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Evaluation of Neuronal Connectivity: Sensitivity of Cross-Correlation

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Cross-correlation analysis of separable multi-unit activity is one of the most commonly used methods to investigate connectivity in neural networks. In the course of development of new analysis techniques which go beyond the study of pairs or triplets of neurons, the need arose for a simple yet versatile simulator to generate spike trains from networks of specified structure. The present paper describes such a simulator and presents some examples of its performance as analyzed by cross-correlation. We noted a distinct asymmetry in the sensitivity of cross-correlation for the presence of excitatory vs inhibitory connections. A theoretical analysis is given from which quantitative criteria for detectability were derived. It appears that indeed the sensitivity of cross-correlation for excitation is larger to an order of magnitude than it is for inhibition. Possible consequences of this finding are indicated, and the relation to commonly used methods to measure strength of interaction are discussed.

INTRODUCTION

Recent years have shown a growing interest in the experimental investigation of activity in neural populations. This is, at least partly, caused by the increased availability of techniques to record simultaneously from groups of neurons and of procedures to separate this activity reliably into the contributions from individual neurons[1,8,10,14,19,32]. This upsurge in experimental techniques has led to an enormous increase in the amount of data produced during an experiment. The subsequent analysis, apart from being very time consuming, is seriously hampered by a relative lack of conceptual and analytical tools necessary for an adequate description of the phenomena associated with groups of interacting neurons. Most analysis at present is based on cross-correlation of simultaneously recorded trains of events from pairs (sometimes triplets) of neurons, introduced as early as 1967 (ref. 26), or methods derived from that[13,27].

Recently it has become possible to detect functional assemblies in populations of neurons by an approach which goes beyond the analysis of firing synchrony between pairs or triplets[15]. In the course of investigating the performance of this new approach it was necessary to simulate spike sequences generated by a network of neurons with specified connections. In view of its use, the simulator had to be simple and had to describe the neurons and their networks by commonly used parameters such as firing rate, synaptic strength and time course of the interaction. The output trains were to show the temporal features usually associated with excitatory and inhibitory connections. Various programs for simulating neural networks are available[22,28,29], and are explicitly built on models of the biophysics underlying synaptic events and spike generation. For our purposes such level of detail represented unnecessary complication; we have chosen a purely functional approach. Our simulator operates entirely in terms of stochastic point processes with prescribed probability density functions.

While studying the behaviour of our simple simulator we noted a peculiar asymmetry in the sensitivity of cross-correlation analysis for excitatory versus inhibitory interactions, the excitation being much more

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readily detected. In this paper, we give a theoretical analysis of the relation between spike train or interaction parameters and the expected shape of the cross-correlation function. This analysis confirms the noted difference in sensitivity which may amount to an order of magnitude. We have been able to derive threshold conditions for a given strength of connection to be visible, expressed in terms of experimental variables (pre- and postsynaptic rates, recording time) and analysis parameters (binwidth). Comparison of these criteria with experimental findings reported in the literature on connectivity analysis in the cortex suggests that the low proportion of inhibition found may, at least partly, be related to this difference in sensitivity of the analytic tools.

MATERIALS AND METHODS

The simulator

The basic components of the simulator are units representing single neurons: each unit generates events, the action potentials, according to some prescribed probability function. For our purposes an action potential is fully described by the moment in time at which it occurs. The units can be treated as unrelated elements, with independent firing statistics. More interestingly, however, is the possibility of 'connecting' the units by having the output events from one unit (the 'driver') influence the probability of occurrence of events from another one (the 'driven'), including possibly the driver itself. This influence can either enhance the probability of firing in the driven unit ('excitation), or it can suppress it ('inhibition'). Each connection can be specified by the amount of this influence ('strength') and its time course ('dynamics'), thus enabling the construction of an interconnected network of units, described by a 'wiring diagram' or 'connectivity matrix'. We will now describe the units and the possible types of connection separately in more detail.

The units

When left to itself, each unit will generate events, following the rules of a renewal process, i.e. fully determined by its interval distribution[3]. The present model uses the so-called gamma distribution[33] specified by the rate ϱ and form parameter γ, with γ an integer number $\geqslant 1$. The actual realization of the model uses a logarithmic transformation of a uniform distribution generated by a standard random number generator algorithm. In order to prohibit unrealistically short or very long intervals, this uniform distribution is mapped on to the interval [0.01, 0.99], rather than on the usual [0,1]. The event sequences generated by the units are either passed to the output (in the case of autonomous units) or can be modulated locally by the effect of incoming events from one or more driver units.

Excitatory connections

The mechanism of an excitatory connection is schematically depicted in Fig. 1a. Upon the occur-

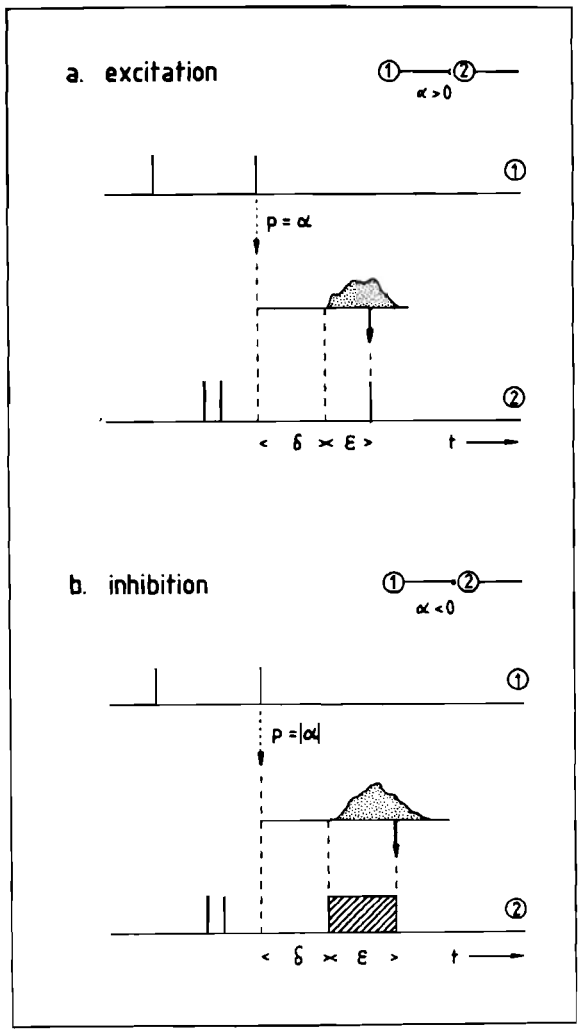

Fig. 1. Mechanism of excitatory (1a) and inhibitory (1b) synapses as implemented in the spike train simulator. Further explanation in text.

rence of an incoming 'presynaptic' event an 'excited' event is inserted into the 'postsynaptic' train with probability α ($0 \leqslant \alpha \leqslant 1$), in the remainder of the cases (probability 1- α) the incoming events pass 'unnoticed'. As a result the post-synaptic train will contain a partial and noisy replica of the pre-synaptic train. The larger α, the higher the chance per driver event to produce an event in the follower train. Hence we may name α the 'synaptic strength'. Note that from the probability definition the strength of a connection is necessarily normalized to the range [0,1]. The timing relation between the excited event and the event giving rise to it is made up of a constant delay δ and a stochastic interval ξ, drawn from a prescribed interval distribution as illustrated in Fig. 1a. The constant δ gives a minimum delay between 'cause' and 'effect', the distribution of ξs describes the stochastics of the coupling dynamics. In the present version of the simulator this distribution was simply chosen to be uniform over a specified range $[0, \sigma]$; any reasonable distribution, however, can be used. Evidently the smaller the width σ, the more precise the temporal coupling between driver and driven events.

Upon insertion of an excited event into the driven sequence, the ongoing interval, generated by the unit's autonomous renewal density, is terminated and a fresh interval is selected and set to start at the point of insertion. This may be taken to correspond to a complete reset of the generator potential as used in so-called (leaky) integrator-type models of neural firing[18]. The insertion of an excited event has no influence on events scheduled to occur before the moment of insertion, neither on the excited events scheduled to occur after it.

Inhibitory connections

The mechanism of the inhibitory connections is illustrated in Fig. 1b, and can be observed to be very similar to the excitatory mechanism. The strength now is defined to be negative ($-1 \leqslant \alpha \leqslant 0$) and is used in the same manner as before: a driver event gives rise, with probability $|\alpha|$, to an insertion into the driven sequence. The item to be inserted now, however, is not an event but a forced 'silent' interval of some variable length ξ generated by a specified probability density function as shown in Fig. 1b. The silent interval starts after a fixed delay δ, following the driver event giving rise to it. In the present model the distribution of ξs, like in the case of excitation, is chosen to be uniform, with some mean μ and width σ. Note that, unlike the case of excitation, there now is no stochastic behaviour as far as the point of insertion (the beginning of the silence) is concerned; this gives rise to a sharp temporal coupling between driver events and the onset of inhibitory effects in the driven train. We will return to this point in the discussion.

The insertion of a silent interval terminates the ongoing interval generated by the driven unit's own event generator, and a fresh interval is selected and set to start (without an intermediate event) at the end of the inserted silence. This corresponds to a complete reset of the driven unit's generator potential followed by a 'hold' at zero level through the silent interval. Moreover, throughout the time span of the silent interval any event, whether autonomous or excited, is prohibited, making this a very strong version of inhibition. The inhibition, throughout its induced silence, effectively prevents all incoming events from possibly eliciting an excited event. The insertion of a silent interval has no effect on events or silences scheduled to occur before the moment of insertion, neither on excited events or induced silences scheduled to occur after its termination.

Implementation

The simulator was implemented in Fortran 77, on a DEC LSI-11/23 computer. The program has been included into a software package (PUNFUN), developed at the Department of Medical Physics and Biophysics at the University of Nijmegen, the Netherlands, and implemented at the authors' laboratory at the University of Pennsylvania. This package contains various procedures for the statistical analysis of series of events arising from single and multi-neuron recordings and was used in the present investigation to analyse the performance of the simulator.

RESULTS

1. *Simulations*

In this section we show some analyses of representative data generated by the simulator. Through this series of simulations we have used parameter values suggested by experiments involving single and multi-unit recordings from cat visual and auditory

cortex; these areas are the subject of experimental investigation in our laboratory[14].

Independent units. We begin by considering the behaviour of autonomous, i.e. non-driven units. Fig. 2 shows the results of statistical analysis of a sequence of events generated by such a unit. The firing rate ϱ was set to be 4 s^{-1}, this value being fairly typical for the 'spontaneous' activity in both the visual[24] and auditory[6,11] cortex. The interval distribution was taken to be Poisson ($\gamma = 1$), which is less realistic but convenient for theoretical purposes. This particular unit was 'recorded' for 256 s, and produced 1038 events (average interval 246.7 ms, standard deviation 236.8 ms, as compared to the theoretically expected value of 250 ms for both quantities). The interval distribution is shown in Figs. 2a (linear scale) and 2b (logarithmic scale). The straight line in Fig. 2b represents the expected interval density for a true Poisson process. The arrows along the time axis indicate the expected average interval of 250 ms.

The temporal structure of the event sequence can be studied by means of the auto-correlation function[25] as shown in Fig. 2c. This basically shows the delta-function at the origin and the flat distribution for non-zero timeshifts, expected for a Poisson process. The expected number of coincident firings per bin (0.5 ms) in this case equals 2 and is indicated by the arrow along the vertical axis of Fig. 2d. It can be observed that the delta peak at the origin is flanked by a trough of 2.5 ms width, caused by the minimum interval in the event generating mechanism (see Methods).

Finally, Fig. 2d shows the similarity between the firings of two non-driven, and therefore statistically independent units as expressed in the cross-correlation function for their respective event sequences[26]. Again we note the basically flat distribution of the number of coincidences per bin around the expected value of 2. The result in this figure should serve as a reference for cross-correlations of event sequences from units where a connection does exist: the variance gives an indication of the statistical reliability of possible features in cross-correlograms using a binwidth of 0.5 ms and event sequences involving some 1000 events each, which is typical for cortical recordings.

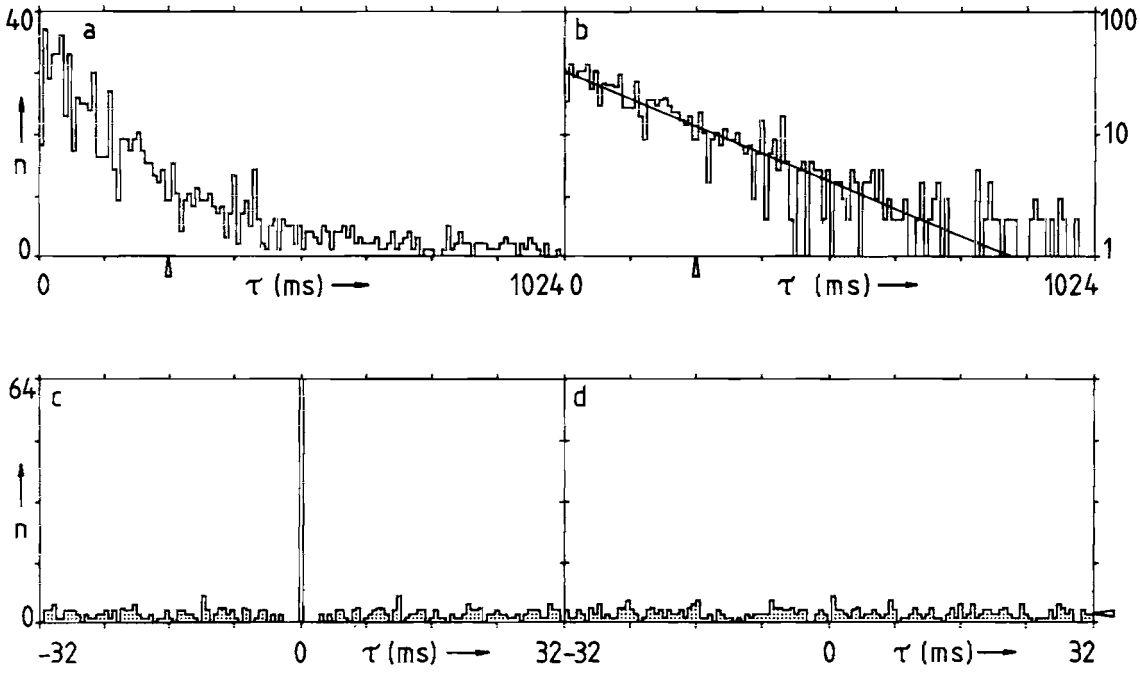

Fig. 2. Statistical analysis of spike train from non-driven unit: interval histogram on linear (2a) and logarithmic (2b) scale; autocorrelation function (2c) and cross-correlation with independent unit (2d). Vertical axes give numbers of counts in all cases. Binwidth: 8 ms (2a, b) resp. 0.5 ms (2c, d). Numbers of events: $N_1 = 1038$, $N_2 = 1096$; observation interval: 256 s. Drawn line in Fig. 2b represents interval distribution for Poisson process. Arrows in Figs. 2a, b indicate expected average interval (250 ms); in Figs. 2c, d arrows indicate expected number of coincidences (2).

Excitatory connections. The parameters used to specify the excitatory connections were inspired by experimental data on intracortical excitatory connections[1,6,24]. Event rates again were set to 4 s^{-1}. Numbers of events per unit were in the order of 1000, the delay δ in all cases was 1 ms, being representative of a monosynaptic delay. We have varied both the 'synaptic strength' α and the width σ of the distribution of excitatory intervals (cf. Fig. 1a). Fig. 3 shows the effect of changing the synaptic strength, while using a fixed width of $\sigma = 2$ ms. Depending on the value of α, we observe a peak of more or less distinct height against an essentially flat background. The a priori expected number of coincidences for uncorrelated sequences (2 per 0.5 ms, cf. Fig. 2d) is indicated by the arrow along the vertical axis in Fig. 3b, d. The extent of the distribution of ξs is indicated by the bar along the time axis, next to the origin. It can be observed that a distinct elevation in the cross-correlogram is present for a value of α as low as 0.05 (Fig. 3b), i.e. in the case that, on the average, only 1 out of 20 presynaptic events induces a postsynaptic event. Even the case of $\alpha = 0.025$ (Fig. 3a) shows at least the suggestion of an elevation at the appropriate time coordinates.

The effect of changing the width σ, while keeping α at a constant level of 0.1 is shown in Fig. 4. The extent of the distribution of ξs is shown by the number in each graph and is indicated by the bar along the time axis. From these results it can be observed, as was to be expected, that increasing σ results in a widening of the peak in the correlogram, at the expense of its height, in such a manner as to keep the product of the two, i.e. the total number of coincident events in the elevation, a constant, the value of which is determined by α. Evidently, with lower values of α this increase in temporal uncertainty may cause the elevation in the correlogram, i.e. the signature of the excitatory connection, to become buried in the variance, especially in preparations with modest firing rates, like the cortex. On the whole, however, using 2–4 ms as a reasonable estimate of σ[1,6,11,24], the cross-correlation function of trains containing some 1000 events each appears to be sensitive to connectivities as low as 0.025.

Inhibitory connections. In the case of inhibitory connections it is much harder to obtain reliable estimates of parameter values from the physiological literature on cortical neurons. They are either not reported or explicitly reported not to have been found. Following Dickson and Gerstein[6] and Michalski et al.[24] we set the delay equal to 2 ms. Event rates were 4 s^{-1} as before, as were the numbers of events 'recorded' (approximately 1000 per unit). The cross-

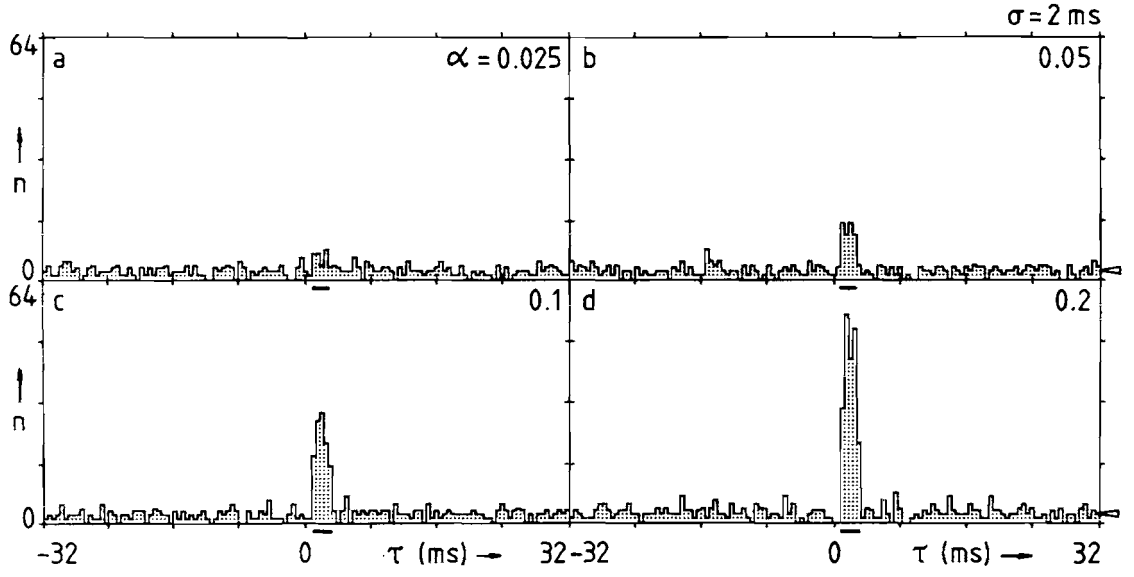

Fig. 3. Cross-correlation analysis of pairs of units with excitatory connection of variable strength α and constant width σ of noisy insertion. Vertical axes give numbers of coincidences. Binwidth: 0.5 ms; observation interval: 256 s; numbers of events: driver (the same in all 4 cases): 1038 (cf. Fig. 2), driven units: 1092 (3a), 1078 (3b), 1115 (3c) and 1254 (3d). Expected number of coincidences for uncorrelated trains (2) is indicated by arrow along vertical axis; extent of distribution of insertions (σ) is indicated by bar along time axis.

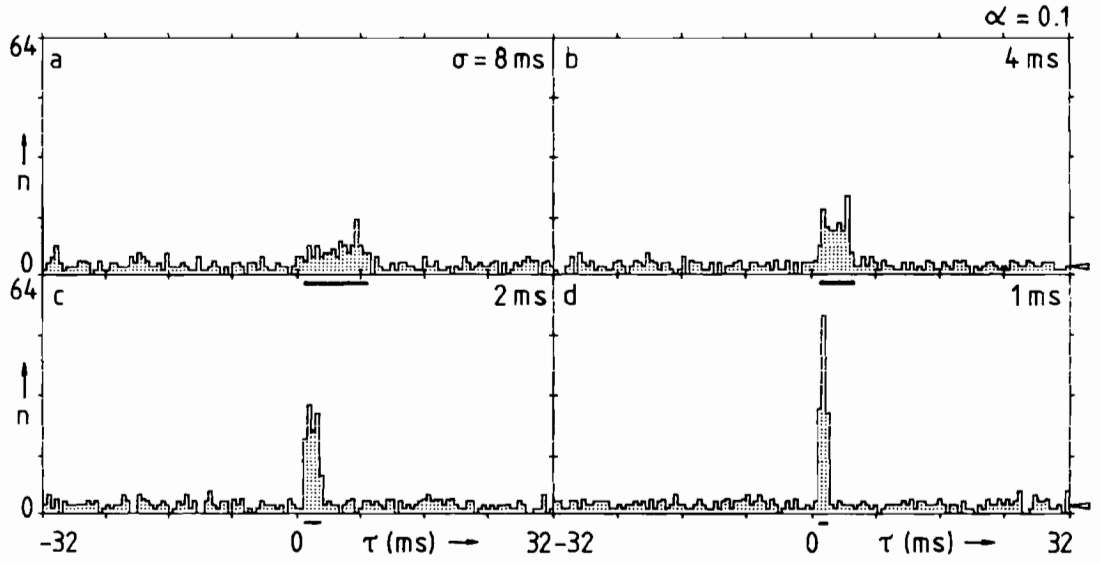

Fig. 4. Cross-correlation analysis of pairs of units with excitatory connection of constant strength α and variable width σ of noisy insertion. Numbers of events: driver (the same in all 4 cases): 1038 (cf. Fig. 2), driven units: 1150 (4a), 1097 (4b), 1130 (4c) and 1210 (4d). Further details as in Fig. 3.

correlation functions for different values of α are given in Fig. 5. The distribution of ξs in these cases was a shifted delta-peak: every inserted silent interval had a fixed length of 4 ms. The extent of this interval is indicated by the horizontal bar along the time axis in the figures.

From these results we observe that an unambiguous sign of inhibition, i.e. a trough at the appropriate position in the otherwise flat cross-correlogram, now is only found in the extreme case of $\alpha = -1$ (Fig. 5d), where *every* incoming event manages to silence the follower unit for the prescribed period of time. Even

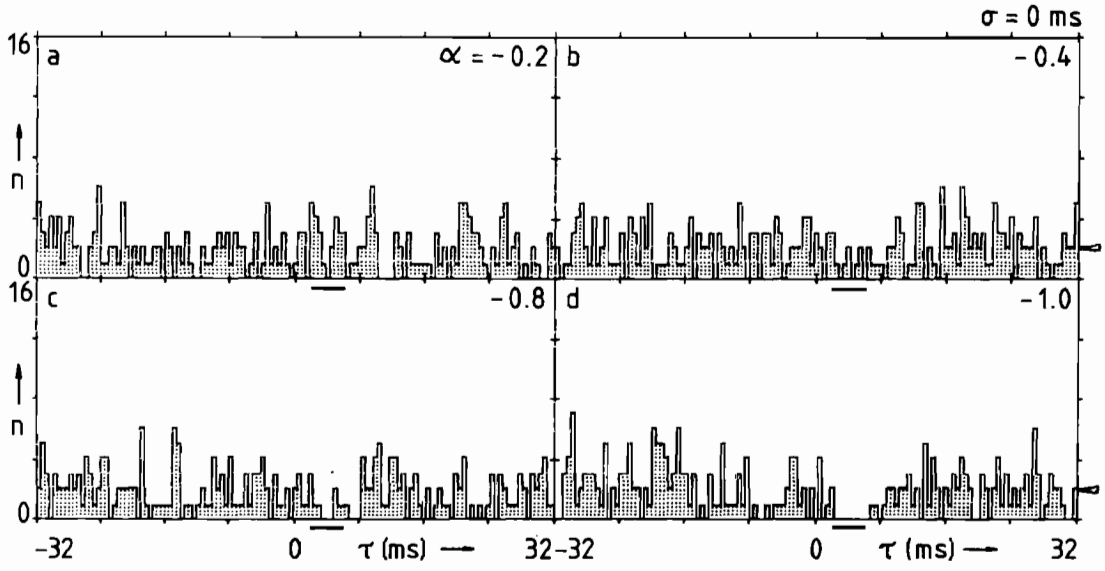

Fig. 5. Cross-correlation analysis of pairs of units with inhibitory connection of variable strength α and constant inserted silence μ. Numbers of events: driver (the same in all 4 cases): 1026, driven units: 1031 (5a), 1003 (5b), 1038 (5c) and 1012 (5d). Extent of inserted silence is indicated by bar along time axis. Further details as in Fig. 3.

in the case of $\alpha = -0.8$ (Fig. 5c), although there is some sign of a trough, it barely manages to escape the variance. Certainly for lower values of $|\alpha|$ (Fig. 5a, b), the inhibition is not reflected by any significant feature in the correlogram, while, on the other hand, valleys that are present (e.g. the trough in Fig. 5a at about $\tau = 12$ ms) are merely due to statistical variation. It should be noted, moreover, that this lack of clear signs of inhibition was obtained despite the use of α-values which are well beyond the range needed for unequivocal demonstration of excitation (cf. Fig. 3).

The major factor determining the bad visibility of inhibition is the variance of the cross-correlogram. In order to reduce this variance appreciably we would have to increase the number of coincidences per bin by an order of magnitude ($\sqrt{N}$-behaviour). One way to obtain this would be to increase the binwidth from the present value of 0.5 ms, e.g. by smoothing. Although something surely could be gained here, an order of magnitude is out of the question if we want to be able to make any sensible statements regarding the time course of the inhibition. The other possibility then lies in increasing the numbers of events involved. Since we do want to continue working with a fairly typical cortical rate of about 4 s^{-1}, this necessarily means increasing the 'recording' time. Fig. 6 shows the results of cross-correlation analysis of event sequences containing between 16000 and 17000 events, all parameters being identical to the ones in Fig. 5. Translated to an actual experiment this would correspond to a recording of well over one hour (4096 s), as compared to the roughly 4 min (256 s) necessary to obtain the data for Fig. 5. One observes that now, at least for $\alpha = -0.8$ (Fig. 6c) and $\alpha = -1$ (Fig. 6d), the correlogram shows the theoretically predicted square well. It may be noted that the well extends some 2.5 ms beyond the width (4 ms) of the inserted silences, as indicated by the horizontal bars. This is caused by the combined effect of (a) the complete reset of the event generating mechanism after insertion of a silence and (b) the minimum interval (in our case 2.5 ms) in the event generator (see Methods). Even with this 4-fold decrease in variance, however, the correlograms for $\alpha = -0.2$ (Fig. 6a) and even for $\alpha = -0.4$ (Fig. 6b) only reluctantly show the sign of inhibition and certainly its time course is not convincingly portrayed by the shape of the well.

2. *Visibility of connections in the cross-correlogram*

The foregoing results demonstrate a remarkable asymmetry in the sensitivity of the cross-correlation function for excitatory vs inhibitory connections.

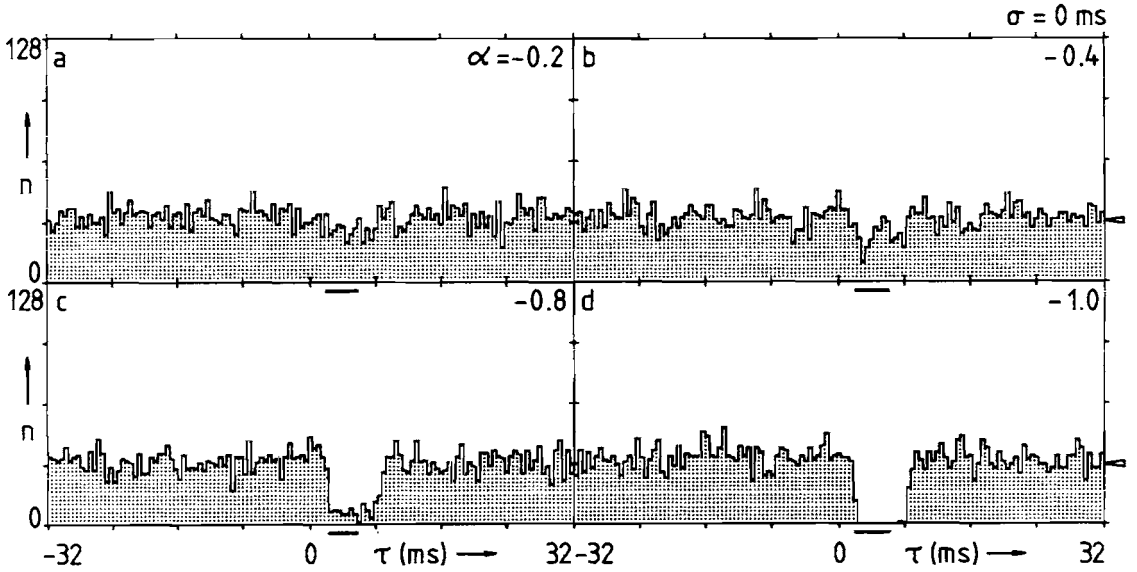

Fig. 6. Cross-correlation analysis of the inhibitory pairs from Fig. 5 with increased observation interval: 4096 s. Numbers of events: driver (the same in all 4 cases): 16,974, driven units: 16,856 (6a), 16,561 (6b), 16,284 (6c) and 16,117 (6d). Expected number of coincidences for uncorrelated trains now equals 32. Further details as in Fig. 3.

This is more or less paralleled by an equally remarkable silence in the experimental literature on intracortical inhibitory connections as revealed by cross-correlation analysis of simultaneously recorded spike trains (with the notable exception of the visual cortex work by Toyama et al.[36,37] to which we will return later). This relative silence is all the more remarkable in view of the abundantly present signs of inhibition as revealed by intracellular recordings both in the auditory[31] and visual[4,35] cortex. We therefore have investigated the theoretically expected sensitivity of cross-correlation, using the present model as a convenient starting-point.

For a uniform distribution of insertions following a driver event, the theoretically expected cross-correlation functions for excitation and inhibition are respectively shown in Figs. 7a and 7b. Quantitative expressions for the background level e and the departure from background, i.e. the signature of the connection, are given in the Appendix. The 'visibility' of the connection is determined by the magnitude of the peak or trough relative to the variance s^2 in the background. If we assume that the event sequences are roughly Poisson, the noise s can be approximated by

$$s = \sqrt{e} = (\varrho_1 \varrho_2' T \Delta)^{1/2} \tag{1}$$

with ϱ_1 and ϱ_2' the average rates of the pre- and postsynaptic trains (ϱ_2' as distinguished from the hypothetical 'unconnected' rate ϱ_2), Δ the binwidth of the correlogram and T the duration of the recording. The binwidth is determined by the time constants of the peak or trough to be investigated: for proper sampling of its time course some minimum number of bins is required (5–10) which sets an upper limit to Δ.

For relatively narrow peaks and wells (narrow with respect to the average interval in the presynaptic train) the amplitudes d_{exc} and d_{inh} of the departure are given by (see Appendix)

$$d_{exc} = \frac{\alpha}{\sigma \varrho_2'} \cdot e \tag{2}$$

and

$$d_{inh} = \alpha \cdot e \tag{3}$$

with α the synaptic strength and σ the width of the distribution of inserted events (cf. Fig. 1a). It is observed that for inhibition the magnitude of the departure relative to the background is equal to the strength of the connection α, whereas for excitation it involves an additional gain factor $(\sigma \varrho_2')^{-1}$ which in most cases will be substantially larger than 1. This discrepancy between (2) and (3) lies at the very heart of the asymmetry in visibility of excitation and inhibition using cross-correlation analysis.

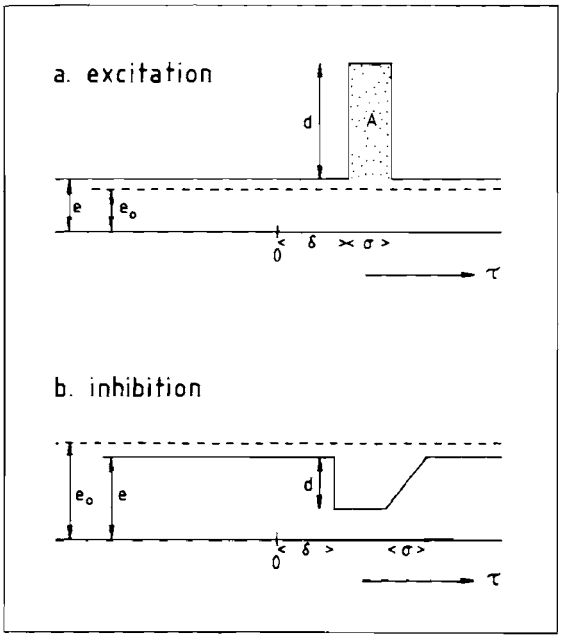

Fig. 7. Theoretically expected cross-correlogram for excitation (7a) and inhibition (7b) for a model using uniform distribution of insertions (cf. Fig. 1). Further explanation in text.

In order to quantify the difference in visibility we may define a *criterion for detectability*: in order for the connection to be noticed we require

$$|d| \geq 2s \tag{4}$$

Like any criterion this one is somewhat arbitrary; numerically it approximates the 0.10-significance limit for PST-histograms given by Dörrscheidt[7]. It leads to the following expressions for the lower limit of $|\alpha|$:

$$\text{excitation:} \quad \alpha_{min}^2 = \frac{4 \sigma^2 \varrho_2'}{\varrho_1 T \Delta} \tag{5}$$

$$\text{inhibition:} \quad \alpha_{min}^2 = \frac{4}{\varrho_1 \varrho_2' T \Delta} \tag{6}$$

The threshold conditions (5) and (6) are shown graphically in Fig. 8a for excitation and Fig. 8b for

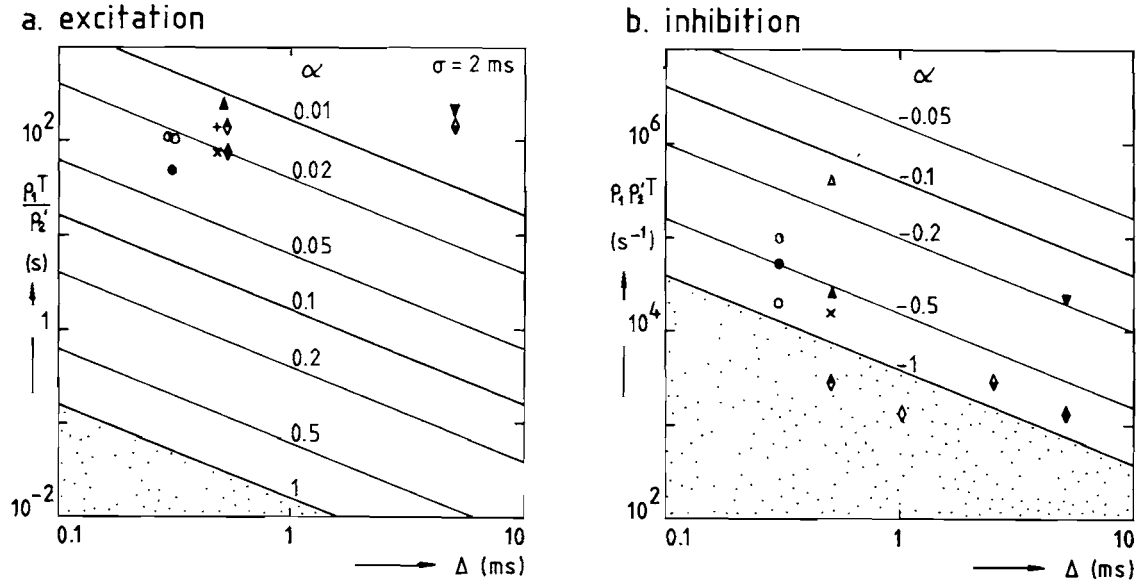

Fig. 8. Thresholds for detectability of connection with varying strength α for excitation (8a) and inhibition (8b), based on Eqns. (5) and (6). Horizontal axis indicates binwidth of correlogram, vertical axis gives combination of pre- and postsynaptic rates and duration of recording; both axes are on log scale. Symbols in the graphs represent data from this paper and from findings in the literature:
Fig. 8a: ▲ = Fig. 3, present paper; ▼ = Fig. 3a in Perkel et al.[26]; ♦, ◊, ◊ = Figs. 4 and 7A, B in Dickson and Gerstein[6]; ×,+ = Fig. 1A, B in Michalski[24]; ○,◑,● = Fig. 5D, E, F in Toyama et al.[36].
Fig. 8b: ▲ = Fig. 5, present paper; △ = Fig. 6, present paper; ▼ = Fig. 3b in Perkel et al.[26]; ♦,◊,◊,♦= Figs. 5A, B, C, D in Dickson and Gerstein[6]; × = Fig. 1c in Michalski et al.[24]; ○,◑,● = Figs. 5D, E, G in Toyama et al.[36].

inhibition. In both graphs the horizontal axis denotes the binwidth Δ, a parameter in the analysis procedure. The vertical axis is a combined measure of the experimental variables ϱ_1,ϱ_2' and T: for excitation we have $\frac{\varrho_1 T}{\varrho_2'}$, for inhibition it is $\varrho_1\varrho_2' T$. This difference again reflects the different forms of (5) and (6). Note that the measure in the case of excitation is asymmetric with respect to which neuron is pre- and which one is postsynaptic. The threshold for excitation still contains the peakwidth σ; in order to conform to the value used in the simulations we set this equal to 2 ms. The value of the synaptic strength α is a parameter in the different graphs, with the area underneath the graph for $|\alpha|=1$ as obviously 'forbidden zone'. Fig. 8 thus can be read as a *recipe*: given the binwidth required for resolving the time course and given the strength of the connection one wants to be able to pick up, it gives an estimate of the minimum amount of experimental data which is required. Or: given the binwidth and the experimental amount of data, it predicts the minimal strength of connection to be discovered. We have indicated the locations corresponding to Figs. 3 (▲ in Fig. 8a), 5 (▲ in Fig. 8b) and 6 (△ in Fig. 8b). It can be observed that indeed there is a fair agreement between the predicted sensitivity and the actual results of cross-correlation in these cases. Furthermore, Fig. 8 explains clearly the impressive difference in sensitivity of cross-correlation for excitation vs inhibition.

Another interesting observation can be made regarding Eqns. (2) and (3). Relation (2) can be written to yield

$$\alpha = \frac{d\,\frac{\sigma}{\Delta}}{\varrho_1 T} = \frac{A/\Delta}{\varrho_1 T} = \frac{N_c}{N_1} \qquad (7)$$

Eqn. (7) simply states that the synaptic strength of an excitatory connection is given by the ratio of the number of correlated events N_c (i.e. the events in the area A of the peak in the correlogram, cf. Fig. 7a) and the number of *pre*synaptic events. This is precisely the quantity that was proposed by Levick et al.[20] as a measure for the '*effectiveness*' or 'dynamic potency of the particular excitatory synapse'. The asymmetry of excitation and inhibition thus is expressed in the difference between (3) and (7): inhibitory strength equals the *ratio* of *ordinate values* inside and

outside the trough while excitatory strength is proportional to the *area* under the peak. Measures involving areas of inhibitory correlograms[36,37] do not so much address the strength of the connection, but rather a combination of the strength and its time course. There is no obvious relation between our model parameters and another measure proposed by Levick et al.[20] the '*contribution*', defined as the ratio $\frac{N_c}{N_2}$ of the number of correlated events relative to the number of *post*synaptic events. It is curious to note that the latter measure has been used in many experimental investigations of connectivity[24,34,36,37] while the former has only rarely[21] been considered. From a theoretical point of view we would expect more detailed information regarding the underlying mechanism of connectivity to result from measuring the strength of the connection using (7), including its possible dependence on stimuli, context, learning[17] and the like. The 'effectiveness' is a detailed measure of a particular synapses' strength, whereas the 'contribution' is a more global measure, which, through N_2, is also affected by the effective strengths of contributions from other projecting neurons, which in turn may be variable, each one in its own way.

DISCUSSION

The simulator

A model was described for simulating spike trains, generated by a population of neurons. The neurons can be chosen to be either independent or interconnected, each connection specified by its strength and time constants. The model essentially is a functional or black box-type model, operating in terms of stochastic point processes with prescribed probability density functions. No attempt was made to incorporate the biophysical mechanisms underlying the generation of action potentials; emphasis was on simplicity and versatility in producing spike trains rather than on microscopic reality. The main purpose of the model is to generate event sequences which can be used as reliable and instructive data for multi-unit analysis procedures, currently being developed[15]. In order for these data to be representative of real neural activity, the stochastics of event generation and synaptic connectivity were modeled on the basis of experimental findings from single- and multi-unit recordings available in the literature.

The mechanism of excitation in the model, i.e. the insertion of an event followed by a reset of the event generator, can also be viewed as a local contraction of time: at certain moments, determined by the driver events, an otherwise unaffected follower train is advanced in time to the point of insertion. A similar arrangement for the inhibition, i.e. local stretching of time, would be to insert a silence into an ongoing interval, without selecting a fresh interval at the end of the silence, thereby effectively retarding the remainder of the follower train. This would correspond to a temporary 'hold' of the generator potential, instead of the current combination of 'reset' and 'hold'. The main reason for the latter choice was that it provides a 'stronger' version of inhibition which should be more readily detectable in statistical analysis of spike trains. The present implementation of inhibition is certainly unrealistic in its deterministic temporal coupling with the driver. Again, this choice was made to obtain the highest degree of visibility of inhibition in the cross-correlogram. Any additional noise in time locking, although certainly more realistic, would have a dispersive effect on the inhibitory trough, rendering it even less conspicuous than it is now.

The basic activity of the units was modelled as a stationary renewal process. More realistic performance can certainly be obtained by relaxing this restriction and adopting certain forms of non-stationarities, e.g. modulation of rate and departures from renewal. This type of temporal manipulation of the event generators could also be used to model 'external' influences on (parts of) the network as exerted for instance, by sensory stimulation of the periphery projecting to the network or global modulation due to efferent projections. The networks considered so far are autonomous, they behave as isolated chunks of brain tissue, not involved in sensory processing of any sort. The incorporation of stimulus-related non-stationarities evidently is among the most necessary and interesting extensions.

Detection of excitation and inhibition

The commonly used framework for investigating connectivity in a neural network is the cross-correlation function of simultaneously recorded spike trains[12,26]. The presence of significant departures

from a flat background is interpreted as indicative of a functional connection, either direct (through one or more synapses) or indirect (shared input, either neural or stimulus-induced). In the present discussion the second possibility can be disregarded since we did not study this form of 'connectivity'. The statistical analysis of spike trains generated by the model, apart from serving as a check for its performance, led us to observe a peculiar asymmetry in the sensitivity of cross-correlation functions for the presence of excitatory vs inhibitory connections. We were able to demonstrate, on the basis of a theoretical analysis of the relation between model parameters and correlogram shape, that indeed the cross-correlation is distinctly less sensitive for inhibition than it is for excitation of comparable strength. Results of this analysis were summarized in a set of threshold curves (Fig. 8) representing the relation between (a) the strength of the connection (system parameter), (b) the binwidth of the correlogram (analysis parameter) and (c) a combination of pre- and postsynaptic rates and the duration of the recording (experiment parameters). Thereby it appeared, apart from the noted asymmetry in sensitivity, that the experimental parameters have to be treated differently for excitation and inhibition: for inhibition we have the product of the rates, for excitation it is the ratio of pre- and postsynaptic rates, the latter combination thus being asymmetric with respect to the order of the units involved.

We have indicated in Fig. 8 the locations corresponding to a number of analyses of connectivity as they appeared in the literature, both from model studies[26] and multi-unit recordings from the cortex[6,24,36]. It appears that for excitation a sensitivity for connections with a strength larger than 0.02–0.05 is fairly commonly obtained. The situation is considerably worse for inhibition: with the exception of part of Toyama's[36] data, inhibition with a strength of less than 0.8 will have failed to be detected in the experimental papers mentioned. The increased sensitivity in Toyama's results is caused by the deliberate enhancement of the firing rates by either visual or chemical (glutamate) stimulation: Even then the sensitivity is not changed by more than a factor of about 2–3: inhibition with a strength of less than 0.2–0.3 will almost surely remain undiscovered. Still the increased proportion of inhibitory connections in Toyama's results compared to other authors' results is remarkable. The approach of going to considerably longer recording times (cf. Fig. 6: approximately one hour) in many cases will be problematic because of variability in either the recording configuration or the network itself[16,23].

Although the criteria for sensitivity as illustrated in Fig. 8 were derived for stationary Poisson sequences, we expect the overall conclusions to be fairly robust for temporal modulations as caused, for instance, by stimulation. Certainly in the case of very strong, almost on–off modulation as present in many experiments using repetitive tone bursts, light flashes and the like, we expect these results to provide a reasonable first order approximation, provided that the effective rates and the relevant recording time over the integrated 'burst'-sequences are used: the remainder of the recording will not contribute substantially to the overall correlogram. More modest and/or complex modulations like the ones caused by continuous stimulation using long and complex stimulus ensembles[9] will probably have a more complicated effect and certainly should be studied further. The derivations of the relations underlying Fig. 8 also were based on the assumption of uniform distributions of insertions; changing the shape of these distributions will affect the final results only marginally.

Intracellular recordings indicate that 'the amount of inhibitory input to cortical neurons is impressive'[4,31,35]. It is thought by some authors that 'the intracortical network is essentially, if not exclusively, inhibitory'[5], with the intracortical inhibition shaping the response characteristics of cortical cells[2]. Cross-correlation analysis of spike trains has so far failed to substantiate this hypothesis. This raises the question whether the relatively low proportion of inhibitory connections detected by cross-correlation of cortical unit activity may simply reflect the disproportionate sensitivity of the analysis tools. Such disproportion would surely distort true connectivity if there is a broad distribution of (inhibitory) α; if the αs are indeed greater than 0.8, no distortion occurs.

Another question concerns the information processing aspects: if cross-correlation is so insensitive to temporal alterations of spike trains produced by inhibition per se, it is not obvious how a neuron would be able to resolve them. In other words: is the principal effect of inhibition to modulate incoming excitation[30] rather than directly to produce silences in the

outgoing spike train. The investigation of such mechanisms surely will require true multi-unit analysis, i.e. going beyond pair analysis as is customary now. Modulation of incoming excitation might also provide an explanation for the observed stimulus-dependence of neural correlations[8,11]; these consist mainly of stimulus-related changes in correlogram peaks. Measures for interaction strength like 'effectiveness' are probably more suited to describe these phenomena than the 'contribution' measure used in most investigations, the latter incorporating also the unknown, possibly varying contributions from unobserved sources.

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APPENDIX

Relation between model parameters and cross-correlogram

The cross-correlation function of spike trains from two independent units with average firing rates ϱ_1 and ϱ_2 is, apart from statistical fluctuations, a constant e_o, the value of which is given by

$$e_o = \varrho_1 \varrho_2 T \Delta \tag{A1}$$

with T the observation interval and Δ the binwidth of the correlogram. The effect on the cross-correlogram of making a connection, either excitatory or inhibitory, between two such units, whereby unit 1 becomes the driver (presynaptic) and unit 2 the driven (postsynaptic), is 2-fold: (1) Due to the overall increase or decrease in the total number of events in the postsynaptic train, the expected number of coincidences in the background level will increase or decrease from its 'unconnected' level e_o to a new level e. (2) Over a certain interval following a driver event there will be a positive or a negative departure from the background-level e, the location, shape and size of which is determined by the distribution of inserted events or silences and the synaptic strength α (cf. Fig. 1).

The theoretically expected cross-correlation functions for an excitatory and an inhibitory connection with uniform distribution of inserted events or silences, are shown in Fig. 7. In the following we will express these expected correlograms in terms of the 'unconnected' rates of the participating units (ϱ_1 and ϱ_2), the connectivity parameters (strength α, average μ and width σ of insertion distribution), the observation interval T and binwidth Δ. We will assume Poisson characteristics for the unconnected event generators and uniform distributions for the insertions. Only the main results will be given, proofs are rather lengthy but straightforward.

Excitation

The expected number of events N_2' in the postsynaptic train during an observation interval T is given by

$$N_2' = N_2 + \alpha N_1 \tag{A2}$$

with N_1 and N_2 the numbers for the unconnected pre- and postsynaptic trains. This implies that for the background level e in the cross-correlogram we obtain

$$e = \varrho_1 (\varrho_2 + \alpha \varrho_1) T \Delta \tag{A3a}$$

or

$$e = \left(1 + \alpha \frac{\varrho_1}{\varrho_2}\right) \cdot e_o \tag{A3b}$$

The departure from background (cf. Fig. 7a) simply reflects the distribution of inserted events, in our case a uniform distribution over the interval $[0,\sigma]$, shifted by the delay δ (cf. Fig. 1a). The width of the elevation consequently is σ, its amplitude d is given by

$$d = \alpha \varrho_1 \left(\frac{1}{\sigma} - \varrho_1\right) T \Delta \tag{A4a}$$

or

$$d = \alpha \frac{\varrho_1}{\varrho_2} \left(\frac{1}{\sigma \varrho_1} - 1\right) \cdot e_o \tag{A4b}$$

Note that (A4) only holds if the width σ of the inserted distribution is smaller than the average interval in the presynaptic train, which is not unrealistic for actual spike trains. Furthermore it has been assumed that the observation grid is considerable smaller than both δ and σ, otherwise we get a blurring of the correlation shape due to undersampling.

As a measure for 'strength' or 'visibility' of the excitatory connection as exhibited in the shape of the cross-correlogram we propose the amplitude of the departure from background relative to the background, in other words, the ratio $\frac{d}{e}$. This definition is analogous to such notions as 'modulation depth' and 'visual contrast'. Using (A3,4) we obtain

$$\frac{d}{e} = \alpha \cdot \frac{\frac{1}{\sigma} - \varrho_1}{\varrho_2 + \alpha \varrho_1} \tag{A5a}$$

or, in terms of the average rates of the connected pre- and postsynaptic units

$$\frac{d}{e} = \alpha \cdot \frac{\frac{1}{\sigma} - \varrho_1}{\varrho_2'} \tag{A5b}$$

For narrow peaks ($\sigma \varrho_1 \ll 1$) this reduces to

$$\frac{d}{e} \simeq \alpha \cdot \frac{1}{\sigma \varrho_2'} \tag{A6a}$$

or

$$\frac{d}{e} \simeq \alpha \cdot \frac{\mu_2'}{\sigma} \tag{A6b}$$

with μ_2' the average interval of the postsynaptic train. We observe from (A5,6) that the 'contrast' in the correlogram is proportional to α, with a gain factor which (for narrow peaks) equals the ratio of average postsynaptic interval and peak width.

Inhibition

Analogous reasoning can be applied to inhibitory connections ($\alpha < 0$). For the number N_2' in the postsynaptic train we now obtain

$$N_2' = (1 + \alpha\mu\varrho_1) N_2 \tag{A7}$$

with μ the average inserted silent interval. This leads to a new, lower background level e given by

$$e = \varrho_1\varrho_2(1 + \alpha\mu\varrho_1) T\Delta \tag{A8a}$$

or

$$e = (1 + \alpha\mu\varrho_1) e_o \tag{A8b}$$

Note that it is assumed that $(1 + \alpha\mu\varrho_1) > 0$, otherwise no events are left in the postsynaptic train. The inhibition causes a trough in the cross-correlogram, the shape of which reflects the integrated version of the distribution of silent intervals. This is shown in Fig. 7b for the case of a uniform distribution over the range $[\mu-\frac{\sigma}{2}, \mu+\frac{\sigma}{2}]$.

Due to inhibition the number of coincidences in the trough drops to the μ-independent level of $(1 + \alpha)e_o$, provided that $\mu > 0$. Therefore the depth d of the well is given by

$$d = \alpha(1-\mu\varrho_1)\cdot e_o \tag{A9}$$

For the 'contrast' in the correlogram we thus obtain

$$\frac{d}{e} = \alpha \cdot \frac{1-\mu\varrho_1}{1+\alpha\mu\varrho_1} \tag{A10a}$$

or, in terms of intervals

$$\frac{d}{e} = \alpha \cdot \frac{1-\frac{\mu}{\mu_1}}{1+\alpha\frac{\mu}{\mu_1}} \tag{A10b}$$

Note that both the depth d and the contrast now are independent of the postsynaptic rate ϱ_2. For narrow troughs ($\mu\varrho_1 \ll 1$) this reduces to

$$\frac{d}{e} \simeq \alpha \tag{A11}$$

where, as in the case for excitation, the contrast is proportional to α, now, however, without an additional gain factor.

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