A method to estimate the effects of parallel inputs on neuronal discharge probability

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Abstract. We here present a method to study the interaction of parallel neural input channels regarding their effects on a neurone. In particular, the method allows to disclose the effects of oligosynaptic pathways that may exist in parallel to direct monosynaptic connections to the cell. Two (or more) inputs (nerves) are stimulated with random patterns of stimuli. The response of the cell to these patterns is evaluated by the computation of peristimulus-time histograms (PSTHs). One of the two stimulus trains is selected as the one to yield reference events for the PSTH computation. From this stimulus train are selected those stimuli as reference events which are preceded, at defined mean intervals, by stimuli in the same or a parallel channel. These “conditioning” stimuli are determined (1) separately from each single stimulus train and (2) concomitantly from the two trains as events occurring simultaneously in both. The effects exerted by these various conditioning events on the effects of the “test” pulses on the cell response yield insights into the interactions between the two (or more) inputs. These methods are demonstrated on spinal Renshaw cells activated by independent random stimulation of two muscle nerves and on dorsal horn neurones responding to cutaneous nerve stimulation.

Key words: Random inputs — Cross-correlation — Nonlinear analysis — Synchronous inputs — Renshaw cells — Dorsal horn neurones

Introduction

In the nervous system, the connection between two neurones may be made via several parallel paths involving different numbers of synapses. For example, one connection could be monosynaptic whilst a parallel one is oligosynaptic (see Fig. 1). It has been suggested that this multiplicity in conjunction with near-synchronous firing in the parallel channels and short-term synaptic modulation plays a role in the rapid build-up of functional cell assemblies (von der Malsburg 1985). More generally, the propagation of excitation or inhibition through more or less long parallel chains of neurones is certainly promoted by synchronous firing on two parallel neural elements which commonly impinge on

one or more subsequent neurones (Abeles 1982; Windhorst 1988).

In the neuromuscular system, the physiological importance of the tendency towards synchronous firing of parallel elements has always been recognized because it may lead to increased muscle tremor (review: Windhorst 1988). For the fine control of posture and movement, this tendency needs to be counterbalanced by intricate mechanisms which until today are little known, however. The way in which components of the motor system handle synchronous firing needs study before understanding and — eventually — cure of disordered tremor can be achieved.

The study of the operation of oligosynaptic connections existing in parallel to monosynaptic ones is difficult. Often use has been made of spatial summation; that is, parallel inputs are activated synchronously to activate commonly excited interneurons which then exert a stronger action on a postsynaptic neurone than activation of one input alone. This technique has been quite successful in disclosing convergence of various input systems on interneurons intercalated in oligosynaptic spinal reflex pathways to motoneurons and other cells (for a review see Baldissera et al.

Fig. 1. Schematic outline of the problem. The output (*O*), here a spike train, of a neurone *c* is recorded in response to parallel random stimulation of (at least) two inputs *I*₁ and *I*₂ (the stimulus trains are symbolized above and below *I*₁ and *I*₂, respectively). These inputs may have monosynaptic excitatory as well as oligosynaptic excitatory and/or pre- and postsynaptic inhibitory effects (the latter via the commonly contacted neurones *a* and *b*). The dashed lines symbolize the open nature of the connections from the neurones *a* and *b* to *c*. If the input *I*₁ and *I*₂ themselves have no excitatory effects on neurone *c*, a third input *I*₃ that has may be substituted as an input yielding test events (see below)

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1981). A disadvantage of this technique as used so far is that the stimuli are usually applied at an unphysiologically low rate so that the whole system is relatively quiet before being thrown into action by each single stimulus. Using higher mean rates poses evaluation problems. However, such an approach will be suggested here using random stimulation of two input channels at more physiological mean rates.

To demonstrate the methods, we have first chosen a relatively simple system for experimental investigation: the connections from motor axons to spinal Renshaw cells. These cells receive their main excitatory input from recurrent axon collaterals synapsing monosynaptically on them. A disynaptic inhibitory connection may be established via other Renshaw cells (mutual inhibition: Ryall 1970, 1981). It is very appealing to believe that this indirect route from two groups of motor axons to a Renshaw cell is opened particularly easily by synchronous impulses in the two input channels. An example from dorsal horn neurones excited by cutaneous suralis input will also be demonstrated.

Materials and methods

Experimental methods. The experimental methods have been described in detail elsewhere (Windhorst et al. 1987; Christakos et al. 1987; Laouris et al. 1988). Briefly, adult cats were anesthetized initially by i.p. injection of pentobarbitone (40 mg/kg). Smaller doses were regularly administered i.v. during the experiment. The cats were spinalized at the L_1/L_2 level. Extracellular recordings were made from Renshaw cells in L_7/S_1 segments. They were activated by random stimulation of two (or three) parallel inputs to the cell. That is, two (or sometimes three) nerves or nerve branches were stimulated concurrently, but with independent random sequences of similar mean rate. Each of these stimulus patterns consisted of brief (0.05 or 0.1 ms) rectangular electrical pulses which occurred randomly like in an atomic decay process, but inter-pulse intervals of less than 5 ms were excluded (thus: "pseudo-Poisson"). Two levels of mean rate were applied: 9.5–13 pulses/s (pps) referred to as "low"; and 20–23 pps referred to as "high". Such a random stimulus pattern has a flat autocorrelogram except at τ 's smaller than 5 ms (for examples see Fig. 3, lower row) and a flat power spectrum (up to 200 Hz corresponding to the inverse of the minimal interval). It is most appropriate for system identification, whether linear or nonlinear (Mannard and Stein 1973; Krausz 1975; Marmarelis and Marmarelis 1978; Windhorst et al. 1983). The stimulus strength for each nerve was set to about twice the value necessary to yield a maximal cell response.

Data analysis. The analysis performed on two input and one output event trains consisted of the computation of two types of peristimulus-time histogram (PSTH), i.e., the cross-correlation of the cell discharges with respect to all stimuli in each input channel or to selected subsets of stimuli (Perkel et al. 1967; Windhorst 1988). The selection of the latter was performed by a computer as follows. The upper two traces in Fig. 2 show the two stimulus patterns labelled S_1 and S_2 . In the case illustrated, S_1 yielded the reference events for the computation of PSTHs. Conditioned (test) stimuli were selected from S_1 and written on reference channels R_1 through R_3 (arrows), if they obeyed the following conditions. Stimuli from S_1 were written on R_1 if they were preceded by stimuli in S_1 (but not S_2) within time windows

Fig. 2. Scheme illustrating the selection of conditioned stimuli. Two parallel stimulus patterns (labelled S_1 and S_2) are used to activate two inputs as in Fig. 1. The patterns are independent of each other and "pseudo-Poisson". That is, the stimuli occur randomly like in an atomic decay process at a selected mean rate; short interpulse intervals below 5 ms are excluded. One of the stimulus trains (here S_1) is chosen to yield reference events for the computation of conditional peristimulus-time histograms (PSTHs). That is, from this train certain stimuli are selected and written on a reference channel which are preceded by stimuli occurring within a time window of 5 ms width at an interval δ before. On channel R_1 are collected those stimuli which are preceded by stimuli in the same channel, i.e., S_1 (auto-conditioning); on channel R_2 those preceded by stimuli in S_2 (cross-conditioning) and on channel R_3 those preceded by stimuli occurring nearly synchronously (within the same time window) in both stimulus trains (simultaneous conditioning)

of 5 ms width, whose centre was located δ ms before ("auto-conditioning"). Stimuli from S_1 were written on R_2 if they were preceded by stimuli in S_2 (but not S_1) within a time window of relative location δ ("cross-conditioning"). And finally, stimuli from S_1 were written on R_3 if they were preceded by stimuli in both S_1 and S_2 within the respective time window ("simultaneous conditioning"). Note that in the first two cases (auto- and cross-conditioning), test stimuli conditioned by simultaneous stimuli were excluded. It is with respect to the five different pulse trains shown in Fig. 2 that the different kinds of PSTH were computed. The first type of PSTH computed is "conventional" in that it was accumulated with respect to all the successive stimuli in one or the other of the two original stimulus trains (S_1 or S_2 ; see Perkel et al. 1967; Windhorst 1988). The other type of "conditional" PSTH was computed with respect to the conditioned (test) stimuli in traces R_1 , R_2 or R_3 and will be referred to as "auto-conditional", "cross-conditional" or "simultaneously conditional", respectively. The bin width for the construction of the PSTHs was always 1 ms.

Results

In the following, the method is demonstrated on results from a single Renshaw cell. More examples will be demonstrated later.

Conventional PSTHs

Figure 3 shows responses of a Renshaw cell activated by random stimulation of the medial gastrocnemius (MG) and the lateral gastrocnemius-soleus (LGS) nerves at mean

Fig. 3. Response of a Renshaw cell to parallel random stimulation of two nerves. The medial gastrocnemius (MG) nerve was stimulated at a mean rate of 12.6 pulses/s (pps) and the lateral gastrocnemius-soleus (LGS) nerve at a mean rate of 9.6 pps. The *top traces* show a 250 ms section of sampled data, with S_1 (MG) and S_2 (LGS) denoting the stimulus trains and RC the spike train. The two *columns* labelled *MG* (*left*) and *LGS* (*right*) show the auto-correlograms of the S_1 (*C*) and S_2 (*F*) stimulus patterns and the respective average cell responses in the form of conventional PSTHs (computed with respect to all the stimuli in each train). Parts *A* and *D* exhibit the entire PSTHs; parts *B* and *E* display enlarged and truncated versions of the PSTHs in *A* and *D*, respectively (note the different ordinate scale). In *B* and *E* the *horizontal line* indicates the mean discharge probability of the cell. The *blackened area* labelled $\bar{a}$ in part *A* comprises the excitatory cell response exceeding the mean discharge probability (henceforth called average Renshaw cell response, here to MG stimulation) and is in the following used as the reference measure for comparison with conditioned responses. It is akin to a firing index. The term *relative frequency* on the ordinate denotes the normalized number of counts (Renshaw cell spikes) per bin (width 1 ms), i.e., the absolute number divided by the number of reference events used to accumulate the PSTH. This measure thus represents an estimate of the probability of Renshaw cell firing (within a bin width) as a function of the time τ from the reference pulses (abscissa). Number of reference events: *A*, 8768; *B*, 6705. Mean cell discharge rate: 33.4 pps

stimulus rates of 12.6 and 9.6 pulses/s (pps), respectively. A 250 ms section of the stimulus and spike sequence is illustrated on top. The bottom row (*C* and *F*) shows the autocorrelograms of the stimulus patterns used to stimulate the respective nerves. They are flat to within random fluctuations except at τ 's smaller than 5 ms, where no stimuli occurred. Parts *A* and *D* show conventional PSTHs calcu-

lated with respect to stimulus train S_1 (MG) and S_2 (LGS), respectively. The flat though noisy line left to the time origin ($\tau \leq -5$ ms) reflects the mean discharge probability. This mean probability is the mean discharge rate (in pps) expressed per ms (see legend). Right to the time origin ($\tau = 0$ ms), at which the test stimuli occurred, this level is exceeded by PSTH peaks which represent the initial excitatory component. In part *A* this response component is enhanced by the black area (labelled $\bar{a}$); it is henceforth called "average Renshaw cell response", $\bar{a}$. It represents the excess number of discharge contributed on average by each stimulus (thus being dimensionless) and will later be used as a quantitative measure. This peak of variable amplitude and duration is usually followed by a small but longer-lasting postexcitatory reduction of firing rate. This reduction is better seen at higher magnification of the PSTH around the mean firing probability as illustrated in Fig. 3 *B* and *E*. Most likely this rate reduction results from postsynaptic excitability changes following a response, among which the summation of afterhyperpolarization stands out (Hultborn and Pierrot-Deseilligny 1979; Walmsley and Tracey 1981).

Linear responses

In order to estimate nonlinear response components, a linear model has to be constructed as a hypothetical reference. This is done, for each conditioning situation, as follows.

Auto-conditioning. If the cell responded linearly to pairs of stimuli in the same train, its response to the test stimuli (occurring at $\tau = 0$ ms) should look like the sum of the conventional PSTH (Fig. 3 *A*) and the same PSTH shifted to the left by δ ms (after subtracting once the mean discharge probability to retain the same as before). In Fig. 4 *A*, such a theoretical linear response is exemplified for $\delta = 12.5$ ms. Note that the linear test response is slightly larger than the preceding conditioning response because the former is superimposed on the tail of the latter. If the test response occurs in the phase of firing rate reduction of the conditioning response (see Fig. 3 *B*), it is smaller.

Cross-conditioning. The linear response to a test stimulus in one channel (here S_1 : MG) conditioned by a stimulus in the parallel channel (here S_2 : LGS) is constructed equivalently to the previous case, but by replacing the conditioning response with the PSTH in Fig. 3 *D*. This response is exemplified for $\delta = 12.5$ ms in Fig. 4 *B*.

Simultaneous conditioning. The linear response to a test stimulus conditioned by near-simultaneous stimuli in both channels is constructed by combining the two procedures described in Fig. 4 *A* and *B*. This is illustrated in Fig. 4 *C*. Whereas this procedure yields the true linear response, another conditioning response should also be taken into account for the following reason. The conditioning response to near-simultaneous stimuli in both input channels needs not itself be a linear sum of the PSTHs resulting from stimuli in both channels. Spatial summation or occlusion may yield nonlinear conditioning responses to the near-synchronous input stimuli. So the aftereffects of this "actual" conditioning response may deviate more or less significantly from those of the theoretical linear one. Therefore, this actual conditioning response was determined by accumulating a PSTH with respect to those stimuli in the reference channel

Fig. 4. Demonstration of linear Renshaw cell responses. To estimate nonlinear response components, a linear reference (model) is needed which is constructed as follows. If the cell behaved linearly, the responses to two successive stimuli would superimpose. For instance, the response to two successive stimuli in the same input channel (here S_1 : auto-conditioning; compare Fig. 2, reference channel R_1) separated by $\delta = 12.5$ ms would look like in part *A*. That is, the response to a (conditioning) stimulus at $\tau = -12.5$ ms would be the average response of Fig. 3*A*, and the response to a second stimulus at $\tau = 0$ ms would again be like in Fig. 3*A*, but superimposed on the tail of the first (conditioning) response (after subtraction of the mean firing probability). Part *B* shows the equivalent procedure for a conditioning stimulus from the other channel (S_2 : cross-conditioning), where the conditioning response is the average cell response to LGS stimulation shown in Fig. 3*D*. Part *C*₁ shows the linear conditioning effect of synchronous stimuli in both channels, i.e., the conditioning response is the sum of the PSTHs in Fig. 3*A* and *D*. Since in fact this conditioning response is not necessarily the algebraical sum of the two separate responses (because of spatial summation or occlusion), another simultaneous control response was calculated by determining as conditioning response that which was actually obtained to near-synchronous stimuli (part *C*₂). That is, the conditioning response is that to stimuli in S_1 , which are accompanied by stimuli in S_2 from $\tau = -2.5$ to $\tau = +2.5$ ms. Same data as in Fig. 3

(here S_1), which were accompanied by stimuli in the parallel channel (here S_2) within a window from -2.5 to $+2.5$ ms. A conventional PSTH superimposed on this actual conditional PSTH shifted by $\delta = 12.5$ ms to the left is shown in Fig. 4 *C*₂.

Conditional PSTHs

Figure 5 shows conditional PSTHs computed with respect to the different kinds of conditioned stimuli (rows labelled R_1 through R_3 as in Fig. 2), for some selected values of δ (columns). Note that all the reference events for the computation of the PSTHs are from the same stimulus train, in this case from that used to stimulate the MG nerve (S_1). That is, the test responses to be conditioned are those to MG input. The cell responses to the conditioning stimuli are represented by the peaks occurring at negative τ 's, and the responses to the test stimuli occur briefly after $\tau = 0$ ms. Note the increasing interval between conditioning and conditioned (test) responses with increasing δ (from left to right). The effect of the preceding activation on the test responses is clearly seen. Its quantification is described below.

Conditioning curves

For quantification, the test responses in Figs. 4 and 5 (after $\tau = 0$ ms) were integrated in the same manner as the average cell response, $\bar{a}$, in Fig. 3*A*, for a wide range of δ 's (7.5–200 ms). This yielded linear $a(\delta)$ from data such as those exemplified in Fig. 4 and actual $a(\delta)$ from data such as those shown in Fig. 5, which were then normalized by division by $\bar{a}$. To display the time course of the conditioning effects, the ratios $a(\delta)/\bar{a}$ were plotted as a function of the conditioning-test interval δ , yielding the “ δ -curves” in Fig. 6. Solid curves represent the actual δ -curves, dots the linear δ -curves. Conforming to the structure of Fig. 4, Fig. 6*A* shows the auto-conditioning, part *B* the cross-conditioning effects etc., as indicated. It is the difference between the linear (dots) and the actual (solid line) δ -curves which express nonlinear effects. Hence, these differences were calculated and plotted

Fig. 5

Actual Renshaw cell responses to various conditioned stimuli. The rows show conditional PSTHs computed with respect to stimuli collected in reference channel R_1 of Fig. 2 (auto-conditioning, see label on the right side), to stimuli in channel R_2 of Fig. 2 (cross-conditioning) and to stimuli in channel R_3 of Fig. 2 (simultaneous conditioning). The three columns display different conditioning-test intervals as indicated on top. The left three PSTHs labelled *A–C* computed for $\delta = 12.5$ ms should be compared with the linear responses labelled correspondingly in Fig. 4. The actual response in *C* has two controls in Fig. 4, i.e., *C*₁ and *C*₂. Same data as in Figs. 3 and 4

Fig. 6

Normalized linear and nonlinear conditioning δ -curves. Conditioned cell responses as exemplified in Fig. 4 (linear controls) and Fig. 5 (actual responses) were determined for 42 values of δ varied between 7.5 and 200 ms and were quantified in the same way as the average Renshaw cell response, $\bar{a}$ (see black area in Fig. 3A). The resulting $a(\delta)$ were normalized by division by $\bar{a}$ and plotted vs. δ . The dots thus represent linear δ -curves, the solid lines actual δ -curves. The structure of the figure is the same as that of Fig. 4. That is, part A shows auto-conditioning, B cross-conditioning, and the two C parts show the different simultaneous controls as shown in the respective parts of Fig. 4, whereas the actual δ -curves are the same. Note that the difference between the related linear and actual δ -curves expresses the true degree of nonlinearity. All the curves were smoothed by sliding three-point averaging, i.e., if y_i denotes the i -th ordinate value prior to smoothing, its new value y'_i is $y'_i = 0.25 y_{i-1} + 0.5 y_i + 0.25 y_{i+1}$. Same data as in Figs 3–5

Fig. 7

Nonlinear δ -curves: differences between linear and actual δ -curves. The curves labelled a plot the difference between the linear and actual δ -curves in Fig. 6A, those labelled b plot the difference between linear and actual δ -curves in Fig. 6B and so forth. In part I are superimposed the nonlinear auto-conditioning (a), cross-conditioning (b) and simultaneous (c_1) conditioning δ -curves. In part II curves a and b were added algebraically assuming linear addition of the conditioning effects from the two input channels. In parts III and IV c_1 is replaced with c_2

in Fig. 7. In part I are superimposed the difference from Fig. 6A as curve a , that from Fig. 6B as curve b and that from Fig. 6C₁ as curve c_1 ; part III reproduces part I with c_1 replaced with c_2 .

Curve a in Fig. 7 I expresses pure depression. Curve b shows an early facilitation which is most probably due to spatial summation of rather long-lasting EPSPs in Renshaw cells (Eccles et al. 1961; Jankowska and Odutola 1980; Walmsley and Tracey 1981; Brink and Suzuki 1987). But after this (at greater δ 's), the curve fluctuates around zero, implying that the activation of the parallel (LGS) input per se did not cause postsynaptic or other changes which might account for the depression in curve a . Therefore, this depression likely is homosynaptic and due to presynaptic depression of transmitter release (see also Laouris et al. 1988). The depression evident in the (thick) curves c_1 and c_2 is

stronger than that apparent in curve a . The effect of the simultaneous conditioning events becomes even more conspicuous if one makes the linearity assumption that the effects of conditioning events in each single channel (S_1 and S_2) just add algebraically when occurring synchronously, which would generate conditioning curves as those labelled $a+b$ in Fig. 7 II and IV. For δ 's up to about 50 ms, the simultaneous conditioning curve (thick line) falls well below the $a+b$ curve. From the previous considerations, it is unlikely that postsynaptic factors account for this fact. A likely candidate for a possible mechanism is mutual inhibition between Renshaw cells (Ryall 1970, 1981), which should be more easily recruited by synchronous events in two input channels than by disjunct events in each single input channel. To assume other possible presynaptic mechanisms such as interactions between axon collaterals impinging on a Ren-

Fig. 8. An example of a Renshaw cell showing linear interaction of two inputs. The medial gastrocnemius nerve was divided into two parts which were stimulated at mean rates of 9.5 pps (conventional PSTH in *A*; number of reference events: 5773) and 12.3 pps (right PSTH in *B*; number of reference events: 7479). The structure of the lower part of the figure (*I–IV*) is the same as that of Fig. 7. Mean Renshaw cell discharge rate: 72.3 pps

shaw cell is more speculative at present (see Collins III et al. 1984).

Further examples

Figure 8 shows a Renshaw cell activated by parallel random stimulation of two parts of the gastrocnemius nerve. The interaction of these two parts on the cell response was such that the auto- and cross-conditioning effects summed nearly algebraically to yield the simultaneous conditioning effects.

Figure 9 shows an example of a dorsal horn neurone activated by parallel random stimulation of two branches of the suralis nerve. This neurone exhibited a stronger (nonlinear) response to simultaneous conditioning stimuli than accounted for by the summed conditioning effects of both input channels separately.

In summary, in 6 runs from 5 Renshaw cells, we found algebraical summation of auto- and cross-conditioning effects as exemplified in Fig. 8 in 2 cases, whereas nonlinear interactions of the kind illustrated in Fig. 7 were found in 3 cases, including two where the same cell was tested twice at low and high mean stimulus rate. In one case of nonlinear interaction, an early facilitation was evident, i.e., the simultaneous δ -curve had positive values higher than the sum $a+b$ for δ up to 40 ms. In 4 runs from 4 dorsal horn neurones, 1 linear and 3 nonlinear interactions occurred, the

Fig. 9. An example of a dorsal horn neurone activated by parallel random stimulation of two branches of the suralis nerve at mean rates of 20.7 pps (conventional PSTH in *A*; number of reference events: 19528) and 21.6 pps (PSTH in *B*; number of reference events: 20242). The structure of the figure is the same as that of Fig. 8. The cell was located 1 mm rostral to the border between L_6 and L_7 segments at a depth of 1.6 mm from cord dorsum. Its mean discharge rate was 34.1 pps

latter including that depicted in Fig. 9 and two cases, in which the simultaneous δ -curve lay above the sum $a+b$. Thus, nonlinear interactions between parallel inputs to a post-synaptic cell are quite frequent and, in part, somewhat complicated.

Discussion

At first glance, the method presented in this paper appears complicated because, in order to arrive at the final result as demonstrated in Figs. 7–9, several intermediate steps including the computation of various controls are necessary. Also, one might argue that this final result could also have been obtained by means of a simpler paradigm in which pairs and triplets of stimuli are repeated at low rate (i.e., three conditioning-test constellations: a test stimulus in one input preceded by a conditioning stimulus in the same, in the parallel and by two synchronous stimuli in both input channels; call it “triple pulse technique”). Thus, the effort may not seem worthwhile.

The pros may be summarized as follows: The present approach with random stimulus patterns at selected mean rates is more natural than the double- and triple-pulse technique. The latter paradigm has often and successfully been used to study, for example, convergence of various inputs onto common spinal interneurons projecting to moto-

neurones (review: Baldissera et al. 1981). But its pattern is very unnatural because, firstly, the mean stimulus rates are very low and, secondly, the synchronous stimuli are excessively abundant (one among three). The random patterns overcome these limitations. The mean rate can be chosen up to high values. This relative freedom in choosing the mean rate is important because the mean rate determines the conditioning effects (Windhorst et al. 1988). This freedom has to do with the fact that, for instance, pairs of stimuli are selected from the original stimulus train(s) irrespective of intervening stimuli (this needs to be avoided in the doublet paradigm). Since in the random pattern the pairs occur randomly also with respect to all other stimuli, these simply proved an average excitatory background. Secondly, synchronous stimuli in two input channels just by chance, buried in the ongoing activity in both channels. Although it may be presumed that spike activities in parallel neural elements are synchronized (or otherwise correlated) to some degree (for review see Windhorst 1988), the chance occurrence of synchronous events in uncorrelated inputs is the most proper control case for estimating the effects of such events on signal transmission through small neural networks.

Random stimulus sequences are also very economical as compared to the triple pulse technique. For, to arrive at the same statistical reliability, the testing by double and triple pulses needs to be repeated many times, but at a low repetition rate. This is very time-consuming.

As to the many steps involved in the calculation, it should be noted that the intermediate results have a value of their own. Auto- and cross-conditioning curves are of interest in their own right (see, for instance, Windhorst et al. 1987, 1988; Laouris et al. 1988).

Whereas in the cases illustrated here the (test) excitation of the cell recorded stemmed, via monosynaptic links, from the same inputs that were also used to activate the oligosynaptic pathway(s), this is no indispensable requirement. This input yielding the test stimuli (and responses) may well be independent (a third channel) of the two inputs yielding the conditioning stimuli. Quite generally, the approach can be generalized to more than two inputs.

As exemplified in Figs. 3–9, we defined that stimulus train as S_1 (yielding the reference events for PSTH computation), which elicited the stronger average cell response. Choosing the other train eliciting the smaller response caused statistical problems, that is noisy conditioning curves. However, if in case of near-equal responses both input trains are successively taken as S_1 , a comparison of the two cross-conditioning effects might yield information on the relative location of the different synapses which elicit the monosynaptic excitatory and oligosynaptic inhibitory effects.

The present paper was designed just to describe a statistical method which allows to study a particular aspect of signal transmission through small neural networks. It was not intended to reveal the specific mechanisms underlying the conditioning effects described. These are best investigated in combination with other methods, e.g., pharmacological ones. The development of higher-order cross-correlation techniques such as those presented here (for others see Windhorst 1988) are of increasing importance if attempts at understanding the complicated signal transmission through neural networks are seriously undertaken. The development of ever more powerful computers is a fortunate circumstance supporting such procedures and should be exploited.

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References

- Abeles M (1982) Local cortical circuits. An electrophysiological study. Springer, Berlin Heidelberg New York
- Baldissera F, Hultborn H, Illert M (1981) Integration in spinal neuronal systems. In: Brooks VB (ed) The nervous system. Handbook of Physiology, vol II, part 1. Am Physiol Soc, pp 509–595
- Brink EE, Suzuki I (1987) Recurrent inhibitory connexions among neck motoneurons in the cat. *J Physiol (Lond)* 383:301–326
- Christakos CN, Windhorst U, Rissing R, Meyer-Lohmann J (1987) Frequency response of spinal Renshaw cells activated by stochastic motor axon stimulation. *Neuroscience* 23:613–623
- Collins III WF, Honig MG, Mendell LM (1984) Heterogeneity of group Ia synapses on homonymous α -motoneurons as revealed by high-frequency stimulation of Ia afferent fibers. *J Neurophysiol* 52:980–993
- Eccles JC, Eccles RM, Iggo A, Lundberg A (1961) Electrophysiological investigations on Renshaw cells. *J Physiol (Lond)* 159:461–478
- Hultborn H, Pierrot-Deseilligny E (1979) Input-output relations in the pathway of recurrent inhibition to motoneurons in the cat. *J Physiol (Lond)* 297:267–287
- Jankowska E, Odutola A (1980) Crossed and uncrossed synaptic actions on motoneurons of back muscles in the cat. *Brain Res* 194:65–78
- Krausz HI (1975) Identification of nonlinear systems using random impulse train inputs. *Biol Cybern* 19:217–230
- Laouris Y, Windhorst U, Rissing R, Meyer-Lohmann J, Kuipers U (1988) Time constants of facilitation and depression in the responses of spinal Renshaw cells to random stimulation of motor axons. *Exp Brain Res* 72:117–128
- Malsburg C von der (1985) Nervous structures with dynamical links. *Ber Bunsenges Phys Chem* 89:703–710
- Mannard A, Stein RB (1973) Determination of the frequency response of isometric soleus muscle in the cat using random nerve stimulation. *J Physiol (Lond)* 229:275–296
- Marmarelis PZ, Marmarelis VZ (1978) Analysis of physiological systems: the white noise approach. Plenum Press, New York
- Perkel DH, Gerstein GL, Moore GP (1967) Neuronal spike trains and stochastic point processes. II. Simultaneous spike trains. *Biophys J* 7:419–440
- Ryall RW (1970) Renshaw cell mediated inhibition of Renshaw cells: patterns of excitation and inhibition from impulses in motor-axon collaterals. *J Neurophysiol* 33:257–270
- Ryall RW (1981) Patterns of recurrent excitation and mutual inhibition of cat Renshaw cells. *J Physiol (Lond)* 316:439–452
- Walmsley B, Tracey DJ (1981) An intracellular study of Renshaw cells. *Brain Res* 223:170–175
- Windhorst U (1988) How brain-like is the spinal cord? Interacting cell assemblies in the nervous system. Springer Berlin Heidelberg New York Tokyo
- Windhorst U, Niemann U, Koehler W (1983) Analysis of nonlinear physiological systems with single or multiple spike inputs and analogue or spike outputs. *Biol Cybern* 48:159–163
- Windhorst U, Rissing R, Meyer-Lohmann J (1987) After-effects of stochastic synaptic Renshaw cell excitation on their discharge probability. *Brain Res* 408:289–294
- Windhorst U, Rissing R, Meyer-Lohmann J, Laouris Y, Kuipers U (1989) Facilitation and depression in the responses of spinal Renshaw cells to random stimulation of motor axons. *J Neurophysiol* 60:1638–1652