A STATISTICAL ANALYSIS OF THE CONTINUAL ACTIVITY OF SINGLE CORTICAL NEURONES IN THE CAT UNANAESTHETIZED ISOLATED FOREBRAIN

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ABSTRACT Statistical analyses are applied to records obtained under conditions of spontaneous activity. These demonstrate that all the cells examined are similar in the respect that their signals are composed of an approximately Poissonian shower gated on and off at random instants. A mathematical model is developed on this basis which characterises the signal of any one neurone with a number of simple parameters. The manner in which these parameters change under various types of stimulation is studied. It is found that the average frequency inside the sections of Poisson shower remains unchanged in all cases examined. Implications as to the types of network involved are discussed.

INTRODUCTION

The succession of spike discharges continually generated by individual neurones in the apparently undisturbed cerebral cortex has been repeatedly observed by workers studying the responses of these units to various forms of stimulation. In most instances this so-called "spontaneous" activity has been regarded as noise and has been ignored as irrelevant to the phenomenon of more direct interest. Recently, however, a few authors have expressed interest in the phenomenon of spontaneous activity *per se* (Smith and Burns, 1958; Gerstein and Kiang, 1960) or have indicated that a study of the spontaneous activity might be helpful in a more accurate evaluation of responses to weak or remote stimuli, (Martin and Branch, 1958; Gerstein, 1962; Burns and Smith, 1962).

A great deal of attention has been focussed lately upon the problem of the properties of large networks of neurone-like elements which are intended to imitate, to a greater or lesser extent, the behaviour of such networks in the brain (*e.g.*, Beurle, 1956; Farley and Clark, 1960; Smith and Davidson, 1962). But the direct applicability of this work has been diminished by a lack of factual evidence concerning the properties and connections of real neurone systems.

With these considerations in view, an examination of the statistics of the maintained activity seemed justified. Firstly, since this signal is the only means at present available for directly observing the activity of single cortical neurones, a greater knowledge of its most striking property seemed essential. Secondly, there was at least a reasonable hope that the statistics of the signal would provide clues regarding the types of mechanism responsible for its genesis.

Our results lead us to conclude that at least two independent mechanisms combine to generate the signal which consists of bursts of random spike activity separated by periods of inactivity of variable duration. The two components are differently affected by external stimulation. Some of these results were summarized earlier in Smith and Smith (1964).

METHODS

Preparation. Most of the records analyzed here were taken from experiments done at the McGill University Laboratory by Burns and Smith (1962). The remainder of the experiments were done according to the methods described by these authors. Cats weighing between 2 and 4 kg were anaesthetized with ethyl chloride followed by ether. Both cerebral hemispheres were exposed over a large area and a cut was made across the midbrain at the tentorium cerebelli, (Bremer, 1935). A hole was made with suction through the cortex into the lateral ventricle on one side to drain the cerebrospinal fluid. A well was made by tying the scalp to a ring above the head and covering the brain with warm mineral oil. The temperature of the animal was kept at 37 ± 0.5°C by a heating pad placed under the abdomen.

The animals were allowed to recover from the anaesthetic and immobilized with gallamine triethiodide, 20 to 40 mg, iv per animal injected every half hour. Respiration was maintained artificially.

Recording. Glass micropipettes of 1 to 5μ internal tip diameters and resistances between 0.2 and 0.8 MΩ where used to record extracellular potentials from cells in all areas of the easily exposed cortex, (Burns, 1961; Burns and Smith, 1962). The electrodes were filled with 9/10 saturated NaCl and supported on a "weightless" microelectrode holder, designed to minimize the effects of brain movements. In early experiments the holder was the one described by Burns and Robson (1960). Later a more convenient arrangement (Fig. 1) was used in which the vertical coil spring suspension element was replaced by a 9 cm piece of 0.18 mm diameter stainless steel wire anchored at one end and held horizontally. The wire was beaten flat at a point about 0.5 cm from the other end and bent to a right angle at that point. A short length of silver wire treated with chloride, soldered onto the end, made contact with the electrolyte solution of the pipette. The spring was enclosed in a glass sleeve and up and down movements of the electrode was restricted by a slit mounted on the end of the glass sleeve. A lead was taken from the stainless steel spring to the grid of a cathode follower, on one side of an R.C. coupled differential amplifier. The other side of the amplifier was referred to a platinum wire electrode placed on the cortical surface immediately above the recording point. The amplifier was set to pass the band between 200 and 5,000 cps so as to include only those frequencies which are major components of the action potential wave form. The signal was recorded on one channel of a two-channel magnetic tape system, the other channel being used for experimental notes and for marking the times of stimulation.

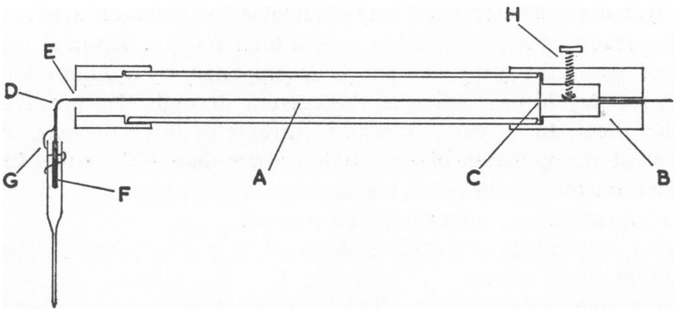


FIGURE 1 Modified "weightless" microelectrode carriage. The electrode is suspended from the stainless steel wire, *A*, which is anchored in a perspex cylinder, *B*, shown in cross-section. The wire is beaten out flat and bent to a right angle at *D* to allow the third dimension of free movement. A silver wire treated with chloride, *F*, makes contact with the electrolyte in the micropipette. The latter is clamped in the looped end of the spring wire, *G*. The wire passes through a small hole in the diaphragm *C* which acts as a fulcrum for the thumbscrew *H* bearing against the wire. The thumbscrew is adjusted so that spring just supports the weight of the pipette and so that its end hangs freely in the center of the slit *E*. The spring is protected within a glass barrel.

We made no attempt to choose specific locations or depths of recording nor did we distinguish cell types from which recordings were made. However, for all of the experiments prolonged steady contact with the cells was required. Hence most of the records were probably taken from the larger neurones of the cortical population, since contact with small structures is easily lost. Furthermore we have generally chosen neurones whose "spontaneous" mean firing rate exceeded sixty impulses per minute since the statistical analyses used required that a fairly large sample be gathered within a reasonable time. The problem of sample size will be discussed later in the paper.

Stimulation. Visual stimuli were applied in the form of simple patterns flashed briefly onto an opal glass screen placed before the animal. The pupil of one eye was dilated with atropine and the eye focussed on the screen with auxiliary lenses. An optically neutral contact glass was placed on the cornea to prevent drying. The unused cornea was covered with an opaque contact glass, (Burns, Heron, and Pritchard, 1962). Electrical stimulation was applied to the cortical surface from platinum wire electrodes placed 1 mm apart and transformer coupled to a Tektronix pulse generator, type 161 (Tektronix Incorporated, Portland, Oregon). Stimuli consisted of weak shocks of 0.5 msec. duration, and strength less than that required to evoke a maximal surface negative response (Adrian, 1936). Short bursts of 10 such pulses 10 msec. apart were regularly repeated usually once per second. Local polarizing current was passed through the microelectrode tip by application of a steady voltage to a 20 MΩ resistance in series with the microelectrode. In these experiments the electrode was coupled to the grid of the cathode follower by a small capacitor and a 20 MΩ grid leak was connected to earth (Burns, 1961). Currents between 0.1 and 0.5.μa, passed through the tip of the recording micropipette and referred to a diffuse earth in the mouth of the animal, result in alterations of average firing rate which depend upon, among other things, the distance between the pipette tip and some part of the neurone. Greatest effects are obtained when the recorded action potential amplitude is greatest (*i.e.*, closest to the membrane) and it is usually not possible to exert any detectable influence on a neurone when the amplitude of the recorded action potential is less than 100 μv. Tip negative polarization results in an increase in the firing rate and is accompanied by a diminution in the spike amplitude both roughly in proportion to the current strength. Tip positive polarization has the opposite effect. In all cases in which currents of this sort were applied only a single action potential population of amplitude greater than 100 μv was being recorded and it is assumed on this ground that the neurone under observation was the only one in the brain which was directly affected by the current.

Analysis. For most of the statistical work it was sufficient to know the timing of action potentials to an accuracy of 10 msec. This fact allowed us to code the signal on punched paper tape acceptable to a digital computer. A binary counter was triggered once by each action potential and the output of each binary stage was fed to a punch operating at 110 rows per sec. The counter was reset after each punch cycle and after a very short delay was permitted to count again. Thus every 9.1 msec. the tape was punched with the number of action potentials which had occurred in the proceding 9.1 msec. period. During the early part of the investigation no counts greater than 6 pulses were observed on any 9.1 msec. period. We, therefore, limited the counter to three binary terms. Using eight-hole paper tape provides room for coding of other information such as the times of stimulation or for a second action potential channel. The action potentials were fed to the counter through a variable threshhold gate which excluded amplifier noise and pulses from distant cells and converted the signal to standard rectangular pulses. The system was used in conjunction with the ACE digital computer of the National Physical Laboratory, Teddington, Middlesex, England, which was programmed to compute the interpulse interval distribution diagram and the autocorrelation diagram of the signal. The other analyses described below were done manually either from photographic film or from the coded paper tape.

RESULTS

Prolonged recordings of signals from single neurones were obtained from widely dispersed locations of the unstimulated cerebral cortex. These records were subjected to a number of statistical analyses the first of which was the interspike interval distribution analysis already described by a number of other workers (Fatt and Katz, 1952; Martin and Branch, 1958; Gerstein and Kiang, 1960). In general form the distributions obtained from all of the 40 neurones tested were similar to those seen by Gerstein and Kiang (1960); *i.e.*, a preponderance of short intervals with a long, right-hand "tail" indicating the presence of a small number of very long intervals. Plotting the distributions on a semilogarithmic scale revealed two striking features. Firstly, all of the distributions possessed a quite definite point of gradient discontinuity and secondly, two groups of diagrams emerged, the larger of which (25 in number) possessed a striking linearity of the right-hand tail (Fig. 2 crosses) while in those of the smaller group the tail was non-linear (Fig. 11). The more important characteristics of the larger group (group I cells) were common to those of group II but for convenience group I will be examined first.

Statistical Structure of the Signals from Group I Neurones. The circled and crossed points of Fig. 2 are typical of the interspike interval distributions ob-

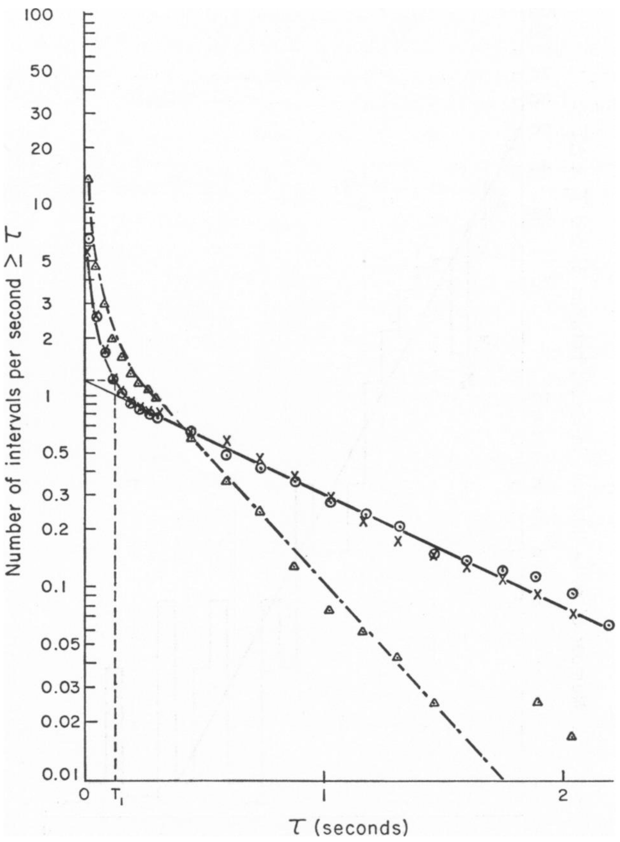


Figure 2 Interpulse interval distributions obtained from a cell in the median suprasylvian gyrus. The crosses (x) are from a 216 sec sample of the "spontaneous" firing in the unstimulated brain. The effect of passing $-0.2\mu a$ for 120 sec through the pipette tip (△) was to increase the average firing rate from 5.5 pulses/sec to 13.4 pulses/sec. After the removal of polarization (⊙) the distribution reverts to the original form (109 sec sample). Lines of maximum likelihood fitted as described in Fig. 3 (cell 30.6.61).

tained from the cells of group I. The data were taken from periods of spontaneous activity in a cell located in the median suprasylvian gyrus. In this diagram each ordinate represents a logarithmic plot of the number of interpulse intervals per second equal to or greater than the value of time at its corresponding abscissa. Two approximately straight sections can be clearly seen. In order to examine this signal structure in greater detail the data were divided into two parts as follows. First the right-hand section was fitted with the maximum likelihood exponential process as shown in Fig. 3. In this figure the data are plotted in the interval density histogram form where each column ordinate represents the logarithm of the number of interpulse intervals falling within its abscissa limits (*i.e.*, the plot of which Fig. 2 is the

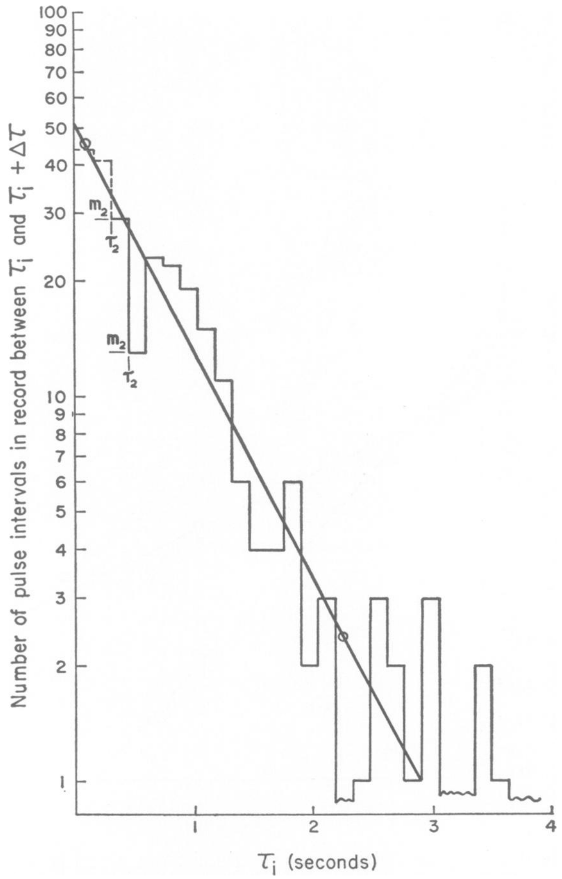


FIGURE 3 Interpulse interval density histogram. The maximum likelihood exponential process (solid line) has been fitted to the tail of the first distribution (x) of Fig. 2. This line also fits the final distribution after polarization. The gradient (λ) of this line was calculated from the expression: $\lambda = 1/\Delta\tau \ln (1 + \Delta\tau\Sigma m_i/\Sigma m_i\tau_i)$. The χ^2 test set the goodness-of-fit at the 15 per cent level.

normalized integral). The conventional χ^2 goodness-of-fit test could then be applied, subtracting an extra degree of freedom for the exponential constant so estimated. In all except one of the cells of group I tested (*i.e.* 24) no significant differences were observed (see Table I). This exponential, extrapolated to zero, was next subtracted from the complete interval distribution histogram giving the result shown by the circled points in Fig. 4. In most of the records tested the region of the curve between 30 and 100 msec. showed no significant deviations from a second exponential, using again the standard goodness-of-fit criterion on the interval density plot (Fig. 5). In general the histograms tended to indicate that the expectation of intervals shorter than 30 msec. is greater than that of an exponential distribution.

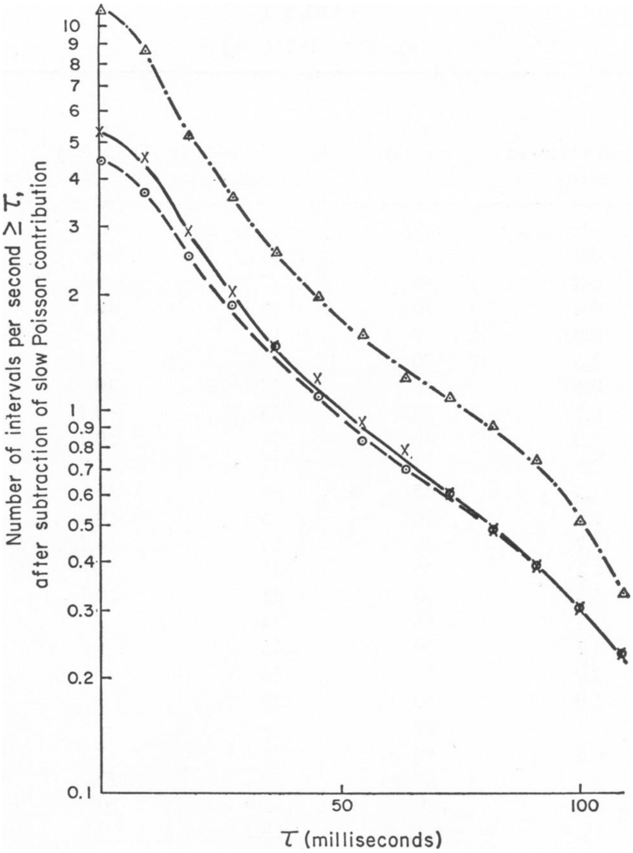


Figure 4 Interpulse interval distribution. Residue after subtraction of maximum likelihood slow process. The difference in ordinates means that the passage of local current has increased the proportion of time spent in the fast process (△) but has not changed the average frequency (gradient) of the fast process.
Compare with the change in gradient of the slow process (Fig. 2).

The timing of action potentials was given by our apparatus only to an accuracy of ± 9.1 msec., but the error in the *cumulative* interval distribution *ordinates* resulting from this rounding process can be shown to be negligible. Some histograms were supplemented by an apparatus which enabled intervals to be recorded in a continuous range from 0 to 20 msec., in order to show up the initial rise and peak of the histogram. Deviations from the double exponential structure in this range appear to be due to the finite recovery time of the cells and a tendency toward double or multiple firing. In extreme cases this tendency can be seen in oscillograph tracings of records in which clusters of up to 5 regularly spaced spikes frequently occur (Fig. 6). The extremes of the range of intervals observed in this investigation were about 10 sec. and 0.9 msec.

TABLE I

(a) GROUP I CELLS

Cell	Intervals between bursts	χ^2 Probability level	Intervals inside bursts	χ^2 Probability level	Time proportion in bursts	Region
	λ(*intervals per sec.*)	(%)	θ(*intervals per sec.*)	(%)	*f*	
14. 4.60	4.8	15	15	60	0.50	MSSG
19. 5.60	0.89	40	7.4	<0.1	0.54	MSSG
7. 7.60 *a*	0.47	40	6.7	10	0.71	MSSG
7. 7.60 *b*	0.95	7	10	60	0.22	MSSG
24.10.60	2.5	30	17	0.1	0.24	MLG
7. 9.60	0.55	15	14	10	0.04	PCG
20. 9.60 *a*	0.73	70	25	0.1	0.01	ASSG
2.11.60 *a*	3.0	60	28	<0.1	0.19	PLG
2.11.60 *b*	0.84	7	11	1	0.12	PCG
2.11.60 *c*	1.5	15	20	15	0.16	PLG
2.11.60 *d*	1.2	70	5.3	<0.1	0.30	MSSG
18. 5.61 *a*	4.7	30	26	0.1	0.05	MSSG
18. 5.61 *b*	1.3	25	31	10	0.07	MSSG
18. 5.61 *c*	2.3	30	25	<0.1	0.21	MSSG
30. 6.61	1.35	15	25	5	0.14	PSSG
13. 7.61	3.1	50	12	5	0.57	PSSG
30. 8.61 *a*	3.0	85	33	1	0.08	MLG
30. 8.61 *b*	1.9	50	20	7	0.06	MLG
30. 8.61 *c*	2.5	60	—*	—	—	MLG
30. 8.61 *d*	6.8	30	25	0.1	0.31	PLG
3. 5.62	2.3	60	16	20	0.03	MSSG
28.11.62 *a*	1.2	30	8.9	20	0.22	PSSG
28.11.62 *b*	1.6	95	15.5	1	0.20	PSSG
12.12.62 *a*	0.82	1	13	2	0.16	MSSG
12.12.62 *b*	0.26	5	9	20	0.08	MSSG

Notes: χ^2 Probability $<$ 5 per cent taken as evidence of significant deviations from Poisson. Figures for the intervals inside bursts do not apply for the region smaller than 30 msec. where there is usually an excess of intervals (see Figs. 4 and 13).

Regions: SSG, suprasylvian gyrus; LG, lateral gyrus; ESG, ectosylvian gyrus; CG, cruciate gyrus; A, anterior; M, median; P, posterior.

*Bursts composed of intervals $<$ 30 msec. only.

The analysis up to this point has demonstrated that the interspike intervals are distributed in the same proportions as a mixture of two approximately exponential processes. The characteristics of the firing patterns are summarized in Table I. Note the wide range of parameters encountered, the high frequency constant ranging from 5 to 33 pulses per second while the low frequency constant ranges from 0.26 to 6 per second. No clear tendency is discernible to grouping of the parameters either with respect to specific regions of the cortex or with respect to individual animals. So far we have no information on the time sequence of the intervals. There are

(b) GROUP II CELLS

Cell	Intervals between bursts	Intervals inside bursts	Region
	λ(*intervals per sec.*)	θ(*intervals per sec.*)	
16. 3.60	4.7	26	MSSG
4. 8.60	3.2	25	ASSG
20. 9.60 *b*	1.5	17	MLG
20. 9.60 *c*	5.4	28	PCG
24.10.60 *a*	5.8	24	MSSG
24.10.60 *b*	1.6	21	PESG
2.11.60 *e*	1.5	20	MESG
13. 7.61	4.1	25	PSSG
17. 8.61	1.9	26	PLG
17. 8.61	2.1	22	PLG
28.11.62 *c*	1.9	12	MSSG
28.11.62 *d*	0.7	11.5	PSSG
12.12.62 *c*	2.6	16	MSSG
15. 1.63 *a*	3.8	—*	MSSG
15. 1.63 *b*	3.4	—	MSSG

*Bursts composed of intervals $<$ 40 msec. only.

many possible ways in which the interval distributions discussed above could be combined, two extreme cases being a homogeneous mixture on the one hand and, on the other, a complete temporal segregation of the intervals belonging to each. One method of distinguishing such cases is to plot the frequencies of pulse counts recorded in an arbitrarily chosen standard time interval. This was done for the cells of group I using a 50 msec. interval and the results obtained for the same record as was analyzed previously are shown in Fig. 7, compared with the frequencies to be expected from the two extreme models. The observed frequencies are clearly closer to those of the second model in which the neurone fires according to the slower exponential law for part of the time, and according to the faster exponential law for the remainder of the time. A small standard interval must be chosen in this analysis in order to minimize the probability of a measurement falling on a transition from the slow to the fast process.

In order to determine if the exponential processes so far observed are Poissonian, it now remained to examine the degree to which the duration of an interval is dependent upon its predecessors. To do this joint occurrence matrices of the form of Fig. 8 were constructed (Rodieck, Kiang, and Gerstein, 1962). The smallest intervals, corresponding to the initial non-linearity of the interval histogram, were first eliminated, and then the remainder of the matrix was divided into two sections bounded by an interval (*e.g.,* T_1 Fig. 2) chosen such that the number of intervals ascribed to the faster process in error was equal to the number of intervals ascribed to the slower process in error (*i.e.,* in statistical terms, to equalize the numbers of type I and type II errors). The underscored figures represent the expected frequencies

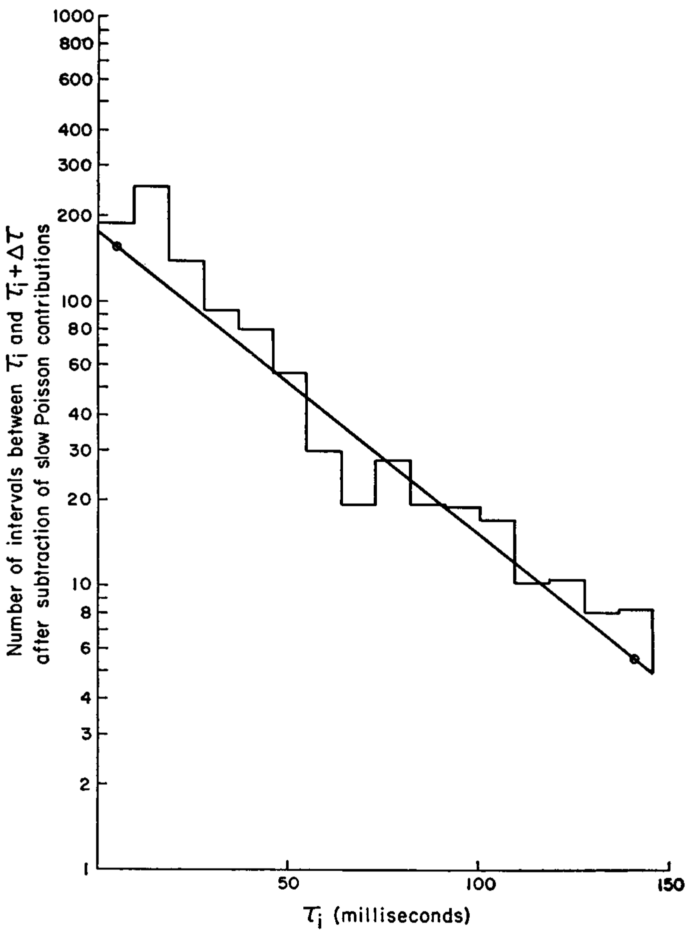


Figure 5 Interpulse interval density diagram—data from circled points (⊙) of Fig. 4. The solid line is the maximum likelihood exponential process fitted as in Fig. 3. Disregarding the first 30 msec. the goodness-of-fit from X^2 was at the 5 per cent level.

computed from the gradients of the interval distribution assuming two Poisson processes switched in time. The significance levels are quoted below the matrix and indicate independence of successive pairs of intervals within each process. Table II gives the results of similar tests done on 10 other records. Where deviations from independence occur they tend to be small and suggest no clear trends. A complete demonstration of independence within each process would of course entail the analysis of runs of greater length in addition, and the absence of significant deviations in all of these could justify the title of Poisson process for each of the respective parts of the signal. In practice sufficiently large samples cannot be obtained, and, while it is clear that non-Poissonian influences are at work in some of these cells,

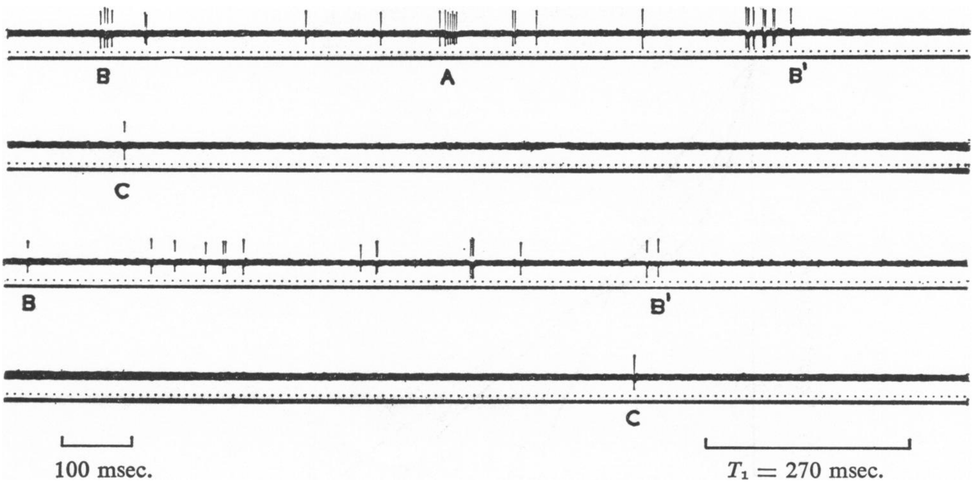


Figure 6 Sequential samples from the record analyzed in Figs. 12 and 13 (cell 7.7.60.*b*). Time marks are 10 msec. apart. At *A* is a cluster of spikes less than 5 msec. apart. Such clusters are responsible for the initial peak in the interval distribution (Fig. 13), *B–B′*, a burst—so defined by the fact that none of the intervals within this period is greater than the interval T_1 (270 msec. in this case). *C* is an isolated pulse, or in terms of the model, a "burst of one." It is separated from adjacent pulses, both before and after, by intervals greater than T_1. In this record the Poisson mean interval length of the fast shower process $1/\theta = 100$ msec., that of the slow process $1/\lambda =$ 1.05 sec.

nevertheless for the purposes of the following analyses the respective processes are treated as if they were Poisson. The constants used are those obtained from the maximum likelihood fits for intervals greater than 30 msec.

The proportion of time spent by a cell in each Poisson process may be estimated from the ratio of the corresponding intercept and gradient of the interval distribution. The difference between the sum of these proportions and unity may be used as an estimator of the abruptness of the transition from one process to the other. In a sample of twenty-four group I neurone records the sum of these proportions had a mean 0.996 and standard deviation 0.028. Thus the time spent in transition is short, probably consuming less than 1 per cent of time.

With the information gained so far we can form a crude picture of the spontaneous discharge pattern. From the distribution of interspike intervals we see that two nearly exponential processes are at work. We discover from the distribution of pulse counts in a 50 msec. interval that these two processes are in some way segragated from one another in time and this observation is further substantiated by the results of the joint occurrence analysis. Moreover we observe from the ratios of intercepts and gradients of the interval distribution components that the transition from the slow to the fast process is very abrupt. Hence we can visualize a signal

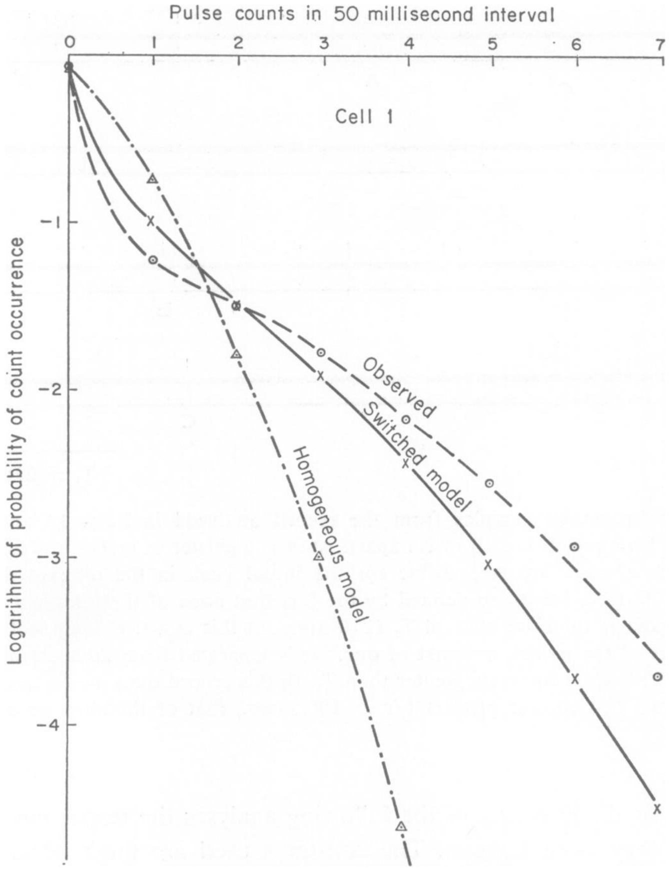
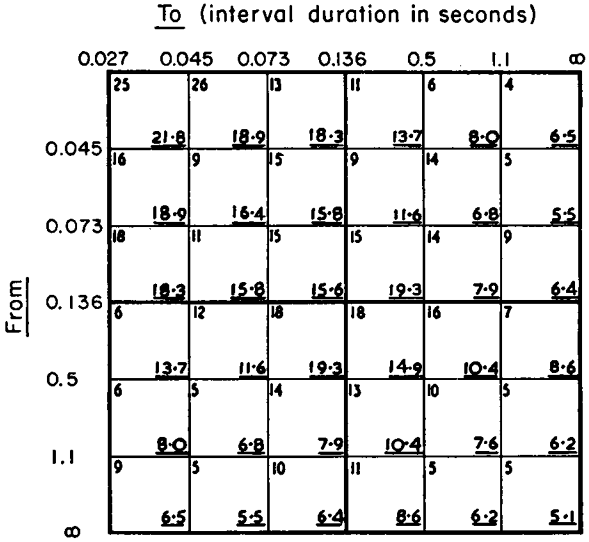


FIGURE 7 Histogram of the distribution of spikes counted in a 50 msec. interval. The circled points (⊙) are taken from the record from which Fig. 2 was derived (cell 30.6.61). During the 216 second sample no stimulus was applied. Each point represents the logarithm of the probability that a 50 msec. interval will contain the stated number of spikes. The results are compared with the predicted distributions assuming (*a*) that the intervals of two Poisson distributions with the constants of the observed signal, are arranged homogeneously in time (△), or (*b*) that the two Poisson distributions are time-segregated (x)—*i.e.*, switched. The points are joined by lines for clarity.

consisting in part of bursts of randomly occurring action potentials, the bursts being of unknown duration and frequency. It is not yet possible to form a clear picture of events taking place between bursts.

The signal described above can be interpreted in a number of statistically equivalent forms. One possibility is that of one random pulse shower gated on and off over another random pulse shower (noise) background. Another possibility is that there is only one random pulse shower, which is gated on and off over a silent



0.027	0.045	0.073	0.136	0.5	1.1	∞
0.045	25 21·8	26 18·9	13 18·3	11 13·7	6 8·0	4 6·5
0.073	16 18·9	9 16·4	15 15·8	9 11·6	14 6·8	5 5·5
0.136	18 18·3	11 15·8	15 15·6	15 19·3	14 7·9	9 6·4
0.5	6 13·7	12 11·6	18 19·3	18 14·9	16 10·4	7 8·6
1.1	6 8·0	5 6·8	14 7·9	13 10·4	10 7·6	5 6·2
∞	9 6·5	5 5·5	10 6·4	11 8·6	5 6·2	5 5·1

Figure 8 Interpulse interval transition matrix (cell 30.6.61) 216 second unstimulated sample. The matrix includes all intervals longer than 27 msec. The matrix is divided into four quadrants by the interval $T_1 = 136$ msec. The numbers in each square represent the number of times an interval in the class on the vertical coordinate was immediately followed by the class on the horizontal coordinate. Observed frequencies are stated first followed by expected frequencies (underlined).

Goodness-of-fit levels from X^2 tests:

Intervals within bursts	20 per cent level
Intervals between bursts	80 " " "
Entry to bursts	7 " " "
Exit from bursts	1.5 " " "

background, the slow process simply corresponding to the "off" periods of the gate. In either case the instants of transition could occur at the actual times of action potentials or could be completely independent of them. All three alternative postulations do in fact lead to the same double exponential form for the interval distribution.

These are essentially hypotheses concerning alternative mechanisms which could generate the signal. Since mathematics is unable to distinguish between them, the only way in which an answer might be obtained is by observing the changes which take place in the signal under various forms of stimulation.

Now, the average rate of firing of a cell may be altered by passing local current through the recording microelectrode, as described in Methods. The effect on the interval distribution of a 2 minute period of cathodal polarization which more than doubled the mean firing rate, is shown in Fig. 2. In terms of the two-Poisson model only the slower process is changed (see Fig. 4 for the invariance of the fast process) and the cell reverts to its original firing pattern soon after the removal of the polariz-

TABLE II

INTERVAL MATRICES

Goodness-of-fit to random hypothesis

Cell	χ^2 probability level	
	Intervals inside bursts	Intervals outside bursts
	%	%
4. 4.60	25	10
7. 7.60 *a*	50	No sample
7. 7.60 *b*	0.1	20
7. 9.60	No sample	60
24.10.60 *a*	No sample	30
18. 5.61 *b*	<0.1	1
18. 5.61 *c*	2	0.1
30. 6.61	20	80
13. 7.61	<0.1	60
3. 5.62	2	70
28.11.62	60	15
No significant deviation in:	4 from 9 tested	8 from 10 tested

ing current. Our general approach is now to attempt to distinguish between the silent or noisy background hypotheses by comparing the changes which occur in the interpulse and interburst intervals when external stimuli are applied. The interval used to separate the transition matrix was employed as a criterion defining a "burst"; *i.e.,* any shorter interval was assumed to lie within a burst. On the basis of this definition, the distribution of intervals between bursts was determined, firstly for bursts of four pulses or more, then for those of three or more, and so on down to bursts of one or more pulses, (the distribution of intervals between bursts of one pulse or more may be taken from the tail of Fig. 2). These distributions (Fig. 9) show no significant deviations from random. The effect of polarization on this distribution of intervals between bursts is shown in Fig. 10. Disregarding the slight nonlinearities introduced by the polarization at the short interval end of these curves, the frequencies of the corresponding burst onset times have all increased by a similar proportion. Anodal polarization has exactly opposite effects. These results suggest that only one slow process is present, namely that representing the time intervals separating the end of one burst and the beginning of the next, and that the signal is best considered in terms of the second model described above; *i.e.*, a nearly random pulse shower gated on to a silent background at random instants for variable lengths of time. In this model isolated pulses, which follow and precede long intervals, consist of "bursts of one." A model of this type is developed in the Appendix. In the succeeding paragraphs we shall replace the terms *fast* and *slow process* by the terms *"Poisson" shower* and *burst onset process* respectively. It is important to

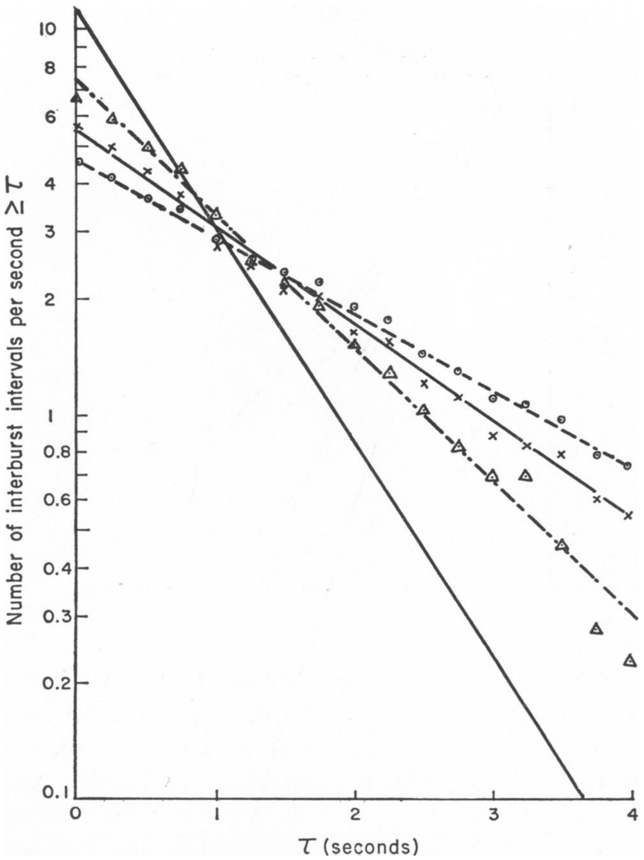


FIGURE 9 Distribution of interburst intervals (cell 30.6.61) unstimulated brain, 216 second sample.

⊙ Intervals between bursts of four spikes or more, gradient = 0.46
x Intervals between bursts of three spikes or more, gradient = 0.59
△ Intervals between bursts of two spikes or more, gradient = 0.79

Solid line is maximum likelihood line from Fig. 2 which is equivalent to the distribution of intervals between bursts of one or more spikes; gradient = 1.28.

note that the intervals between bursts are substantially independent of the characteristics of the burst preceding them. This may be demonstrated by performing a regression analysis, see Cramer, 1945 (*a*), on the two random variables: number of pulses in each burst, and duration of interval until the next burst. For example such an analysis performed on cell (30.6.61) gave a sample correlation coefficient of −0.006, and a probability level of 90 per cent given that the parent distribution was completely uncorrelated.

Statistical Structure of Group II Neurones. A second group of cells is typified by the histogram of Fig. 11. These cells differ from those of the first group in

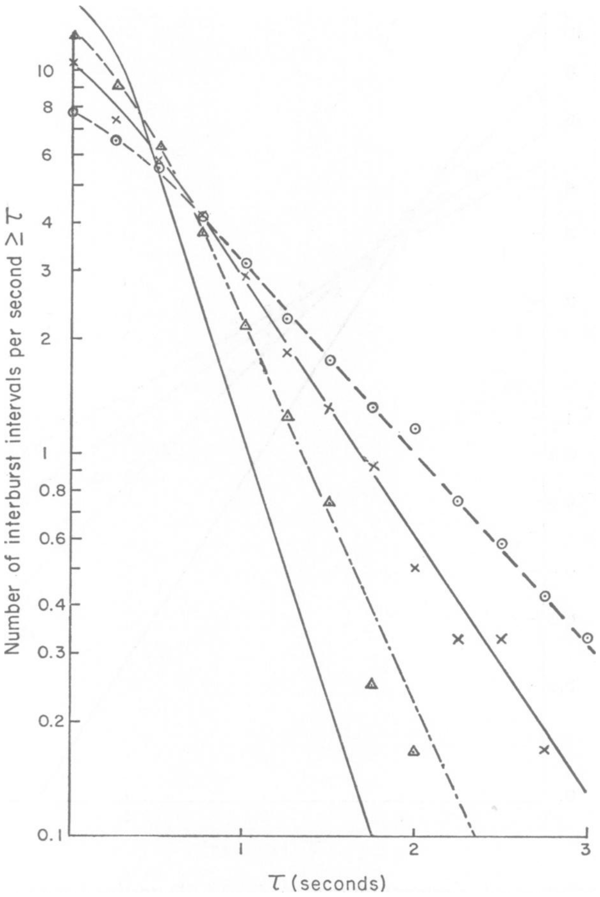


FIGURE 10 The effect of local cathodal current on the distribution of interburst intervals. (cell 30.6.61 during the passage of $-0.2\mu a$ for 120 seconds). Symbols as in Fig. 9. The ratios of the gradients before and during polarization Gradient (polarized)/Gradient (control) are as follows:

For bursts of four or more 1.13/0.46 = 2.46
For bursts of three or more 1.55/0.59 = 2.63
For bursts of two or more 2.27/0.79 = 2.87
For bursts of one or more 3.11/1.28 = 2.43

that the slow process is non-exponential and resembles that of the first group when their average frequency has been artificially increased (compare the triangle points of Fig. 2 and Fig. 11). It is thought that these cells are not qualitatively different from those of the first group, since they can be represented by means of a similar model having a non-random burst onset process, and in other respects the properties appear to be similar to those of group I.

Implicit in all of the analyses up to this point has been the assumption of a long

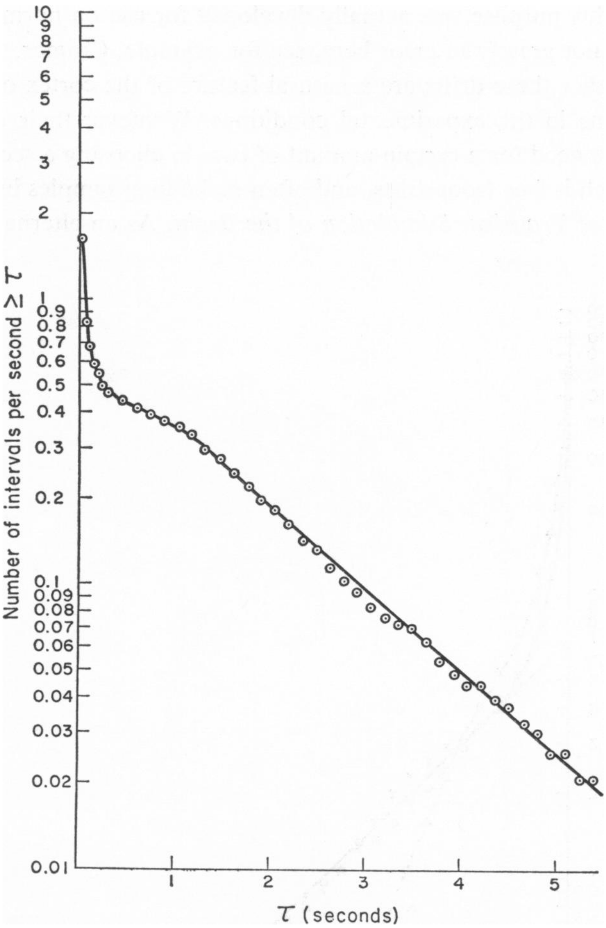


Figure 11 Interpulse interval distribution of group II cell (28.11.62).

term stationarity of the spontaneously firing neurone signal. The "stationarity" is qualified here because the switched nature of the signal obviously implies that it is not stationary over short periods. Some confidence may be gained from the fact that interval distributions for successive samples of record fall closely together; see for example the two unstimulated curves of Fig. 2. However, a statistical test of the hypothesis that such histograms derive from the same parent distribution is inapplicable here since the signal is in fact a compound distribution. In order to test the long term stationarity therefore, a series of pulse counts over succesive 50-second intervals was taken and analyzed for drifts of the mean and variance. Such tests showed that the signals do in fact remain stationary for periods as long as 15 minutes with variances close to those predicted for the switched Poisson structure. Nevertheless, definite changes in the mean are quite frequently encountered [the "Student" *t* test used for this purpose was actually developed for use on normal distributions, but is probably not greatly in error here, see for example, Cramer, 1945 (*b*)]. It is not known whether these drifts are a natural feature of the cortex or due to uncontrolled alterations in the experimental conditions. Whatever their cause, however, they indicate the need for a certain amount of care in choosing a section of a record for analysis which is free from drifts, and often make long samples impracticable.

Effects of Transient Stimulation of the Brain. As an alternative to polariza-

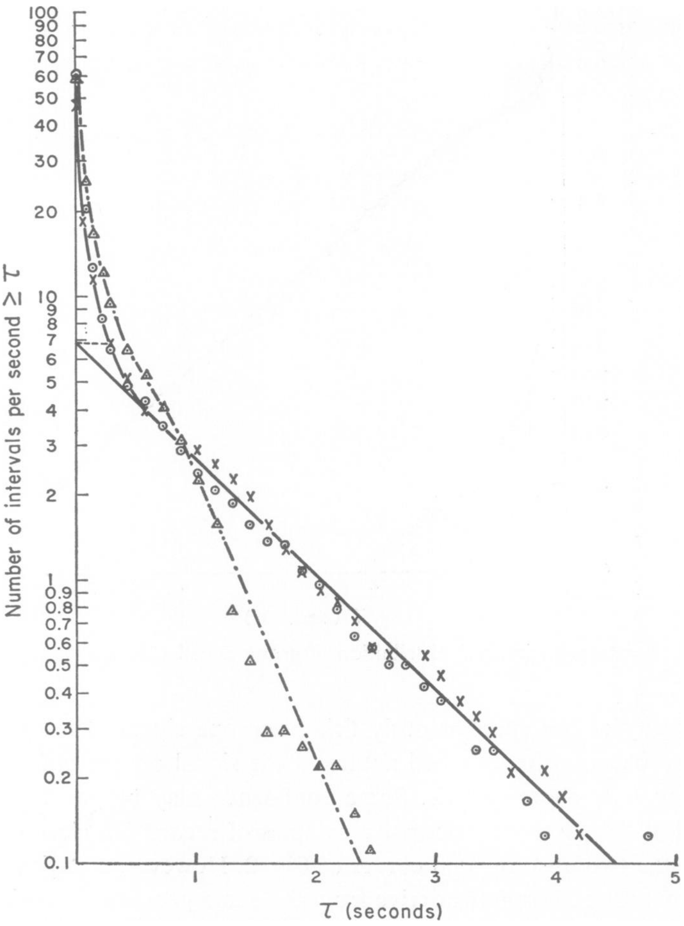


FIGURE 12 Interpulse interval distributions showing the effect of transient stimulation (cell 7.7.6.*b* in the median suprasylvian gyrus).

x, 300 second sample from unstimulated brain. △, 300 second sample during gross stimulation of cortical surface at a point about 10 mm from the recording point on the same gyrus. The stimulus consisted of clusters of 10 shocks applied once per second as described in Methods. ⊙, 300 second sample after stimulation was removed.

tion for artificially changing the firing patterns of cells a number of experiments were performed using regular stimulations by patterned light flashes or through surface electrodes (see Methods). In most cases the cells from which recordings were made were remote from the region of stimulation (*i.e.,* remote from the visual cortex in the case of the light flash); in all of these cases the timing of pulses relative to the time of stimulation is measurably altered (Burns and Smith, 1962). The effect on the interval distribution of a 4 minute period of surface stimulation is shown for a typical cell in Fig. 12. As for the polarized cells, the interval distribution reverts to its original form upon the removal of stimulation, and, also in common with the polarized cells, the Poisson-shower constant remains invariant (Fig. 13). In contrast, analyses similar to those of Figs. 9 and 10 show that the proportions of bursts of different lengths are not preserved.

As an indicator of the time dependence of successive pulse intervals the auto-

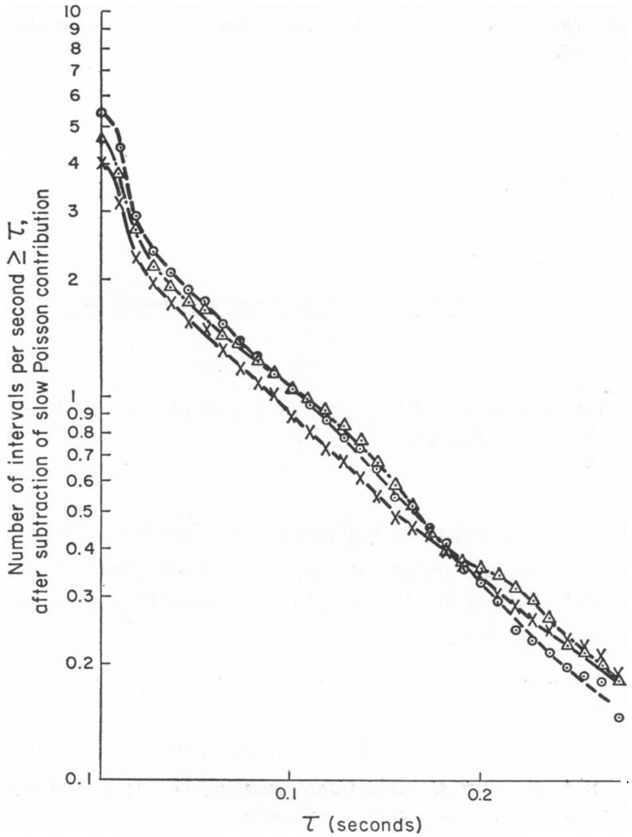


Figure 13 Interpulse interval distribution. Residue after subtraction of the maximum likelihood slow processes of Fig. 12. Symbols as in Fig. 12. Stimulation has had no effect on the fast process.

correlogram is a more sensitive, if in this case a more ambiguous, alternative to the transition matrix. To obtain a measurable correlogram the action potentials must be artificially broadened in some manner (Burns, Mandl, and Smith, 1963). The autocorrelograms of Figs. 14 and 15, from the same cells as analysed above, were

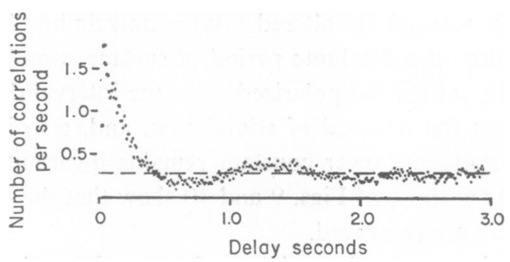


Figure 14 Autocorrelogram of unstimulated cell record (cell 30.6.61). Note that the initial point at zero delay does not include the correlations of each spike with itself. The spikes were widened to 9.1 msec. The dashed line is the level corresponding to linear independence.

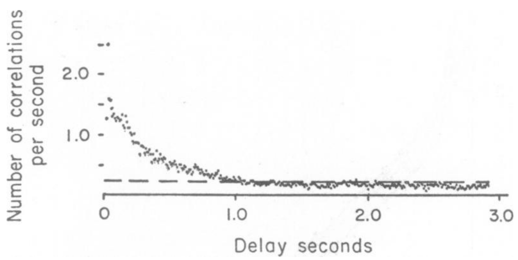


Figure 15 Autocorrelogram of unstimulated sample of cell 7.7.60.*b* (300 seconds). Analytical method as for Fig. 14.

obtained by widening the pulses to 9.1 msec. The slow initial falling phase of these autocorrelograms is due, of course, to the switched structure. Note that deviations from the calculated asymptote are sometimes observed to occur with delays of as much as 2 seconds (Fig. 14).

DISCUSSION

Of the forty neurone records examined in this project none have been found in which the continual activity cannot be satisfactorily described by the model we have proposed. Some of the records have shown characteristics which in one respect or another were significantly different in the statistical sense from the predictions of the model. However, an examination of the interval density histograms of such signals reveals that these deviations reflect preferences toward certain interval ranges within an essentially random context rather than suggest a different distribution. Indeed all of the tests applied in all cases were unambiguous in so far as they distinguished among alternative models. Where there was disagreement between the best-fitting model and the data it was such as to suggest that the model was too naive rather than that it was wrong (*cf.* Fig. 7). Hence we assert that all of the neurone signals examined in this project possess statistics which are adequately described either by the proposed model or by some more sophisticated version of it. Although the neurones are remarkably uniform in the general pattern of their firing, they do show wide variations in the values of the parameters. The significance of this is best seen by comparing this range of variation with the ease with which the parameters can be changed in any given neurone. The slow (burst onset) process is easily changed by polarization or stimulation, and in addition spontaneous drifts of average frequency can most often be ascribed to changes in this parameter. The fast process on the contrary seems to be extremely non-labile and more characteristic of the individual cell. In these circumstances therefore, the range of variation of the fast process (from 6 to 33 pulses per second) must be ascribed to real differences among the cells.

From these results it would appear that only one neurone population has been sampled, the properties of which are uniform in all regions in the neocortex. The question of sample bias has already been raised but even if another important functional type has been overlooked the presence of a virtually homogeneous population extending throughout the cortex suggests that widespread functional uniformity must also exist. This observation is related to the demonstration of widespread responses to local stimulation (Buser *et al.,* 1959; Burns and Smith, 1962) and lends weight to the view that from point to point in the cortex the similarities are more important than the differences. This view suggests that the most profitable first approach to cortical mechanisms may be to regard the tissue as composed of a small number of functional elements reiterated uniformly and to regard the various classical subdivisions of the cortex as regions in which connections to or from other parts of the brain occur with greatest density. While it is recognized that to ignore the obvious histological and physiological differences from one area to another may lead to dangerous oversimplification, nevertheless such an approach might well reveal general properties of cortical physiology which would otherwise have been overlooked, and lead to generalizations which can be applied to the specific conditions of connection in each part of the cortex.

The available evidence regarding the effects upon single neurones of the passage of local currents indicates that depolarization of neurone membrane lowers the threshold to incoming excitation (Eccles, 1957). If this is assumed to be the case with cortical neurones the results of the polarization experiments reported here suggest that alteration of this threshold affects only the times of onset and the durations of bursts leaving the activity within them unchanged. This is the principal fact which must be satisfied by any physiological explanation advanced to account for the switched Poisson signal. A number of general physiological hypotheses will now be suggested for their credibility examined in the light of the experimental results.

Let us first suppose that the Poisson shower is generated in part by processes external to the cell. The simplest possibility of this type would be local, uniformly interconnected networks composed only of the type of cell we have recorded, and which were excited above their ignition points periodically by external excitation (Smith and Davidson, 1962). Such a theory would demand that if the threshold of an individual cell were changed the average frequency at which it responded to the reverberation of its local network would alter, while the times of onset of the reverberation would remain constant. This is the exact opposite of what we observe and this theory must be discarded.

If the shower does originate in other cells, therefore, the situation must be more complex. One possibility is that the fast activity originates in cells which are inaccessible to our electrodes, either because of their small size or perhaps because they are located subcortically. This view is consistent with the observed effects of transient gross stimulation—that the shower process remains invariant while changes take place in the poststimulus histograms of all neurones tested by these recording techniques (Burns and Smith, 1962). In addition the cells we have recorded must have two thresholds, one controlling the onset and duration of bursts, which is sensitive to polarization, and another controlling the actual onset of action potentials within the bursts, and which is not sensitive to polarization. A basis for this condition might lie in the distinctly different configurations of somatic and dendritic synapses, resulting in different influences of these structures upon the output of the neurone (Rall, 1962).

A third hypothesis of this general class has been suggested in which the neurone fires in response to external excitation, but has a period of hypersensitivity which follows each action potential resulting in the burst structure observed. Since the bursts consist of a Poisson process which is independent of the quiescent threshold, the theory implies that the hypersensitivity after each action potential follows a rectangular time course. This is therefore logically similar to a gating process controlled by some part of the incoming excitation.

Another general hypothesis is that the Poisson shower originates entirely within the cell. This might be the case for example if the cell membrane were to become unstable when the potential across it reached a critical level of depolarization, and was easily triggered by disturbances within the cell. In this case it is unnecessary to postulate any type of cell other than those from which we have recorded, but only that incoming activity from time to time raises the total excitation on the neurone above the critical level. Against this hypothesis must be set the fact that the Poisson shower process, with an average firing rate as low as six pulses per second, is rather slow compared with the time properties usually associated with neurone dynamics.

The above may be summarized as follows: the results we have observed are consistent with both of the two general exhaustive hypotheses:

A. That the Poisson shower is dependent wholly or in part on influences external to the cell.

B. The Poisson shower originates entirely within the cell.

If (*A*) is the case then the results imply:

(*i*) The cells recorded possess at least two functionally different channels for incoming activity.

(*ii*) A class of cells exists which is inaccessible to our electrodes.

In any event cell properties are implied which are more complex than the classical picture of the neurone as a simple threshold switch.

Further work now in progress may narrow these hypotheses more satisfactorily. This includes a study of the extent to which burst onsets in different cells are interdependent, and a study to determine if the Poisson shower characteristics of single cells are affected by their firing history. Perhaps the key question is that of the mechanism of genesis of the Poisson shower activity and this is also under investigation.

SUMMARY

Statistical analyses have been applied to the signals recorded from continually active cortical neurones in the isolated forebrain of the unanaesthetized cat. The results of these analyses have been used to develop a mathematical model of the activity and to infer some properties of cortical neurones.

1. From the analysis of interspike intervals the signals generated by the majority of neurones have been shown to be composed of two roughly Poissonian components.

2. These distributions are shown, by analyses which are sensitive to interval sequence, to be segregated in time, *i.e.* intervals which are members of the higher frequency distribution tend to occur in succession, resulting in "bursts" of these intervals separated by intervals which are members of the lower frequency distribution.

3. The distribution of intervals between successive bursts is shown to have the same form as the distribution of long interspike intervals. When the average frequency of firing of the neurone is artificially altered by local cathodal polarization the alteration of the distribution of interburst intervals is of the same form and by the same ratio as is the alteration of the distribution of the long interspike intervals. It is concluded that the signal can best be conceived as a high-frequency activity following an approximately Poissonian law and gated on at random instants for

variable times. A minority of neurones have burst onset processes which are not random.

4. A mathematical model of the signal is developed on this basis. The signals of all the neurones examined were consistent with this model.

5. No tendencies toward grouping of neurone signal properties were observed either with respect to location in the cortex or to individual animals.

6. In general the signal parameters of a given neurone in the unstimulated brain tend to be stationary although drifts occur from time to time which, however, are short lived and are followed by further periods of stationarity.

7. Gross stimulation of the cortex and the passage of short periods of local currents are capable of altering the frequency of occurrence and the form of the distribution of "bursts" but neither appears to alter the fast activity within the bursts. It is concluded that two separate mechanisms are active in generating the signal, the one more liable to these manoeuvres than the other.

8. Some of the implications of these results are discussed in relation to the properties of the neurones and the networks of which they are a part.

APPENDIX

STATISTICAL MODEL OF NEURONE SIGNAL

As stated earlier, many alternative sets of postulates consistent with the observed neurone signal are possible, all leading to the same form for the interval distribution. The set of postulates below have been chosen because they appear plausible from the results on stimulated cells and are convenient from a measurement viewpoint. The mathematical argument is here carried through for this case only, as an example and point of comparison.

Postulates

The signal is considered to be composed of a Poisson shower gated on and off such that:

1. Average frequency of Poisson process is C.
2. Probability density function for the duration of the "on" periods of the gate is $g(t)$.
3. Probability density function of the duration of the "off" periods of the gate is $h(t)$

It is assumed that these parameters remain stationary in time in any unstimulated section of the record, and that successive "on" and "off" times of the gate are independent. Evidence for these assertions is given in the Results section. In addition we will assume that:

4. $h(t)$ has the form e^{-At} (true for group I cells).
5. $g(t)$ has the form e^{-Bt} (difficult to measure but probably approximately true).
6. Each gate onset occurs at the instant of some action potential.

This is convenient for measurement since bursts of no pulses are avoided. Some retrospect evidence will be mentioned later. A model where the gate goes off at the instants of action potentials and commences independently is equivalent.

Starting at one action potential, the probability that after an interval *t*, another action potential occurs in time *dt and* the gate has not turned off in the meantime is:

$$p_{CB}(t) = e^{-Bt}\cdot e^{-Ct}\cdot C\,dt$$

with the characteristic function:

$$M_{CB}(jv) = \int_0^\infty e^{-ivt} p_{CB}(t)\,dt = \frac{C}{B + C + jv}$$

Similarly, the probability that the gate goes off after an interval *t*, without a pulse in the meantime is:

$$p_{B\bar{C}}(t) = e^{-Ct}\cdot e^{-Bt}\cdot B\,dt$$

with:

$$M_{B\bar{C}}(jv) = \frac{C}{B + C + jv}$$

Starting where the gate turns off, the probability that after an interval *t* it goes on again for the first time is:

$$P_A(t) = e^{-At}\, A.\ dt$$

with:

$$M_A(jv) = \frac{A}{A + jv}$$

The cumulative characteristic function for the whole signal is therefore:

$$\begin{aligned} M(jv) &= M_{BC} + M_{BC}\cdot M_A \\ &= \frac{C}{B + C + jv} + \frac{AB}{(A + jv)(B + C + jv)} \\ &= +\frac{1}{j(B + C - A)}\left\{\frac{AB}{v + jA} + \frac{(C - A)(B + C)}{v + j(B + C)}\right\} \end{aligned}$$

The cumulative probability density function for intervals of length *t* is obtained by contour integration in the complex plane.

$$\begin{aligned} p(t) &= \frac{1}{2\pi}\int_{-\infty}^{\infty} e^{+ivt} M(jv)\,dv \\ &= \frac{1}{B + C - A}\{ABe^{-At} + (C - A)(B + C)e^{-(B+C)t}\} \qquad (1) \end{aligned}$$

By integration the distribution function of intervals equal to or greater than τ is:

$$F(\tau) = \frac{1}{B + C - A}\{Be^{-A\tau} + (C - A)e^{-(B+C)\tau}\} \qquad (2)$$

The expected interval length is:

$$E(t) = \int_0^\infty tp(t)\,dt = \frac{A + B}{A(B + C)} \qquad (3)$$

The expected number of intervals per second equal to or greater than τ is:

$$N\{I \geq \tau\} = \frac{A(B + C)}{(A + B)(B + C - A)} \{Be^{-A\tau} + (C - A)e^{-(B+C)\tau}\} \tag{4}$$

This is the form of the interval distributions of the text.
Using the transformation:

$$\left.\begin{aligned} \theta &= B + C \\ \lambda &= A \\ f &= \frac{A(C - A)}{(A + B)(B + C - A)} \end{aligned}\right\} \tag{5}$$

gives:

$$N\{I \geq \tau\} = f\theta e^{-\theta\tau} + (1 - f)\lambda e^{-\lambda\tau} \tag{6}$$

The transformed parameters (5) refer to a model where the gate both begins and ends at the instants of some action potentials, and have the significance of average frequency inside bursts, inverse of expected interval between bursts, and fraction of time consumed by bursts, respectively. The values of these parameters for any given cell may be obtained directly from the gradients and intercepts of the observed distributions and are the ones listed in Tables I and II. Other formulations are possible, leading to the same distribution form (6), (text above; also Miss V. Cane, private communication.)

The effects of the various forms of stimulation used in the experiments may now be simply summarized in terms of the above model. Polarization of the cell alters the burst onset process $h(t)$, causing an increase or decrease in the mean burst onset frequency according to whether the current is negative or positive respectively. The burst duration process $g(t)$ and the interval pulse shower mean C are left sensibly unchanged. This separation is one argument in favour of postulating that one of the gate transitions tends to synchronize with action potentials.

Measurements on cells stimulated periodically from the surface or through natural channels have shown that both $h(t)$ and $g(t)$ are changed. Since θ remains invariant, the parameters A, B, and C, are uneconomical, an argument in favour of postulating that both transitions of the gate tend to synchronize with action potentials (equation 5).

Group II cells have a non-exponential gate onset process $h(t)$, but are similar in other respects to the above.

Pulse Counts Variance

When examining the stationarity of the signal parameters over long periods of time it is useful to have an estimate of the variance of pulse counts taken from the gated Poisson model. We have used an approximation which is based on the assumption that the random variable which is the number of pulses occurring during unit time can be represented as the product of three simpler random variables *viz.*:

1. The number of gate onsets per second
2. The duration of a gate
3. The number of pulses per second of gate

Then, assuming these to be independent, the second moment of the product can be obtained as the product of the second moments of the components.

Using the postulates advanced earlier the expected number of burst onsets per second would be:

$$\frac{AB}{A+B}$$

Treating this as a Poisson process (a further approximation) the second moment would be:

$$\frac{AB}{A+B}\left(1+\frac{AB}{A+B}\right)$$

The duration of a single gate has a first moment $1/B$ and a second moment $2/B^2$, while the number of pulses per second of gate has a first moment C and a second moment $C(1+C)$.

We can now write down the first and second moments of the product signal, adding terms due to our postulate that every gate begins with a pulse: Expected number of pulses per second

$$= \frac{AB}{A+B}\left(1+\frac{C}{B}\right) = \frac{A(B+C)}{A+B} \qquad \text{(compare form 3)}$$

Second moment of number of pulses per second

$$\approx \frac{AB}{A+B}\left(1+\frac{AB}{A+B}\right)\left(1+\frac{2C(1+C)}{B^2}\right)$$

Therefore variance of number of pulses per second

$$\approx \frac{AB}{A+B}\left(1+\frac{AB}{A+B}\right)\left(1+\frac{2C(1+C)}{B^2}\right) - \left\{\frac{A(B+C)}{A+B}\right\}^2 \qquad (7)$$

Values for A, B, and C for any given cell may be obtained from the interval histogram, using the transformation equation (5).

Autocorrelograms

Predictions for the autocorrelograms (Figs. 14 and 15) may also be obtained from the model. In practice, however, the fact that most of the neurone signals deviate from the model for intervals in the region $\tau < 30$ msec. results in discrepancies. For this reason the interval histogram has been used as the main vehicle of analysis in this investigation.

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