Source Mechanisms for Unit Activity in Isolated Crayfish Central Nervous System

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ABSTRACT As a tool for the identification of source mechanisms underlying the activity of single interneurons in the isolated crayfish abdominal cord, pulse trains from a sample of such neurons are statistically described (shape, mean, standard deviation, and serial correlation coefficient of interval distributions). These statistics were measured under four independent means of obtaining different average frequencies: (*a*) naturally occurring frequencies under standard conditions, (*b*) temperature control, (*c*) dc polarization, and (*d*) electrical stimulation of presynaptic fibers. 40 of 44 units studied had unimodal histograms, symmetrical when the mean interval was small and positively skewed with large means. Under standard conditions these units had SD = k (mean)^n, with n approximately 2. Fifteen single units were caused to fire at varied frequencies by cooling or dc stimulation. The resulting SD-vs.-mean plots for single units showed: (*a*)all points for a given unit fell approximately on a single line, regardless of whether temperature, dc, or neither was used to vary frequency, and (*b*) the average value of n (slope of log SD vs. log mean) for the fifteen units was 1.9 ± 0.2. A paradox arises from interpretation of these data via gamma distributions. It is concluded that most of the spontaneous activity in the isolated abdominal cord may result from pacemaker activity within each cell and does not require a network of active units. Finally, the fact that the SD-mean relation was found not to depend measurably on temperature is interpreted as a useful restriction on models for neuronal noise processes.

The abdominal nerve cord of the crayfish contains units which, when the cord is completely isolated from the rest of the animal, continue firing for some time—the impulse trains of different units varying widely in pattern. Those units which fire with extreme regularity appear to be analogous to the "pacemaker" cells in, for example, crustacean cardiac ganglion (Maynard, 1954; Bullock and Terzuolo, 1957) and central ganglia of *Aplysia* (Arvanitaki and Chalazonitis, 1955; Tauc, 1955). In these cases intracellular recordings have

shown that production of the impulse train involves a series of membrane depolarizations of constant form, not associated with synaptic potentials or other evidence of influence by other neurons. The question which then arises will be a main theme of this paper: do the outputs of cells with less regular discharge imply some more complicated origin of their impulse trains (e.g., several presynaptic cells), or are they simply more "noisy" pacemakers?

"Spontaneous" activity has been recorded in crayfish abdominal cord in vivo (Preston and Kennedy, 1962; Kennedy and Preston, 1963) with micropipette penetration of fibers in the connective between the fifth and sixth ganglia, and in the sixth ganglion neuropile. The most regular units were not discharged by afferent stimulation, while those showing greater variability responded in general to nerve root stimulation; in the latter, evidence was obtained that the source of background discharge was or was not accessible (in different units) to spikes of synaptic or antidromic origin. The commonest recording site in these investigations was presumably not suitable for observation of postsynaptic potentials, and no such potentials were reported.

In a previous paper (Biederman, 1964) some evidence was presented which suggested a pacemaker-type source of the activity in isolated crayfish abdominal cords. In the present study the question of possible source mechanisms for spontaneous activity was approached through analysis of the extracellularly recorded discharge of partially dissected fibers. Certain statistical measures of this discharge are used for comparison with theoretical models (in the literature) of noisy impulse source mechanisms which may be intrinsic in a single neuron, as well as with data on the discharge properties of certain tonic receptors. The latter are considered here as model pacemakers, since the repetitive process is intrinsic in the receptor cell and changes its mean rate in response to a change in the level of steady membrane depolarization.

METHODS

The statistical descriptions applied are the interval histogram, mean interval, standard deviation of interval, and serial correlation coefficient r_k (Burington and May, 1953). The latter is a measure of correlation between the size of interval I_j and that of interval I_{j+k}, where $j = 1, 2, \cdots, N$ for a train of N intervals, and $1 \leqslant k \leqslant 99$ (selected in each case as required, always so that $N \gg k$). As part of this last computation, a joint interval histogram was constructed. This is a plot of the frequency of occurrence of the joint event, "an interval of length a has, as its kth following interval, one of length b;" in the present case I_j was plotted along the abscissa, I_{j+k} was plotted along the ordinate, and the joint frequency was indicated by the symbol printed in each (square) bin.

The state of a partially dissected neuron must necessarily change in time; however, ordinary statistical analysis can be usefully interpreted only if an assumption of stationarity is adequately fulfilled. It is therefore necessary to strike a compromise between samples (interval sequences) so short that the number of data points is

inadequate and those so long that long term changes of state are reflected in an appreciable drift in the variables being measured. In the present study, the mean interval was taken as an indicator of such drift, and the following criteria were established. Those sequences in which average frequency was clearly drifting (judged by listening to the audio system and watching the oscilloscope) were not further analyzed. Of those not rejected on this basis, several were subsequently rejected because they failed the following test: in the joint interval histogram of interval I_j vs. I_{j+99}, the means of the x (I_1 through I_{N-99}) and y (I_{99} through I_N) distributions are given. The difference between these means gives the net rate of change of interval length per 99 intervals. This value was divided by the standard deviation and the unit was rejected when the resulting ratio exceeded the arbitrarily selected value 0.2. This method was selected over, for example, comparison of means of the first and last halves of the sequence, because the rate-of-change measure used here is independent of N (the number of intervals in the sample), and because it was easily obtained with the computer program used. In cases of doubt, a plot of sequential interval magnitudes was made and inspected by eye for evidence of excessive drift.

The computations were done on an IBM 7094 computer. About one-quarter of the data was converted from analog (nerve impulses recorded directly on magnetic tape) to digital form by electronic digitizing equipment (CDC computer and accessory devices). The rest was transferred from the original magnetic tape to photographic or polygraph paper; measuring of intervals and recording on IBM cards could then be done manually in one operation by use of the Benson-Lehner oscillograph reader, OSCAR Model J.

The dissection of ascending fibers from abdominal connectives of the isolated cord, and stimulation of the cord by dc, were done as previously described (Biederman, 1964). Units were commonly held in an apparently stationary state of activity for 10 to 30 min, and usually failure of a unit was marked by diminution of spike amplitude without sudden change in frequency or pattern, indicating that degeneration from the cut end rather than deterioration at the spike-originating end was at fault. Isolated cords usually yielded good units for 3 hr or longer. Temperature control of the saline bath surrounding the cord was provided by a semiconductor thermode approximately $2\frac{1}{2} \times 1\frac{1}{2}$ in. sealed with silicon stopcock grease to a thin cover glass which formed the bottom of the preparation dish. A small glass-bead thermistor monitored the solution temperature in the region of the nerve cord. Temperature was held constant to within ± 0.5°C during periods of recording at a given temperature.

RESULTS

The fifty-eight units to be discussed here had discharge patterns which required primarily stochastic description; that is, casual inspection did not indicate any systematic ordering of interval lengths within the sequence. The remaining minority of the units recorded from fired in bursts of short intervals (two to many spikes) separated by relatively long silent periods; such units will not be treated here. The "stochastically firing" units were

observed under different conditions of temperature, extracellularly imposed current, and presynaptic stimulation.

1. *Shape of Interval Histogram*

Fig 1 illustrates typical interval histograms under various conditions. The number of intervals per histogram in all cases ranged from 100 to over 1000.

The "initial conditions" (first sequence recorded after dissection of the fiber) comprised a saline temperature of 24–27°C and absence of imposed

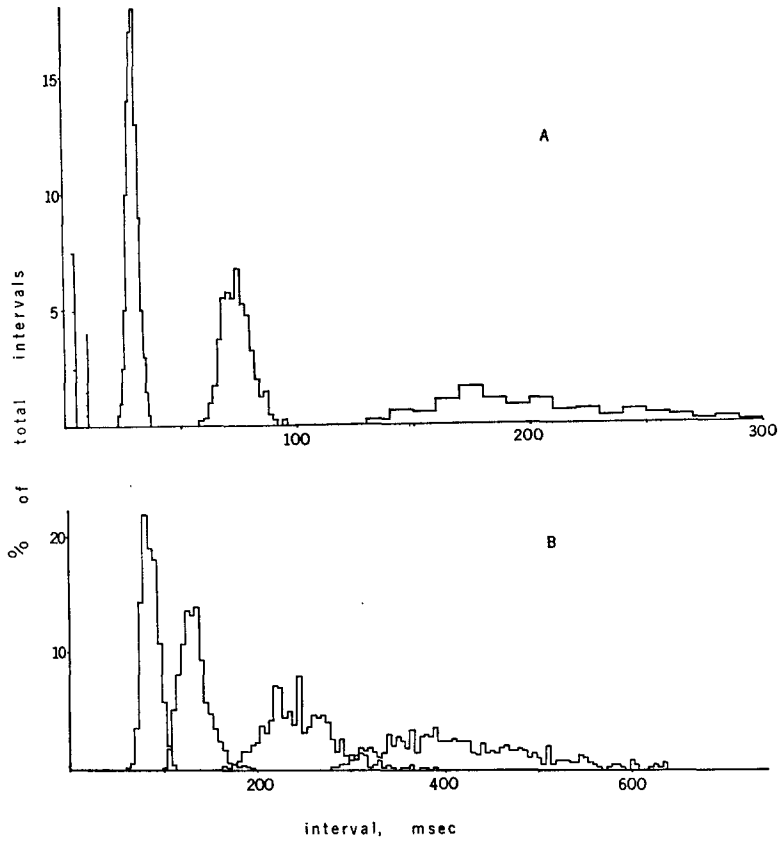

FIGURE 1. A, interval histograms of three different units, immediately after dissection from isolated crayfish abdominal cords. Temperature, 25°C. Since the three graphs use different bin sizes, equality of area under the curves was preserved by scaling the vertical axis: the left axis belongs with the left histogram, middle with middle, and right with right (the divisions indicate 5, 10, and 15% in all cases). N = 2000, 861, and 265, respectively (left to right). B, interval histograms from a single unit at different temperatures. From left to right, 25°C (N = 497), 19° (1292), 16° (495), 13° (503). The increase in SD and skewness with increased mean is typical for neurons under the influence both of temperature change and of dc stimulation.

dc or presynaptic stimulation. Under these conditions, it was generally found that units firing at high discharge rates had symmetrical, narrow interval distributions, while more slowly firing units had broad, more positively skewed distributions (Fig. 1A). Forty out of forty-four units could be thus described.

When the temperature was lowered or dc was applied to the cord, the units changed their mean firing rate. Thus different interval distributions,

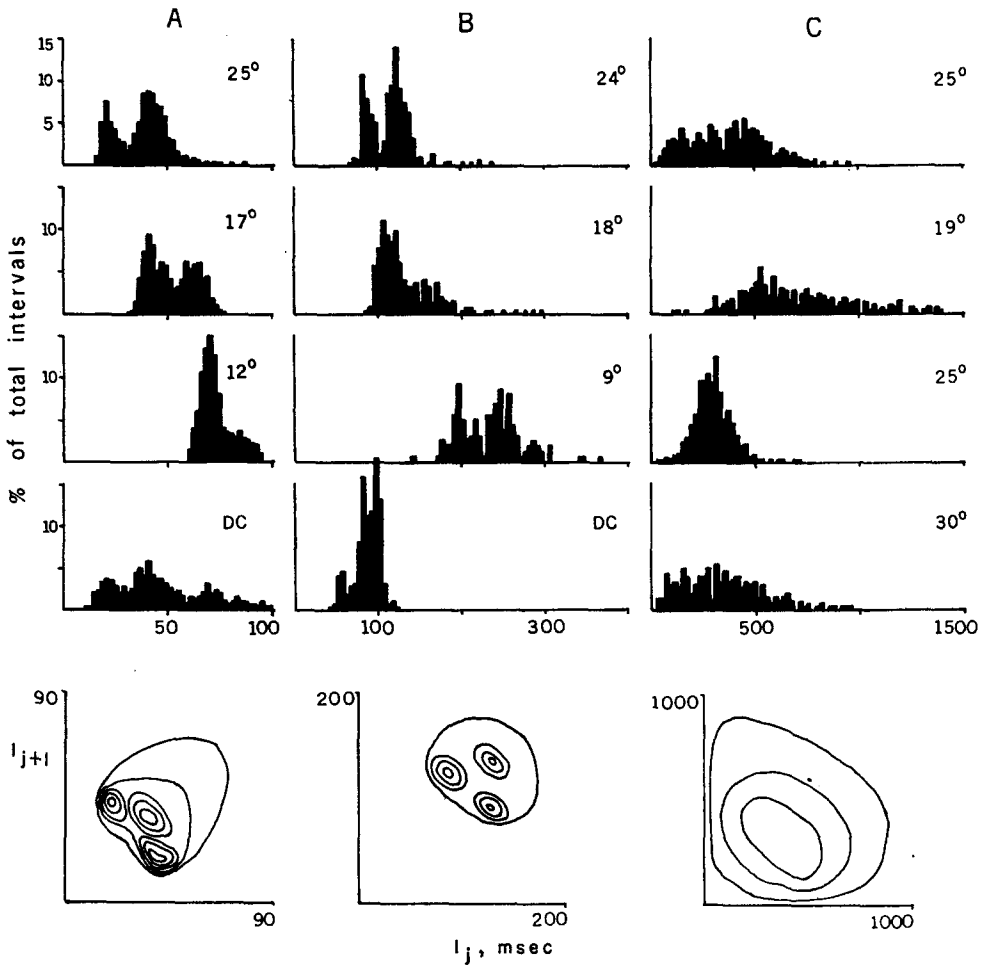

FIGURE 2. Examples of multimodal histograms of four units (two units gave essentially identical results, shown in C. They were from different animals and therefore may have been the corresponding neuron). Beneath each set of histograms is a diagram (approximate constant density lines) of the first-order joint-interval histogram corresponding to the initial condition (24–25°C). All three of these distributions show fairly large negative serial correlation (r_1).

reflecting different states of the source process, could be obtained from a single unit (Fig. 1B). Histograms thus obtained from the forty "unimodal" units were found to be symmetrical and narrow when the mean interval was small, and broad and positively skewed when the mean was large, under all conditions.

The remaining four units are shown in Fig. 2: their histograms under initial conditions had several modes or were negatively skewed. The histograms of these units changed in an apparently nonsystematic manner as temperature and current were changed.

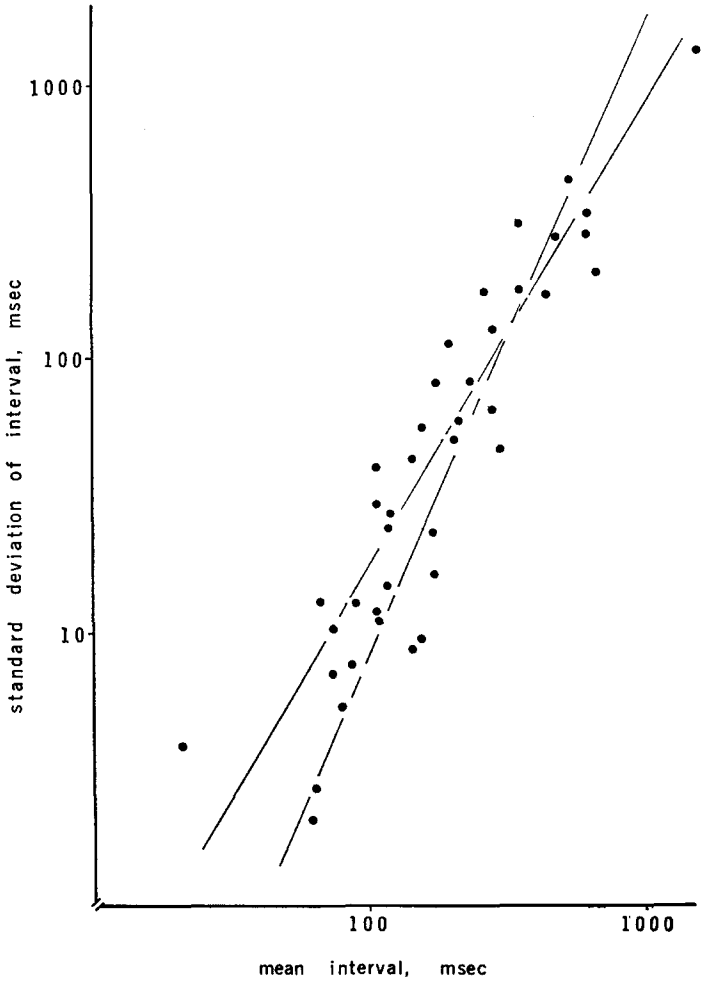

FIGURE 3. Log-log plot of standard deviation of interval against mean interval for forty units with unimodal histograms. The reference lines show the extreme range of conceivable fits to the data points (they have slopes of 1.7 and 2.3). The slope of the best fit line, which corresponds to the exponent of the power relation, would be between these, or approximately 2 (i.e., SD = k(mean)^2).

2. *Standard Deviation vs. Mean Interval*

A more quantitative measure of the way in which the spread of interval distributions changes with changed mean is given by plots of standard deviation vs. mean. Fig. 3 shows such a plot for the interval distributions under initial conditions for the forty unimodal units. The points appear to lie around

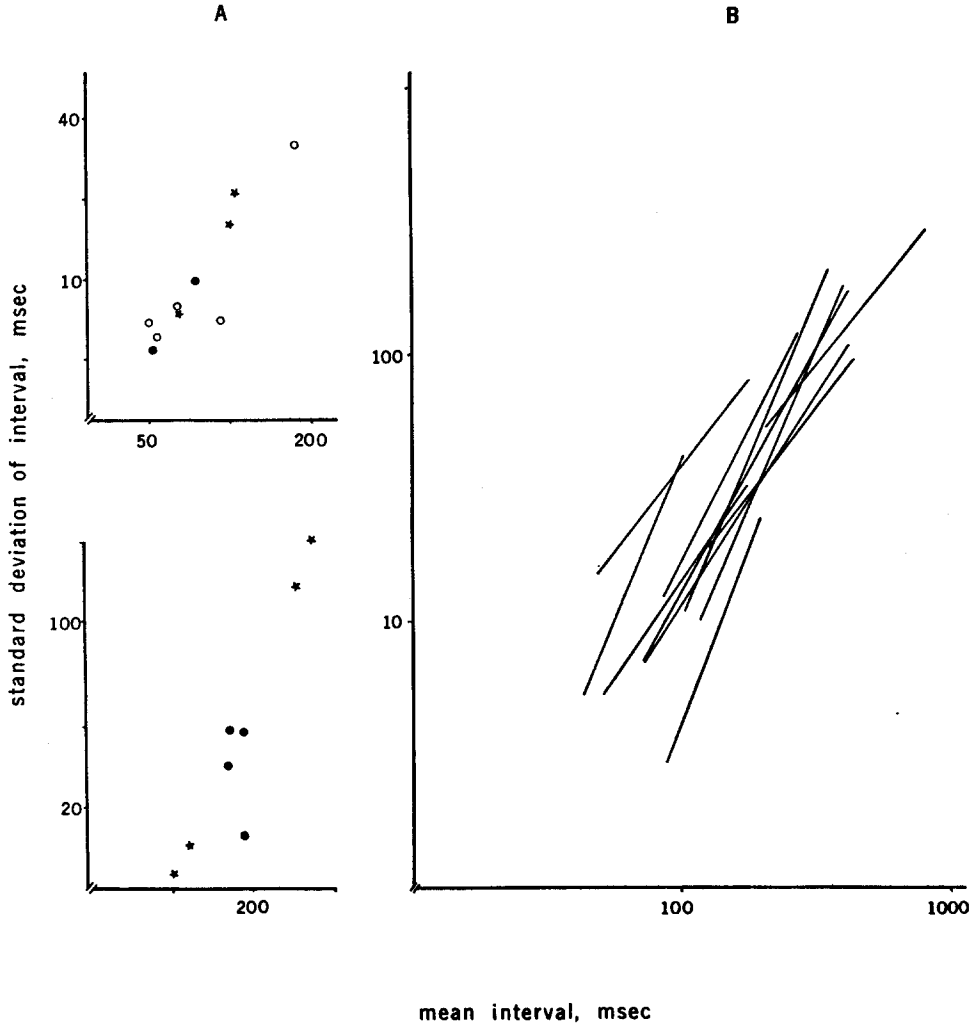

FIGURE 4. A, double logarithmic plots of SD vs. mean interval for two different units, each under a variety of conditions. Closed circles, standard conditions (25°, no dc stimulation); open circles, temperature lowered; stars, during stimulation with inhibitory or excitatory dc. Quite often the cells, after stimulation, stabilize at a discharge rate different from the original one; this leads to the presence on many graphs of several closed circles in different locations. B, slopes and positions of ten such graphs, estimated by eye as in Fig. 2. The length of line indicates the range of frequencies covered. The distribution of slopes overlaps the distribution of single-points-per-unit shown in Fig. 2.

a straight line, which in a log-log plot indicates a power relation. The slope of the best fitting straight line is approximately 2. Thus, at the moderate levels of precision required and justified here, the relation between mean interval and standard deviation for different units under standard conditions may be described by

$$SD = k\ (\text{mean})^2.$$

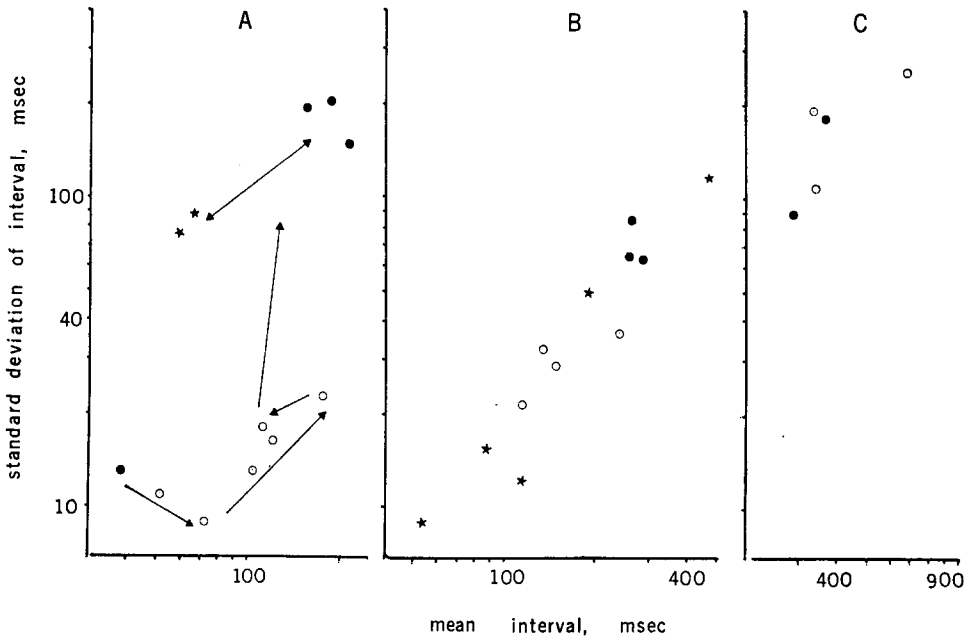

FIGURE 5. Log-log plots of SD vs. mean for the three types of multimodal units shown in Fig. 2. Symbols as in Fig. 4. The arrows in A show the order in which samples were taken.

Fig. 4 shows some corresponding plots for single units under different conditions. Owing to the necessity of avoiding steady drift and to a sometimes irreversible effect of extreme cooling, it was difficult to get an adequate number of points for single units. However, in fifteen units it was possible to record four or more acceptable impulse sequences of different mean frequency. Plots from these units had two noteworthy features. First, the points for a given unit tended to fall on a straight line, regardless of the temperature or level of imposed current (Fig. 4A). Second, the (eye-fit) slopes of these lines averaged 1.9, with SD = 0.2 (Fig. 4B).

Unit standard-deviation-vs.-mean plots for the four "multimodal" neurons are shown in Fig. 5. One of these has a linear distribution of points similar to those of the unimodal units (Fig. 5B, the same unit as in Fig. 2B); the others are more complex.

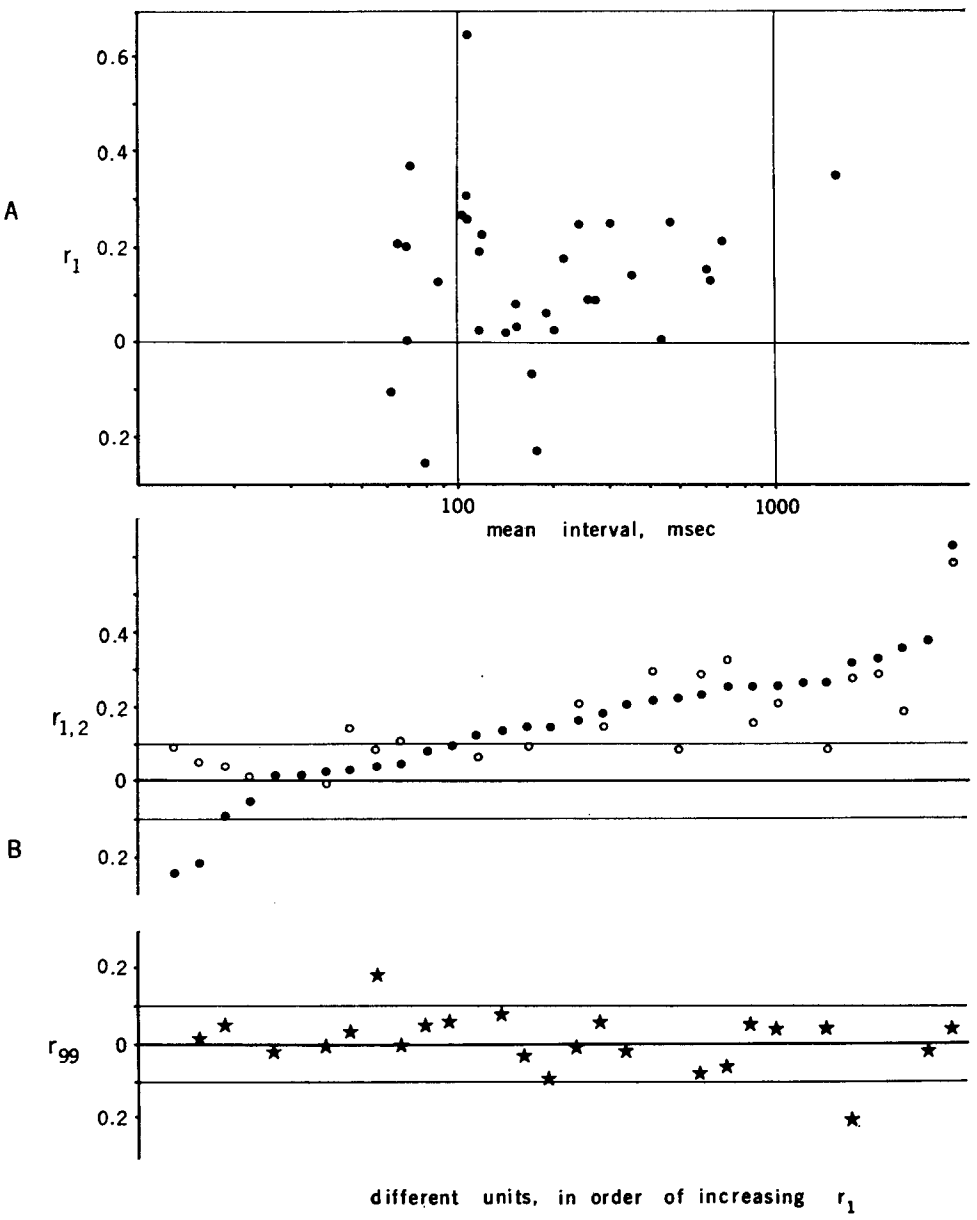

FIGURE 6. A, r_1 as a function of mean interval; each point represents a different unit. There is no apparent correlation between r_1 and mean. B, comparison of different order r. Each vertical column of points represents a single unit for which r_1 (solid circles), r_2 (open circles), or r_{99} (stars) was calculated; for some units all three coefficients were computed. The units are arranged in order of increasing r_1 ; r_{99} shows no correlation in magnitude with r_1 , but r_2 tends to increase with r_1 . Confidence limits may be computed for the correlation coefficients from a normal distribution, based on the number in the sample; by such criteria, the r_1 greater than 0.1 are, with two exceptions, significantly different from zero ($p < 0.01$, according to Table A-30a in Dixon and Massey, 1957).

3. *Serial Correlation Coefficient*

Fig. 6 presents the results of computations of r_1, r_2, and r_{99} for thirty-one of the unimodal units under initial conditions. The coefficients for adjacent intervals (r_1) were mostly positive, ranging from about -0.2 to $+0.6$; there

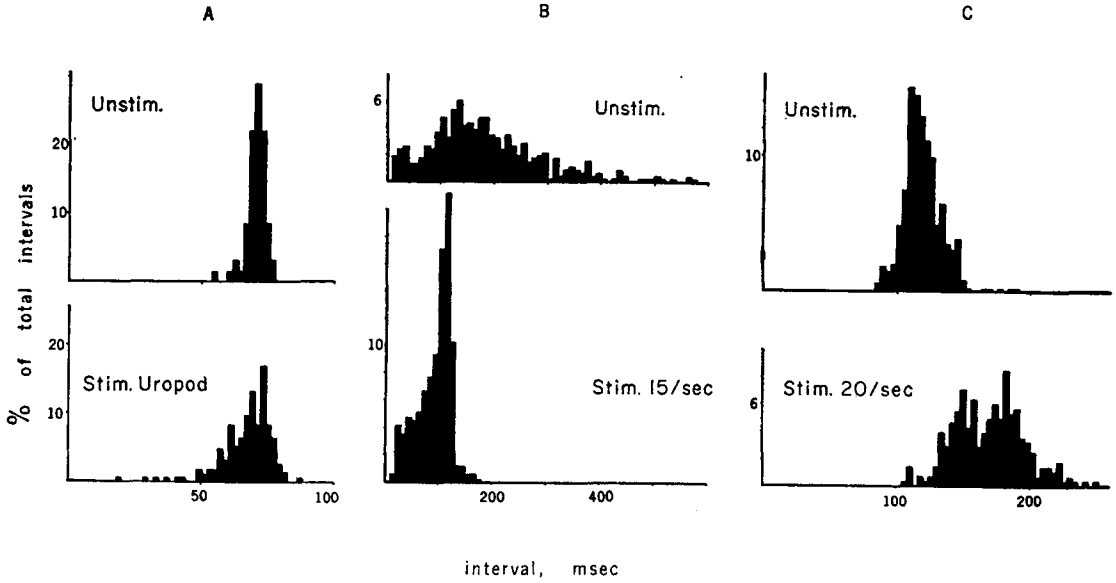

FIGURE 7. A, change in interval histogram of a pacemaker when phasic synaptic input is applied. The upper histogram is from the caudal photoreceptor neuron, recorded with abdominal cord *in situ*, lighted room; no intentional stimulation. The lower is a histogram of activity during a period when a uropod was flexed and released several times, stimulating mechanoreceptors which synapse with the photoreceptor neuron. B and C, changes in interval histogram of ascending neurons produced by stimulating electrically the abdominal-thoracic connectives. B, a unit which appears from its "unstimulated" histogram to be a pacemaker with some synaptic input. Under stimulation the histogram acquires a strong negative skewness; when the ganglion nearest the recording site was progressively isolated by cutting away other parts of the cord, the discharge rate became higher, (either by removal of inhibition or by stimulation via injury discharges following cutting) and the histogram was more symmetrical. C, another unit which was inhibited by presynaptic stimulation; the bimodality of the histogram, which developed during stimulation, disappeared slowly in subsequent unstimulated histograms.

was some tendency for r_2 to increase with r_1. In all but two cases r_{99} fell between ± 0.1. There was no apparent correlation between the magnitudes of r_1 and mean interval (Fig. 6A). The multimodal units had relatively large negative r_1 under initial conditions; their joint interval histograms are shown in Fig. 2.

4. *Effects of Presynaptic Stimulation*

The response of ascending neurons with intact afferents to mechanical stimulation of the integument was markedly transient in nature. For example, in some spontaneous neurons bending of one uropod produced a brief burst at each movement, the mean frequency of the basic spontaneous discharge remaining approximately constant between bursts. To illustrate that the mode of the unstimulated histogram could be approximately preserved but a negative skewness introduced, the uropod was stimulated repeatedly during a sample of record for which the interval histogram was computed. Fig. 7A compares such a histogram with that for a sample in which no stimulation was done. (The slight increase of long intervals in the stimulated case may be due to a simultaneous inhibitory component in the stimulus.)

However, for comparison with the temperature and dc data, a sustained level of synaptic input was desired. The nerve roots were so short that spread of stimulus current applied to them might easily have affected the ganglion directly. Therefore the anterior end of the cord was stimulated while activity in a single ascending neuron (3–4 connective) was monitored. With 1 cm or more of grounded solution between stimulating and recording sites, effects of current spread were minimized, and the fact that the ascending axon recorded from was broken anterior to the recording site further reduced the possibility of direct stimulation of a part of that unit.

Only four out of fourteen units tested showed a measurable response to such stimulation. One of these (not illustrated) responded in a discrete manner to the stimulus: each stimulus pulse was followed by a silent period lasting 250 to 400 msec. The unstimulated interval histogram had a pronounced positive skewness with the form of a very long, flat tail, and its SD was large, well outside the SD range for units with similar mean interval shown in Fig. 3. In the other three responding units there was no apparent preferred phase relation between stimulus pulses and recorded spikes. Fig. 7B and C shows histograms for two such units, one excited and the other inhibited by the descending stimulation. With this form of stimulation the mode as well as the shape of the histogram was changed. In standard-deviation-vs.-mean plots for each unit the points fall very nearly on a straight line, and in all three cases this line has a slope of about 2.

DISCUSSION

Summary of Data

Three aspects of the data from unimodal units are most relevant to the question of source mechanisms for the activity:

1. The histogram shapes and standard-deviation-vs.-mean plots for single

units under varied conditions were similar to those for all units under initial conditions. Said in another way, the differences in these measures between units under standard conditions tend to disappear when the conditions are adjusted so as to make the *discharge rates* of the units the same.

2. It might have been expected that the total population of units would separate into distinct subgroups with respect to shape of histogram, location on the "initial conditions" SD-vs.-mean plot, or slope of single-unit SD-vs.-mean curve. This would have suggested the presence of different source mechanisms associated with the different subgroups. Such a result was not found, however: the unit-to-unit variation in all three of these measures was *continuous* over the entire range found, from regularity of discharge to extreme irregularity.

3. On the single-unit SD-vs.-mean plots about the same relation between these variables was maintained regardless of temperature, imposed polarization, or intrinsic variation in the state of the neuron. That is, the "noise"-generating elements of the source mechanism do not seem to be *specially dependent* on any of these conditions.

Implications of Data; Pacemaker vs. Synaptic Driving

Points 1 and 2. If one assumes that those impulse sequences which are extremely regular are produced by an intrinsic relaxation oscillation, there is no evidence that a different mechanism is involved in the other units; rather, the properties of the most regular units are found also in the most irregular ones.

Point 3. The effect of temperature on different cells in the cord varies (in both magnitude and direction), and the effect of dc is to some extent dependent on the orientation within the cord of the stimulated cell (Biederman, 1964). Therefore this point argues against a source hypothesis in which the monitored cell reflects the superposition of input trains from several presynaptic cells. That is, it is not to be expected that the stochastic properties of the discharge of a cell driven by several other cells would be the same regardless of which of these parameters (temperature, dc, or neither) is varied. It is therefore suggested that the unimodal units—a majority of the spontaneous units in the cord—are driven by a pacemaker mechanism intrinsic to the cell monitored.

Consistency with Pacemaker Models

Comparison with models in the literature shows that the data obtained can be expected from current notions of an intrinsic pacemaker process. Several models of spike-generating processes have been examined with respect to the stochastic properties of their output (see particularly Hagiwara, 1954; Viernstein and Grossman, 1960; Gerstein and Mandelbrot, 1964; Goldberg,

Adrian, and Smith, 1964). Fundamentally such models have consisted of a nonlinear function of time ("recovery" function, usually taken as having the form e^{-ct} or $e^{c/t}$) intersecting a "threshold" function (constant or time-varying) to produce a spike, with noise of a variety of descriptions superimposed on one of the functions. In some of the papers cited the noise was considered presynaptic and in others, intrinsic in origin. Many varieties of this basic model can produce a sequence of interval histograms qualitatively similar in shape to those presented here, if the mean spike frequency is varied by changing the level of the threshold with respect to the recovery (or "pacemaker") function.

Another initially appealing notion is as follows: postsynaptic potentials arising according to the rules defining a Poisson process, or intrinsic events following the same rules (e.g., possible random intracellular activation of acetylcholine), will give rise to an output distribution of intervals corresponding to a gamma distribution, if every rth event produces a spike (r = constant). This type of model has been extensively developed by Stein (1965), who showed that suitable choice of the three parameters p (rate of the Poisson process), r (threshold or cell "scale factor"), and t_o ("refractory period", off-time of the scaler following each spike) allowed fitting of gamma distributions to the output interval distributions of a variety of neurons. Symmetrical and positively skewed distributions of the type presented here could also be fitted approximately by gamma distributions; however, one encounters the following difficulty in attributing physical significance to the parameters. If the mean of a gamma distribution is changed by varying r, SD $= k(\text{mean})^{1/2}$; if it is changed by varying p, SD $= k(\text{mean})$. Therefore both initially plausible notions, that the mean interval of the neuron output is changed by varying either the rate of a Poisson process or the relative threshold alone, are not compatible with the finding that SD $= k(\text{mean})^2$. Both must be changed whenever frequency is changed, to fit the crayfish and receptor data.

The way that these parameters must change may be computed from the equations for mean and variance of a gamma distribution (Stein, 1965):

$$\text{mean} = r/p + t_0 \qquad \text{variance} = r/p^2$$

If the refractory period is neglected, for example, it follows immediately that

$$p = kr^{3/2}$$

is the required constraint to produce a quadratic relation between standard deviation and mean. That is, as frequency increases the parameter r must increase, providing the observed increase in symmetry; but since r represents the threshold the paradox arises that threshold must increase at higher frequencies rather than decrease. Inclusion of t_o complicates the above equa-

tion but does not change the direction of the dependence: r must still increase at higher frequencies.

Since the threshold concept properly involves several membrane variables (conductances, voltage, $d(\text{voltage})/dt$), it is conceivable that there may be such a relation between p, t_o, and r as the "gamma distribution" hypothesis suggests. Some intrinsic constraint between these variables would then be suggested, since it is unlikely that the effect of temperature upon three *independent* variables would give the same SD-vs.-mean relation as that of dc or of any of the other factors operating in these experiments; the observed result would be more plausible on the assumption that temperature, dc, etc. each act upon one of the variables, with an intrinsic constraint leading to the invariance of the SD-vs.-mean relation. The events in the supposed Poisson process (when the model is applied to the crayfish data) are not likely to be presynaptic spikes, since their rate p would often have to be higher (on the order of 1/msec) then would be expected considering the small number of active fibers estimated in this preparation (Biederman, 1964) and the rarity of fibers firing more than 1 spike/100 msec. The experimental data cannot rule out the suggestion of a system in which an intrinsic noise process, refractory period, and threshold interact in the particular way corresponding to the gamma distribution hypothesis; however, the previously cited "recovery function" hypothesis seems a more heuristically valuable interpretation of the present data, in light of the above difficulties.

Verveen and Derksen (1965) have found that the noise (voltage fluctuations) inherent in frog axon produces probabilistically describable variations in latency of the spike evoked by a constant stimulating pulse. Comparison of the latency distributions obtained with pulses of different intensities revealed a quadratic relation between standard deviation and mean, and a change in histogram shape from symmetry at small mean to positive skewness at large mean. A quadratic relation between mean interval and SD was also found to hold, over a range of frequencies (excluding the low-frequency samples), for impulse trains from frog stretch receptor (Hagiwara, 1954; Buller, Nicholls, and Ström, 1953) and carotid-body chemoreceptor neurons (Biscoe and Taylor, 1963). The possibility is therefore suggested that a process similar to that present in Verveen's axonal preparation may exist at the neuronal loci of intrinsic relaxation oscillators.

If one assumes intrinsic noise and nonlinear recovery processes in the neurons studied here, there remains the question whether the setting of the relative levels of threshold and recovery process is intrinsic as in the classical pacemaker-cell concept, or is the result of smoothed synaptic activity. The common observation in crayfish ventral cord is that presynaptic activity has a discrete, brief effect on the postsynaptic discharge (for examples see above and Kennedy and Preston, 1960; Preston and Kennedy, 1962; Kennedy, 1963).

This would suggest that the cells studied were indeed driven by an intrinsic pacemaker mechanism. However, when the whole anterior cord was stimulated with electrical pulses, three cells showed a response very similar to that of other cells to cooling and dc stimulation (Fig. 7), even though the stimulus rates were fairly low (15 to 20 pulses/sec). This indicates that a massive enough input can produce a smoothed effect on discharge pattern in some crayfish units. The possibility that many of the cells studied were receiving such input cannot be excluded but seems unlikely in view of the previously mentioned limited total activity in the isolated cord.

Departures from Model Predictions

Neither the models nor the receptors mentioned above showed any serial correlation. If the values of r_{99} in the present experiments are taken as an estimate of the correlation-coefficient values which might arise by chance in these data, about half the neurons have positive r_1 values of "significant" magnitude (greater than approximately 0.1). Positive serial correlation has occasionally been found in interval sequences from other neurons (Rodieck, Kiang, and Gerstein, 1962; Poggio and Viernstein, 1964); such correlation could be the result of slow fluctuations in the relative trigger level or of low frequencies in the supposed noise spectrum.

The four multimodal units suggest the occasional occurrence of more complicated source mechanisms. Several plausible schemes can be constructed to account for their discharge characteristics (e.g., one pacemaker cell receiving input from another, or one neuron with multiple pacemaker loci), but since experimental constraints are not available to test these models they will not be discussed in detail here.

Other Implications of the Data

The statistics of neuron discharge have not previously been compared under varied temperature and applied dc. The use of these techniques in the present study provides certain constraints on the mechanisms of noise production. These mechanisms are unknown, although several candidates have been suggested (for a summary see Verveen and Derksen, 1965). In the present experiments no *special* effect of temperature on the noise process was detected. If the noise power were proportional to absolute temperature, this is what might be expected. The expected small change would not be noticed here. The experiment does, however, exclude all models of the spike generation in which appreciable temperature dependence (Q_{10} appreciably different from unity) of the noise process is reflected in the SD-vs.-mean relation.

Finally, it was argued above that the only way in which presynaptic activity could affect the discharge of the majority of the neurons studied here is by summing to exert a dc bias on the postsynaptic cell. That is to say, the

discharge of the postsynaptic cell reflects nothing of the temporal structure of the presynaptic discharges. These results support the useful notion that wherever histograms and SD-vs.-mean relations like those reported here are found in neuronal statistics, they may reflect properties of the cell recorded from rather than those of possible presynaptic elements.

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