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Temporal Control of Voltage-Clamped Membranes: An Examination of Principles

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Summary. The theoretical principles governing both speed and fidelity in the temporal control of voltage-clamped, excitable membranes are developed. A generalized equivalent circuit for voltage-clamping various biological preparations is analyzed. For critical damping response, the amplifier time constant must either be much smaller (as with puffer neuron) or much larger (as with squid axon or eel electroplaque) than the time constant of the membrane-solution-electrode "load". In the latter case, speed of response can be significantly increased with a new type of feedback designed to decrease the effects of current electrode and solution resistances. This new feedback, in addition, minimizes the lack of fidelity caused by the membrane time varying conductances. The analysis has been verified by experiments on eel electroplaque and with analogues that simulate an excitable membrane.

The electrical potential difference across a voltage-clamped membrane is forced to follow a time course chosen so that the resultant transmembrane current will yield information about membrane permeability (*see* Hodgkin, Huxley & Katz, 1952; Moore & Cole, 1963; Moore, 1971). This technique has been extremely useful and the voltage clamp has therefore become an important tool for the study of excitability in biological membranes.

Control of the membrane potential is possible only if two constituent problems can be resolved; one is spatial and the other is temporal. Spatially, the entire membrane region within which measurements are made must be forced to respond as a single, uniform unit. The attendant difficulties have

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been extensively discussed (Taylor, Moore & Cole, 1960; Chandler, Fitzhugh & Cole, 1962). Temporally, accurate conformity to the chosen time course must be obtained, but this problem has, until now, been treated in only a comparative cursory manner (Moore & Cole, 1963; Moore, 1971). We shall examine it more systematically in this paper.[1]

The transmembrane current has two components: ionic (due to membrane permeability), and displacement (due to membrane capacitance). Ionic current is both voltage and time dependent, reflecting the behavior of the underlying permeabilities; displacement current exists only during voltage transients. The time course that one desires the potential to follow is fixed by a suitable command pulse in the control circuit (Figs. 1 and 2). If this pulse is rectangular, this displacement current will be confined to the "on" and "off" edges of the pulse. Away from these edges, the potential imposed on the membrane should, ideally, be constant. These features of a rectangular command pulse simplify the separation from each other of the displacement and ionic components of the total current as well as of the voltage and time dependence of the ionic current. For this reason, such command potentials are very useful, and most often utilized.

With a rectangular command potential, successful separation of the ionic and displacement current requires that the capacitative charging process be essentially complete prior to the onset of the excitable ionic conductance phenomena. The clamp must therefore shift the membrane potential very rapidly from its initial to its final value. Rapidity of clamping is thus essential. However, a different clamp property is necessary to separate time dependence from voltage dependence in the ionic current itself. For this, the membrane must be held at the command potential with great fidelity in spite of excitation.

Adequate temporal control thus requires both rapidity and fidelity. A properly designed voltage clamp must satisfy these two demands. The problem is, how to achieve this. The answer can be obtained from a study of the differential equation that describes the response to the command pulse of the clamp-electrode-membrane assembly.

Theory and Discussion

The Basic Configuration and Its Equation

Different preparations often require different electrode arrangements both for monitoring the membrane potential and for passing current (Fig. 1).

1 Preliminary reports of this work have appeared (Katz & Schwartz, 1967; Schwartz & Katz, 1973).

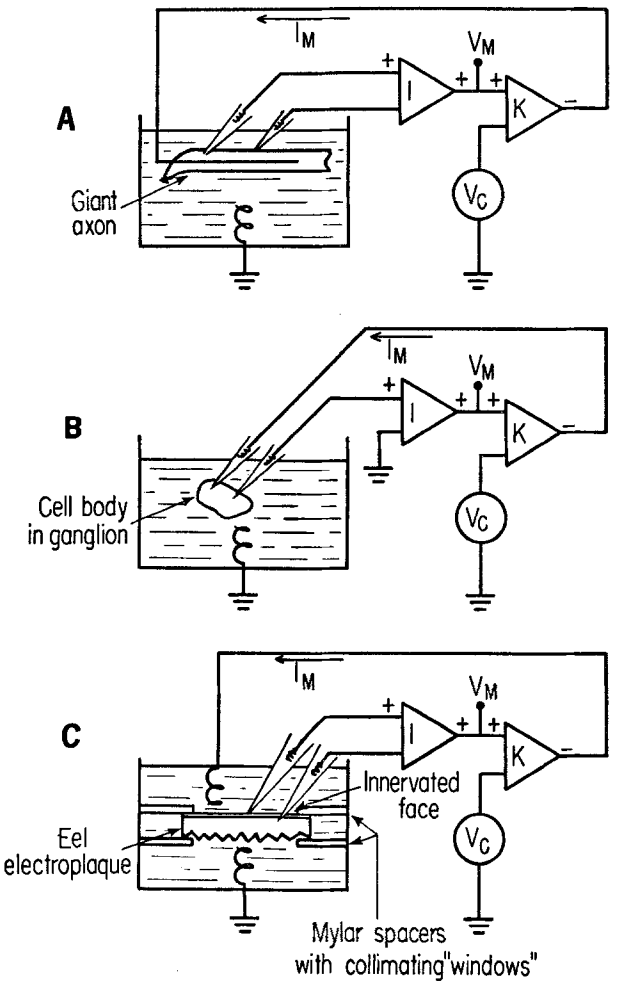

Fig. 1 *A–C*. Three typical arrangements for the voltage clamping of different cellular membranes. The membrane potential is denoted by V_M and the command potential by V_c. The amplifiers have gains of unity and K, as shown. (*A*) Clamping the membrane of a giant axon. An axial wire electrode has been inserted to pass current across the membrane. The membrane potential is, in this case, measured by a pair of straddling microelectrodes; other techniques have been discussed in the literature. For simplicity, the guard system used to solve the space clamp problem is not shown. (*B*) Clamping a small neuron. A micropipette is used to inject current into the cell. The membrane potential is measured between a second microelectrode and ground. (*C*) Clamping the innervated face of the eel electroplaque. The membrane is straddled with a pair of microelectrodes to measure its membrane potential. Current is passed across both the electrogenic innervated face and the low resistance, inexcitable, noninnervated face. The current is confined to an appropriate region of membrane by a matched pair of collimating mylar insulators

But they are all characterized by a common sequence of events; the difference between the membrane potential and the command pulse is amplified, and the resulting voltage is used to drive current across the membrane.

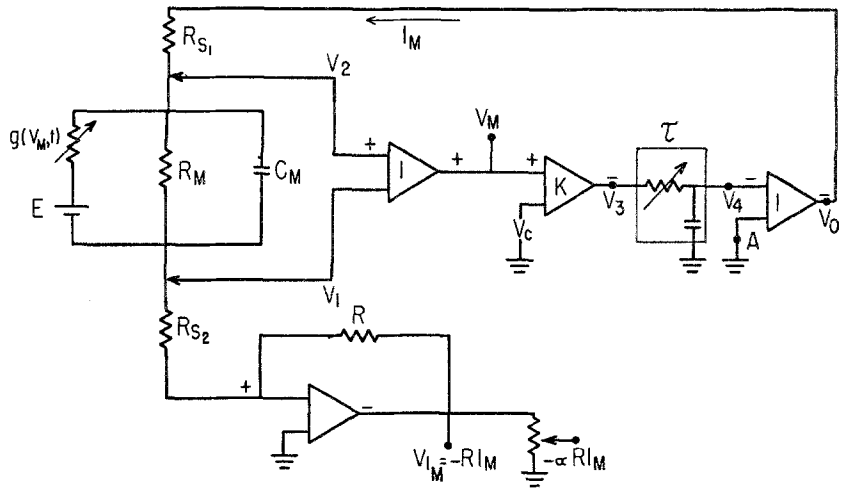

Fig. 2. Equivalent circuit for a voltage-clamped membrane. The membrane is represented by an inexcitable resistance R_M; an excitable voltage and time dependent conductance $g(V_M, t)$ and its associated "battery" E, and a capacitance C_M. The transmembrane current is denoted by I_M. All amplifiers are assumed to be ideal, and the adjustable bandwidth control of the clamping circuit lies in the time constant τ. For simplicity of analysis, the entire gain K of the clamp has been confined to its second stage, and the impedances associated with the current passing electrodes and their interposed solutions have been treated as resistances (R_{S1} and R_{S2}). The use of the voltage ($-\alpha RI_M$) derived from the current monitoring amplifier is discussed in the text

Circuit relationships are arranged so that this transmembrane current is precisely that required to hold the membrane potential close to that of the command.

One typical arrangement (Fig. 1*A*) is commonly used with the squid axon (Moore & Cole, 1963). The clamping of smaller cells requires a modified technique (Fig. 1*B*): the membrane potential is monitored with one microelectrode while current is driven into the cell through another (Hagiwara & Saito, 1959). The eel electroplaque – a large cell shaped like a rectangular wafer with only one excitable face – requires a third technique (Fig. 1*C*). In this case, current is passed across the entire cell (Nakamura, Nakajima & Grundfest, 1965).

The essential features of all of these clamping configurations can be described by a single equivalent circuit (Fig. 2). The membrane is assumed to be space clamped so that our attention can be confined to the problem of temporal control. Ionic permeability is represented by a parallel branched equivalent circuit (Hodgkin & Huxley, 1952; Finkelstein & Mauro, 1963). The "battery" associated with the inexcitable resistance R_M can be omitted

with no loss in generality. The excitable branch is assumed to be nonconducting when the membrane is unexcited and at rest. The preparation in its chamber usually has an additional resistance in series with the membrane but located between the voltage measuring electrodes that is due to interposed solution and connective tissue. This series resistance is omitted because its effects can be eliminated (Hodgkin *et al.*, 1952). In this circuit

$$V_M = V_2 - V_1 \tag{1}$$

$$V_3 = K(V_c - V_M) \tag{2}$$

$$V_4 = V_3 - \tau \frac{dV_4}{dt} \tag{3}$$

and

$$V_O = V_4. \tag{4}$$

The clamp is related to its membrane-solution-electrode "load" by the relationship

$$V_M = V_O - I_M R_S = V_4 - I_M R_S \tag{5}$$

where

$$R_S \equiv R_{S_1} + R_{S_2}. \tag{6}$$

The membrane imposes the requirement that

$$I_M = C_M \frac{dV_M}{dt} + g_{(V_M,t)}(V_M - E) + \frac{V_M}{R_M}. \tag{7}$$

It is convenient to define

$$\begin{aligned} \tau_S &\equiv R_S C_M, \\ \tau_i &\equiv R_M C_M, \\ \text{and} \qquad & \\ \tau_e &\equiv \frac{C_M}{g}. \end{aligned} \tag{8}$$

These time constants are associated with the electrode-solution resistances, the inexcitable membrane regions, and the excitable membrane regions, respectively. Combining these relationships yields

$$\begin{aligned} &\frac{d^2 V_M}{dt^2} + \left\{\frac{1}{\tau_e} + \frac{1}{\tau_i} + \frac{1}{\tau} + \frac{1}{\tau_S}\right\} \frac{dV_M}{dt} \\ &\quad + \left\{\left(\frac{1}{\tau_e} + \frac{1}{\tau_i} + \frac{K+1}{\tau_S}\right) \frac{1}{\tau} + \frac{d}{dt}\left(\frac{1}{\tau_e}\right)\right\} V_M \\ &= \frac{K}{\tau \tau_S} V_c + \left\{\frac{1}{\tau \tau_e} + \frac{d}{dt}\left(\frac{1}{\tau_e}\right)\right\} E. \end{aligned} \tag{9}$$

This equation describes the response of the entire clamp-electrode-membrane assembly. Since the conductance g of the electrically excitable membrane regions is a function of both voltage and time and since τ_e is a function of g [Eq. (8)], Eq. (9) will generally be nonlinear and will have time dependent coefficients and a time dependent forcing function. Useful analytic solutions for this general case are not available. But sufficient insight can fortunately be obtained without resort to an analytic solution.

Rapidity of Clamping

Critical Damping. The conductance of an electrically excitable membrane does not generally respond instantaneously to a voltage change (Hodgkin & Huxley, 1952). With a step command, the membrane potential can usually be brought from its initial to its final value before conductance changes begin. Consequently, during this initial interval the membrane remains unexcited so that

$$\frac{1}{\tau_e}=0$$

and (10)

$$\frac{d}{dt}\left(\frac{1}{\tau_e}\right)=0.$$

Eq. (9) then reduces to the linear expression

$$\frac{d^2V_M}{dt^2}+\left\{\frac{1}{\tau_i}+\frac{1}{\tau_S}+\frac{1}{\tau}\right\}\frac{dV_M}{dt}+\left\{\left(\frac{1}{\tau_i}+\frac{K+1}{\tau_S}\right)\frac{1}{\tau}\right\}V_M=\frac{K}{\tau\tau_S}V_c \qquad (11)$$

where both the coefficients and the forcing function are constant.

The potential can arrive at its final state in three ways, corresponding to the three possible kinds of solution to Eq. (11). It may undergo a damped oscillation, it may respond slowly in an exponential-like manner, or it may respond quickly while just avoiding oscillation (Ford, 1933). These three possibilities are often referred to as being underdamped or "ringing", overdamped, or critically damped, respectively.

Although one usually adjusts the clamp for a slightly underdamped condition since the speed of response is improved, simplicity is preserved if the critically damped case is analyzed. Critical damping will occur only when[2]

$$\left(\frac{1}{\tau_L}-\frac{1}{\tau}\right)^2=\frac{4K}{\tau\tau_S}, \qquad (12)$$

2 The solutions of this differential equation can be found in textbooks on linear differential equations (*see* Ford, 1933).

Table 1. Approximate resting membrane parameters for three types of voltage-clamped preparations

Cell	$\tau_i = R_M C_M$ (sec)	$\tau_S = R_S C_M$ (sec)	$\tau_L = \frac{\tau_i \tau_S}{\tau_i + \tau_S}$ (sec)	β	K	τ (for critical damping) (sec)	
						$\tau_L \gg \tau$	$\tau \gg \tau_L$
Squid axon (1)	1.25×10^{-3}	6.3×10^{-6}	6.3×10^{-6}	1	200 to 1,000	7.9×10^{-9} to 1.6×10^{-9}	5.0×10^{-3} to 25.0×10^{-3}
Eel electroplaque (2)	114×10^{-6}	100×10^{-6}	53×10^{-6}	0.53	1,300	19.2×10^{-9}	146×10^{-3}
Puffer supramedullary ganglion cells (3)	5×10^{-3}	85×10^{-3}	4.7×10^{-3}	0.057	5,000	4.1×10^{-6}	5.4

Calculations were made from data in (1) Cole & Moore (1960), (2) Nakamura *et al.* (1965) and Schwartz *(unpublished)*, and (3) Hagiwara & Saito (1959).

(1) 0.1-cm^2 membrane in central chamber.

(2) 0.05-cm^2 "window".

(3) 200-μ cell.

where

$$\frac{1}{\tau_L} \equiv \frac{1}{\tau_S} + \frac{1}{\tau_i} \tag{13}$$

defines τ_L the time constant presented to the clamp by the membrane-electrode-solution load during this initial period. It does not matter for critical damping, which is the larger of the two time constants, τ_L or τ, because only the magnitude of their difference is involved; but the clamp will ring if the difference is smaller than that specified.

Eq. (12) can be cast into a more useful form. We will see in the next section, that the gain K is usually made large (Table 1) to obtain conformity between the membrane and command potentials. If critical damping is maintained the difference between $\frac{1}{\tau_L}$ and $\frac{1}{\tau}$ must also be large [Eq. (12)]. This can occur in two ways: either $\tau_L \gg \tau$ or $\tau \gg \tau_L$. Eq. (12) therefore reduces to the two conditions

$$\tau_L \gg \tau; \quad \frac{\tau_L}{\tau} = 4\beta K, \tag{14a}$$

$$\tau \gg \tau_L; \quad \frac{\tau}{\tau_L} = 4\beta K, \tag{14b}$$

where

$$\beta \equiv \frac{\tau_i}{\tau_i + \tau_S}. \tag{15}$$

The parameter β has the significance that βK is the closed-loop gain of the clamp-load system at zero frequency.

Voltage clamp design for different biological preparations will depend upon which of Eq. (14) is applicable. For example, with squid axon, eel electroplaque, or other preparations having similar characteristic parameters, it is not feasible to construct a voltage clamp in which $\tau_L \gg \tau$ [condition (14a)]. Amplifiers with sufficient bandwidth (i.e., small enough τ) are not available (Table 1). Therefore, in these cases condition (14b) must be satisfied for critical damping to be achieved. Amplifiers with sufficient bandwidth are available, however, for preparations with characteristics similar to the supramedullary neuron of the puffer fish. In this case, condition (14a) can be satisfied. In feedback terminology: with "fast" squid or eel, the amplifier must be designed to provide the limiting time constant ($\tau \gg \tau_L$), but with the "slow" neuron, the neuron itself can provide the limiting time constant ($\tau_L \gg \tau$).

An arrangement like that shown in Fig. 2 is presently being used to clamp the eel electroplaque (T. L. Schwartz, *unpublished*). In practice, the magnitude of τ is manually adjusted for critical damping after the membrane has been brought into clamp in an initially overdamped state. Measurements made both with a membrane (Fig. 3) and with a resistive-capacitive analogue (Figs. 4 and 5) have verified the above criteria for critical damping.

Rate of Membrane Potential Change. Critical damping yields the most rapid membrane potential change possible without attendant ringing. But how rapid is this change? The critically damped response of the potential to a command pulse deduced from Eq. (11) is

$$\tau_L \gg \tau; \quad V_M = \frac{\beta K}{1+\beta K} V_c \left\{1 - \left(1 + \frac{t}{2\tau}\right) \varepsilon^{-\frac{t}{2\tau}}\right\} \tag{16a}$$

$$\tau \gg \tau_L; \quad V_M = \frac{\beta K}{1+\beta K} V_c \left\{1 - \left(1 + \frac{t}{2\tau_L}\right) \varepsilon^{-\frac{t}{2\tau_L}}\right\}. \tag{16b}$$

Although these equations cannot be explicitly solved for time, it can be shown numerically that the membrane potential will reach 90% of its final value when

$$\tau_L \gg \tau; \quad t = 7.78\,\tau \tag{17a}$$

or

$$\tau \gg \tau_L; \quad t = 7.78\,\tau_L. \tag{17b}$$

Both the puffer ganglion [Eq. (17a)] and the squid axon [Eq. (17b)] could be clamped rapidly in a straightforward manner; the ganglion because τ is only 4.1 μsec, and the axon because τ_L is only 6.3 μsec (Table 1). The eel electroplaque, however, presented a more difficult problem: the 90% point would not be reached for 412 μsec [Eq. (17b) and Table 1] even when the current-electrode and solution resistances were decreased as far as possible in order to minimize τ_S and, thus, τ_L. This response is much too slow, particularly since some potassium conductance changes seem to be completed in this membrane during the first 100 to 200 μsec (Nakamura *et al.*, 1965; Morlock, Benamy & Grundfest, 1968). We have developed a new feedback technique to solve this problem. It decreases τ_L by electronic means, and its use promises improved clamping of other preparations as well.

If the voltage, $-\alpha RI_M$ (Fig. 2) is fed back to point A (disconnected from ground), Eq. (4) must be modified to

$$V_O = V_4 + \alpha R I_M. \tag{18}$$

Eq. (5) then becomes

$$V_M = V_4 - I_M(R_S - \alpha R) = V_4 - I_M R'_S \tag{19}$$

where $R'_S = R_S - \alpha R$ is the effective resistance in series with the membrane. This effective resistance can be decreased by adjusting α.

The previous discussion [Eqs. (1)–(17)] then remains valid provided that τ_S and τ_L are replaced by τ'_S and τ'_L, respectively, where

$$\tau'_S \equiv R'_S C_M \tag{20a}$$

and

$$\frac{1}{\tau'_L} = \frac{1}{\tau'_S} + \frac{1}{\tau_i}. \tag{20b}$$

As α is increased towards the point $\alpha = \frac{R_S}{R}$, τ'_S and τ'_L are decreased. A faster response for the critically damped condition can then be obtained.

In the theoretical case that the current and output amplifiers have infinite bandwidths, α can be set to $\frac{R_S}{R}$. Then R'_S and, hence τ'_L are both zero and Eq. (9) as well as Eq. (11) reduce to

$$\frac{dV_M}{dt} + \left(\frac{K+1}{\tau}\right) V_M = \frac{K}{\tau} V_c. \tag{21}$$

This expression describes the response of the clamp-load assembly under this new condition. It is first order, linear, and independent of the load. The solution to this equation is

$$V_M = \frac{K}{K+1} V_c \left\{ 1 - \varepsilon^{-\left(\frac{K+1}{\tau}\right)t} \right\}. \tag{22}$$

No ringing is possible, and the rate of approach to the final potential is governed by a time constant $\frac{\tau}{K+1}$ which depends only on the characteristics of the clamp. Theoretically, then, this feedback technique should enable the construction of a clamp whose rapidity is not limited by the nature of either the membrane or the current passing electrodes. It should be emphasized that this feedback system is designed to eliminate the effects of the resistance in series with the membrane but *not* located between the voltage measuring electrodes. The additional feedback is applied to the point A of Fig. 2. This differs from that feedback used by Hodgkin *et al.* (1952) both in purpose and in point of insertion. The technique used by Hodgkin *et al.* was designed to counter the effects of a resistance in series with the membrane, but located between the voltage measuring electrodes. For this latter resistance correction, feedback is applied prior to the point where V_M is measured[3].

In practice, the eel electroplaque as well as an analogue of the squid axon can, indeed, be clamped more rapidly in this manner. The theoretical limit $R_S' = 0$ cannot be achieved both because of the nonideality of the amplifiers and, because, as a result of polarization at the current electrode-solution interface, R_S is not really a simple resistance. If the feedback voltage, $-\alpha R I_M$ is modified by the addition of a term proportional to $\frac{dI_M}{dt}$ the nonideality of the amplifiers (i.e., finite rise time) could be partially overcome and the theoretical limit approached more closely. Indeed, with this type of feedback the electroplaque membrane reaches 90% of its final value in 30 to 50 μsec; a considerable improvement over the 412 μsec which is the rate that can be achieved by ordinary techniques.

Fidelity in Clamping. The gain K is commonly made larger in an attempt to force the membrane potential to follow the command in spite of excitation (Cole & Moore, 1960; Moore, 1971). This technique is not always as effective as desired; the potential may vary somewhat during the period

3 The Hodgkin, Huxley and Katz (1952) feedback has sometimes, however, been improperly inserted (Cole & Moore, 1960, Fig. 13; Moore & Cole, 1963, Fig. 6).

of sharpest excitation (*see* Moore & Cole, 1963, Fig. 8) and further increase in K cannot always solve this problem.

The source of this lack of fidelity can be identified. In the previous section, the rate of membrane potential change was analyzed for a passive unexcited membrane $\left(\frac{1}{\tau_e}=0, \frac{d}{dt}\left(\frac{1}{\tau_e}\right)=0\right)$. If the membrane continued to behave in this manner, a steady state governed by Eq. (11) would be reached. During this steady state

$$V_M=\frac{\frac{K}{\tau_S}V_c}{\frac{1}{\tau_i}+\frac{K+1}{\tau_S}} \tag{23}$$

so that for large K

$$V_M\cong V_c. \tag{24}$$

But the membrane is excitable and its long term behavior is therefore described by Eq. (9), and not Eq. (11). We may no longer ignore the time and voltage dependence of both the forcing function and the coefficients.

The effects of membrane excitation can be examined by recasting Eq. (9) to yield

$$V_M=\frac{V_c+\frac{\tau\tau_S}{K}\left\{\left[\frac{1}{\tau\tau_e}+\frac{d}{dt}\left(\frac{1}{\tau_e}\right)\right]E-\frac{d^2V_M}{dt^2}-\left(\frac{1}{\tau_e}+\frac{1}{\tau_i}+\frac{1}{\tau}+\frac{1}{\tau_S}\right)\frac{dV_M}{dt}\right\}}{\frac{\tau\tau_S}{K}\left\{\left(\frac{1}{\tau_e}+\frac{1}{\tau_i}+\frac{K+1}{\tau_S}\right)\frac{1}{\tau}+\frac{d}{dt}\left(\frac{1}{\tau_e}\right)\right\}}. \tag{25}$$

Since critical damping must be maintained, the relationship between τ and K is fixed by the appropriate constraint [Eqs. (14a) and (14b)]. For the puffer supramedullary ganglion

$$\tau_L\gg\tau; \quad \frac{\tau_L}{\tau}=4\beta K \tag{14a}$$

so that

$$\tau=\frac{\tau_L}{4\beta K}. \tag{26}$$

Thus, as K is increased τ should be decreased. Substituting with Eq. (25) under the condition of large K, we obtain

$$V_M\cong V_c+\frac{\tau_L\tau_S}{4\beta K^2}\left\{\frac{4\beta K}{\tau_L\tau_e}E-\frac{d^2V_M}{dt^2}-\frac{4\beta K}{\tau_L}\frac{dV_M}{dt}\right\} \tag{27}$$

and finally

$$V_M\cong V_c. \tag{28}$$

For the puffer ganglion increasing K is therefore an adequate method for achieving fidelity in voltage clamping.

In the case of the eel electroplaque or the squid axon, however, the situation is not as fortunate. For critical damping to be maintained we must have

$$\tau \gg \tau_L; \quad \frac{\tau}{\tau_L} = 4\beta K \tag{14b}$$

so that

$$\tau = 4\beta K \tau_L. \tag{29}$$

As K is increased, τ must also be increased. Substituting, again, with Eq. (25) under the condition of large K, we find that

$$V_M \cong \frac{V_c + 4\beta\tau_L\tau_S\left\{\frac{d}{dt}\left(\frac{1}{\tau_e}\right)E - \frac{d^2V_M}{dt^2} - \left(\frac{1}{\tau_e}+\frac{1}{\tau_i}+\frac{1}{\tau_S}\right)\frac{dV_M}{dt}\right\}}{1+4\beta\tau_L\tau_S\frac{d}{dt}\left(\frac{1}{\tau_e}\right)}. \tag{30}$$

The error terms that prevent the correspondence of V_M and V_c cannot in this case be eliminated by increasing K. The cause of fidelity loss with these cells during the period of sharpest excitation thus becomes clear; it is due to the terms containing $\frac{d}{dt}\left(\frac{1}{\tau_e}\right)$.

Fortunately, the new feedback technique discussed in the preceding section (*Rate of Membrane Potential Change*) can be used not only to increase the speed of response but also to improve fidelity. In the presence of this feedback, τ_S in Eq. (25) must be replaced with τ_S'. As the condition $\tau_L' = 0$ is approached, Eq. (25) would then yield

$$V_M = \frac{V_c - \frac{\tau}{K}\frac{dV_M}{dt}}{\frac{K+1}{K}} \tag{31}$$

so that, for large K

$$V_M \cong V_c. \tag{32}$$

The response is entirely independent of the load so that excitation has no effect on the fidelity of the clamp.

The effect of this kind of feedback was explored in experiments with both the eel electroplaque and an analogue of the membrane. The results of one experiment with the electroplaque are shown in Fig. 3. Marked

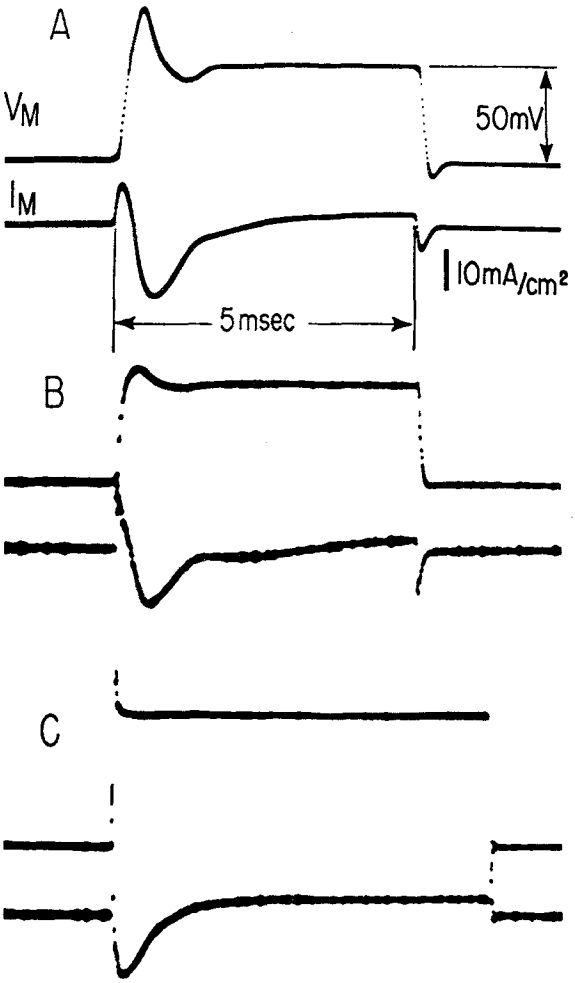

Fig. 3. Fidelity and the use of feedback to reduce the effects of τ_S. Three records obtained with a single voltage-clamped eel electroplaque. In each record, the upper trace is the membrane potential and the lower is the transmembrane current. Feedback to reduce the effects of τ_S was absent in (*A*), and introduced in (*B*). Both (*A*) and (*B*) are of an overdamped state, but in (*C*), τ has been decreased to produce critical damping

improvement was evident, even though the clamp circuit was not able to completely cancel the high series resistance R_S which increased the error terms that degrade the fidelity of the clamped membrane potential [*see* Eq. (28)]. However, in this experiment, the result of applying the additional feedback stood out more clearly.

In the membrane analogue (Fig. 4) an FET was used to generate a time dependent conductance channel, $g(t)$. This conductance varied between zero and a maximum during a command pulse. Fidelity is dependent on

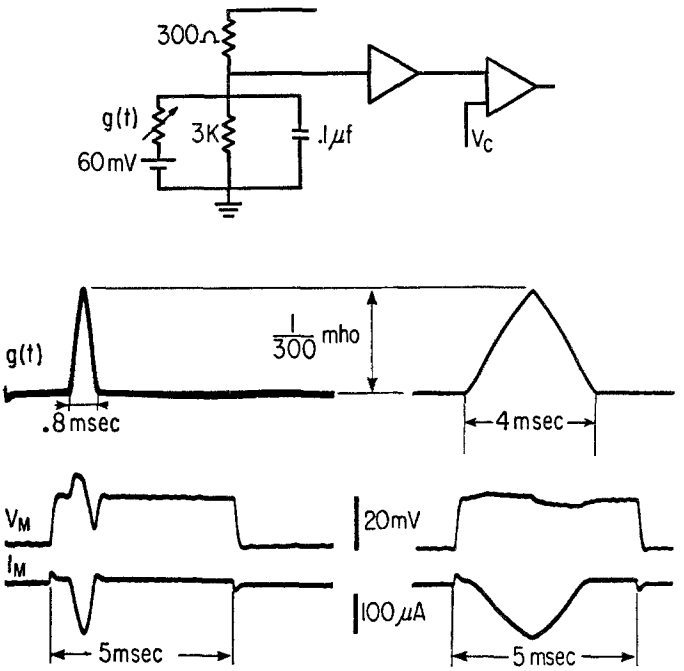

Fig. 4. Fidelity loss caused by a time varying conductance. The circuit used as a membrane analogue with an artificially generated time dependent conductance $g(t)$ is shown at the top. This conductance varied between 0 and a maximum. The clamp was adjusted for critical damping with $\tau \gg \tau_L$ and, in the absence of additional feedback to minimize the series resistance, $g(t)$ causes a loss of fidelity that is dependent on $\frac{dg}{dt}$

$\frac{dg}{dt}$ [Eq. (28)], and it is seen that the degradation of the clamped "membrane" potential is more pronounced for the more rapidly changing conductance. Fig. 5 shows the dramatic improvement in fidelity when the same membrane analogue is clamped, but this time τ_s is reduced with additional feedback.

Summary and Conclusions

1. The effectiveness of a voltage clamp depends upon the accuracy with which it can compel the membrane potential to follow a command pulse. This pulse is commonly rectangular. Accuracy must then be equated both with rapidity of membrane-response at the leading edge of the pulse, prior to excitation, and fidelity during the pulse, in the face of excitation when the conductance changes as a function of potential and time.

2. The clamp-membrane-electrode system can be described by a second-order differential equation. Rapidity of response depends on the achievement

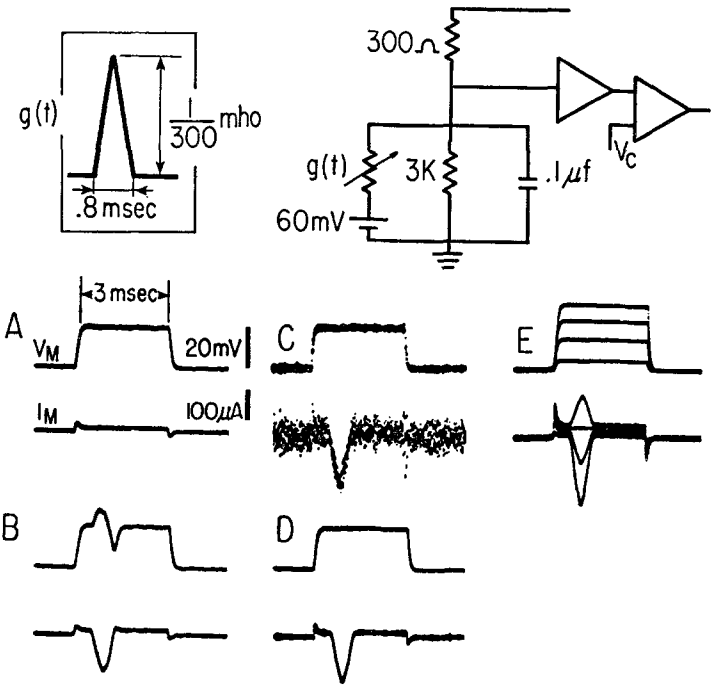

Fig. 5. Fidelity and the use of feedback to reduce the effect of τ_S in clamping a membrane analogue. (*A*) Voltage and current recordings for the passive ($g(t)=0$) membrane model brought into clamp with critical damping under the condition $\tau \gg \tau_L$ (circuit in upper right). (*B*) Recordings when $g(t)$ changes as shown in insert (upper left), but without feedback to reduce τ_S. (*C*) Feedback is introduced to cancel the series resistance. (*D*) The increased noise has been suppressed by decreasing the oscilloscope bandwidth. (*E*) The magnitude of the clamping potential was varied. The analogue behaves like a natural membrane and shows a reversal potential

of critical damping. This requires that a specified relationship between the time constant of the clamp τ and the time constant of its membrane-current-electrode load τ_L be satisfied. Depending on both τ_L and available amplifiers, one must have either $\tau \gg \tau_L$ as with the squid axon and eel electroplaque or $\tau_L \gg \tau$ as with puffer supramedullary ganglion. It is only in the former case that τ, the amplifier time constant, should be made available for adjustment by the investigator.

3. Speed of response, measured as the time required for the membrane potential to reach 90% of its final value, is given by 7.78 times τ_L or τ, whichever is the smaller. In the case that τ_L is smaller, speed of response may be materially improved by using another feedback loop which decreases τ_S and thus τ_L by electronic means.

4. Lack of fidelity results from conductance changes upon membrane excitation. Increasing the clamp gain reduces the error between the membrane potential and command pulse; but the requirements of critical damping set a limit to this. Fidelity can be significantly improved when feedback is used to decrease the effects of τ_S. However, since these effects cannot in practice be completely cancelled, increasing the gain and using this type of additional feedback should be viewed as complementary methods.

5. Experiments with the eel electroplaque as well as analogues of various membranes have confirmed the salient features of this analysis. However, several secondary problems were neglected for the sake of simplicity. For example, the amplifiers are really not ideal. The nonideality of each amplifier may be represented by an additional time constant; but each additional constant increases the order of the governing differential equation by one. Therefore, these time constants must be made small enough to be negligible. This constraint also applies to the additional time constants, introduced when microelectrodes (even if capacitatively compensated) are used to monitor the membrane potential. That this can be achieved is confirmed by the fact that such electrodes, or their analogues, were used in many of the confirmatory experiments. However, it was found that microelectrodes can create a different problem in the presence of current in the bath. An artifact is then generated that does interfere with successful clamping. This problem and its cure have been discussed elsewhere (Schwartz & House, 1970).

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