SYNAPTIC INTEGRATION MECHANISMS

Theoretical and Experimental Investigation of Temporal Postsynaptic Interactions Between Excitatory and Inhibitory Inputs

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ABSTRACT The effect of temporal activation of two closely adjacent synaptic inputs upon the postsynaptic output (voltage amplitude and time integral) is analyzed theoretically and experimentally. It is shown that (*a*) under certain conditions, maximal nonlinearity in the summation of postsynaptic potentials is obtained with asynchronous activation of the two synaptic inputs rather than with simultaneous activation; (*b*) the time integral of the voltage is more sensitive to the timing of the synaptic inputs than is the voltage amplitude; (*c*) an input, which by the classical definition is inhibitory, under defined conditions can and does increase the amplitude (and area) of an excitatory synaptic potential, and thus acts as an excitatory input.

INTRODUCTION

Nervous integration at the cellular level is affected by the nature of the synapses (excitatory, inhibitory, electrical) (Furukawa, 1966; Burke et al., 1979), the geometry of the postsynaptic cell (Rall, 1964; Rall and Rinzel, 1973), the spatial organization of synaptic inputs (Rinzel and Rall, 1974; Jack et al., 1975), and the temporal relations between different inputs. While theoretical and experimental analysis is available for some of these problems (Rall, 1962; Jack and Redman, 1971 *a*, *b*; Barret and Crill, 1974; Jack et al., 1975; Torre and Poggio, 1978), the analyses of temporal aspects of closely adjacent synaptic inputs have received little attention.

Previous theoretical studies analyzing temporal aspects of postsynaptic interactions only dealt with the simpler cases where the synaptic inputs were represented either by delta function conductance changes (MacGregor, 1968) or by small, constant amplitude PSP, i.e., unaffected by nonlinear summations (Segundo et al., 1968, and see also Barnwell and Cerimele, 1972). Thus, the nonlinear interactions between different adjacent synaptic inputs, resulting from both conductance changes and changes in the driving forces were not considered. Moreover, in these studies, only the temporal effects of synaptic inputs on the final amplitude of the synpatic response were taken into account, and attention was not given to the area of the postsynaptic potential that is of physiological significance (Calvin, 1975).

In the present paper we present both a theoretical analysis and experimental demonstrations of the postsynaptic effects of the interaction between two closely adjacent synapses, paying particular attention to the influence of timing. We treated the case of an isopotential cell with two synaptic inputs located at the same point. In the theoretical analysis, one synapse was taken as excitatory in the sense that its reversal potential is above threshold. The second is taken as inhibitory, in the sense that its reversal potential is below threshold (Ginsborg, 1967). For the experimental section of this study, we selected a system that approaches this condition: the crustacea neuromuscular system. Its polyneural and multiterminal innervation (Takeuchi and Takeuchi, 1965; Atwood, 1967), the spread of inputs all along the muscle fiber (Atwood, 1967), and the electrical proximity of its inhibitory and excitatory terminals (Atwood and Bittner, 1971) make this system appropriate for such analysis.

We show that (*a*) maximal nonlinearity (minimal response or maximal inhibition) in the summation (amplitude and area) of the postsynaptic potentials (PSPs) is not necessarily obtained when both inputs are activated simultaneously; (*b*) the nonlinearity in the summation of the two synaptic potentials is larger for the area than for the amplitudes; and (*c*) a classically defined IPSP (inhibitory postsynaptic potential) may increase the EPSP (excitatory postsynaptic potential) amplitude (and area) even when the two inputs are activated simultaneously. Thus, a single synapse may have two modes of effect (inhibitory or excitatory) upon a second synapse, depending on the relative timing of their activation and on the parameter of the postsynaptic potential that is examined.

The temporal nature of postsynaptic interaction and its physiological significance is discussed, in view of these results.

Appendix by I. Segev.

THEORETICAL ANALYSIS

A schematic representation of an isopotential cell with two inputs is given in Fig. 1 *A*. The equivalent circuit is given in Fig. 1 *B* in which g_0 is the resting conductance (the resting potential taken to be zero) and g_1, E_1, g_2, and E_2 are the conductances and electromotive forces of the synapses S_1 and S_2, respectively. We assume that both g_1 and g_2 are positive constants for the duration of t_1, i.e., that the synaptic inputs are represented by a transient step conductance increase (Hubbard et al., 1969).[1]

In the following analysis g_2 appears with a delay ΔT with respect to g_1 as shown in Fig. 1 *C*. The differential equations that govern the change in the voltage $V(t)$, produced at the circuit of Fig. 1 *B* is

$$cdV/dt = g_0V + g_1(V - E_1) + g_2(V - E_2). \qquad (1)$$

The solution of Eq. 1 is given in Appendix A.

The Voltage Amplitude

The amplitude of the postsynaptic potential is an important parameter for neuronal integration. It is the most frequent parameter examined both theoretically (Rall, 1960; Rall, 1964; Jack and Redman, 1971 *a*; Torre and Poggio, 1978), and experimentally (Rall et al., 1967; Jack and Redman, 1971 *b*). For example, the effect of the neuron geometry (Rinzel and Rall, 1974; Poggio and Torre, 1977), membrane properties (Barret and Crill, 1974), and the location of synaptic inputs (Rall, 1964; Rall et al., 1967) on the amplitude of the synaptic potential along the cell structure have been analyzed (see also Burke et al., 1979).

We first analyzed the voltage amplitude produced by the two inputs as affected by their temporal relation.

Minimal Amplitude

Proposition 1. Suppose that both synaptic inputs (g_1 and g_2) depolarize the postsynaptic cell so that the mutual steady-state potential of S_1 and S_2 ($[g_1E_1 + g_2E_2]/[g_0 + g_1 + g_2]$) is bigger than E_2. Assume that $E_1 > E_2 \geq 0$. Under these conditions, minimal amplitude is obtained if S_1 is activated first, and S_2 is activated at the time when the voltage of S_1 reaches E_2.

The formal treatment of the proposition is given in Appendix A. The physiological implications of this proposition are as follows:

(*a*) Minimal amplitude is obtained when S_2 contributes only inward (negative) current during its activation.

(*b*) For $E_2 > 0$, to obtain minimal amplitude the two synapses should be activated asynchronously. In such a case, the delay t^* by which S_2 should follow S_1 is independent of both the duration (t_1) and the magnitude of the conductance g_2 (see below, Eq. 3).

(*c*) For $E_2 = 0$, simultaneous activation of both inputs (i.e., the time when S_2 reaches E_2) results in minimal voltage amplitude.

A logical extension from the proposition is that simultaneous activation is the preferred timing to obtain minimal amplitude also for cases where $E_2 < 0$ (hyperpolarizing synapse). This is intuitively true because in this case, S_1 voltage is reached closest to E_2 at $t = 0$, i.e., at the beginning of S_1 activation (see Appendix A).

[1]In our treatment we simulate the synaptic conductance changes by step functions rather than by the more conventional "alpha" functions (Rall, 1967; Jack and Redman, 1971 *a*). This is done only to obtain analytic solutions of the equations. However, because the alpha functions resemble step functions in a certain sense, the solution with an alpha function will resemble the solution with a similar step function. The mathematical reason for this is that small perturbations in a differential equation cause usually only small changes in the solution. Indeed, we have solved Eq. 1 numerically, using several alpha functions, and the results concerning the timing effects were essentially the same as with step functions (<5% difference).

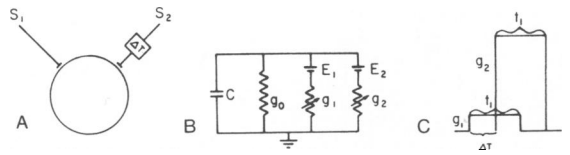

FIGURE 1 *A*, schematic representation of an isopotential cell with two synapses, S_1 and S_2. *B*, equivalent circuit of the case described in *A*. The resting conductance is g_0 (the resting potential is taken as zero) and g_1, E_1 and g_2, E_2 are the conductances and the driving forces of the synapses S_1 and S_2, respectively. *C*, an example of a possible timing between the activation of the two inputs. Here, $\Delta T > 0$, i.e., S_2 activation follows S_1 by a delay of ΔT. Both conductance changes continue for a duration of t_1.

Using Proposition 1, we compute the best timing t^* (the time for the S_1 potential to each E_2) to obtain the minimal amplitude as follows. The development of S_1 potential in time is

$$V(t) = g_1E_1[1 - e^{-(g_0+g_1)t/c}]/(g_0 + g_1). \qquad (2)$$

According to Proposition 1, t^* is such that $V(t^*) = E_2$. Thus, from Eq. 2 we get

$$t^* = [c/(g_0 + g_1)]\ln[1 - (1+g_0/g_1)(E_2/E_1)]^{-1}. \qquad (3)$$

For example, if we use the parameters $g_1/g_0 = 1.5$, $E_2 = 5$ mV and $E_1 = 100$ mV, we find that $t^* = 0.0349\tau$ ($\tau = c/g_0$). (Fig. 2, arrow on abscissa.)

The preferred timing depends on E_2 (t^* increases as E_2 becomes more positive), and the value of E_2 determines the degree of the difference between the minimal amplitude (obtained at t^*) and that obtained at simultaneous activation. Thus, for small E_2 the difference is small and it becomes significant (a few milivolts) for larger positive E_2 (Fig. 3).

From Fig. 3 it can be seen that with simultaneous activation, S_2 acts as an inhibitory synapse (reduces S_1 amplitude) for $E_2 < 8.5$ mV (arrow 1). For E_2 greater than this value, simultaneous activation of both synapses (dashed line) results in a larger amplitude than produced by S_1 alone (dotted line). Hence, for the conditions set in the calculations, when $E_2 >$

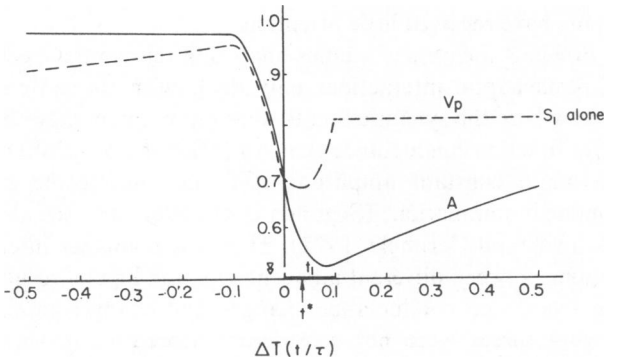

FIGURE 2 Effects of the timing (ΔT) of S_2 activation with respect to S_1 on the PSP amplitude (V_p) and area (A). Linear summation is at $V_p = A = 1$. The arrow at t^* indicates the delay that yields minimal amplitude (Eq. 3). Between -0.03 (double arrow) and t_1, S_2 acts as an inhibitory synapses. Above $\Delta T = t_1$, there is no effect, while below $\Delta T = -0.03$ it adds to the amplitude of S_1. For A, between $\Delta T = -0.03$, and 0.97 (not shown), S_2 acts as an inhibitory synapse and outside this interval, S_2 increases S_1 area. The minimal area is obtained at $\Delta T = 0.09$ and is significantly smaller than the one produced by simultaneous activation. Parameters were: $g_1/g_0 = 1.5$, $g_2/g_0 = 10$, $E_1 = 100$ mV, $E_2 = 5$ mV, $t_1 = 0.1\ \tau$.

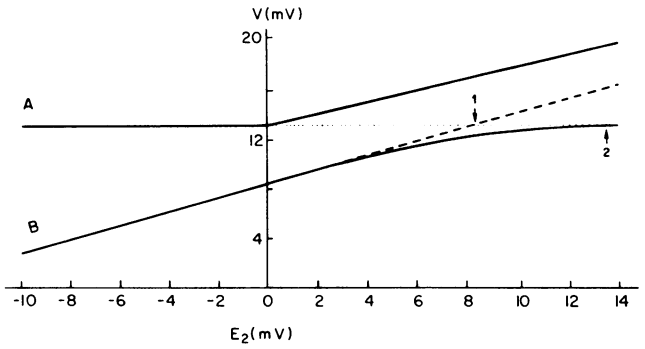

FIGURE 3 PSP amplitude as a function of E_2. *A*, the maximal amplitude that may be obtained from the two synapses S_1 and S_2. *B*, the minimal one. ---, the amplitude obtained in the case of simultaneous activation of the two synapses. $\cdots$, the amplitude of S_1 when active alone. For simultaneous activation, S_2 inhibits S_1 for E_2 below 8.5 mV (arrow 1). It may continue to inhibit S_1 up to $E_2 = 13.27$ mV (arrow 2) if it is activated at the preferred timing t^* (see text). Parameters were $g_1/g_0 = 1.5$, $g_2/g_0 = 10$, $E_1 = 100$ mV, $t_1 = 0.1\ \tau$.

8.5 mV, a synapse (S_2), which by the classical definition is inhibitory (its reversal potential lies below threshold), now adds to the potential produced by S_1 alone, possibly reaching value above the threshold. This is true even though the values of E_2 (between arrow 1 and 2) are below S_1 amplitude (13.27 mV), so that one may intuitively have expected S_2 rather to reduce S_1 amplitude (to act as an inhibitory synapse). However, it should be noticed that it is the steady-state value of S_1 together with S_2 (and not that of E_2), toward which the voltage tends to develop. This value is more positive than that of the S_1 amplitude alone, for the parameters we have chosen (g_1/g_0, g_2/g_0, E_1, E_2).

However, as can be seen from Fig. 3, at E_2 values above 8.5 mV and below 13.27 mV (arrows in Fig. 3), S_2 may continue to inhibit S_1 if it is activated at the preferred (t^*) timing (continuous line). At arrow 2, E_2 is equal to S_1 amplitude and no inhibition exists. A corollary of these results is that the mode of the postsynaptic effect of one synapse upon a second postsynaptic potential depends on their timing. It may have an excitatory effect at certain time intervals and an inhibitory effect at other intervals (see Discussion and Experimental Results).

Maximal Amplitude

Using the same arguments of Proposition 1, it is possible to show which timing is the necessary one for obtaining the maximal amplitude of the summed postsynaptic potential.

Proposition 2. For the same conditions of Proposition 1, maximal amplitude is obtained in the case where S_2 is activated first, and S_1 is turned on as the S_2 conductance change is turned off. The proof is given in Appendix A.

The physiological implications of this result are that when the reversal potential of both synapses is positive, maximal potential amplitude is obtained for their successive rather than for simultaneous activation (Fig. 2, V_p). When E_2 is negative, the maximal amplitude is that of S_1 alone; it increases as E_2 becomes positive (Fig. 3 *A*).

A detailed description of the dependence of the PSP amplitude (with respect to linear summation) on the timing between S_1 and S_2 is given in Fig. 2 (V_p). For $\Delta T > t_1$ (0.1) no inhibition exists, and the PSP amplitude is that of S_1 alone. Inhibition does exist for $-0.03 > \Delta T > 0.1$ (double arrows to t_1) where a decreased amplitude, compared with that of S_1 alone, is obtained. The minimum of V_p (maximal inhibition) is reached at $\Delta T = t^* = 0.0349$ (Eq. 3).

However, only a small decline in V_p is found between the case of simultaneous activation of both synapses (at $\Delta T = 0$, $V_p = 0.7$) and the minimal one (at $\Delta T = t^*$, $V_p = 0.68$). For $\Delta T < -0.03$, S_2 starts to add to S_1 amplitude and the maximum of V_p is obtained at $\Delta T = -t_1$ (Proposition 2) where $V_p = 0.96$. It continues to add to S_1 amplitude also for more negative ΔT's. As ΔT becomes more negative, S_2 potential decays towards zero before S_1 activation, and the PSP amplitude tends to reach that of S_1 alone.

The Voltage Time Integral

The time integral of the postsynaptic potential is another important parameter to evaluate synaptic efficacy. In certain cases it is a better measure for describing the efficacy and the contribution of the synaptic input to the summed postsynaptic potential than is the voltage amplitude (Rall, 1959; Calvin, 1975). In barnacle muscle, Ashley and Ridgway (1970) showed that prolongation of a constant amplitude depolarizing pulse increases the muscle tension. Others (Connor and Stevens, 1971; Sokolov and Cooke, 1971) showed an increase in the number of action potentials when the duration of a constant amplitude pulse increases (Barret and Crill, 1974; Rinzel and Rall, 1974; Jack et al., 1975; Calvin and Graubard, 1979; Gardner, 1980).

Thus, we found it important to analyze the effect of the timing between the two inputs on the PSP area.

Minimal Area

Proposition 3. For the same synapses of Proposition 1, to obtain minimal area of the PSP, S_1 and S_2 conductance changes should overlap (i.e., $0 \le \Delta T \le t_1$). A formal treatment of this proposition is given in Appendix B.

In Fig. 2 we plot the effect of the timing between S_1 and S_2 upon the normalized area (A). The nonlinearity in the summation of the areas of S_1 and S_2, as a function of their timing, is much more pronounced than in the summation of the amplitudes (compare V_p and A in Fig. 2). At the best timing ($\Delta T = 0.09$), the PSP area reaches a minimum that is 0.53 of the linear one ($A = 1$). This is in agreement with the statement (Proposition 3) that minimal area is reached for timing such that $0 \le \Delta T \le t_1$.

It should be noted that unlike the voltage amplitude, the area at the best timing is significantly smaller than the one obtained at $\Delta T = 0$ (where $A = 0.7$). In other words, for the voltage time integral parameter, simultaneous activation of the synaptic input is never the preferred timing for obtaining minimal area. Note also that, unlike the PSP amplitude, its area is affected also for timing in which $\Delta T > t_1$. In such cases, the activation of S_2 accelerates the decay of the S_1 voltage (see Appendix B), and as a result, the PSP area is reduced.

EXPERIMENTAL RESULTS

Methods

The abdominal superficial flexor nerve-muscle preparation of the shrimp *Macrobrachium rosenbergi* was used. This neuromuscular system in crayfish is without presynaptic inhibition (Atwood, 1967), and the same seems to hold for *Macrobrachium* (Segev and Parnas, unpublished). For intracellular recording, 5–10 M Ω microelectrodes filled with 3 M potassium acetate were used. Fine glass suction electrodes, 10–20 μm in diameter, were placed on the nerve to stimulate selectively the excitatory and the inhibitory axons (Atwood et al., 1967). At each time interval between the inhibitory and the excitatory inputs, the postsynaptic potential (PSP) at the muscle was measured, and up to 32 sweeps were averaged and recorded on a digital tape, using the Nicolet Instrument Corporation (Madison, WI) 1074 averager. The values of the PSP amplitude and time integral were digitally measured, using the data analyzing programs of the Nicolet. We define simultaneous activation ($\Delta T = 0$) with reference to the case in which the inhibitory and the excitatory voltage peaks (as measured when each alone is active) appear at the same time.

RESULTS

Fig 4 shows that the experimental preparation is indeed appropriate for the theoretical model. Using the Nicolet digital measurements, the decay of the membrane potential that results from intracellular current pulse (*a*) and that of the EPSP (*b*) as a function of time are plotted. As can be seen, the EPSP decays exponentially with $\tau = 82$ ms, although the decay of the membrane potential that results from intracellular current pulse is faster and not exponential. (It approaches the error function of $\sqrt{t/\tau}$.) This result suggests that the excitatory input is homogeneously distributed over the muscle length; i.e., the cell is isopotential for this input (Rall, 1960). The decay time course of the IPSP was found to be close to that of the EPSP (not shown). Hence, as far as these two synaptic inputs are concerned, this experimental system approaches the conditions assumed for our theoretical analysis.

Examples of the EPSP and a depolarizing IPSP are shown in Fig. 5. In each of the cases *A–D*, the potential is shown in the lower trace and its time integral on the upper one. In *A–C*, two different timings of the IPSP (filled arrows) on the EPSP (empty arrows) are shown. For example, in *A*, both timings are such that the activation of the inhibitory input precedes that of the excitatory one, but in *C* both follow it. (In Fig. 5 *D*, the IPSP alone is shown.) Timing the IPSP to appear at different intervals along the decay phase of the EPSP, a reversal potential of +3 mV (above the resting potential) was found for the IPSP (not shown). A quantitative analysis of the same experiment is given in Figs. 6 and 7, which should be compared to Fig. 2, above. In Fig. 6, the effect of the timing between the two

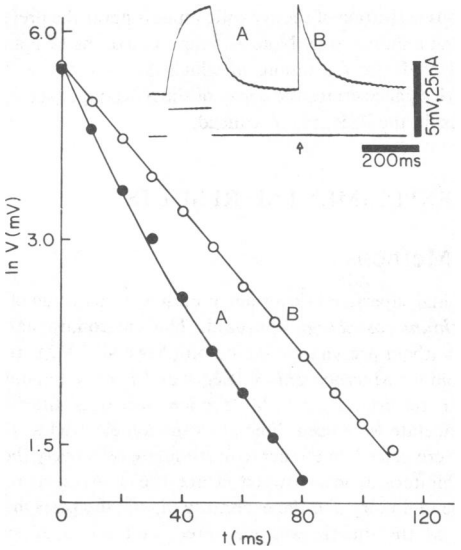

FIGURE 4 The decay of membrane potential induced by intracellular current pulse (*A*) and that of an EPSP (*B*) in the same cell. *Inset*, experimental results. The points in the graph were taken from the *inset*. As can be seen, the EPSP decays exponentially with time constant $\tau = 82$ ms although the potential induced by the current pulse decays faster and nonlinearly. Empty arrow (*inset*) indicates EPSP activation.

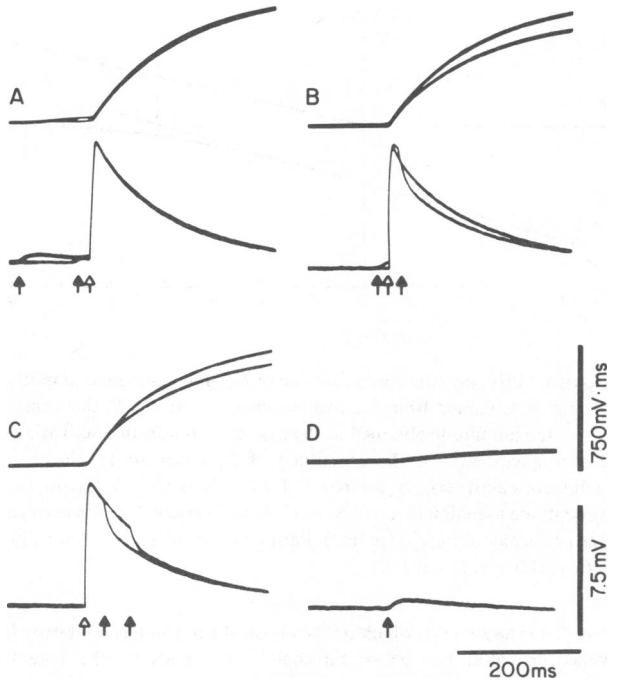

FIGURE 5 *A–C*, effect of the timing of a depolarizing IPSP with respect to the EPSP on the amplitude of the PSP (lower traces) and its time integral (upper traces). In *A–C*, two timings of the IPSP (filled arrows) on the EPSP (empty arrows) are superimposed. Each response is an average of 32 sweeps. *D*, the IPSP and its time integral.

inputs on the postsynaptic voltage amplitude is shown. As expected for this case, linear summation of the amplitudes result in a greater potential than that of the EPSP alone.

As predicted above, the minimal amplitude is not obtained with simultaneous activation. It seems to occur with $\Delta T = 0.08$, the time when the EPSP reaches a value of 3.5 mV, which is close to the IPSP reversal potential. The

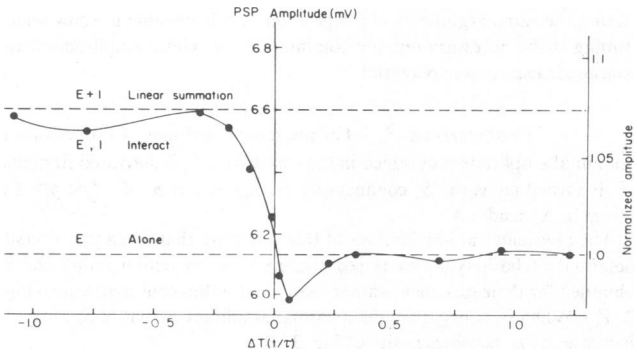

FIGURE 6 Effect of the timing of a depolarizing IPSP (*I*) interacting with an EPSP (*E*) on PSP amplitude. The graph was composed from a series of measurements of which part is shown in Fig. 5 *A–C*. At $\Delta T = 0$, the peaks of the IPSP and that of the EPSP appear simultaneously. The time scale (abscissa) was normalized in units of the resting time constant as found from the decay of the EPSP. Right ordinate shows the normalized amplitude with respect to the amplitude of the EPSP alone. Note that the maximal reduction of the peak is not at zero interval. (Compare with Fig. 2.)

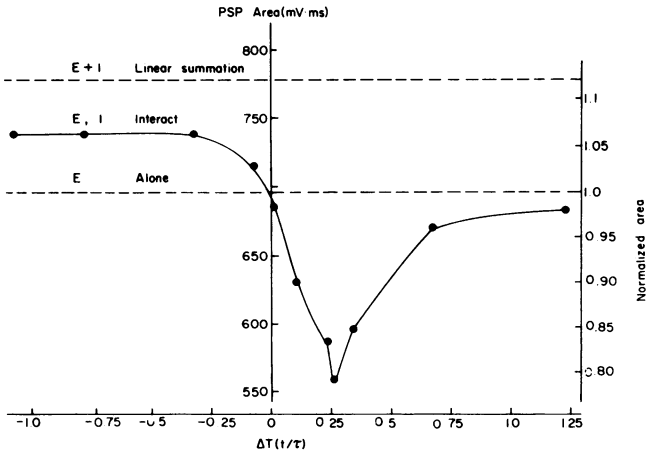

FIGURE 7 Effect of the timing of a depolarizing IPSP (*I*) with respect to an EPSP (*E*) on the time integral of the PSP. Same preparation of Figs. 5 and 6. ΔT is the same as defined in the previous figure. The right ordinate shows the normalized area with respect to the area of the EPSP alone. Note the marked nonlinearity (minimal area) that is obtained at a positive $\Delta T = 0.25$. (Compare with Fig. 2).

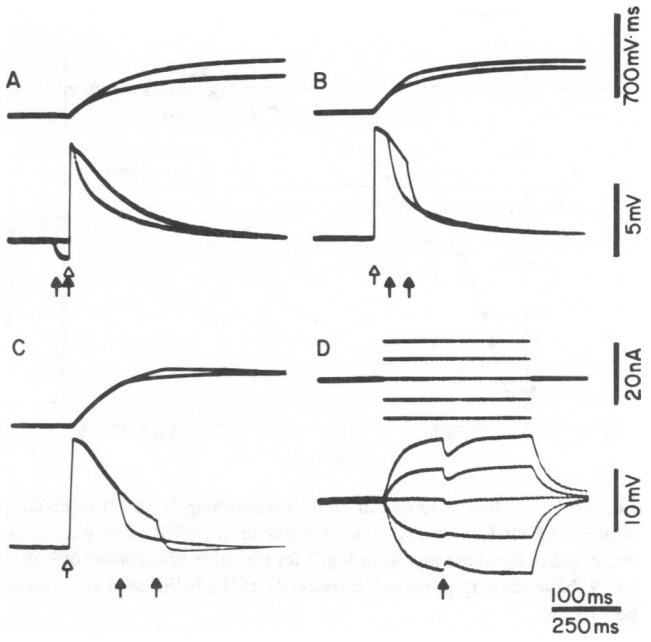

FIGURE 8 Effect of the timing of an hyperpolarizing IPSP with respect to the EPSP on the PSP time integral (upper traces) and amplitude (lower traces). *A–C*, as in Fig. 5. *D*, reversal of the same IPSP of *A–C*, by passing current with a second intracellular microelectrode (upper traces). Calibration, *A–C*, 100 ms, 5 mV, and 700mV · ms. *D*, 250 ms, 10 mV, 20 nA. Filled arrows, inhibitory stimulation. Empty arrows, excitatory stimulation. The reversal potential of the IPSP is −3.5 mV relative to the resting potential (see text).

maximal amplitude, which is bigger than that of the EPSP alone, occurs when the inhibitory input precedes the excitatory one.

The effect of the inhibitory input on the voltage time integral is examined in Fig. 7. A pronounced (>20%) reduction in the EPSP area is found: the EPSP area of 695 mV·ms is reduced to a minimum of 560 mV·ms, at $\Delta T = 0.25\tau$. The IPSP affects the EPSP area for a wider range of time intervals than in the case of its amplitude. For this case, the IPSP doesn't always reduce the EPSP area. For timings such that $\Delta T < 0$ the IPSP area is added to that of the EPSP up to a maximum that reaches 740 mV·ms.

In another cell the IPSP was hyperpolarizing, and its effects were examined (Fig. 8). In this case, the reversal potential was determined by passing current with a second intracellular electrode (Fig. 8 *D*). The value of −3.5 mV found in this way, is a slight over-estimation, because in this cell the synapses are distributed although the current is injected at one point in the middle of the cell.

Figs. 9 and 10 show the quantitative analysis for the cell of Fig. 8. In Fig. 9, the PSP amplitude is plotted. Here, in contrast with Fig. 6, the peak of the EPSP alone is higher than that which results from a linear summation of the IPSP and the EPSP amplitudes. The minimum is obtained at $\Delta T = 0$. Because the minimal amplitude is close to the value of linear summation, it seems that the reduction of the EPSP amplitude, due to the activation of the IPSP, is almost entirely explained by the hyperpolarizing effect of the IPSP. This finding implies that here the IPSP conductance increase is relatively small in comparison with the one that was taken for the theoretical computation.

Here too, the area of the postsynaptic potential (Fig. 10) was found to be affected to a greater degree than the voltage amplitude; only the positive area was taken. Minimal area is obtained near $\Delta T = 0$, where a reduction of 46% (from 660 to 358 mV·ms) is obtained. Unlike the voltage amplitude, the area is reduced much below of that expected from a linear summation of the two inputs.

As in the case of Fig. 7, the behavior of the PSP area as a function of the time interval ΔT fits well with the theory. The inhibitory effect is marked; it acts over a wide range of time intervals and its maximal effect appears near $\Delta T = 0$ (see Discussion).

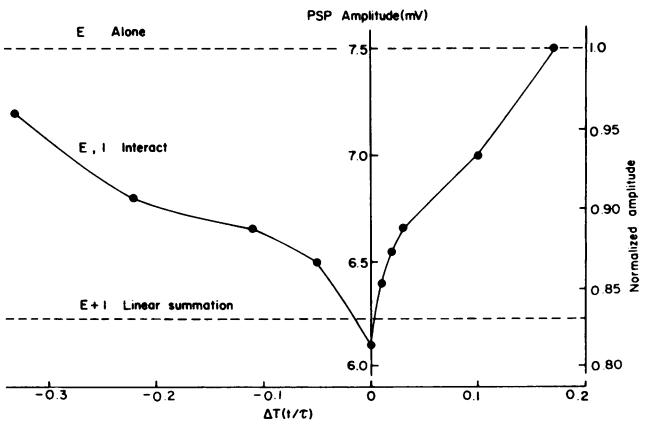

FIGURE 9 Effect of the timing of an hyperpolarizing IPSP (*I*) with respect to the EPSP (*E*) on the PSP amplitude. The graph was composed in the same way as in Fig. 6 from a series of measurements of which part is shown in Fig. 8. Note that the minimum is at $\Delta T = 0$.

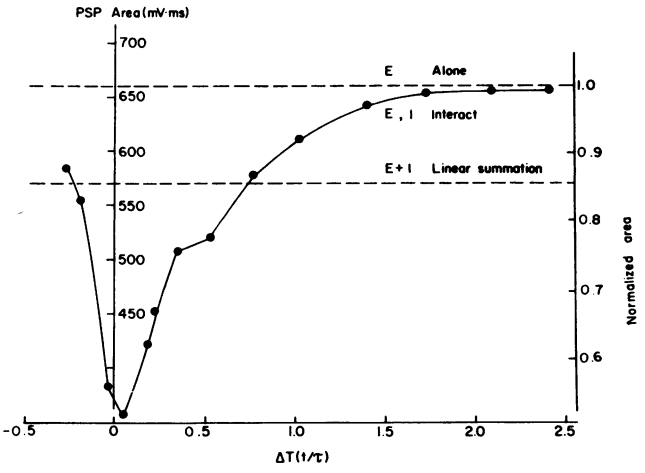

FIGURE 10 Effect of timing of an hyperpolarizing IPSP (*I*) interacting with an EPSP (*E*) on the time integral of the PSP. The graph was composed in the same way as in Fig. 7 for the same preparation of Figs. 8 and 9. Note the very pronounced reduction of the EPSP area at a slightly positive ΔT.

DISCUSSION

In recent studies, it has been suggested that the temporal pattern of the synaptic activation can be of major importance for nervous integration. Rall (1964) was the first to show that the somatic potential is critically dependent on the timing of synaptic inputs, which are arranged in an orderly sequence of distances from the soma. On the basis of this finding, Erulkar et al. (1968) explained the ability of a neuron in the cochlear nucleus to detect the direction of an auditory stimulus. Torre and Poggio (1978) suggest that nonlinear summation and the timing between the postsynaptic inputs may be responsible for the direction selectivity shown by the visual system. Abeles (1982 *a*, *b*) argues that the temporal pattern of the synaptic inputs is the main parameter that determines whether a neuron in the auditory system will fire. Although several studies have theoretically treated temporal aspects of postsynaptic integration (MacGregor, 1968; Segundo et al., 1968), neither theoretical nor careful experimental study of the temporal aspects of postsynaptic interactions between closely adjacent inputs on the level of a single neuron, have been performed. As a result, there exists a general agreement that simultaneous activation of adjacent synapses results in the minimal output (Iansek and Redman, 1973; Jack et al., 1975).

In the present paper, we analyzed the simplest case, the isopotential cell in which no spatial effects exist. In this way, we could separate both theoretically and experimentally the temporal aspects of the postsynaptic interaction from spatial effects. Furthermore, we chose to analyze the case where only two inputs interact postsynaptically. Despite these restrictions, this analysis is of physiological interest and is not only applicable to the crayfish neuromuscular junction as demonstrated here, but is also relevant to the case of closely adjacent synapses located on the same dendritic branch, where the PSP is affected by both the shunting effects of the synaptic conductance changes and by changes in the driving forces for the synaptic currents (Iansek and Redman, 1973; Torre and Poggio, 1978).

The Dual Effect of a Synapse

Our analysis is concerned with two parameters of the postsynaptic output. We found conditions where a synapse, which is classically defined as inhibitory (namely, a synapse whose reversal potential is below threshold, e.g., Ginsborg, 1967) may have two modes of effect upon the potential produced by another excitatory synapse. At some timings between the two synaptic inputs it may reduce the potential area or amplitude of the other synapse while at others it adds to it (Figs. 2, 6, 7). As was shown in Fig. 3, such an addition may take place even when the two inputs are activated simultaneously. This will happen only when both inputs produced by conductance increase and are depolarizing (see Figs. 6, 7). Depolarizing inhibitory potentials were found in various cells, and are summarized by Ginsborg (1967, Table II). For such cases, the synapse may be defined as excitatory or inhibitory according to its most pronounced effect and not by its reversal potential relative to threshold. Thus, in the case shown in Fig. 7, the maximal inhibition (minimal area) is larger than the maximal excitatory effect, and this synapse may be defined as an inhibitory one. However, according to the same criterion, the same synapse should be defined as excitatory when the potential amplitude parameter is considered (Fig. 6). This calls for attention to the fact that the nature (inhibitory or excitatory) of a synapse depends on the postsynaptic parameter that is examined.

PSP Amplitude vs. Time Integral

In this study, it was shown theoretically and experimentally that the potential time integral is affected to a larger extent by the timing of the synaptic inputs than is the amplitude of the synaptic potential (Figs. 2, 6, 7, 9, 10). (See also Jack et al., 1975, p. 199, for a related problem). In contrast to the amplitude, the time integral is affected also at times following the potential peak in which the two inputs may interact. Because most of the EPSP area appears at times that follow its peak, the shortening of its decay time, due to the activation of the IPSP, results in a pronounced reduction of its area (Figs. 2, 7, 10). Furthermore, when the conductance change of the excitatory input is brief, its amplitude is expected to be affected only slightly by the change in the input resistance, which results from the activation of the inhibitory input (Hubbard et al., 1969).

Physiological EPSP conductance changes also continue at times following its peak. We found that the EPSP rise time is only 0.025–0.1 of τ and that its exponential decay starts only 0.075–0.2 τ following the peak. Because the

onset of exponential decay signals the end of the conductance change, the relatively long period between the potential peak and the onset of exponential decay represents a period in which the input conductance of the cell decreases towards its resting value. In the theory, however, we took conductance changes of the synapses to be transient step functions, where the rise time of the PSP is equal to the duration of the conductance change. In Fig. 2, we set g_1 duration to the relatively large value of 0.1 τ, and thus more pronounced nonlinearity in the peak summation is found in this figure compared with the experimental ones (Figs. 6, 9). Although our analysis is only qualitatively comparable to the experimental results, and though we did not intend to attempt to obtain a quantitative fit, the results concerning the timing effects also hold for synaptic conductances other than step functions (see footnote 1).

The difference between the behavior of the EPSP amplitude and area, as affected by the activation of the IPSP, is even more pronounced for the many experimental cases in which the duration of the inhibitory input was found to be much longer than that of the excitatory one.

The Preferred Timing for Minimal Postsynaptic Response

A careful analysis of the effect of the timing between the two inputs upon the postsynaptic output reveals that the common view that minimal output is obtained when the two inputs are activated simultaneously (Jack et al., 1975, p. 194) is not always correct. Thus, simultaneous activation yields minimal amplitude only in cases where both inputs are of opposite signs (depolarizing vs. hyperpolarizing) accompanied by a conductance increase (Fig. 9). When both inputs are of the same sign, minimal amplitude is obtained when the two inputs are activated asynchronously (see Eq. 3 and Figs. 3, 6). However, the difference in the amplitude obtained with simultaneous activation and the minimal amplitude is large only when the reversal potential of the inhibitory synapse is not close to the resting potential, as can be seen in Fig. 3. On the other hand, the minimum of the potential time integral is more pronounced and is always at asynchronous activation of the two input. These conclusions are general and do not depend on the shape of the synaptic conductance changes. The experimental results (Figs. 7 and 10) agree with the theoretical prediction (Proposition 3 and Fig. 2) that the minimal area is obtained when the conductance changes of the two synaptic inputs overlap.

Implications of Results to Integrative Mechanisms

Our results suggest that the area of the PSP is the parameter that is most affected by temporal interactions between adjacent inhibitory and excitatory synaptic inputs. For cases where PSP area is indeed the important parameter for integration processes, the concept that the postsynaptic inhibition has a fixed effect of reducing the postsynaptic response by a constant amount should be modified. Rather, its effect should be viewed as that of a fine modulator, which may be activated at different timings and thus regulate the postsynaptic response. This is in addition to other known processes (summation, facilitation, desensitization, presynaptic inhibition, etc.) that enable the modulation of the postsynaptic response.

Our analysis describes the nonlinear interaction in the case where the inhibitory and the excitatory conductance changes occur at the same site. However, significant nonlinearities may occur also when the two inputs are spatially remote. We found marked nonlinearities in the PSP response at the soma, when the inhibitory synapse is located on the way between the distant excitatory one and the soma (Segev, unpublished calculation). For this case, the inhibitory input should be activated when the attenuated EPSP arrives to the IPSP initiation site (see also Rall et al., 1967).

APPENDIX A

Amplitude of the Postsynaptic Potential

The development of the potential (V) that is produced by the activation of the two synapses (S_1, S_2) of Fig. 1 *B* is described by the following equation:

$$c\frac{dV}{dt} + g_0V + g_1(V - E_1) + g_2(V - E_2) = 0 \qquad \text{(A1)}$$

where g_0, g_1, and g_2 are positive constants. The general solution of Eq. A1 for $V(t = 0) = V_0$ is

$$V(t) = (V_0 - V_s)e^{-g\cdot t/c} + V_s \qquad \text{(A2)}$$

where $g = g_0 + g_1 + g_2$ and $V_s = (g_1E_1 + g_2E_2)/g$. The conductance g_1 (of S_1) is always activated at $t = 0$ while g_2 (of S_2) may be activated at a delay of ΔT (Fig. 1 *C*). In the following analysis we find the value of $t = \Delta T$ for which the amplitude of V is minimal.

Proposition 1. Assume that $E_1 > E_2 \geq 0$, $g_0, g_1, g_2 > 0$ and $g_1E_1 + g_2E_2/g_0 + g_1 + g_2 > E_2$.[2] Then the amplitude of the potential V is minimal if S_1 is activated first and S_2 is activated when $V = E_2$.[3]

In the proof of Proposition 1 we will use the following lemma: Suppose that $X' = f(X)$, $Y' = g(Y)$. Assume that $f(s) > g(s)$ for all $s > 0$. If $X(t_0) = Y(t_0) > 0$ then $X(t) > Y(t)$ for all $t > t_0$.

The proof of the lemma follows: Let $Z(t) = X(t) - Y(t)$. To prove the lemma we have to show that $Z(t) > 0$ for $t > t_0$. Because $X(t_0) = Y(t_0)$, $Z(t_0) = 0$. For small $t - t_0 > 0$, $Z(t) > 0$ because $Z'(t_0) = f[X(t_0)] - g[Y(t_0)] = f[X(t_0)] - g[X(t_0)] > 0$. If the result is false there must be a

[2]This assumption implies that the steady-state value of the two synapses is larger than the reversal potential (E_2) of the inhibitory synapse S_2. It holds if $E_1/E_2 > 1 + g_1/g_0$. In most physiological cases $g_0 \approx g_1$ and $E_1 >> E_2$ (Rall, 1967), and, therefore, in these cases the condition holds. Note that the assumption implies also that $E_2 < g_1E_1/g_0 + g_1$, i.e., the steady-state value of S_1 alone is larger than E_2.

[3]It is implicitly assumed that S_1 is activated long enough so that V crosses the value E_2, which is by assumption smaller than the steady-state value of S_1.

first value $t_1 > t_0$ for which $Z(t_1) = X(t_1) - Y(t_1) = 0$ while $Z(t) > 0$ for $t_0 < t < t_1$. This implies that $Z'(t_1) \leq 0$ in contradiction to $Z'(t_1) = f[X(t_1)] - g[Y(t_1)] = f[X(t_1)] - g[X(t_1)] > 0$. Q.E.D.

Proof of Proposition 1

Let $W(t)$ be the potential produced by S_1 alone. The maximum is obtained at t_1, the duration of the g_1 activation (Fig. 1 *C*). Let t^* be the time where $W(t^*) = E_2$ and let $\Delta T > 0$ be the time at which S_2 is activated (Fig. 1 *C*).

Case 1: $\Delta T < t^*$ (Early Activation). Suppose we activate S_2 at $\Delta T = t'$ such that $0 < t' < t^*$. Let $U(t)$ be the solution of Eq. A1 for this case. Let $V(t)$ be the solution for the case where $\Delta T = t^*$ (correct activation) (Fig. 11 *A*).

For U the following equation holds:

$$c\dot{U} = -g_0U + g_1(E_1 - U) + g_2(E_2 - U) \qquad \text{(A3)}$$

where the last term of the right side is positive as long as $U(t) < E_2$.

In the case where S_2 is not activated the following equation continues to hold

$$c\dot{V} = -g_0V + g_1(E_1 - V). \qquad \text{(A4)}$$

At t', $V(t') = U(t')$ thus, according to the lemma $V(t) < U(t)$, as long as $U(t) < E_2$. Let t'' be such that $U(t'') = E_2$ (Fig. 11 *A*). For $t \geq t''$ U satisfies Eq. A3 where $U(t'') = E_2$. V satisfies now

$$c\dot{V} = -g_0V + g_1(E_1 - V) + g_2(E_2 - V) \qquad \text{(A5)}$$

where $V(t^*) = E_2$.

Because U and V are now the solutions of the same differential equation and $U(t'') = E_2 = V(t^*)$, it follows from the uniqueness of the solution and the autonomity (g_0, g_1, g_2 are independent of t) that $U(t) = V(t + \delta)$ where $\delta = t^* - t''$ and $t > t''$ (Fig. 11 *A*). Because V and U are increasing functions[4] on the interval $[t', t_1]$ it follows that $U(t) > V(t)$ for every t in the interval and in particular $U(t_1) > U(t_1 - \delta) = V(t_1)$.

Case 2: $\Delta T > t^*$ (Late Activation). Let $W(t)$ be the solution of $cW = -g_0 \cdot W + g_1(E_1 - W)$ with $W(0) = 0$. Suppose that S_2 is activated at $\Delta T = t' > t^*$. For $t > t'$, the potential U in this case satisfies Eq. A3, where $U(t') = W(t')$.

From the lemma we get that $V(t) < W(t)$ for $t > t^*$ (Fig. 11 *B*). Because $V(t)$ increases and has a maximum at $t = t_1$, two possibilities exist: either $U(t') > V(t_1)$, which implies that $\max U(t) > \max V(t)$, or $V(t'') = U(t')$ for $t' < t'' \leq t_1$ (Fig. 11 *B*). In the second case it follows from the uniqueness of the solution and the autonomity of the equations that $U(t) = V(t + \delta)$ for $\delta = t'' - t' > 0$. Hence the maximum of V is smaller than that of U.

From cases 1 and 2 it follows that the amplitude of the potential is minimal if S_2 is activated when the potential of S_1 reaches E_2.[5] This ends the proof of Proposition 1.

[4]Because U satisfies Eq. A3 for $t \geq t'$ and $U(t') < E_2 < g_1E_1 + g_2E_2/g_0 + g_1 + g_2$ it follows by Eq. A3 that $\dot{U}(t) > 0$ for all $t \geq t'$. Because V satisfies Eq. A4 for $t' \leq t \leq t^*$ and $V(t') < E_2 < g_1E_1/g_0 + g_1$ it follows from Eq. A4 that $\dot{V}(t) > 0$ for $t' \leq t \leq t^*$. For $t > t^*$ V satisfies Eq. A3 and because $V(t^*) = E_2 < g_1E_1 + g_2E_2/g_0 + g_1 + g_2$ it follows from Eq. A3 that $\dot{V}(t) > 0$ for $t \geq t^*$.

[5]For $E_2 = 0$ (the reversal potential of S_2 is at the resting potential), the potential of S_1 reaches E_2 at $t = 0$. Thus according to the proposition, a minimal amplitude for this case is obtained for simultaneous ($\Delta T = 0$) activation of the two synapses. From the analytic solution for t^* (see Eq. 3) it can be shown that simultaneous activation yield minimal amplitude also for $E_2 < 0$.

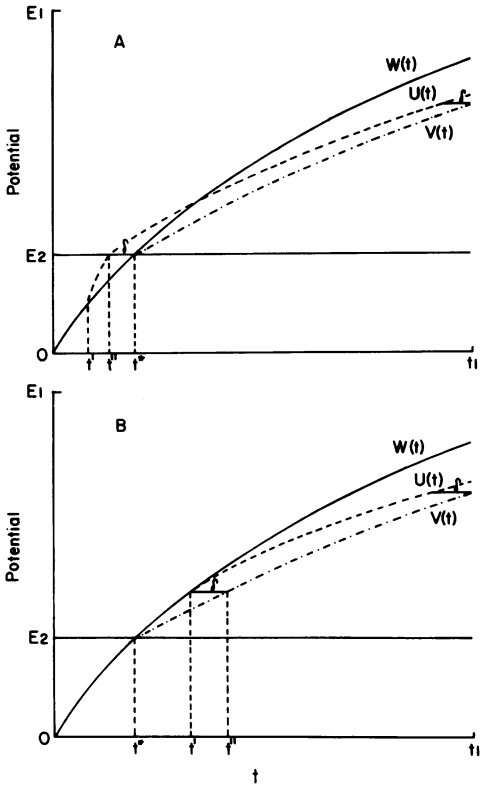

FIGURE 11 Schematic representation of the effect of the timing between the synapses S_1 and S_2. In both *A* and *B*, $W(t)$ represents the potential of S_1 when activated alone, and $V(t)$ the potential for the case where S_2 is activated at t^* — the time at which S_1 potential reaches E_2. The duration of the conductance changes of both S_1 and S_2 is t_1. *A*, the case in which S_2 is activated at $t' < t^*$ and as a result the potential $U(t)$ is obtained. *B*, the cases in which S_2 is activated at $t' > t^*$ and as a result the potential $U(t)$ is obtained. For both cases *A* and *B*, the minimal amplitude is reached by $V(t)$ at $t = t_1$ (see text).

The proof of Proposition 2 follows from similar arguments. Under the assumptions of Proposition 1, the maximal amplitude is obtained when S_2 is activated first and S_1 is activated at the end of the activation of S_2 (i.e., $\Delta T = -t_1$).

APPENDIX B

Time Integral of the Postsynaptic Potential

In the following analysis we find the range of ΔT that gives a minimal time integral of the solution V of Eq. A1.

Proposition 3. Under the assumptions of Proposition 1, the timing ΔT which yields the minimal time integral is obtained for $0 \leq \Delta T \leq t_1$.

□ PROOF OF PROPOSITION 3 First we show that for every $\Delta T > t_1$, the time integral of the potential V is larger than the one obtained at $\Delta T = t_1$.

Let S_2 be activated at $\Delta T > t_1$. As shown in Fig. 12, there exist three distinct time intervals following the peak potential V_0:

(*a*) For $t_1 < t < t_1 + \Delta T$, the potential of S_1 decays exponentially with the (resting time) constant g_0/c. Hence, the potential at $t = t_1 + \Delta T$ is

$$V(t_1 + \Delta T) = V_0e^{-g_0 \cdot \Delta T/c}$$

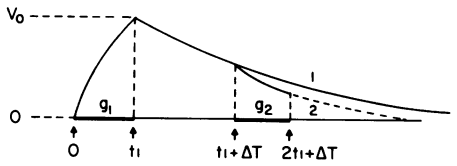

FIGURE 12 Schematic representation of the cases where S_2 activation follows the peak potential (V_0) of S_1. Case 1, S_1 alone is activated at $t = 0$ for the duration of t_1 (g_1, marked line). Case 2, S_2 is activated also at $t = t_1 + \Delta T$ for the duration of t_1 (g_2, marked line). Using the graph, it can be seen that the minimal area of the PSP is obtained when g_1 and g_2 overlaps (see text).

(*b*) For $t_1 + \Delta T < t < 2t_1 + \Delta T$ the synapse S_2 is activated. From the solution of Eq. A1 given in Eq. A2 for the initial condition $V(t_1 + \Delta T)$ we get at $t = 2t_1 + \Delta T$

$$V(2t_1 + \Delta T) = [V(t_1 + \Delta T) - V_2]e^{-(g_0+g_2)\cdot t_1/c} + V_2$$

where $V_2 = g_2E_2/g_0 + g_2$, is the steady-state value of S_2.

(*c*) For $2t_1 + \Delta T < t$ the potential decays exponentially with the (resting time) constant g_0/c; therefore

$$V(t) = V(2t_1 + \Delta T)e^{-g_0[t-(2t_1+\Delta T)]/c}.$$

Combining the three time intervals we get that for every $t > 2t_1 + \Delta T$ the potential $V(t)$ is

$$V(t) = [(V_0e^{-g_0\cdot\Delta T/c} - V_2)e^{-(g_0+g_2)t_1/c} + V_2]\, e^{-g_0(t-(2t_1+\Delta T))/c}$$

or

$$V(t) = V_0e^{-(g_2\cdot t_1+g_0t)/c} + V_2e^{-g_0(t-2t_1)/c}\,(1-e^{-(g_0+g_2)t_1/c})\, e^{g_0\cdot\Delta T/c}.$$

Because the first term on the right side of the last equation is independent of ΔT, and the second one is positive, we get that for any fixed $t > 2t_1 + \Delta T$, $V(t)$ increases as ΔT increases, i.e., the potential (and hence its time integral) is minimal for $\Delta T = 0$. It is easy to see that this result holds also for $t_1 < t < 2t_1 + \Delta T$.

From the results concerning the potential amplitude (Propositions 1 and 2) it is clear[6] that the minimal area cannot be obtained when the activation of S_2 precedes that of $S_1(\Delta T < 0)$. Hence the minimum is obtained for $0 \le \Delta T \le t_1$.

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[6]From Propositions 1 and 2 it can be seen that the early activation of S_2 ($\Delta T < 0$) yields a potential that has a larger amplitude than the minimal one. As a result, the time integral for this case is larger than the one obtained in the case where a minimal amplitude is obtained.

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