MUTUAL TEMPORAL RELATIONSHIPS AMONG NEURONAL SPIKE TRAINS

STATISTICAL TECHNIQUES FOR DISPLAY AND ANALYSIS

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ABSTRACT We describe a statistical technique, the joint peristimulus time (PST) scatter diagram, for the analysis of data from simultaneously recorded neurons subjected to repeated stimulation. Distinguishable features in the scatter diagram are related to effects of the stimulus on the observed neurons and to functional relations among the neurons. Properties of this measure and its variants are described and practical aspects of its application to experimental data are discussed.

INTRODUCTION

In recent years, increasing numbers of studies have been performed using simultaneous recordings of the activity of several neurons, thus opening a new level of neural integration to direct experimental investigation (Bullock and Horridge, 1965, p. 257). The interactive role of groups of neurons in carrying out integrative functions was stated by Sherrington: "It may be objected that it is not nerve impulses which are likely to tell us what we want to know of the brain. . . . Activity of the brain involves great numbers, not to say, vast numbers, of nerve cells cooperating. Yet the means of securing that cooperation is by impulses via the nerve fibers connecting cells" (Sherrington, 1951, p. 219). Although these ideas may be traced back even beyond Descartes, there continues to be only indirect evidence suggesting that the nervous system does indeed operate by means of relatively small functional groups of neurons.

The basic problems requiring investigation are those of identifying such functional groups and of characterizing their activity in terms of information processing or production of behavior. A higher order problem is that of plasticity of such functional neural groups.

Because functional grouping implies some degree of temporal organization in the activities of the constituent neurons, it follows that experimental evidence for the existence of such groups may be furnished by quantitative characterizations of the mutual temporal relationships in the electrical activity of the involved neurons. The further understanding of the operating principles of neuronal groups, once identified, is crucially dependent on detailed knowledge of the underlying circuitry.

Detailed description of functional neuronal connectivity is only partially furnished by classical neuroanatomical techniques, or by a combination of electrophysiological signal tracing and such newer methods as fluorescent dye injection and scanning electron microscopy. The approach described in this paper is intended as an adjunct to these more familiar methods; it can yield functional "circuit diagrams" of the connections among the observed neurons. Such a circuit diagram may, of course, not be unique, but rather may represent one member of a class of equivalent circuits.

These techniques, statistical in nature, must be imbedded in an appropriate experimental design in order to allow inferences of biological significance to be drawn. Application of these techniques to isolated fragments of data is likely to be a futile exercise.

The measures we describe involve the simultaneous recording of spike trains from several neurons, typically in conjunction with repetitive stimulation. As such, we treat the nerve impulses as point events (Perkel et al., 1967 *a*, *b*). The trains of events may be derived from extracellular recordings, or through intracellular recordings in which classes of postsynaptic events are resolved. It is important to realize that data obtained simultaneously from several neurons inherently contain kinds of information not available from an equivalent body of observations obtained serially from the same neurons.

In this paper we first introduce a class of statistical measures for multiple-neuron impulse data. We illustrate the effects of various assumed connections, describe control calculations, discuss various quantitative aspects, and extend these ideas to larger numbers of neurons. Examples are drawn both from computer simulation and from appropriate animal experiments; the latter are identified in the legends to the figures.

JOINT PST SCATTER DIAGRAM

The simplest statistical measure of the type under discussion is the joint PST scatter diagram (Gerstein and Perkel, 1969). This is a two-dimensional representation, in the plane, of the temporal relations among three simultaneously recorded sets of events: a train of periodic, identical stimuli and two trains of action potentials (A and B). We represent these data as a scatter diagram of the joint occurrences of spikes in trains A and B relative to the times of stimulation.

The construction of this scatter diagram from the data is illustrated in Fig. 1. The ordinate of each point plotted corresponds to the time between a stimulus event and a spike in A; the abscissa corresponds to the time between the same stimulus event and a spike in B. These coordinate values are indicated by the time intervals x_i and y_j in the figure. For the interstimulus interval shown we plot the six

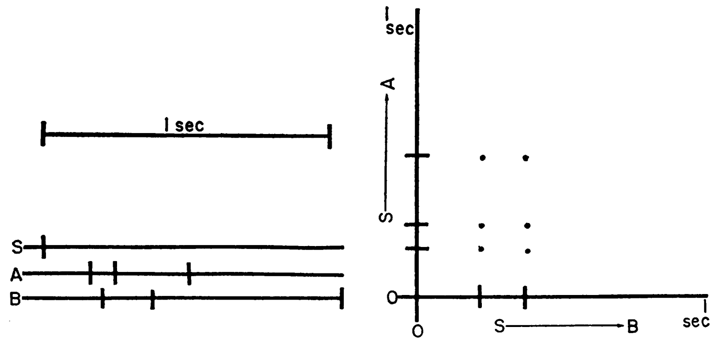

FIGURE 1 Method for constructing the joint peristimulus scatter diagram. The times of occurrence of impulses from neurons A and B during one stimulus interval are shown schematically at left; the corresponding contributions to the scatter diagram are shown at right. See text.

points (x_1, y_1), (x_1, y_2), (x_1, y_3), (x_2, y_1), (x_2, y_2), and (x_2, y_3); these are indicated by dots. This process is continued for all stimulus intervals in the record.

Clearly each spike gives rise, in general, to more than one point in the scatter diagram. The exact number depends on the number of spikes in the other train that occur during the same stimulus interval. In particular, if during one stimulus interval one of the neurons should fail to fire, no points will be added to the scatter diagram.

The resulting scatter diagram represents an estimate of an underlying joint PST density, defined as follows:

$$\xi_{AB}(t, u) = \lim_{\substack{\Delta t \to 0 \\ \Delta u \to 0}} [1/(\Delta t \Delta u)] \text{ prob } \{\text{spike in } A \text{ in } (t, t + \Delta t)$$

$$\text{and spike in } B \text{ in } (u, u + \Delta u) \mid \text{stimulus at zero}\}, \quad (1)$$

where the two time variables t and u are measured from a stimulus event. These variables may take negative values corresponding to the prestimulus period.

Tallies of points along various directions in the scatter diagram estimate certain well-known functions that are used to characterize unit activity. For example, the set of point tallies made in columns parallel to the principal diagonal (45° line), taking proper account of boundary effects, corresponds to the cross-correlation histogram between neurons A and B. Because of obvious geometrical relations, the time scale of this cross-correlation histogram is in the ratio $1:\sqrt{2}$ to that of the PST scatter diagram.

The sets of point tallies made in vertical and horizontal columns do not in general correspond to the PST histograms for the individual neurons. This may be shown by writing the joint density as the product of a conditional density and a marginal density:

$$\xi_{AB}(t, u) = \xi_{A|B}(t, u)\xi_B(u), \quad (2)$$

$$= \xi_{B|A}(u, t)\xi_A(t), \quad (3)$$

where the marginal densities $\xi_A(t)$ and $\xi_B(u)$ are the ordinary PST densities for the neurons indicated by the subscripts. The conditional density is defined as:

$$\xi_{A|B}(t, u) = \lim_{\Delta t \to 0} (1/\Delta t) \text{ prob } \{\text{spike in } A \text{ in } (t, t + \Delta t) \mid \text{spike in } B \text{ at } u \text{ and stimulus at zero}\}. \quad (4)$$

This function and the analogously defined $\xi_{B|A}(u, t)$ are functions of both time variables. Only when one neuron's firing probabilities are completely independent of the firings of the other neuron does its conditional density revert to the corresponding marginal density:

$$\xi_{A|B}(t, u) = \xi_A(t) \qquad \text{(under independence)}. \quad (5)$$

Comparison of equation 5 with equations 2 and 3 shows that independence implies mutual independence. In the case of independence, the joint PST density is the product of the corresponding marginal (ordinary PST) densities. This forms the basis for a control calculation described below. Moreover, only with such independence are the vertical and horizontal tallies proportional to the individual PST histograms.

In the ensuing examples of PST scatter diagrams, we conjoin the corresponding cross-correlation and PST histograms, computed from the original data.

SIGNS OF CONNECTIVITY IN THE SCATTER DIAGRAM

Each of the several possible types of functional connection to and between the observed neurons produces a characteristic signature in the joint PST scatter diagram. We illustrate these relationships with a series of examples of increasing complexity, drawn in these instances from computer simulations.

The null case of two neurons each of which fires entirely independently of the other and of the stimulus is shown in Fig. 2 *a*. Although the underlying density function is uniform over the plane, the scatter diagram, derived from a finite sample, frequently shows a clothlike texture. This effect is particularly evident when the number of spikes per stimulus interval is relatively large, in which case each spike gives rise to a set of points strung out along a line parallel to one of the coordinate axes. In the null case, both PST histograms and the cross-correlation histogram are flat to within the usual sampling fluctuations.

If the two neurons continue to fire independently, but if one is affected by the stimulus, we obtain the scatter diagram shown in Fig. 2 *b*. The vertical band of greater point density corresponds to an excitatory influence of the stimulus on neuron *B*. The latency of this effect is measured by the displacement of the vertical band to the right of the ordinate; the duration of the excitatory effect is measured by the width of the band. These stimulus effects appear in the corresponding PST histogram as the position and width of a peak. The uniform density of the band along its length is a consequence of the lack of any influence of the stimulus on neuron *A*, as shown by the flatness of the corresponding PST histogram.

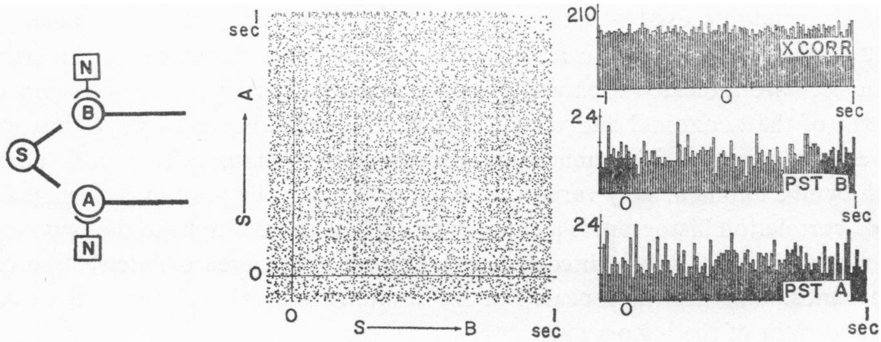

FIGURE 2 *a*

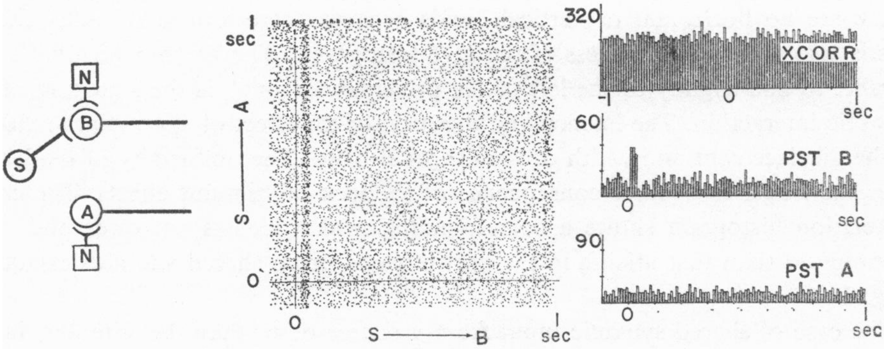

FIGURE 2 *b*

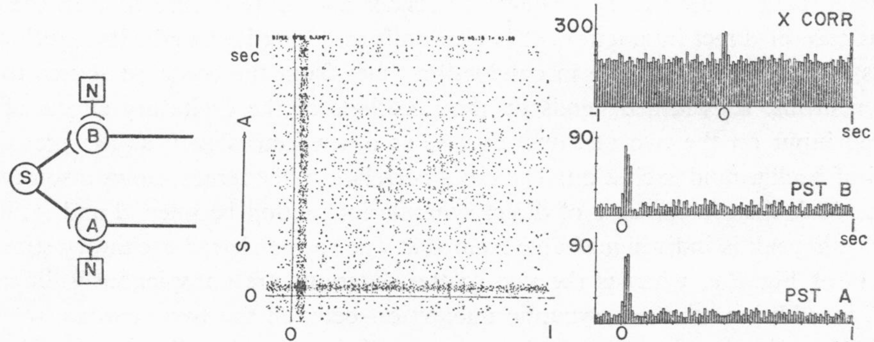

FIGURE 2 *c*

FIGURE 2 Stimulus effects. For each connectivity diagram, the corresponding joint peristimulus scatter diagram, cross-correlation histogram, and the two PST histograms are shown. The observed neurons are *A* and *B*. (*a*) Control case; stimulus has no effect and neurons are independent. (*b*) Stimulus excites neuron *B* only. (*c*) Stimulus excites both neurons.

If the stimulus excites both neurons, which otherwise fire independently, the scatter diagram of Fig. 2 *c* results. The latencies and durations of the stimulus influences are indicated separately for the two neurons by the displacements and widths of the horizontal and vertical bands. The peaks in the two PST histograms have corresponding interpretations. Because the stimulus affects both neurons, each band exhibits density variations along its length. The peak in the center of the cross-correlation histogram is a consequence of the shared input to the two neurons from the stimulus; its width corresponds to that of the area of intersection of the two bands. This peak is repeated at the stimulus interval, as seen at the extreme left and right of the histogram.

We now consider the case in which the stimulus has no effect on either neuron, but in which neuron *B* excites neuron *A* through a synaptic connection (Fig. 3 *a*). There are no horizontal or vertical bands because there are no stimulus effects; this is also seen in the flatness of the PST histograms. The narrow diagonal band, parallel to and slightly to the left of the principal diagonal, is the signature of the synaptic interaction. The latency and duration of the effect of *B* on *A* are reflected in the displacement and width of the diagonal band. The uniformity of its density along its length is another consequence of the lack of stimulus effects. The cross-correlation histogram shows a corresponding peak which is narrower and more pronounced than that arising in the previous case from shared stimulus excitation (Fig. 2 *c*).

The case of shared synaptic input from a source other than the stimulus, in the absence of stimulus effects and of direct interaction, is shown in Fig. 3 *b*. Again, there are no horizontal or vertical bands, and the PST histograms are flat. The diagonal band is seen to be somewhat broader and more diffuse than in the previous case of direct interaction. It is also uniform along its length. Its position reflects the effective difference in conduction times from the common source to the two neurons. Its width depends on the durations of the excitatory effects of the shared input on the two neurons, as well as other factors such as the prevailing level of background excitation. The cross-correlation histogram shows a somewhat wider peak than in the case of direct synaptic interaction between *B* and *A*; however, this peak is indistinguishable from that due to the shared excitatory stimulus effects of Fig. 2 *c*, whereas the two scatter diagrams are conspicuously different.

If, in addition to direct synaptic interaction between the two neurons, we also allow the stimulus to affect both neurons, all features described previously are visible in the scatter diagram and associated histograms (Fig. 3 *c*). The horizontal and vertical features, representing stimulus effects, are clearly distinguishable from

FIGURE 3 Interaction effects. (*a*) No stimulus effect; neuron *B* excites neuron *A*. (*b*) No stimulus effect; neuron *C* (not directly observed) excites neurons *A* and *B*. (*c*) Stimulus excites both neurons; neuron *B* excites neuron *A*. (*d*) Stimulus excites *A* and *B*; neuron *B* excites neuron *A* via interneuron *C*; stimulus inhibits neuron *C*.

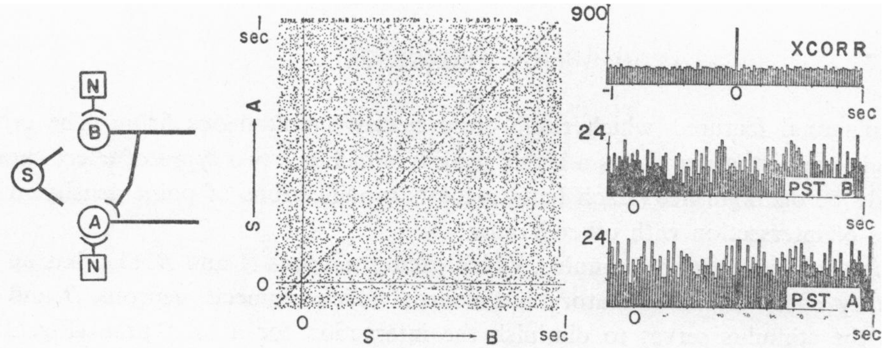

FIGURE 3 *a*

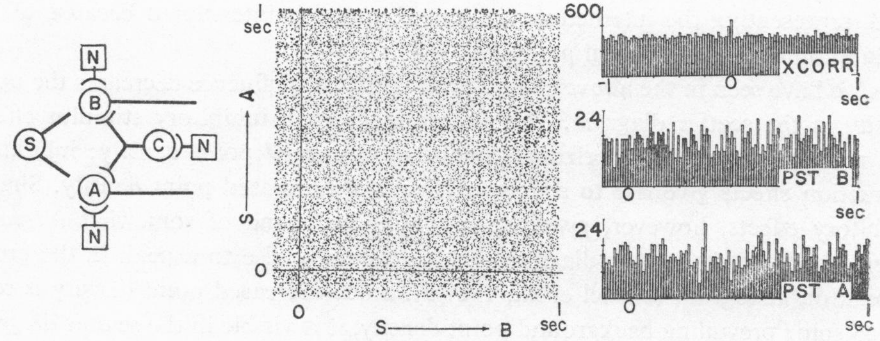

FIGURE 3 *b*

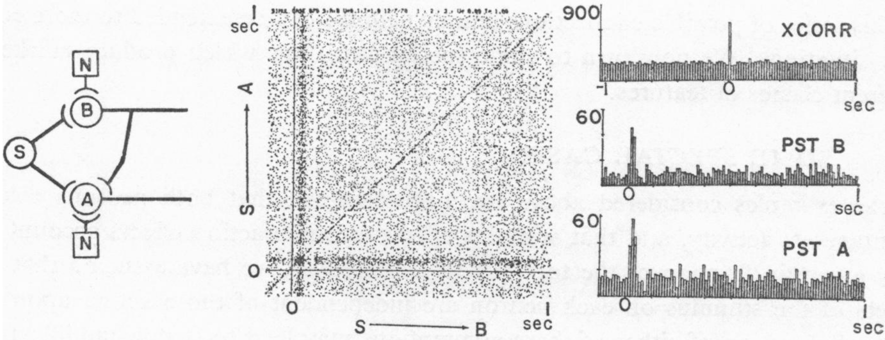

FIGURE 3 *c*

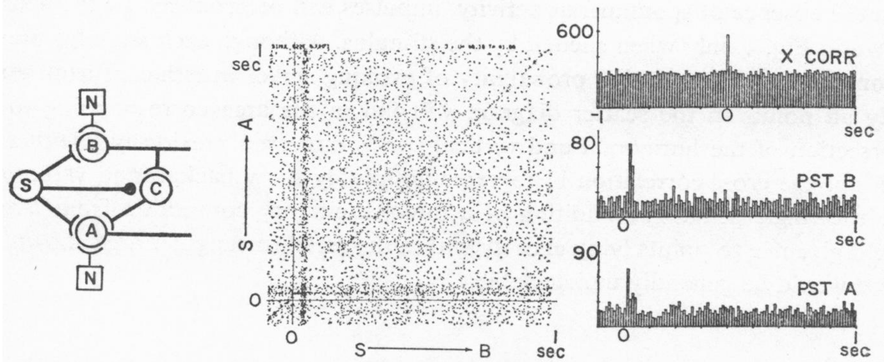

FIGURE 3 *d*

the diagonal features, which result from nearly simultaneous firing. The cross-correlation histogram shows a single peak in which the two types of effect cannot clearly be distinguished. Each band exhibits modifications of point density in regions of intersection with other bands.

Suppose now that the stimulus, which excites neurons A and B, also has an inhibitory effect on an excitatory interneuron which connects neurons B and A; thus the stimulus serves to diminish the interaction for a brief time (Fig. 3 *d*). Clearly distinguishable in the scatter diagram are the horizontal and vertical bands of increased density that result from the direct stimulus effects. The diagonal band, representing the interaction between B and A is interrupted because of the modulation of the interaction pathway.

As we have seen in the above example, an inhibitory influence decreases the point density in the scatter diagram. This is true in general: inhibitory stimulus effects give rise to vertical and horizontal bands of *decreased* point density; inhibitory interaction effects give rise to a diagonal band of decreased point density. Shared inhibitory effects, however, give rise to a diagonal band of somewhat *increased* point density in the scatter diagram, just as they give rise to a peak in the cross-correlation histogram (Perkel et al., 1967 *b*). Since decreased point density is relative to some prevailing background point density, it is visible in the scatter diagram only when the spontaneous activity is sufficiently great to provide the necessary contrast.

This series of possible connections among neurons can be extended to more complex situations. We now turn to some special situations which produce markedly different classes of features.

SOME SPECIAL CASES

In the examples considered above, we have assumed that both neurons exhibit spontaneous activity, and that stimulus effects and interaction effects account for only a certain fraction of the total activity. Moreover, we have assumed that the effects of the stimulus on each neuron are independent of those acting upon the other. Relaxation of either of these assumptions may lead to strong modifications in the scatter diagram.

In the absence of spontaneous activity, impulses will be produced by the neurons shown in Fig. 4 only when elicited by the stimulus. Although each stimulus presentation need not have a high probability of evoking spikes in either neuron, essentially all points in the scatter diagram will fall in the area corresponding to the intersection of the horizontal and vertical bands discussed previously. Neither the PST nor the cross-correlation histograms noticeably show background values outside the single peak. The addition of a direct excitatory connection from B to A would give rise to points lying entirely within the small rectangular area shown and hence would be generally undetectable.

If only one of the neurons, say *B*, exhibits spontaneous activity, then a horizontal band will be produced, while the vertical band remains in its "collapsed" state. Only if both neurons fire spontaneously will there appear the typical distribution of points over the plane.

When the responses of the two neurons to the stimulus are not independent,

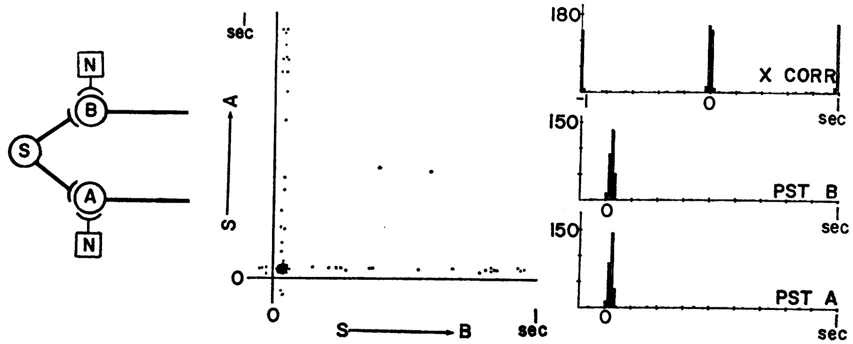

FIGURE 4 Stimulus excites both neurons in the absence of nearly all spontaneous activity.

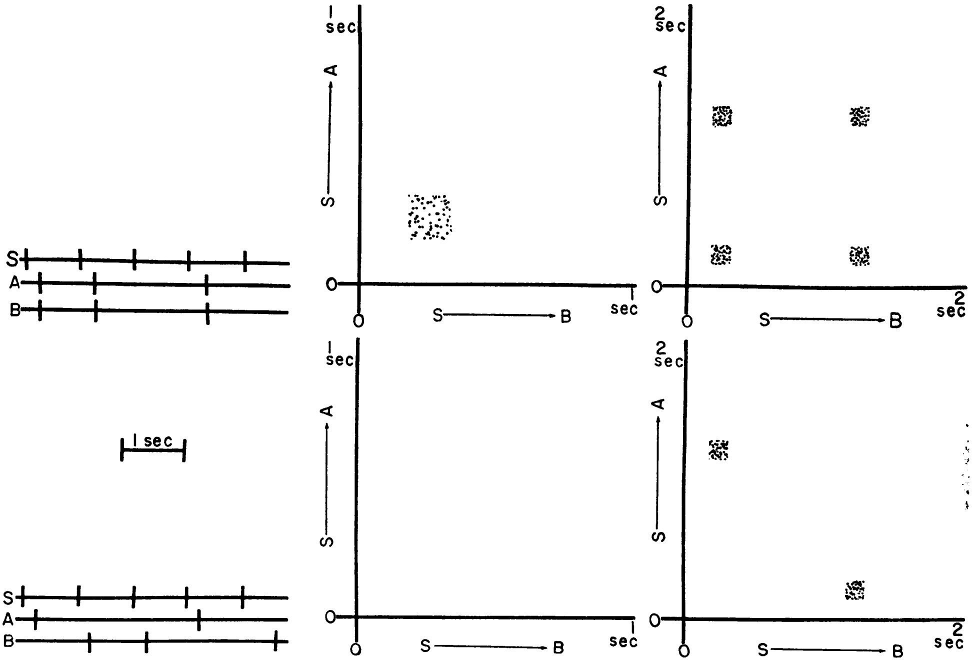

FIGURE 5 Correlated responses to the stimulus (semischematic). Upper row: in response to stimulus both neurons either fire at constant latencies or else both remain silent. The right-hand scatter diagram covers twice the time span of the left-hand diagram. Lower row: in response to the stimulus, one or the other neuron fires at its characteristic latency; both neurons never fire during the same stimulus interval.

the scatter diagram is modified in a different fashion. We illustrate this with two extreme cases. In the first situation, suppose that each stimulus presentation either causes both neurons to fire, or neither neuron to fire. As seen in the upper set of schematic records of Fig. 5, the firings of neurons *A* and *B* are maximally correlated. The corresponding scatter diagram shows a dense concentration of points at a location corresponding to the peaks in the two PST histograms and in the cross-correlation histogram, as also seen in Fig. 4. If, on the other hand, each stimulus presentation causes only one or the other neuron to fire, then no points whatever appear in the scatter diagram (Fig. 5, bottom). The two PST histograms, however, would be indistinguishable from those of the previous situation; the cross-correlation histogram would be empty. This somewhat surprising result may be clarified by extending the range of the scatter diagram to include two (or more) stimulus intervals, as illustrated at the right of Fig. 5.

Diagonal features in the 135° direction are also possible, under circumstances in which the poststimulus latency of one neuron is negatively correlated with that of the other. Suppose, for example, that two neurons fire fairly regularly and out of phase. If the stimulus is presented at roughly the same frequency, a small latency in one neuron is then associated with a large latency in the other neuron, and vice versa. This produces a band of increased point density oriented at 135°. Direct stimulus effects on these neurons will complicate this picture.

Complications of another sort can arise through serial correlation of stimulus effects. These topics are covered in some detail in the section on control calculations for the joint PST scatter diagram.

DIFFERENTIATION BETWEEN INTERACTION AND COMMON INPUT

A region of altered diagonal density in a PST scatter diagram, like the corresponding peak or valley in the cross-correlation histogram, represents coordination in the firing of the two neurons. Two major physiological mechanisms can produce such coordination: direct interaction between the neurons, typically synaptic (Fig. 3 *a*), and shared input from a common source (Fig. 3 *b*). On the basis of the PST scatter diagrams and the cross-correlation histogram it may be difficult to distinguish between the two mechanisms unless additional information is available, such as that provided by intracellular electrodes.

As previously illustrated (Perkel et al., 1967 *b*), the peak in a cross-correlation histogram is broader and lower when due to shared input of a given strength than when due to direct synaptic interaction of the same strength. A similar difference is seen in the diagonal bands of the PST scatter diagram; Fig. 6, drawn from computer simulation, illustrates these differences. In the network, one neuron provides excitatory synaptic input to two neurons each of which has additional independent excitatory input. For clarity, the intensity of the periodic stimulus was reduced to negligible values. In the left-hand diagram, measurements were taken from the

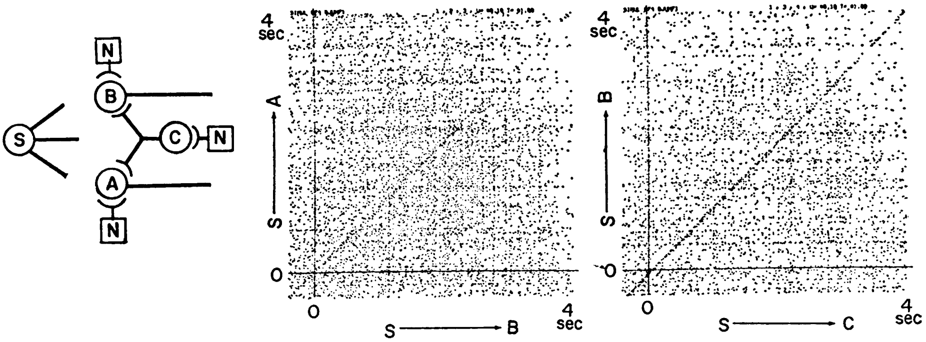

Figure 6 Comparison of effects of shared input with those of direct interaction. The left-hand scatter diagram is for neurons *A* and *B*, which share input from neuron *C*; the right-hand scatter diagram is for neurons *B* and *C*, which are directly connected.

two postsynaptic neurons. The diagonal band corresponding to the shared input is relatively broad and diffuse. In the right-hand diagram, taken from a pre- and a postsynaptic neuron, the diagonal band is narrower and more intense. Diagonal bands of both kinds have been observed in physiological experiments, and sometimes even in the same scatter diagram (Gerstein and Perkel, 1969).

In one particular situation the ambiguity is completely resolved. A narrow diagonal band that is *precisely* centered on and symmetric about the principal diagonal can only be the result of common input over conduction paths with identical delays. If, however, this band does not have a symmetric profile, some admixture of direct interaction is indicated.

The ambiguity between the effects of shared input and direct interaction are exacerbated by the introduction of interneurons in the direct pathway. Such interneurons will, in general, broaden and weaken a diagonal band in the scatter diagram, thus making it resemble even more closely the diagram for shared input. A sufficiently long chain of interneurons will wash out the observable features of a scatter diagram.

CONTROLS

In order to draw more quantitative conclusions from the features observed in the joint PST scatter diagram, we introduce control calculations. These controls exhibit just those features of the scatter diagram that arise from independent stimulus effects on the two neurons, i.e. those effects that are predictable in principle solely from the two PST histograms, which estimate the marginal PST densities. The difference between the joint PST scatter diagram and its control therefore represents all other factors influencing the joint firing of the two neurons, including direct interaction and shared input.

In principle, the appropriate control could be constructed simply by cross-multiplying the two PST histograms; however, because of their finite bin structure, the resulting two-dimensional histogram is difficult to compare directly with the original scatter diagram.

An equivalent control statistic, which consists of a scatter diagram rather than a two-dimensional histogram, is constructed as follows. One of the two spike trains is segmented at the instants of stimulus presentation, and these segments are subjected to a random shuffle. This shuffled train is used together with the unaltered train (of the other neuron) to construct a joint PST scatter diagram, in the usual manner. It is an estimate of the product $\xi_A(t)\xi_B(u)$. As seen in the example (Fig. 7), the control diagram retains the vertical and horizontal features of the original diagram with virtually no alteration. The diagonal features, however, are eliminated as such; their constituent points are rearranged and may take the form of vertically or horizontally oriented configurations.

In some situations the effects of the stimulus on one neuron may depend on the response of the other neurons. If this is the case, the control scatter diagram will also alter vertical and horizontal features of the original. One example is shown in the bottom row of Fig. 5.

In the majority of experimental situations, a simpler method suffices for constructing the control. Rather than segmenting and shuffling one spike train, we merely shift it along the time axis by one or more complete interstimulus intervals, after which we construct a scatter diagram as usual. Examples of the "shift control" are given by the off-diagonal squares in the extended scatter diagrams of Fig. 11. The shift control differs significantly from the shuffle control only if there is significant serial dependence in the effects of successive stimulus presentations. The most likely source of such serial dependence encountered experimentally is a long-term trend in the firing rates of both neurons. Thus, at the beginning of such an experiment, a stimulus may elicit more spikes in each train than at the end. In the presence of such trends, care should be exercised in the choice and interpretation

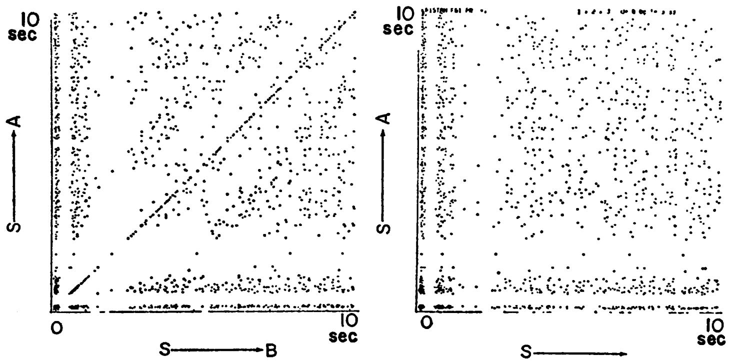

FIGURE 7 Original (left) and control (right) scatter diagrams. For the control, the nerve impulse times of one neuron have been shifted by one stimulus interval as described in the text. Note the disappearance of all diagonal features. Data from isolated pleural ganglion of *Aplysia californica* (Kristan, 1971).

of the control. Various gating mechanisms, unlikely to be encountered in the laboratory, can give rise to more pronounced differences between the two types of control.

To recapitulate, control scatter diagrams wash out all diagonal features of the original. If at a given stimulus presentation the response of one neuron depends on the response of the other, then, in addition, vertical and horizontal features are altered in either type of control. If stimulus effects are serially dependent, the two types of control will differ. All these effects may occur in a given experiment.

A particularly simple statistic for the comparison of actual and control joint PST scatter diagrams is the ratio of the actual number of points plotted to that predicted on the basis of the independence hypothesis. The predicted number is $N_A N_B / N_S$ (Kristan and Gerstein, 1970), where N_A and N_B are the numbers of spikes recorded from the two neurons, and N_S is the number of stimuli. Approximate significance levels may be derived from Poisson counting statistics. Refractory and other physiological phenomena undermine some of the assumptions underlying Poisson statistics, but this is rarely a practical problem unless stimulus presentations are too frequent.

A somewhat more complicated numerical comparison between actual and control joint PST scatter diagrams can be used to make inferences about neurons not under direct observation and even to estimate synaptic effectiveness. For example, suppose that an unobserved neuron is suspected to be interposed between the stimulus and the two observed neurons (Fig. 8). All three synapses are excitatory, act independently, and have the spike-eliciting probabilities P_i, P_a, P_b, as indicated. We observe both a horizontal and a vertical band in the scatter diagram and its control together with corresponding peaks in the PST histograms. In the control scatter diagram, the expected number of points within the horizontal band is equal to the product $N_S P_i P_a$; the expected number in the vertical band is $N_S P_i P_b$. In the actual scatter diagram, the number of points in the rectangular area of band intersection is given by the product $N_S P_i P_a P_b$. The three indicated point counts determine estimates of all three probabilities. If, for example, $P_i = 1$, then the postulated interneuron probably does not exist. If $P_i < 1$, the indicated diagram of neuronal connections is reasonable. If $P_i > 1$, the indicated diagram is not correct, and probably should include a stimulus-modulated interaction be-

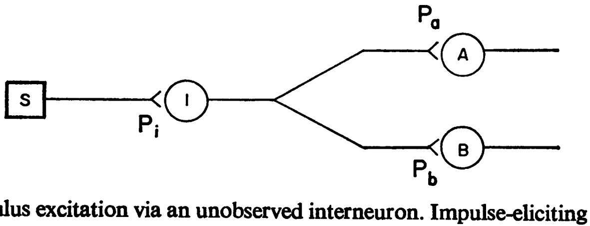

FIGURE 8 Stimulus excitation via an unobserved interneuron. Impulse-eliciting probabilities are indicated by P_i, P_a, P_b for the corresponding synapses. See text.

tween the two observed neurons. Spontaneous activity calls for a simple modification of this procedure.

TIME-COURSE OF SYNCHRONOUS FIRING

As we have seen, the joint PST scatter diagram depicts the direct effects of the stimulus as vertical and horizontal bands, whereas all other effects of interaction

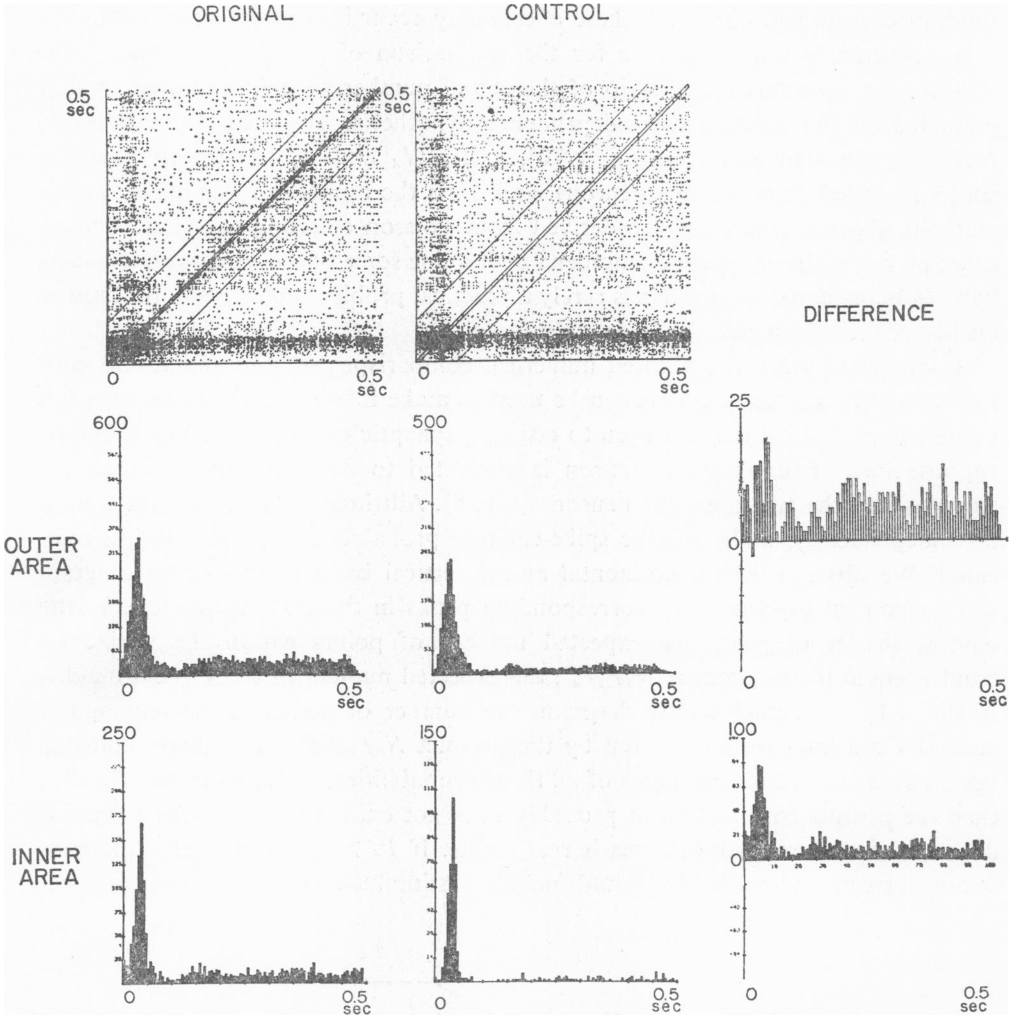

FIGURE 9 PST coincidence histograms. Upper row: original and control scatter diagrams. Superimposed lines delineate outer and inner areas about the principal diagonal. Middle row: PST coincidence histograms derived from the outer areas of the corresponding scatter diagrams; the difference histogram is shown at the right. Lower row: same as middle row, but for the inner areas of the scatter diagrams. Auditory cortex of unanesthetized cat; stimuli were periodic noise bursts.

or shared input appear as diagonally disposed structures. It is useful to examine the time-course of the latter effects relative to the stimulus.

The statistical measure we use corresponds to the marginal density that is estimated by the set of tallies along the direction perpendicular to the principal diagonal. The range with respect to the principal diagonal over which tallies are accumulated may be chosen so as best to study a particular interaction. The resulting histogram is called the PST coincidence histogram, because the events that contribute to it are not spikes, but rather are near coincidences of spikes from the two neurons.

As an example, we turn to experimental data from the auditory cortex of an awake cat (Fig. 9). We interpret the joint PST scatter diagram as implying both a direct interaction between the two neurons (narrow diagonal line) and a shared input to the two neurons (broad, less dense diagonal band), in addition to the direct stimulus effects (vertical and horizontal bands). The PST coincidence histograms shown are calculated using different regions about the principal diagonal. One histogram is taken from the outer area indicated in the scatter diagram; it excludes the inner region. In the other histogram, the region encompasses only the narrow diagonal feature attributed to direct interaction.

These histograms suggest that the phasic effects of the stimulus may be different for the broad and narrow bands; however, a more quantitative statement depends on the comparison with a control coincidence histogram, taken from the corresponding region of the control PST scatter diagram. Such control PST coincidence histograms are also shown in Fig. 9, together with histograms of the algebraic difference between corresponding originals and controls. From these, it is evident that the time-course of the process responsible for the narrow diagonal band is significantly different from that responsible for the broad band. This conclusion cannot be reached by mere inspection of the original joint PST scatter diagram.

The PST coincidence histogram measures only one aspect of the train of near coincidences. In general, this train may be treated by statistical methods appropriate to any train of point events (Perkel et al., 1967 *a*); for example, the interval histogram and autocorrelogram of this train are usually essential for detailed interpretations.

THREE OR MORE NEURONS

The joint PST scatter diagram can also be constructed for three neurons. A three-dimensional cartesian coordinate system is set up in which each axis corresponds to the time from stimulus presentation to the firings of one of the neurons. Positions of points are defined in a completely analogous way to that of the two-dimensional case; the resulting cloud of points is most conveniently visualized by plotting a stereoscopic pair of perspective views (Fig. 10), produced by a standard computer program "package" for graphic display, using well-known results from analytic geometry to provide the slightly discrepant projections.

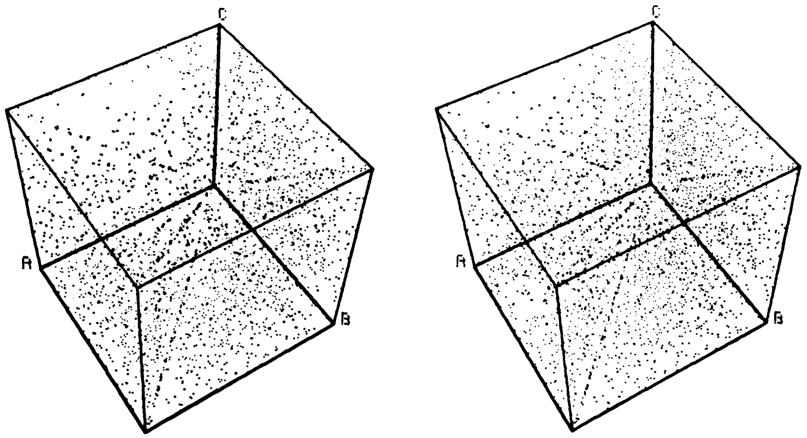

FIGURE 10 Joint peristimulus scatter diagram for three neurons; stereoscopic pair. Origin is the most distant vertex from the viewer. Note point concentrations along diagonals of three faces of the cube as well as along the principal diagonal of the cube. The left-hand cube should be viewed by the left eye. Data from isolated pleural ganglion of *Aplysia californica* (courtesy of W. B. Kristan, Jr.).

If the stimulus has no effect on the firing of any of the neurons, the cube corresponding to one stimulus cycle is uniformly filled with points. If one or more of the neurons fire several times during each stimulus cycle, a clothlike texture results as in the two-dimensional case.

Each neuron that responds with fixed latency to the stimulus gives rise to a plane of altered density. For example, if neuron B responds, the corresponding plane of points is parallel to the AC plane. If two neurons respond, two such planes will be generated, and their line of intersection will be particularly visible. If neurons A and B respond to the stimulus, this line of points will be parallel to the C axis. If all three neurons respond, the three resulting planes will intersect pairwise in three mutually perpendicular lines, and a local concentration of points will be seen around their locus of common intersection. Where the effects of stimulus are inhibitory rather than excitatory, regions of decreased point density will appear instead of regions of increased point density.

Coordinated firings of the neurons give rise to various diagonal features in a systematic manner. If neurons B and C tend to fire synchronously, the resulting plane of increased point density passes through the A axis and through the bisectrix (45° line) of the BC plane. If all three neurons tend to fire synchronously, the three corresponding planes will intersect on the line which forms the principal diagonal of the cube. Fixed latencies of coordinated firing give rise to a similar system of planes which lie parallel to those of the strictly synchronous case.

In typical physiological cases, all the above features may be present simultaneously, and become apparent in the stereoscopic view (Fig. 10). In a manner analogous to the two-neuron case, modulating effects of the stimulus are reflected by systematic changes of point density as a function of the distance from the origin.

If four or more neurons are included, direct visualization of the corresponding joint PST scatter diagram is unfortunately impossible. Examination of the N-dimensional space may be made in a number of indirect ways with the help of a computer. Without recourse to visualization, the computer program can search for altered point densities in the relevant hyperplanes, planes, lines, and points in N-space. Nearly synchronous firings of two or more neurons may themselves be treated as a single series of events (as in the calculation of the PST coincidence histogram above). That series can be used with two other spike trains to generate three-dimensional scatter diagrams of the usual sort. Estimation of probabilities through tallies and construction of controls may then be done in a manner completely analogous to the two-dimensional case.

NONPERIODIC STIMULUS

In most of the above examples, the domain of scatter diagrams and PST histograms has been exactly one stimulus interval. If we extend the domain to encompass more than one stimulus interval, as previously mentioned, a checkerboard-like pattern will emerge. Ignoring the minor contributions of end effects, the original square is reproduced in all its detail at successive squares that straddle the principal diagonal of the plane. The new squares that appear at off-diagonal locations are the control scatter diagrams that have been described above as being produced by shifting one spike train an integral number of stimulus intervals with respect to the other. Each such square is itself replicated along the direction parallel to the principal diagonal. Thus the entire diagram is invariant with respect to stepwise translation along the principal diagonal.

This invariance depends critically on the strict regularity of stimulus presentations. If there is minor departure from strict regularity, there will be a gradual blurring of stimulus-related features with increasing distance from the origin. In the case of highly irregular stimuli, clarity is preserved only in that portion of the original scatter diagram which lies within the time of the minimum interstimulus interval. These properties are illustrated in Fig. 11. For this reason, as well as for computational convenience, strictly regular stimulation should be used whenever possible in studying mutual temporal relations among neurons.

The blurring which we have described can be characterized mathematically, assuming that each stimulus presentation has independent effects. In the strictly periodic case we may interpret the replication of the pattern as the convolution of the primary square with the autocorrelation of the stimulus train, which in this case is a sequence of delta functions. In this context, the primary square delineates the "basic" response to the stimulus. With relaxation of strict regularity, we may continue to describe the resulting pattern in terms of convolution of the basic response with the autocorrelation of the stimulus train. The autocorrelation now consists of a series of increasingly broad peaks (except for the single delta function

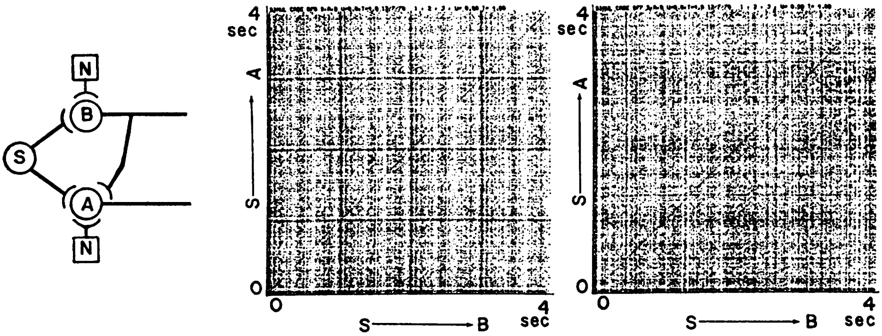

FIGURE 11 Effects of a nonperiodic stimulus. The left-hand scatter diagram extends over four stimulus cycles under conditions of strictly regular stimulus presentation. In the right-hand scatter diagram, which extends over the same time span, the stimulus was presented at random intervals with a mean of 1 sec and a standard deviation of 0.1 sec.

at the origin), which is responsible for the observed blurring. Convolution equations of this type are described by Perkel (1970).

In the extended "checkerboard" diagram, coordinated firings will produce their characteristic diagonal features only in the primary square and its replications along the principal diagonal of the plane. If stimuli are irregular, the consequent blurring will affect the representation of coordinated firing only with regard to stimulus modulation effects. In such a case, squares on the diagonal far removed from the primary square will show no variation of density along the direction of the principal diagonal. Variations of density along the orthogonal direction (135° line) continue to reflect the features of the cross-correlation histogram between the two spike trains.

The irregular stimulus train is conceptually not different from the spike train of a third neuron. Thus an experiment in which three spontaneously active neurons are monitored (ignoring any stimulation) may also be analyzed with joint PST scatter diagrams. The choice as to which spike train plays the role of the stimulus train is arbitrary; the three possible scatter diagrams contain equivalent information.

The asymmetry inherent in using one neuronal spike train in lieu of the stimulus for plotting a scatter diagram can easily be removed by the use of triangular coordinates. The axes of such a system, which intersect at 120° angles, represent the time differences $T_A - T_B$, $T_B - T_C$, and $T_C - T_A$; each point corresponds to such a triplet, and stimulus times (if any) are ignored. This variant of the joint PST scatter diagram can be understood equivalently as the projection of the PST cube (stimulus plus three spike trains) onto a plane perpendicular to the principal diagonal, taking proper account of boundary effects. In such a diagram (Fig. 12), synchronized firings between pairs of neurons give rise to radial concentrations of points midway between coordinate axes, while stimulus effects produce concen-

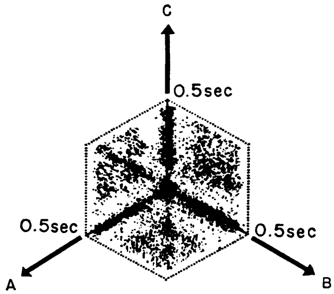

FIGURE 12 Triangular coordinate scatter diagram of the joint impulse activity of three neurons without reference to stimulus. This is also equivalent to the projection on a plane perpendicular to the principal diagonal of the three-dimensional joint PST scatter diagram. The three axes shown are those of the joint PST scatter diagram; the corresponding axes of the triangular coordinate system defined in the text are at right angles to the former. Cat auditory cortex. See text.

trations along lines parallel to the coordinate axes. Synchronous firings of all three neurons give rise to concentrations of points about the origin; systematic latency patterns displace the region of concentration. This hexagram is the natural generalization of the cross-correlation histogram to the case of three simultaneously observed spike trains. The generalization to the case of four spike trains can be plotted with tetrahedrally disposed coordinate axes. Such generalizations of the cross-correlation histogram directly indicate multiple interactions among the observed neurons. These interactions cannot be measured by combinations of pairwise comparisons alone.

DISCUSSION

As we have pointed out above, in order to infer detailed connectivity by analysis of spike timings, it is essential to make use of data recorded simultaneously from two or more neurons. Serial recordings from the same neurons provide only that information which is equivalent to the control calculations we have described; these contain only effects of the stimulus itself on each neuron individually. On the other hand, the full power of the joint PST scatter diagram for investigating interactions is only realized when it is used in conjunction with cross-correlation histograms and PST histograms, together with the appropriate controls. Such additional information as provided by autocorrelation histograms, interval histograms, serial correlograms, and the like is a desirable adjunct. Moreover, intracellular recordings may furnish a complementary set of basic data. These may serve as an independent corroboration of some aspects of the inferred neuronal connectivity, and also may help resolve ambiguities between direct interaction and shared input.

In fact, given sufficient stability of recording conditions, it is possible to generalize the joint PST description of spikes to the case of two or more continuous recordings (e.g., obtained through intracellular or gross electrodes). Interpretation of vertical, horizontal, and diagonal features is analogous to that for the scatter diagrams (C. E. Molnar, private communication). The time relations between a spike train and a simultaneously recorded continuous process (from the same or a different source) may also be analyzed through the same basic technique. Measures of this kind, in the absence of repetitive stimulation, have been used by Frost and Elazar (1968). Such measures delineate the time structure of correlation, with respect to the stimulus, and at the same time allow quantitative comparison with appropriate controls.

The principal strength of the joint PST scatter diagram over and above the cross correlation is the ease with which stimulus effects are abstracted. Isolation of stimulus effects through cross correlograms and PST histograms alone is cumbersome (Perkel et al., 1967 *b*); however, since the cross correlation is a marginal density of the joint PST distribution, it follows that the cross-correlation histogram is typically a more sensitive indicator of weak interactions than is the scatter diagram. Interpretation of features of the cross-correlation histogram has recently been reviewed (Moore et al., 1970). The ambiguity in the statistical signs of direct connection vis-à-vis shared input is common to the cross-correlation histogram and the joint PST scatter diagram. Although in particular situations this ambiguity can be readily resolved (Moore et al., 1970), a generally applicable technique has not yet been devised.

On the theoretical side, there are two principal directions in which further development is needed to improve the quantitative application of these measures. The first of these concerns sampling statistics, both for the original scatter diagram and for its various controls. There are at present no rigorous ways of evaluating the statistical significance of departures from the control diagram. The difficulties involve the choice of sampling time and the assignment of elemental areas in which to compare tallies.

The second direction for further development anticipates the availability of substantially larger numbers of simultaneously recorded spike trains. Although the methods we have described can in principle be extended indefinitely, the practicality of the associated computations decreases rather rapidly as the number of spike trains increases. A radically new approach is needed in order to define and measure a time-dependent "state function" characterizing the activity of a large group of neurons. Interesting attempts along these lines have appeared (Caianiello et al., 1968; Cowan, 1968). It would seem that phenomenological descriptions of neural activity are most usefully formulated within the context of the informational and behavioral function of the neural population under observation.

Despite these limitations, the joint PST scatter diagram and its associated measures are being applied in a number of current investigations of sensory coding, of motor pattern generation (Smith, 1970; Zucker, 1971), and of neural plasticity (Kristan and Gerstein, 1970; Kristan, 1971). These measures are particularly applicable to studies of plasticity arising through modifications of neural connectivity, not only because they provide quantitative measures of effective connectivity, but also because the necessary data can be provided through relatively nondestructive extracellular recording methods.

Note Added in Proof. An essentially identical scatter diagram has been described by Lyamin (1971).

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REFERENCES

Bullock, T. H., and G. A. Horridge. 1965. Structure and Function in the Nervous System of Invertebrates. W. H. Freeman and Co., Publishers, San Francisco, Calif.

Caianiello, E. R., A. de Luca, and L. M. Ricciardi. 1968. *In* Neural Networks. E. R. Caianiello, editor. Springer-Verlag KG., Berlin, W. Germany.

Cowan, J. D. 1968. *In* Neural Networks. E. R. Caianiello, editor. Springer-Verlag KG., Berlin, W. Germany.

Frost, J. D., Jr., and Z. Elazar. 1968. *Electroencephalogr. Clin. Neurophysiol.* **25**:499.

Gerstein, G. L., and D. H. Perkel. 1969. *Science (Washington).* **164**:828.

Kristan, W. B., Jr. 1971. *J. Neurophysiol.* **34**:321.

Kristan, W. B., Jr., and G. L. Gerstein. 1970. *Science (Washington).* **169**:1336.

Lyamin, E. A. 1971. *In* Mathematical Models of Biological Systems. G. M. Frank, editor. Izdatelstvo "Nauka," Moscow.

Moore, G. P., J. P. Segundo, D. H. Perkel, and H. Levitan. 1970. *Biophys. J.* **10**:876.

Perkel, D. H. 1970. *In* The Neurosciences: Second Study Program. F. O. Schmitt, editor-in-chief. The Rockefeller University Press, New York. 587.

Perkel, D. H., G. L. Gerstein, and G. P. Moore. 1967 *a*. *Biophys. J.* **7**:391.

Perkel, D. H., G. L. Gerstein, and G. P. Moore. 1967 *b*. *Biophys. J.* **7**:419.

Sherrington, C. S. 1951. Man on His Nature. Cambridge University Press, London, England. 2nd edition.

Smith, D. O. 1970. Motor control of the crayfish claw. Ph.D. Dissertation. Stanford University, Stanford, Calif.

Zucker, R. S. 1971. A neuronal circuit mediating escape behavior in the crayfish. Ph.D. Dissertation. Stanford University, Stanford, Calif.