursting neuron based on a modified form of the FitzHugh-Nagumo equations (*5*). In this approach we do not model individual action potential spikes but consider a cell membrane potential v with action potentials either removed or averaged over. In the particular case of the STG, this is a good approximation because its neurons release neurotransmitter as a graded function of membrane potential, and action potentials contribute little (*6*). In the general case, it is reasonable because the integrated contribution of a given action potential spike to the current through the resistive coupling is quite small.

To construct the model, we divide the total current entering or leaving the cell into four parts, the capacitive current $C\,dv/dt$, an external current I, a fast component of the membrane current $f(v)$, and a slow membrane current s. By current conservation,

$$C\frac{dv}{dt} = -f(v) - s + I \qquad (1)$$

The slow component of the membrane current is determined by another differential equation

$$\tau\frac{ds}{dt} = \alpha v - s \qquad (2)$$

The parameter τ determines the time scale for variations of s. In contrast to the usual approach (*5*), our fast current $f(v)$ is purely resistive but at intermediate membrane potentials it has a negative-resistance region that connects two positive-resistance regions at low and high potentials.

Depending on the exact form of $f(v)$ and on the values of the parameters C, α, and τ, neurons modeled by these equations can be oscillatory, can display plateau properties, can be tonically active, or can be silent. For the oscillatory case, the neuron can be predominantly hyperpolarized (top left of Fig. 1) or predominantly depolarized (top right of Fig. 1) during its cycle, depending on the value of an additive constant in the expression for $f(v)$.

To explore the effects of electrical coupling, we take for the "external" current

$$I = g(v_p - v) \qquad (3)$$

where g determines the strength of the electrical coupling and v_p is the membrane potential of a passive cell to which the oscillator is coupled. The membrane current for the passive cell is modeled as purely resistive and fast, so including the electrical coupling to the oscillator we have

$$C_p\frac{dv_p}{dt} = -G_p(v_p - \bar{v}_p) + g(v - v_p) \qquad (4)$$

Here $\bar{v}_p$ is the resting potential and G_p is the conductance of the passive cell in the absence of electrical coupling.

Figure 1 shows the result of increasing the coupling conductance between a hyperpolarized passive cell and two different oscillators. It is clear that the effect of electrical

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coupling is quite different for the two wave forms shown. In the left panel, the oscillator period increases as the coupling strength increases, as one might expect. In contrast, the oscillator shown in the right panel speeds up when coupled to a silent neuron until it reaches a maximal frequency after which a further increase in the coupling strength causes it to slow down. If we had taken the passive cell to be tonically firing ($\bar{v}_p$ depolarized relative to v), these results would be reversed, with the oscillator at the left in Fig. 1 initially speeding up and then slowing down as g increases while the one at the right simply slows down. Thus, within this model the change in the oscillation frequency depends on the state of the passive cell, on the strength of the electrical coupling, and, in addition, on details of the oscillator wave form.

To calculate the effect of electrical coupling on the period of an oscillator, we divide the oscillatory cycle into segments, each characterized by a monotonically changing membrane potential. We determine how each segment is modified by coupling to a passive cell and then sum these results to get the total effect. Consider a segment during which the membrane potential changes from an initial value v_i to a final value v_f. We assume that during this portion of the cycle the oscillatory neuron can be modeled by Eqs. 1 and 2 and that v_i and v_f do not change with g, at least for small g. For the fast component of the current in this section of the cycle we use a simple resistive form

$$f(v) = Gv - (G + \alpha)v_R \quad (5)$$

The peculiar notation for the reversal potential is used because the parameter v_R corresponds to the reversal potential of the steady-state current in the model. The parameter α can actually be removed from the model because $G + \alpha$ is just the steady-state conductance with G the instantaneous conductance. Both of these results can be obtained if the time derivatives in Eqs. 1 and 2 are set equal to zero and s is eliminated. In the absence of electrical coupling, the portion of the cycle we are discussing takes a time t to be completed. When a small electrical coupling of strength g is included, this time changes to $t + \Delta t$. Using an approximate analytic solution of the model, we find

$$\Delta t = t\left[\frac{g}{(G+\alpha)}\right] \times \left[\frac{\alpha}{G} + \frac{(v_i - v_f)(\bar{v}_p - v_R)}{(v_i - v_R)(v_f - v_R)\ln\left(\frac{v_i - v_R}{v_f - v_R}\right)}\right] \quad (6)$$

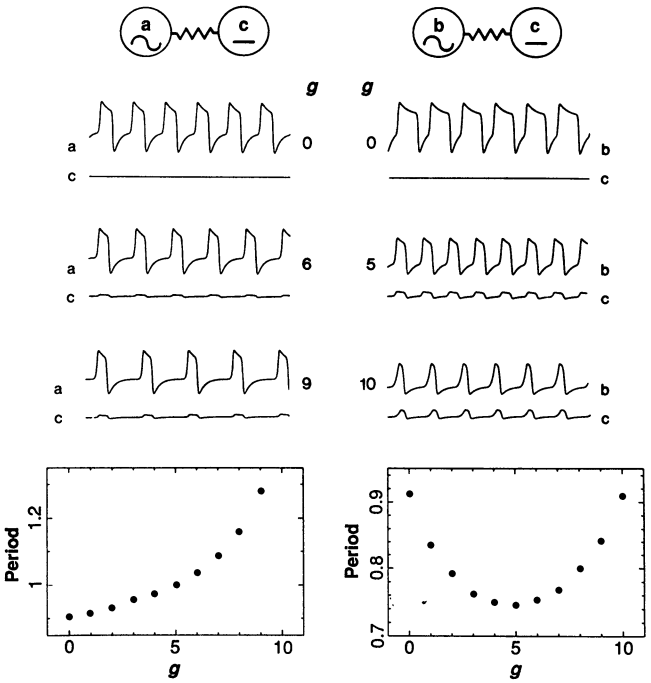

Fig. 1. Effect of coupling conductance on the frequency of model oscillators. The cycle of neuron a is dominated by a hyperpolarized phase, whereas neuron b has a longer depolarized phase. Neuron c is passive and hyperpolarized; its resting potential equals the minimum potential during oscillation in the uncoupled cells a and b. As the coupling conductance g is increased, the network containing neuron a slows down while that containing neuron b first speeds up and then slows down as shown in plots of period against coupling conductance. All units are arbitrary.

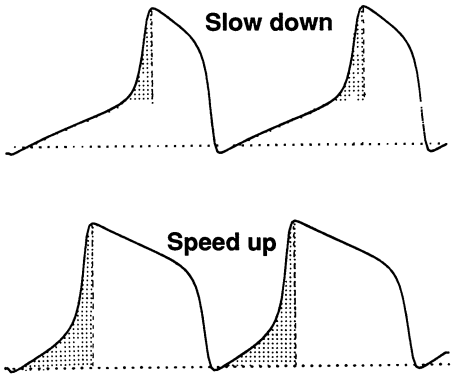

Fig. 2. Inward and outward current portions of the oscillator bursts. Portions of the cycle with inward currents are stippled. (**Top**) A model neuron in which the cycle is dominated by an inward current phase. Coupling this cell to a hyperpolarized passive cell adds an outward current, which slows down the depolarization of the cell, increasing its period. (**Bottom**) A model neuron in which the cycle is dominated by an outward current phase. Coupling this cell to a hyperpolarized passive cell adds an outward current, terminating the plateau and thus decreasing the period. For larger coupling, the inward and outward portions of the cycle become equal, at which point this neuron behaves like the one shown on top.

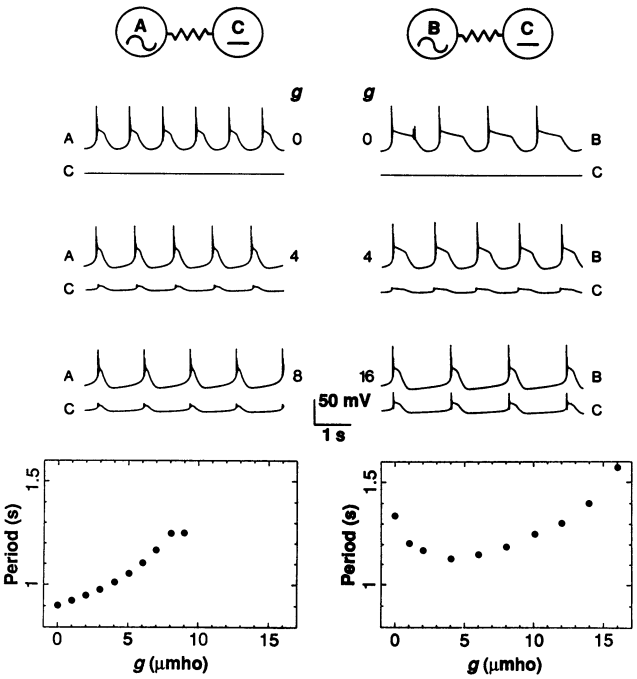

Fig. 3. Effect of coupling conductance on more realistic model neurons. Neurons A and B are constructed from differential equations describing five membrane currents (7) and differ only in the value of the Ca^{2+} conductance and the Ca^{2+}-activated K^+ conductance, both of which are larger in neuron B than in neuron A. Neuron C has simple Hodgkin-Huxley (8) characteristics. Its resting potential is equal to the minimum potential during oscillation in the uncoupled cells A and B. The action potentials of neuron C in the lower right trace have been clipped for ease of presentation.

This expression for Δt is fairly complicated, but its basic features can be understood intuitively. The first term in the bracket (which is always positive) tends to slow the oscillator down regardless of its wave form or the state of the passive cell. On the other hand, the second term in the bracket depends on the shape of the wave form through ν_i and ν_f and on the state of the passive cell through $\bar{\nu}_p$. If the cell oscillates, the denominator of this term is always positive, so its sign depends on whether ν is rising ($\nu_f - \nu_i > 0$) or falling ($\nu_f - \nu_i < 0$) and on whether $\bar{\nu}_p$ is depolarized or hyperpolarized relative to ν_R. To explain this dependence, let us take the passive cell to be hyperpolarized. Then, this term tends to slow down a portion of the oscillator cycle characterized by a net inward current ($\nu_f > \nu_i$) and to speed up one with net outward current ($\nu_f < \nu_i$). This makes sense because for $\nu_p < \nu_R$ the resistive current is itself outward. Of course, for a depolarized passive cell ($\nu_p > \nu_R$) the situation is just reversed.

One can estimate the complete effect of a small electrical coupling on the oscillator wave form by summing the result of Eq. 6 over all the different segments making up the complete cycle. The time change for any section is proportional to its duration. Thus, the overall effect will depend on whether the complete cycle is dominated by portions that are lengthened or shortened when the coupling is changed. This is illustrated in Fig. 2, where we have indicated regions of inward and outward current flow for the oscillators of Fig. 1. Roughly, an oscillator with a wave form dominated by periods of inward current will slow down when electrically coupled to a hyperpolarized passive cell and speed up when the passive cell is depolarized. An oscillator dominated by periods of outward current flow will experience reversed effects.

The previous explanation, while intuitively reasonable, was developed with the use of a very simplified oscillator model. To assure ourselves that this treatment is applicable to a neural oscillator, we consider a more realistic model based on differential equations that describe the voltage and time dependences of five membrane conductances (*7*). By varying the conductances of this model we can construct oscillatory neurons with different wave forms (see Fig. 3) (*7*). We then electrically couple the model oscillator to a neuron with simple Hodgkin-Huxley characteristics (*8*).

Figure 3 shows that this mechanistically realistic model produces behavior similar to that of the simplified model previously described. The period of the oscillator on the left increases as the strength of the electrical coupling increases, whereas the period of the oscillator on the right first decreases to a minimal value and then increases. Again we have taken the passive cell to be silent.

The results described here have important implications for networks in which neurons with oscillatory properties are electrically coupled to other neurons. We have shown that the period of an oscillating neuron may be either increased or decreased by its electrical coupling to another neuron. Thus, neuromodulatory substances that change the electrical coupling strength (*9*) may have complex effects on the emergent frequency of an oscillatory network. In addition, any neurotransmitter or other modulator that changes the shape of the burst of an oscillating cell by modulating the underlying voltage and time-dependent conductances (*10*) may change the effect that other neurons electrically coupled to the oscillator have on its period. In the particular case of the STG, many substances modulate the AB neuron (*11*). If, as has been suggested (*12*), the ionic mechanisms underlying the AB burst are widely variable, then the effect of other electrically coupled neurons on the frequency of the AB neuron may be substantially different in the presence of various modulators. Earlier work demonstrated the effect of simultaneous electrical and chemical coupling to an oscillatory neuron (*13*). The flexibility demonstrated here adds to our understanding of how the output of a neural network can be modulated in a behaviorally useful manner.

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