NEURONAL SPIKE TRAINS AND STOCHASTIC POINT PROCESSES

II. Simultaneous Spike Trains

DONALD H. PERKEL, GEORGE L. GERSTEIN, *and* GEORGE P. MOORE

From the Department of Mathematics, The RAND Corporation, Santa Monica, California 90406, the Departments of Biophysics and Physiology, School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania 19104, and the Departments of Physiology and Engineering and the Brain Research Institute, University of California, Los Angeles, California 90024

ABSTRACT The statistical analysis of two simultaneously observed trains of neuronal spikes is described, using as a conceptual framework the theory of stochastic point processes.

The first statistical question that arises is whether the observed trains are independent; statistical techniques for testing independence are developed around the notion that, under the null hypothesis, the times of spike occurrence in one train represent random instants in time with respect to the other. If the null hypothesis is rejected—if dependence is attributed to the trains—the problem then becomes that of characterizing the nature and source of the observed dependencies. Statistical signs of various classes of dependencies, including direct interaction and shared input, are discussed and illustrated through computer simulations of interacting neurons. The effects of nonstationarities on the statistical measures for simultaneous spike trains are also discussed. For two-train comparisons of irregularly discharging nerve cells, moderate nonstationarities are shown to have little effect on the detection of interactions.

Combining repetitive stimulation and simultaneous recording of spike trains from two (or more) neurons yields additional clues as to possible modes of interaction among the monitored neurons; the theory presented is illustrated by an application to experimentally obtained data from auditory neurons.

A companion paper covers the analysis of single spike trains.

INTRODUCTION

In a companion paper (Perkel, Gerstein, and Moore, 1967), we discuss the statistical analysis of the single spike train. The mathematical framework used is the theory of stochastic point processes, that is, of random processes whose realizations may be described as series of point events occurring in time. In that paper and in a recent review (Moore, Perkel, and Segundo, 1966), it is shown how analysis of spike trains

by means of these statistical techniques can shed light on such neurophysiological questions as the mechanisms of spike production, integrative, i.e. input-output, properties of nerve cells, and theories of information processing by the nervous system.

It is, however, in the comparison of two or more simultaneously observed spike trains that we believe lies the greatest potential usefulness for the statistical analysis of the data provided by precise measurement of times of spike events. It has become increasingly feasible to record spike trains simultaneously from several neurons (Gerstein and Clark, 1964; Simon, 1965). These experimental techniques have been motivated by the promise that intercomparison of spike trains will reveal details of synaptic connections and other sources of interaction among the observed neurons. The statistical techniques for analyzing simultaneous spike-train data, however, are not in as satisfactory a state of development as the experimental techniques for obtaining these data. The present paper is offered as a contribution to the statistical theory so that the experimental techniques may be more thoroughly and properly exploited.

There are two fairly conspicuous difficulties associated with two-train analyses. The first has to do with sampling distributions: in the principal statistical measures, e.g. the cross-correlation histogram, the distribution of tallies is not known in general, and except in the special case of two independent Poisson processes, the observations do *not* constitute Bernoulli trials. Hence, confidence limits and tests of hypotheses based on binomial statistics are not valid, and their use, although reported in the neurophysiological literature, may yield erroneous conclusions from the data.

The second difficulty is involved with the interpretation of the statistics when dependencies are found. It is not always clear whether a given cross correlation is better explained by a synaptic connection between the two neurons, mediated perhaps by one or more interneurons, or alternatively by a shared source of input to the two cells. Interpretation of simultaneous spike-train measurements must take cognizance of the several alternative possible explanations for observed statistical signs of dependence and acknowledge the difficulty which arises from the paucity of our quantitative knowledge of the statistical consequences of each of the several postulated alternative possibilities.

In both of the difficulties mentioned and with regard to complications arising from nonstationarities, we have found the use of simulation of interacting neurons on a digital computer to be a powerful and indispensable tool. Many of the examples and illustrations presented, therefore, are drawn from computer simulations (Perkel, 1965).

The interpretation of data taken simultaneously from three sources of spikes is at present considerably more difficult than that of two-train data. One somewhat special case is furnished by experiments in which the third source of point events consists of the times of presentation of a brief stimulus. When these presentations

are sufficiently infrequent, the intercomparison of the three trains of events (two spike trains and the stimulus-presentation train) can yield much more information about the underlying functional relationships between the two neurons than can be obtained from analysis of their spontaneous activity alone.

In this paper, then, we discuss first the consequences of the independence hypothesis for two spike sequences. The statistical measures are based on the idea that, for independent trains, spikes in one cell represent random instants in time with respect to the other. We then describe their use in tests for independence and show the effects on the statistical measures of some of the typical types of dependence. We next discuss and illustrate the effects of nonstationarities, in the form of rate trends, on these statistical measures. Finally, we develop a simple theory for dealing with brief, repetitive stimuli in conjunction with the observation of two spike trains; we apply these methods to an example based on experimental data from auditory neurons.

TWO SIMULTANEOUS SPIKE TRAINS

The Problem of Functional Relationship

In the interpretation of spike trains recorded simultaneously from several neurons, the spike trains are ordinarily compared by pairs.[1] The first question to be asked is whether the two trains of spikes are independent. More specifically, we wish to make a statistical test of the null hypothesis that the two trains are drawn from independent point processes. This would imply that the two neurons are functionally unrelated. The test is accomplished, in principle, by computing a histogram, which estimates a suitable function, e.g. either the cross-density or the cross-correlation function described below, and comparing the estimate with the predicted function as based on the assumed independence of the two trains. If the observed and predicted functions differ significantly, the null hypothesis is rejected, and the trains are considered to be dependent.

An observed dependence between two spike trains can arise from one (or both) of two sources: (*a*) functional interaction and (*b*) common input. By functional interaction, we mean any mechanism by which the firing of one neuron influences the probability of firing by the other neuron. Such mechanisms could be synaptic (whether direct or mediated through interneurons), ephaptic, or due to "field effects." By common input we mean any mechanism that simultaneously modulates the firing patterns of both neurons. Such mechanisms could involve synaptic contact from branches of the same axon or field effects from a source other than the two neurons. It must be emphasized that long-term concomitant changes in firing rates, if shared wholly or in part by two neurons, constitute a form of dependence and if

[1] Statistical measures for the concurrent intercomparison of three or more trains are currently under investigation.

sufficiently pronounced, will be detected by statistical test procedures (see discussion below on Types of Dependence).

Independence: Consequences of the Null Hypothesis

According to the null hypothesis, spike trains A and B are independent in the mathematical sense. This means that spikes in train A occur at moments taken at random with respect to train B. In relating the two spike trains we may therefore use some mathematical results about single point processes observed from random moments in time.

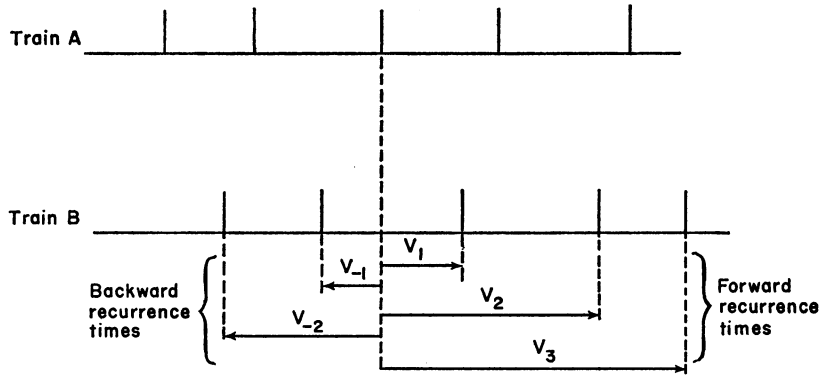


FIGURE 1 Recurrence and waiting times. Train B is a train of spikes. If the events in train A are random instants in time, then V_1 and V_{-1} are the (first-order) forward and backward *recurrence times*, respectively; V_2 and V_{-2} are the corresponding second-order recurrence times, etc. If train A is a spike train, then (in the usage of this study) the corresponding time intervals are called *waiting times*, and are designated W_1, W_{-1}, W_{-2}, etc. See text.

One such result concerns the distribution of so-called *recurrence* times, which are defined as follows. From a random instant in time, we denote by V_1 the time until the next event in a point process and by V_{-1}, the time backward to the most recent event in the point process; V_1 and V_{-1} are known as the *forward* and *backward recurrence times*, respectively (Fig. 1). Each of these times has the same distribution with the pdf

$$g_1(\tau) = [1 - F(\tau)]/\mu = \mathfrak{F}(\tau)/\mu, \tag{1}$$

where μ is the mean interspike interval; $F(\tau)$, as usual, is the cumulative probability distribution of the intervals; and $\mathfrak{F}(\tau)$ is the survivor function. This result is true for both renewal and nonrenewal processes (Cox, 1962; McFadden, 1962).

In terms of two spike trains, this result means that the time from a randomly selected spike in A to the immediately following, or to the immediately preceding,

spike in B is distributed with the pdf

$$\eta_1(\tau) = \eta_{-1}(\tau) = [1 - F_B(\tau)]/\mu_B \tag{2}$$

if the two spike trains are independent. A similar relationship holds, of course, for the times between any spike in B and the neighboring spikes in A, with the appropriate change of subscripts.

Generalizing this notion, we may make the corresponding measurements from two simultaneous spike trains, whether or not they are in fact independent. We designate as W_1 the "waiting time" from a spike in train A to the next subsequent spike in train B and as W_{-1}, the time backward to the most recent spike in train B. The distributions of these random variables are specified by their pdf's, $\eta_1(\tau)$ and $\eta_{-1}(\tau)$, which we call the *forward* and *backward cross-interval densities*, respectively. These densities, as usual, are estimated by histograms constructed from the observed spike trains. If the trains are independent, the cross-interval histograms will agree with their predicted forms, according to Equation 2. It is to be noted that this prediction is based only on the interval distribution in train B. If the observed histograms agree with the predictions, however, it can be concluded that the trains are independent only insofar as adjacent spikes are concerned. Effects of one neuron's spikes on the other's spikes may well be delayed to the extent that they will not be revealed in the cross-interval histograms. Departures of the cross-interval histograms from their predicted form may often indicate the type of dependence between the two trains. Furthermore, in cases of indicated dependence, the forward and backward cross-interval histograms will in general be different.

The second mathematical result deals not only with the first-order recurrence times (forward and backward to the adjacent events), but with the sum of these for all orders. Letting V_2, V_3, $\cdots$ refer to the times from a random moment to the second, third, $\cdots$ events encountered in the process B, we may define in general $g_i(\tau)$ to be the pdf of recurrence time V_i. If these pdf's exist (as they do for most physically realizable processes, including spike trains), then it follows from a result of McFadden (1962, Equation 2.21) that the sum of the recurrence densities of all orders is a constant:

$$G_B(\tau) \equiv \sum_{i=1}^{\infty} g_i(\tau) = 1/\mu_B . \tag{3}$$

Here μ_B is the mean interval between events in the process B, which is not necessarily a renewal process. Since the backward recurrence times V_{-i} of all orders have the same distributions as the corresponding forward recurrence times, their sum also has the same distribution as that for the forward times.

We proceed as before to generalize to the case of not necessarily independent spike trains, and we define the *forward* and *backward waiting times of order i*, W_i and W_{-i}, as the time measured from a spike in train A to the ith subsequent spike en-

countered in train B, or backward to the ith previous spike in train B, respectively (Fig. 1). The corresponding *cross-interval densities of order i* are denoted by $\eta_i(\tau)$. We further define the *cross-correlation function* $\zeta_{AB}(\tau)$ as the sum of all orders of cross-interval densities:

$$\zeta_{AB}(\tau) = \sum_{\substack{i=-\infty \\ i\neq 0}}^{i=\infty} \eta_i(\tau). \tag{4}$$

Now, reasoning as we did with Equations 10 and 11 in Perkel et al. (1967) for the autocorrelation, we realize that for a given spike in train A, the time, backward or forward, to any spike in train B must be the waiting time of some order i ($i = \cdots, -2, -1, 1, 2, \cdots$). Therefore, the cross correlation represents the probability of encountering any event in train B as a function of time before or after an actual event in train A:

$$\zeta_{AB}(\tau) = \lim_{\Delta t \to 0} \text{prob}\,\{\text{an event in } B \text{ in } (t_0 + \tau,\ t_0 + \tau + \Delta\tau) \mid \text{an event in } A \text{ at } t_0\}/\Delta t. \tag{5}$$

Functions of this kind are called "cross intensity functions" by Cox and Lewis (1966, p. 247).

The same reasoning applies, of course, if train A consists of random instants of time, and so we may write

$$\begin{aligned} G_B(\tau) &= \lim_{\Delta t \to 0} \text{prob}\,\{\text{an event in } B \text{ in } (t_0 + \tau,\ t_0 + \tau + \Delta t) \mid \text{random instant } t_0\}/\Delta t \\ &= 1/\mu_B\,. \end{aligned} \tag{6}$$

There is a parallelism between the function $G_B(\tau)$ and the renewal density (autocorrelation) $h(\tau)$. If we observe a stochastic point process starting at a *random event*, then the probability of encountering spikes at any time thereafter is measured by $h(\tau)$. For large τ, i.e. at long times after the initial observation point, this density becomes a constant, equal to the mean firing rate $1/\mu$ (Equation 12 in Perkel et al., 1967). On the other hand, if we start our observation at a *random instant* in time, rather than at a particular event, the probability density $G_B(\tau)$ of encountering a spike at some later time is already a constant. Heuristically, this means that if we know that a spike has occurred, we can predict (from the statistical properties of the spike train) when subsequent spikes will occur; our prediction becomes worse the farther removed we are from the initially observed spike, until our prediction is based solely on the mean firing rate. But if we start at a random moment, we do not even know the position of one spike, and we can do no better at predicting subsequent spike locations than by using the mean rate. These considerations apply to both renewal and nonrenewal point processes.

The cross-correlation function $\zeta_{AB}(\tau)$ has a useful symmetry relationship. Consider the compound event "a spike in record A is followed at a time X by a spike in record B"; this event may be equivalently described as the event "a spike in record B is preceded at a time X by a spike in record A." The probability of this compound event may be expressed in either of two ways, implying the following symmetry relationship for the cross-correlation:

$$\zeta_{AB}(\tau)/\mu_A = \zeta_{BA}(-\tau)/\mu_B \,. \tag{7}$$

This identity holds even if the two trains are not independent. For this reason, the cross-correlation function is customarily measured in only one direction, from train A to train B, and for both forward and backward waiting times, i.e., for both positive and negative values of τ.

Application to Experimental Data

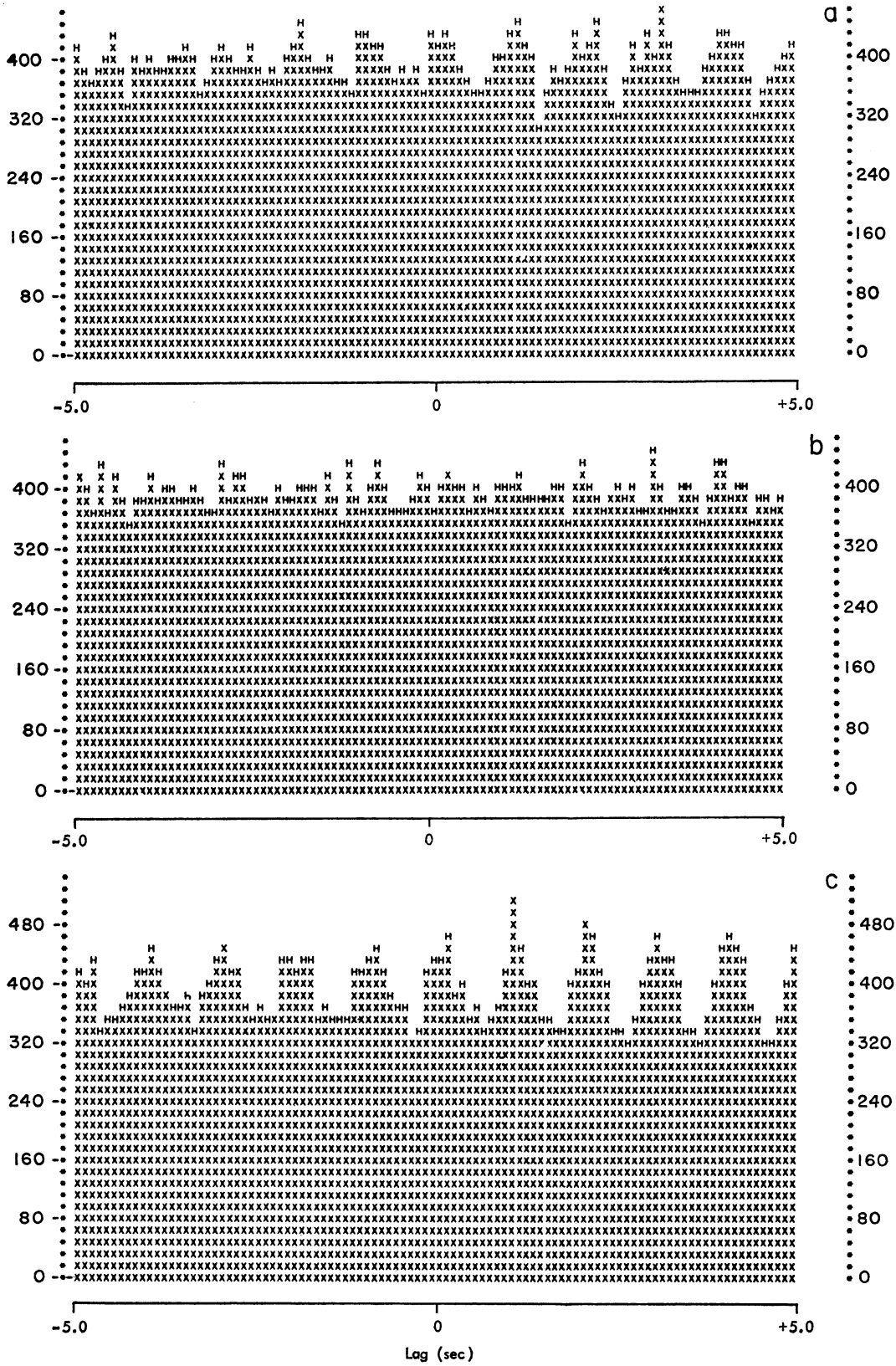
Thus, in order to test whether two spike trains are independent, we may make the following measurements:

(*a*) The *cross-interval histogram.* We select spikes in train A and construct a histogram of the times to the *nearest* spikes in train B. This histogram can then be compared with the estimated backward and forward recurrence-time densities $\eta_1(\tau)$ and $\eta_{-1}(\tau)$, which in turn are estimated from the interspike-interval histogram of train B, using Equations 4 *b* in Perkel et al. (1967) and Equation 2 above. This procedure must be repeated after interchanging the rôles of the trains.

(*b*) The *cross-correlation histogram.* We select spikes in train A and construct a histogram of the times to *all* spikes in train B, both forward and backward, out to some specified time. This histogram provides an estimate of the cross-correlation function $\zeta_{AB}(\tau)$, which can be compared with the predicted constant value $1/\mu_B$.

If the two spike trains are independent, then the forward and backward cross-interval histograms will be equal to the prediction $[1 - F_B(\tau)]/\mu_B$ and hence equal to each other, within sampling effects. Since these equalities are a necessary but not sufficient condition for independence of the spike trains, the cross-interval histograms have two major uses: (*a*) as a corroboration of independence when indicated by a flat cross correlation, and (*b*) as a means of exploring suspected short-latency interactions, i.e., those occurring with a latency smaller than the mean interspike interval in train B. Cross-interval histograms must be computed separately for train A against train B and vice versa. These measurements are related to the phase histograms of Wyman (1965).

Satisfactory statistical tests of the null hypothesis of independent firings have not yet been completely developed (see Cox and Lewis, 1966, pp. 247–248). There are two sources of difficulty. The first arises from the lack of independence of successive bins in the cross-correlation histogram, as was mentioned for the PST histogram in Perkel et al. (1967). An observation contributing to one bin of the histogram is pro-


a
b
c
400
320
240
160
80
0
-5.0
0
+5.0
480
Lag (sec)

duced by the firing of a neuron; the refractoriness of the neuron will then lower the probability of a contribution to the next bin. The magnitude of this effect depends on the degree and duration of refractoriness, as well as on bin width. If two neurons are in fact independent, consider a bin that has a larger number of spikes than average due to a random fluctuation. The next bin is then more likely to have a smaller number of spikes than average. Thus, the contribution to the sum of squares of deviations from the mean will be larger than if bins represented independent observations. This can lead to false attributions of dependence to cells that are, in fact, firing independently.

A related source of the same type of error arises from the sampling requirements for spike trains of neurons that are pacemakers. Two independently firing pacemakers of nearly the same frequency typically exhibit peaks in the cross-correlation histogram, which oscillate with the period of the pacemakers. The magnitudes and phases of these peaks vary randomly from sample to sample, and they may disappear only with extraordinarily long samples. In Fig. 2, we show examples of cross-correlation histograms taken from two simulated independent pacemakers (normally distributed intervals with standard deviation of interval set at 10% of the mean). Each sample consisted of 4000 spikes in each neuron. The examples show the appearance and occasional disappearance of the peaks, which may be interpreted as a run of phase "locking" arising by chance. It is consequently very difficult to decide from reasonably sized samples whether two pacemakers are independent.

Thus, there are appreciable risks of falsely attributing dependence to independent spike trains, particularly when both neurons are pacemakers. The other possible type of error is false attribution of independence to trains that are in fact dependent. This is much less likely and can arise in either of two ways: (*a*) The dependence may be so weak that its effects are indistinguishable from "noise" in the sample taken. (*b*) A simultaneous combination of positive and negative interactions may coincidentally combine in such a fashion as to cancel each other. If this should happen, however, the two neurons may still be regarded as firing independently since, in an operational sense, the firing of one cannot be used to predict firing times of the other.

Types of Dependence

Dependence may arise from a number of physiologically important processes. We have already distinguished *interaction* and *common input*. The combinations of possibilities are virtually endless.

FIGURE 2 Cross-correlation histograms for two independent pacemakers. Each train has independently drawn normally distributed intervals, with mean 1.0 sec, standard deviation 0.1 sec; each train has 4000 spikes. a, unshuffled data; b, after prolonged random shuffling of intervals in train B; c, after further prolonged random shuffling of intervals in train B. See text.

Two cells may interact through synaptic connections that may be either direct, e.g. an axon collateral of one of the cells forming a synapse with the other, or indirect, i.e., mediated through one or more interneurons. The effects of such interactions on the cross correlation cannot be predicted precisely without a detailed knowledge of the intracellular processes, including postsynaptic potentials, which underlie the production of spikes by each cell. In simple cases, however, the gross effects are intuitively obvious. For example, if cell A makes a single excitatory synaptic connection with cell B, with a mean conduction time w, then we would expect the probability that cell B fires to be enhanced during a period starting at a time w after the occurrence of every spike in cell A. Therefore, we observe a peak in the cross-correlation function $\zeta_{AB}(\tau)$ near the point $\tau = w$. The shape of the peak depends on the details of the synaptic interaction (Fig. 3 a). If the connection is inhibitory, a depression rather than a peak will be observed (Fig. 3 b).

Two cells may receive input from a common source either directly or indirectly. In a simple example, the source might be a cell that makes excitatory synapses with cells A and B at latencies z_A and z_B, respectively. We then expect a peak in the cross-correlation function $\zeta_{AB}(\tau)$ at or near $\tau = z_B - z_A$. The magnitude of the peak will depend on the firing rate of the source cell as well as on the neurophysiological characteristics of the observed cells.

We have examined a number of cases by means of computer simulation to obtain guidelines for the interpretation of dependence observed in physiological experiments. A few representative examples are illustrated in Fig. 3.

When networks are constructed of neurons with basically similar properties and parameters, then the following generalizations may be made on the basis of simulation studies.

(*a*) Common sources of input are more difficult to detect than direct or indirect connections.

(*b*) Indirect connections are more difficult to detect than direct connections.

(*c*) Several different arrangements of functional interaction may lead to the same cross correlation; unique inferential conclusions concerning the anatomy and physiology of the interneuronal dependence may therefore be impossible. Furthermore, moderate degrees of nonstationarity will not appreciably mask the statistical signs of neuronal interaction, nor in general will they lead to false inferences of interaction when none, in fact, is present, as discussed in the next section.

Effects of Nonstationarity

It is shown in the companion paper on single spike-train analysis (Perkel et al., 1967) that the effects of nonstationarities in the form of trends, or rate changes, in a single spike train are most apparent in the serial correlogram, and that they are far less pronounced in an irregularly firing cell than in a pacemaker-like neuron. We confine our discussion of nonstationarities in the comparison of two spike trains to

a class of cases in which the nonstationarities show little effect on the single-train statistics, namely that of Poisson processes with time-varying rate parameters. It should be borne in mind that trends exacerbate the problem of interpreting cross correlations between regularly firing cells (Fig. 2).

If rate changes are shared by two otherwise independent neurons, the cross correlation will typically display, more or less symmetrically in the neighborhood of its origin, an elevation above its "null" level (that expected for independent cells). Rather large rate changes must be present (for irregularly firing cells) in order for this elevation to be readily apparent in the histogram. It can be shown, for example, that for two Poisson processes with time-varying parameters, the maximum fractional expected departure of the cross correlation from its "null" level is less than the square of the maximum fractional rate variation. If, for example, the shared rate changes of the two processes vary within 20% of their respective means, then the cross correlation will depart by at most 4% from its predicted value for independent stationary processes based upon observed firing rates (Equation 3). The actual amount of departure depends upon the precise nature of the temporal variation of the rate parameters of the Poisson processes; details will be presented elsewhere.

These results are illustrated in Fig. 4, in which cross-correlation histograms are shown for pairs of Poisson processes subjected to monotonic and oscillatory rate changes. The elevation in level of the cross-correlation histogram near its origin is seen to be much more pronounced under more drastic rate changes. Linear trends result in a uniformly elevated cross correlation, which remains flat. Oscillating trends give rise to an oscillating cross-correlation histogram, if the range of the latter is great enough.

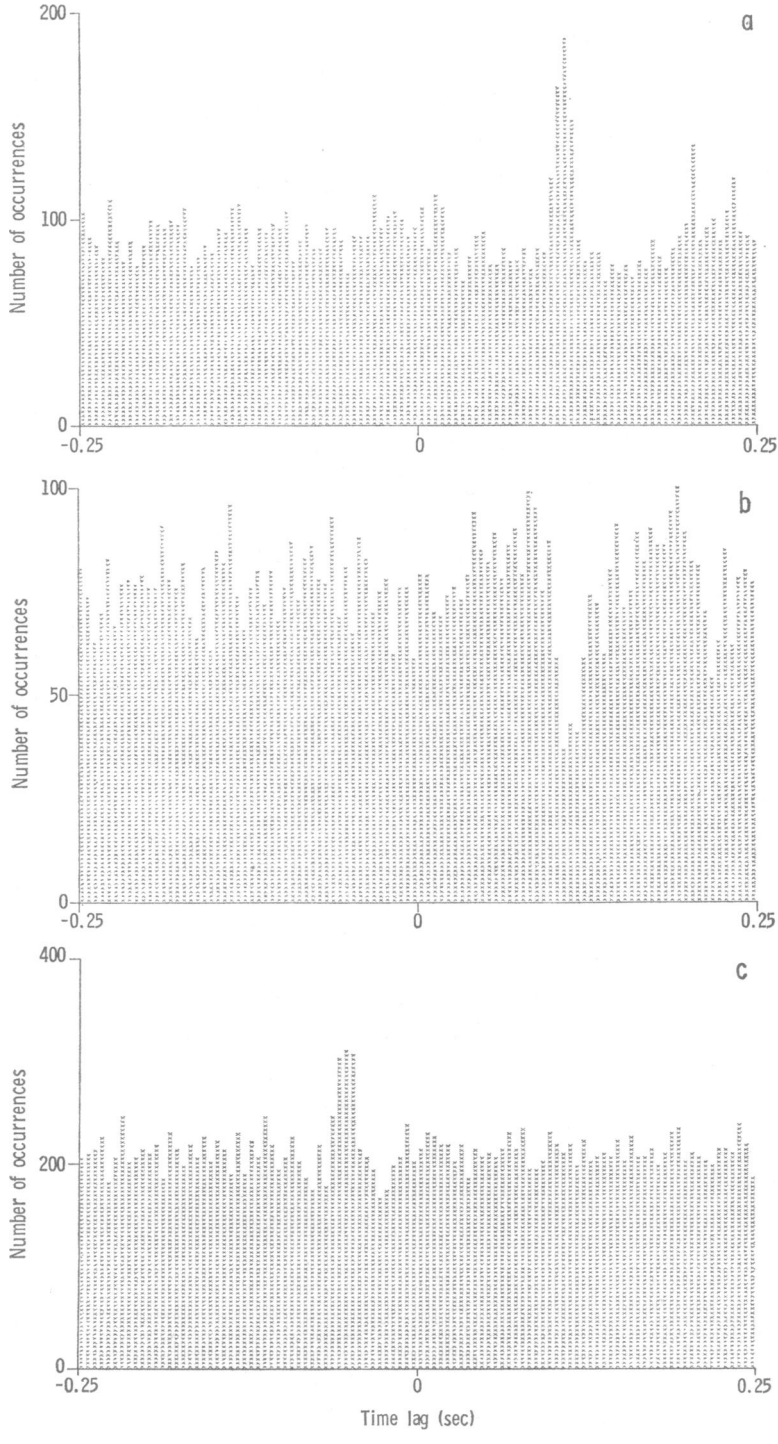
Because the effects of nonstationarity on the cross correlation are of second order, the statistical indications of interaction between two cells (discussed earlier) are not severely affected by rate changes in one or both of the cells, even if they are fairly severe. Inasmuch as stationary conditions are often difficult to maintain in an experiment, this fact is an encouragement to the attempt to elucidate functional interconnections of neurons through statistical comparison of their spike trains.

A special case of nonstationarity, deliberately introduced, is the use of experimentally controlled stimulation of the organism. This provides a new experimental variable, which can markedly enhance the utility of spike correlation measurements, as discussed in the following section.

TWO SPIKE TRAINS IN THE PRESENCE OF STIMULATION

Functional Effects of Stimulation

Interactions between neurons may be altered by presenting a stimulus to the animal; these changes are generally reflected in the cross-correlation histogram. The effects on the cross correlation may arise (*a*) through changed firing rates of one or both cells, (*b*) through direct or indirect synaptic input to both cells from a common


a
200
100
0
Number of occurrences
-0.25
0
0.25
b
100
50
0
Number of occurrences
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c
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Number of occurrences
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Time lag (sec)

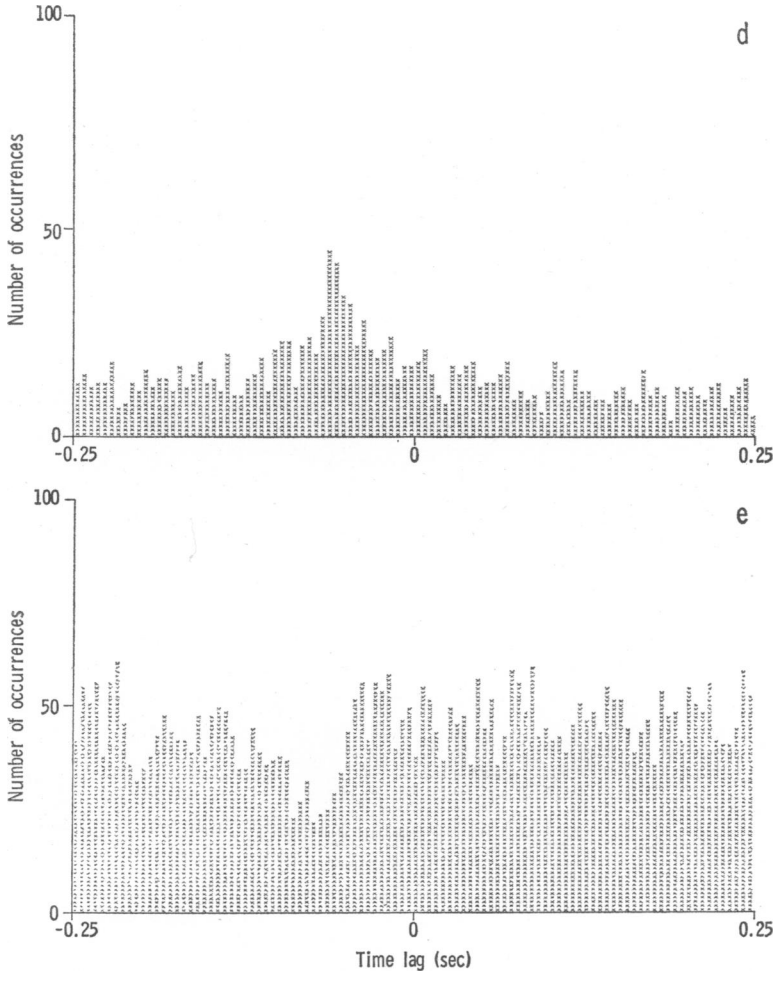

FIGURE 3 Cross-correlation histograms for interacting neurons; data from computer simulations. a, *direct excitation.* Neurons with characteristics similar to those of Fig. 2 b of Perkel et al. (1967). Each neuron excited by an independent Poisson source of EPSP's, amplitude normally distributed with mean 7.5 mv, standard deviation 1.0 mv; mean arrival rate 100/sec. Cell A makes excitatory synapse on cell B with latency of 100 msec; EPSP mean amplitude 10 mv, standard deviation 1 mv. In 250 sec, 2053 spikes produced by cell A, 2286 spikes by cell B. b, *direct inhibition.* Same as Fig. 3 a except A-B synapse is inhibitory (mean amplitude −10 mv, etc.). In 250 sec, 2068 spikes produced by cell A, 1858 spikes by cell B. c, *shared excitation.* Simulated neurons similar to those above, but with no synaptic connections between them. Each independent Poisson source now produces 10-mv EPSP's and is reduced in rate to 25/sec. An additional Poisson source with a mean rate of 150/sec delivers 10-mv EPSP's to both cells, each arriving at cell A 50 msec earlier than at cell B; i.e., each cell receives an average of 175 EPSP's per sec, of which 150 (86%) are shared with the other cell. Each cell produced about 2500 spikes in 150 sec. d, *shared inhibition.* Similar to previous example. Each independent excitatory input produces 10-mv EPSP's at a mean rate of 175/sec, shared channel produces 30-mv IPSP's at a mean rate of 100/sec. Both cells depolarized by DC input to produce spikes. About 550 spikes produced by each cell in 150 sec. e, *shared inhibition and excitation.* Similar to previous cases; cell A is depolarized and receives IPSP's from shared 100/sec channel as in case 3 d; cell B is excited as in example 3 c. Cell A produced 732 spikes and cell B, 2600 spikes in 200 sec.

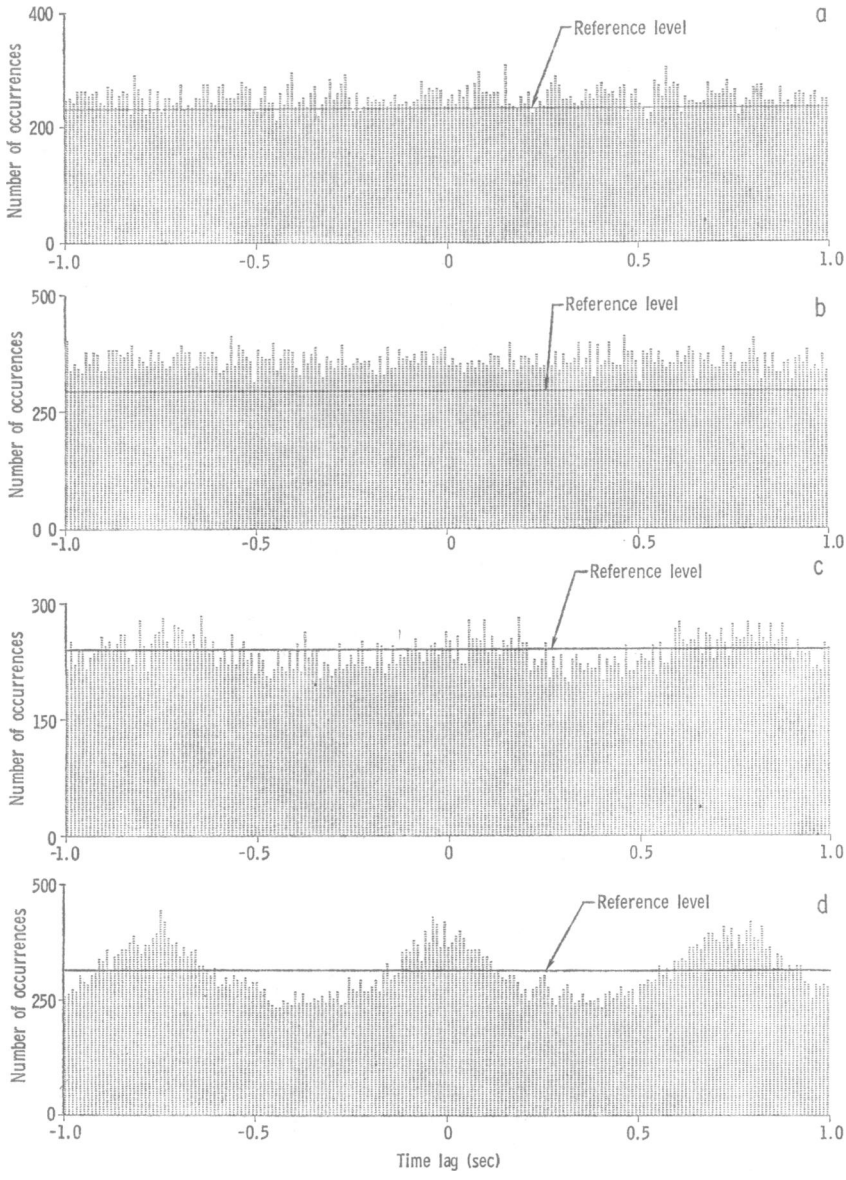

FIGURE 4 Effects of slow rate changes on cross correlations. Cross-correlation histograms between pairs of trains drawn from Poisson processes with time-varying rate parameters. Each member of a pair of trains has identical time variation of mean rate, but each train is generated independently. Reference level in cross correlation is predicted expected level for stationary independent processes, based upon observed mean firing rates (see text, Equation 3). Rate variations same as in Fig. 12 of Perkel et al. (1967). a, deceleration, ±50%. b, deceleration, ±70%. c, oscillation, ±50%. d, oscillation, ±70%.

source (or a set of parallel sources) that responds directly to the stimulus, (*c*) through the effects of the stimulus on interaction pathways between the two observed cells, or (*d*) through any combination of these. It is shown below that the changes in the cross-correlation function can be predicted when the stimulus is repeated periodically, and when mechanisms (*a*) or (*b*), or both, are operative. If the observed changes from unstimulated to stimulated situation agree with those predicted, it may be concluded that mechanism (*c*) is not operative, i.e., that any interaction pathways between the two neurons are not significantly affected by the stimulus.

We now consider the case in which an identical stimulus is presented at regular intervals of duration P.[2] These intervals are long with respect to conduction times and the duration of postsynaptic effects; i.e., the observable effects of a given stimulus have essentially died away by the time the next stimulus is presented.

The post-stimulus-time (PST) histogram discussed in Perkel et al. (1967) is an estimate of the firing probability of a neuron as a function of time since the onset of the stimulus. (It is clear that this is a special case of the cross-correlation function, $\zeta_{SA}(\tau)$, where "cell" S always fires at the onset of each stimulus.) It has two additive components: the relatively constant background component, which is due to firings of the observed cell that are not necessarily related to the stimulus, and departures from this level, which are due to the effects of the stimulus, whether directly or indirectly mediated. The background level may be affected by the stimulus, since ρ'_A, the mean firing rate of the cell with (repetitive) stimulus "on," may be different from ρ_A, the mean rate with stimulus "off," accordingly as the stimulus has a net excitatory or inhibitory effect on cell A.

It is clear that the observed cross-correlation function between two cells A and B will, in general, be different under "stimulus-on" and "stimulus-off" conditions. The major classes of effects possible are illustrated schematically in Fig. 5.

In the simplest situation, shown in Case a, cells A and B are independent, but both receive input from the stimulus. (Each arrow represents one or more synaptic connections, with the possible interposition of interneurons; the sketches in Fig. 5 are functional rather than anatomical diagrams.) Cells X and Y represent independent sources of synaptic activity supplying cells A and B, respectively. Cells X and Y may be influenced by the stimulus, as indicated by the broken lines. With the stimulus off, in this situation, the only contribution to the cross correlation $\zeta_{AB}(\tau)$ consists of a different background component (due to rate changes) and a superimposed, nonuniform component due to shared input.

In a more complicated situation (Case b), the two cells may interact. The interaction may be from A to B, from B to A, or in both directions; it may be direct or indirect, single or multiple. There may also be shared input from sources other than the stimulus. All these possibilities are subsumed in the square box I in the figure. With the stimulus off, the background effects, interactions, and possible

[2] Irregularly spaced stimulus presentations lead to irrelevant complications in the calculations.

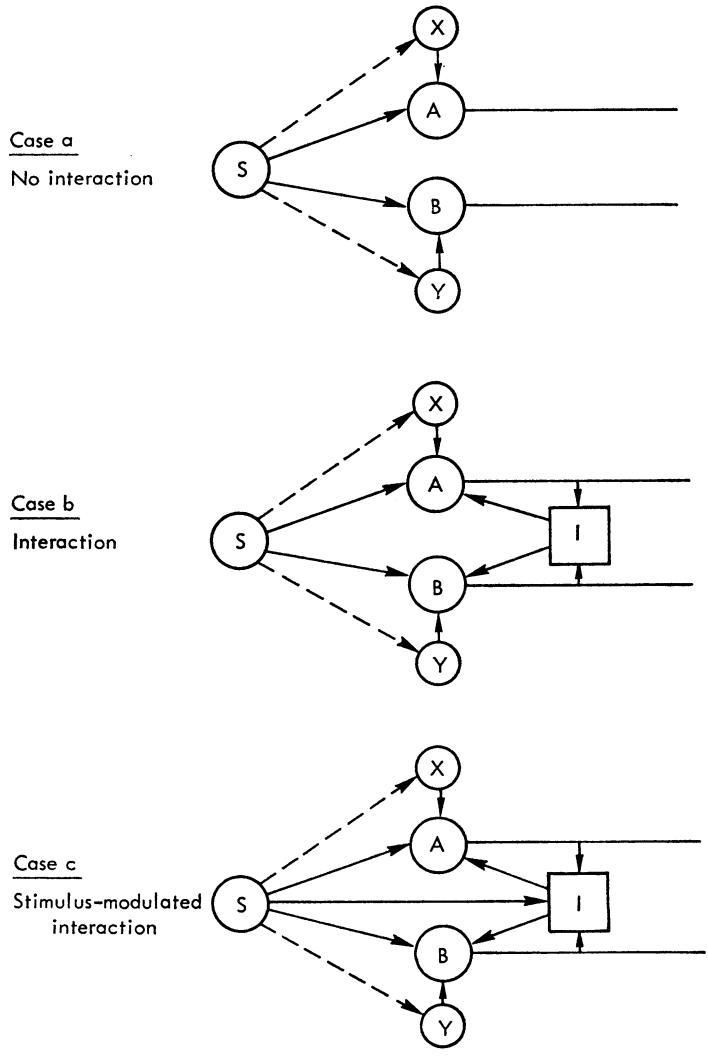

FIGURE 5 Functional relationships between stimulus and observed neurons; schematic. Stimulus *S* affects neurons A and B either directly or (dotted lines) through intermediate networks *X* and *Y*. In Case a, there is no interaction between cells A and B. In Case b, there are interaction pathways *I* for possibly reciprocal interaction between neurons A and B. In Case c, interaction pathways *I* are themselves affected by the stimulus *S*. See text.

shared input contribute to the cross correlation (which need not be flat). When the stimulus is on, this function is affected by (*a*) a changed background level, (*b*) "first-order" shared input, represented by the arrows going directly from the stimulus *S* to the cells A and B, and (*c*) "second-order" shared input, represented by the pathways from *S* through A and *I* to B, and conversely.

A still more complicated situation is shown in Case c, in which the interaction itself is modified by the stimulus. Such modifications, or "modulation," may result from activation of excitatory or inhibitory synaptic connections from S on interneurons in I, causing increased or reduced effectiveness in the transmission of interactions between cells A and B.

Prediction of the Cross-Correlation

In the two simpler situations described above, it is possible to predict the cross correlation in the stimulus-on condition, on the basis of the following measurements: (*a*) the mean firing rates of both cells under both stimulated and unstimulated conditions, (*b*) the observed cross-correlation function with the stimulus off, and (*c*) the observed PST histograms for both cells.

The basic assumption involved in this prediction is that these various modifications of the cross correlation, produced by the stimulus, are sufficiently independent to be additive. This assumption breaks down, for example, when firing rates are high, so that refractory characteristics of the neurons become important.

With stimulus off, the cross-correlation histogram is composed of "background" and "interaction" components. The background component is predicted on the basis of expected random coincidences, as follows.

With a bin width of δ in the cross-correlation histogram, we define a lagged coincidence (with lag time τ) as the occurrence of an event in A at t_o together with the occurrence of an event in B in the interval $t_o + \tau \pm \delta/2$. The lag times τ correspond to midpoints of bins: $\tau_k = \pm k\delta/2; k = 1, 2, \cdots$. Then the expected number $N(\tau, \delta)$ of lagged coincidences in the corresponding bin, arising from a segment of A-record of length T, is given by

$$E[N(\tau, \delta)] = \rho_A T \int_{\tau-\delta/2}^{\tau+\delta/2} \zeta_{AB}(x)\, dx, \tag{8}$$

where $\rho_A = 1/\mu_A$ is the mean firing rate in record A. Thus, the estimate of the theoretical density $\zeta_{AB}(\tau)$ is given by

$$\hat{\zeta}_{AB}(\tau) = \frac{N(\tau, \delta)}{n_A \delta}, \tag{9}$$

where n_A is the observed number of spikes in A during T; its expected value is given by $E[n_A] = \rho_A T$, so that we have

$$\zeta_{AB}(\tau) = N(\tau, \delta)/(\rho_A T \delta). \tag{10}$$

If, however, the stationary point processes of the two cells are independent, so that an event in record A occurs without any knowledge of the sequence of events in record B, we have already pointed out that the cross-correlation function is a

constant: $\zeta_{AB}(\tau) = \rho_B$. Therefore, for records of finite length, the observed histogram for such independent cells has an expected value of $\rho_A\rho_B T\delta$ in each bin, with "noisy" fluctuations that are reduced in relative magnitude as the sample size increases. This "random," or background, level, produced through randomly occurring lagged coincidences, is the sole component of the cross-correlation function for independent cells and is a contributing component to this function for dependent cell pairs. (See Cox and Lewis, 1966, pp. 246–248).

Rewriting Equation 10, we have

$$N(\tau, \delta) = \rho_A T\delta\zeta_{AB}(\tau) = \rho_A\rho_B T\delta + \rho_A T_\delta\xi_{AB}(\tau), \tag{11}$$

where $\xi_{AB}(\tau)$ represents the departure from background level or the "interaction" component:

$$\xi_{AB}(\tau) \equiv \zeta_{AB}(\tau) - \rho_B . \tag{12}$$

With stimulus on, and with new firing rates ρ_A' and ρ_B', the corresponding contributions to the new lagged coincidences $N'(\tau, \delta)$ are given by two terms:

$$G = \rho_A'\rho_B' T'\delta + \rho_A' T'\delta\xi_{AB}(\tau), \tag{13}$$

taking into account that the new observation time T' may differ from the previous time T.

A third component of the stimulus-on correlation histogram, which is due to shared input from the source, is obtained from the post-stimulus-time histograms. By subtracting out the background components as in Equation 12, we have the net PST density functions

$$\xi_{SA}(\tau) = \zeta_{SA}(\tau) - \rho_A', \tag{14 a}$$

$$\xi_{SB}(\tau) = \zeta_{SB}(\tau) - \rho_B', \tag{14 b}$$

which represent the departures in spike densities of cell A or B from the new mean levels at times τ after the onset of a stimulus.

Now the enhancement (or reduction) of the probability of a lagged coincidence that occurs between cells A and B can come about by an enhancement (or reduction) in delayed coincidences between S and B at a time $t + \tau$, making a contribution $\xi_{SA}(t)\xi_{SB}(t + \tau)$. We must integrate this over the entire period of the stimulus to obtain the enhancement (or reduction) per stimulus presentation of the lagged-coincidence density. Thus, we evaluate the correlation integral

$$[\xi_{SA} \times \xi_{SB}](\tau) = \int_0^P \xi_{SA}(t)\xi_{SB}(t + \tau)\, dt. \tag{15}$$

Because the stimulus is periodic, with period P, we take the arguments modulo P in the correlation integral.[3]

An alternative way of predicting the contribution of shared input from the stimulus to the cross correlation is to isolate those effects that are time-locked to the stimulus. This may be done as follows. One of the records, e.g., record B, is divided into equal segments, each equal to the interval P between stimulus presentations. These segments are thoroughly shuffled, so that their new order is effectively random. Then the cross correlation is recomputed between record A and shuffled record B. The shuffling has destroyed all significant time relationships between the two trains except those related to stimulus presentations. The cross-correlation histogram after shuffling and after subtraction of the background contribution estimates the same quantity as the correlation integral of the PST's:

$$\zeta'_{AB,\text{shuf}}(\tau) - \rho'_B \approx [\xi_{SA} \times \xi_{SB}](\tau). \tag{16}$$

In practice, shuffling need not be performed, but rather the two records are offset in time by an amount sufficient to destroy direct temporal relationships; the amount of offset is, of course, an integral multiple of the interstimulus interval P.

The expected number of lagged coincidences produced by the shared input from the stimulus is given by

$$H = (T'_A/P)\delta[\xi_{SA} \times \xi_{SB}](\tau). \tag{17}$$

The total predicted number of lagged coincidences with stimulus on is given by the sum of terms G and H (Equations 13 and 17):

$$N'_{\text{pred}}(\tau, \delta) = T'\delta\{\rho'_A\rho'_B + \rho'_A\xi_{AB}(\tau) + (1/P)[\xi_{SA} \times \xi_{SB}](\tau)\}. \tag{18}$$

Hence the predicted cross-correlation function is given by

$$\zeta'_{AB,\text{pred}}(\tau) = \xi_{AB}(\tau) + \rho'_B + (\rho'_A P)^{-1}[\xi_{SA} \times \xi_{SB}](\tau). \tag{19}$$

Application to Experimental Data

Comparison of the predicted and observed cross correlations enables us to distinguish among the three situations shown in Fig. 5.

If the cross-correlation function with stimulus off is flat, within statistical limits, we may conclude that there is no interaction (Fig. 5 a). In this case the expected value of $\xi_{AB}(\tau)$ is 0. If the observed cross-correlation function with stimulus on

[3] When the densities ξ are obtained from PST histograms, they represent averages over a bin width. If the cross-correlation histogram has the same bin structure as the PST histograms, there is an imprecision in the approximation of the integral by a sum, as well as a shift in the argument τ by half a bin; the latter may be compensated for by an averaging procedure.

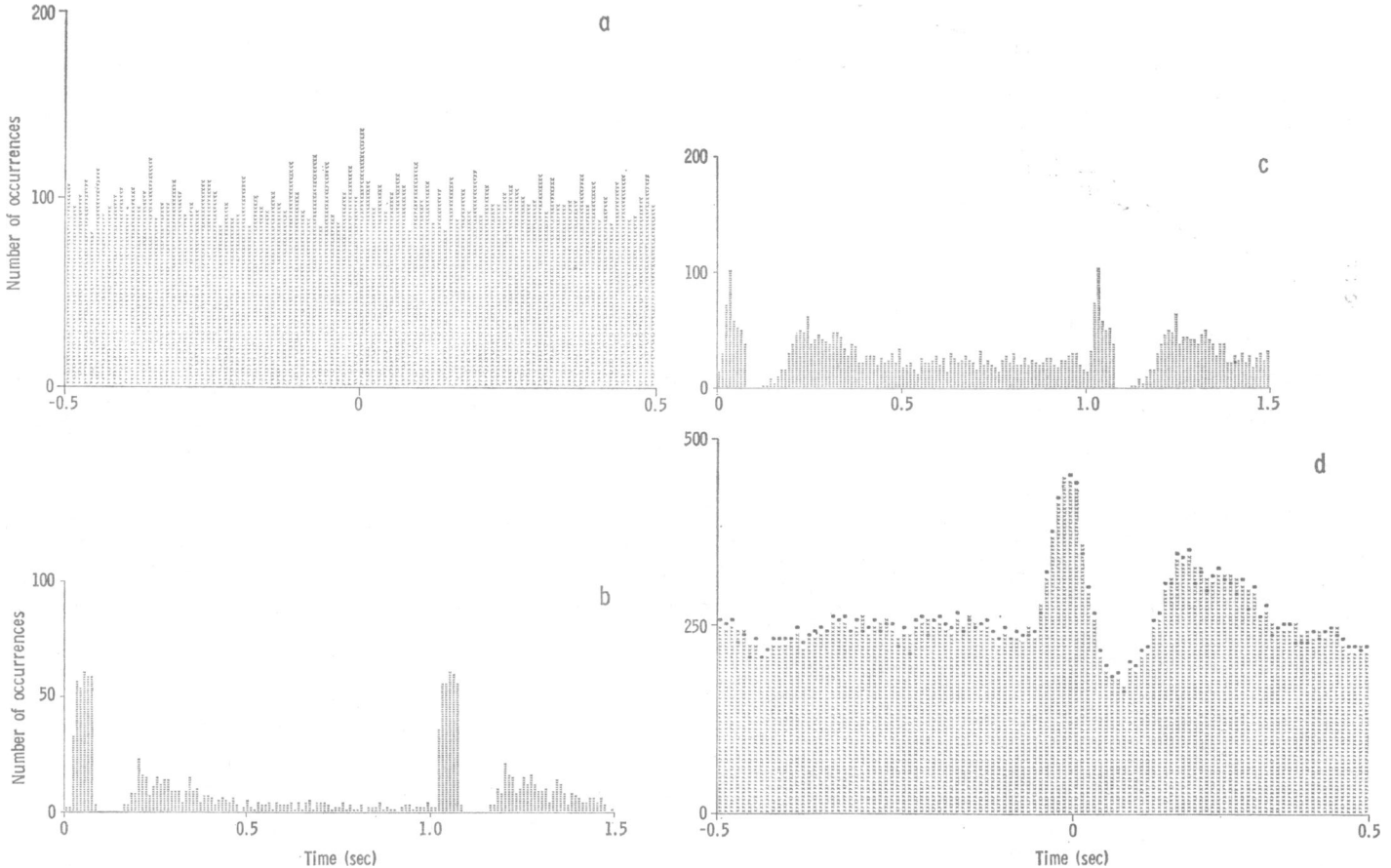

FIGURE 6 Effects of repeated stimulation on the cross correlation. a, cross-correlation histogram between spike trains recorded from two neurons in the cochlear nucleus of the cat; unstimulated. b, post-stimulus-time histogram, cell A. Stimuli consisting of brief tones were presented at 1-sec intervals. c, post-stimulus-time histogram, cell B. d, cross-correlation histogram under repetitive stimulation. Plotted histogram is observed; blackened points correspond to predicted cross correlation (method described in text) based on assumption of absence of potentiating effect on interactions by the stimulus. See text.

does not then agree with that predicted using Equation 19, the appropriate conclusion is that we actually have the situation of Fig. 5 c, where I represents an interneuronal network that is ineffective unless potentiated by the stimulus.

If the cross correlation with stimulus off is not flat, we may conclude that there is interaction, i.e., that I is functioning. If the prediction for the stimulus-on cross correlation agrees with the actual measurement, we may conclude that we have unmodulated interaction (Fig. 5 b). If there is statistically significant disagreement, we may conclude that the interaction is modulated by the stimulus (Fig. 5 c). It may then be possible to distinguish among several different modes of such modulation.

The application of these techniques to the detection of interactions has been investigated through digital computer simulations, and the results have been presented elsewhere (Perkel, 1964).

An example of this technique, drawn from experiments in the cochlear nucleus of the cat, is shown in Fig. 6. The agreement between predicted and observed cross-correlation histograms with the stimulus on indicates that the complex shape of this histogram can be completely explained by stimulus-imposed changes in firing patterns of both units.[4]

In summary, we may distinguish among the three possibilities as follows: (*a*) When there is no interaction, the cross-correlation histogram is flat with stimulus off, and periodic with the period of the stimulus with stimulus on; and the cross correlation can be predicted from mean rates and PST histograms. (*b*) When there is interaction, the cross-correlation histogram with stimulus off is not flat; with the stimulus on, it may not be strictly periodic, but the correlation function can still be predicted. (*c*) When the interaction is modulated by the stimulus, the predicted cross correlation does not agree with observation.

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[4] The possibility of a small excitatory influence of cell A on cell B, with a latency of about 4 msec, however, cannot be excluded, but this would have a negligible effect on the remainder of the cross-correlation histogram, which extends to ½ sec.

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