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Identification of Synaptic Interactions*

David R. Brillinger
Department of Statistics, University of California, Berkeley, California

Hugh L. Bryant, Jr., and José P. Segundo
Department of Anatomy and the Brain Research Institute, University of California, Los Angeles, California USA

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Abstract

This paper studies the influence exerted by the presynaptic spike train on the postsynaptic one. It applies to synaptic exploration a novel method for characterization of point-process systems (Brillinger, 1974, 1975a), and draws from it physiologically meaningful conclusions. The departure point was a large data set of action potential trains from an *Aplysia* network whose neurons are connected by monosynaptic inhibitory or excitatory PSP's, and either discharged spontaneously or were driven by intracellular pulses. First, a sequence of "kernels" is estimated, each with a physiological connotation relevant to synaptic transmission. The kernel independent of time – of zero-order – measures the postsynaptic rate with no presynaptic discharge. That of a single time argument – of first-order – relates to the rate effect of the average PSP. Those of two, three, or more time arguments – of second, third or higher-order – relate to interactions between two, three, or more postsynaptic potentials (e.g. to facilitation) and/or spikes (e.g. to refractoriness). Then successive models are constructed recursively and based on the kernel of zero-order, on the kernels of zero and first order, on those of zero, first and second order, and so forth, until a desired approximation is achieved. The plausibilities of each kernel estimate and of each model are evaluated separately by way of spectra and coherences. The "linear" model based upon the zero and first-order kernel was tested (after that based exclusively on the zero-order one was proven inadequate). When presynaptic discharges are very irregular and at intermediate or low rates, it provides satisfactory description and prediction, and the first-order kernel is an uncontaminated display of the rate effects of the average presynaptic spike: this constitutes the "linear" domain. When presynaptic discharges are bursty, regular or very fast, the linear model is unsatisfyctory: this is referred to as "non-linear" domain. Reasons for non-linearity lie in PSP facilitation and anti-facilitation, conversion of membrane current into firing rate, after-spike excitability oscillations, and special pacemaker interactions. The model can be extended to three-neuron networks where partial coherences extract interactions between followers, even while submitted to a common driver. The basic and ubiquitous issues of spike train description and stability were discussed. The counting and the interval statistics of spike trains provide equivalent descriptions and their current opposition is conceptually meaningless. Concomitant short-term fluctuations in spike generation intensity at pre- and postsynaptic levels have functional significance beyond changes in the overall average rate or interval: they are made precise by parameters whose definition, estimation and physiological interpretation are presented here. Some stability of the experimental preparation is presupposed by investigators, but variations (e.g. from cycles or deterioration) always exist. Hence, decisions as to the preparation's evolution and as to tolerable changes must be made, and based upon pre-existing knowledge, educated guesses and practical considerations. This study provided basic knowledge of the individual synapse considered the elementary building block of the nervous system when viewed as a network of interacting nerve cells. It also contributed generally applicable mathematical techniques which were illustrated by application to relatively well studied and simple networks.

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Introduction

The present communication describes how action potential (AP) data can be used as indices of synaptic function, allowing the investigator to describe transsynaptic relations and to model or "identify" synaptic operation through an expansion that relates physiologically meaningful variables.

System identification rests upon a vast quantitative methodology (e.g. Astrom and Eykhoff, 1971; Nieman *et al.*, 1971), but most of it refers to linear operations where the input and output functions are continuous or defined at discrete equispaced points. Living systems, however, often are non-linear and express themselves by point-like processes (Marmarelis and and Naka, 1973a, b; Segundo, 1971). The methods used here for synaptic identification, based in part upon procedures developed by Brillinger (1974, 1975a), are designed specifically for point process systems and make no assumption as to whether or not they are linear. Related approaches are described by Perkel *et al.* (1970), Terzuolo (1970), Marmarelis and Naka (1973a), Knox and Poppele (1975, and Krausz (1975).

The synapse is "identified" when certain relevant functions are derived from corresponding pre- and postsynaptic trains. The acceptability of each model is measured by coherence functions. The first approximation claims that the rate or probability of

the postsynaptic spike relates only to a function that reflects the postsynaptic rate change after each presynaptic arrival, and to the rate were there no input. Better approximations are achieved when complexities due to PSP interactions, to postsynaptic after-firing excitability shifts, etc. are considered too. The model admits extension to multicell networks with, say, one driver cell and two followers, where partial coherences measure the degree of association of the followers with the driver's effects removed.

A special section defines and explains the parameters used in these studies with AP trains, indicating their estimation procedures and physiological interpretations. Also, it discusses briefly the basic issues of spike train description and experimental stability. A preliminary report has appeared recently (Segundo *et al.*, 1975).

Experimental Methodology

The preparation (abdominal ganglion of *Aplysia californica*), experimental procedure, and electrophysiological recording and stimulation techniques were identical to those in a previous communication (Bryant *et al.*, 1973). The present report re-examines much of the data used therein. In summary, the isolated abdominal ganglia of small specimens were perfused in a buffered *Aplysia* saline at a servo-controlled temperature between 14 and 21° C. Intracellular recordings and passage of current were obtained with potassium citrate-filled electrodes, a bridge circuit and conventional amplification, display and analog tape storage devices. In a typical case, the presynaptic cell L 10 was impaled with one or two "followers" (e.g. L 3, L 2). In "spontaneous" experiments, activity was recorded with no deliberate stimulation (or, at most, with a slight DC accelerating or slowing bias). In "driven" experiments, the presynaptic cell was stimulated intracellularly with 50 msec duration rectangular pulses, each eliciting one AP. Each interspike interval was drawn independently from a prespecified density: different presynaptic discharge "forms" reflected different densities which were approximately exponential, narrow Gaussian or uniform and are referred to as "Poisson", "pacemaker", or "uniform", respectively. All densities had mean intervals between 0.1 and 2 sec (implying rates between 0.5 and 10 per sec), and were truncated at a lower bound of 50 msec (because of refractoriness). The L 10 junctions with EPSP's (e.g. at R 15 or R 16) either are hard to find or are labile: hence, the relatively stable EPSP elicited by a single shock to right viscero-pleural connective (RVP) in R 15 was analyzed too.

A total of 47 cases were studied. Each case implied observation of two, or sometimes three corresponding and stationary AP trains, one presynaptic and one or two postsynaptic. The presynaptic discharge form was "Poisson" in 19 cases, "uniform" in 13, "pacemaker" in 8 and "bursty" in 7. To illustrate this communication we have deliberately chosen cases that were representative, i.e. that reflected the majority in their class, and were not necessarily the most striking (e.g. had the highest coherences, see below). Whenever possible, we deliberately chose data used for the figures of Bryant *et al.* (1973), considering this an economic way of comparing different methods.

Statistical Methodology

Model Description; Basic Issues; Parameter Definition, Estimation and Physiological Interpretation

The purpose of this communication is to provide a quantitative method for an exhaustive and physiologically meaningful characterization of the synapse as a system. Hence, the core of this section is the modelling and "identification" approach with the definition, estimation procedures and physiological implication of certain statistics, including methods not available elsewhere in complete form and laid down here in enough detail to be of use to physiologists.

Two general issues are pertinent, spike train description and stability. They are, in fact, very basic to a large number of publications, imposing decisions which, deliberate or unknowingly, are implied always though rarely made explicit. Their relevancy and generality are so great that it was felt that they should not remain undiscussed. The first refers to the *representation of AP trains.* The empirical observation of the importance of the times of occurrence of AP's, commonly all-or-nothing, brief with respect to the interval between them, justifies for certain purposes the assimilation of a train to a point process along a line. Such a process is described fully by an ordered sequence of the times $\ldots < \sigma_{-2} < \sigma_{-1} < \sigma_0 < \sigma_1 < \sigma_2 < \ldots$ of occurrence of each point (Cox and Lewis, 1966). Inherent and central in the intuitive notion of any such process is whether the points along the line are abundant and closely packed, or sparse and widely separated, i.e. the "intensity" of their generation. Also intuitive is the recognition that the intensity is linked directly with the likelihood of encountering a point. A first representation of a point process is through the "counting process" $N(t)$, i.e. the number of points between time 0 and time t. As t increases, this variate jumps by one at each point. The overall intensity is reflected by the mean rate of the process, estimated by

$N(T)/T$ for large T. A second representation of the point process, intuitively less natural, is provided by the "ordered sequence of intervals" ..., $i_{01}=\sigma_1-\sigma_0$, $i_{12}=\sigma_2-\sigma_1$, ... between successive points. The overall intensity is reflected reciprocally by the average interval estimated by $T(n)/n$, where n is the number of intervals observed and $T(n)=\sigma_0+i_{0,1}+\ldots+i_{n-1,n}$ is the time from the origin to the last point. The mean rate itself may be estimated by $n/T(n)$. Both of these exhaustive representations are essentially equivalent (Cox and Lewis, 1966); however in practice, when simple properties are studied, both can be informative and the approaches complementary. Two conclusions can be drawn. First, that the opposition of counting versus interval statistics, as implied in for example Terzuolo and Bayly (1968), is not meaningful conceptually since it contraposes equivalent descriptions. Indeed, any question and answer can be expressed interchangeably in count or interval language. Second, that in a practical situation either counting or interval statistics may provide the more appropriate quantification and parsimonious description for the central notion of firing intensity.

Neurophysiologists often have been satisfied with observing whether there were a lot or only a few spikes over a relatively prolonged period, using the overall mean rate as a measure; important concepts have been clarified through this approach. An experiment usually provides a number of trains, collected so as to appreciate whether changes in the discharge and in some sensory or motor variable can be matched appropriately. There usually are, however, within these relatively prolonged periods, shorter term variations in AP intensity. It is here that the really meaningful physiological question arises, asking whether the overall mean rate suffices to infer or predict the corresponding cause or consequence (e.g. sensory stimulus or movement), or the shorter term rate or interval fluctuations, i.e. the spike timing and pattern, are meaningful and informative too (e.g. Segundo and Perkel, 1969). The latter are revealed by a running display of rates over shorter periods, or of successive intervals, and can be summarized by, say, an auto-intensity function or an interval standard deviation. Experimental evidence supports the intuitive belief in the second alternative (e.g. Segundo *et al.*, 1963; Segundo, 1970). Thus, a second important question arises: namely, that of how fluctuations are transferred in terms of amplitudes and time courses. Efforts to analyze these questions in the sensory sphere are abundant, since the early work of Pringle and Wilson (1952). The same questions can be asked a propos of synaptic transfer, and paragraphs below make precise the notion of short term discharge intensity variations, concomitant in pre- and postsynaptic neurons, describing a method for identification of point process systems.

The second issue, *stability*, refers to the extent to which preparations depart as time goes on from the characteristics exhibited initially. The investigator assumes, on the one hand, that the system realized by the preparation has invariant features that allow for general conclusions; the statistician formalizes this in a requirement of stationarity (Cox and Lewis, 1966). There are, however, inevitable instabilities in living matter; for example, aging and the deterioration of the experimental preparation. All systems vary during the observation time by trends and/or fluctuations and each instance raises such questions as whether an observed change is small enough that the preparation can still be considered stable, whether a progressive change is part of a trend or of a cycle that eventually would have reverted to the initial conditions, and so forth. The unavoidable judgement concerning acceptable stability thus requires an a priori practical decision as to the magnitude and quality of tolerable changes (La Salle and Lefschetz, 1961; O'Leary *et al.*, 1975; Weiss and Infante, 1967) and as to the expected form of the preparation's evolution. These decisions with their important connotations are reached for each case on the basis of pre-existing knowledge, educated guesses and practical considerations.

The *model* arises from methods recently formalized and made practical (Brillinger, 1975a) for identification of point process systems, that is of systems whose input and output are point processes (the synapse and spike trains, respectively, in this case). The synapse is said to be "identified" when an acceptable model is found. One model involves an expansion based upon functions referred to as "kernels", and is a point process analog of the Volterra-Wiener expansion of ordinary time series (Marmarelis and Naka, 1973a, b). The kernels are i. expressed as functions of time arguments, ii. meant to be invariants of the system that retain the same essential characteristics even when the presynaptic discharge varies (other commonly used functions do not have this property, e.g. Bryant *et al.*, 1973; Knox, 1974; Moore *et al.*, 1970), and iii. estimated from corresponding pre- and postsynaptic spike trains.

The first step of the identification is to estimate certain conditional rate functions, each of which is a physiological connotation relevant to synaptic transmission. The one of zero-order μ, i.e a constant, simply measures the mean rate. The one of first-order,

a function of a single time argument, relates to the average effect of a presynaptic spike and the PSP it elicits. The one of second-order, a function of two time arguments, relates to the interactions between pairs of spikes or PSP's. Those of higher orders, functions of several time arguments, relate to interactions between more than two events. The second step is to construct recursively the successive models, i.e. that based on the zero-order kernel, that based on the zero- and first-order, that based on the zero-, first- and second-order, and so on until it makes sense to add no more. The acceptance of a kernel estimate as plausible does not necessarily mean that the series up to the corresponding term is a good predictor. The coherence provides a measure of predictability.

The kernel of first-order $a(u)$ relates to the effect of a single presynaptic spike or PSP. It is defined as the best linear predictor of the average change of the instantaneous rate at time u in a spike train B, when a single spike occurs at time 0 in a spike train A. It is useful, therefore, in predicting whether there will be a B spike u time units away from an A spike, and will be positive, negative or zero, if B accelerates, slows or does not change, respectively. $a(u)$ satisfies

$$m_{AB}(u)-m_B=a(u)+\int a(u-v)[m_{AA}(v)-m_A]dv \qquad (1)$$

where $-\infty<u<\infty$. $m_{AB}(u)$ is the AB cross-intensity function (CIF), a first-order conditional rate function that measures, for one cell B and close to any particular time t (i.e. between t and $t+h$, with h positive and tending to zero), the average instantaneous rate or the likelihood of generating an AP, conditional on an A spike u time units away (e.g. Bryant *et al.*, 1973; Knox, 1974; Moore *et al.*, 1970). Its profile reflects the timing and the rate effects of A spikes, among other issues (as the correlation of A with a third cell C which also acts upon B). It is defined by

$$m_{AB}(u)=\lim_{h\downarrow 0}\text{prob}\{B\text{ spike in }(t,t+h)\mid A\text{ spike at }t-u\}/h\,, \qquad (2)$$

$m_{AA}(u)$ is the auto-intensity function of A, i.e. the CIF of the A train with itself. The constants m_A and m_B are the overall mean rates.

The integral Eq. (1) can be derived and justified in two distinct manners, both relevant to synaptic characterization. Suppose that $\sigma_j(j=0,\pm1,\pm2,\ldots)$ denote the times of the presynaptic spikes and $\tau_k(k=0,\pm1,\pm2,\ldots)$ those of the postsynaptic spikes. In the first derivation, we set about modelling the change in the likelihood of a postsynaptic AP very close to t by the expression

$$\text{prob}\{B\text{ spike in }(t,t+h)\mid A\text{ spikes at }\sigma_j\}\sim\left[\mu+\sum_{j=-\infty}^{\infty}a(t-\sigma_j)\right]h\,. \qquad (3)$$

The constant μ represents B's rate with A inactive. The rationale for (3) follows: if there were no A spikes, the probability of a B spike close to t would be μh, μ being B's rate with A silent; if the A train consisted of a single spike at time σ, it would be $[\mu+a(t-\sigma)]h$ for some function $a(.)$; if it consisted of two spikes at times σ_1, σ_2, and they did not interact (see below), the probability would be $[\mu+a(t-\sigma_1)+a(t-\sigma_2)]h$.

Extending this reasoning to a train having many non-interacting spikes, the probability would be

$$\left[\mu+\sum_{j=-\infty}^{\infty}a(t-\sigma_j)\right]h \qquad (4)$$

as $h\downarrow 0$. The summation is extended from minus to plus infinity for reasons given below. Averaging expression (3) over all possible A trains leads directly to the first-order relationship

$$m_B=\mu+m_A\int a(v)dv\,. \qquad (5)$$

Multiplying Eq. (3) through by the differential increment $dN_A(t-u)$ of the counting process, averaging and using the identity (5) leads to the integral Eq. (1). A second derivation of (1) comes about from seeking to predict the instantaneous rate of the B train from the times of A spikes by an expression of the form (4), i.e. without interactions. If $N_B(t,\ t+h)$ denotes the number of B spikes in $(t,\ t+h)$, we ask for the μ and $a(u)$ that lead to the smallest separation (measured by the average of the squared differences) between the instantaneous rate, $N_B(t,\ t+h)/h$, and a postulated function of the form $\mu+\Sigma a(t-\sigma_j)$: i.e. that lead to the minimum of

$$\lim_{h\downarrow 0}E\mid N_B(t,t+h)/h-\mu-\Sigma a(t-\sigma_j)\mid^2\,. \qquad (6)$$

Evaluating expression (6) and using the calculus of variations leads again to the integral Eq. (1).

The first-order kernel $a(u)$ is, in all cases, the best linear predicter in the sense of expression (3), and one might anticipate that it would be zero for negative times when A acts trans-synaptically on B. It is necessary to understand, however, that the procedure under discussion is designed to fit associations and, therefore, no particular kernel will necessarily suffice to describe certain effects. Hence, though this may indeed happen, in other cases unexpected features may appear; for example, when studying pacemaker cells

there may be "predictive" features which seem to ignore causality like non-zero $a(u)$ values to the left of the origin. This, and the fact that the direction of causation is sometimes unknown, is the reason why the summation in Eq. (4) is from $-\infty$ to ∞.

Both derivations of $a(u)$ used the assumption that the effects of separate A spikes are additive and not interactive in their influences upon B rates. This clearly holds when, for example, the presynaptic discharge is slow and successive spikes are so far apart that the effects of each have died down by the time the next arrives. Under these conditions, model (3) is plausible and the first-order kernel can be referred to as "average impulse response function" of the system. On the other hand (see Results), $a(u)$ is subject to limitations that arise from not allowing for common and often powerfully interactive issues (Segundo *et al.*, 1963) like the after-effects of earlier presynaptic arrivals, e.g. after-firing fluctuations of exitability. Hence, it may be necessary to extend the model to include higher-order terms. A conditional rate function of second-order allows in part for these issues:

$$m_{AAB}(u, v) = \lim_{h \downarrow 0} \text{prob}\{B \text{ spike in } (t, t+h) \mid A \text{ spikes at } t-u \text{ and } t-v\}/h. \quad (7)$$

This function represents the postsynaptic rate close to time t, conditional on the presynaptic cell having fired u and v seconds earlier. [It may be estimated by expressions analogous to (13) below, and the large sample properties remain including the advantages of taking square roots, Brillinger, 1975b.] When, as in Eq. (7), two presynaptic spikes are taken into account, the probability of a postsynaptic spike close to t, given a presynaptic train of spikes at time σ_j, can be modelled by

$$\left[\mu + \sum_j a_1(t-\sigma_j) + \sum_{j \neq k} a_2(t-\sigma_j, t-\sigma_k)\right] h. \quad (8)$$

$a_2(u, v)$ is the second-order kernel whose arguments are the times to distinct presynaptic spikes. It relates to the rate effects of two presynaptic spikes combined at any given relative timing, incorporating into the identification the influence that one presynaptic spike (say, at $t-u$) exerts upon the rate effects of another (at $t-v$). It thus is sensitive to PSP facilitation and antifacilitation.

A new term is added to (8) for each new presynaptic spike (or PSP) one wishes to account for. Thus, higher order kernels incorporate the consequences of PSP facilitation or anti-facilitation that occur after two, three, ... events with any conceivable given timing. The expression is expanded until a suitable description of the synapse's behavior over a reasonable domain of inputs has been achieved. "Suitability" is evaluated by means of the coherence, a general measure of association applicable to models of any order (see below). The general model based upon presynaptic spikes (or PSP's) is of the form

$$\left[\mu + \sum_{j=-\infty}^{\infty} a_1(t-\sigma_j) + \sum_{j_1 \neq j_2} a_2(t-\sigma_{j_1}, t-\sigma_{j_2}) + \ldots + \sum_{\substack{j_1 \neq j_2 \neq \ldots j_k \\ \text{distinct}}} a_k(t-\sigma_{j_1}, \ldots, t-\sigma_{j_k}) + \ldots\right] h \quad (9)$$

where the generic kernel a_k incorporates the interaction at time t of the effects of k distinct presynaptic events at times $\sigma_{j_1}, \ldots \sigma_{j_k}$. When $k=1$, (9) reduces to (4), which is referred to as the "linear" model.

Another set of conditional rate functions and kernels takes into account postsynaptic firings with their resulting excitability shifts (e.g. refractoriness). Yet another takes into account combinations of pre- and postsynaptic firings. For example, a useful "mixed" function of two time arguments is provided by:

$$m_{ABB}(u, v) = \lim_{h \downarrow 0} \text{prob}\{B \text{ spike in } (t, t+h) \mid A \text{ spike at } t-u, B \text{ at } t-v\}/h. \quad (10)$$

This function represents the postsynaptic rate close to t, conditional on the presynaptic cell and the postsynaptic cell having fired u and v seconds earlier, respectively. It and the corresponding kernel are useful for identification when there exists an influence of a postsynaptic spike (at $t-v$) upon the rate effects of a presynaptic one (at $t-u$); or, equivalently, an effect of a single A firing (at $t-u$) upon B's rate at two instants $(t-v, t)$. Thus, they are sensitive to the joint after-effects of postsynaptic potentials and spikes.

The zero- and the first-order kernel of (1) are examined in this paper. A forthcoming publication shall explore higher orders (Brillinger, Bryant and Segundo, in preparation). Models of all orders require essentially the same methodology; computational problems may arise mainly because of the large number of operations and storage requirements needed in their evaluation.

A requirement important for ease of interpretation is that each successive term in an expression for the probability not be affected greatly by factors accounted for by earlier terms. Other procedures (e.g. Bryant *et al.*, 1973; Knox and Poppele, in preparation; Perkel, 1970; Perkel *et al.*, in the press) allow at best a partial separation of issues. Models (3), (8) and (9) for the probability of a postsynaptic spike conditional on the presynaptic train are direct

expansions which, in essence, imply that the influences of the various issues are combined by an additive process. It is conceivable, however, that a more reasonable description could be obtained by other expansions implying combination by, for example, a multiplicative process (so that the logarithm of the probability and not the probability itself is given by a sum). This is an empirical question, and research is in progress to see which expansion is most suitable (Brillinger, Bryant and Segundo, in preparation).

There are other related efforts in the literature approaching the same questions. For example, Perkel (1970) proposed that, since pre- and postsynaptic correlations depend on the presynaptic rhythmicity and on the primary effects, the cross-covariance could be the convolution of the presynaptic auto-covariance and of a time function $\varepsilon(u)$ called the "synaptic response". The latter in turn depends on a term $\sigma(u)$ that reflects primary PSP effects, and on the resetting of the postsynaptic rhythmicity. The author points out correctly that the functions ε and σ may depend on the form of the input train, and that this dependence is an empirical question. The same basic issues (i.e. presynaptic rhythmicity, primary effects) are incorporated, though in a somewhat different manner, into the formulation in expression (10). Between-PSP interferences and after-spike excitability are not accounted for in these models, at least not explicity and separately. Other efforts take a somewhat different tack, but still are related. The instantaneous rate, if scaled appropriately, is a measure of firing probability; hence, the present analysis of how the postsynaptic rate is influenced by presynaptic spikes and postsynaptic firings at one, two, ... delays relates conceptually to the earlier analysis of how the probability of a postsynaptic spike is influenced by one, two, ... presynaptic spikes and recent postsynaptic firings (Segundo *et al.*, 1966). The conclusion at that time was that at a junction between two neurons, say one with excitatory postsynaptic potentials, the probability of a postsynaptic AP at a particular instant relates to the timing of a limited number of presynaptic spikes (PSP's) which occur within a recent time period. At such a junction, the probability of a postsynaptic spike is maximized when the presynaptic rate surpasses a certain minimum and is sustained, or even if this condition is not quite met, when a favorable pattern is formulated. This general rule is modified by special modes of PSP summation (facilitation or anti-facilitation) and of postsynaptic threshold recovery.

The proposed modelling procedure requires definition, estimation and physiological interpretation of certain *parameters*. Suppose that A is a stationary spike train. Let $N_A(t)$ denote the number of A spikes between time 0 and time t. The mean intensity of the A train is defined by

$$m_A = \lim_{h \downarrow 0} \text{prob}\,\{A \text{ spike in } (t, t+h)\}/h\,. \tag{11}$$

If the train is observed for the time interval $0<t<T$, then m_A may be estimated by

$$\hat{m}_A = N_A(T)/T\,. \tag{12}$$

The cross-intensity function, $m_{AB}(u)$, between two simultaneously occurring spike trains A and B was defined above by expression (2). Because of stationarity this function does not depend on t. It gives the short-term intensity of the B train u time units after an A spike. If the trains are independent, then $m_{AB}(u)=m_B$ for all u. If B spikes are independent of "later" A spikes, then $m_{AB}(u)=m_B$ for all $u<0$. Deviation of $m_{AB}(u)$ from m_B is suggestive of dependency of the B train on what happened in the A train u time units earlier. Specifically it relates both to the primary rate effects of the average A spike and to the form of the A train. All spike train CIF's will be flat and essentially equal to m_B, at large $|u|$ values because inevitably any real-life correlating influence will be ephemeral. The auto-intensity function (AIF), $m_{AA}(u)$, is defined for $u\neq 0$ by expression (2) with the B spike train identical to the A spike train.

Suppose $\sigma_1, \sigma_2, \ldots$ and $\tau_1, \tau_2, \ldots$ denote the times of the A spikes and of the B spikes, respectively, that occur in the time interval $0<t<T$. Let b denote a bin width and "#" stand for "the number of": then $m_{AB}(u)$ may be estimated by the cross-correlation histogram (CCH)

$$\hat{m}_{AB}(u) = \frac{\#\left\{u-\frac{b}{2}<\tau_k-\sigma_j<u+\frac{b}{2}\,;\,\tau_k\neq\sigma_j, j, k=1,2,\ldots\right\}}{bN_A(T)}\,. \tag{13}$$

The tally in each bin is divided by the product $bN_A(T)$. The result is expressed in numbers of B spikes per unit time and per A spike, so it can be compared from one case to another. For large T, the distribution of $\hat{m}_{AB}(u)$ may be shown to be approximately that of $(bTm_A)^{-1}P$, where P denotes a Poisson variate with mean $bTm_Am_{AB}(u)$. Its mean will be approximately $m_{AB}(u)$ and variance approximately $(bTm_A)^{-1}m_{AB}(u)$. These results suggest the graphing of the estimate $\sqrt{\hat{m}_{AB}(u)}$ of $\sqrt{m_{AB}(u)}$. Its variance is approximately stable for all u at $(4bTm_A)^{-1}$. Doing this constitutes a general

statistical advantage since it quantifies rigorously the uncertainty in the rejection of the null hypothesis of independence. Earlier workers (e.g. Bryant *et al.*, 1973) had to rely upon empirical procedures. In the case of independent trains, Griffith and Horn (1963) approximated the distribution by a multiple of a binomial or a normal. We feel that the Poisson will provide a better approximation provided b is not too large, nor is the span of dependence of the process large. Throughout the paper we have set approximate 95% confidence limits corresponding to plus and minus 2 standard deviations. The auto-intensity function $m_{AA}(u)$ may be estimated by the cross-correlation histogram between the train A and itself. This estimate, called the auto-correlation histogram (ACH), is denoted by $\hat{m}_{AA}(u)$, and is given by expression (13) with $\tau_k \equiv \sigma_j$. When ACH's correspond to a train of AP's, they tend to exhibit low values for short times, reflecting the fact that refractoriness prevents very close firings. The large sample variance of $\sqrt{\hat{m}_{AA}(u)}$ will be approximately $(4bTm_A)^{-1}$, as it was for $\sqrt{\hat{m}_{AB}(u)}$.

We now pass to relevant identification details. Were one able to drive the presynaptic cell A with Poisson noise, $m_{AA}(v)$ would equal m_A for all v, and so the solution of Eq. (1) would be

$$a(u) = m_{AB}(u) - m_B \tag{14}$$

i.e. the corresponding CIF up to an additive constant, and it could be estimated by the corresponding CCH. Unfortunately, and mainly because cells cannot fire at short intervals, the A train cannot be Poisson in all important respects. The presynaptic form referred to as "Poisson" in Bryant *et al.* (1973) and in this paper, is similar to the output of a non-paralyzable Geiger counter with a jittery dead-time submitted to a long-lived radioactive source. It is a renewal process whose intervals correspond to the sum of a short dead-time, with small variability, and an exponential variate, whose mean is much larger than the dead-times. The AIF of this form is flat, nearly equal to the mean rate of all lags u, except for having near 0 values around the origin followed by a small peak (see Figs. 1, 3, 9 in Bryant *et al.*, 1973).

We turn to the problem of constructing a solution $a(u)$ of Eq. (1). Let

$$f_{AB}(\lambda) = (2\pi)^{-1} \int_{-\infty}^{\infty} m_A [m_{AB}(-u) - m_B] \exp\{-i\lambda u\} du \tag{15}$$

denote the cross-spectrum of the A and B trains. This parameter is proportional to the covariance of the component of frequency λ of the A train with the corresponding component of the B train, hence it may be interpreted as reflecting how a certain frequency component in the A train is associated with one in the B train. Similarly let

$$f_{AA}(\lambda) = (2\pi)^{-1} m_A + (2\pi)^{-1} \int_{-\infty}^{\infty} m_A [m_{AA}(u) - m_A] \exp\{-i\lambda u\} du \tag{16}$$

denote the power spectrum of the A train. This is proportional to the variance of the component of frequency λ of the A train, hence it may be interpreted as reflecting the power in each frequency component of that train. For pure Poisson noise, it is identically constant and equal to $(2\pi)^{-1} m_A$. For the "Poisson" form employed in the experiments of this paper, it dips from this value in the neighborhood of $\lambda = 0$. In the case of a pacemaker train of period τ, the power spectrum $f_{AA}(\lambda)$ is composed of a series of spikes at equal multiples of $2\pi/\tau$. In real life, because correlating influences always are ephemeral, $f_{AA}(\lambda)$ will be near $(2\pi)^{-1} m_A$ for large $|\lambda|$. Finally define

$$A(\lambda) = \int_{-\infty}^{\infty} a(u) \exp\{-i\lambda u\} du$$

to be the Fourier transform of the solution $a(u)$. The gain at frequency λ is the absolute value of the Fourier transform of $a(u)$, i.e. $|A(\lambda)|$. Taking the Fourier transform of equation (1) and using definitions (15), (16) now leads to the simple relationship

$$f_{AB}(\lambda) = A(\lambda) f_{AA}(\lambda). \tag{17}$$

It follows that, if $f_{AA}(\lambda) \neq 0$ for $-\infty < \lambda < \infty$, the solution of Eq. (1) may be written

$$a(u) = (2\pi)^{-1} \int_{-\infty}^{\infty} f_{AA}(\alpha)^{-1} f_{AB}(\alpha) \exp\{iu\alpha\} d\alpha. \tag{18}$$

Equations (17) and (18) may be used to construct and estimate of $a(u)$ based on estimates of $f_{AA}(\lambda)$ and $f_{AB}(\lambda)$. The steps above are also described in Brillinger (1974).

Turning to the construction of an estimate of $a(u)$, let $\hat{m}_A$, $\hat{m}_B$, $\hat{m}_{AA}(u)$, $\hat{m}_{AB}(u)$ all be formed in the manner of expressions (11), (12). Let $u_j = jb$ for $j = 0, \pm 1, \pm 2, \ldots$ and let $k_T(u)$ be a window function (that is, a stretch of multipliers introduced into an empirical Fourier transform in order to improve its convergence properties (Brillinger, 1975c). The needed spectra may now be estimated by

$$\hat{f}_{AA}(\lambda) = (2\pi)^{-1} \hat{m}_A + (2\pi)^{-1} b \hat{m}_A \sum_j [\hat{m}_{AA}(u_j) - \hat{m}_A] \cdot \exp\{-i\lambda u_j\} k_T(u_j) \tag{19}$$

$$\hat{f}_{AB}(\lambda) = (2\pi)^{-1} b \hat{m}_A \sum_j [\hat{m}_{AB}(-u_j) - \hat{m}_B] \cdot \exp\{-i\lambda u_j\} k_T(u_j) \tag{20}$$

respectively. It is usual to plot $\log_{10}\hat{f}_{AA}(\lambda)$, whose variance is given approximately by $(0.4343)^2T^{-1}\cdot\int k_T(u)^2du$ for $\lambda\neq 0$. In figures presented later, we indicate approximate 95% confidence limits by graphing amounts of ± 2 standard errors. Other procedures for estimating the spectra of point processes are described in Brillinger (1975b).

The Fourier transform, $A(\lambda)$, may now be estimated by

$$\hat{A}(\lambda)=\hat{f}_{AA}(\lambda)^{-1}\hat{f}_{AB}(\lambda)\,. \tag{21}$$

The variance of $\log_{10}A(\lambda)$ is approximately $(0.4343)^2T^{-1}\int k_T(u)^2du[|R_{AB}(\lambda)^{-2}-1]$ for $\lambda\neq 0$, where

$$|R_{AB}(\lambda)|^2=|f_{AB}(\lambda)|^2/|f_{AA}(\lambda)f_{BB}(\lambda)| \tag{22}$$

is the coherence at frequency λ, a measure of the degree of association of the trains (see below). It is never less than zero or greater than one, and defined to be zero if $f_{AA}(\lambda)$ or $f_{BB}(\lambda)$ equal 0. The expression for the variance of $\log_{10}\hat{A}(\lambda)$ shows that the estimate is better, the nearer the coherence is to one. Stein and associates (e.g. Stein *et al.*, 1972) have applied the coherence usefully to neurophysiological data in hybrid situations where a point process and an ordinary time series are matched.

Let $\lambda_j=jc$ for $j=0,\pm 1,\pm 2,\ldots$ and some $c>0$. Let $K_T(\lambda)$ be a second window function. The function $a(u)$ may now be estimated by

$$\hat{a}(u)=(2\pi)^{-1}c\sum_j\hat{A}(\lambda_j)\exp\{iu\lambda_j\}K_T(\lambda_j)\,. \tag{23}$$

The variance of this estimate is approximately

$$c(2\pi)^{-1}T^{-1}\int k_T(u)^2du$$
$$\int[1-|R_{AB}(\alpha)|^2]f_{BB}(\alpha)f_{AA}(\alpha)^{-1}K_T(\alpha)^2d\alpha\,. \tag{24}$$

This estimate will also be better the nearer the coherence is to one.

The coherence defined by expression (22) is an extremely useful measure of the degree of relationship of the A and B trains. Direct manipulations show that the minimum of the mean squared error of prediction (6) may be written

$$\lim_{h\downarrow 0}\int\left(\frac{\sin h\alpha/2}{\sin\ \alpha/2}\right)^2 f_{BB}(\alpha)[1-|R_{AB}(\alpha)|^2]d\alpha$$

showing one sense in which this is true. A coherence of one would imply perfect linear prediction of the B train by the A train. A coherence of zero results if the two trains are statistically independent or if $f_{AA}(\lambda)$ or $f_{BB}(\lambda)=0$ at the frequency under consideration. It can also result if the two trains are related in certain nonlinear manners. The coherence may be estimated by

$$|\hat{R}_{AB}(\lambda)|^2=|\hat{f}_{AB}(\lambda)|^2/|\hat{f}_{AA}(\lambda)\hat{f}_{BB}(\lambda)|\,. \tag{25}$$

The 95% point of the distribution of this estimate, in the case that $|R_{AB}(\lambda)|^2=0$, is given approximately by $1-(0.05)^{1/n}$ where $n=T/\int k_T(u)^2du$, (5). This level is indicated in figures provided later.

We next consider the case of three simultaneously occurring stationary spike trains A, B, C. Suppose that there exists some association between A and B, between B and C, and between A and C as revealed by the measures discussed above. It may be physiologically interesting to enquire whether B and C, say, are truly connected with each other or whether their apparent association is simply due to the common influence of A. One tool for investigating such a question is partial coherence analysis. Consider a situation where the counting processes in any particular interval are related by

$$\begin{aligned}N_B&=N_{B'}+N_{B''}\\ N_C&=N_{C'}+N_{C''}\end{aligned} \tag{26}$$

where the trains B' and C' depend on the A train, but the pair (B'', C'') is independent of A, B', C'. In particular suppose that

$$\begin{aligned}&\text{Prob}\{B'\ \textit{spike in}\ (t,t+h)|A\ \textit{spikes at}\ \sigma_j\}\sim\sum_j b(t-\sigma_j)h\\ &\text{Prob}\{C'\ \textit{spike in}\ (t,t+h)|A\ \textit{spikes at}\ \sigma_j\}\sim\sum_j c(t-\sigma_j)h\end{aligned} \tag{27}$$

as $h\downarrow 0$, for some functions $b(u)$, $c(u)$. For this model the coherence between the B'' and C'' processes is given by $|R_{BC.A}(\lambda)|^2$ where

$$R_{BC.A}(\lambda)=\frac{f_{AA}f_{BC}-f_{BA}f_{AB}}{(f_{AA}f_{BB}-f_{BA}f_{AB})(f_{AA}f_{CC}-f_{CA}f_{AC})} \tag{28}$$

in terms of the basic spectra. (In this last expression, dependence of the right hand side on λ has been suppressed for notational convencience.) The parameter $|R_{BC.A}(\lambda)|^2$ is called the partial coherence of the processes B and C given A. It will be zero in the case that the processes B'' and C'' are statistically independent, that is, the total tying together of the processes B and C is through B' and C' depending on the A processes. The partial coherence may be estimated by substituting estimates of the power and cross-spectra into expression (28). The above sort of partial analysis is standard for ordinary time series and may be found in Brillinger (1975c) for example.

Results

This section is organized around the purposes of the communication as listed in the introduction. The various functions considered (i.e. the CIF, spectra, coherences, kernels) obviously cannot be displayed since they are population parameters. All graphs are therefore estimates, though sometimes referred to as functions for brevity. Figure 1 illustrates the domain over which the model (3), based upon the first-order kernel $a(u)$ and the rate μ were there no input, is plausible, and when $a(u)$ gives a faithful representation of the rate effects of the single average presynaptic spike (or PSP). The presynaptic cell L 10, driven by a "Poisson" form, was very irregular and (for *Aplysia*) at a moderate rate of 1.96 spikes/sec; correspondingly, the postsynaptic discharge in cell L 3, where IPSP's were elicited, also was "Poisson". Graphs *A* and *B* depict time domain statistics. The square root (*A*) of the CCH shows the typical polyphasic effects associated with the single, average PSP, including here a pronounced slowing of L 3 for about 0.5 sec after the reference L 10 spike (at time zero), followed by a brief "rebound" acceleration. The solid horizontal lines indicate the limits of the approximate 95% confidence band, set a plus and minus two standard deviations about the estimate (dotted line) of the square root of the overall mean rate of L 3. These lines confirm the statistical significance suggested in Bryant *et al.* (1973) of the polyphasic effects of the average PSP, whether "inhibitory" as here or "excitatory" as in Fig. 4. The advantage of having a formal confidence procedure is clear. The square root of the ACH's of L 10 (*B*) and L 3 (not shown) have the character expected for "Poisson" forms, depressed near the origin partly because of refractoriness, but otherwise constant. Graphs *C* and *D* give the logarithm to the base 10 of the estimated power spectra of L 10 and L 3, respectively. The broken line is the level they would have were the trains Poisson at the same rate, or the asymptotic value as $\lambda \to \infty$; the solid horizontal lines are approximate 95% confidence limits. The estimates are small at low frequencies, suggesting that the spikes of the trains are farther apart than those of the comparable Poisson train. The highest frequency appearing in these and later estimates of spectra is 4 cps; after some experimentation, we found that power spectra did not deviate much from the Poisson level beyond this frequency. Graph *E* is the estimated coherence (with the 95% point of the null distribution indicated by the broken line): it is significantly high up to about 1.6 cps, suggesting that the two cells are tied together strongly in their slow and the moderately rapid behaviors. Finally, the estimate $\hat{a}(u)$ of the time function $a(u)$ calculated in the manner of Eq. (24) is shown (Graph *F*). As indicated earlier, this function attempts to unfold or deconvolve the postsynaptic L 3 train by removing the character of the presynaptic L 10 train in order to reveal the underlying "primary effect" of the

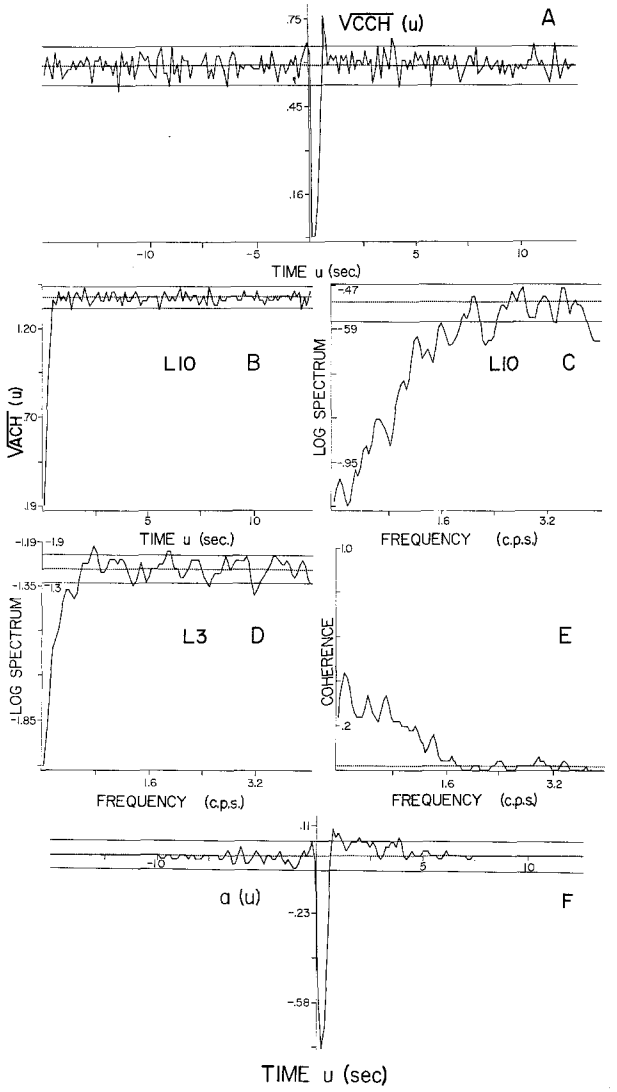

Fig. 1. Linear domain in the transformation from pre- to postsynaptic spike trains at the L 10-L 3 synapse (IPSP). Time and frequency statistics. All figures are described in detail in the text. The presynaptic discharge was very irregular and at a moderate rate, and so was the postsynaptic one. The corresponding square root of the ACH's and the spectra are shown in *B*, *C* and *D*. The coherence estimate (E) is significantly high up to about 1.6 cps. Hence, the model based upon the $a(u)$ function in *F* is considered acceptable; compare *F* with the square root of the CCH in *A*. The L 10 spike train had $N = 1745$ action potentials; its interval statistics were mean μ 0.509 sec, standard deviation σ 0.294 sec, and coefficient of variation 0.578. L 3 spike train: N 301; μ 2.947 sec; σ 1.546 sec; CV 0.525. These graphs were constructed with the same data as Fig. 1 in Bryant *et al.* (1973)

average presynaptic spike on L 3's rate. The confidence band (plus and minus two standard deviations) for the estimate is indicated. As shown, the primary effect is a biphasic slowing-accelerating sequence with a prolonged (about 3 sec) tapering off acceleratory phase. The anticipatory peak, apparent in the CCH, can disappear when the data is deconvolved in this manner. At very high L 10 rates, L 3 was silent most of the time.

Figure 2-I illustrates the domain over which the model based exclusively upon $a(u)$ and μ is not sufficient, and where $a(u)$ does not represent faithfully the rate effects of the single presynaptic spike (or PSP). The presynaptic cell L 10 produced approximately once every 25 sec a spontaneous burst with an intraburst accelerando pattern. The postsynaptic cell L 3 also was very bursty, completely shut off during the L 10 bursts but firing between them. The estimate (Graph *A*) of the autospectrum of L 10 shows a concentration of power at the low frequencies (from 0 up to about 0.2 cps), suggesting a propensity of the cell to continue doing whatever it is presently doing. The power at 0 frequency is greater than it would be for a Poisson with the same rate, suggesting that the spikes of the train are more clustered than in the latter. The power of most frequencies is smaller by about 0.55 logarithmic units or decibels, i.e. 3–5 times smaller, than that of the Poisson, and by about 2.5 units, i.e. 320 times smaller, than those of frequencies close to zero. Graph *B* is the autospectrum of L 3 and exhibits substantial power at the very low frequencies; it has, however, characters of its own, including another maximum at about 2 cps. The coherence estimate (Graph *C*) is remarkably high at 0 frequency and then drops to insignificance almost immediately. The very small coherences at virtually all frequencies that were present in reasonable amounts in the autospectra of both cells indicates that the simple model is insufficient. A high coherence value exclusively at zero suggests that only the very slowly evolving behavior of the two cells is related.

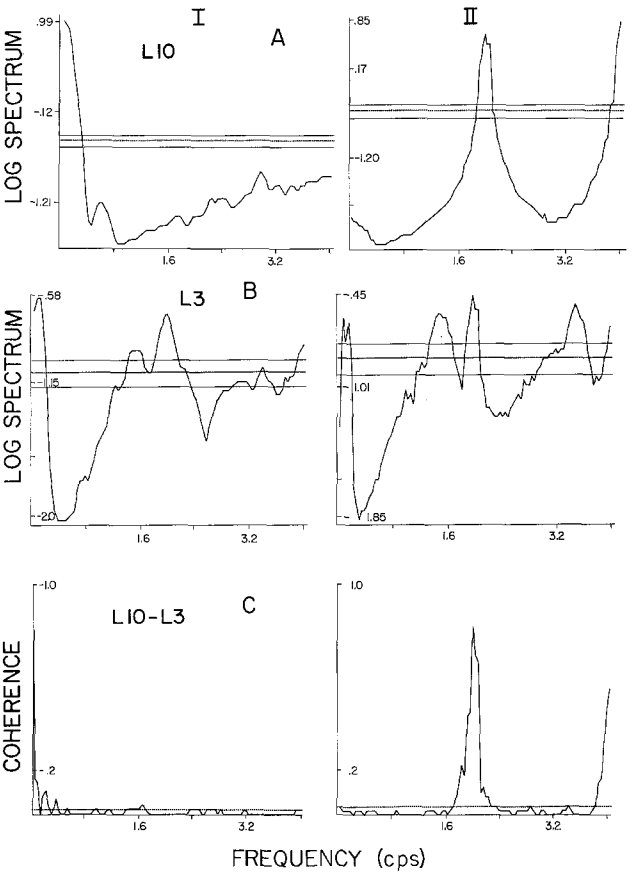

Fig. 2. Frequency statistics for the L 10-L 3 synapse. I. Non-linear part in the transformation domain. The presynaptic discharge was very "bursty" (A) and at a moderate rate. The coherence (C) was low throughout all frequencies except at those close to zero. II. Impossibility of estimating the kernels meaningfully. The presynaptic discharge was extremely regular at 2 spikes/sec. Its power (A) as well as the coherence (C) concentrated at that frequency and its harmonics. I. L 10 train: N 1422; μ 0.501 sec; σ 0.059 sec; CV 0.118. L 3 train: N 624; μ 1.132 sec; σ 1.141 sec; CV 1.008. II. L 10 train: N 1630; μ 0.476 sec; σ 2.368 sec; CV 4.976. L 3 train: N 416; μ 1.837 sec; σ 3.335 sec; CV 1.815. Data as in Fig. 5-A, B, C of Bryant *et al.* (1973) for I and in Fig. 4-D, E, F of Bryant *et al.* (1973) for II

Figure 2-II illustrates the commonplace situation where extreme presynaptic pacemaker-like regularity precluded a meaningful estimation of the kernels. Cell L 10 was driven regularly at 2 spikes/sec; L 3 fired in bursts with usually evenly spaced AP's. The frequency domain statistics are especially useful in this case. Graph *A*, the estimate of the autospectrum of L 10, shows peaks at the fundamental frequency of about 2 cps and at the harmonics. The power at most other frequencies is smaller by about 2 decibels than of the Poisson at the same rate, and by about 3.5 than that at the pacemaker frequency (or its harmonics): i.e. the former power is about 100 and 3200 times smaller than the other two, respectively. Graph *B*, the estimated autospectrum of L 3, also shows peaks at the 2 cps frequency and its harmonics; in addition, L 3 has its own spontaneous behavior with strong frequency components at about 0.08, 1.48 and 3.48 cps (the latter possibly caused by the beating together of the 1.48 and 2.00 cps components). The coherence estimate (Graph *C*) is very high at the driving frequency of 2 cps (and its harmonics): hence, the IPSP ties the cells together at that frequency. However, because $\hat{f}_{AA}(\lambda)$ is near 0 for so many frequencies, the variance expression (24) indicates that one cannot construct a meaningful estimate of $a(u)$.

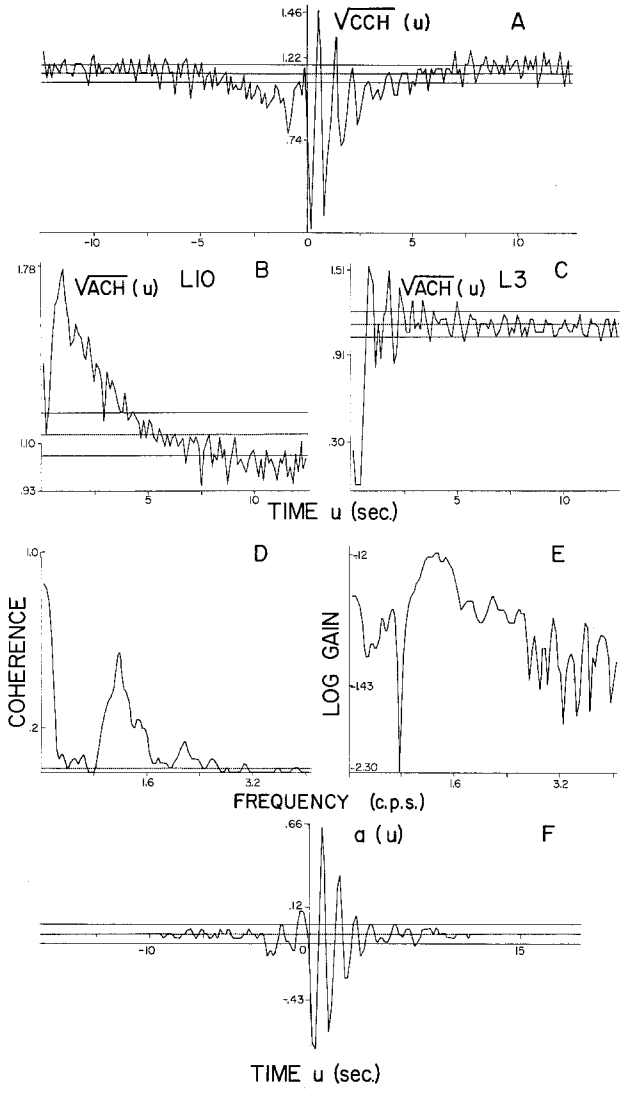

Fig. 3. Non-linear domain at the L 10-L 3 synapse. Time and frequency statistics. L 10 and L 3 had a somewhat bursty pattern, as illustrated by the square roots of the ACH's (*B*, *C*) (and by the autospectra which are nor shown). The coherence (D) is high only around the O and 1.2 cps frequencies. The linear model does not suffice, and the $a(u)$ function (F) does not provide an acceptable description (e.g. has oscillations over negative times) of rate effects: it still constitutes an improvement over the time domain statistic (A). The gain (*E*) is maximum between 0.8 and 1.6 cps. L 10 train: N 1018; μ 0.780 sec; σ 1.369 sec; CV 1.755. L 3 train: N 992; μ 0.800 sec; σ 0.582 sec; CV 0.727. Data as in Fig. 5-D, E, F in Bryant *et al.* (1973)

Figure 3 illustrates the domain where the linear model (3) does not satisfy but where $a(u)$, though not representing faithfully the rate effects of the single presynaptic spike, still represents an improvement with respect to the ordinary time function. In this case, L 10's spontaneous bursting was less pronounced than in Fig. 2-II, and correspondingly L 3 was never silenced for long intervals. Graph *A*, the square root of the CCH, contains (mainly for positive times) a clear oscillation of period about 0.75 sec, superimposed upon a slower downward drift (roughly from -3 to 5 sec) due to L 10's bursts. Graphs *B* and *C* are the square roots of the L 10 and L 3 ACH's, respectively. The uniformly high values of *B* up to about 3.5 sec are consistent with L 10's bursting behavior; the lack of significantly low values close to the origin relates to the fact that successive AP's in the burst were very close to each other, indicating a short refractory period. The estimate of the autospectrum of L 10 (not shown) has, by comparison to a Poisson process of the same rate, excess power up to 0.24 cps, deficient power to about 1.0 cps, and the same power beyond 1.0 cps. The autospectrum estimate for L 3 (not shown) has more power in the immediate neighborhood of 0 than at the next interval of frequencies. Graph *D*, the coherence estimate, shows two pronounced but narrow peaks, one at 0 frequency, the other at about 1.2 cps, indicating that only the slowly evolving behavior of the two trains and that at 1.2 cps are associated. Graph *E*, the estimated gain, shows a boosting in the L 10 to L 3 transformation of frequency components from 0.8 to 1.6 cps, i.e. of periods from 0.65 to 1.25 sec: apparently, the L 10–L 3 synapse enhances particularly that oscillatory behavior. The $\hat{a}(u)$ in Graph *F* exhibits successive slowings and accelerations over a 5 sec interval; these include some to the left of 0 which, since they anticipate the presynaptic AP, cannot be attributed to a causally determined "primary effect". A simple comparison of Graphs *A* and *F*, however, does suggest that even in this case where the estimate is not plausible there are advantages in the deconvolution operation to form $\hat{a}(u)$: for example, whereas the CCH exhibits its oscillations superimposed upon a pronounced dip (caused by the bursting character of L 10), the graph in *F* oscillates about a constant level.

In a case where L 10 was "Poisson"-driven at 1/sec, and L 3 fired quite irregularly, many (but not all) L 10 spikes produced L 3 slowing for periods of up to 4 sec, i.e. there was "inhibition of long duration" (e.g. Fig. 6 in Bryant *et al.*, 1973). The coherence estimate was high up to about 0.7 cps. The $\hat{a}(u)$ dropped off very rapidly as u increased from 0, and then recovered slowly, reaching zero level only at close to 3.0 sec.

Figure 4 illustrates the domain of plausibility for the linear model and for $a(u)$ as a rate effect description at the stable EPSP junction between fibers in the right viscero-pleural connective RVP and R 15. The junction was driven by a "Poisson" form whose consequences only partially disrupted R 15's typical

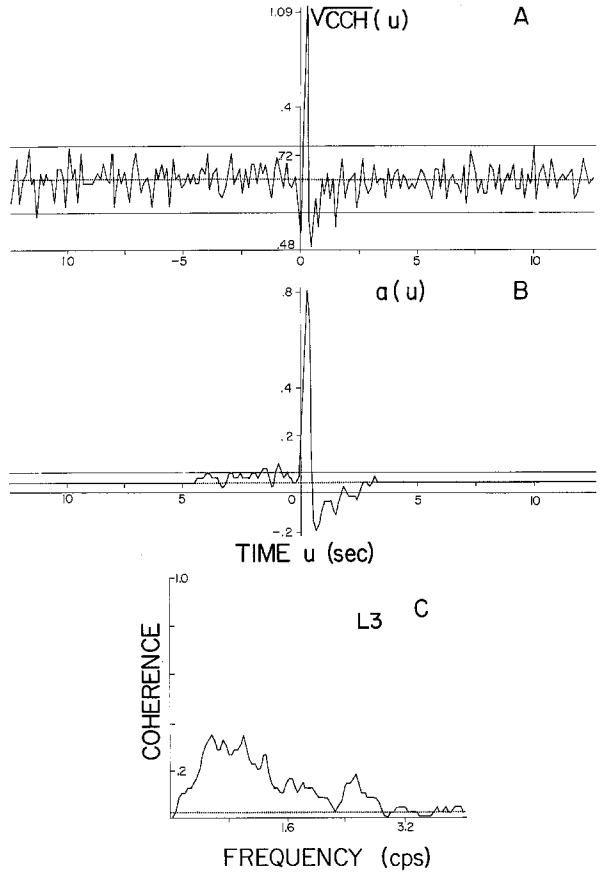

Fig. 4. Linear part of the transformation domain for a right-viscero-pleural to R 15 synapse with an EPSP. Time and frequency statistics. Coherences (C) are high up to about 3 cps. The square root of the CCH (A) shows the anticipatory dip followed by a peak by a rebound dip. The $a(u)$ (B) shows the peak and the rebound dip, thus eliminating the anticipatory dip attributable to a characteristic of the presynaptic discharge. Viscero-pleural train: N 1606; μ 0.200 sec; σ 0.117 sec; CV 0.585. R 15 train: N 440; μ 0.730 sec; σ 1.150 sec; CV 1.575. Data as in Fig. 14-A of Bryant *et al.* (1973)

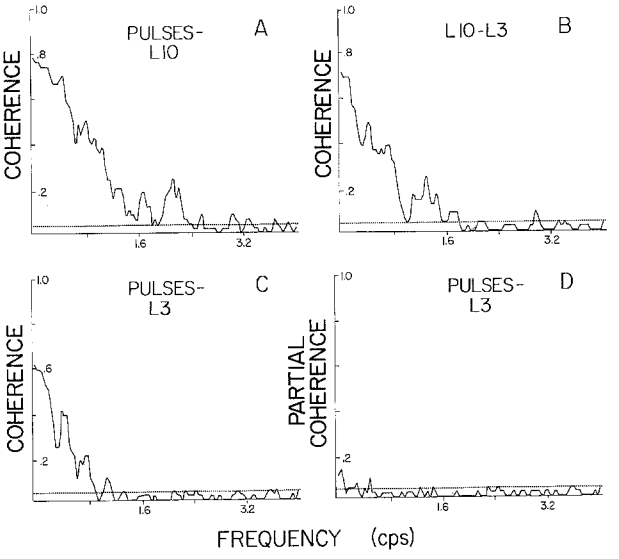

Fig. 5. Value of partial coherences. Confirmation of the a priori knowledge that there is no influence. An irregular ("Poisson") pulse train of hyperpolarizing pulses drove L 10 which in turn acted on L 3: there was no direct pulse influence on L 3. Pairwise coherences (pulses-L 10, *A*; L 10-L 3, *B*; pulses-L 3, *C*) were high for the smaller frequencies. On the basis of *C* alone, there is no way of telling how much of the pulses-L 3 association is due to the L 10 mediated effect on L 3 and how much to a direct influence. The partial coherence D with L 10 eliminated was not significant however, and this is compatible with the a priori knowledge. Pulse train: N 729; μ 1.020 sec; σ 0.560 sec; CV 0.549. L 10 train: N 702; μ 1.060 sec; σ 0.670 sec; CV 0.632. L 3 train: N 613; μ 1.210 sec; σ 0.290 sec; CV 0.240. Data as in Fig. 16 of Bryant *et al.* (1973)

bursting. Graph *A*, the square root of the CCH, has polyphasic character showing an anticipatory dip, followed by an acceleration and then another dip. The coherence estimate in Graph *C* is low at very low frequencies and then of some magnitude approximately from 0.2 up to 3.0 cps. The $a(u)$ estimate, Graph *B*, reveals a strong brief acceleration (about 60 msec) followed by a less marked slowing lasting about 2.5 sec. The anticipatory dip does not appear, confirming that it was introduced by the particular character of the input train (see above and Brillinger, 1975b). This $\hat{a}(u)$ is, to a degree, a mirror image of the $\hat{a}(u)$ of Fig. 1-*E*.

The validity of partial coherence analysis as a tool for confirming a real, or for discarding a spurious, interaction is illustrated in Fig. 5. In that experiment, "Poisson"-driven inward-current pulses were injected into L 10 which in turn elicited IPSP's in L 3: obviously the association between the pulses and L 3 was due to L 10's intervention and there was no direct effect of the pulses upon L 3. The estimated pairwise coherences are all high for low frequencies, Graph *A* being pulses-L 10, *B* L 10-L 3 and *C* pulses-L 3. The estimated coherence is least in the last case as was to be expected. Graph *D* gives the estimated partial coherence of the pulses with L 3 eliminating L 10: it is essentially zero, confirming the self-evident fact of no influence other than that mediated by L 10 from the pulses to L 3.

Figure 6 illustrates a practical application of the partial coherence which leads to a conclusion involving functional connectivity. In this case L 10 fired spontaneously as a pacemaker, and entrained two followers L 2 and L 3 via large IPSP's. The estimated coherences for L 10-L 3 in Graph *A*, for L 10-L 2 in *B*, and for L 3-L 2 in *C* are all high around 1 cps, the pacemaker frequency. The high coherences involving L 10 can be explained by the recognized and powerful IPSP's it elicits in the other cells. The high L 3-L 2

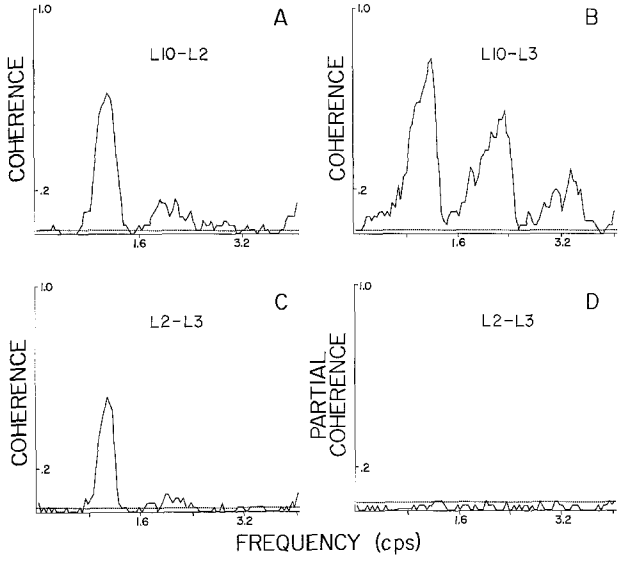

Fig. 6. Value of partial coherences. Rejection of the hypothesis of a direct connection between two neurons (L 2, L 3) influenced through IPSPs by a common source (L 10). All pairwise coherences are high: i.e. those between the driver L 10 and each of the followers L 2 (A) and L 3 (B) and that between the followers (C). The latter is compatible with the idea of an influence between L 2 and L 3, but this hypothesis is rejected by the low value of the partial coherence (D). L 10 train: N 767; μ 0.970 sec; σ 0.121 sec; CV 0.125. L 3 train: N 742; μ 1.000 sec; σ 0.130 sec; CV 0.130. L 2 train: N 523; μ 1.380 sec; σ 0.524 sec; CV 0.380. Data as in Fig. 15 A, B, C of Bryant *et al.* (1973)

coherence either can reflect exclusively the common drive L 10 exerts on both L 2 and L 3, or can depend on a hypothetical interconnection between L 2 and L 3. There is no electrophysiological evidence of such a connection, but this could be due to an electrode location in the soma remote from where the corresponding PSP's are generated. The existence of such an unrecognized influence was investigated using partial coherence analysis of L 2 and L 3 with L 10's influence eliminated. The partial coherence (Graph *D*) is not significantly different from zero. This leads to the physiologically interesting conclusion that, if an additional pathway exists, it is inactive under the present conditions.

Figure 7 is a final example of the power of the frequency domain approach. L 10 fired spontaneously with a strong pacemaker rhythm whose frequency $1/T$ was 2.34 cps, corresponding to a period T of 0.428 sec. The coherence estimate between L 10 and L 3 (not shown) was very high at this frequency. However, Graph *A*, the square root of the time domain statistics (ACH) of L 3 shows no clear sign of the period T corresponding to this frequency; indeed, the interval between the origin and the first mode (estimating the "dead time" of the cell) is about 0.750 sec, i.e. longer than T and corresponding to a frequency of 1.33 cps. Contrastingly, Graph *B*, the estimate of the power spectrum of L 3, does have a peak (indicated by the arrow) at 2.34 cps, from which the L 10 pacemaker period and frequency might have been identified were they unknown. L 3 reveals its frequency $1/T$ with period T by firing at periods $2T$, $3T$, ... that are integral multiples of T. There is, in addition, a peak at 1.33 cps.

Discussion

The present communication explains a quantitative model that explores and describes the influence exerted by a presynaptic train upon the corresponding postsynaptic one; the conceptual entity that allows this influence can be referred to as "synaptic operator" (Bryant *et al.*, 1973). The procedure used derives from a mathematical method for identification of point-process systems (Brillinger, 1974), and permits interpretations and conclusions in qualitative and physiological terms.

The general plan of the model is based upon functions referred to as "kernels" that relate rate changes to one or more time arguments and reflect biologically meaningful issues. That (μ) of zero-order is simply the overall average rate of the postsynaptic

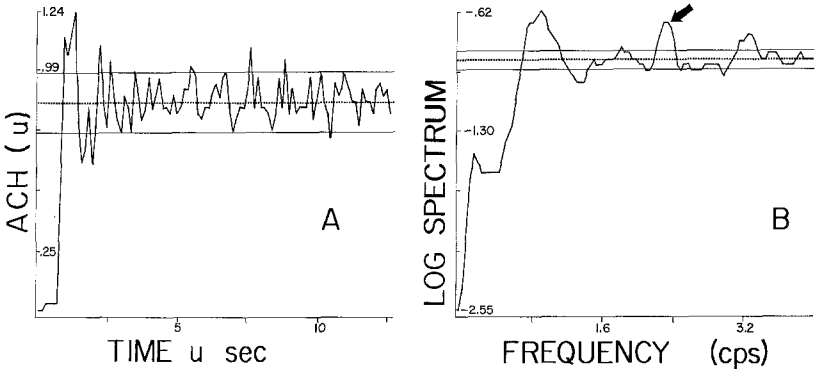

Fig. 7. Usefulness of the frequency domain. The presynaptic (L 10) pacemaker rhythm (2.34 cps, period 0.428 sec) was not apparent in the time domain statistic A of L 3, but did show up in the autospectrum B, revealing that it was indeed transferred. L 10 train: N 1537; μ 0.430 sec; σ 0.017 sec; CV 0.040. L 3 train: N 509; μ 1.290 sec; σ 0.420 sec; CV 0.326. Data as in Fig. 4-A, B, C of Bryant *et al.* (1973)

cell when the specific presynaptic neuron is silent. A first-order kernel relates to the rate changes elicited by the single average presynaptic spike, or PSP. The higher order kernels (which will be analyzed separately, Brillinger *et al.*, in preparation) relate to interactions between two or more pre- and/or postsynaptic spikes and measure these joint effects for any possible timing of the conditioning events. For example, a kernel of second-order will evaluate how the PSP facilitation at any time after any pair of PSP's is reflected by the postsynaptic rate.

Results added specific details of biological interest, apart from confirming the generalization that every regular system in Nature is in some way non-linear, but under certain conditions can be approximated satisfactorily by a linear model. Concretely, they showed that a domain of presynaptic discharges reasonably close to that of natural performance has two substantial and separate portions. In one, where presynaptic discharges are irregular and at intermediate rates (e.g. Figs. 1, 4), an acceptable description of synaptic transfer is provided by a model (3) and (4) based upon the rate when the presynaptic cell is silent and upon linear sum of appropriately timed first-order kernels. In this part of the domain, the function $a(u)$ becomes an adequate representation of the rate effects of the average presynaptic spike, i.e. becomes the average impulse response function. Hence, this part can be referred to as "linear". It should be noted that the assumption underlying expressions (3) and (4), i.e. that only the expectations are so related, is far weaker than the linearity assumption of ordinary system analysis, where the output y is essentially linear on the input x except for the error term. The large number of cases where this model was plausible, as revealed by substantial coherences in a broad frequency range, was remarkable.

The appropriateness of $a(u)$ as an uncontaminated description of single spike effects allowed the confirmation of the idea that both "inhibitory" or "excitatory" synapses generate biphasic effects. The unexpected strength of the late effects (e.g. acceleration with IPSP's) were confirmed, an issue which together with their genesis and implications have been discussed elsewhere (Brillinger, 1975b).

The "contamination" due to the presynaptic discharge form could thus be minimized and, in favorable cases, eliminated. Contrastingly, the CIF, even with the "Poisson" form, exhibits an "anticipatory" event (Bryant *et al.*, 1973) whose genesis is explained heuristically as follows for IPSP's (and similarly for EPSP's). The instant when the presynaptic cell A fires is the time origin for both the AB cross-intensity function, $m_{AB}(u)$, and the A auto-intensity function, $m_{AA}(u)$. The latter has a trough around 0 and is flat elsewhere. Now at any particular time u, $m_{AB}(u)$ depends on complex summation of "primary" effects that have invariant time courses with respect to the evoking A spikes and are elicited with characteristic relative timings that reflect, on the average, $m_{AA}(u)$. At long delays (large u's), $m_{AA}(u)$ is flat, so that primary effects are elicited in complete asynchronicity. Hence, their average consequences arise from uniformly staggered spikes and therefore produce no CIF deviation from flatness. At small delays (small u's), $m_{AA}(u)$ has a trough so that primary effects are elicited less than on average. Hence, to obtain $m_{AB}(u)$, one must subtract from the average amounts that depend on the primary effect and on the low $m_{AA}(u)$. An important portion of the primary effects of IPSP's involves slowings, and subtracting negative amounts implies increased $m_{AB}(u)$ values. Increases from the dead time preceding the A spike appear clearly as the "anticipatory" peak. Increases from that following the A spike combine with its primary effects and therefore are less apparent. Other presynaptic forms have more drastic effects. The CIF, revealing the postsynaptic rates around each presynaptic spike, does not provide an adequate representation of the rate effects of each. The solution $a(u)$ of Eq. (1) suffers considerably less from such effects.

It is desirable to stress the differences between several current functions of time. The postsynaptic rate changes occurring around the single presynaptic spike (or PSP) reflect in a complex way several issues like the statistical properties of the presynaptic train, and the rate effects of the single presynaptic spike. They are revealed by the CIF (Bryant *et al.*, 1973; Knox, 1974; Moore *et al.*, 1970). The rate effects of the single presynaptic spike reflect, in turn and also in a complex way, issues like PSP shapes and spike generation mechanisms. They are best predicted by the first-order kernel $a(u)$: in the linear portion of the domain, $a(u)$ provides a realistic and accurate description (referred to as average impulse response function), but in the non-linear portion (see below) it cannot be considered necessarily as realistic, though it still remains a part of expansion (9). Therefore, the rate changes around the presynaptic spike, those resulting from it, or the best linear predictor of the latter have profiles that are partly independent from one another and from the PSP shape, though they do relate because the latter is a common determinant. It is of interest to study each curve independently and understand its genesis, as well as to analyze their

differences. For example, those between CIF and PSP's were pointed out by Moore *et al.* (1970), Bryant *et al.* (1973) and Knox (1974), all of whom noted that they relate but neither linearly nor simply; those between the CIF and $a(u)$ are stressed here (e.g. Figs. 1, 3).

In the other portion of the domain, where presynaptic discharges are either bursty or very rapid (e.g. Figs. 2-I, 3), acceptable descriptions require other models, which can be referred to as non-linear. These incorporate kernels of several time arguments that account for interactions between two or more AP's, either presynaptic (i.e. implying PSP facilitation or anti-facilitation), postsynaptic (e.g. implying refractoriness), or both. Strictly neurophysiological work has demonstrated interactions, recovery cycles, and their dependence on timing; earlier results (e.g. Segundo *et al.*, 1963), however, often were neither exhaustive nor completely satisfying, providing few examples and using preparations where monosynapticity was questionable. These important issues can now be explored more precisely, thoroughly and convincingly, using higher-order kernels in a more suitable preparation such as this one (Brillinger, Bryant and Segundo, in preparation). The non-linear portion includes also pacemaker-like presynaptic discharges, even though the present results (e.g. Fig. 2-II) did not allow conclusions in this respect: indeed, when pre- and postsynaptic discharges are regular, their correspondence is complex, involving zigzag relations between rates and other effects (Perkel *et al.*, 1964; Schulman, 1969; Segundo, 1970).

Several neuronal properties (e.g. Segundo, 1970; Segundo *et al.*, 1969), e.g. spontaneous activity, PSP shapes and interactions, current-to-rate conversion refractoriness, are well known and recognized as mediators of the influence that presynaptic spikes exert upon postsynaptic spikes, i.e. the influences whereby fluctuations of generation intensity are transferred in terms of time courses and amplitudes. The model proposed here "handles" – i.e. accounts for and describes quantitatively and largely separately – the effects upon the postsynaptic rate of each of these properties as affected by any number (i.e. zero, one, two, and so on) of pre- and/or postsynaptic firings at any given relative timing. Several of these properties cause non-linear effects (e.g. Granit *et al.*, 1966): the relative magnitude of their respective contributions will arise from construction of higher order models. Our present tentative interpretation of the genesis of non-linearities, based upon observation of the transmembrane potential records where PSP interactions are not large, attributes the main responsibility to limiter behavior, post-firing excitability changes, and pacemaker interactions.

The idea of a network of neurons influenced trans-synaptically and generating AP's is the conceptual framework for an important approach to the nervous system where the elementary building-block is the transformation at an individual junction from pre- to postsynaptic activities. The dynamic features of the synapse is an important biological question, since an opinion that is plausible (though not immune to epistemological criticisms, Efron, 1967; Thom, 1972) claims that complete understanding of higher functions reduces to the behavior of membranes and molecules; knowing how the neuronal network operates is an indispensable intermediary prerequisite. Regardless of the language in which it is posed (quantitative and physiological, or sophisticated and mathematical), or of its aims (clues to integration or to local physico-chemical mechanisms), synaptic identification is always based upon a joint description of pre- and postsynaptic activities. Matched pairs can simply be listed, but a catalog provides little insight by itself. Heuristic extraction of qualitative conclusions has provided most progress in the field so far. More quantitative use of data and construction of mathematical representations as discussed here are also of value (Marmarelis and Naka, 1973a) for qualitative conclusions are often based upon quantified arguments. Indeed, models provide precise and concise canonical descriptions which imply a conceptualization of transformations and can serve as substitutes for experiments that are preliminary, technically difficult, or predictive.

The present work developed rigorous and exhaustive mathematical techniques and provided interpretations inferred from anatomically simple situations. Examples of such contributions are the following. The synaptic influence can be characterized via functions that do not depend on the presynaptic discharge and that are meaningful in terms of well-known biological realities (e.g. spontaneous spike generation, the effects of a single spike, and the interactions between several pre- or postsynaptic ones at all possible relative timings). The model that best predicts the postsynaptic discharge corresponding to any input can be constructed, as an expansion, to any degree of pre-specified accuracy. The physiological circumstances where a "linear" model is acceptable and where it is not were recognized: where it is plausible, the kernel of the first-order provides the uncontaminated rate effects of the single presynaptic spike. The partial coherence allowed conclusions as to connectivity, evaluating mutual influences between two post-

synaptic cells while submitted to a common presynaptic drive: we know of no other manner in which this may be done, apart from favorable electrophysiology. The practical value of rigorous confidence procedures for physiologically interesting estimators (so far used without them) seems self-evident.

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Dr. J. P. Segundo
Dept. of Anatomy
Center for the Health Sciences
University of California
Los Angeles, Calif. 90024, USA