STATISTICAL SIGNS OF SYNAPTIC INTERACTION IN NEURONS

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ABSTRACT The influence of basic open-loop synaptic connections on the firing of simultaneously recorded neurons has been investigated with auto- and cross-correlation histograms, using experimental records and computer simulations. The basic connections examined were direct synaptic excitation, direct synaptic inhibition, and shared synaptic input. Each type of synaptic connection produces certain characteristic features in the cross-correlogram depending on the properties of the synapse and statistical features in the firing pattern of each neuron. Thus, empirically derived cross-correlation measures can be interpreted in terms of the underlying physiological mechanisms. Their potential uses and limitations in the detection and identification of synaptic connections between neurons whose extracellularly recorded spike trains are available are discussed.

INTRODUCTION

It has now become routine in many neurophysiological laboratories to characterize spontaneous or stimulus-driven unit activity of neurons by a variety of quantitative measures; the required calculations have been facilitated by the availability of high speed digital computing machinery. These statistical measures of spike trains are of value not only in characterizing patterns of discharge in single neurons, but also in answering questions about input-output relations, information transmission, and mechanisms underlying the impulse-generating mechanism. Discussions of these measures and their applications have appeared recently (1–6).

With the development of improved multiple microelectrode techniques and "window" techniques which discriminate individual spikes from multiunit recordings on the basis of amplitude or waveform (7–10), and techniques which allow intracellular synaptic potentials to be detected and identified (11, 12), it is possible to derive measures for several neurons observed simultaneously, on the basis of these

measures to search for possible correlations between neurons, and to use indications of correlation systematically in the elucidation of neuronal interactions and network structure.

In the present paper we discuss some elementary types of neuronal synaptic connections whose existence and basic properties have been demonstrated in a variety of nervous systems. These functional connections are considered in terms of the temporal correlations they induce in the respective spike trains of the corresponding neurons. We demonstrate some specific statistical measures which are useful in detecting and characterizing a synaptic connection, and describe how prototype connections are reflected in these measures. These studies are to be regarded as a step in the development of methods for the direct investigation of neuronal synaptic interactions and network structure by means of extracellular measurements.

The theory by which synaptic connections can be detected from statistical analysis depends on the basic result (13) that if two spike trains are independent, i.e. if the occurrence of a spike in one cell is not correlated with the subsequent activity of the other cell, and vice versa, the cross-correlation histogram between those spike trains will be flat, i.e., the cross-correlation function is a constant. When the cross-correlation is not flat, we can assume some functional correlation between these cells. In what follows, we consider some important "primitive" or basic types of dependence which may arise.

There are a number of distinct classes of physiological phenomena which might induce a correlation between the spike trains of two neurons. For example, both cells may actually be connected synaptically, with one cell producing either excitatory or inhibitory postsynaptic potentials (EPSP's or IPSP's) in the other. Again, both cells may be functionally related within a common neuronal system, as, for example, are respiratory neurons, which experience correlated periods of greater or lesser activity. This would occur if the cells received common input from presynaptic sources, or if they were correlated by a common dependence on some factors like blood CO_2, temperature, blood pressure, etc. Other influences promoting coherence in firing might arise from diffuse potential fields, ephaptic effects, and similar electrical phenomena which have been postulated to govern excitability in cell populations (14, 15, 15 *a*).

A study of the structure of the cross-correlogram between two spike trains could be used to indicate the mechanisms underlying the correlation. In the case of synaptic interaction, the cross-correlogram might be used to infer the existence and nature of connections that involve the observed nerve cells, and therefore, indicate neuronal network structure.

The aim of the present paper is to describe how certain elementary prototypical synaptic connections are reflected in the structure of the cross-correlation function and the extent to which an understanding of this relationship can be exploited in the investigation of network structure when only extracellular recordings are

available. It is a relatively simple matter to explore the relationship between known network organizations and the statistical measures of the spike train records these circuits generate. Whenever the network relationships are formulated with suitable precision, it is possible to employ computer simulations, calculate appropriate statistical measures, and study their dependency on various network parameters. In many experimental situations however, the network structure is largely unknown: the process must therefore be reversed, and empirical calculations on the spike train records must be used to draw inferences about network connections. Then, when hypotheses about functional connections are quantitatively formulated, computer simulation can again be used to test the appropriateness of the model or hypothesis.

In the present study, we are largely concerned with the much easier problem of showing how certain kinds of synaptic connections lead to characteristic features of the cross-correlation function. Specifically, we deal only with direct or shared open-loop excitatory and inhibitory synaptic coupling. In addition, we discuss some of the difficulties of the inverse problem.

METHODS

The basic interactions studied in this paper were all initially observed in one or more neurons from the isolated visceral ganglion of the marine gastropod *Aplysia californica*. Simulation of the interactions reported here was based on a computer model with parameters appropriate to *Aplysia* neurons.

The details of the computer program have been discussed elsewhere (16). In some cases, considerable experimentation was required in order to arrive at a choice of parameters which would adequately illustrate the effects. Each computer run was terminated when at least one neuron in the network had generated a total of 5,000 spikes, or when the run exceeded some fixed time limit. Clock times of the firing of each cell in the network were stored in the computer memory and used to construct the autocorrelation histogram for each cell and the cross-correlation histogram for each pair of cells. These measures have been described previously (3–5). In all figures, the cross-correlation histogram measuring the time-varying probability of cell Y firings relative to cell X firings is indicated as XY cross-correlation or simply "XY." The YX cross-correlation is the mirror image of XY reflected about the ordinate axis (5). Each histogram for a simulation run or experimental run displays as an ordinate the number of firings observed in cell Y at various times τ (the abscissa), before and after all cell X firings.

RESULTS

Statistical Signs of Excitatory Synaptic Coupling

The most elementary interaction is that exhibited by two neurons A and B connected by an excitatory synapse (Fig. 1). Every firing of cell A is followed, after the conduction and transmission delay, by an EPSP in cell B. The size of this EPSP may normally be influenced by factors such as the postsynaptic membrane potential, the recent history of activity in the same or other synaptic sources, etc. Essentially,

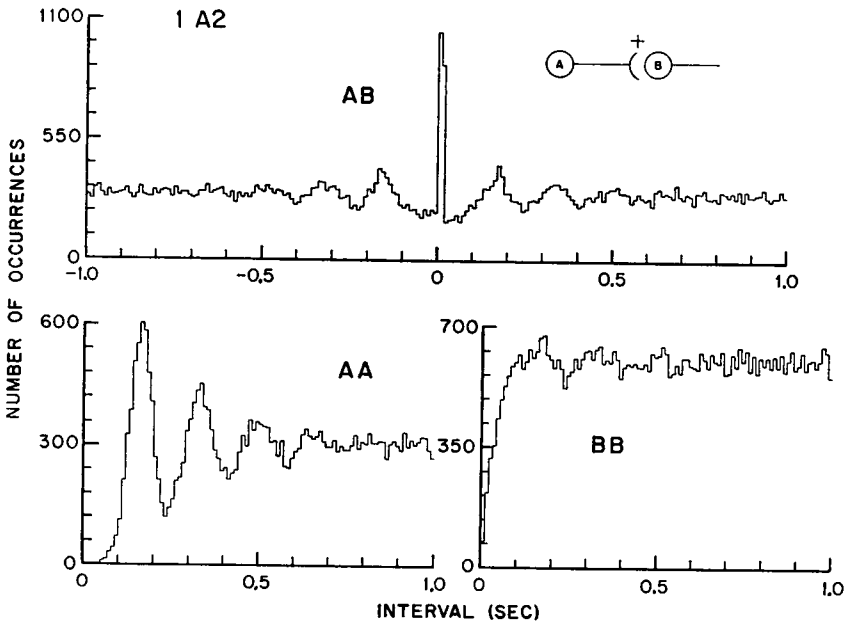


FIGURE 1 Monosynaptic excitation. Periodic presynaptic cell. In this and Figs. 2–6 the *A* cell is always the presynaptic cell, *B* the postsynaptic cell. Top: *AB* cross-correlation. Bottom, left: cell *A* autocorrelation. Right: *B* autocorrelation (run 1A2).

however, one would expect the arriving spike from cell *A* to produce a depolarization in cell *B* and this, on the average, increases the likelihood that cell *B* will produce a spike within the effective duration of the EPSP. The cross-correlation histogram between cells *A* and *B*, which is a measure of the expected past and future spiking probability of *B* relative to firing times of *A*, will reflect this enhanced likelihood. A sample histogram for two monosynaptically coupled cells thus shows an increased frequency of observed firings with a central peak close to the origin (e.g. at about 10 msec). Far from the origin (e.g. at about 1000 msec), on the other hand, the cross-correlation is flat to within statistical fluctuations, signifying that cell *A* firings are not influential in determining the behavior of cell *B* that far in the future. Using the actual figures in the correlogram, this means that if 2,512 moments are picked at random and the number of *B* spikes occurring in a 10 msec period following each selected moment are counted, an average of 270 spikes will be observed. But, if those 2,512 moments coincide with cell *A* spikes, then about 1100 *B* spikes will be observed in the 10 msec that follow, i.e. about one-half of the time that cell *A* fires, cell *B* will fire between 0 and 10 msec later. (In this example, 2,512 was the total number of cell *A* spikes, 10 msec was the bin width, 270 was the average number of spikes in any 10 msec bin located in the flat part of the histogram; and 1100 was the actual number of spikes in the 10 msec bin beginning at 0 msec. Approximately 5000 postsynaptic *B* spikes were produced.)

Clearly, the direct excitatory synaptic coupling in this situation accounts for the appearance of the central peak, commencing from the time of arrival of the presynaptic spike ($\tau = 0$) and declining after several time constants of synaptic-potential decay ($\tau_M = 3$ msec). We term this effect the "*primary effect.*" We employ this term not only because of the direct temporal relation of this peak to the arrival of the input spike but also because the shape of this early peak is directly related to the actual waveform of the postsynaptic potential.

We can also see (Fig. 2 from an intracellular *Aplysia* record) that the shape of the primary peak is strongly influenced by the waveform of the synaptic potential. This is true because to a first approximation, the instantaneous firing probability of a neuron is inversely related to the instantaneous distance between membrane potential and threshold. That is, the probability of firing during a postsynaptic potential is a monotonic function of the amount by which the synaptic currents raise the membrane potential toward the threshold level. The EPSP waveform evoked in an *Aplysia* cell is superimposed on the same time scale in Fig. 2 in registry with the initial peak in the cross-correlation of the same two cells.

In general, the shape of the central peak will be influenced by other factors as well; for example, by additional correlated phenomena such as those due to interneurons (see below) and repetitive bursts in the pre- and postsynaptic cell (e.g. 17, 18) as well as by fluctuations in conduction delay (e.g. 19).

Additional *secondary peaks* may be evident in the cross-correlation, and in general are due to statistical features of the pre- and postsynaptic discharges themselves. For example, in Fig. 1, the presynaptic cell displayed a fairly pronounced rhythmicity that was reflected in its autocorrelation histogram (Fig. 1, bottom

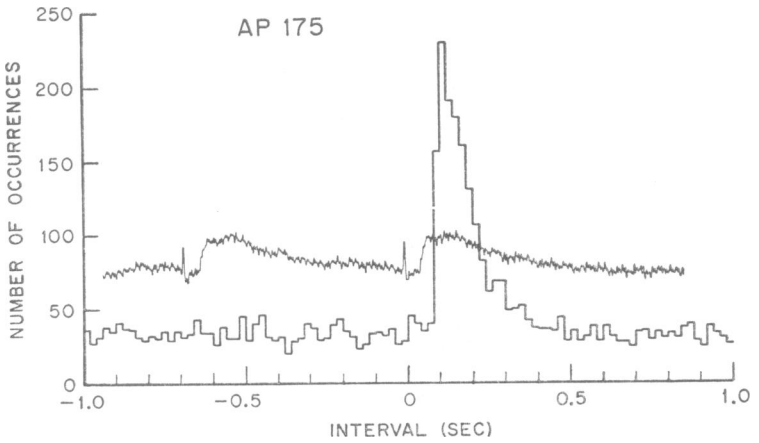


FIGURE 2 Synaptic excitation. Aperiodic cells; *Aplysia* data. The excitatory synaptic input was generated by a connective stimulated by a Geiger-driven stimulator. Central-peak waveform of *AB* cross-correlation shown with expanded time base. EPSP waveform from intracellular record of cell *B* shown superimposed for comparison (experiment AP 175).

left). Such rhythmicity might arise if the cell were a pacemaker neuron, or because of its own input (20, 21). As a result, if the presynaptic cell has a tendency to induce postsynaptic discharges shortly after its own spikes, then we can expect enhanced numbers of postsynaptic firings to occur with greater likelihood at the "preferred" times corresponding to past and future presynaptic firings. Thus, to the extent that the postsynaptic cell is a "follower" of the presynaptic cell, the cross-correlation histogram will reflect more or less strongly the characteristics of the driver cell's autocorrelation histogram. A direct comparison of both correlograms in Fig. 1 shows that the peaks on either side of the central peak in the cross-correlation histogram can be directly attributed to this periodicity in the presynaptic spike train. The paracentral peaks generated by this effect are generally more diffuse than the primary one, and this is due to the spreading of the times of occurrences of the next presynaptic firings.

A third source of peaks in the cross-correlation histogram arises in a similar manner from periodicities in the postsynaptic cell *B*. For example, *B* may fire rhythmically, even in the absence of input from the presynaptic cell *A*, because of inherent pacemaker properties and/or its other input. Any such behavior determines peaks in the *B* autocorrelation function, and may be reflected in the cross-correlation. Again, as noted above, some presynaptic spikes trigger a postsynaptic spike, and there is a peak near the origin. If there is sufficient time between presynaptic spikes, there may be a tendency for the next postsynaptic spike to occur at its usual interval. By this *resetting* of the output spike train, the postsynaptic cell autocorrelation is replicated in the cross-correlation. Fig. 3 (top) shows the cross-correlation in a simulated case of strong but infrequent driving of cell *B* by cell *A*. The strong central peak depends on the excitatory input. Cell *B* receives other sources of input, however, causing it to fire quasiperiodically, as shown by its autocorrelation function (Fig. 3, lower right). The peak in the cross-correlogram to the right of the central peak clearly corresponds in time to the first peak in the *B* autocorrelation. However, there is no corresponding peak to the left of the origin, since, with randomly occurring input from *A*, their spike times are uncorrelated in the past (i.e. cell *B* does not influence *A*). If the input-triggered spike does *not* reset the input spike train, then the effect described here will not occur. This would be the case, for example, if the postsynaptic cell rhythmicity were due to its being a follower of another strong, periodic, independent driving cell.

These examples suggest the general conclusion that features of both the presynaptic and postsynaptic cell autocorrelation functions are reflected in the cross-correlation function. We term their influence "*secondary effects.*" Features of the presynaptic autocorrelation histogram will be mapped symmetrically onto positive and negative values of the delay variable τ, while the features of the postsynaptic autocorrelation will appear only for positive τ values, providing there is no feedback pathway from *B* to *A*. Thus, if both cells are periodic, only the periodicity of the

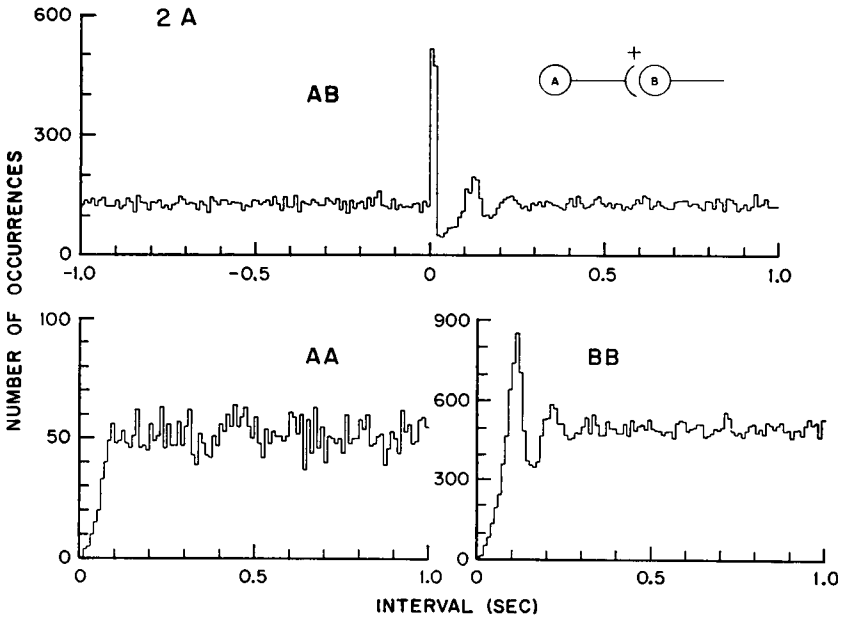


FIGURE 3 Monosynaptic excitation. Periodic postsynaptic cell. Postsynaptic rhythmicity reflected for positive values of τ. Top: *AB* cross-correlation. Bottom: cell A and cell B autocorrelations (run 2A).

input cell will appear to the left of the primary peak, while both periods will appear to the right.[1] When neither cell is periodic, the cross-correlation is flat except for the primary peak (e.g. Fig. 2).

There are, of course, many possible complications which can obscure the fundamental pattern of excitatory synaptic couplings as revealed by the cross-correlation function: here we mention only those which involve additional neurons.

(*a*) *Correlated input.* Two or more of the excitatory inputs to a neuron may themselves be correlated by means of synaptic connections. This tends to impose a repeated temporal pattern on the arrival of impulses at the common postsynaptic cell. For example, Fig. 4 A shows an intracellular record from an *Aplysia* neuron in which clusters of EPSP's can be seen to arrive at regular intervals, as if the several cells (interneurons) generating them were themselves being triggered periodically. Fig. 4 B also shows a computer simulation of this case in which a common presynaptic pacemaker source (cell *A*) produces EPSP's in three interneurons after a conduction delay of 10 msec. Each such EPSP has about a 0.5 probability of producing a spike in the corresponding interneuron. These spikes in turn arrive at the

[1] This is not meant to preclude even more complex patterns arising when multiple pacemakers are involved. Under suitable conditions, peaks arise in the cross-correlation which correspond to differences in pacemaker periods and multiples of these differences.

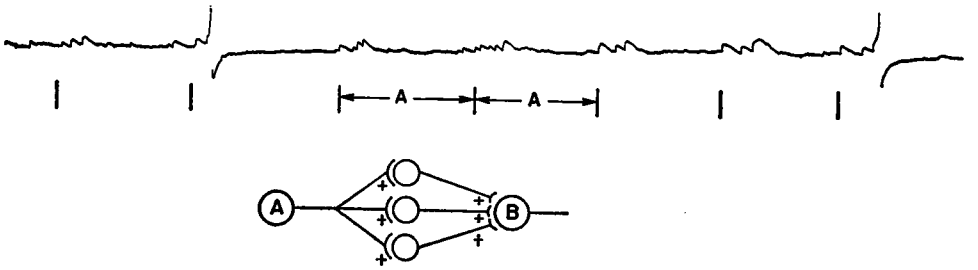


FIGURE 4 A

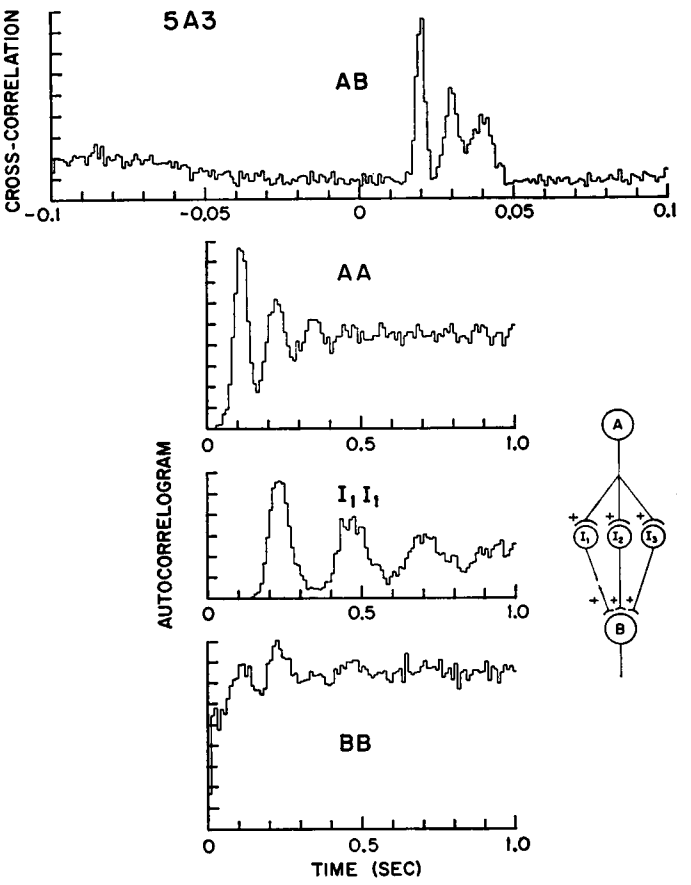


FIGURE 4 B

FIGURES 4 A and B Excitation via interneurons. Effect of correlated input on primary peak. 4 A Intracellular recording from *Aplysia* showing clusters of EPSP's probably elicited by several interneurons driven regularly at approximately 1 second intervals from a common pacemaker source. 4 B Cross- and autocorrelations of cells in network simulating record of Fig. 4 A. Top: *AB* cross-correlation. Bottom: *A*, I_1, and *B* autocorrelations. Model network has 10 msec delay from *A* to I_1, I_2, I_3 and 10, 20, and 30 msec delays from I_1, I_2, I_3 to *B*, respectively. Note expanded time base which excludes the secondary effects (run 5A3).

final common cell *B* after delays of 10, 20, and 30 msec, respectively. A number of different network configurations could have produced the clustering of synaptic potentials observed in the *Aplysia* record (Fig. 4 A). The autocorrelations of cell *A*, cell *B*, and a typical interneuron, and the *AB* cross-correlation are shown in Fig. 4 B.

(*b*) *Synaptic coupling through an interneuron.* It can also be shown that when an observed cell makes an excitatory connection with an interneuron which in turn is excitatory to a final cell, there will still be an increased probability of a firing in the final cell for a period of time following the generation of an impulse in the initial cell. This probability change, however, will now reflect the interneuron probability relations as well, and this may induce a considerable number of changes in the cross-correlation between initial and final cells. The initial primary peak will be attenuated (relative to the peak in the cross-correlation between the interneuron and the final cell) and there may be a similar attenuation in the secondary features of the cross-correlation. Indeed, despite the fact that the synaptic potentials at each junction are relatively large, the over-all transmission ratio may be quite small. Since these are both less than unity, attenuation can be quite rapid when a succession of synapses is involved, and indirect connections of this sort may be detectable only with large sample sizes. In most cases it will also be impossible to distinguish a strong indirect interaction from a weak direct coupling.

Statistical Signs of Inhibitory Coupling

Inhibitory coupling between two cells introduces quite a different element into the firing statistics of the inhibited cell. By a natural analogy with the excitatory case, we can say that following an impulse in the presynaptic cell, there will be a reduced probability of firing in the postsynaptic cell, directly attributable to an increasing polarization of the postsynaptic cell membrane potential or to a "clamping" tendency of the membrane potential away from threshold. Thus, the primary effect of inhibition on the cross-correlation is a trough near the origin. For this interaction to be visible in the cross-correlation, there must be presented a background of postsynaptic spiking against which the inhibitory effect may be exercised.

As in the case of excitatory coupling, the autocorrelations of both the input and output cells contribute their structure to the cross-correlation. If both pre- and postsynaptic cells have flat autocorrelations, the cross-correlation will show only the primary trough effect. If either cell is periodic, its period will be reflected in the cross-correlation. For example, in Fig. 5 we see the influence of the rather strongly periodic autocorrelation of the inhibiting input cell. After the initial decrease in postsynaptic firing probability generated by all input spikes (at $\tau = 0$), there is a repolarization period until the expected time of arrival of the next inhibitory spike, which is given by the distribution of first-order interspike intervals of the input train. That is, the *deletions* imposed on the otherwise flat cross-correlation may be

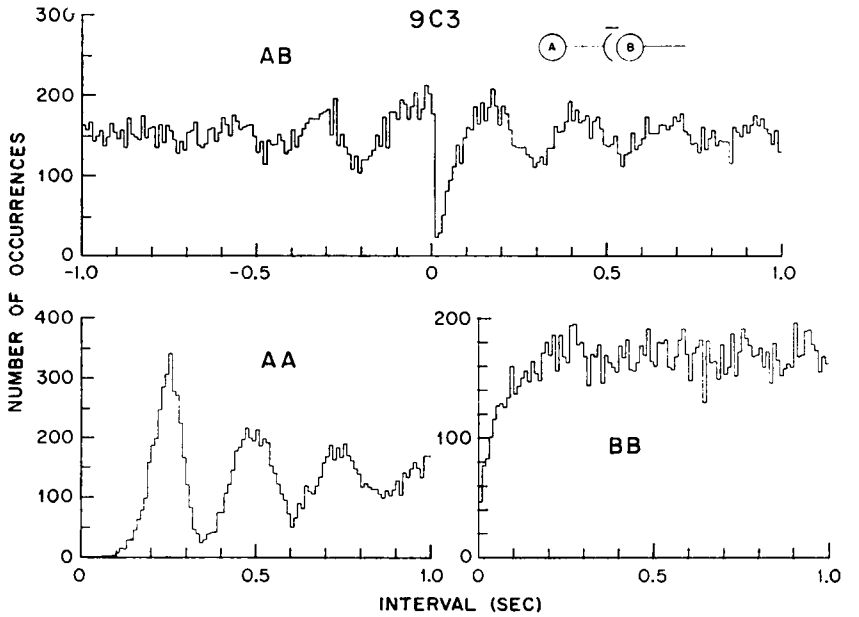


FIGURE 5 Monosynaptic inhibition. Periodically firing presynaptic cell. Top: *AB* cross-correlation. Initial trough is primary sign of monosynaptic inhibition. Bottom: *A* and *B* cell autocorrelations. Note appearance of input cell's periodicity in cross-correlation for positive and negative τ (run 9C3).

expressed as the resultant of successively convolving the first-order cross-channel waiting time distribution (which includes the central trough) with the first-order interspike interval distribution of the input train. If the duration of the primary trough is short compared with the presynaptic interspike intervals, then effectively the input autocorrelation will be mapped *negatively* onto the cross-correlation (Fig. 5) for positive and negative values of τ.

Fig. 6 shows the case of an inhibitor input cell with flat autocorrelation acting on a periodic postsynaptic cell. Following each input spike there is a reduced postsynaptic firing probability, giving rise to a primary trough. The coherence of the inhibited periods induces a coherence of subsequent firing periods, and therefore tends to lock the positive τ cross-correlation into a shape resembling the postsynaptic autocorrelation function. There is, however, some distortion of the mapping.

Fig. 5 shows that the initial trough in the cross-correlation bears a striking resemblance to the shape of the IPSP itself. In a later figure (Fig. 11), taken from recordings in *Aplysia*, we can see both the actual IPSP and the initial trough. In the inhibitory case, the trough will be present even when the IPSP equilibrium potential is near the resting potential of the cell and hence when the IPSP might be difficult to observe in intracellular recordings. For that reason we prefer to characterize excitatory and inhibitory input by means of their influence on firing prob-

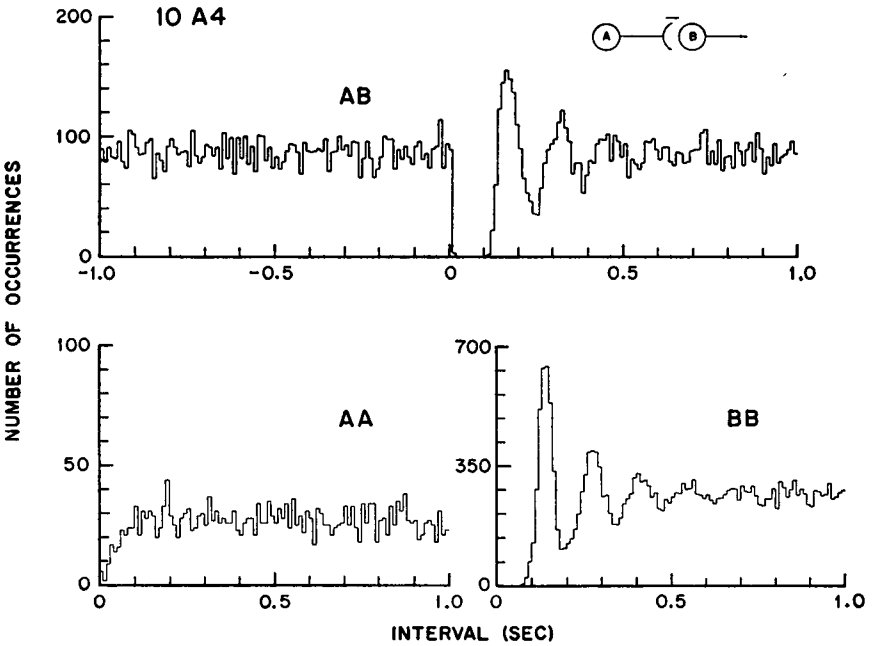


FIGURE 6 Monosynaptic inhibition. Periodically firing postsynaptic cell. Top: *AB* cross-correlation. Bottom: *A* and *B* cell autocorrelations. Note *B* cell periodicity in cross-correlation for positive τ values only (run 10A4).

abilities, as outlined here, rather than by the changes in membrane potential which they may induce.

As before, we can consider briefly the effect of simple complications introduced into the basic inhibitory coupling by the presence of interneurons in the forward path. Two effects not easily distinguishable from direct (monosynaptic) inhibition will be considered: (*a*) a configuration in which the *A* cell excites an inhibitor to *B*, and (*b*) where the *A* cell inhibits an interneuron excitatory to *B*. In both situations increased activity in *A* tends to reduce activity in *B* either by decreasing direct excitation or by increasing direct inhibition. From our previous discussion we can predict the general features of the cross-correlations between the directly coupled cells. Computer simulations have shown that in some cases, the parameters of the interacting cells can be chosen so that weak monosynaptic inhibition can be confused with multisynaptic inhibition because attenuation again plays a dominant role.

Shared Synaptic Input

Shared synaptic input occurs widely in neuronal networks, being an inevitable consequence of the fact that most axons divide into many branches, each of which may carry identical or very similar pulse trains (22). In simultaneously recorded intra-

cellular activity in *Aplysia* neurons, one often observes synaptic potentials arriving nearly simultaneously in several cells, presumably from axon branches originating from a common presynaptic cell, or from activity from a common cell relayed through interneurons (23). Such common input will induce correlations in the discharges of those neurons influenced by the common source. Obviously, a common driver will introduce complexities not found in direct connections, which poses additional problems in interpreting cross-correlations between the postsynaptic cells. We consider three basic prototypes.

Shared Excitation. In the configuration of Fig. 7, the common excitatory input received by both cells is reflected in a tendency toward synchronous firing that generates a peak near the origin of the postsynaptic cells' cross-correlation. This is the *primary effect* of shared excitation, and obviously reflects the fact that the simultaneous appearance of EPSP's in both postsynaptic cells momentarily increases their probability of firing nearly simultaneously. We are ignoring here the fact that if the shared excitatory input is received by two cells after different delay times,

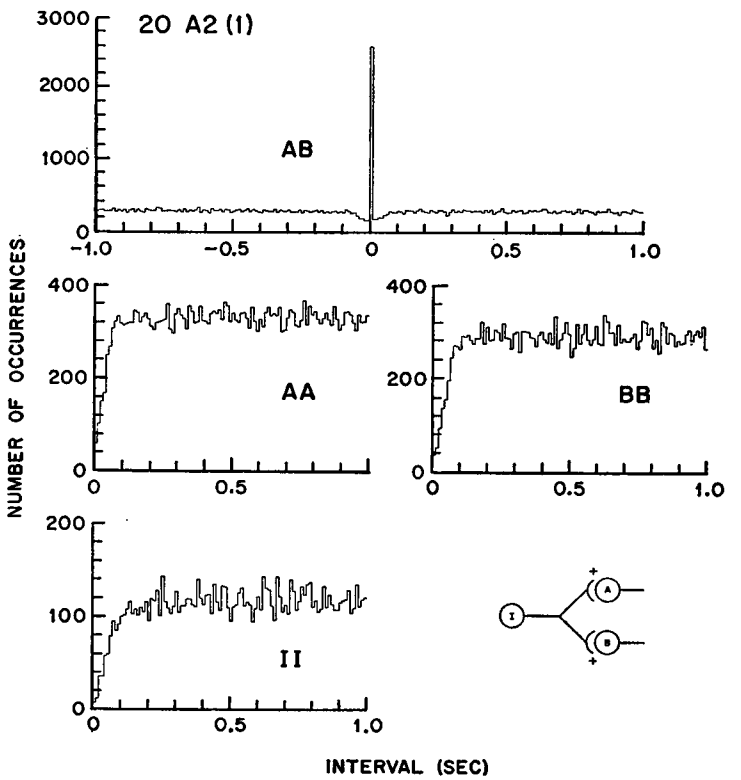
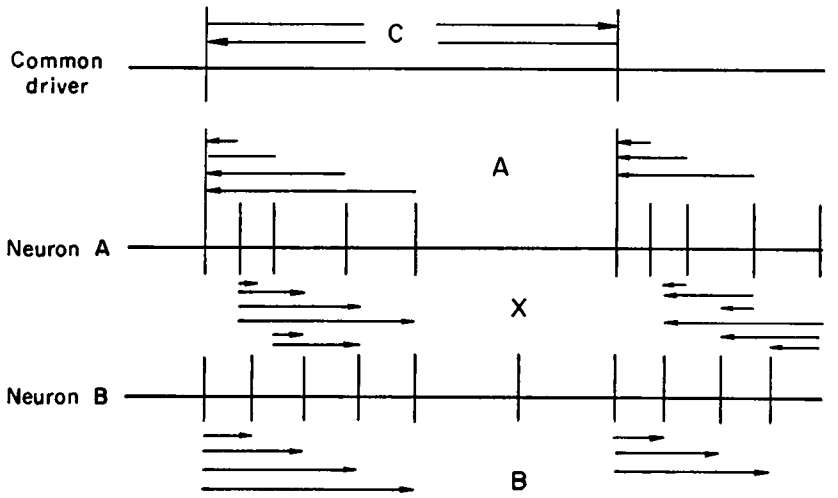


FIGURE 7 Shared excitatory synaptic input. Basic case in which common excitatory source and both postsynaptic cells have flat autocorrelations.

the primary peak will be shifted by an amount equal to the difference in conduction times.

Correlations in the firing of *B* with respect to firings at *A*, which contribute features (departures from flatness) in the cross-correlation histogram between *A* and *B* arise in several ways. Referring to the schematic diagram, we note the following four principal contributions to the cross-correlation.



(*a*) Firings of the common driver *C* will, with high likelihood, trigger nearly simultaneous firings (or bursts) in both driven cells, *A* and *B*. This will produce a peak in the cross-correlation histogram centered on or near the origin. The autocorrelation of the common driver *C* may be mapped symmetrically and bilaterally by the same mechanism as described above in the discussion of the cross-correlation between pre- and postsynaptic neurons. Thus some secondary peaks arise because the firing of the driver at $\tau = 0$, which contributes to the peak at the origin, implies that the driver has fired at preferred earlier and later times; its autocorrelation function maps the intensity and time course of these firing "preferences." These contributions are indicated by the arrows labeled "*C*" in the diagram.

(*b*) Firings of *B* induced by the common driver *C* (and occurring nearly simultaneously with firings of *A*) tend to be followed (but not preceded) at preferred intervals by subsequent firings of *B*; these preferred intervals (indicated by the right-going arrows labeled "*B*" in the diagram) correspond to the autocorrelation of *B*. Thus, *B*'s autocorrelation is mapped unilaterally, to the right of the central peak in the *A*-*B* cross-correlation histogram.

(*c*) By a similar argument, firings of *A* subsequent to those driven directly by *C* (and causing an *A*-*B*-*C* coincidence) correspond to the preferred intervals described by the autocorrelation of *A*. But these are *B*-*A* intervals, whereas the cross-

correlation histogram tallies A-B intervals; therefore, these will be negative intervals, mapped to the left of the origin only. They are indicated by the left-going arrows labeled "A" in the diagram.

(*d*) Finally, the intervals between "secondary" firings (as in a burst, e.g. references 24, 25) of A and B will contribute more complex and diffuse patterns to the cross-correlation, on both sides of the central peak. The corresponding intervals are indicated by the arrows labeled "X" in the diagram.

Thus, the autocorrelations of all three cells contribute to the cross-correlation histogram: that of the common driver is mapped bilaterally; that of cell A is mapped unilaterally to the left; that of cell B is mapped unilaterally to the right; and a complicated resultant of those of A and B is mapped both to the right and left. It follows that if all three autocorrelations are relatively flat, then the AB cross-correlation will also be flat except close to the origin (Fig. 7).

To illustrate this further, consider the example in Fig. 8, in which cells A and B receive relatively strong input from a common source. Additional input to cell A, however, has been provided so that the output of A has obvious periodicities while that of B does not. From the strength of the central peak our previous arguments would lead to the conclusion that if the connection is direct, and in the direction of A to B, then the periodicities of cell A should appear in the AB cross-correlation.[2] In fact, as can be seen in Fig. 8, none appear, leading to the conclusion that the correlation results from shared excitatory input from a nonperiodic source, not from direct excitation as might be supposed.

Conversely, in the event periodicities appear in the AB cross-correlation in the absence of obvious periodicities in the A and B cell autocorrelations, one would be forced to conclude that the periodicity arises from a common excitatory cell which itself possesses such periodicity.

These two examples suggest a feature of great importance in the interpretation of cross-correlation histograms, namely, that in certain cases, by careful study of the cross-correlation and autocorrelations, information about cells other than those under direct observation can be gained, and indeed information about both their existence and their pattern of activity may be inferred from statistical analysis.

Shared Inhibition. The converse case involving two postsynaptic cells receiving simultaneous IPSP's is shown in Fig. 9, and illustrates the basic feature that common inhibitory input tends to synchronize postsynaptic cell periods of nonfiring. This leads to the perhaps paradoxical result that, to the extent that their periods of nonfiring overlap, their periods of firing must also overlap, and hence they tend to fire in a synchronous, i.e. correlated, way. The central peak in the cross-correlation shown in Fig. 9 is a measure of this coherence, and is the primary effect of shared

[2] In experimental situations, of course, one may not know which of two cells may be the input cell except when A is a stimulus train. The argument presented here applies to either hypothesis (A input to B or vice versa).

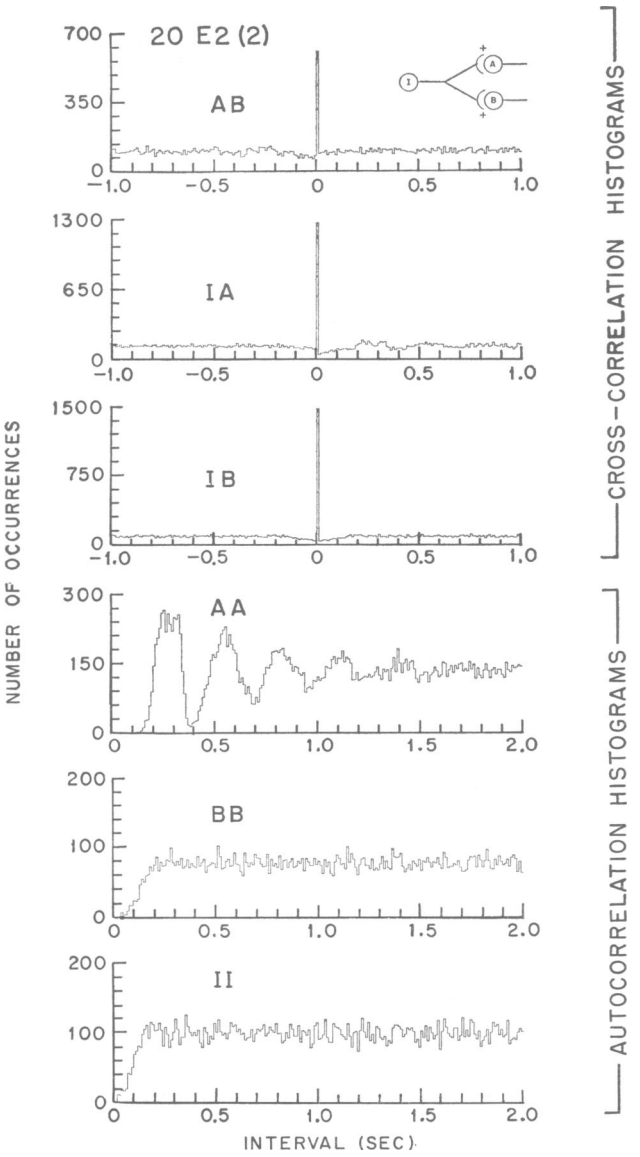


FIGURE 8 Shared excitatory input. Lack of periodicity in the cross-correlation precludes possibility that *A* and *B* are monosynaptically connected despite presence of central peak.

inhibition. The amplitude of the peak is dependent on the strength, duration, and rate of each of the respective IPSP's. The effect, however, is exerted in a manner quite different from that of shared EPSP's, and may be explained qualitatively in the following terms. Each time the common inhibiting cell *C* fires, it is more likely that the time of the firing of *A* is either earlier than or more than a characteristic

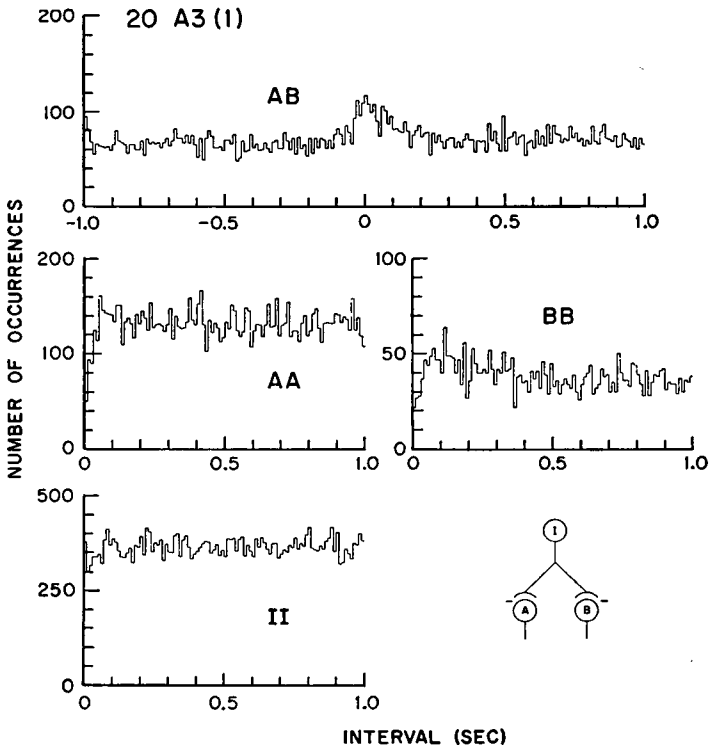


FIGURE 9 Shared inhibitory synaptic input. Basic case of shared inhibition in which all three cells have flat autocorrelation functions. Central broad peak is primary characteristic of the shared inhibition.

interval later than a firing of C. But then, in the temporal vicinity of a firing by A, it is also highly likely that cell B is likewise uninhibited by A. This means that in the vicinity of the origin of the AB cross-correlation histogram we are selecting periods of time when cell B will be uninhibited by C, and hence near-simultaneous coincidences between A and B will be more likely than usual. This coincidence peak is usually not large and may have considerable spread. (Indeed, the central peak may be difficult to detect if the shared IPSP's are small and infrequent.) When the three cells of the configuration all have flat autocorrelations, the broad central peak is the only prominent feature in the AB cross-correlation (Fig. 9).

Since the A and B firings have been forced into relative synchrony, then in the AB cross-correlation (with A firings at $\tau = 0$) the future B firings will occur at a frequency greater than that expected from random occurrence at those times which correspond to natural recurrent firing periods of the B cell in the absence of inhibition (unless such times also correspond to expected times of occurrence of the shared inhibitory input). Hence, for positive τ, the AB cross-correlation will reflect the periodicity of the B autocorrelation. By the same reasoning the BA cross-cor-

relation (using B spikes at $\tau = 0$) should show peaks at positive τ corresponding to the natural A period. Since the BA cross-correlation is identical with the AB cross-correlation except for a reversal in the sign of τ, we therefore expect the A periodicity to appear in the AB cross-correlation for negative τ.

By hypothesis, the times of occurrence of shared inhibitory input are unlikely in the region of $\tau = 0$ and hence are displaced to the right and left of the origin. It the common inhibitory cell is periodic, then periodic dips in the AB cross-correlation should appear at times displaced from the two paracentral troughs of the AB cross-correlation (Fig. 10). Thus, in this sense there will tend to be a displaced, distorted, bilateral, negative mapping of the common inhibitory autocorrelation on the AB cross-correlations. When any periodicity (due to this "occult" cell) appears

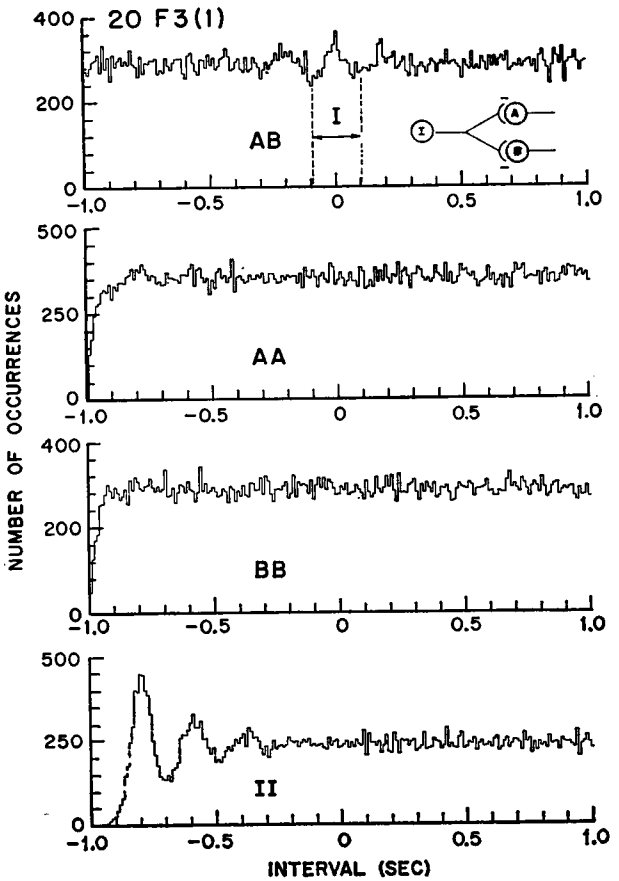


FIGURE 10 Shared inhibition arising from periodic source. Periodicity in AB cross-correlation which does not appear in A or B autocorrelation is indication of an occult periodic inhibitor.

in the *AB* cross-correlation but is weak or absent in the *A* and *B* autocorrelations (Fig. 10), the existence and periodicity of an unobserved inhibitor cell may be inferred. On the other hand, when the periodicity of one or more of the cells *A* and *B* fails to appear in the cross-correlation, as would be expected from monosynaptic coupling, the existence of a third cell must also be suspected.

When all three cells exhibit independent periodicities, there may be complex peaks in the *AB* cross-correlation. Fig. 11 shows an actual intracellular *Aplysia* record of shared inhibition superimposed on the *IA* and *IB* cross-correlations, together with the three cross-correlations. The correspondence between the IPSP waveform and the central trough of the *IA* and *IB* cross-correlations should be noted.

Reciprocal Synaptic Input. Finally, we consider the situation in which an IPSP in one cell occurs in conjunction with an EPSP in the other. This may be because the common source activates at least one interneuron in a chain, or because it can produce simultaneously and directly excitation and inhibition (26, 27).

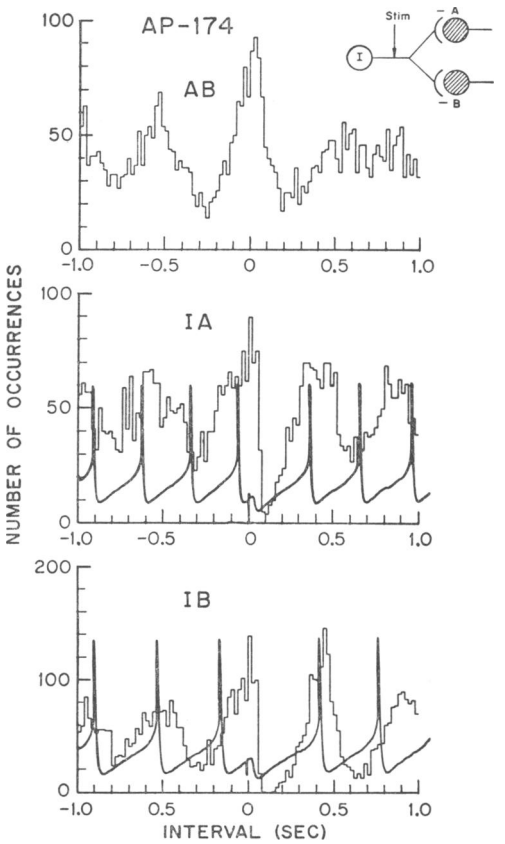


FIGURE 11 Shared inhibitory synaptic input. Two *Aplysia* pacemaker cells *A* and *B* with inhibitory postsynaptic potentials driven by a stimulator. *I* is the stimulating shock to the connective trunk. On the *IA* and *IB* cross-correlations are superimposed (at the same time scale) the *A* and *B* cell intracellular record showing stimulator-triggered IPSP's: the time course of the initial trough of the cross-correlation can be seen to follow the IPSP wave form, and the process by which the paracentral peaks are generated can be seen.

In Fig. 12 is shown a cross-correlation function for two postsynaptic cells A and B reciprocally driven by a common source. As one might expect, the arrival of an IPSP in one cell simultaneously with an EPSP in another tends to generate an anti-synchrony of firing in the postsynaptic neurons. The primary sign of reciprocal input will therefore appear as a dip in the cross-correlation at $\tau = 0$. In general, the depth of the trough relative to the base line is a function of the two synaptic potential amplitudes. In shape, the trough resembles the IPSP and is directed toward positive or negative τ depending on which cell is inhibited, and which cross-correlation (AB or BA) is being computed.

As before, when all three cells have flat autocorrelations, the primary sign is the only distinctive feature in the cross-correlation. In some cases this can indeed be misinterpreted as direct inhibition and some caution must be exercised. However, in other cases it may again be possible to infer the existence of an unobserved third cell by observing the presence or absence of a periodicity in the AB cross-correlation which is inconsistent with the hypothesis of direct inhibition. Conversely, cases can arise when periodicity in either the A or B cell fails to appear as predicted by the

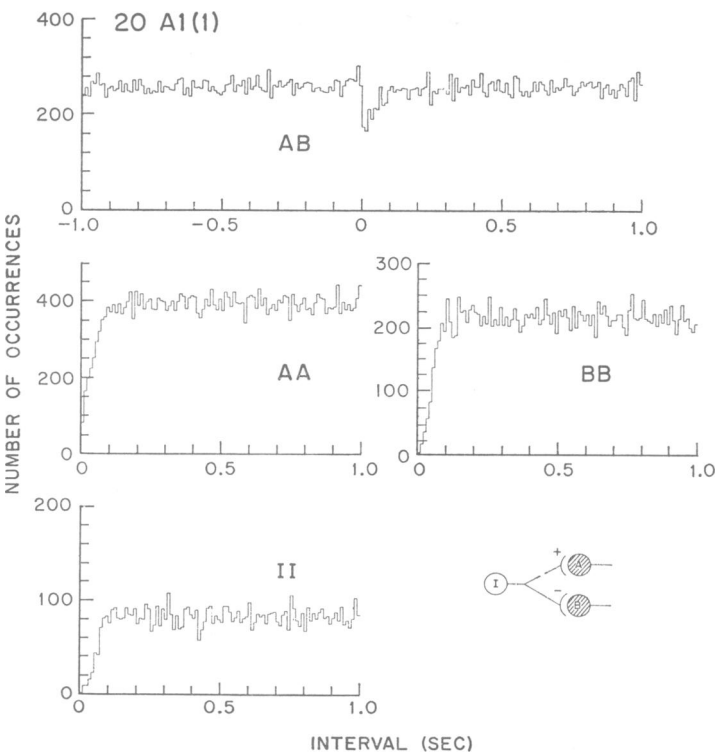


FIGURE 12 Shared reciprocal synaptic input. Basic case of simultaneous arrival of an EPSP in cell A and an IPSP in cell B. All three cells have flat autocorrelation histograms. Central trough is primary sign of reciprocal synaptic input.

hypothesis of direct excitation or inhibition, again suggesting that the correlation is induced by an occult cell. When all three cells are periodic, rather more complicated *AB* cross-correlations can arise.

SUMMARY OF RESULTS

We can summarize the results of these studies best by distinguishing two features of the cross-correlation function, namely the primary and secondary effects.

(*a*) The *primary effect* of a given synaptic arrangement is the peak or trough near the origin, shifted according to the conduction delay between the pre- and the postsynaptic spike-recording loci. Its amplitude, direction, and form reflect mainly the synaptic potentials involved, relatively unaffected by the over-all firing characteristics of the pre- and postsynaptic cells.

i. PRIMARY PEAKS can arise directly from monosynaptic or polysynaptic excitatory connections and from shared excitatory input. Typically broader primary peaks can also arise from shared inhibition, or shared rate changes (5).

ii. PRIMARY TROUGHS may arise from monosynaptic or polysynaptic inhibition, and from shared inhibition-excitation. Broader troughs may arise from nonstationarities in the same way as peaks arise, in those cases where a rate change in one cell is opposite in direction to the rate change in the other (5).

(*b*) *Secondary effects* are the structural features of the cross-correlation function occurring further from the origin. These are due mainly to the temporal structure of the pre- and postsynaptic cell spike trains. The autocorrelation function of the input spike train is mapped onto the cross-correlation function for both positive and negative τ values, and the strength of this mapping is a measure of the strength of the synaptic connection. The input autocorrelation mapping also reflects the polarity of the connection since inhibitory sources have their autocorrelations mapped negatively on the cross-correlation. Conversely, postsynaptic statistics are reflected only for positive values of τ in monosynaptic connections, while for shared input each postsynaptic cell contributes only to one half-plane. This asymmetry allows us to distinguish some otherwise ambiguous situations. For example, a sharp positive primary sign could arise either from direct connection between the recorded cells or from a shared excitory input: if a periodicity which belongs to neither observed cell is present for negative as well as positive τ values, the most likely interpretation would be a common excitatory source.

These general rules permit inferences to be drawn about the structural relations between empirically observed cells based on correlation analyses. Furthermore these rules indicate that at least in some instances two kinds of information may be available which are not directly observable. First, one can make fairly precise statements about the waveform, amplitude and polarity of the *intracellular* synaptic potential which couples the correlated cells, from purely extracellular measurements. Secondly, one can infer the existence of other neurons present in the network (for

example, a common source), and make rather precise statements about their firing characteristics, *even when no samples of their activity are observed directly.*

DISCUSSION

In the preceding sections we have explored several elementary open-loop network interactions and the quantitative two-neuron statistical relations to which they give rise. With each type of synaptic coupling there is associated first a characteristic central form of the cross-correlation function (the *primary effect*) whose details reflect the amplitude, polarity, and waveform of the synaptic potential by which the respective electrical histories of the two neurons are coupled. In addition, the cross-correlation function reflects certain aspects of the total average statistical impulse history of each of the participating neurons, and these aspects are reflected in what we term *secondary effects.*

The examples chosen have been simple and obvious ones, and where possible we have utilized intracellular records from animals to indicate the validity of the model and the mechanisms operating to induce correlations. The computer simulation program used was developed previously on the basis of observations from *Aplysia* neurons (16) and has not been modified in these computations to approximate known variations as occur, for example, in some mammalian preparations.

The utilization of the detailed information derived from purely extracellular pulse-train measurements in the study of internal events of the neuron is a technique which may have great importance when used in conjunction with the already proven techniques of the neurophysiologist and neuroanatomist. Much of the value of these statistical measures lies in their usefulness in detecting and characterizing various modes of synaptic coupling: they can, therefore, be instrumental in the development of new hypotheses about neuronal network structures, or as a means for validating existing hypotheses.

In practice, the process of hypothesis development will often require a reversal of the steps that we have utilized in this paper. Ordinarily, one proceeds from multiple direct extracellular measurements to computation of the autocorrelation of each observed cell and the cross-correlation of all pairs of cells. When significant correlations are present, the general conclusions presented here can be used in their interpretation, and they may lead to the development of a tentative hypothesis about network structure. At this point, the putative networks can be simulated, and the corresponding auto- and cross-correlations can be computed and compared with those derived from the real data.

There are, however, limitations to this procedure. Firstly, since we are comparing measures derived from two networks, at best we can only say that the *measures* either are or are not significantly different. This leaves open the question as to whether the *networks* are, in fact, equivalent or different. Significant differences between the measures imply that the two networks are not equivalent, but it may not

be possible to determine the reasons for this failure by simply examining the correlation measures. For example, the connections may be correct, but the values attributed to some parameters (e.g. PSP amplitudes) may be inappropriate.

It is important to point out also the serious limitation imposed upon this technique by the nonuniqueness of the inferential process about network structure. For example, an isolated central peak in the cross-correlation can arise from either monosynaptic excitation, polysynaptic excitation, common excitatory input, and even common inhibitory input. Practically it may be impossible to distinguish among these cases on the basis of the available data, and the cross-correlation histograms may serve only to restrict the number of possible explanations by excluding some.

Finally, there is the problem that, given a hypothesis about the network structure, techniques have not yet been developed which can estimate the large set of parameters necessary for the explicit representation of the model in a stimulation. Optimizing the choice of parameters by comparing *measures* of the model with *measures* of the real data is a truly formidable problem (28).

Although in this paper we have not been concerned with determining the statistical significance of observed features in the cross-correlation histogram, clearly this is a critical problem when one wishes to draw structural inferences from correlation measures. It should be pointed out that no generally satisfactory statistical tests for significance of features in the cross-correlation histogram are known by us. One useful expedient is to shuffle the intervals in one of the spike trains to generate a control cross-correlogram (5).

Until now, the use of cross-correlation analysis in the study of multiple units has been quite limited (29–41) due partly to the limited availability of suitable computing machinery and even more to the absence of any theoretical foundation for the interpretation of cross-correlations in terms of meaningful physiological mechanisms.

In some of these studies, positive correlations have been observed, usually presented without interpretation, but the corresponding autocorrelations, without which real analysis is impossible, have not been presented. Nor have any systematic reports been made of stationarity in the component records (but see reference 42); again, without these, interpretation of peaks or troughs in the cross-correlation, which can arise from nonstationarities (5), is impossible. Nonstationarities may arise from gradual deterioration of an animal preparation, changes in anesthesia level, or from periodically active influences such as respiration or blood pressure. For example, broad, nearly symmetrically located central peaks, accompanied by bilaterally located broad secondary peaks of progressively diminishing amplitude, were observed by Holmes and Houchin (32) in widely separated cortical units. Such correlations may be entirely attributed to nearly periodic brief increases in excitability of both cortical cells, which in fact exhibited pronounced, nearly simultaneous bursts at fairly regular intervals which corresponded to the separation between peaks in the cross-correlograms (33).

Recently, Rodieck (39) exhibited cross-correlations from three pairs of ganglion cells from the cat retina. Two of these showed significant correlation which the author did not interpret in functional terms. Examination of Rodieck's Fig. 10 B in comparison with our own simulations (Fig. 13) suggests that his two cells might be receiving input simultaneously from a common source, excitatory to one and inhibitory to the other (with a suggestion of periodicity in one of the three cells). This hypothesis has recently been suggested on the basis of other evidence (43). The question could be resolved only by examining the autocorrelations of the two cells, which were not published. It should be emphasized that autocorrelations must accompany cross-correlations as an indispensible aid to their interpretation. Rodieck's Fig. 10 C suggests a model of direct inhibition consistent with a number of our simulations and consistent with his own hypothesis of lateral inhibition, with neither cell apparently being strongly periodic. Wyman's (41) cross-correlations between motor neurons in the dipteran flight muscle system are similar in form. He interprets these also as evidence of reciprocal inhibition; our results are entirely in accordance with this view.

Recently, Noda, Manohar, and Adey (37) have reported that in the cat hippocampus cross-correlations between pyramidal neurons were essentially flat during wakefulness and rapid eye movement sleep, but showed strong peaks during intermediate and deep sleep. The authors tentatively interpret these findings in terms of a model in which both shared excitatory input and shared synchronized inhibitory input (followed by postinhibitory rebound) participate. Comparison of their cross-correlation histograms (Fig. 2 of Noda et al.) with Figs. 7 and 11 of this paper lends credence to this interpretation.

While we have stressed here the use of neuronal correlation in the study of network structure, we also wish to stress the additional usefulness of such information in the

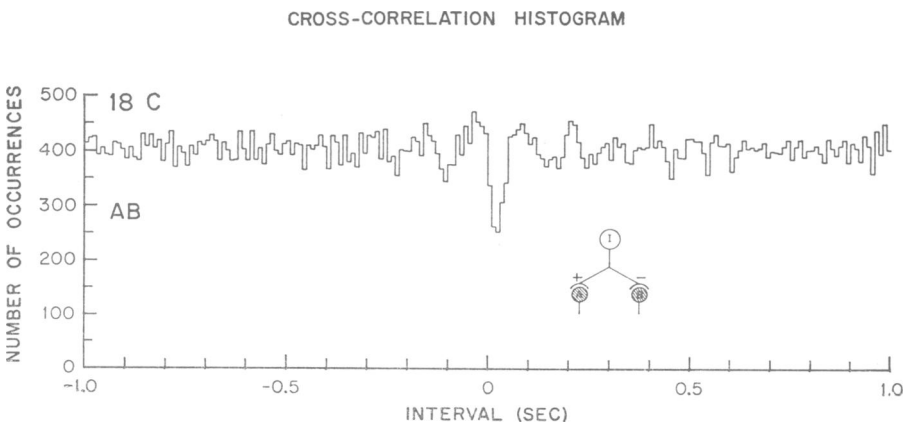


FIGURE 13 Shared synaptic input model of retinal ganglion cell interactions based on data of Rodieck (6). See text.

more general study of networks, since any quantitative theories of communication and control by the brain must include information about correlation or redundancy in firing among neurons. It has been shown recently that within physiological limits, variations in the degree of correlation among presynaptic terminals may qualitatively change the output of a postsynaptic cell (44, 21).

SUMMARY

The influence of basic open-loop synaptic connections on the firing of simultaneously recorded neurons has been investigated with auto- and cross-correlation histograms, using experimental records and computer simulations. The basic connections examined were direct synaptic excitation, direct synaptic inhibition, and shared synaptic input. To the cross-correlation histogram derived from each configuration there corresponds a primary sign, characteristic for that connection, and certain secondary signs which arise from the connection and from each of the component neurons as revealed by their autocorrelation histograms. The primary sign is a higher-than-average value for excitatory connections and for shared inputs of identical signs. It is a lower-than-average value for inhibitory connections and for shared inputs of opposite signs. These cross-correlation measures can be interpreted in terms of the underlying physiological mechanisms. Their potential uses and limitations in the detection and identification of synaptic connections between neurons whose extracellularly recorded spike trains are available are discussed. Certain statistical limitations are pointed out.

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