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Presynaptic Irregularity and Pacemaker Inhibition*

A. F. Kohn**, A. Freitas da Rocha***, and J. P. Segundo

Department of Anatomy and Brain Research Institute, University of California, Los Angeles, CA 90024, USA

Abstract. It is known (e.g., Perkel et al., 1964) that when a pacemaker neuron elicits IPSP's in another, there are domains called "paradoxical segments" where in the steady-state i) faster inhibitory discharges determine faster inhibited ones, and ii) pre- and postsynaptic spikes are "locked" in an invariant forward-and-backward positioning in time, spikes alternating in the ratios 1:1 (1 pre for 1 postsynaptic), 1:2, 2:1..., that are also the slopes of the synaptic rate-transformation. The present project examined the matter further in the inhibitory synapse upon the crayfish tonic stretch receptor neuron, confirming the above. In addition it showed that locking and alternation existed also in the segments interposed between the 1:2, 1:1 and 2:1 paradoxical segments, even though they were not as marked and apparent, and that when tests were close to each other their order became influential and hysteresis-like phenomena appeared. The main finding was that paradoxical rate-relations, locking and alternation persisted when the presynaptic train was irregularized up to interval coefficients of variation of around 0.20 (Figs. 2–5). Therefore, both phenomena may not simply be laboratory curiosities, but also have a role in natural operation where probably a substantial population of neurons exhibits that kind of irregularity. As presynaptic irregularity increased, the paradoxical segment slopes and widths decreased and locking and alternation became less clear-cut. With CV's of about 0.20, only a relatively narrow 1:1 paradoxical segment with about O slope and little locking and alternation remained (Figs. 2b, 3g, 4right, 5third row). With larger CV's, the rate relation decreased monotonically and there was no locking nor alternation (Figs. 2e, 3h, 5 bottom row). The postsynaptic discharge was more regular and had fewer changes in the number of presynaptic spikes per postsynaptic interval within paradoxical segments (particularly in their centers) than in segments interposed between them (left vs. right-hand columns in Figs. 5, 6; Fig. 7): the contrast, remarkable for regular stimuli, attenuated as variability increased. The following conclusions are relevant to coding of spike trains across a synapse with IPSP's. i) With fairly regular discharges, the same postsynaptic rate may result from several presynaptic ones (e.g., may result from rates in the 1:1 and 2:1 paradoxical segments and in the interposed one, Fig. 2): in some cases but not others, the precise presynaptic rate can be identified on the basis of postsynaptic CV's, interval histograms and cycle slips. ii) A small rate change in a regular presynaptic discharge will have very different postsynaptic consequences depending on where it happens: if across a paradoxical-interposed boundary, for instance, it will cause remarkable rate, pattern and correlation changes. iii) The trans-synaptic mapping of variability involves an increase for the more regular presynaptic discharges and a decrease for the more irregular ones. iv) The postsynaptic discharge was slower with IPSP's than without in most cases; however, when the control discharge was weak or absent, IPSP's accelerated it. Results are relevant also to the operation of periodically performing systems that involve neuronal correlates, indicating that it is necessary in every case to ask whether zigzag relations and locking occur. The "delay function" plots the arrival time of an IPSP (or IPSP burst) relative to the last postsynaptic spike, i.e., the "phase" (Φ in Fig. 1b), against the interval lengthening produced, i.e., the "delay" (δ). In all cases, most points clustered around a straight line (Fig. 8), whose slope and ordinate intercept were in the 0.43–0.87 and the 0.02–0.52 ranges, respectively, for single IPSP's.

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** Supported by FAPESP (Sao Paulo, Brazil). Present address: Esc. Politecnica, Dee, Universidade de Sao Paulo, CP8174, Cid. Univ., Sao Paulo, S.P., Brazil

*** Present address: Departamento de Fisiologia e Biofisica, Universidade Estadual de Campinas, CP 1170, Cid. Univ., Campinas, S.P., Brazil

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The slope reflects how the IPSP effectiveness depends on when it arrives in the cycle; the intercept reflects the IPSP effectiveness. Large phases often showed "aberrant" points whose ordinates were either large (and having special formal implications), or very small (perhaps reflecting conduction and synaptic delays), or clustered around a second straight segment with a large negative slope (when spontaneous rates were low) (Fig. 8c). Delay functions for widely separated pairs of IPSP's could be multi-valued, points clustering around 2 or 3 parallel straight lines. A mathematical model of pacemaker inhibitory synaptic interactions (Segundo, 1979) agreed with this embodiment insofar as some postulated properties are concerned (e.g., regular discharge, interval lengthening by IPSP's, linear delay functions with slopes around 0.7) and as to the main aspects of the preparation's behavior (i.e., zigzag rate relations and locking), but not in terms of some aspects of the postulates (e.g., interval variability, rebound) or behavior (e.g., segment boundaries, jitter in the locking, and hysteresis). The model was judged to be on the balance satisfactorily realistic.

Introduction

When pacemaker neurons are subjected to pacemaker-like IPSP-eliciting presynaptic spike trains (Kohn, 1980; Moore et al., 1963; Perkel et al., 1964; Schulman, 1969; Segundo and Perkel, 1969), the plot of postsynaptic rate as a function of the IPSP rate in the steady state, though decreasing in the overall, is interrupted by wide ranges where an increase in IPSP rate accelerates the pacemaker rate. This behavior contrary to a naive expectation was dubbed "paradoxical" and the ranges, "paradoxical segments". Within them, there was a fixed time relationship between IPSP's and pacemaker spikes, i.e., a "phase-locking", that occurred with no anatomical feedback loops.

The present work, carried out in the inhibitory synapse of a crayfish stretch-receptor neuron, has the following goals. First and foremost, an assessment of the natural significance of the paradoxical effects and the locking, since so far experiments have involved unnatural trains of extreme regularity almost exclusively [excepted are some simulations by Moore et al. (1963)]. For this assessment, various degrees of presynaptic discharge variability were used and paradoxical effects and locking looked for; the domain of their existence and that of neuronal variabilities in nature were compared and a judgment was made as to their relative breadths. Second, a better analysis [pursued further in an electronic neuromine, Kohn and Segundo (in preparation)] of the ranges referred to as "interposed segments" between paradoxical segments since little is said about them in the earlier literature. Third, an evaluation of the validity of a formal model (Segundo, 1979) by comparing its assumptions and behavior with the reality of the living preparation according to criteria explained elsewhere (Segundo and Kohn, 1980).

Materials and Methods

Experiments were performed on adult *Procambarus clarkii* of either gender from April to December (see also last paragraph of Results). The thorax and two abdominal segments of the abdomen were anchored to a bath filled with van Harreveld's solution maintained at about 14 C, oxygenated, and usually renewed continuously. The eighth thoracic segment stretch-receptor organs and eighth dorsal nerve were dissected following standard procedures (e.g., Segundo et al., 1976). While the caudal insertion of the slowly adapting or tonic receptor muscle (RM 1) remained in place, the cephalic end was freed and held by a micrometrically controlled forceps whose position served (with limitations explained in Results) to set the afferent firing intensity. Stimulating pulses were delivered via a hook-electrode that held the phasic receptor muscle (RM 2) near the receptor cell and inhibitory axon terminals; pulses drove the latter antidromically, and through an axon reflex, produced IPSP's in the tonic receptor cell. Action potentials were recorded with another hook-electrode on the cut dorsal nerve; each shock produced one inhibitory action potential. The experimental data were recorded on analog tape for later processing. The inhibitory fiber was driven with different pulse sequences whose intervals were all greater than 5 ms so as to respect refractoriness, and independent of one another.

Different stimulus trains, i.e., presynaptic train forms with different variabilities, were used. Sequences referred to as "Poisson" consisted of exponentially distributed intervals and were obtained from a special generator. Sequences referred to as "gamma of order k" were obtained by feeding the "Poisson" into a digital frequency-divider that allowed the passage of only every k^{th} pulse ($k = 2, 4, 8, \ldots$). Sequences referred to as "periodic" or "regular" exhibited evenly spaced pulses and were generated by conventional stimulators. The average firing intensity of the sequence, as measured by the mean rate, was set using generator or stimulator controls.

The theoretical Poisson point process has exponentially distributed independent intervals, and all are possible (Hoel et al., 1971). If only every k^{th} point is preserved, the resulting process will have intervals whose distribution is that of the sum of k independent

exponentially distributed variables and is called gamma or Erlang of order k. The gamma of order 1 is the exponential. As k increases, the interval average and standard deviation increase with k and $\sqrt{k}$, respectively, but the coefficient of variation (CV), i.e., the ratio of the standard deviation to the average decreases with $(\sqrt{k})^{-1}$. So as to obtain a "gamma" stimulus of order k with mean rate f, the "Poisson" at the input of the digital frequency-divider must have a mean rate of $k \cdot f$. Increasing variability or irregularity, i.e., decreasing regularity, was obtained by passing from the "regular" sequence, through "gammas" of decreasing order (128, 64, 32, ..., 2) to the "Poisson". This methodology guaranteed the setting of the presynaptic CV, i.e., its degree of irregularity, in an easy manner repeatable over all experiments. Gamma-like interspike interval densities are encountered in numerous nerve cells (e.g., Skvaril et al., 1971).

The first step in the experimental paradigm was to view simultaneously on a memory oscilloscope the stimulus and postsynaptic trains and, using a periodic stimulus, determine the presynaptic rate bounds for the 1:1, 2:1, and 1:2 "paradoxical" segments (Moore et al., 1963; Perkel et al., 1964; Schulman, 1969). These bounds guided the choise of rates of the gamma forms delivered during the experiment in random order. For each given postsynaptic spontaneous rate, several mean stimulation rates were used, usually for gammas of two orders. Inhibitory trains lasted for at least one minute; they were initiated when the aftereffects of previous ones (see below) had subsided, usually from one to several minutes. Whenever possible, a different postsynaptic spontaneous rate was obtained and the same gammas were tested. Experiments usually lasted for several hours.

Data analysis in a PDP 8/E minicomputer started by identifying the times of occurence of the individual spikes (the inhibitor followed the stimulus by a few milliseconds) and then displayed the "running" or "bin" rate over periods of 1 s or 4 s as a function of ongoing time as explained by Segundo et al. (1976). Presynaptic discharges were of course stationary. The characteristic postsynaptic response (see Results) usually exhibited a late stationary segment whose duration was 20s to 30s. Stationarity [in the mean rate sense, Cox and Lewis (1966)] was evaluated either by naked-eye examination or by using a Kendal rank correlation test at a significance level of 0.05 (Gibbons, 1971). Subsequently the computer calculated over the stationary segments the "overall" pre and postsynaptic statistics (the word "overall" is omitted hereafter): number of spikes, mean rate, and interval means, standard deviations, coefficients of variation and histograms; additionally it computed auto-correlation and cross-correlation histograms. Our examination of this data concentrated upon the relation between mean rates, referred to as "mean rate transformation" (MRT), on the evaluation of "locking" and cycle slips in the stationary period, and on the "delay function." The "*mean rate transformation*" was a plot of the presynaptic mean rate on the abscissa with the corresponding postsynaptic mean rate on the ordinate.

We say that two spike trains are "*locked*" when an action potential (AP) in one of them A is usually both preceded by an AP in the other at a fixed interval t_1 and followed by one at a fixed interval t_2 (which need not be equal to t_1). This means that from one spike train it is possible to make statements about the other, into either the past or the future. Though there may be other definitions, this seems the most adequate for this study. The cross intensity function (CIF) between two trains A and B at time t measures the rate of spike B at time t conditioned on the occurence of a spike in A at time O (Bryant et al., 1973; Cox and Lewis, 1966). Properly scaled, it measures the probability of B firing around t given that A fired at O. Locking implies that the CIF, and therefore its estimator the cross-correlation histogram (CCH), will exhibit a peak at $-t_1$ and another at t_2. [The "anticipatory" deviation close to the origin is excluded from these considerations (Bryant et al., 1973).] Locking can be considered "perfect" when the probability of B firing is 1 in an arbitrarily small vicinity of certain positive and negative t values and zero elsewhere. Locking (in the sense defined above) is not present when the CIF (and CCH) either is flat as when both trains are independent, or exhibits peaks only on one side, i.e., when an AP in one train is followed at a consistent interval, but not preceded, by an AP in the other (e.g., Fig. 5). The auto-intensity function (AIF) and the autocorrelation histogram (ACH) are the CIF and the CCH of the train with itself. Theoretically it is possible to have a CIF with one peak at each side of the origin even when one point process shows no periodicity, i.e., is Poisson. In practice, the presynaptic train always exhibits some periodicity, i.e., the AIF and the ACH have peaks: these appear in the CIF and CCH characteristically to both sides of the origin (Bryant et al., 1973), thus implying some degree of locking. This means that a quantitative measure of locking (e.g., the count cross-spectrum, Cox and Lewis, 1966) is useful for comparisons. In the present work, the presence of locking and its magnitude were accepted on the basis of a visual inspection of the record itself and particularly of the CCH in terms of the number and size of the peaks (and throughs) that both to the left and right of the origin departed appreciably from the average value [for significance tests of CCH peaks, see Brillinger et al. (1976)]. If departures occured only on one side, or were absent, locking was rejected.

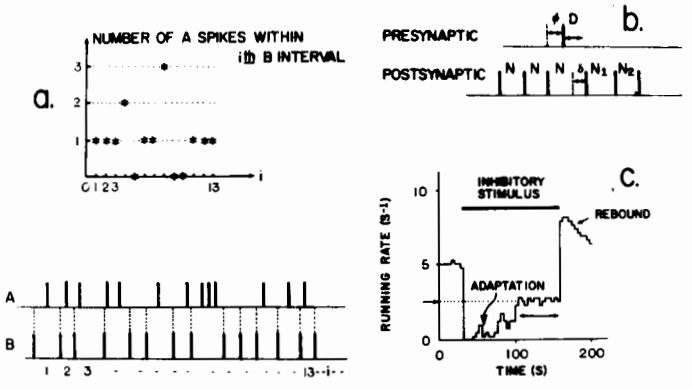


Fig. 1a Cycle slipping. Below: presynaptic train *A* and postsynaptic train *B*. Above: on the abscissa, postsynaptic interval serial order *i* and on the ordinate, number of presynaptic spikes in the i^{th} interval. **b** Phase Φ, delay δ, silent interval *D* and natural intervals N, N_1, N_2. A single presynaptic spike (above) at a time or "phase" Φ from the last postsynaptic one (below) lengthens the natural postsynaptic interval *N* by the "delay" δ, creating a "silent interval" *D* and perhaps causing aftereffects with $N_1, N_2, \ldots$ different from *N*. The dependence of the delay on the phase is the "delay function" (Fig. 8). **c** Postsynaptic "running" or "bin" rate and inhibitory fiber stimulation. On the abscissa, ongoing time divided in 4 s "bins"; on the ordinate, postsynaptic rate measured over individual bins. Spontaneous prestimulus rate about 5.0/s. The inhibitory train (mean rate 6.0/s, CV = 0.20) applied during the horizontal bar produced a temporary arrest of postsynaptic firing followed by a partial recovery or adaptation, and a stabilization during the period indicated by the horizontal double arrow around a stationary rate of about 2.5/s (indicated by the dotted line). At the stimulus "off" the postsynaptic rate overshot the spontaneous value by about 2.5/s ("rebound"), and slowly adapted back to 5.0/s (shown incompletely)

Locking can occur in different pre to postsynaptic ratios: it is called $s:r+1 (s=1,2,\ldots; r=0,1,2\ldots)$ when for each *s* presynaptic spikes there are $r+1$ postsynaptic ones. This report concentrates almost exclusively upon ratios of types $1:r+1$ or $s:1$.

The alternation of pre and postsynaptic firing implied by the locking was evaluated using the concept of *cycle slipping* used in communication theory (Lindsey, 1972) and extended to point processes. The algorithm illustrated in Fig. 1a consisted in plotting the number of presynaptic *A* spikes comprised within each postsynaptic *B* interval against the order of the latter. A perfect $1:r+1$, or $s:1$, alternation would show the periodic repetition of *r* zeroes and a single 1, or all points at an ordinate *s*, respectively. Figure 1a shows a hypothetical result with some degree of 1:1 alternation (and locking) where usually one presynaptic pulse was contained in a postsynaptic interval suggesting a 1:1 alternation. The latter was disrupted occasionally by either gain or loss of presynaptic spikes: a point at an ordinate *k* larger than 1 (e.g., the 4^{th} and 8^{th} in Fig. 1a) is said to correspond to $k-1$ positive cycle slips; a zero ordinate (e.g., points 5, 9, 10 in Fig. 1a) to 1 negative cycle slip. As the number of positive and negative cycle slips is meaningful only when compared to the total number of presynaptic spikes, the "degree of slipping" (DS) is defined as the ratio of the former to the latter. A low DS indicates few skippings and a well-preserved alternation.

The effect of an IPSP (or IPSP burst) on the pacemaker interval was characterized by the "*delay function*" (Fig. 1b). The time or phase Φ of IPSP arrival after a postsynaptic action potential is plotted on the abscissa against the corresponding lengthening of the interspike interval on the ordinate (Perkel et al., 1964; Segundo, 1979; Segundo and Perkel, 1969). The delay function was determined usually at the beginning of the experiment, and sometimes also later, by delivering about 250 pulses of a low mean rate (e.g., 0.6/s) "Poisson" train with a dead time around 5 ms. The computer calculated and displayed the delay function; both variables were normalized with respect to the average of the last three intervals without IPSP's, or "natural" ones, preceding that with IPSP's. The delay function tells us about the likelihood of the postsynaptic cell firing again after a certain time given an IPSP with phase Φ, i.e., the interval probability density function (estimated by the interval histogram) conditional to an IPSP at Φ.

Results

The tonic or slowly-adapting stretch receptor neuron, when not disturbed, behaved as a pacemaker, firing regularly with interval coefficients of variation (CV) from 0.01 to 0.05, and impulse rates from 2.5 to 11/s; CV's; similar to those reported by Firth (1966), were higher for low rates. The undisturbed discharge remained stationary for lengths of time that depended on the spontaneous rate (and the preparation). When spontaneous rates were intermediate, from, say, 3/s to 10/s as was the rule, stationarity persisted practically for up to several hours; slow trends existed in practically every case however (as reported by Burkhardt, 1958) and were compensated for by adjusting the receptor length. Extremely high or low rates outside of the 3–10/s range were difficult to either preverse if observed initially or to produce if not, by adjusting the length: indeed, even though stretch or relaxation was followed by high peaks or long pauses, adaptation usually returned the activity to the 3–10/s range. A few times it was possible, by releasing the receptor muscle and/or cleaning away adjacent tissues, to suppress the receptor's discharge.

A presynaptic inhibitory train influenced the postsynaptic bin rate (Fig. 1c) by producing, in succession, an immediate drop ("undershoot") followed by an exponential-like upward recovery ("adaptation") that eventually settled to a stationary level ("steady state")

lower than the non-inhibited rate. The turning off (after over 20 s of steady state) was followed by an increase beyond the pre-stimulus level ("rebound overshoot") which then decayed slowly to the pre-stimulus value.

The main features of the graph of pre versus postsynaptic overall mean rates, i.e., of the "*mean rate transformation*" (MRT), for regular (periodic) inhibitory trains (Fig. 2a) were i) an overall decreasing trend, ii) a "zigzag" profile which included segments of positive slope and segments of negative slope; and iii) a somewhat abrupt decrease to zero postsynaptic rate at high presynaptic rates. These characteristics had been found by Moore et al. (1963) in computer simulations and by Perkel et al. (1964) and Schulman (1969) in stretch receptor neurons. The positive-slope segments are called "paradoxical" and those between them, with overall negative slopes, "interposed". The most conspicuous paradoxical segments exhibited pre to postsynaptic rate ratios of 1:1, 2:1, and 1:2. Table 1 shows in its left column the bounds found experimentally for the 1:1 and 1:2 regions for several preparations whose spontaneous rates are indicated in the rightmost column. Usually fairly high periodic stimulation rates were needed to suppress the receptor's firing for prolonged periods: for example, one cell with a 7.7/s spontaneous rate submitted to a 15.5/s stimulus was silenced for 2 min but then escaped and began firing slowly; a 17/s stimulus silenced it throughout its 4.5 min duration. Strong rebound followed the inhibitory trains.

The decreasing trend and the zigzag profile [features i) and ii) above] persisted when the presynaptic train was irregularized by passing from the stimulator-driven periodic form whose CV was practically *0* to the "gammas" of high and intermediate orders with CV's up to around 0.20, as shown in Fig. 2 where the vertical dashed lines indicate the 1:2, 1:1, and 2:1 regions determined using periodic stimuli. The slopes of the paradoxical segments decreased for increasing stimulus CV. In some cases (for example in the 1:1 region with 0.20 CV) practically horizontal segments or "plateaus" were found, also paradoxical in the sense that they lacked the negative slope predicted naively. The presynaptic rate range covered by a paradoxical segment (e.g., the 1:1) with periodic inhibition contained the ranges of those encountered with gammas; that of each gamma contained those of higher CV's. When the presynaptic discharge became very irregular, using gammas of lower orders with CV's of 0.30 up to 1.00, the MRT decreased monotonically and the paradoxical segments disappeared (Fig. 2e open triangles).

The paradoxical segments at ratios other than 1:1 were not clear for stimulus CV's from 0.10 to 0.20 in every preparation. In Fig. 2d (filled triangles) for example, the MRT for a 0.10 CV gamma had clear paradoxical segments in the 1:1 (i.e., where with regular discharges pre- and postsynaptic rates were equal) and 1:2 regions; the existence of 1:3 and 2:1 segments, though suggested, requires more points for an affirmative answer. A 0.13-gamma (Fig. 2e, filled squares) had a paradoxical segment in the 1:1 region and plateaus in the 2:1 and 1:2 regions. A 0.20-gamma

Table 1. Experimental and theoretical bounds for the paradoxical [segments 1:1 (a) and 1:2 (b)]

	Experimental bounds lower–upper (spikes/s)	Theoretical bounds lower–upper (spikes/s)	Postsynaptic spontaneous rate (spikes/s)
(a)	1.60 – 2.10	1.23 – 1.67	2.50
	1.90 – 2.70	1.75 – 2.68	2.90
	2.10 – 3.40	2.02 – 3.14	3.60
	3.50 – 5.40	3.52 – 5.29	5.70
	3.00 – 4.90	3.13 – 5.11	5.80
	4.10 – 6.00	4.22 – 5.80	6.80
	4.25 – 6.00	4.17 – 5.80	7.00
	3.90 – 5.75	3.13 – 4.92	7.50
	4.40 – 6.50	3.58 – 5.83	7.80
	4.85 – 7.05	4.55 – 6.81	8.00
	4.50 – 7.50	4.17 – 6.23	8.50
	6.80 – 10.10	5.10 – 8.11	11.00
(b)	2.50 – 3.20	2.21 – 2.97	7.50
	3.30 – 3.80	2.90 – 3.68	8.00

Table 2. Postsynaptic inter-spike interval coefficient of variation (CV). The pacemaker was subjected to the inhibitory trains indicated on the left column. Each group of rows corresponds to a different experiment. CV values in each of these rows correspond to points within the regions indicated by the column headlines as determined using regular presynaptic trains

	Interposed between 1:2 and 1:1	Paradoxical 1:1	Interposed between 1:1 and 2:1
Periodic or regular	0.190	0.073	0.250
		0.081	0.220
			0.260
Periodic or regular	0.280	0.015	
Gamma CV 0.10	0.150		
	0.176		0.250
	0.176	0.125	0.180
	0.150		
Gamma CV 0.13		0.130	0.260
		0.140	0.266
		0.130	0.260
Gamma CV 0.20		0.190*	0.266
		0.135*	0.310
		0.235	0.286
		0.225	0.320

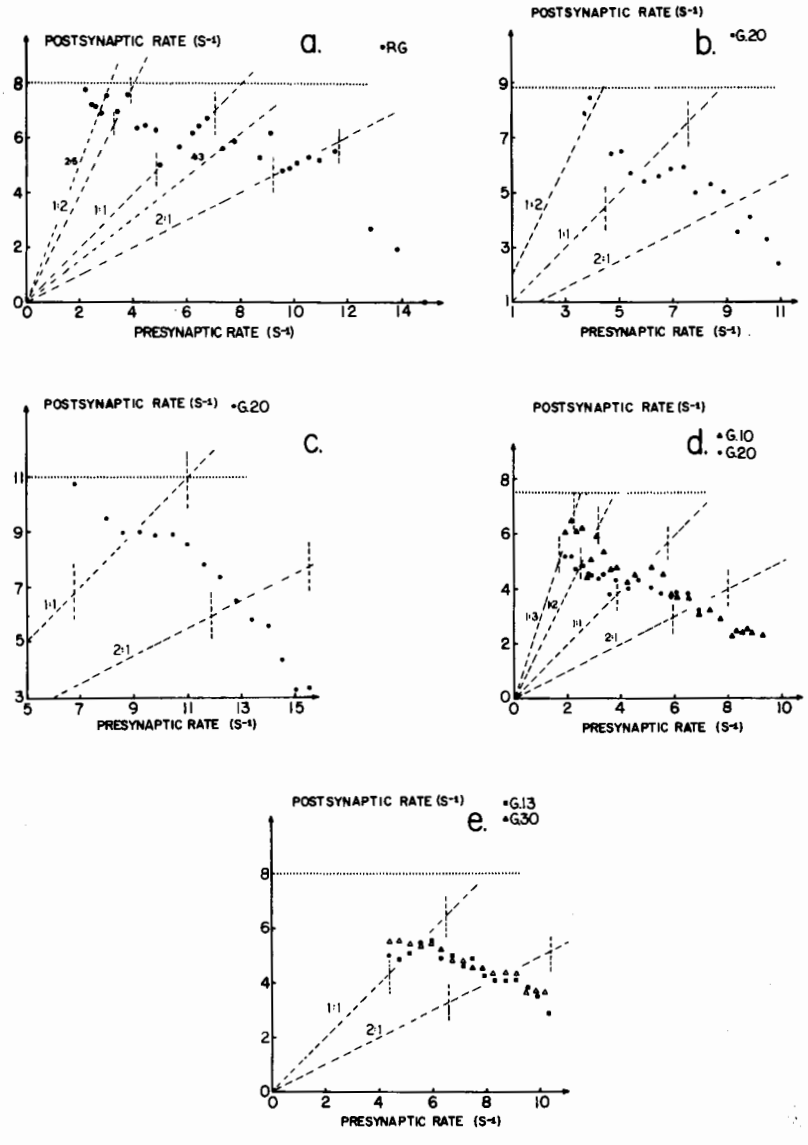


Fig. 2a–e. Mean rate transformation (MRT). On the abscissa and the ordinate, corresponding pre- and postsynaptic rates over a stationary period. The bounds of the paradoxical segments at the 1:1, 2:1, and 1:2 rate relations were determined with regular trains in each case: the appropriate slopes and bounds are indicated by broken lines: spontaneous or natural postsynaptic rate without IPSP's is indicated by a dotted horizontal line. Different graphs illustrate different presynaptic irregularities measured by their coefficients of variation (CV). **a** *Regular* (RG, black circles). A presynaptic rate of about 14.8/s silenced postsynaptic firing. **b, c** Gamma with 0.20 CV (*G* 0.20, open circles). Note that the axes do not intersect at the origin. **d** Gammas with 0.10 and 0.20 CV's (*G* 0.10, black triangles; *G* 0.20, open circles). Data from a single preparation. **e** Gammas with 0.13 and 0.30 CV's (*G* 0.13, black squares; *G* 0.30, open triangles). Data from a single preparation

(Fig. 2b–d, open circles) had either positively or zero sloped segments in the 1:1 region, but usually a monotonic decreasing curve in the 2:1 region. For 0.30-gamma stimuli, the only departures from monotonic decreases were in a few cases a plateau and in a single case a positive slope in the 1:1 region.

The postsynaptic discharge was more regular within paradoxical segments than within interposed ones (Table 2): the contrast was remarkable for regular stimuli, attenuating as the CV increased and disappearing around 0.30. For example, in one case (first row), the postsynaptic CV for periodic stimuli increased from 0.073 to 0.250 on passing from the 1:1 to the interposed segment on its right. For a 0.13-gamma (fourth row), the corresponding increase was about from 0.13 to 0.26. For CV's up to 0.20, the postsynaptic CV in the 1:1 region was usually larger than the presynaptic CV, exceptions being indicated by asterisks in Table 2. The higher the CV, the harder it was to arrest the discharge for prolonged periods.

"*Locking*" was a constant feature within paradoxical segments using regular presynaptic discharges, as illustrated by Fig. 3 showing the trains of presynaptic stimuli (lower traces) and of postsynaptic action potentials (upper traces) locked in 1:1 (a), 1:2 (b), and 2:1 (c) relations. Figure 4a, from the same trains as Fig. 3a, shows superimposed oscilloscope sweeps triggered either by the postsynaptic spike (top) or the stimulus (lower): the locking is implied by the occurence very close to a precise instant after the beginning of the sweep of the stimulus (lower beam) and the action potential (upper beam), i.e., with very small jitter around a particular relative position to the other event. In some preparations, other locking ratios as 2:3, 3:2

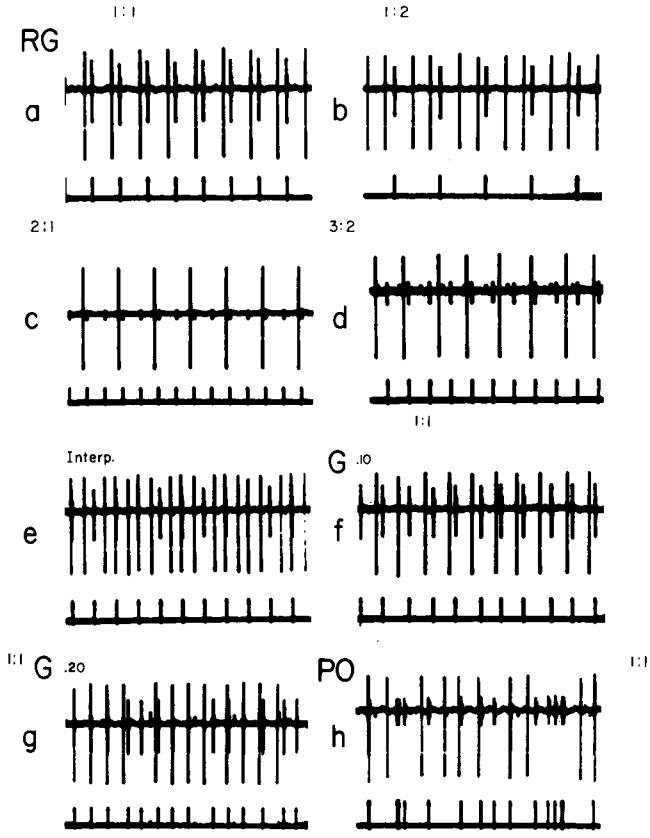


Fig. 3a–h. Corresponding spike trains I. Single slow sweep. For Figs. 3 and 4, the presynaptic discharge is represented by the stimulus train in the lower record and by the smaller action potentials in the upper record, either from the inhibitory fiber alone in **c, d, h** or from both the inhibitory and the phasic receptor fiber in **a, b, e–g** and in Fig. 4. Presynaptic discharges: regular (**a–e**), gamma 0.10 **f**, gamma 0.20 **g**, Poisson **h**. Presynaptic rates in the following segments of the MRT for regular discharges: 1:1 (**a, f–h**), 1:2 (**b**), 2:1 (**c**), 3:2 (**d**), interposed between 1:1 and 2:1 (**e**). Total sweep durations: 2070 ms (**a–c, e**); 5180 ms (**d, f, g**); 2590 ms (**h**)

(Fig. 3d), 2:5 were found as well, usually at a single stimulus rate; occasionally, two adjacent values suggested the existence of a paradoxical segment at that ratio (e.g., a 4:3 in Fig. 2a).

Figure 5a shows a cross-correlation histogram (CCH) for regular train in a 1:1 paradoxical segment: the multiple peaks to the left and right of the origin illustrate the locking. The postsynaptic inter-spike interval histogram (ISIH, Fig. 6a) evidenced the unconditioned regularity of the discharges. When the presynaptic train was regular but at an interposed region, the naked-eye observation of the records (Fig. 3e), and especially the CCH (Fig. 5b) showed that there still were preferred postsynaptic firing times although they weren't as consistent and did not happen with a regular alternation pattern, i.e., there were cycle slips. Nevertheless, it is legitimate to state that locking existed in every case with regular IPSP's, regardless of their rate. The ISIH (Fig. 6b) showed a preferred firing interval, but additional peaks revealed some irregularities. When the presynaptic irregularity reached 0.10 CV, locking was found in the 1:1 range (Figs. 3f, 4b, and 5c) with practically no evidence of cycle slipping; the postsynaptic ISIH (Fig. 6c) was narrow except for the minute peak at the right and resembled the stimulus ISIH. In the interposed range locking was present still (Fig. 5d): the postsynaptic ISIH (Fig. 6d) had a large peak and some smaller ones. For a 0.20-gamma, the raw data in a 1:1 paradoxical segment (Figs. 3g and 4c) suggested some preferred time relationship, or locking, between the pre and postsynaptic spikes. This was confirmed by the CCH (Fig. 5e), not too different from that in an interposed segment (Fig. 5f); the ISIH's (Fig. 6e and f) differed clearly, however, that in the 1:1 segment being far less multimodal and much narrower, suggesting different

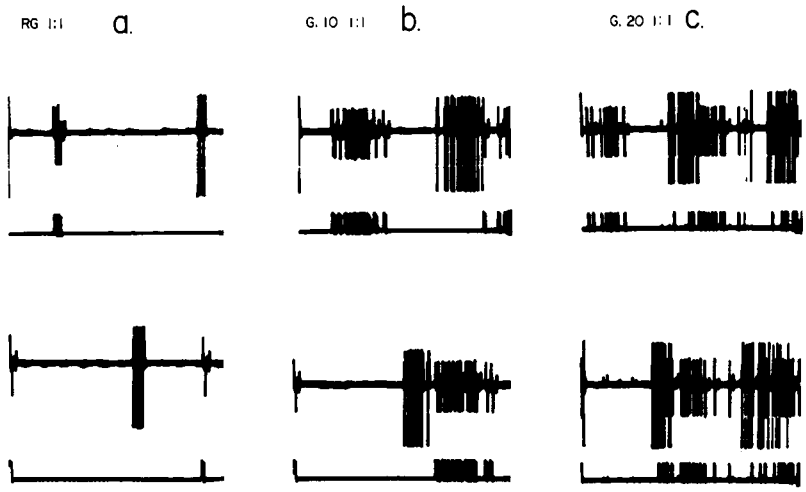


Fig. 4. Corresponding spike trains II. Superimposed fast sweeps. Sweeps are triggered internally by either the postsynaptic spike (upper frames) or the presynaptic stimulus i.e., spike (lower frames). Presynaptic discharges all in 1:1 region: regular (left column), gamma 0.10 (center, same as in Fig. 3a); gamma 0.20 (right, same as in Fig. 3g). Total sweep durations: 260 ms

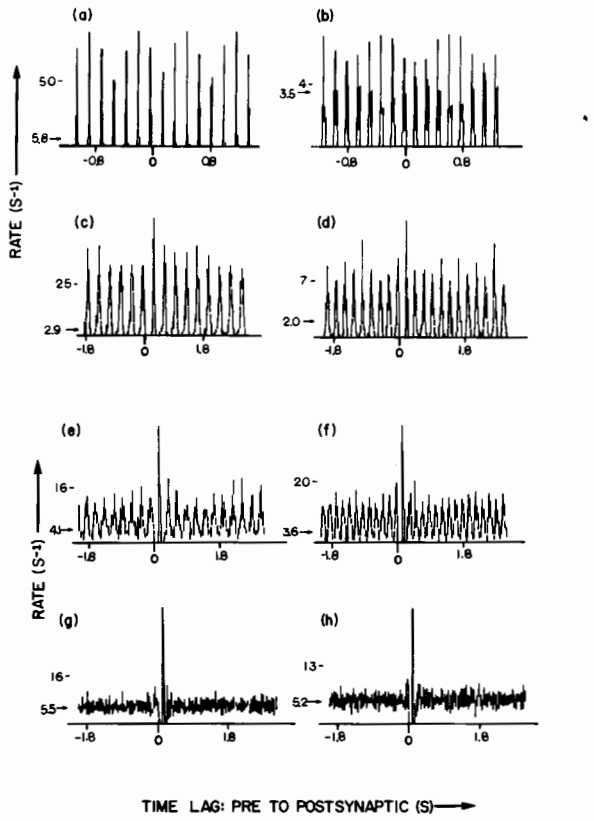


Fig. 5. Locking. Cross-correlation histograms. On the abscissa time from a presynaptic stimulus, i.e., spike, divided into 10 ms bins; on the ordinate, average postsynaptic rate over each 10 ms bin. Presynaptic discharges: regular (top row), gamma 0.10 (second row), gamma 0.20 (third row), gamma 0.30 (botton row). Presynaptic rate regions: 1:1 paradoxical segment (left column), interposed segment between 1:1 and 2:1 (right column). Both records in each row are from the same preparation except for those in the top one. Evidence for locking is seen in all rows except for the bottom one

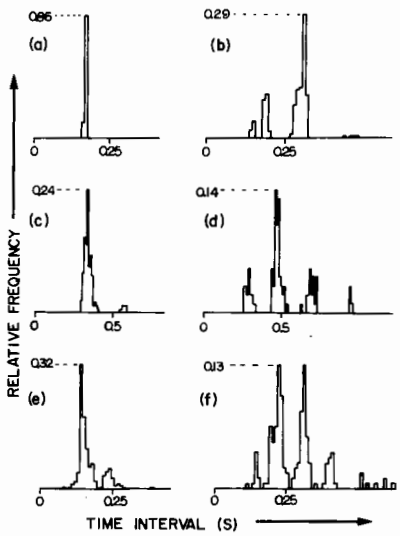


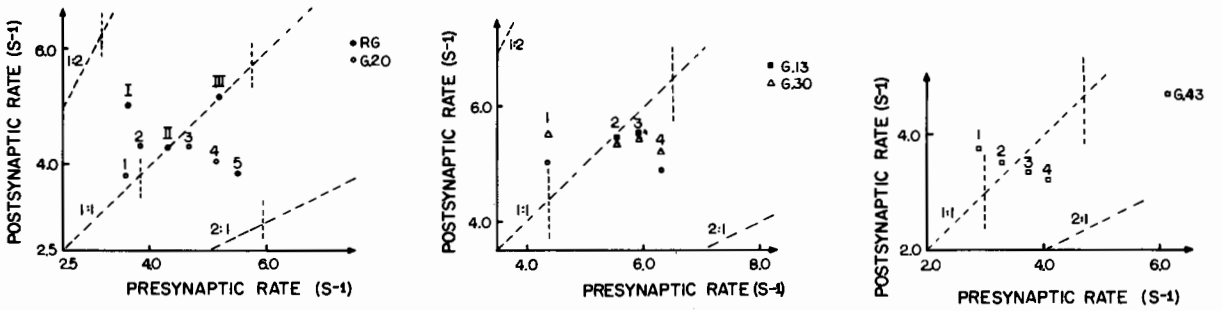
Fig. 6. Inter-spike interval histograms of postsynaptic spike trains. On the abscissa, interval durations classified into 10 ms bins; on the ordinate, relative frequency of each class. Histograms *a* to *f* correspond to the crosscorrelation histograms *a* to *f*, respectively, in Fig. 5. The essentially unimodal and narrow shapes in the 1:1 segment (left column) contrast with the clear multimodal and dispersed histograms in the interposed segment (right column)

lockings or alternations. For presynaptic CV's of 0.30 or larger, no locking was seen, as illustrated in Figs. 3h and 5g, and h).

Alternation was quantified by counting cycle slips and computing the degree of slipping (DS), i.e., the ratio of the total number of slips (positive, negative) to the number of presynaptic spikes (see Methods). For periodic stimuli (Fig. 7a, black circles) the DS was practically zero in the 1:1 region (II, III), and considerably larger (0.39) in the interposed segment (I). For gammas with CV's up to 0.20 (open circles), the DS values increased close to the borders of the 1:1 range (e.g., row 5) so that low DS's (under 0.1) occurred over smaller ranges (e.g., row 3). Negative, or positive, cycle slips predominated near the lower, or upper, ends of the paradoxical segments, respectively. Figure 7b shows DS's for CV's at both sides of 0.20. For gammas with high CV's (e.g., 0.30. open triangles), DS's were large for points anywhere in the 1:1 region (e.g., Fig. 7c). The consequences of positive and negative slips tended to cancel each other in the long run, so that the average postsynaptic rate remained close to that of the presynaptic train even though the 1:1 alternation was disrupted.

The "*delay function*" is a normalized plot of delay δ versus phase Φ (see Methods). In all cases, the majority of experimental points (Fig. 8 I) clustered rather closely around a straight line with positive slope. The estimates for the slope A, and the intercept B, of the linear regression $\delta = A\Phi + B$ resulted in the ranges 0.43–0.87, and 0.02–0.52, respectively. Higher values were found with higher postsynaptic spontaneous rates, in either the same or different preparations. For example, in one case A was 0.472 and 0.676 and B, 0.207 and 0.365 for rates of 6.3/s and 8.5/s, respectively (Fig. 8I a and c).

Aberrant points, though present in most cases, usually were few. Some were at the lower right, i.e., with phases close to the natural interval and small delaying effects: these could be due to tonic and inhibitory axonal propagation and synaptic delays which could lead to underestimates of Φ by about 5 ms. so that the IPSP's judged to just precede the spike actually just followed it. In about a third of the cases, at least one point at a phase near 1 had a delay that exceeded 1 by an amount equal to the delay at practically zero phase: this has consequences as to the type of the resulting phase-resetting curve (see Discussion). Far less frequently and only associated



a.

		+CS	-CS	STIM.	D.S.
•	I	0	41	106	0.39
	II	0	0	100	0.00
	III	11	0	208	0.05
○	1	2	6	54	0.15
	2	2	15	115	0.15
	3	10	1	116	0.09
	4	25	0	103	0.24
	5	48	0	165	0.29

b.

		+CS	-CS	STIM	D.S.
■	1	1	19	131	0.15
	2	0	0	100	0.00
	3	5	0	88	0.06
	4	23	0	95	0.24
△	1	6	31	108	0.34
	2	25	17	167	0.25
	3	23	11	147	0.23
	4	44	9	190	0.28

c.

		+CS	-CS	STIM	D.S.
□	1	15	42	86	0.66
	2	18	26	99	0.44
	3	30	16	113	0.41
	4	23	10	60	0.55

Fig. 7a–c. Degree of slipping. Below, degree of slipping (DS); above, MRT. **a** Regular stimuli (filled circles): points (II, III) on the 1:1 paradoxical segment showed small degrees of slipping (DS); a point (I) in an interposed segment had a much larger DS. Gamma 0.20 (open circles): close to the center of the 1:1 segment a point (3) had a low DS; near the upper or lower boundaries of the 1:1 region (points 1, 2, 4, 5) the number of cycle slips increased. **b** Gamma 0.13 (filled squares): points (2.3) clearly on the 1:1 showed low DS's as compared to points (1,4) close to its borders. Gamma 0.30 (open triangles): all points of the plateau in the 1:1 region had large DS. **c** Gamma 0.43: all points showed a high DS

with low spontaneous rates (5.0/s) and/or less steep delay functions, there were points with phases greater than about 0.8 clustered around a second segment of marked negative slope (Fig. 8I b).

The delay functions corresponding to bursts of several regular IPSP's (at a particular inter-IPSP interval) usually were acceptably linear over most of their range; slopes and intercepts were larger than in the single IPSP case. For example, in one experiment A and B were 1.85 and 0.58 for 5 IPSP's (interval, 11 ms) and 0.83 and 0.18 for one IPSP, respectively. Finally, in certain experiments the delay function corresponding to bursts of 2 IPSP's, i.e., to pairs, separated by an interval that was a large fraction (e.g., 0.8) of the natural interval was multivalued (Fig. 8d). Points clustered around 2 or 3 lines with approximately the same slopes (e.g., 0.544, 0.535, 0.516) but different intercepts (e.g., 1.23, 0.75, 0.14, respectively): the smaller phases tended to yield points in the intermediate cluster, and longer ones in the other two. Another way of expressing this is by saying that the likelihood of the postsynaptic cell firing again after a pair of IPSP's (separated by I) has arrived with a phase Φ, as expressed by the interval density conditional to an IPSP at Φ and another at $\Phi+I$, has three modes: i.e., the cell will fire obligatorily at one of them, but not necessarily at the first or the second.

Figure 8II plots the same data as 8–I but, whereas the abscissa still is Φ, the ordinate is the "silent period" D that (Fig. 1c) extends from the last IPSP to the next pacemaker spike (see Schulman, 1969 and Figs. 172, 173 in Segundo and Perkel, 1969). D equals $-\Phi+N-(s-1)I+\delta$ when there are s IPSP's separated by I($s=2$ in Fig. 8d); when there is a single IPSP it equals $-\Phi+N+\delta$ (Fig. 8IIa–c). D would coincide with the "co-phase" (as defined by Winfree, 1980) if the natural intervals were re-established immediately. The reason for the apparent redundancy of Fig. 8 is to express the data both in the format used so far by us (Kohn, 1980; Moore et al., 1963; Perkel et al., 1964; Segundo, 1979; Segundo and Kohn, 1980; Segundo and Perkel, 1969) and in the stimulus-referenced one used currently and explained by Winfree (1980) who correctly points out the desirability of a standardized format. This data display leads easily to "new phase-old phase" discussions (e.g., Segundo and Kohn, 1980; Winfree, 1980).

As analyzed in three experiments, an IPSP in one interval could affect the following ones, even up to the fifth. The first interval N_1 (Fig. 1b) without IPSP's tended to be larger than, equal to, or smaller than the natural one, depending on whether the spontaneous rate was low (under 4/s), intermediate or high (over 6/s), respectively. There was no clear dependence of this on the phase, although for phases less than 0.2 the effect decreased somewhat; according to Schulman (1969) the slope of the N_1 versus Φ curve was generally between 0.0 and -0.3.

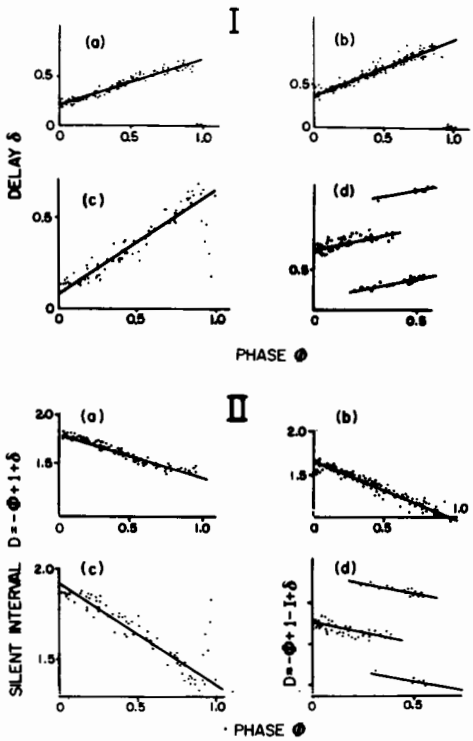


Fig. 8a–d. Delay functions. Experimental points and fitted regression lines. On the abscissa, the phase Φ (see Fig. 1b) and on the ordinate the corresponding delay δ, both normalized to the average of the last three natural intervals prior to the IPSP. **a–c** Singles IPSP. **a** and **b** are from the same preparation, spontaneous rates being around 6.4/s in **a** and 8.5 in **b**. **c** is a less common delay function type with a steep decreasing segment for phases near 1. Regression lines (aberrant points omitted): **a** $\delta=0.47\Phi+0.21$, **b** $\delta=0.68\Phi+0.36$, **c** $\delta=0.57\Phi+0.08$, **d** Pair of IPSP's (separation 100 ms). This multivalued type was seen for pairs only if their separation was close to the natural intervals (about 125 ms in this case). Regression lines for individual clusters: $\delta=0.54\Phi+1.25$, $\delta=0.53\Phi+0.75$, $\delta=0.52\Phi+0.14$. II. Silent interval functions. Experimental points and filled regresson lines. Same data as in I. On the abscissa, the phase Φ and on the ordinate, the corresponding silent interval D, both normalized as in I

In the few cases where the receptor was silenced by releasing it from its cephalic insertion and adjacent tissues, it was possible to reactivate it by stimulating the inhibitory fiber as shown for the phasic stretch-receptor by Kuffler and Eyzaguirre (1955). For example, in one case one IPSP elicited a receptor AP with a latency of 60 ms; a regular stimulus at 2.5/s kept a 1:1 locking during its application; a regular stimulus at 3.0/s caused an initial 1:1 locking followed by an increase in the firing rate of the receptor that continued for a short period after the stimulus was turned off. Inhibitory stimuli, singly or in bursts, caused an overstretched and silent receptor to fire not only on each IPSP but also for a short time after the stimulus cessation.

The influence of one steady-state regular stimulus appeared to extend beyond its duration and affect subsequent ones. So as to test for this, several presynaptic stationary regular trains at rates that spanned (and extended somewhat below and above) the segment interposed between the 1:1 and 2:1 lockings were delivered, passing immediately, i.e., without the usual wait, from one to the next and going from one extreme value to the other and back. The expectation was that interposed segments would reveal possible differences more clearly than paradoxical segments which at most would just change their boundaries. Results indicated that each stimulus train did influence subsequent ones, and that the delivery-order was significant: indeed, within the interposed segment hysteretic-like cycles became apparent involving postsynaptic rate separations of up to 1.0/s, and presynaptic bound differences of, say, 0.5/s. Dissimilarities were not simple, however, perhaps reflecting the inherent complexity of interposed segment behavior: little can be said without further examination, beyond recognizing the fact of historical influences and their importance. Hysteretic-like cycles of several types were found when the presynaptic discharge was modulated periodically (Segundo et al., 1976).

During the spring and around the molting season, it became difficult to obtain a preparation that worked, and those that did were short-lived: none of them is included in this series. It is worthwhile noting, however, that the organ's operation may be quite different at that time: in two of them, for example, repetitive inhibitor stimulation, after initially producing the effect illustrated in Fig. 1c, quickly came to produce one where the rate increased at the "on," settled down to a stationary higher than control level, again overshot at the "off" and finally settled down to a rate close to the control one.

Discussion

Earlier work had indicated that in the steady state when one pacemaker neuron elicited IPSP's in another, there were domains where "paradoxical" accelerations interrupted the decreasing trend in the coding of firing intensities and where the pre- and postsynaptic discharges were "locked" (Moore et al., 1963; Perkel et al., 1964; Schulman, 1969; Segundo and Perkel, 1969). The present work confirmed these observations, first extending them to the finer detail allowed by larger samples and by measurements as cross correlation histograms and degrees of slipping (CCH's, DS's). It is now possible to make statements about the cells' variability, interval histograms and correlations in both the paradoxical and the interposed segments. Particularly interesting is the suggestion that paradoxi-

cal segments (in ratios other than $1:r$ or $s:1$) exist within the interposed segments, and that locking is present everywhere. These features are found also in an electronic neuromime (Kohn, 1980; Kohn and Segundo, in preparation), and predicted on a formal basis for sinusoidal modulations (Glass and Mackey, 1979; Rescigno, 1978) or for synaptic relations (Segundo and Kohn, 1980).

The central finding of the present study, however, was that both paradoxical effects and locking subsisted even when presynaptic regularity deteriorated and was substituted by irregularities of substantial degrees involving interval coefficients of variation (CV) of up to about 0.20. The main implication of this is that, since irregularities within this range are exhibited by many neurons in experimental situations and probably in nature, such phenomena may well be relevant facets of natural synaptic performance and not just laboratory curiosities. In fact, a zigzag graph of tonic receptor rate as a function of tail flexion occurred in a quasi-intact crayfish preparation (Nja and Walløe, 1975) and, providing the discharges have appropriate rates and regularities, the paradoxical segment could be explained by the EPSP-eliciting connections from abdominal stretch receptors to the rostral inhibitor (Eckert, 1961), through the causal sequence "greater flexion, faster caudal receptor, EPSP's, faster rostral inhibitor, IPSP's, faster rostral receptor".

Paradoxical intensity relations at inhibitory synapses, as well as the smoothing influence of presynaptic irregularity, have been encountered both under steady-state conditions with stationary discharges when mean rates were estimated over many seconds (as described here), and under dynamic conditions with periodically modulated discharges when mean rates were displayed as cycle histograms (Segundo et al., 1976). The influence of other, or several, deviations from the rigorous conditions of earlier experiments will be described later, as observed in an electronic neuromime (Kohn and Segundo, in preparation).

The main results discussed here have implications both in the study of how the presynaptic train characteristics exert their transynaptic influence, i.e., are "coded" or "read" (Segundo and Perkel, 1969), and in the study of the temporal relationships between neuronal systems with periodic behaviors. The conclusions relevant to synaptic coding are the following, all implying a joint dependence of such a transformation on the presynaptic average intensity and variability, as measured across a prolonged train (Segundo and Perkel, 1969). i) The mean rate transformation (MRT) was either zigzag-locked, monotonic-unlocked, or intermediate, depending on the presynaptic regularity or irregularity. When zigzag-locked or intermediate, the coding was not one-to-one, as the same postsynaptic rate can result from several presynaptic ones (i.e., there is no inverse function): these may lie either in separate paradoxical or interposed segments (Fig. 2a), or within a plateau (Fig. 2c). When the observed postsynaptic rate may correspond to a paradoxical or to an interposed segment, other postsynaptic statistics aid this retrospective differentiation (Table 2; Figs. 5–7) since in the paradoxical segment the CV and degree of slipping (DS) are small and the interval histogram is unimodal and sharp, but in the interposed segment the CV and DS are large and histograms broad. However, when the postsynaptic rate may correspond to different paradoxical segments (e.g., 1:1 and 2:1), the retrospective differentation is not possible, even using other postsynaptic statistics. ii) The effects of a small presynaptic change in the intensity of a fairly regular train depends on where it happens. If, on the one hand, it is across the paradoxical-interposed boundary, it leads to special and remarkable changes in postsynaptic rate, CV and histograms, as well as in locking and alternation (Figs. 2a, 3a and e, 5a and b, 6a and b, and 7a; Table 2); if, on the other hand, it is within a paradoxical segment, there will be mainly a proportionate rate change. Variations in locking, implying as they do those in correlation, can be "read" by a third cell submitted jointly to the discharges of the pair (Segundo et al., 1968). iii) Variability is larger post- than pre-synaptically (e.g., upper rows of Table 2) within the 1:1 paradoxical segment and when presynaptic discharges are fairly regular, suggesting some perturbing noise in synaptic and/or postsynaptic (e.g., triggering) events. However, variabilities are about equal or may even be smaller postsynaptically (e.g., rows with asterisks in Table 2) when presynaptic discharges are irregular (CV 0.20 or greater): the occurrence of positive and of negative cycle slips allows the postsynaptic cell to "escape" from presynaptic jitter (Fig. 7). iv) Finally, the postsynaptic discharge intensity, in general lower during IPSP arrivals than without them, could be higher when the control discharge was weak or absent. The observation of Kuffler and Eyzaguirre (1955) that IPSP's force the silent phasic receptor neuron into firing was thus extended to the tonic one. This is yet another substantial limitation to the idea that IPSP effects, or "inhibition", is equivalent in an unqualified way to less firing: other limitations are, for example, the paradoxical effects discussed above (Fig. 2), the acceleration that follows the trough to the right of the origin in the CCH (e.g., Fig. 5g and h; Fig. 5 of Bryant et al., 1973), and rebound (Fig. 1c). This mixed effect of IPSP's is not surprising for several known mechanisms can contribute: for instance, depolarizing IPSP's [unlikely in this case because of the long latency of the evoked action potential, e.g.,

Grundfest (1974)], a release from hyperpolarization (e.g., Bryant et al., 1973; Hartline, 1976; Hartline and Gassie, 1979), multiple transmitter-receptor interactions (e.g., Shimahara and Tauc, 1975) and shifts in the topology from the stable equilibrium point assumed during overstretch to be an unstable one encircled by a limit cycle in the models of Teorell (1971) or of Cooley et al. (1965).

The time course (i.e., undershoot, adaptation, steady-state, rebound, adaptation, Fig. 1c) of the postsynaptic rate during and after the inhibitory train was similar to that in other preparations (e.g., Maynard, 1961). The partial recovery from the minimum inhibited rate and the rebound are due at least in part to a sodium electrogenic pump (Sokolove and Cooke, 1971), a negative feedback mechanism "trying" to keep the rate constant: if, for example, the cell depolarizes and its rate increases, more Na^+ enters the cell, activating the pump that then tends to hyperpolarize and decelerate. In intact crayfish, another negative feedback derives from a recurrent inhibition whereby the receptor neuron activates its inhibitor (Eckert, 1961).

The second main implication of this work is in the study of the relationships between periodic or rhythmic systems with neuronal correlates, systems that are more common than appears at first sight (Segundo and Kohn, 1980): indeed, it is apparent from the above that it is necessary to ask whether zigzag relations and locking occur in such cases, and if so, what are their consequences, advantages and disadvantages. A few exemples from physiology or pathology will be mentioned. In Aphysia ganglia, a shared presynaptic influence imposes a between-follower correlation that, if regular (evenly spaced spikes or bursts), implies a three-way phase-locking (Bryant et al., 1973; Pinsker, 1977a, b). Swimeret motoneurons in adjacent abdominal segments in the crayfish can phase-lock (1:1, 2:1 or 3:1), conceivably because of similar mechanisms (Stein, 1974). The lobster pyloric rhythm is controlled by stomatogastric ganglia networks (Ayers and Selverston, 1979; Hartline and Gassie, 1979), and locking between pre- and postsynaptic cells occurs when pacemakers are driven externally. In mammalian primary spindle afferents, the plot of rate vs. length modulation frequency is formed by positively-sloped segments with locking and negatively-directed jumps (Grüsser and Thiele, 1968). Paradoxical effects and locking have been found in the mammalian controls of the heart by the vagus (Levy et al., 1969) and of respiration by lung inflation (Glass et al., in press; Vibert et al., in press). Certain patients still show stable phase relationships between atrium (controlled by the SA node) and ventricle in spite of a complete block between them (Segers et al., 1947): the proposed explanation involves an ultimately "inhibitory" pathway from one pacemaker to another, i.e., ventricular contraction, systolic pressure peak, baroreceptor afferent volleys, medulla, vagal efferents, SA node.

The delay function is a quantitative confirmation and extension of the observation by Kuffler and Eyzaguirre (1955) that the interval lengthening by IPSP's was greater with arrivals late in the pacemaker cycle. The best linear regression $A\Phi + B$ quantifies by way of its slope A the phase sensitivity to IPSP's, and by way of its intercept B the IPSP "strength" at small phases. Delay functions of SRO's subjected to hyperpolarizing pulses (Hartline, 1976) resemble those for IPSP's, a similarity suggesting that inhibitory effects depend more on hyperpolarization than on clamping by a conductance increase. The consequence of a single IPSP extends little beyond the interval it occupies, in contrast to that of a prolonged train (Fig. 1c): therefore, the delay function usually gives sufficient information about synapse-pacemaker dynamics for prediction of locking (e.g., Segundo, 1979; Winfree, 1980): the same is not true in, say, vagus-heart preparations where inhibitory effects have long latencies and durations (Spear et al., 1979). Theoretically, for given pre- and postsynaptic rates, the maximum number of stable locked phases will be one for the observed delay functions and two for functions whose slopes were between 1 and 2 (Kohn, 1980).

Both slope and intercept increased with the spontaneous postsynaptic rate (see Results and Schulman, 1969). This could be explained as follows: higher rates usually correspond to less negative transmembrane potentials (caused, for example, by greater generator currents developed at the dendrites), and therefore to deeper and more prolonged IPSP's close to the synapses (Eyzaguirre and Kuffler, 1955; Florey and Florey, 1955; Craelius, 1976). Furthermore, PSP propagation along dendrites and soma depends on the membrane potential, theoretically at least (Swigert, 1970), and with more depolarization the propagation mode passes from the classical "passive cable pulse diffusion" to a "damped wave propagation" with attenuation, and finally to a "wave propagation" with practically no attenuation: this would imply that, when the cell is depolarized and firing faster, IPSP's are reduced less from dendrites to trigger-zone, and are therefore more effective.

The most common delay function (Fig. 8a) was best approximated by a positively-sloped segment. One-third of all functions, however, also had a few points at phases close to the natural period with delays equal to the latter plus that caused by almost zero phases. Delays were of this kind in pacemaker simulations using the Hodgkin-Huxley equations (Best, 1979), and a single small IPSP stopped the pacemaker entirely if at a critical phase: conceivably such IPSP's could

occur naturally (during the molting season, for example see last paragraph of Methods). Yet other delay functions had inverted-V shapes (Fig. 8b). If delay functions are juxtaposed so that the end of the abscissa in one cycle coincides with its beginning in the next, there will be from one cycle to another a discontinuous jump for the commonest type (which results, however, in a continuous new phase – old phase plot), and continuity for the inverted-V kind. It is not clear which biological factors determine the type of delay function in each case, though a high spontaneous postsynaptic rate favors the first one: subsequent work will pursue these issues further, examining and discussing the descriptions and the fundamental topological issues explained by Kawato (1980), Kawato and Susuki (1978), and Winfree (1980).

The reality of a mathematical model of pacemaker inhibitory synaptic interactions (Segundo, 1979) was evaluated by comparing it with the crayfish synapse, applying criteria discussed elsewhere (Segundo and Kohn, 1980). It is not the only applicable model, since Moore et al. (1963) and Perkel et al. (1964) examined computer-simulated variations of the "leaky-integrator" based upon mathematical formulations that predicted stable lockings and segment boundaries, and Schulman (1969) treated mathematically the factors (i.e., delay and resetting) that determine the main features, and those (postsynaptic variability, rebound) that disrupt them. The postulates of the Segundo (1979) model agreed with the experimental preparation in terms of pre- and postsynaptic regularity, interval lengthening by IPSP's and close-to-linear delay functions with 0.4–0.9 slopes for 1 or 2 IPSP's. Postulates departed from nature in three respects, however. Firstly, spontaneous postsynaptic intervals were not all equal, having some dispersion (CV's up to 0.05). Secondly, during IPSP arrivals, intervals without IPSP's were not all equal, since particularly at high spontaneous rates the after-effect was a shortening of the next interval for a single IPSP (e.g., $N_1 < N$, Fig. 1b), and a remarkable rebound for several (e.g., Fig. 1c). Thirdly, delay functions were not perfectly linear, showing some scatter around best-fitted lines and clearly aberrant points, or being multivalued. The two main features of the model's behavior, i.e., zigzag rate relations with paradoxical and interposed segments and locking, were clear-cut experimental findings. Discrepancies between the model and real life appeared firstly in the paradoxical-interposed boundaries that differed by under 8% for the lower rates and by more for higher ones (Table 1), and secondly in that even the best locking involved some jitter (e.g., Figs. 4a and 5a). Improvements in boundary calculations were obtained by correcting the formulae when the delay function was an inverted V (as explained by Kohn and Segundo, in preparation), by using a shorter value N' instead of N if rebound was pronounced, etc. For example, in the case (Table 1) with a 7.5/s spontaneous rate and an N of 133.3 ms the real bounds were 3.90–5.75/s: the ones calculated were 3.13–4.92/s. on the basis of the model as published, but 3.75–5.85/s. when rebound, i.e., an N' of 112.0 ms, was accounted for. These experiments defined the domain over which this particular embodiment of the model could depart from complete presynaptic regularity and still exhibit certain modeled behaviors. The domain was substantial, probably including, as discussed above, numerous natural situations: hence, the model was judged a satisfactory representation, from the particular viewpoint of this embodiment and the presynaptic regularity postulate.

The order in which tests were carried out influenced the results causing hysteretic-like behavior if between-test intervals were too short. Matthysse (1976) deduced that such phenomena should exist in any GABA synapsis: his argument was based upon the idea that, because of the way the receptors associated with the ionic channels behave, the GABA-induced conductance change should depend not only on present presynaptic activity, but also on its history. The prediction may apply also to the different dose-response curves observed in crustacean muscles when GABA perfusion with increasing concentrations is performed without or with washing (Feltz, 1971; Takeuchi and Takeuchi, 1967).

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Prof. J. P. Segundo
Department of Anatomy
School of Medicine
University of California
Los Angeles, CA 90024
USA