A SUPERPOSITION MODEL OF THE SPONTANEOUS ACTIVITY OF CEREBELLAR PURKINJE CELLS

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ABSTRACT Based on physiological evidence for multiple firing zones in the dendritic arborizations of cerebellar Purkinje cells, a superposition model is proposed for spike triggering in these cells. Spike trains from 10 Purkinje cells were analyzed in terms of independence of interspike intervals and the properties of their variance-time curves. The results of this analysis were found consistent with the hypothesis that the spike train of a cerebellar Purkinje cell is the pooled output of a relatively large number of independent component processes. Simplifying assumptions as to the statistical nature of these processes lead to a very rough estimate of the number of firing zones.

INTRODUCTION

The mode of neuronal spike triggering plays a central role in determining how a neuron processes afferent information, and presumably relates to its functional role. On the one hand, some neurons in the anteroventral cochlear nucleus, for example, are innervated by about three cochlear nerve fibers per neuron, and it is believed that a single impulse in any of these fibers is sufficient to initiate a spike (Molnar and Pfeiffer, 1968). On the other hand, each granule cell of the cerebellar cortex receives synaptic inputs from about four mossy fibers (Szentágothai, 1968), but summation of the excitatory actions of two or more mossy fiber impulses is required to trigger a granule cell spike (Eccles, Sasaki, and Strata, 1967; Eccles, Táboříková, and Tsukahara, 1970). In neurons having many weak excitatory inputs, summation of the synaptic action of even a larger number of afferent impulses is required to initiate a spike. It is not certain if the spike is initiated at a single site in each neuron; multiple dendritic firing zones have, in fact, been postulated for hippocampal pyramidal cells (Spencer and Kandel, 1961) and cerebellar Purkinje cells (Eccles et al., 1966).

The present study is aimed at characterizing spike triggering in cerebellar Purkinje cells in terms of a superposition model, according to which the output spike train is

considered to arise from the pooling of a number of individual component processes. Probabilistic and statistical aspects of superposed stochastic point processes have been discussed by several authors (Cox, 1962, p. 71–79; Cox and Lewis, 1966, p. 210–222; Cox and Miller, 1965, p. 362–365; Cox and Smith, 1953, 1954; Gnedenko, 1967, p. 425–432; Grigelionis, 1963; Khintchine, 1960, p. 49–56; Ososkov, 1956; ten Hoopen, 1967). Nonetheless, both the theory and applications of superposed processes are at an early stage of development. The present investigation of Purkinje cell spike trains in terms of superposed processes can therefore only be exploratory in nature. It is hoped that an appreciation of the practical importance of superposition models and of the limitations of available statistical techniques will spur further development of the theory of superposed processes and its application to problems of spike triggering in some types of neurons.

METHODS

34 adult cats weighing between 2.5 and 3.5 kg were either decerebrated surgically under ether at the intercollicular level, or lightly anesthetized with sodium thiopental or pentobarbital (Murphy and Sabah, 1970 *a*). During the experiment the animal was paralyzed with gallamine triethiodide and securely held in a rigid stereotaxic frame (AB Transvertex, Vårby, Sweden). Temperature, end-tidal CO_2, and electrolytic balance were maintained within physiological ranges. Recordings were made from tracks in the vermis or pars intermedia of the anterior lobe of the cerebellar cortex, using 2–8 MΩ glass microelectrodes filled with 2 M NaCl. Respiratory movements and pulsations were minimized by covering the exposed cortex with a solution of 3.5% purified agar and 10% sucrose, and, occasionally, by pneumothorax and cisternal drainage at the level of the foramen magnum. The mechanical stability of the experimental setup was such that the activity of a single Purkinje cell could sometimes be recorded for several hours.

Purkinje cells were identified either by their antidromic response to juxtafastigial stimulation or by their characteristic, spontaneous or evoked, climbing fiber responses (Eccles, Ito, and Szentágothai, 1967, p. 71–73 and 157–158). Additional evidence was provided by the size, polarity, and characteristic pattern of spike discharges, and by location in the Purkinje cell layer, as indicated by field potential depth profiles of mossy fiber and climbing fiber activity (Eccles et al., 1968).

Before the spike activity of a Purkinje cell was recorded it was ascertained that the level of discharge was not affected by small movements of the microelectrode, thereby minimizing the possibility of mechanical injury contributing to the spontaneous discharge. For the same reason, largely positive spikes or "giant" spikes were ignored. The spike activity was processed on-line by means of a Fabri-Tek 1062 special purpose computer (Fabri-Tek Instruments, Inc., Madison, Wis.) which was used to obtain interspike interval histograms and their associated cumulative frequency distributions. It was thus possible to detect gross trends in firing frequency or changes in the firing pattern. Spike trains satisfying the following requirements were recorded for subsequent detailed analysis:

(*a*) Comparative rarity of spontaneous climbing fiber responses. These responses generally occur at a slow rate (0.5–3/sec) and consist of about four spikes followed by a silent period of variable duration (Murphy and Sabah, 1970 *b*). For the purpose of this preliminary investigation it was deemed advisable to exclude as much as possible the complicating effects of the climbing fiber response on the background activity of Purkinje cells.

(*b*) Absence of gross trends in firing frequency or pattern of discharge over time periods including at least 1000 consecutive spikes.

Detailed statistical analysis was performed on the CDC 6400 computer (Control Data Corp., Minneapolis, Minn.), using SASE IV program (Lewis et al., 1969) modified as appropriate to suit the requirements of this investigation and to make it compatible with CDC 6400 Fortran.

Spike trains that showed no significant trends in firing at the 5% level according to SASE IV program (Lewis et al., 1969) were tested for independence of interspike intervals (see below). All the spike trains so tested exhibited a high degree of independence of intervals. Stationarity was therefore assessed by testing for significance at the 5% level as follows:

(*a*) Three samples of 150–250 intervals each were taken from the beginning, middle, and end of the spike train under consideration. A two-sided Kolmogorov-Smirnov test was applied to the three samples (Fisz, 1963, p. 448).

(*b*) All the intervals in the spike train were used to test for a monotone trend in the rate of firing by means of a permutation type test based on exponential ordered scores and a linear orthogonal polynomial (Cox and Lewis, 1966, p. 54–58).

Only those spike trains which satisfied these criteria were considered to be adequately stationary (Cox and Lewis, 1966, p. 59–69; Perkel et al., 1967) and therefore suitable for the type of statistical analysis described in this paper. Because of these requirements, it was found that out of the recorded output of 116 Purkinje cells, spike trains from only 10 Purkinje cells were suitable for detailed analysis. Each of these samples contained 700–1500 consecutive interspike intervals.

A limitation of the techniques employed in this investigation is that they are only valid for stationary processes. Seemingly, the 10 spike trains analyzed constitute, from the statistical point of view, a sample that is biased in favor of stationarity. But it may be argued that nonstationary is a consequence of changing inputs to the cell and would not invalidate the applicability of a superposition model. In principle, it should be possible to filter out trends in firing and consider the filtered events. However, such problems are still not well understood (Lewis, 1970).

SYMBOLS

C Coefficient of variation of interspike intervals (standard deviation/mean).
m Mean rate of occurrence of events.
n Number of intervals in a sample of interspike intervals.
p Number of component processes in a pooled output process.
t_o Total duration of spike train.
$V(t)$ Variance of the number of intervals in an interval of time $(0, t)$.
δ Absolutely refractory period.
λ Mean rate of occurrence of events in a Poisson process.
ρ_j Serial correlation coefficient of order j.

$\sim$ placed over a symbol denotes the finite sample estimator for the quantity represented by the symbol. A superscript (p) refers to the pooled output of p component processes.

SPONTANEOUS DISCHARGE OF PURKINJE CELLS

The spontaneous activity of cerebellar Purkinje cells has been described in a variety of preparations (Bell and Grimm, 1969; Bindoni et al., 1967; Braitenberg et al., 1965; Crepax and Infantellina, 1957; Eccles et al., 1966; Granit and Phillips, 1956;

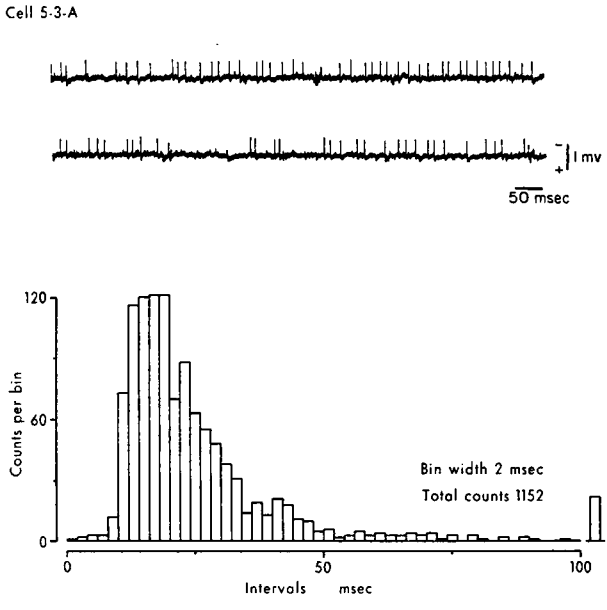

FIGURE 1 Interspike interval histogram of a Purkinje cell. In the last bin are lumped all the intervals larger than the maximum value of the horizontal scale of the histogram. Samples of the spike activity of the cell are shown.

Laget and Delhaye-Bouchaud, 1966; Schlapfer, 1969; Snider et al., 1967; Thach, 1968). A study has recently been made of the statistics of the spontaneous discharges of Purkinje cells in decerebrate cats and in cats anesthetized with thiopental or pentobarbital (Murphy and Sabah, 1970 *a*). As far as the results of this investigation are concerned, no appreciable differences were found between Purkinje cells from unanesthetized decerebrate or lightly anesthetized cats. Because of the smallness of the sample, however, no firm conclusions can be drawn as to significant differences between these preparations and no further distinctions will be made on this basis.

Cerebellar Purkinje cells generally discharge irregularly and continuously at mean frequencies in the range 10–70 imp/sec (Murphy and Sabah, 1970 *a*). Approximately 80% of the 116 Purkinje cells studied had interspike interval distributions with exponential or longer than exponential tails, as illustrated in Figs. 1 and 2. The interspike interval distributions of the remaining cells were mostly broad and irregular, a few being of diverse shapes. Spike trains of Purkinje cells usually have a number of relatively long intervals of up to 2 or 3 sec, in which case the coefficient of variation of the intervals may exceed 3.0. When these long intervals are absent, or are deleted from the sample of intervals, the coefficient of variation is usually in the range 0.5–1.0. Autocorrelograms obtained in the usual way (Perkel et al., 1967; Bell and Grimm, 1969) are illustrated in Fig. 3 and are similar in shape to the auto-

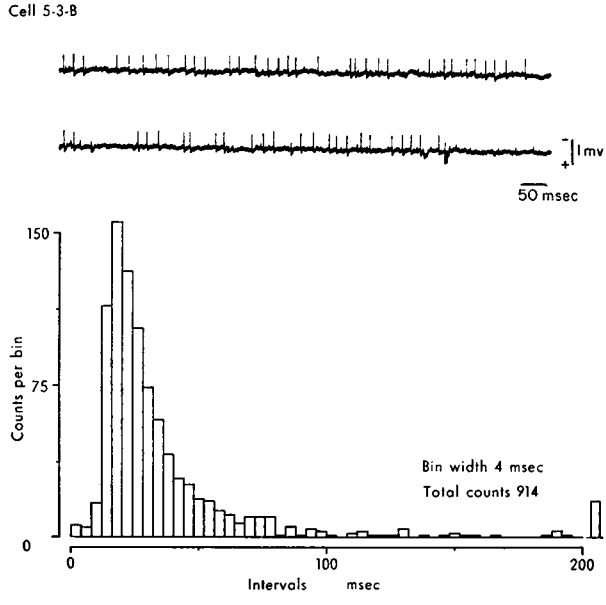

FIGURE 2 Interspike interval histogram of a Purkinje cell, illustrated as in Fig. 1.

correlograms of some irregularly firing neurons (Perkel et al., 1967; Segundo et al., 1968).

FIRING ZONES OF PURKINJE CELLS

There is physiological evidence that at least the larger dendrites of Purkinje cells are capable of supporting spike conduction (Eccles et al., 1966; Eccles, Faber, and Táboříková, 1970; Fujita, 1968; Llinás and Nicholson, 1969). The analysis by Eccles et al. (1966) of field potential profiles following antidromic stimulation of Purkinje cells in the cat has revealed that the antidromic impulse invades almost synchronously, approximately 100 μ out of a total height of about 300 μ, for the dendritic expanse of a Purkinje cell. This invasion has been attributed to trigger zones of low threshold in the dendrites. A remarkable feature of the antidromic invasion of the soma-dendritic region of Purkinje cells is a relatively refractory period of 3–4 msec at all depths between 200 and 400 μ (Eccles et al., 1966). The absolutely refractory period is presumably about 2 msec or less. In fact, Marchesi and Strata (1970) report interspike intervals as short as 3 msec for mossy fiber–driven activity of Purkinje cells in awake, unanesthetized cats.

Llinás and Nicholson (1969) cited evidence for preferred centripetal conduction of dendritic spikes and for collisions of these spikes following stimulation of the surface of alligator cerebellar cortex.

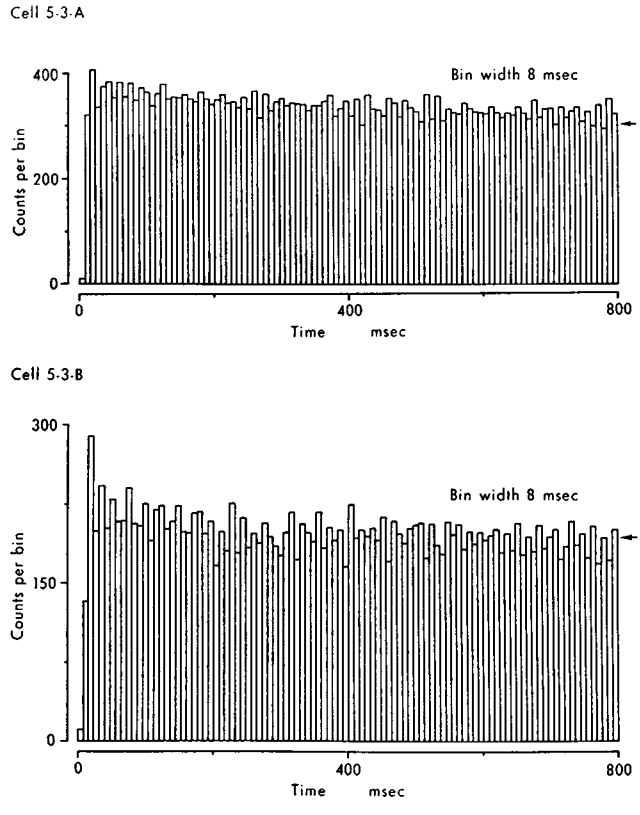

FIGURE 3 Autocorrelograms of the same two Purkinje cells of Figs. 1 and 2. The arrows on the right mark the asymptotic level corresponding to the mean firing frequency.

SUPERPOSITION MODELS OF NEURONAL SPIKE TRIGGERING

Based on the physiological data, the dendrites of a Purkinje cell may be assumed to have multiple firing zones separated by high threshold stretches of dendritic membrane. Excitatory postsynaptic potentials (EPSP) and inhibitory postsynaptic potentials (IPSP) summate, in the usual manner, at each firing zone and discharge it when threshold is reached. It follows that the spike train of a Purkinje cell arises from some form of pooling, or superposition, of the activities of the firing zones. The difficulty with superposition models of neuronal activity is that refractoriness may cause deletion of spikes, so that the output of the cell would not be the strict superposition of the activities of the firing zones. For example, if spikes from different firing zones propagate down confluent dendritic branches and arrive at the junction within the refractory period, then some of the spikes will in general be annihilated and will not contribute to the pooled output. The effects of refractoriness do not arise if whenever any firing zone discharges, it discharges in turn the remain-

ing firing zones. If the dendritic firing zones of Purkinje cells are located within 100 μ of the soma, it follows from the configuration of the dendritic tree (Eccles, Ito, and Szentágothai, 1967, p. 195) that the longest possible distance between firing zones is about 0.4 mm. Assuming an average conduction velocity of about 1 m/sec for the dendritic spikes (Eccles, 1960), all the firing zones may be discharged within a fraction of a millisecond. The whole dendritic tree therefore "explodes" and a spike is propagated down the Purkinje cell soma. Following a refractory period, firing is again initiated by a firing zone and so on. In this explosion type of model, the times at which a given firing zone *initiates* firing will define a component stochastic point process, and the output spike train will be the superposition of such components as illustrated schematically in Fig. 4. The number of component processes will be equal to the number of firing zones. It should be noted, however, that in this type of model the component processes cannot be strictly independent since they are subjected to a common refractory period.

In practice, it is possible that the firing of a given zone will discharge neighboring firing zones and those in the centripetal path of the spike towards the cell soma, but not necessarily all the firing zones in the dendritic tree. The explosion condition will not be fulfilled and a superposition model will not be strictly valid. It becomes necessary, therefore, to estimate the fraction of triggered spikes that are lost, on the average, in the pooled output. Clearly, this fraction will have an upper bound, h, in the case where the component processes have no dead time and each firing zone does not discharge any other zone. h can be estimated readily if the component processes are assumed to be identical, independent Poisson processes. Then the pooled output is also a Poisson process (Cox and Miller, p. 364), and if this output is subjected to a dead time equal to an absolutely refractory period δ, then it can be shown that $h = [\delta m^{(p)}]/[1 + \delta m^{(p)}]$, where $m^{(p)}$ is the mean firing frequency of the pooled output (cf. Goldberg et al., 1964, Appendix 2). If $m^{(p)}$ is 70 imp/sec and δ

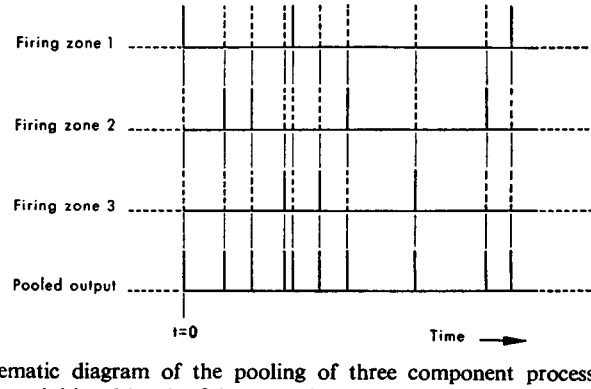

FIGURE 4 Schematic diagram of the pooling of three component processes. The solid "spikes" are those initiated by the firing zone itself, while the dotted spikes represent the discharge of the firing zone due to spike initiation in other zones. The solid spikes for each zone define a component stochastic point process.

is 0.002 sec, then not more than about 12% of the spikes will be lost, on the average, in the pooling process. It cannot be readily assessed how serious is the loss of even a larger fraction of spikes, because this depends, ultimately, on the configuration of the neuronal network and the manner in which it processes information. In the case of Purkinje cells, there is a redundancy of input via the parallel fiber system and redundancy of output on account of the convergence of 20–50 Purkinje cells onto their target neurons (Eccles, Ito, and Szentágothai, 1967, Chap. XIII).

It may be noted that in the case of Purkinje cells, basket cell inhibition could cause some disparity between the pooling of the activities of firing zones and the output spike train that propagates down a Purkinje cell axon. The lower part of the Purkinje cell body and the initial part of the Purkinje axon are the sites of inhibitory synaptic input from the basket cells (Eccles, Ito, and Szentágothai, 1967, p. 94–105). These inhibitory synapses could conceivably exercise their effect in two ways: either by contributing a hyperpolarization to the firing zones of the lower parts of the dendritic tree, or by "gating" the spike train propagating down the initial part of the axon, thereby deleting spikes from it. As there is no information available at present as to the precise mode of action of basket cell inhibition, this matter will not be considered further.

STATISTICAL CONSIDERATIONS

If a spike train is the pooled output of a relatively large number of stationary, independent component processes, then interspike intervals in the pooled output would be expected to be independent, and the pooled output approaches a Poisson process locally, i.e., over time intervals that are short compared with the mean interspike interval in the component processes (cf. references cited in the Introduction). These aspects of the sample spike trains of Purkinje cells will be considered next.

Independence of interspike intervals was investigated mainly by means of two types of tests, one based on serial correlation coefficients and the other on product-moment statistics of exponential scores (Cox and Lewis, 1966, p. 165–167; Lewis et al., 1969). Fig. 5 *a* illustrates a serial correlogram for a Purkinje cell spike train, based on an asymptotically unbiased estimator, $\tilde{\rho}_j$, that tends to correct for the effects of nonstationarities (Cox and Lewis, 1966, p. 89–92; Lewis et al., 1969). When assessed by the value of the standard normal variable, $\tilde{\rho}_j \sqrt{n-j}$, where n is the sample size, all the serial correlation coefficients of Fig. 5 *a* were found to be nonsignificantly different from zero at the 5% level except those of orders 23, 24, 40, and 43. Even for a renewal process a few of the estimators for the serial correlation coefficients may be expected to be significantly nonzero due to chance variations. In fact, after the intervals pertaining to Fig. 5 *a* were randomly shuffled, the recomputed estimators of serial correlation coefficients of orders 18, 28, 32, and 70 were found to be significantly nonzero (Fig. 5 *b*). Exponential product moment statistics of orders 1, 2, and 3 were computed for the interspike intervals and com-

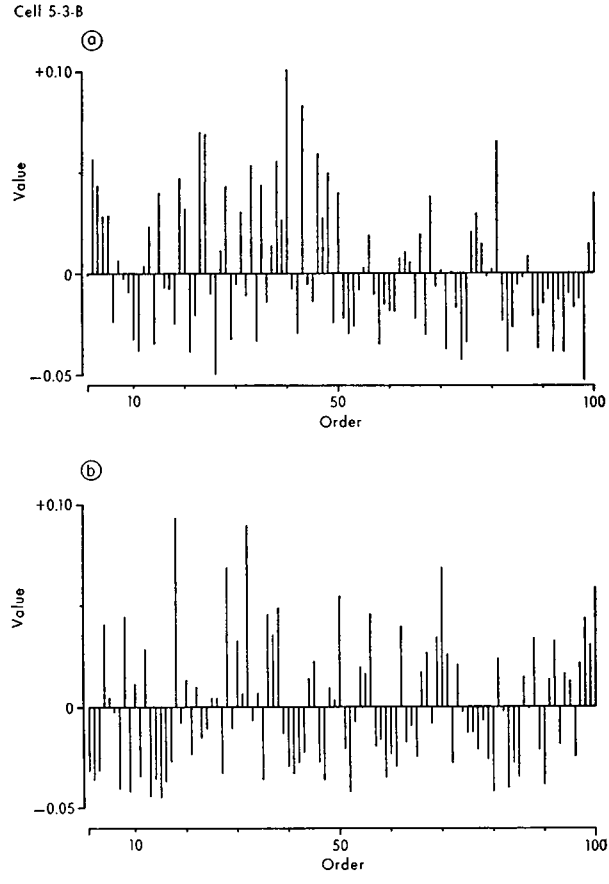

FIGURE 5 Serial correlogram of a Purkinje cell. In (*a*) are shown the first 100 serial correlation coefficients for a sample of 914 intervals. After random shuffling of these intervals the serial correlation coefficients are changed to those in (*b*).

pared with the tabulated permutation distributions of these statistics (Lewis and Goodman, 1968). None of the product-moment statistics were found significant at the 5% level, and not more than 6 of the serial correlation coefficients of orders 1–100 for any sample were significantly different from zero at the 5% level. It was concluded, therefore, that the interspike intervals in the samples examined were independent.

The local Poisson property of the spike train may be examined by means of the variance-time curve, $V(t)$, which shows how the variance of the number of events in a fixed interval of time varies with the length of the interval (Cox and Lewis, 1966, p. 72; Cox and Smith, 1953, 1954). Fig. 6 illustrates a variance-time curve for a Purkinje cell spike train estimated by the covariance method (Cox and Lewis, 1966, p. 117–118; Lewis et al., 1969).

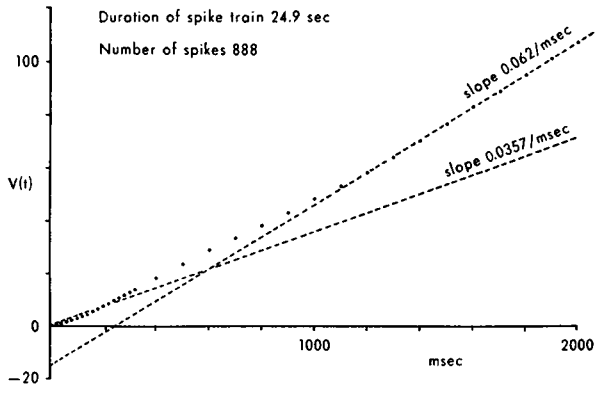

FIGURE 6 Variance-time curve of the spike train of a Purkinje cell. The coefficient of variation of the interspike interval distribution is 1.35. The large-time asymptote of the variance-time curve should, according to equation 1, have a slope of 0.065/msec and an intercept of −12.0. The asymptote of the experimental points has a slope of 0.062/msec and an intercept of −15.0. The line of slope 0.0357/msec represents the mean firing rate.

The relevant characteristics of the variance-time curve, $V(t)$, are the following:

(*a*) In the case of renewal processes, it can be shown that for large t,

$$V(t) \simeq C^2mt + A \tag{1}$$

where C is the coefficient of variation of the interval distribution and A is a constant (Cox, 1962, p. 58; Cox and Miller, 1965, p. 342–346; Cox and Smith, 1954). The value of A depends upon whether the renewal process is of the ordinary or equilibrium type, i.e., whether the starting point for the observation of the process is at an arbitrary event or an arbitrary time (Cox, 1962, p. 25–28 and p. 58). For the curve illustrated in Fig. 6, the asymptote for large t has a slope of 0.062/msec and an intercept of −15, as compared to 0.065/msec and −12.0 predicted from equation 1 for an ordinary renewal process. In general, deviations from theoretical values are to be expected due to sampling fluctuations, local nonstationarities, and departures from ideally complete independence of intervals.

(*b*) For small t, it can be shown that under fairly weak conditions:

$$V(t) \simeq mt \tag{2}$$

where m is the mean rate of occurrence of events (Cox and Lewis, 1966, p. 75, 213) In general, equation 2 may be expected to hold for $t \ll 1/m$. For a Poisson process of mean rate, λ, $V(t) = \lambda t$ for all $t \geqslant 0$. When a process of rate $m^{(p)}$ is the pooled output of a large number, p, of independent, identical component processes each of mean rate m, then it follows from the local Poisson property, as well as from relation 2 for the component processes and the pooled output, that the slope of the variance-

time curve will be $m^{(p)}$ for times large compared with $1/m^{(p)}$ but still small compared with $1/m$ (Cox and Lewis, 1966, p. 213).

The variance-time curve of Fig. 6 conforms to this pattern. For small t the experimental points fall below the line $V(t) = \lambda t$ for a Poisson process of the same mean rate, because of comparative rarity of short intervals on account of nerve refractoriness. As t increases, $V(t)$ runs almost parallel to λt, then deviates for large t. The deviation from a Poisson process may be approximately assessed from the standard error of the covariance estimate, $\tilde{V}(t)$, of the variance-time curve for a Poisson process:

$$\text{var}\,[\tilde{V}(t)] = \left(\frac{2}{3} + \frac{4\tau}{3t}\right)(\lambda t)^2\left(\frac{t}{t_o}\right) + (\lambda t)\left(\frac{t}{t_o}\right),$$

(Cox and Lewis, 1966, p. 118). In the case illustrated in Fig. 6 deviations from a Poisson process, at the 5% significance level, occur at $t \simeq 320$ msec, or approximately 11.4 times $m^{(p)}$, the mean interspike interval. The variance-time curves for the spike trains of eight other Purkinje cells behaved in a similar way, although in two cases deviations from a Poisson process at the 5% level occurred for values of t that were approximately five times the mean interspike interval. In one case, the variance-time curve did not depart significantly at the 5% level from the line λt over the range $(0, t_o/10)$, indicating a close approach to a Poisson process.

It follows that the statistical properties of the spike trains examined are consistent with the hypothesis that they arise from the pooling of a relatively large number of independent component processes. Conceivably, if there were a substantial degree of dependence between the component processes, the pooled output might not approach a Poisson process even locally, and the variance-time curve will not behave in the manner to be expected for a pooled output of independent component processes. To our knowledge, there are no practical methods available at present for testing in a more definitive manner for the validity of a superposition model or for assessing the identity or independence of the component processes.

ESTIMATION OF THE NUMBER OF FIRING ZONES

In principle, it should be possible to estimate the number of component processes from the statistical properties of the pooled output. However, methods for doing this have only been worked out in practice for the case of strictly periodic component processes (Cox and Smith, 1953) and for the case where the component processes are identical, independent renewal processes (Cox and Smith, 1954). The independence of interspike intervals and the behavior of the variance-time curve seem to justify the assumption of independence between component processes. There is no information available at present as to whether or not the component processes can be justifiably considered as identical renewal processes. It seems in fact unlikely that the component processes are identical; but it is not known how different they

might be, or what error such difference would introduce into the estimation of the number of firing zones.

If the component processes are considered to be identical renewal processes, then the method recommended by Cox and Lewis, and applied by them to Fatt and Katz nerve impulse data may be used for estimating the number, p, of component processes (Cox and Lewis, 1966, p. 216, 220–222). A gamma distribution of unspecified parameters is assumed for the component processes, which then enables estimation of p from the slope and intercept of the asymptote of $V(t)$ for large t. Application of this method gave values of p ranging between 15 and 150 for the 10 Purkinje cells studied. In view of the explicit and implicit assumptions involved, however, these estimates can at best be regarded as very rough.

When a large number of component processes are superposed, the mean interval between events in the superposed output will be much smaller than that in the component processes. Conceivably, the long intervals usually observed in the spike trains of Purkinje cells may in fact be the intervals between events in a single component process or at most a few of these processes.

DISCUSSION

An important aspect of studying the spontaneous activity of Purkinje cells is ascertaining the origin of this activity. It is uncertain whether or not the spontaneous activity of Purkinje cells is wholly synaptic in origin, because activity of these cells has been observed in the chronically deafferented cerebellum (Eccles et al., 1966; Eccles, Ito, and Szentágothai, 1967, p. 189–190) and in chronically isolated cortical slabs (Crepax and Infantellina, 1957; Bindoni et al., 1967; Snider et al., 1967). These observations suggest that Purkinje cells and/or granule cells may have an "intrinsic rhythm" of their own, although other explanations are possible, such as mechanical injury to cells or nerve fibers, and increased excitability of membranes of these cells in the chronically deafferented state. The absence of periodicities in the autocorrelograms (cf. Fig. 3) seems to preclude "pacemaker" type of activity in Purkinje cells. The few cases of pronounced regularity in firing that we have observed were attributable either to cell injury or to a high level of barbiturate anesthetic (Murphy and Sabah, 1970 *a*). Studies on the effects of these anesthetics reveal that the spontaneous activity of Purkinje cells can be suppressed at least temporarily by appropriate doses of barbiturates, presumably on account of a depressing effect at the mossy fiber–granule cell synapses or at preceding relays (Murphy and Sabah, 1970 *a*; Körlin and Larson, 1970). Moreover, it is not an uncommon observation in our experiments that some Purkinje cells are silent, unless activated by stimulation of cerebellar afferents. The suggestion is therefore at hand that the spontaneous activity of Purkinje cells in the normal cerebellum is due to synaptic input from the parallel fibers, and that this activity represents under stationary conditions a steady level of input. Climbing fibers discharge at a frequency that is generally

low compared to the mean firing frequency of the spontaneous activity of Purkinje cells and their contribution to this activity will be small.

Independence of interspike intervals implies that the precise sequence of intervals in the spike train does not carry information under stationary conditions. Rather, information must be carried by some order-independent property of the spike train, such as the mean firing rate or other properties (Perkel and Bullock, 1968). The target neurons in the cerebellar nuclei and Deiters' nucleus (Eccles, Ito, and Szentágothai, 1967, p. 227–234) receiving spike trains of Purkinje cells should therefore ignore particular sequences of intervals that arise by chance, but must respond to these or similar sequences that are caused by dynamically changing inputs to the cerebellar cortex. Moreover, studies on natural arm movements in the unanesthetized monkey reveal that the changes in firing patterns of both Purkinje and cerebellar nuclear neurons can take place over periods not exceeding 100 msec or so (Thach, 1968). Hence the target neurons should be capable of distinguishing within a fairly short period of time between random fluctuations in intervals and variations due to dynamically changing inputs. Two factors may be involved in making such a distinction. Firstly, the target neurons receive collaterals from both types of afferents of the cerebellar cortex: the mossy fibers and the climbing fibers (Eccles, Ito, and Szentágothai, 1967, Chap. XIII). Apart from providing an excitatory input (Eccles, Ito, and Szentágothai, 1967, p. 257) these collaterals would also signal to the target neuron changing inputs to the cerebellar cortex. Secondly, each target neuron is innervated by 20–50 Purkinje cells (Eccles, Ito, and Szentágothai, 1967, Chap. XIII). This convergence may imply that a target neuron does not respond to each Purkinje cell individually, so that the concerted action of a number of Purkinje cells may be required to produce a significant effect on the output of the target neuron.

When considering the mechanism of spike triggering in neurons, it is well to keep in mind that there is probably no such thing as a typical neuron. Neurons vary widely in size, shape, and the number and distributions of synapses terminating on them; there is no a priori reason to assume that spikes are triggered in the same way in all neurons. Moreover, the mechanism of spike triggering is inextricably tied to the deeper problem of what the function of the neuron is and how it processes the afferent signals. Consequently, it seems much more meaningful to consider spike triggering in particular cell types and in relation to possible functional roles of these cells.

Clearly there is much scope for further experimental and theoretical work relating to the characterization of the spike trains of Purkinje cells in terms of a pooled output of a number of component processes. For example, it will be very desirable, if the number of component processes is 100 or so, to be able to record at least 50 events on the average from each component processes. However, it is not easy to record spike trains of 5000 interspike intervals or more, satisfying stationarity requirements, even if the recorded data from a single cell is segmented. It will also be desirable, at least in preliminary investigations, to be able to study the background

discharge of Purkinje cells without the complicating effects of climbing fiber activation (Murphy and Sabah, 1970 *b*). This is probably best accomplished by eliminating the climbing fiber input by means of a lesion in the inferior olive or along the olivocerebellar pathway. On the theoretical side it will be worthwhile investigating the statistical properties of the pooled output of nonidentical and/or partially dependent component processes, developing statistical tests with high powers of discrimination between superposition and nonsuperposition models of spike activity, and establishing confidence intervals for numerical estimates of model parameters. This will enable investigation of such problems as the constancy of the number of firing zones in a given Purkinje cell and the possible correlation between the number of firing zones and some statistical measures of Purkinje cell spike trains.

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