PHYSIOLOGY OF ELECTROTONIC JUNCTIONS*

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INTRODUCTION

A number of different kinds of electrical interactions between cells are known. This paper is concerned with the numerous instances where cells behave essentially as if they are part of the same core conductor, that is, they are "electrotonically coupled". In many of these cases a distinct morphological relationship between the coupled cells can be demonstrated. As discussed in the preceding paper (Pappas & Bennett, 1966), there are regions where the membranes of the coupled cells come together, occluding the extracellular space. The correlation between electrotonic coupling and regions of membrane fusion allows the inference that these regions are the sites of coupling between cells. Thus, they may be considered electrotonic junctions. In this paper, two idealized cells connected by a resistance and capacity will be taken as a model of an electrotonic junction. The properties of this model will first be explored, and then its relationship to real examples considered.

ANALYTICAL TREATMENT OF THE MODEL

Definition and Steady-state Behavior

The model for electrotonic coupling is shown in FIGURE 1*A*. Two cells have a portion of their surface membranes in contact. This region connects the cells and allows no current to leak to the external medium. The resistivity of the cytoplasm and external medium are negligible. In each cell, voltage can be measured and current applied as symbolized in the diagram by the four electrodes. An equivalent circuit of this model is shown in FIGURE 1*B*. The two cells and the junction are each represented by a lumped resistance and capacity. V_1 will be considered prejunctional and V_2 postjunctional.

If a constant current is applied on one side of the junction, steady state voltages will be reached in the two cells. The ratio of voltage to current on the polarized side may be defined as the input resistance, r_{11} or r_{22}, and is simply related to the resistances in the circuit (cf. Watanabe & Grundfest, 1961). On the prejunctional side:

$$r_{11} = \frac{V_1}{i_1} = \frac{r_1 (r_c + r_2)}{r_1 + r_c + r_2} \tag{1}$$

On the postjunctional side:

$$r_{22} = \frac{V_2}{i_2} = \frac{r_2 (r_1 + r_c)}{r_1 + r_c + r_2} \tag{2}$$

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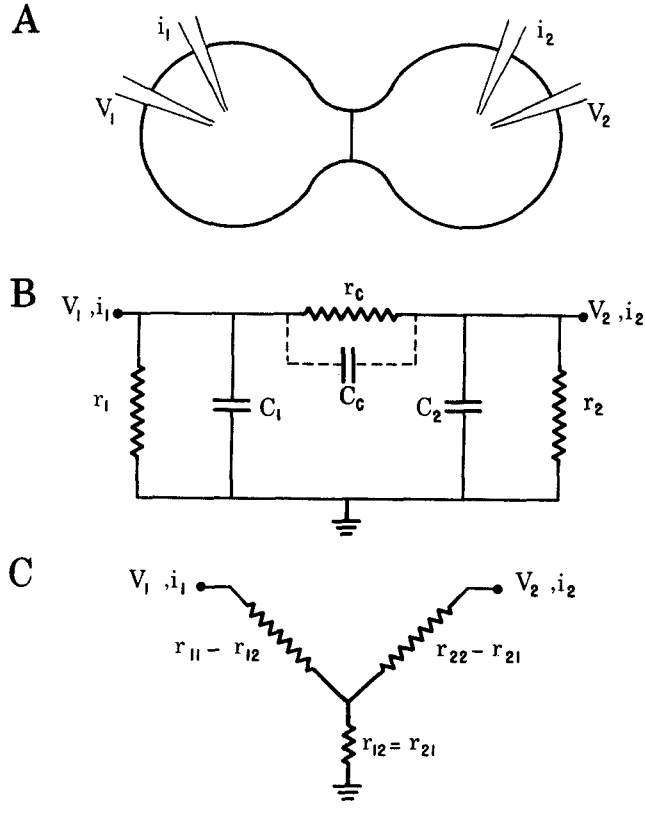

FIGURE 1. The model for an electrotonic junction. *A*. Diagram of the cells. *B*. An equivalent circuit for the cells. The junctional capacity is connected in by dotted lines to signify that it is usually negligible. *C*. A wye equivalent of the resistors in *B* which are in a delta configuration.

The ratio of voltage in the opposite cell to the applied current, defined as the transfer resistance, r_{12} or r_{21}, is also a simple function of the circuit resistances.

For polarization on the prejunctional side:

$$r_{12} = \frac{V_2}{i_1} = \frac{r_1 r_2}{r_1 + r_c + r_2} \tag{3}$$

For polarization on the postjunctional side:

$$r_{21} = \frac{V_1}{i_2} = \frac{r_1 r_2}{r_1 + r_c + r_2} \tag{4}$$

Thus, the transfer resistances in the two directions are equal.

It is well known that the resistors of FIGURE 1*B* have an equivalent of the form in FIGURE 1*C*; that is, for each delta network there is a corresponding wye network and vice versa (cf. Guillemin, 1953). The transformation makes obvious the equality of the transfer resistances in the two directions, since the common resistance in the wye form is the transfer resistance. The relationship between the

input resistances and the other two resistances in the wye equivalent can also be seen by inspection of the circuit. The circuit constants in a delta network are ordinarily calculated from the measured input and transfer resistances. These relations are essentially the inverse of the delta-wye transformation and are as follows:

$$r_1 = \frac{r_{11}\, r_{22} - r_{12}^2}{r_{22} - r_{12}} \tag{5}$$

$$r_2 = \frac{r_{11}\, r_{22} - r_{12}^2}{r_{11} - r_{12}} \tag{6}$$

$$r_c = \frac{r_{11}\, r_{22} - r_{12}^2}{r_{12}} \tag{7}$$

If current can be applied on both sides simultaneously and adjusted so that the voltage across any one of the resistances is zero, the relations become much simpler.

If $V_1 - V_2 = 0$, then:

$$r_1 = \frac{V_1}{i_1} \tag{8}$$

$$r_2 = \frac{V_2}{i_2} \tag{9}$$

This procedure was used on the crayfish septate axon by Watanabe and Grundfest (1961).

If $V_1 = 0$ (for which i_1 and i_2 must be of opposite sign):

$$r_c = \frac{V_2}{i_1} \tag{10}$$

$$r_2 = \frac{V_2}{i_2 + i_1} \tag{11}$$

Corresponding equations hold if $V_2 = 0$.

It is useful to consider the relation between a potential produced on one side by a given potential on the other. The ratio of the former to the latter may be defined as the coupling coefficient in the particular direction, k_{12} or k_{21}. In the "forward" direction, that is, when the potential is generated by a current on the prejunctional side:

$$k_{12} = \frac{V_2}{V_1} = \frac{r_{12}}{r_{11}} = \frac{r_2}{r_2 + r_c} = \frac{1}{1 + r_c/r_2} \tag{12}$$

In the "reverse" direction:

$$k_{21} = \frac{V_1}{V_2} = \frac{r_{12}}{r_{22}} = \frac{r_1}{r_1 + r_c} = \frac{1}{1 + r_c/r_1} \tag{13}$$

It is clear that $k_{12} \to 1$ as $\frac{r_c}{r_2} \to 0$ and $k_{21} \to 0$ as $\frac{r_1}{r_c} \to 0$

Thus, forward coupling that is arbitrarily close to unity can be achieved with arbitrarily small coupling in the reverse direction. However, one may define the transmissional efficiency as the ratio of energy dissipated on the posttjunctional side to the total energy dissipated. Efficiency so defined becomes very small as the reverse coupling approaches zero.

It may be useful to consider the relation between coupling, membrane area, and membrane resistivity. If ρ represents areal resistivity and A area, then from Equation **12**

$$k_{12} = \frac{1}{1 + \frac{\rho_c A_2}{\rho_2 A_c}} \tag{14}$$

Solutions for Transient Inputs

Several important properties are derivable from consideration of the model under nonsteady state conditions. It may be assumed for simplicity that V_1 can be clamped to an arbitrary V_1 (t) and effectively $r_1 = 0$. This assumption is not unreasonable for action potentials which in general involve large increases in conductance. The differential equation for the equivalent circuit becomes:

$$\frac{V_2}{r_2} + C_2 \dot{V}_2 = \frac{1}{r_c}(V_2 - V_1) + C_c (\dot{V}_1 - \dot{V}_2) \tag{15}$$

or

$$V_2 + \tau \dot{V}_2 = k V_2 + \frac{C_c}{C_c + C_2} \dot{V}_1 \tag{16}$$

where $\tau = \frac{r_c r_2}{r_c + r_2} (C_c + C_2)$ and $k = k_{12} = \frac{r_2}{r_c + r_2}$

and the dot denotes differentiation with respect to t. The quantity τ may be defined as the coupling time constant, and a normalized value of time, $T = \frac{t}{\tau}$, can be used. Then:

$$V_2 + \dot{V}_2 = k (V_1 + \beta \dot{V}_1) \tag{17}$$

where

$$\beta = \frac{r_c C_c + r_2 C_c}{r_2 C_2 + r_2 C_c} \tag{18}$$

An integrating factor for the left side is e^T and, if V_2 (0) = 0, a solution is:

$$V_2 = k e^{-T} \int_0^T e^{T'} (V_1 + \beta \dot{V}_1)\, dT' \tag{19}$$

A useful further step may be taken if V_1 (0) = 0:

$$\begin{aligned} V_2 &= k e^{-T} \int_0^T e^{T'} (V_1 + \dot{V}_1 + (\beta - 1) \dot{V}_1)\, dT' \\ &= k V_1 + k (\beta - 1) e^{-T} \int_0^T e^{T'} \dot{V}_1\, dT' \end{aligned} \tag{20}$$

Solutions Neglecting Junctional Capacity

As will be discussed below, the time constant of junctional membranes is usually short compared to the postjunctional time constant. In the extreme case of $C_c = 0$ the equivalent circuit is identical to that used to represent voltages recorded by an electrode closely applied to a patch of inactive membrane on an excitable cell (Freygang & Frank, 1959; Werman, 1963). The inactive membrane is equivalent to the postjunctional cell, and the resistance of the external medium is equivalent to the junction, but the voltage of primary interest is that across the

resistor-capacitor pair rather than the single resistor. Since when $C_c = 0$, then $\beta = 0$, the differential equation for the simplified network is:

$$V_2 + \dot{V}_2 = k\dot{V}_1 \tag{21}$$

and a form of solution is:

$$V_2 = kV_1 - ke^{-T}\int_0^T e^T \dot{V}_1 \, dT \tag{22}$$

From the equivalent circuit, it is clear that V_2 can exceed V_1. If V_1 is positive over some interval and then rapidly goes to zero, V_2 remains positive for some time afterwards, because the capacitor C_2 discharges exponentially through r_2 and r_c, that is, with the coupling time constant for $C_c = 0$. The criterion for V_2 to exceed V_1 is that at some time when $V_2 = V_1$, V_1 should decrease more rapidly than exponentially with the time constant of the postjunctional cell, $r_2 C_2$. This relationship is also obvious from the equivalent circuit, since if V_1 is clamped at the value of V_2, V_2 falls exponentially with the time constant $r_2 C_2$. A similar criterion holds for there to be a diphasic potential external to inactive membrane, for when $V_2 > V_1$, the potential across the junction is reversed in sign.

If V_1 increases monotonically to a maximum and then monotonically decreases again to zero, a maximum will occur in V_2 which will be delayed compared to that in V_1. Assuming continuity of V_2 (which follows from the differential Equation **21** if V_1 is continuous), $\dot{V}_2 = 0$ at the maximum of V_2 and

$$V_{2\,max} = kV_1 \tag{23}$$

But this would be the voltage if V_1 remained at its maximum indefinitely. Thus V_1 must have decreased from its maximum at the time of the maximum of V_2. Also, in Equation **22** the integral term must be positive up until the maximum of V_1 and for some period thereafter, if $\dot{V}_1$ is finite. Thus, V_2 cannot equal kV_1 until sometime after the maximum of V_1. (Note, however, that if the maximum of V_1 is immediately followed by a negative step, $\dot{V}_1 = -\infty$ and a maximum will occur in V_2 at this time).

Differentiation of Equation **21** does not change its form. Therefore it can also be shown that the maximum rates of rise and fall (inflection points) in V_2 are delayed compared to those in V_1 provided the corresponding continuity conditions hold.

Assuming thresholds for detection of V_1 and V_2 are equal, the onset of V_2 will appear delayed with respect to V_1, since V_2 is necessarily less than V_1 until sometime after the maximum of V_2. It is also obvious from Equation **21** that if $V_1(0) = V_2(0) = 0$, then $\dot{V}_2(0) = 0$. Thus, linear extrapolation of V_2 to zero from any point on the rising phase up to where the rate of rise has a maximum will indicate a delay from $T = 0$. Similarly if $\dot{V}_1$ is continuous, extrapolation of V_1 to zero from the earliest point where the slope equals or exceeds that chosen for extrapolation of V_2 will indicate that V_1 began before V_2, since $\dot{V}_1 > \dot{V}_2$ until after the maximum of $\dot{V}_1$. A minor difference is that $\dot{V}_1(0)$ may exceed the chosen value at $T = 0$ and then decrease. Extrapolation would then give a time of onset for V_1 that was less than zero.

The change in shape of V_2 compared to V_1 that the model produces when $C_c = 0$ may be illustrated for several functions which have a shape approximating that of action potentials. The function $V_1 = \alpha T e^{-\alpha T+1}$ rises to a maximum of unit value at $\alpha T = 1$ and then slowly decreases towards zero (the uppermost curve, labeled $\alpha = 0$, in FIGURES 2 and 3). The maximum positive slope is at $T = 0$, and there is an inflection on the falling phase at $\alpha T = 2$. The final decay approaches exponential with a time constant $1/\alpha$. This form of function was first

used to mimic an action potential by Werman (1963). A function with a shape rather more like that of a nerve impulse is $V_1 = \alpha^2 T^2 e^{-2\alpha T+2}$ which also has a maximum of unit value at $\alpha T = 1$ (the uppermost curve, labeled $\alpha = 0$, in FIGURES 4 and 5). The initial slope, however, is zero and there are inflections on the rising and falling phases when $\alpha T = 1 \pm \frac{1}{2}\sqrt{2}$. The final falling phase approaches exponential with a time constant equal to $1/2\alpha$.

For both functions, α may be considered the ratio of the coupling time constant to the rise time or time to peak of the impulse function. The solutions of the differential equations for these two impulse functions are as follows:

$$\text{For } V_1 = \alpha T e^{-\alpha T+1} \tag{24}$$

$$V_2 = \frac{k\alpha}{(1-\alpha)^2}\left\{ e^{-\alpha T+1}[T(1-\alpha)-1]+e^{-T+1} \right\} \tag{25}$$

which if $\alpha = 1$, reduces to:

$$V_2 = \frac{k}{2} T^2 e^{-T+1} \tag{26}$$

$$\text{For } V_1 = \alpha^2 T^2 e^{-2\alpha T+2} \tag{27}$$

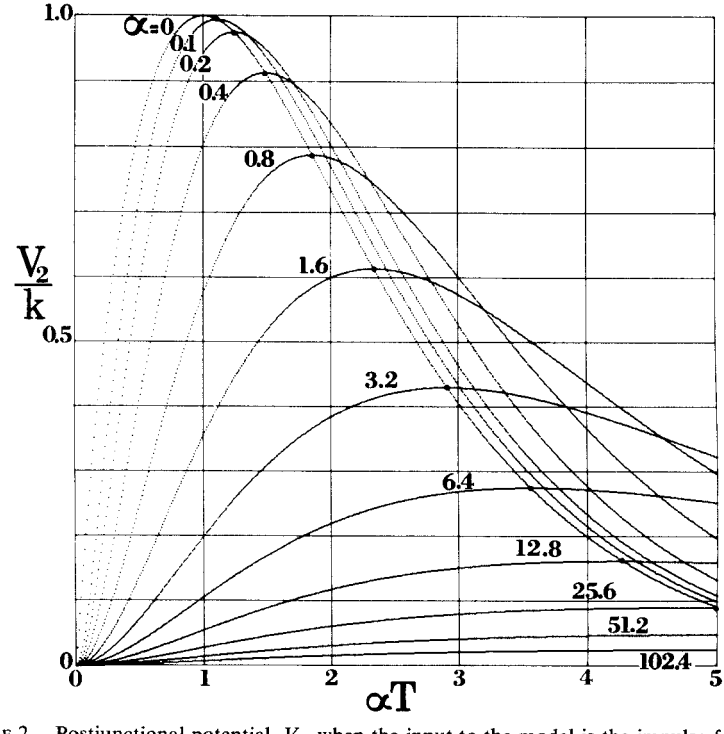

FIGURE 2. Postjunctional potential, V_2, when the input to the model is the impulse function $V_1 = \alpha T e^{-\alpha T+1}$. Solutions are shown for $0 \leq \alpha \leq 102.4$, each successive value after 0.1 being double the previous one. The abscissa is in units of T and extends from 0 to 5. The ordinate is in units of V_2/k. The value of α for each curve is indicated. The curve for $\alpha = 0$ is identical to V_1. The intersections of this curve with those for larger α are indicated by larger dots. For the figure V_2 was evaluated at intervals of $\Delta \alpha T = 0.01$ by digital computer, and the data were recorded by a General Dynamics 4020 curve plotter.

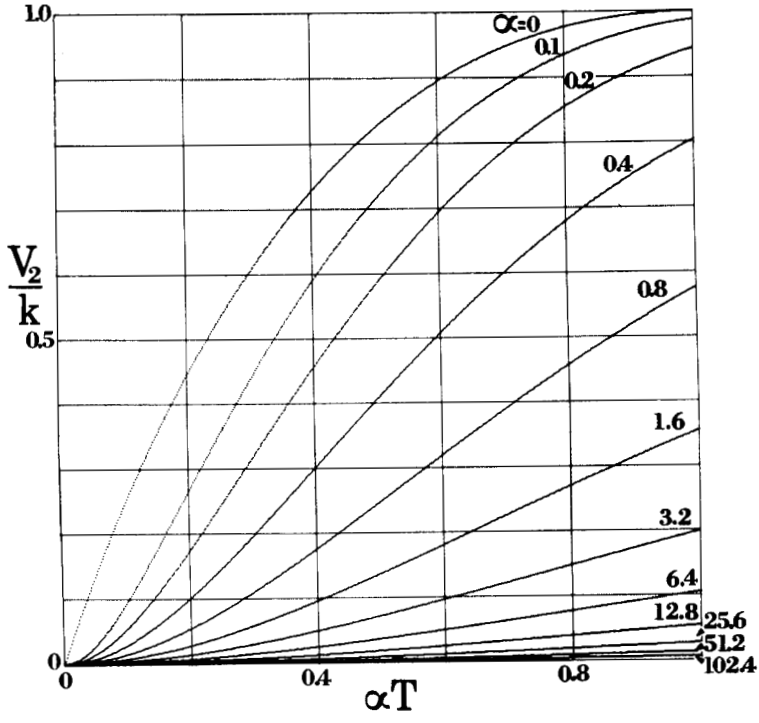

FIGURE 3. Postjunctional potential, V_2, as in FIGURE 2 but shown for αT from 0 to 1 to display the initial part of the curves more clearly ($\Delta \alpha T = 0.002$).

$$V_2 = \frac{k\alpha^2}{1-2\alpha}\left\{ e^{-2\alpha T+2}\left[T^2 - \frac{2T}{1-2\alpha} + \frac{2}{(1-2\alpha)^2} \right] - e^{-T+2}\frac{2}{(1-2\alpha)^2} \right\} \quad (28)$$

which if $\alpha = 1/2$, reduces to:

$$V_2 = \frac{k}{12}T^3 e^{-T+2} \quad (29)$$

These functions can be conveniently graphed in terms of αT and V_2/k as a family of curves with the parameter α. They are shown in FIGURES 2–5 for ranges of αT from 0 to 5 and 0 to 1. Essentially this procedure normalizes voltage in terms of steady state coupling and time in terms of the rise time of the impulse. When α becomes very small, that is, the functions become slow compared to the coupling time constant, Equations **25** and **28** approach $V_2 = kV_1$. For any value of α, the maximum of V_2 occurs when $V_2 = kV_1$, as seen from Equation **23**. The value of αT at which the maximum occurs for a particular α can be determined from this equation. Sufficiently long after the maximum, V_2 approaches an exponential decay with whichever time constant of the two exponentials is larger.

From inspection of FIGURES 2 and 4, the maxima of the transmitted potentials are about ¾ of what would be predicted from the steady state coupling when $\alpha = 1$, but they decrease to about ⅕ when $\alpha = 10$. For the simpler impulse function, Equation **25** predicts that, if $\alpha > 1-k$ then $V_2 > V_1$ for large α, that is V_2 outlasts V_1. This criterion is equivalent to the time constant of fall of V_1 being

less than the time constant of the postjunctional membrane. The time that V_2 "crosses over" V_1 can be obtained from Equations **24** to **26**. When $\alpha = 1$, crossover occurs at $T = 2/k$, and for smaller or larger α, the crossover occurs at smaller or larger values of αT respectively. Similarly, from Equation **28**, for the second impulse function, crossover occurs if $2\alpha > 1 - k$. When $\alpha = \frac{1}{2}$ crossover occurs at $T = 3/k$ and at smaller or larger values of αT for smaller or larger α respectively.

The apparent delays at the model junction for these impulse functions may be estimated from FIGURES 3 and 5. For example, for $\alpha = 0.8$ in FIGURE 5, the delay would be about 0.15 times the rise time of the input pulse if the threshold were taken as 0.01 k. Extrapolating from the most rapid rate of rise would give a somewhat larger delay, about 0.3 times the rise time. Note that the delay is a rather smaller fraction of the rise time of the postjunction potential.

Solutions Including Junctional Capacity

Solutions for the model when $C_c > 0$ are simply related to that when $C_c = 0$, as may be seen from Equation **20**, where only β and the units of T are affected by C_c. If $r_c C_c = r_2 C_2$, then $\beta = 1$ from Equation **18**, and Equation **20** reduces to $V_2 = kV_1$. In this case, the model is equivalent to a capacity-compensated voltage divider. If $r_c C_c < r_2 C_2$, then $B < 1$ and for an impulse input function simi-

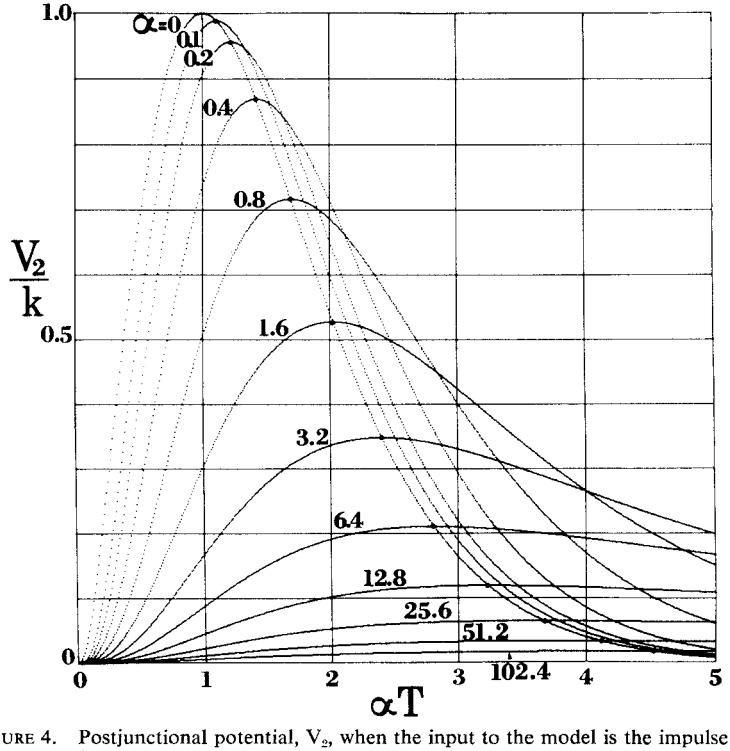

FIGURE 4. Postjunctional potential, V_2, when the input to the model is the impulse function $V_1 = \alpha^2 T^2 e^{-2\alpha T+2}$. Display as in FIGURE 2, the curve for $\alpha = 0$ is identical to V_1.

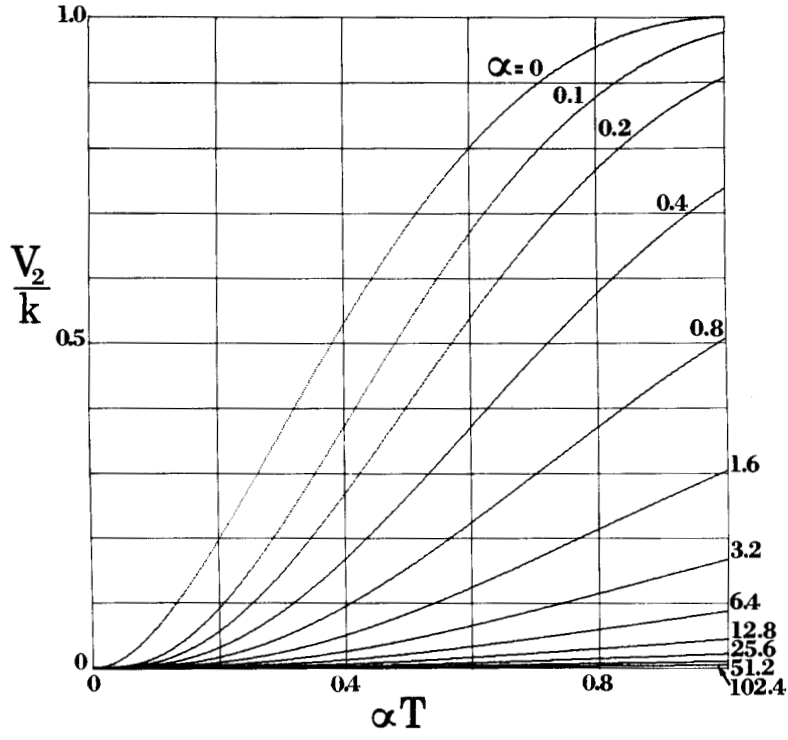

FIGURE 5. Postjunctional potential, V_2, as in FGIURE 4 but for αT from 0 to 1 to display the initial part of the curves ($\Delta \alpha T = 0.002$).

lar to those above, the maximum of V_2 is delayed compared to that of V_1. This may be seen from Equation **20**, in which the integral is decreasing at the time of the maximum of V_1 (assuming continuity of V_1), and, since $\beta - 1 < 0$, V_2 must be increasing. Similarly, it can be shown that the inflection points of V_2 are delayed if $\dot{V}_1$ is continuous. If $r_c C_c > r_2 C_2$, then $\beta > 1$ and $\beta - 1 > 0$. Thus V_2 must be decreasing at the time of the maximum of V_1 and have had its maximum earlier. Also, the inflection points of V_2 are advanced compared to those of V_1 if $\dot{V}_1$ is continuous. If C_c is increased without limit, $\beta \to 1/k$, $\tau \to \infty$, $e^{\pm T} \to 1$, and therefore Equation **20** becomes $V_2 = V_1$, as is physically obvious. For $C_c = \infty$ and $\beta = 1$, there is no advance in the maxima and inflection points, so there must be a maximum advance for some intermediate value of C_c.

When V_1 is a monotonic increasing and then decreasing function, and $\beta < 1$, V_2 can be equal to V_1 if V_1 decreases sufficiently rapidly, since the term with the integral in Equation **20** increases after the maximum of V_1. If after such a time when $V_1 = V_2$, V_1 decreases more rapidly than the postjunctional time constant V_2 will exceed V_1, as may be seen from the equivalent circuit. If $\beta > 1$ on the other hand, the same statement holds for the potential across the junction by symmetry and V_2 then will be negative. Also, if $\beta > 1$, V_2 cannot equal V_1, for if $V_2 > kV_1$, $\dot{V}_2 < \beta \dot{V}_1$ from Equation **17** and therefore V_2 must decrease more rapidly than V_1.

If the sum of C_c and C_2 is kept constant and only their relative values changed, the coupling time constant does not change. This is a meaningful case, for the time constant measured by applying current pulses on the postjunctional side is the coupling time constant, assuming $r_1 << r_c$. In order to determine the relative time constants of junctional and postjunctional membranes, it is necessary to apply a known potential on the prejunctional side and compare it to the potential on the postjunctional side. Holding $C_c + C_2$ constant, Equation **20** can be rearranged to:

$$V_2 \mid_{\beta>0} = \beta kV_1 + (1-\beta)(kV_1 - ke^{-T}\int_0^T e^{T'} \dot{V}_1 \, dT')$$

which, from Equation **22**, simplifies to:

$$V_2 \mid_{\beta>0} = \beta kV_1 + (1-\beta)V_2 \mid_{\beta=0} \qquad (30)$$

The limits of β are 0 when $C_c = 0$ and $1/k$ when $C_2 = 0$. In the latter case, Equation **30** becomes

$$V_2 \mid_{\beta=1/k} = V_1 + (1-1/k)V_2 \mid_{\beta=0} \qquad (31)$$

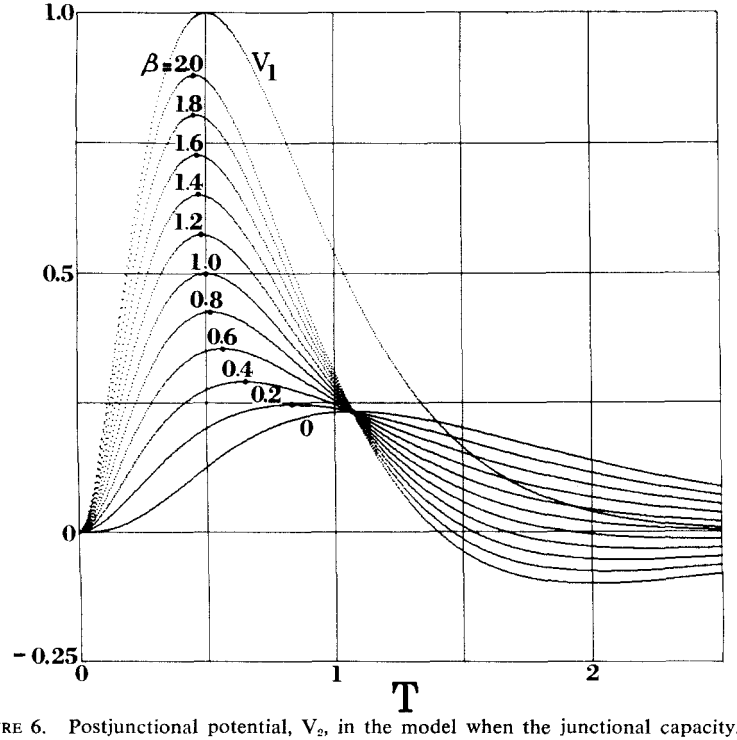

FIGURE 6. Postjunctional potential, V_2, in the model when the junctional capacity, C_c, is not negligible. The input was the impulse function, $V_1 = 4Te^{-4T+2}$, that is, as in Equation **27** with $\alpha = 2$. The points were obtained from Equation **30**, with $V_2 \mid \beta = 0$ as in Equation **28** for $\alpha = 2$. The value of k was taken as 0.5 and solutions are shown for $0 \leq \beta \leq 2$ in steps 0.2 as indicated. The input function, V_1, is also shown. The abscissa is in units of ($\Delta T = 0.005$) and the ordinate is in pure numbers. The maxima of the solutions are shown by dots and are earlier than that of V_1 when B>1 and later when B<1. Data computed and plotted as in FIGURE 2.

The effect on V_2 of changing β can be seen from the example in FIGURE 6, for which $V_1 = 4T^2e^{-4T+2}$, $k = 0.5$ and β is varied from 0 to $1/k = 2$ in steps of 0.2. The uppermost curve is V_1, the curve when $\beta = 1$ is $V_2 = kV_1$, and the curve when $\beta = 0$ is obtained from Equation **28**. All V_2 solutions intersect at the maximum of $V_2 \mid_{\beta=0}$, as can be shown from Equations **23** and **30**. It is clear from the figure that if $0 < \beta < 1$, then V_2 lies between the curves for $\beta = 0$ and $\beta = 1$, as can also be shown from Equation **30**. Similarly, if $1 < \beta < 1/k$, then V_2 lies between the solutions for $\beta = 1$ and $\beta = 1/k$. Since $k = 0.5$ in FIGURE 6, Equation **31** becomes:

$$V_2 \mid_{\beta=0} + V_2 \mid_{\beta=1/k} = V_1$$

as is also obvious from symmetry. In the solutions for $\beta < 1$, V_2 exceeds V_1 for large enough T, because V_1 decreases more rapidly than the postjunctional time constant. Correspondingly, for $\beta > 1$, V_2 becomes negative for larger values of T.

From the preceding it may be seen that for $\beta < 1$, the model is an integrating circuit. When the input voltage consists of repetitive pulses, the postjunctional potential will, in effect, show temporal summation, provided the time between pulses is sufficiently short compared to that for discharge of the postjunctional capacity. For a given coupling time constant, the closer β is to zero the greater the smoothing of the input voltage. In the extreme case, a train of brief pulses is transformed into a steady potential, the magnitude of which depends on the frequency of the pulse train. It is but a slight generalization to have a number of junctions connected to the same postjunctional cell whereby spatial, as well as temporal, summation becomes possible.

One further property of the model is relevant to the subsequent discussion. Consider an "impulse" on the prejunctional side which starts from an initial potential level determined by a constant current applied on the postjunctional side. The impulse is assumed to reach the same maximum and have the same shape as in the absence of current. This impulse approximates the behavior of an action potential in the presence of small polarizations, because the conductance during the latter response is much greater than at the resting level. In the model the amplitude of the impulse would be changed by the current, and the potential change that the impulse produced on the postjunctional side would also be altered. The value of polarization on the postjunctional side that would reduce the potential change to zero would be that which produced a potential on the prejunctional side equal to the impulse height, V_s, in the absence of current. This potential would also be that at which the postjunctional response would become zero, and may be called the (apparent) reversal potential, V_{rev}, by analogy with the behavior at junctions where transmission is chemically mediated (cf. Eccles, 1964; Grundfest, 1961). The amplitude of V_{rev} is inversely related to the coupling coefficient in the reverse direction, and is simply:

$$V_{rev} = \frac{1}{k_{21}} V_s \qquad (32)$$

Relevance of the Model to Real Junctions

The Equivalent Circuit

As far as steady-state behavior is concerned, the circuits of FIGURE 1*B* and *C* are necessarily valid for any three-terminal network. A network involving more than three resistances can be transformed into an equivalent involving only three (cf. Guillemin, 1953), and the proposition that a network consists of a certain number of elements is more of a morphological statement than an electrical one.

The measurement of coupling between the interiors of two cells is essentially a three-terminal measurement, the terminals being the indifferent electrode and the intracellular sites of electrode placement, whether or not separate voltage and current electrodes are used. Thus, that the interiors of two cells are coupled in the steady state does not imply the existence of a single element like r_c in FIGURE 1. Moreover, if two cells lie in a volume conductor, there will be a common transfer resistance between their interiors merely due to volume resistivity, and a correction for this factor has to be made before the existence of a junction between them can be inferred.

In practice, the coupling between electrodes should be closer, that is, the transfer resistance greater, when measuring between intracellular sites than when measuring between intracellular and nearby extracellular or between two extracellular sites. Alternatively, coupling should be observed with differential recording across the post-junctional membrane. Such criteria are equivalent to having a network with more than three terminals. It is also implicit that the extracellular sites are neither very close together nor in the junctional region. The latter is a reasonable assumption, because in most cases the volume of junction is small. The coupling due to volume resistivity in general is nearly purely resistive and is set up without measurable delay. If the intracellular time constants are fairly large, the transmembrane potentials will be slowly rising and falling. It may therefore be possible to subtract the potential due to volume resistivity from the intracellular potential without extracellular measurement, because this component appears as a sudden step at the onset and termination of the current pulses. An additional complication is that coupled recording sites may be in different parts of the same cell or in different parts of a syncytium. The exclusion of these possibilities is primarily morphological, since the axial resistance of a core conductor can be electrically very similar to a junctional resistance. Usually the site of electrode placement is inferred from a combination of morphological and physiologilcal observations. There are a few known instances of electrical interactions between neurons where coupling, as operationally defined above, would not occur (Furukawa & Furshpan, 1963; Grundfest & Magnes, 1951; cf. Bennett, Pappas *et al.*, 1966). These examples will be considered more fully in a subsequent publication.

When recording from two cells in a multiply connected pool of coupled neurons, input and transfer resistances can be measured and from them values for r_1, r_2, and r_c calculated. However, the latter resistances can not be assigned to actual membranes without additional knowledge of the network, and in general each calculated resistance will be a function of both junctional and nonjunctional resistances. Probably FIGURE 1*A* directly applies in very few instances. For example, occasional pairs of toad motoneurons appear to be electrotonically coupled, although the existence of junctional membranes between them has not been established (Washizu, 1960; cf. Bennett, Aljure *et al.*, 1966). Under the artificial conditions of tissue culture, cardiac muscle cells can remain electrotonically coupled (Crill *et al.*, 1959; Fänge *et al.*, 1956), and if two cells were isolated from others, they would probably approach the condition of FIGURE 1*A*. Pairs of epithelial cells might also be coupled in tissue culture (cf. Loewenstein *et al.*, 1965). A fertilized egg during its first division passes through an electrical state like that of two cells coupled by a resistance, but whether there is at any time junctional membrane has not been determined (Ashman *et al.*, 1964). As far as the junction itself is concerned, FIGURE 1*A* probably does apply to septate axons (Watanabe & Grundfest, 1961; Goldman, 1964) and to motor giant synap-

ses of the crayfish (Furshpan & Potter, 1959), where the junctional membranes are approximately isopotential although the pre- and postjunctional cells are not. While the giant ganglion cells of the leech constitute a two-cell system, the coupling occurs along the axons, and the actual junctional properties have not been determined (Eckert, 1963; Hagiwara & Morita, 1962). The two giant neurons of the electric catfish are coupled by way of prejunctional fibers ending on both cells and, again, the actual junctional properties cannot be determined (Bennett, Nakajima & Pappas, 1966*b*). A few single cells in the Mormyrid electromotor nuclei probably approximate a half of FIGURE 1*A*, but identification of these cells experimentally would be difficult (Bennett, Aljure, *et al.*, 1966).

Occasionally special circumstances allow the inference of electrotonic coupling, although direct measurement of electrotonic spread may be difficult or impossible. For example, in the pacemaker nuclei of the Gymnotids, graded hyperpolarization or depolarization of a single cell gradedly decelerates or accelerates the electric organ discharge (Bennett, Pappas, *et al.*, 1966). The influence of recurrent collaterals, or extracellular current flows, can be ruled out, and the only simple explanation is that the cells are electrotonically coupled. Graded short latency depolarizations can be evoked in a number of nuclei by antidromic stimulation of their efferent pathways. In a coupled pool of neurons where an impulse in one cell is unable to actively propagate to the other cells, graded antidromic stimuli should produce graded subthreshold potentials in cells with higher threshold axons (FIGURE 10*B* cf. Bennett, Giménez, *et al.*, 1964, Bennett, Pappas, *et al.*, 1966; Pappas & Bennett, 1966). In the absence of direct measurement of electrotonic spread, the existence of very short latency antidromic depolarizations constitutes good evidence of coupling. The requirement for short latency is to exclude chemical transmission which is characterized by a definite delay (Eccles, 1964; Grundfest, 1961; Katz & Miledi, 1965). In addition, electrotonic coupling between afferent fibers and postjunctional cells may be inferred when the postjunctional potentials are of sufficiently short latency (Bennett, Nakajima & Pappas, 1966*b*; Furshpan, 1964).

The question of current flow from the junctional regions into the extracellular space, or taking the anatomical data into account, from between the two closely apposed, or fused, unit membranes, is one that is difficult to resolve experimentally. If the circuit of FIGURE 1*B* is modified to include such a leakage path as in FIGURE 7*A*, a wye-delta transformation of the central three resistors leads to the circuit of FIGURE 7*B*. Thus, leakage from the junction is equivalent to a shunt in the membrane right beside the junction, and the measurement of leakage requires a high degree of spatial resolution. In no neuronal junction has this degree of resolution been obtained, not even in the septate axons, where the accessibility is relatively great. Even if much greater resolution could be obtained, it could not be assumed that membrane resistivity were uniform.

If coupling is very close, leakage must be small compared to current flowing across the junction. This fact may be seen by inspection of FIGURE 7*A*, because if little voltage drop occurs across the junction, the leakage resistance must be large compared to the resistance from cell to cell. Nevertheless, the presence of close coupling does not itself allow the inference that the leakage current is small compared to the current through the adjacent pre- and postjunctional membranes.

The only coupled cells where leakage from the junctions seems excluded are the giant salivary gland cells of Diptera. These cells are joined by a septate desmosome (Wiener *et al.*, 1964), a structure quite different from the regions of membrane fusion associated with electrotonic coupling elsewhere (cf. Wood,

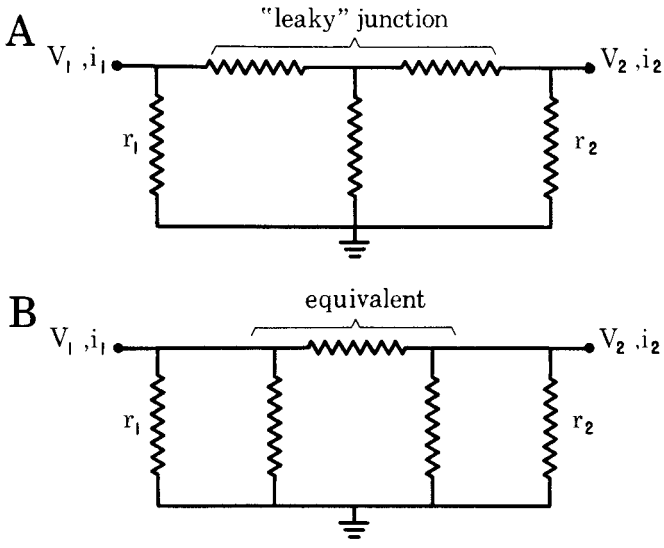

FIGURE 7. Equivalent circuits of a "leaky" junction. *A*. The circuit where the current path out of the middle of the junction into the extracellular medium is represented by a resistance. *B*. A circuit equivalent to that in *A* obtained by a wye-delta transformation of the central three resistors. The "leak" is electrically equivalent to additional resistances in parallel with the pre- and postjunctional resistances.

1959; Locke, 1965; Pappas & Bennett, 1966). When a potential decreases along the gland, there is virtually no extra voltage drop across the junctions, but rather a uniform gradient along the cells (Loewenstein & Kanno, 1964). That leakage is small compared to current across the junction is shown by the close coupling. That leakage is small compared to the current through the nonjunctional membrane would be inferred from the uniform voltage drop along the cells. In fact, the data suggest that membrane resistivity is increased at the convoluted regions of cell apposition that are adjacent to the junctions. The increased membrane area in these regions would otherwise cause a decreased resistance of a unit length and a larger axial voltage gradient. Another possible explanation of the uniform gradient is that current flow out of the cleft in the convoluted regions is in some way restricted.

Inexcitability of the Junctional Membrane

The model assumes that the junctional membrane is linear, or in other words, that it is electrically inexcitable in the most rigorous sense. This property has been best demonstrated for the septum of the crayfish septate axon. Potential differences of 25 mV. in either direction produce no departure from linearity (really only tested in the steady state) and preliminary data indicate that this behavior continues over a much larger range (Watanabe & Grundfest, 1961). At the motor giant synapse of the crayfish, the junctional membrane has been shown to rectify strongly, the only known junction where this property occurs (Furshpan & Potter, 1959). The junctional membrane at rest is of relative high resistance, but when the prejunctional side is about 20 mV. positive to the post-

junctional side, the resistance greatly decreases. The function of this junction will be further discussed below.

There have been few corresponding experiments in other systems, for the most part because the cells cannot be penetrated under visual control or the junctions are distant from the visible cell bodies. However, where the input and transfer resistances are linear, the effective coupling resistance is linear, and linearity of the junctional membrane is highly probable. Experimentally, input resistances are usually linear for a moderate amount of hyperpolarization, and measurement of the two transfer resistances for hyperpolarization involves potential gradients across the junction of both signs. Under these conditions, equality of the transfer resistances implies linearity of the junctional membrane. In the best case in Mormyrid spinal electromotor neurons, the transfer resistances were equal for potential differences between cells of about 15 mV. in either direction (Bennett, Aljure, *et al.*, 1966). As the cells were close together, there was probably a direct dendro-dendritic connection between them, and much of the potential drop would have been across this junction. The supramedullary neurons may be taken as an example showing difficulties in demonstration of linearity. These cells, num-

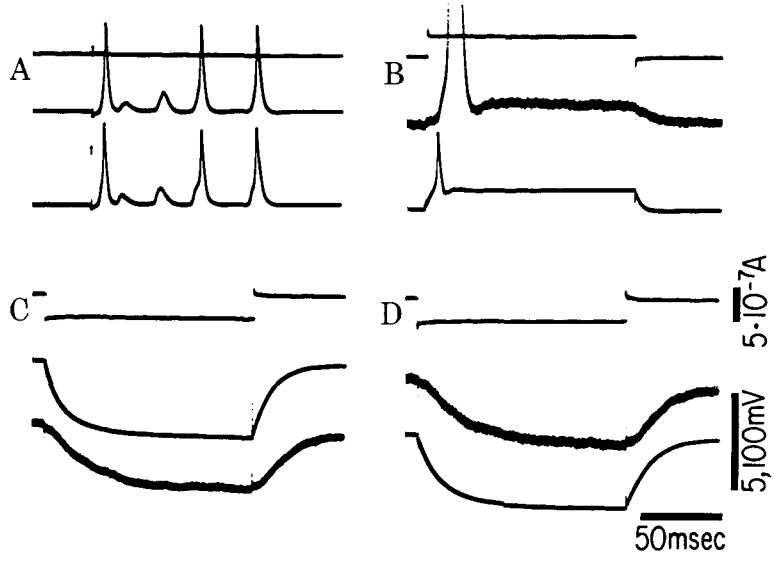

FIGURE 8. Electrotonic coupling of supramedullary neurons of the puffer (*Spheroides*). Experimental procedures and display for this and subsequent figures as described in Bennett *et al.*, 1959, and Bennett, Aljure, *et al.*, 1966. *Upper trace*, polarizing current. *Middle and lower traces*, intracellular recordings from adjacent neurons. *A*. A multispike response to spinal stimulation. The same number of responses occurred in each cell although the somata were not invaded during two of the responses. *B*. A depolarizing pulse was applied to the cell of the lower trace, (cell 2) which was adequate to evoke a spike. A somewhat delayed spike was observed in the cell of the middle trace (cell 1). There was also appreciable electrotonic spread of the maintained depolarization. Higher gain recording in cell 1. *C*, *D*. Electrotonic spread of hyperpolarization polarizing in cells 1 and 2, respectively. Higher gain recording in the unpolarized cell. The electrotonically spread potentials were slowed, and the most rapid rise and fall occurred somewhat delayed from the onset and termination of the current pulses. Thus, these potentials had a sigmoidal shape in contrast to the potentials in the polarized cells, where the most rapid rise and fall occurred immediately after the onset and termination of the pulses.

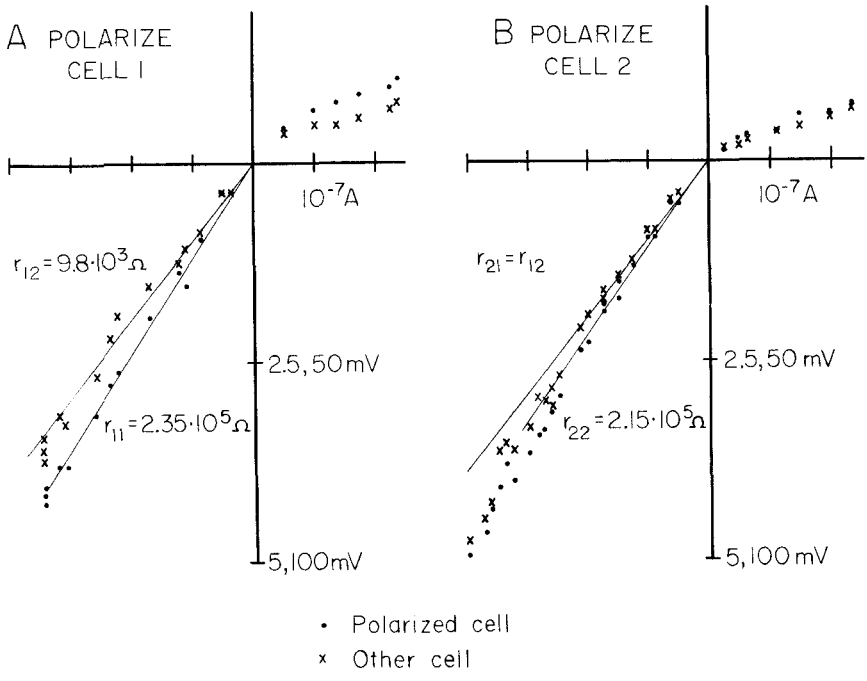

FIGURE 9. Input and transfer resistances for electrotonic coupling of the cells in FIGURE 8. Cell 1 corresponds to the cell of the middle trace. Hyperpolarization in the lower left quadrant. The larger units on the ordinate are for potentials in the polarized cell. The input resistances of the two cells were linear for moderate hyperpolarization and differed slightly. Over the same range, the transfer resistances were approximately equal, and the same resistance line was drawn for both sets of data. For larger hyperpolarizations and for depolarizations, the input resistances became nonlinear, but the amount of electrotonic spread was not changed in proportion to the change in resistance.

bering about 50 to 100 in the puffer, are coupled along their axons and constitute a large nonisopotential pool of neurons (Bennett *et al.*, 1959; Bennett, Nakajima & Pappas, 1966*a*). Representative records showing the spread of hyperpolarization between two adjacent cells are shown in FIGURE 8*C* and *D*, and additional data from these cells was used for the graphs of FIGURE 9. For hyperpolarizations up to about 50 mV., the input resistances in the two cells were linear and differed slightly. However, the transfer resistances in the two directions were equal within the experimental error. The input resistances increased for larger hyperpolarizations and decreased for depolarizations. The amount of coupling did not change by a corresponding amount, suggesting that the changes in input resistances were largely due to changes in the polarized cells and did not involve the connecting path or the postjunctional cells. It is therefore likely that the effective junctional resistance remained linear over a wider range than produced by 50 mV. hyperpolarization in either cell. Nevertheless, the potentials across the actual junctions must have been smaller and by an indeterminate amount.

The actual measurement of symmetry of the junctional membranes appears in many cases relatively unimportant because of functional considerations. In most known systems, coupled cells are functionally equivalent (cf. Bennett,

Pappas, *et al.*, 1966), and symmetry of the junctional membrane may be inferred from this alone. If the input and transfer resistances for hyperpolarization applied in any one cell are linear, linearity of the junctions between cells is also implied. By this means, one may infer the linearity of the junctions between giant ganglion cells of the leech (Eckert, 1963; Hagiwara & Morita, 1962), giant electromotor neurons of the electric catfish (Bennett, Nakajima & Pappas, 1966*b*), cardiac muscle cells (Weidman, 1952), and probably also pacemaker and relay neurons in the Gymnotids (Bennett, Pappas, *et al.*, 1966).

Although in most known systems, functional symmetry would tend to exclude rectification of the type seen at the motor giant synapses, rectification might be advantageous at the club endings on the Mauthner cell. If the eighth nerve input is to be solely a function of the number of eighth nerve fibers activated in the periphery, antidromic invasion of these fibers should not occur. Graded stimulation of the eighth nerve produces smoothly graded depolarizations in the Mauthner cell, but there also appears to be considerable antidromic spread of depolarization into the eighth nerve fibers (Furukawa *et al.*, 1963; Furshpan, 1964). Although these data are not necessarily contradictory, further investigation appears desirable.

While electrical symmetry may be inferred from functional symmetry, it should be emphasized that symmetry is not the same as linearity. The actual potentials produced across junctions when impulses are present on only one side are usually considerably larger and briefer than can be tested experimentally. This is both because of limitations of current that can be passed through microelectrodes and because of difficulty in placing electrodes at the junctions themselves. Thus, linearity remains unproved in a significant time and voltage range, and either increased or decreased resistance might well occur.

Time Constants of Junctional and Postjunctional Membranes

In general it can be said that postjunctional time constants are greater than junctional. Two factors in this property can be related to the anatomy of most known electrotonic junctions. The junction itself involves two unit membranes which are fused (cf. Pappas & Bennett, 1966), and the capacity of the fused membranes would therefore be about one-half the normal value for a single membrane. The junctions cover a relatively small part of the postjunctional cell, and it is often possible to show that the observed steady-state coupling requires a greatly lowered membrane resistivity of the two junctional unit membranes (cf. Equation **14**). Indeed, wherever it has been measured, junctional resistivity is lower than resistivity of surrounding membrane (refs. below). Thus, the junctional time constant would be lower because its resistivity, and, to a smaller extent, its capacity, were lower.

The septate axon may be taken as an example. Assuming the septum to be an ellipse with the major axis five times the minor, the resistivity is about 75 Ω cm.^{2} from the measurements of Watanabe and Grundfest (1961). The resistivity of the junctional membrane itself must be considerably lower, for only a small part of the total area of the septum appears to be involved (Hama, 1961). Taking the junctional membrane capacity as 0.5 μf/cm.^{2}, the time constant would be less than 40 μsec. The nonjunctional membrane time constant was given as 1 to 2 msec., and as a rough approximation the effective "input" time of the axon on either side of the junction may be taken as one-half to one-third the true membrane time constant (Rall, 1960). Thus, the input time constant would be considerably greater than the calculated value for the junctional membrane. The

calculated resistivity of the junctional membrane is also lower than that of the postjunctional membrane in supramedullary neurons (Bennett, Nakajima & Pappas, 1966*a*), neurons of the Mormyrid electromotor system (Bennett, Aljure, *et al.*, 1966), electromotor neurons of the electric catfish (Bennett, Nakajima & Pappas, 1966*b*), electromotor relay neurons of some Gymnotids (Bennett, Pappas, *et al.*, 1966), motor giant synapses (Furshpan & Potter, 1959), cardiac muscle (Weidman, 1952), and a number of epithelia (Loewenstein *et al.*, 1965).

It may be simpler to determine the relative magnitude of junctional and postjunctional time constants by measuring the time relations between pre- and postjunctional potentials. Where maxima of the postjunctional potentials are delayed, the postjunctional time constant is the greater. Delays of this kind are observed in nearly all coupled systems, but in few are the potentials measured sufficiently close to the junctions for one to be sure that the delay occurs at the junctions themselves, e.g., the septate axon (Watanabe & Grundfest, 1961), and motor giant synapses (Furshpan & Potter, 1959) of the crayfish and the Mormyrid spinal electromotor neurons (Bennett, Aljure, *et al.*, 1966). In other instances, the delay of maxima may be a result merely of electrotonic spread along an axonal or dendritic pathway, and relative time constants may be more reliably determined from measured coupling and relative junctional area.

Another commonly observed property equivalent to the delay of maxima is that the attenuation of steady-state potentials should be greater than that of brief potentials. However, in septate axons (Watanabe & Grundfest, 1961), supramedullary neurons (Bennett, Nakajima & Pappas, 1966*a*), and the giant ganglion cells of the leech (Hagiwara & Morita, 1962), an impulse can produce an electrotonic potential proportionally larger than the spread of brief hyperpolarizations, sometimes even larger than the spread of steady-state polarizations. This difference can be readily explained as a result of impulses propagating part way along the pathway connecting the cells. Hyperpolarization decreases along the entire pathway, while impulses are decrementally propagated only beyond the junctional regions.

Delay at Electrotonic Junctions

Delayed onset of postjunctional electrotonic potentials is difficult to detect when currents are extrinsically applied through microelectrodes, for the artifacts generated by the large voltages that are required obscure the early events. With action potentials, the delays are more readily measured and are about 0.05 to 0.1 msec. in the septate axon (Watanabe & Grundfest, 1961) and the motor giant synapses (Furshpan & Potter, 1959). These delays are of the order of what would be predicted from the model. Taking T as 0.5 msec., α is about 0.8 for an impulse. If the detection threshold is 0.5 mV. or about 0.01 k, a delay of 0.06 msec. is estimated from FIGURE 5. At the motor giant synapses, rectification would tend to introduce some delay, because the resistance of the junction does not decrease until there is considerable potential across it. The time required for the conductance increase to occur would have to be short for it to act in impulse transmission, but this latency has not yet been determined.

In the electromotor neurons of the catfish and in the Mauthner cell, the electrotonic potentials due to afferent nerve stimulation close to the cells have a minimum latency of about 0.1–0.2 msec. (Bennett, Nakajima & Pappas, 1966*b*; Furshpan, 1964). Electrotonic potentials are produced in toadfish swim bladder motoneurons by antidromic activation of neighboring cells, and these potentials are delayed about 0.3–0.4 msec. in relation to the antidromic spikes when the ipsilateral efferent nerve is stimulated (FIGURE 10A, Pappas & Bennett, 1966). Somewhat

longer latencies are observed when the contralateral nerve is stimulated (FIGURE 10D). An unknown portion of these latencies is in conduction time in the prejunctional fibers or dendrites. The values of these latencies are to be compared to the 0.5 msec. or greater delay observed at neuromuscular junctions of cold-blooded forms where transmission is chemically mediated (Dudel, 1963; Katz & Miledi, 1965). In principle, postjunctional potentials due to constant current polarization on the prejunctional side should be sigmoidal where the postjunctional time constant exceeds the junctional time constant. In general, potentials of this shape are not observed. Apparently the early slowly rising phase is, like the delay, obscured by the stimulus artifact. However, a sigmoidal shape of electrotomically spread potentials can be seen in cardiac muscle (Weidman, 1952) and occasionally in supramedullary neurons (FIGURE 8*C*, *D*). Again, however, some or all of this effect could be due to electrotonic conduction along the muscle cells or axons rather than to distortion produced only at the junctions.

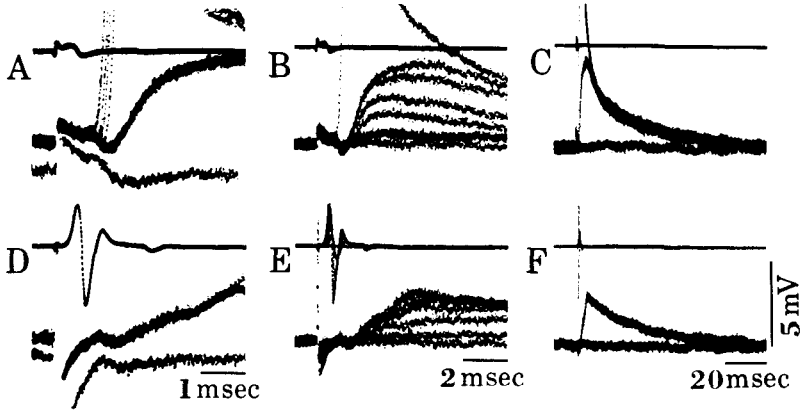

FIGURE 10. Antidromic depolarizations in motoneurons of the swim bladder nucleus of the toadfish (*Opsanus tau*). *Upper trace*: antidromic volley recorded monopolarly at the edge of the spinal cord contralateral to the penetrated cell (consequently stimulation of the contralateral nerve produced a larger potential). Dorsal roots sectioned. Several superimposed sweeps in each record. *Lower trace*: intracellular recording. Added traces in *A* and *D*: recordings in a just extracellular position at the same stimulus strength as for the corresponding intracellular records. Subtraction of the extra- from intracellular potentials gives the transmembrane potentials. *A* to *C*. Stimulation of the ipsilateral nerve to the swim bladder muscle. *D* to *F*. Contralateral stimulation. *A*. Stimulation threshold for the axon of the penetrated cell. The latency of the spike varied somewhat, and when it did not occur, there was a small negative potential followed by much larger positive potential. Subtraction of extra- from intracellular potentials indicates that transmembrane depolarization began about 0.3 msec. after the earliest onset of the antidromic spike. B. The antidromic depolarization was smoothly graded in amptitude when graded antidromic stimuli were given. This result represents spatial summation of electrotonic postjunctional potentials. *C*. Threshold stimulation as in *A*, but at a slower sweep speed to show the duration of the antidromic depolarization. A baseline without stimulation is also shown. *D*. As in *A* but a maximal stimulus was given to the contralateral nerve. Depolarization occurred which was at a somewhat longer latency than with ipsilateral stimulation. *E*. The depolarization due to contralateral stimulation was also smoothly graded with antidromic stimulus strength. There appeared to be a small component of somewhat longer latency when stronger stimuli were given (cf. FIGURE 13). *F*. As in *C* but with a maximal contralateral stimulus.

Unidirectional Conduction

From consideration of a purely passive or linear model, it is obvious that electrotonic junctions can transmit impulses effectively in only one direction. The asymmetry in the model arises not in the junctional membrane itself but in the relative resistances of the pre- and postjunctional structures (cf. Equations **12** and **13**). As far as impulses are concerned, other kinds of asymmetry that did not involve the junction might also be revelant, such as differences in pre- and postjunctional time constants and thresholds. At a number of electrotonic junctions impulses are conducted in only one direction under normal or experimental conditions. The motor giant synapses of the crayfish are an interesting example as the only known rectifying junctions (Furshpan & Potter, 1959). Antidromic conduction does not occur from motor fibers to either lateral or medial giant fiber. However, the lateral giant is considerably larger than the motor fiber, and antidromic conduction would probably not occur even in the absence of rectification. The medial giant is smaller, at least in some species, and perhaps rectification is necessary to block antidromic conduction. In any case, the rectification prevents appearance of large subthreshold potentials in either giant fiber when the motor fiber is excited by the other giant or by any of the numerous other afferents to it. Such subthreshold potentials might be significant in the lateral giant by interacting with segmental inputs that are found in each ganglion (Wiersma, 1952).

At other junctions, unidirectional conduction does not appear to result from rectification. In the electric catfish, excitatory afferents to the electromotor neurons form electrotonic junctions on them (cf. Bennett, Nakajima & Pappas, 1966*b*). An impulse in a single afferent will not excite either motoneuron, but an impulse once initiated in either giant neuron will excite the prejunctional fibers. Linearity of input and transfer resistances, symmetry, and bidirectional action indicate that the junctional membrane does not rectify. The unidirectional conduction of impulses is simply a result of the great difference in size of the pre- and postjunctional structures, and it is in what would classically be the antidromic direction. However, the discharge frequency is set in these cells apparently by summation of a large number of (excitatory) electrotonic inputs, and for this functional reason, orthodromic activity in one or a few axons should not excite the neurons. The prejunctional fibers also serve to couple the cells together electrotonically and thereby to synchronize their firing. When an impulse arises in either cell, it is rapidly conducted to the other cell, and conduction in this, initially the antidromic, direction appears to proceed with a high safety factor. Although these junctions are morphologically clearly polarized anti- and orthodromic conduction in the usual sense cannot be defined for them.

In the chick ciliary ganglion, unidirectional electrotonic transmission probably occurs in a number of cells just after hatching. In later life conduction appears to become bidirectional in most if not all cells (Martin & Pilar, 1963*a*, *b*).

Under normal conditions of activation, the supramedulary neurons fire approximately synchronously, and the same number of impulses occurs in each cell (FIGURE 8*A*) or impulses occur in no cells. Synaptic depolarizations are widely distributed throughout the cluster, and apparently under these conditions an impulse initiating in one cell is able to propagate to all unexcited cells. However, if a single cell is directly excited in the absence of afferent stimulation, such spread of excitation is infrequent but nevertheless occurs from a few cells (FIGURE 8*B*; cf. Bennett *et al.*, 1959). Conduction from one of these cells to another from which this spread of excitation does not occur is also an example of unidirectional conduction, although it does not appear to have any physiological signifi-

cance. As there probably is no rectification at the junctions between these cells, the unidirectional conduction would appear to result from other kinds of asymmetry at the junctions.

In principle, any symmetrical junction that normally conducts impulses in either direction can be made unidirectional by an experimental procedure that acts asymmetrically. Hyperpolarization on one side of the septum of the crayfish septate axon can block conduction from the unpolarized side without blocking conduction in the opposite direction (Watanabe & Grundfest, 1961). Although this junction is not quite symmetrical, unidirectional conduction could no doubt be obtained in either direction with suitably placed electrodes. Also a uniform experimental procedure may cause unidirectional conduction in an asymmetrical system. A possible example is the atrioventricular node which becomes unidirectional when treated with acetylcholine (Sano *et al.*, 1960).

Several instances are known where an impulse in a number of cells coupled to one can excite that cell, but where activity of the one will not excite the others. The junctions themselves do not appear to be rectifying, nor need they be asymmetrical because an adequate asymmetry may result solely from the difference in numbers of pre- and postjunctional cells. In the electromotor relay nucleus of the Gymnotid, *Sternopygus*, a maximal pacemaker volley excites all the relay cells, but a maximal antidromic volley in the relay cells does not excite the pacemaker cells (Bennett, Giménez *et al.*, 1966). In the related species *Gymnotus* and *Steatogenys* larger or smaller relay volleys respectively are able to excite the pacemaker axons antidromically. These effects in pools of neurons will be further discussed in relation to spatial summation.

Effects of Postjunctional Hyperpolarization on Postjunctional Potentials

There are only a few examples of this kind of experiment, but they illustrate virtually the entire range of possible behavior. At the crayfish septum, postjunctional hyperpolarization greatly augments the postjunctional potential following block of transmission (Watanabe & Grundfest, 1961). The potentials increase nearly linearly with an extrapolated reversal potential about 100 mV. positive from the resting potential. The reversal potential that would be predicted from the known coupling and spike amplitude is two or three times as great (cf. Equation **32**). Apparently the hyperpolarization tends to lower the potential at the spike peak, although if this be true, the high degree of linearity observed is somewhat surprising.

The postjunctional potentials in the electromotor neurons of the catfish tend to be augmented by small hyperpolarizations but are greatly reduced by quite large ones (Bennett, Nakajima & Pappas, 1966*b*). This result is explained by block of impulses in the afferents at some distance from the junctions. Similar behavior is shown by antidromic impulses in these cells which normally fail to invade the cell bodies, and thus provide an analog of a unidirectional conduction at an electrotonic junction. Small hyperpolarizations augment the antidromic responses, but larger ones reduce them. The degree of reduction is much smaller than that observed with the postjunctional potentials.

The electrotonic potentials in the chick ciliary ganglion are little affected by moderate hyperpolarization, probably reflecting a rather small degree of coupling in the antidromic direction (Martin & Pilar, 1963*a*).

At the motor giant synapses of the crayfish, the postjunctional potentials are greatly augmented by moderate hyperpolarization. The effect would be primarily due to decreasing the junctional resistance rather than to increasing the prejunctional spike height (Furshpan & Potter, 1959).

Spatial Summation

The primary requirement for spatial summation to occur is that many cells should be coupled to a single cell, but that the single one should not be excited by an impulse in any one of the others. Under these conditions, postjunctional potentials from two or more inputs can sum to bring about excitation of the postjunctional cell.

Spatial summation appears to occur under physiological conditions in the electromotor neurons of the electric catfish (Bennett, Nakajima & Pappas, 1966*b*), in the Mauthner cell (Furukawa *et al.*, 1963; Furshpan, 1964), and in the cardiac ganglion of the lobster (Hagiwara *et al.*, 1959). In the first two, inputs from afferent fibers sum in the postjunctional cells. In the latter, the junctions between "follower" cells serve a synchronizing function, and there is probably not a preferred direction. There also appear to be electrotonic junctions between the pacemaker and follower cells as well as chemically transmitting junctions in the orthodromic direction (Watanabe & Bullock, 1960). The role of these electrotonic junctions is undetermined.

In several pools of electrotonically coupled neurons where normal activity is synchronous, spatial summation can be demonstrated experimentally. An example is shown in FIGURE 10 from the swim bladder nucleus of the toadfish, where the neurons appear to be coupled directly and by way of the afferent fibers (Pappas & Bennett, 1966). Activity of one cell in this nucleus will excite neither the other motoneurons nor the prejunctional fibers. The electronic potentials produced by single cells are small, and smoothly graded potentials are obtained from graded antidromic stimuli to either ipsilateral (*B*) or contralateral nerve (*E*). If enough motoneurons are active, the impulses may invade the prejunctional fibers and other inactive motoneurons in the nucleus (cf. Pappas & Bennett, 1966; FIGURES 8 & 9). In supramedullary neurons, simultaneous stimulation of two cells can cause the impulse to spread to all the cells where stimulation of

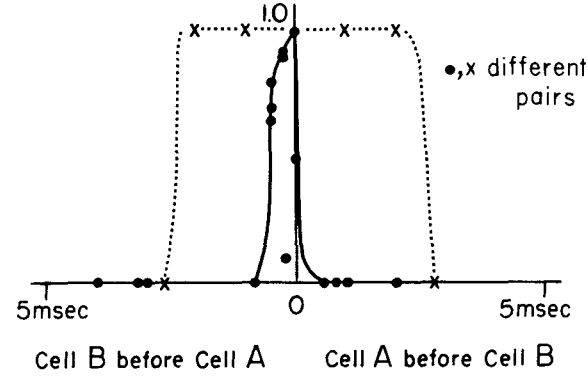

FIGURE 11. Spatial summation in facilitation of spread of impulses through the cluster of supramedullary neurons (puffer). In two different experiments, a pair of adjacent cells were directly stimulated with varying intervals between stimuli as indicated on the abscissa. On the ordinate is shown the fraction of trials in which spread occurred throughout the cluster as determined by the presence of the characteristic efferent volley in a dorsal root (cf. Bennett *et al.*, 1959). Stimulation of any single cell alone did not cause spread, but when a pair were stimulated in the proper time relations, spread was observed. In the experiment indicated by dots, the stimuli had to be nearly simultaneous. In the experiment indicated by crosses, a somewhat longer interval between stimuli could be used.

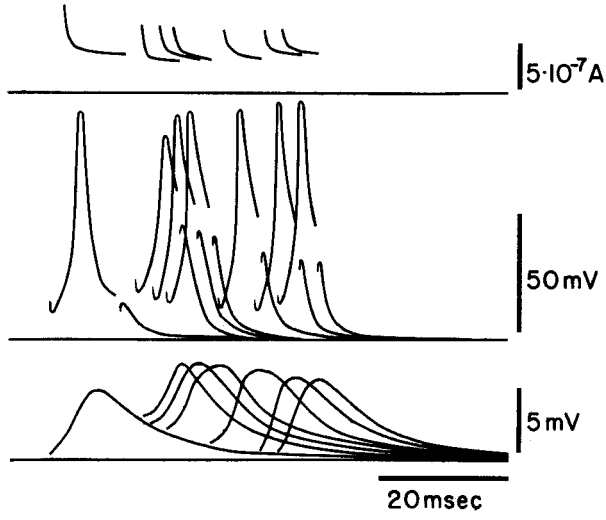

FIGURE 12. Temporal summation of electrotonic postjunctional potentials in supramedullary neurons (puffer). Superimposed tracings of different sweeps. *Upper tracings*: paired stimulating pulses to one cell; the first stimulus was given in each case and the time of the 2nd was varied. *Middle tracings*: potential in the stimulated cell. *Lower tracings*: potentials in an adjacent cell. The postjunctional potentials in the adjacent cell due to the second spike summed with those due to the first. At intermediate intervals between stimuli, the second postjunctional potential was somewhat augmented, probably due to a component of local response.

either cell alone will not (FIGURE 11). Spatial summation of electrotonic potentials explains why an impulse will spread to all cells, when once it has started propagating along the cluster. At the front of activity, depolarizations from a number of cells sum in as yet unexcited cells, and cause excitation where depolarization from any single one of the active cells would not.

In relay and pacemaker nuclei of Gymnotids, a spike in a single cell will not spread to the other cells of the nucleus. However, if all the cells surrounding a single cell are active, it may require very large hyperpolarizations to block that cell's firing (Bennett, Pappas *et al.*, 1966).

Temporal Summation

The criteria whereby electrotonic junctions should show temporal summation are similar to those for spatial summation. A single prejunctional impulse should produce a subthreshold postjunctional potential that outlasts the refractory period on the prejunctional side. As quite marked slowing is observed in several instances where the postjunctional potentials are small, temporal summation is readily demonstrated. In FIGURE 12 from the supramedullary neurons repetitive stimulation of one cell produced electrotonic potentials in a neighboring cell that summed. At the intermediate intervals between stimuli, the second postjunctional potential was even somewhat larger than the first, probably due to a component of local response. However, there are few physiological examples of temporal

summation. In the electromotor neurons of the catfish, the degree of temporal summation is slight, for the single postjunction potentials are of fairly short duration (Bennett, Nakajima & Pappas, 1966*b*). Probably the same consideration applies at the Mauthner cell (Furshpan, 1964). Somewhat more temporal summation could occur in the cardiac ganglion of the lobster, but this point has not yet been carefully studied (cf. Hagiwara *et al.*, 1959).

Two instances where temporal summation is quite long-lasting can be demonstrated under experimental conditions. The electrotonic potentials produced by antidromic stimulation of toadfish swim bladder motoneurons are greatly prolonged compared to the antidromic spikes. These potentials are much longer than the refractory period for antidromic stimulation and thus can sum when repetitive stimulation is used. In FIGURE 13*A*, when the ipsilateral motor nerve was stimulated a second time after an interval of c. 10 msec., the electrotonic potential summed with the potential remaining from the first stimulus. In *B* to *D*, weak paired stimuli were given to the contralateral nerve, and appreciable summation occurred even when the interval between stimuli exceeded 25 msec. When stronger contralateral stimuli were used (*D* to *F*) an early brief component sometimes appeared on the response (*D*, *F*). With shorter intervals between stimuli, this brief component showed marked facilitation. The brief component is ascribable to excitation of the prejunctional fibers, which can be directly shown to be invaded by antidromic activity of the motoneurons (Pappas & Bennett, 1966;

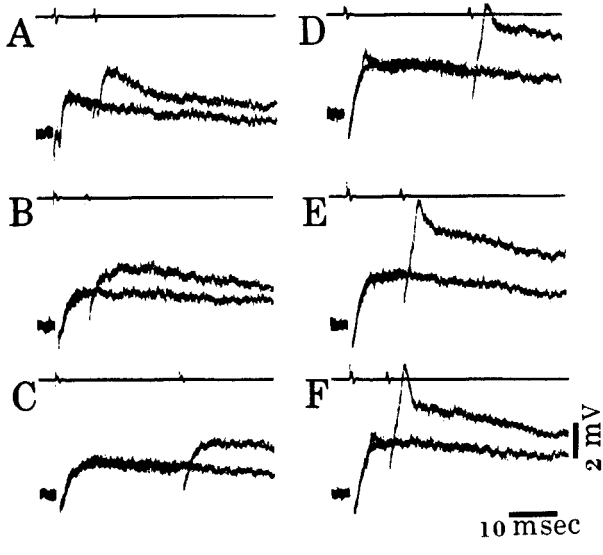

FIGURE 13. Temporal summation of electrotonic postjunctional potentials in swim bladder motoneurons of the toadfish. *Upper trace*: antidromic volley recorded monopolarly at the edge of the spinal cord ipsilateral to the penetrated cell, dorsal roots sectioned. *Lower trace*: intracellular recording from a motoneuron. Superimposed sweeps in each record with and without the 2nd antidromic stimulus. *A*. Paired antidromic stimulation of the ipsilateral nerve subthreshold for the axon of the penetrated cell. The electrotonic potentials summed. *B*, *C*. Paired submaximal stimuli to the contralateral nerve at different intervals. *D* to *F*. Paired maximal stimuli to the contralateral nerve. An early small and brief component sometimes occurred, superimposed on the response to the first stimulus. A much larger brief component was obtained in the response to the second stimulus. This component was larger when the interval between stimuli was shorter.

FIGURE 8). This component could not be due to excitation of neighboring motoneurons, for it was shown in other experiments not to be accompanied by activity in the efferent nerve. It is difficult to explain the difference in duration of the two components, since the coupling time constants must be about the same. Most probably the difference is due to a difference in the prejunctional potentials.

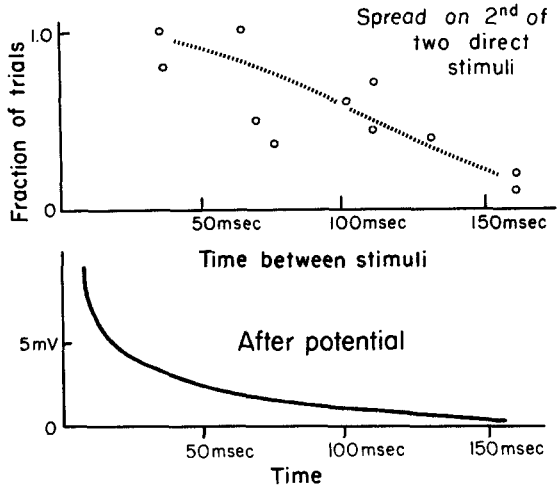

FIGURE 14. Long lasting temporal facilitation of spread of impulses obtained with paired stimulation of a single supramedullary neuron (puffer). *Upper graph*: Two spikes were evoked by brief direct stimuli to a single cell at intervals indicated on the abscissa; spread of impulses to other cells followed the second spike but not the first. The fraction of ten trials in which spread occurred is indicated on the ordinate. Spread was obtained when the interval between stimuli was up to 160 msec. *Lower graph*: Time course of the after-potential in the cell following a directly evoked spike. The magnitude of the after-potential is correlated with the frequency of occurrence of spread.

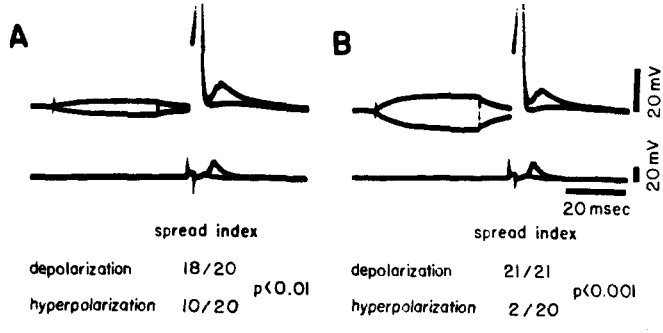

FIGURE 15. Facilitation of spread of impulses from a single cell by preceding directly applied polarization. *Upper trace*: stimulated cell. *Lower trace*: a distant cell used to monitor the occurrence of spread. Superimposed sweeps are shown where a directly evoked spike was preceded by a small hyperpolarizing pulse and an equal depolarizing pulse; the pulses were smaller in *A* than in *B*. The fraction of trials in which spread occurred ("spread index") following either sign of polarization is indicated for each strength of polarization in the tables. The incidence of spread was significantly greater following depolarizing pulses, and the probabilities that these differences could have arisen as a result of chance are indicated.

Motoneuronal spikes have longer lasting after-potentials than those of the prejunctional fibers, and the actual prejunctional spike in the former case might be a dendritic spike which was even longer lasting.

The longest lasting temporal effects known at electrotonic junctions are those in the supramedullary neurons. The spread of excitation from a single cell to all the others may be facilitated by paired stimulation, that is, it may occur on the second but not the first of a pair of stimuli. The interval between stimuli with which the effect is demonstrable may exceed 150 msec. (FIGURES 14, 16). Initially, before demonstration of electrotonic coupling of the cells, this duration was considered indicative of chemically transmitting junctions between the cells (Bennett *et al.*, 1959). That interaction between cells is solely by electrotonic coupling is indicated by the single peak in the electrotonic potentials associated with a prejunctional impulse (FIGURE 12; cf. Bennett, Nakajima & Pappas, 1966*a*). Therefore, a dual mode of transmission such as in the ciliary ganglion (Martin & Pilar, 1963*a*) does not appear to be present. The long duration of the facilitation may be explained by the long-lasting depolarizing after potentials that are observed in these cells (FIGURE 14). That potentials of the small size of the after-potentials can influence the spread of excitation was shown by stimulating a single cell after a small conditioning polarization was applied. In the experiment of FIGURE 15, the probability of spread from the directly stimulated cell was significantly increased when the cell was depolarized by 5 or 10 mV., compared to when it was hyperpolarized a similar amount. As the junctions between cells are along the axons at some distance from the cell bodies, the poten-

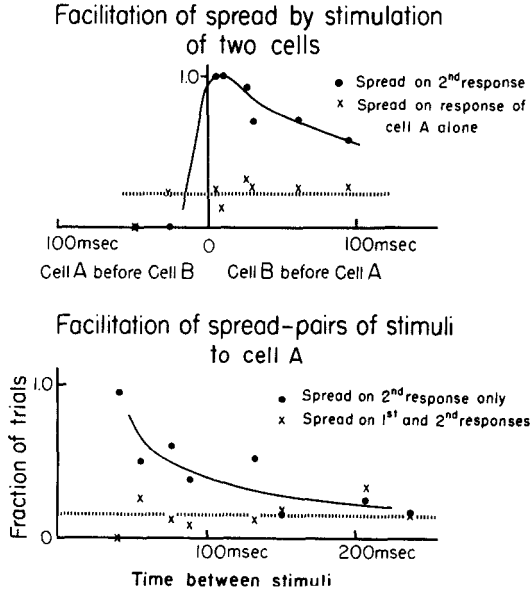

FIGURE 16. Long lasting temporal facilitation of spread of impulses obtained with direct stimulation of two different cells and with paired stimulation of one. *Upper graph*: as in FIGURE 11, except that stimulation of cell A alone was occasionally followed by spread as indicated by the dotted line. Spread from cell A was facilitated for at least 100 msec. following stimulation of cell B. Stimulation of cell A was inadequate to cause spread from cell B. *Lower graph*: facilitation of spread by paired stimulation of cell A as in upper graph of FIGURE 14.

tials at the junctions themselves would have been considerably smaller. If after-potentials are responsible for the facilitation of spread of excitation across the electrotonic junctions, it would be expected that long-lasting "hetero-junctional" facilitation would be also observed, that is, direct stimulation of one cell should facilitate spread from much later stimulation of a nearby cell. Such facilitation is uncommon but was observed in one instance (FIGURE 16). In this experiment, the action was markedly asymmetrical in respect to which cell was stimulated first, as might be expected. Probably the rarity of this kind of facilitation is due to difficulty in finding the right combination of pre- and postjunctional cells to stimulate.

COMMENTS

The above data indicate that electrotonic junctions can mediate most kinds of known neural interactions. Indeed, it may be difficult to identify a postjunctional potential as electrotonically or chemically mediated if the prejunctional elements cannot be penetrated by microelectrodes (cf. FIGURES 10, 13). As far as the appearance of postjunctional potentials as graded, nonpropagated events, these properties are solely a result of there being many inputs, each subthreshold, and do not reflect the mode of transmission. To be sure, hyperpolarizing postjunctional potentials are difficult or impossible to explain without assuming chemical transmission, and, if a postjunctional potential can be reversed by adequate polarization, there is little doubt that it is chemically mediated (cf. Bennett, 1964; Furshpan & Potter, 1959; Grundfest, 1961). As far as excitatory potentials are concerned, reversal may be experimentally difficult in the face of delayed rectification. Moreover, in nonisopotential systems, reversal should occur well above the actual membrane reversal potential (Bennett, Freeman, & Thaddeus, 1966), and, if mixed excitatory and inhibitory potentials are involved, an intermediate reversal potential could be measured whether or not a true reversal potential for the excitatory action existed. Probably inhibitory action could be excluded, at least in vertebrates, by showing that Cl^- injection did not alter the response (cf. Eccles, 1964, but also Gershenfeld & Chiarandini, 1965). At electrotonic junctions there may be apparent reversal potentials obtained by extrapolation that are in the range predicted for nonisopotential chemically transmitting systems. At electrotonic junctions hyperpolarization may also produce an effect opposite to that usually occurring at chemically transmitting junctions, that is, a large reduction in postjunctional potentials. If such a reduction occurs, the implication is strong that transmission is electrical. Absence of refractoriness produced by a postjunctional spike can occur at either class of junction, for it only implies that the postjunctional activity does not invade the prejunctional element. If a spike does block a postjunctional potential, and inhibition on the prejunctional path can be excluded, at least a component due to electrotonic coupling can be inferred. Temporal effects of quite long duration can be observed at either class of junction. However, the large increases in amplitude that can be produced by repetitive activation of chemically transmitting junctions (cf. Eccles, 1964) have not been observed at electrotonic junctions.

In summary, there are a number of ways to show by postjunctional tests that a given potential is electrotonic. Is it blocked by a spike, is it greatly reduced by hyperpolarization, or is it of very short latency? There are different tests for a chemically mediated response. Is it hyperpolarizing? If it is not hyperpolarizing, can it be reversed by depolarization, and if so can the influence of an admixture of inhibition be excluded? Does the response show postactivation potentiation with little change in shape? The failure of any of the above tests for one mode

of transmission does not necessarily mean that the other mode occurs, nor can the possibility of exceptions be excluded. If more is known about a junction morphologically or pharmacologically, other tests can be applied. Considering only physiological data, known instances of excitatory chemical transmission are fewer in number than known instances of electrotonic transmission. On the other hand, morphological data suggest that chemical transmission is far more common than electrotonic. There does remain a slight possibility of artifact, since in some instances fixation appears to pull apart the fused membranes of electrotonic junctions (cf. Bennett, Pappas *et al.*, 1966).

There are three functional properties which in general separate electrical from chemical transmission: speed, ability to transmit graded subthreshold potentials, and an intrinsic reciprocity. Where there is a functional requirement for a high degree of synchrony between cells, electrotonic coupling may be required because of its greater speed. From an evolutionary point of view, it would appear easy for electrotonic junctions to develop under these conditions, and at least eight separately evolved instances are known: two in Mormyrids (Bennett, Aljure *et al.*, 1966), one in the electric catfish (Bennett, Nakajima & Pappas, 1966*b*), three in the Gymnotids (Bennett, Giménez *et al.*, 1964, 1966), all of which are in electromotor systems, and one in the toadfish and one in the sculpin, both in sonic muscle systems (Pappas & Bennett, 1966, and unpublished). In less highly synchronized systems, the speed of electrical transmission would not be required. The observed synchrony of supramedullary neurons and of the lobster cardiac ganglion could probably be mediated by reciprocal chemically transmitting junctions. In these less synchronized systems, the significant properties of electrotonic junctions may be their reciprocity and ability to transmit graded, even subthreshold, potentials of either sign. These properties could be particularly important in coupled nuclei where there were graded excitatory and inhibitory inputs. In effect, electrotonic junctions would mediate positive feedback between the cells, and communication could occur without impulse generation, which would not be possible in a system coupled by typical chemically transmitting junctions. However, where impulses do not occur, the distances between interacting cells cannot be great. In view of the functional characteristics of electrotonic junctions, it is reasonable to suggest that they may occur in any synchronously active system.

The speed of electrotonic junctions would appear important in various neurons involved in escape systems like septate axons and motor giant synapses of the crayfish and Mauthner cells. Even in slower conducting systems like cardiac muscle where very many cells are connected in series, a small increase in speed at each junction might be a factor.

Chemical transmission is undoubtedly better adapted to mediating inhibition. While one instance of electrical inhibition is known (Furukawa & Furshpan, 1963) it is mediated by a complex structure which is probably inefficient in the sense defined for the model junction. The adaptive value of the electrical action appears in this case also to be in speed. Chemical transmission might be considered better adapted to efficient unidirectional transmission. Large postjunctional potentials cannot be efficiently generated at electrotonic junctions without increasing the degree of antidromic coupling to the point where antidromic excitation becomes probable. However, chemically mediated postjunctional potentials produced by single fibers are usually quite small (Kuno, 1964). They are so small that it seems highly likely that a moderate-sized prejunctional element could be made to produce an equal electrotonic potential with a negligible probability of antidromic conduction of impulses. Long-lasting postactivation

effects are also more prominent and perhaps more stable at chemically than at electrically transmitting junctions (cf. Eccles, 1964). Long-lasting effects on conductile membrane are also known, and, in illustration, maintained hyperpolarization was for a long time used to explain postactivation potentiation. There is no obvious reason why similar or opposite effects could not play a role at electrotonic junctions under normal conditions.

From a superficial point of view, electrotonic transmission is simpler than chemical and the widespread occurrence of regions of membrane fusion and electrotonic coupling between epithelial cells might be an argument for the greater primitiveness of electrotonic junctions (cf. Pappas & Bennett, 1966; Loewenstein *et al.*, 1965). On this basis, given the functional near-equivalence for (short term) integration of chemical and electrical transmission, it might be wondered why chemical transmission developed at all. However, chemically mediated interactions between cells must be very common in development, and recent evidence indicates that neurosecretory cells occur in coelenterates (Burnett & Diehl, 1964; Lentz & Barnett, 1965).

From these examples it would appear that both chemical and electrical interactions are extremely old in metazoa, and a characterization of either as more primitive is not likely to be relevant to its occurrence in higher forms. In any case, most instances of electrotonic transmission in nerve cells seem to be secondarily evolved, in that they occur in highly specialized systems. A qualification is that it is easier to demonstrate coupling in these systems, and the greater frequency of occurrence in them may only reflect experimental simplicity. But granting the difference in frequency, it would be inferred that there must be a survival advantage to chemical transmission where it remains. Whatever this advantage might be, it is not obvious that it is in the mediation of short term excitatory interactions between cells.

Summary

In an electrical model of electrotonic coupling, pre- and postjunctional cells and junctional membranes are each represented by a resistance and capacity. Steady-state and transient behavior are considered. Large coupling in one direction with small coupling in the reverse direction can occur as a result of differences in pre- and postjunctional resistances. The onset of postjunctional potentials is delayed. In the case where the junctional time constant is negligible, solutions are given and plotted for several input functions having the shape of action potentials. For such impulse functions, maxima and inflection points of postjunctional potentials are delayed compared to those of the prejunctional potentials, if the junctional time constant is less than the postjunctional time constant. The maxima and inflection points are advanced if the junctional time constant is greater than the postjunctional time constant.

Probably the model applies literally in few cases, for often pre- and postjunctional cells are complexly interconnected. Although circuit values corresponding to those in the model can be obtained, they may not correspond to actual membranes. It is difficult to exclude current flow directly out of the junction into the extracellular medium. In all but one known case, junctional membrane appears to behave linearly, at least over a moderate range of polarization of either sign. In general, junctional time constants appear to be smaller than postjunctional as determined by estimates of junctional resistance and capacity or inferred from the delay of maxima in the postjunctional potentials. Hyperpolarization of postjunctional cells may augment, decrease, or little change postjunctional potentials.

The following properties can be readily demonstrated experimentally and in a few instances are also found under normal conditions. Undirectional conduction of impulses occurs, and in all but one case is ascribable to differences in the ore- and postjunctional cells. Spatial summation occurs where there are a number of prejunctional cells, an impulse in any one of which will not excite the postjunctional cell. Temporal summation can be obtained where the refractory period of the prejunctional cell is short enough compared to the duration of a single postjunctional potential. Temporal summation may be quite long lasting, and facilitation of spread of impulses can be observed with intervals between stimuli in excess of 150 msec. The longer durations are apparently a result of long-lasting afterpotentials.

The functional properties that distinguish electrotonic from chemical transmission are speed, intrinsic reciprocity, and ability to transmit graded potentials without impulse initiation. In most cases of electrotonic coupling, speed appears to be the significant feature. However, it is not obvious that chemical transmission has any functional advantage over electrotonic transmission in the mediation of short term excitatory interactions.

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