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PERIODIC COMPONENTS IN STEADY-STATE ACTIVITY OF CUNEATE NEURONES AND THEIR POSSIBLE ROLE IN SENSORY CODING

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SUMMARY

1. Individual cuneate touch, touch-hair and proprioceptive neurones often display periodic components in their steady-state resting or evoked discharges. The periods may be visible in the interspike interval distribution or may be revealed only by the expectation density function. Several levels of complexity were identified; from one to four mutually prime, periodic components may be present, with or without an aperiodic component.

2. The periodic components usually depend on the peripheral input, as shown by their introduction or modification by peripheral stimulation and by their disappearance following deafferentation.

3. The coefficient of variation of periodic discharges by touch and touch-hair neurones was 0·024–0·23 (aperiodic, driven discharges usually had CV's of 0·64–0·77).

4. The occasional recording extracellularly of a periodically occurring, unitary prepotential preceding the spike and the intracellular recording of a periodic, 'giant', unitary e.p.s.p. imply that an individual periodic impulse in an afferent fibre may elicit a post-synaptic discharge. Even with threshold stimulation, the timing of post-synaptic discharge was quite precise.

5. The periodicity did not appear to reflect a cycle of subnormality intrinsic to the post-synaptic neurone, because a current pulse injected through a micro-electrode lying just external to the membrane could block the next expected periodic discharge, but did not continuously vary its waiting time.

6. A mathematical model that fits some discharge patterns predicts

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the interval distribution from an over-all probability of response to the periodic input: each periodic afferent impulse is treated as an independent trial resulting in success (post-synaptic discharge) or failure. Some other interval distributions are fitted by a 'conditioned' model in which a success reduces the probability of response at the next periodic trial.

7. The discussion includes a hypothesis of tactile discrimination, in which the output of a sensory relay, e.g. cuneate neurone, signals both the number of afferent fibres converging upon it and the afferent period in each.

INTRODUCTION

The dorsal column nuclei (Amassian & De Vito, 1957; Andersen, Eccles, Schmidt & Yokota, 1964*a*; Andersen, Eccles, Oshima & Schmidt, 1964*b*) share with a number of other mammalian sensory relay nuclei (Bishop, Burke & Davis, 1962; Eide, Fedina, Jansen, Lundberg & Vyklický, 1969*a*, *b*) the following properties: (1) a discrete afferent input with activity in individual presynaptic fibres that is readily sampled, (2) an intranuclear synaptic organization which is at least partially elucidated and (3) an output tract which is readily stimulated, permitting the identification of projection neurones by demonstrating antidromic invasion. In common with the Clarke's column relay, the *major* excitatory input to the dorsal column nuclei is the primary afferent fibre, whose temporal patterns of discharge have been well defined. However, not all the excitatory input to the dorsal column nuclei is from unrelayed impulses. The dorsal column relays (Hursh, 1940) may excite cuneate neurones (Amassian & De Vito, 1957; Andersen *et al.* 1964*a*); furthermore, Uddenberg (1968) identified synaptically relayed impulses in deep, dorsal column fibres, but did not determine whether they influenced the dorsal column nuclei. Descending projections from cerebral cortex have also been extensively studied (Andersen *et al.* 1964*a*, *b*; Gordon & Jukes, 1964*b*; Jabbur & Towe, 1961).

A major step in the typing of dorsal column neurones was the demonstration of classes responding to different stimulus modalities, presumably reflecting modality specific afferent fibres (Gordon & Paine, 1960; Kruger, Siminoff & Witkovsky, 1961; Perl, Whitlock & Gentry, 1962; Gordon & Jukes, 1964*a*; Winter, 1965). During maintained stimulation or even during the 'resting state,' at least two of the major classes of afferent fibres projecting to the dorsal column nuclei yield slowly adapting discharges, whose pattern is periodic. Proprioceptive afferent fibres recorded close to the cuneate nucleus discharge periodically (Amassian, Macy, Waller, Leader & Swift, 1964). Type II touch afferent fibres have a remarkably periodic discharge (Chambers & Iggo, 1967; Iggo & Muir, 1969) and, in

contrast to the Type I, project directly in the dorsal column to high cervical levels (Petit & Burgess, 1968). A quantitative analysis of the firing patterns of proprioceptive cuneate post-synaptic neurones suggested a model in which the output discharges by the post-synaptic neurone resulted from a mixture of a *small* number of periodically discharging proprioceptive afferent fibres, each delivering a *powerful* excitation (Amassian *et al.* 1964). The general description of these patterns was confirmed by Galindo, Krnjević & Schwartz (1968). Ten Hoopen (1966) provided a mathematical description of a model in which periodic and aperiodic discharges were mixed. Somewhat similar patterns of activity were recorded in dorsal spino-cerebellar tract (DSCT) cells by Jansen, Nicolaysen & Rudjord (1966) and were treated quantitatively by Walloe (1968). A deficiency in these experimental studies was the failure to extract periodic components using the expectation density function, as employed by Poggio & Viernstein (1964) on discharges by ventroposterior units of thalamus.

The hypothesis that individual afferent fibres may exert powerful excitatory effects on sensory relay neurones is supported by intracellular recording. Andersen *et al.* (1964*b*) and Eide *et al.* (1969*b*) recorded e.p.s.p.s of many millivolts amplitude in cuneate and Clarke's column cells, respectively; these e.p.s.p.s were attributed to individual afferent impulses. Recording extracellularly the field potentials associated with the e.p.s.p.s of lateral geniculate neurones, Bishop *et al.* (1962) suggested that activity in a single optic fibre could excite a lateral geniculate neurone.

This paper presents a quantitative analysis of periodic components in the steady-state discharge patterns of certain classes of cuneate neurones, and tests of the hypothesis that the discharge patterns of such neurones reflects periodic activities of individual afferent fibres. A preliminary account of these findings has been presented to the Physiological Society (Amassian & Giblin, 1968).

METHODS

The data presented below were obtained from twenty-eight cats, all but one of which were anaesthetized with Na pentobarbitone with an initial I.P. dose of 42 mg/kg. Subsequent maintenance doses were given I.V. (One cat was decerebrated at the intercollicular level.) Cats were usually immobilized by I.V. injection of gallamine triethiodide and were artificially ventilated with oxygen. Arterial pressure was routinely monitored and an electrocorticogram was recorded from sensorimotor cortex. The rectal temperature was maintained between 35–40° C by a shielded infra-red heating lamp.

The dorsal column nuclei were conventionally exposed (Amassian *et al.* 1964). Most units were recorded at levels between the obex and 4–5 mm below. For extracellular recording, adequate control of cardiorespiratory pulsations of the brain stem was secured by a free cisternal drainage of c.s.f. and double pneumothorax.

Residual pulsations of the brain stem usually prevented us from obtaining intracellular records adequate to establish the presence of periodic components. Therefore, a technique was used in which both the pulse pressure and brain stem pulsations were drastically reduced by alternately lowering and raising the aortic pressure during systole and diastole, respectively (Giblin & Amassian, 1966). The method included the following steps: (1) the vagi were ligated to increase and to stabilize heart rate, (2) the right common carotid artery was cannulated to monitor the pulse pressure, (3) the abdominal aorta was ligated distally and the largest possible polyethylene catheter (e.g. PE 320 gauge) was inserted centrally up to the level of the arch (the ligation resulted in ischaemia of the hindquarters), (4) the cannula was

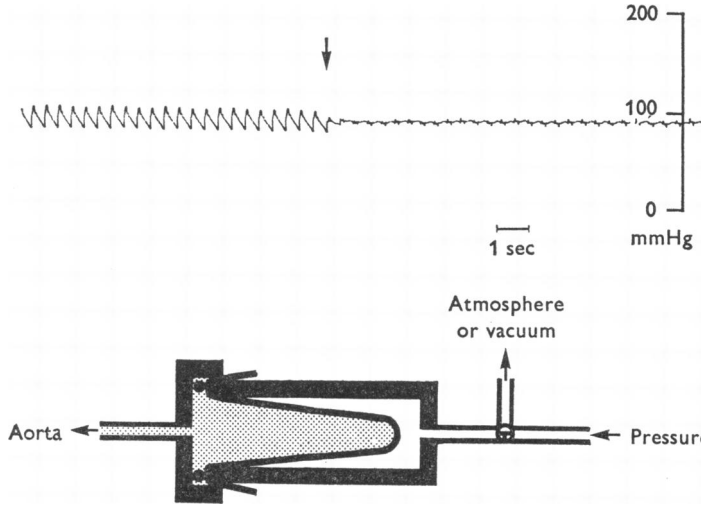

Fig. 1. Method used to facilitate cuneate intracellular recording by reducing the arterial pulsation. Upper record shows pulse pressure in carotid artery before and after opening (at arrow) the aortic cannula to device shown in diagram, below. Details of operation in Methods.

connected to a chamber (Fig. 1) with a total capacity of 15 ml. in which heparinized blood (stippled) and air were separated by a polyethylene membrane (conveniently, the thumb of a plastic glove). During most of the cardiac cycle, the air compartment was connected through an electromagnetically operated valve to a high pressure reservoir. Following the P wave of the e.c.g., the valve was actuated after an adjustable delay (e.g. 45–60 msec), thereby connecting the air compartment for a brief period (e.g. 10 msec) early in systole to a source of low pressure. Optimal reduction of pulse pressure was secured when the pressure source was high enough (e.g. 3–5 psi) to keep the polyethylene bag nearly collapsed and when a screw clamp on the aortic cannula (not shown in Fig. 1) was critically adjusted. All parameters were best adjusted when the arterial pressure was displayed on oscilloscopic traces synchronized with the e.c.g.

The great majority of the units were recorded extracellularly with glass-insulated tungsten micro-electrodes. These were prepared as previously described (Amassian *et al.* 1964), except that the final cathodal polishing of the tip was omitted. All intracellular recordings were obtained with glass micropipettes filled with 2 M-K citrate;

occasionally, extracellular recordings were obtained with such micropipettes or with micropipettes filled with 2–3 M-NaCl. A conventional bridge circuit was used to record unit activity during passage of current either with intracellular or with extracellular placement of the micropipette. The resistance of the input limb of the bridge was 10^9 Ω. With extracellular placements of the micropipette and with the tip negative, relatively large currents (e.g. 2×10^{-8} A) had to be passed to block the unit discharge. Although excellent for recording from both somata and axons, glass-insulated tungsten micro-electrodes could not pass such currents without gross imbalance of the bridge and failure of recording because of a rapidly increasing impedance of the electrode. Micro-electrode signals were led through a negative-capacity input stage, were amplified conventionally and recorded on a multichannel tape recorder at a tape speed of 15 in./sec.

The peripheral stimuli used in gathering data on periodic components were exclusively mechanical. The modality of neurones responding to cutaneous stimulation was identified as either hair or touch by using a light probe, an airjet or a maintained weight on the skin. Neurones which failed to respond to cutaneous stimulation but which responded to bending of joints were classified as proprioceptive. The response to palpation of the muscle belly at different limb positions occasionally permitted distinction between muscle and joint inputs, but in no instance was this confirmed by limb dissection. The computer analyses performed required that neuronal discharge patterns be in a relatively steady state. Therefore, tape recordings were begun many seconds after any change in condition, e.g. after placing a weight on the paw or changing the joint angle.

In some instances, ongoing afferent inputs during steady-state peripheral stimulation were reversibly excluded by anodically blocking the nerve in a neurovascular bundle which was the *only* connexion between the forelimb and the rest of the cat (Schwartz, Giblin & Amassian, 1964). (The neurovascular bundle was surrounded by a polyethylene catheter containing Locke solution and an Ag-AgCl electrode which was made the anode. The cathode was a similar electrode inserted distally into the limb.) Currents of 0·3–1·7 mA were effective in blocking both the cuneate population response and the summed somatosensory area I primary response to test peripheral stimuli. A late cortical response (latency 17–35 msec) persisted, implying that A delta fibres projecting rostrally outside the dorsal columns had escaped the block. Recordings from individual dorsal column afferent fibres showed cessation of discharge during successful blocks. Other cuneate neurones were irreversibly deafferented by nerve ligation.

Cuneothalamic projection neurones were identified by stimulating the contralateral medial lemniscus through bipolar electrodes at the level of caudal thalamic nucleus ventralis posterior.

Data processing

The taped runs were played back and spikes derived from an individual neurone were converted into 100 μsec rectangular pulses which were rerecorded on a second tape recorder. For most units, a simple amplitude discriminator served to detect reliably discharges by a neurone. For other units and especially when two neurones were recorded simultaneously by the same electrode, a 'coincidence' detection procedure proved more reliable than a simple amplitude criterion: This method depends on the fact that spikes of different neurones may differ not only in amplitude but also in the sequence and in the durations of their positive and negative phases. For example, by arranging that positive and negative phases separately set up 50 and 10 μsec pulses, and by appropriately delaying the pulse set up by the positive phase, coincidence between these pulses occurs with a positive-negative

spike but not with a negative-positive spike of similar amplitude. The coincidence of pulses triggers a pulse which is recorded on one channel and also cancels pulses set up by positive-negative spikes on a common channel, leaving pulses due to the other neurone.

The pulse trains were played back into a suitably programmed Control Data Corporation 160A digital computer. Intervals between successive discharges were measured with a precision of 100 μsec and were stored serially. Subsequent analyses performed on the 'interval stack' include (1) the interval distribution between spikes (ID of spikes), (2) the ID of spikes in successive time slices (this is used as a measure of stability of the run), (3) the expectation density function (ED) (this proved a valuable method of extracting periods in complex spike trains; Poggio & Viernstein, 1964). Cuneate neurones, especially those of cutaneous modalities tend to fire in high frequency bursts of 2–3 spikes (Amassian *et al.* 1964; Galindo *et al.* 1968). Periodic sequences of such doublets or triplets would lead to multiplication of the contents of periodic modes in the ED (e.g. a triplet following a triplet). Such multiplication would make an estimate of the probability of response to a periodic input impossible without knowing the precise *sequence* of triplets, doublets and single discharges in the run. Therefore, high frequency bursts and isolated discharges were treated equivalently as individual events, which were timed either from first discharges of bursts or from isolated discharges; after this transformation, the ED and ID of events were used in computing probabilities in complex spike trains. The duration of the interspike interval (within the burst) which determined that a burst would be treated as a single event was chosen after inspection of the ID of spikes; it typically ranged from 2 to 10 msec in different runs.

RESULTS

Patterns of periodic discharge

Even in the absence of deliberate peripheral stimulation, post-synaptic cuneate neurones often show a resting discharge (Schwartz *et al.* 1964). The resting discharge of hairfield neurones usually consists of brief high frequency bursts and single discharges at irregular intervals; in only four out of 164 neurones was a periodic component identified. Furthermore, we did not observe periodic discharge by hairfield neurones during maintained driving by a continuous airjet. By contrast, the resting discharge of twenty-six out of seventy-two touch field, thirteen out of thirty-six touch-hair (convergent) and twelve out of fifty-two proprioceptive field neurones showed periodic components.

The simplest pattern of periodic discharge is shown in the top row of Fig. 2. The discharges were recorded from a touch field neurone while the paw was elevated off its receptive field. The ID of spikes (left column) shows a tall peak in the first and second one msec bins, which contain 60% of the intervals: the neurone fired almost exclusively in 2–3 spike bursts. The remaining intervals are nearly all dispersed narrowly around a single mode (57 msec in this sample), indicating a high degree of periodicity of the bursts. In the ID of events (middle column), brief intervals are practically eliminated, but the periodic intervals are retained. The ED

function of events (right column) shows a large number of peaks at multiples of the basic period. Typically, successive modes show increasing dispersion.

Another run was recorded from neurone *A* many seconds after the paw was lowered on to the receptive field and weighted. The middle row shows that, as in the previous run, the neurone fired in periodic bursts, but the period had decreased slightly from 57 to 54 msec. However, the IDs both of spikes and of events show numerous intervals of intermediate value; those > 2 msec and < 50msec are approximately equiprobably

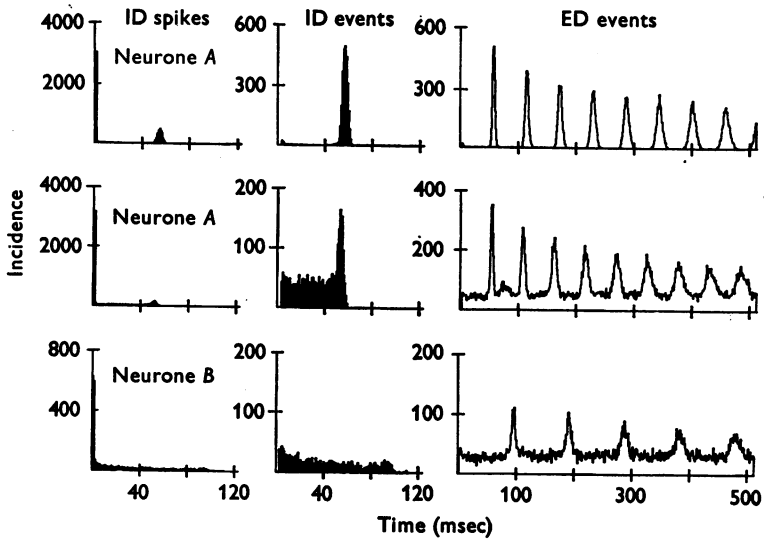

Fig. 2. Distributions of intervals between spikes, between events and expectation density functions of events derived from two cuneate neurones. Top and middle rows are derived from same touch neurone under resting and stimulated conditions, respectively. In all histograms, the plot is suppressed when the count per bin in all subsequent bins is less than 4. In all Figures, time scales in each column are identical unless indicated otherwise.

distributed. Such intervals were also present in the previous run, but were very few in number. An interval of intermediate duration could result from either of two processes. In the first, an *aperiodic* discharge by the neurone in response to another type of input splits a periodic interval into two shorter intervals. The number of intervals having the duration of the period thereby would be decreased, but the first mode of the ED would be essentially unaffected because it reflects the sums of successive intervals. In the second process, the interval terminated by the aperiodic discharge is followed by an interval approximately equal to the basic

period, that is, the periodic process is 'retimed'. When the incidence of intervals of intermediate duration is low the two processes may be readily distinguished by examining the sequence of intervals between events. In most cases, the event sequence shows long series of modal intervals interrupted by pairs of intervals whose sum equals the modal interval (Fig. 3, top). That is, the periodic process is not retimed. Splitting was apparent even when the modal interval drifted (Fig. 3, middle). An

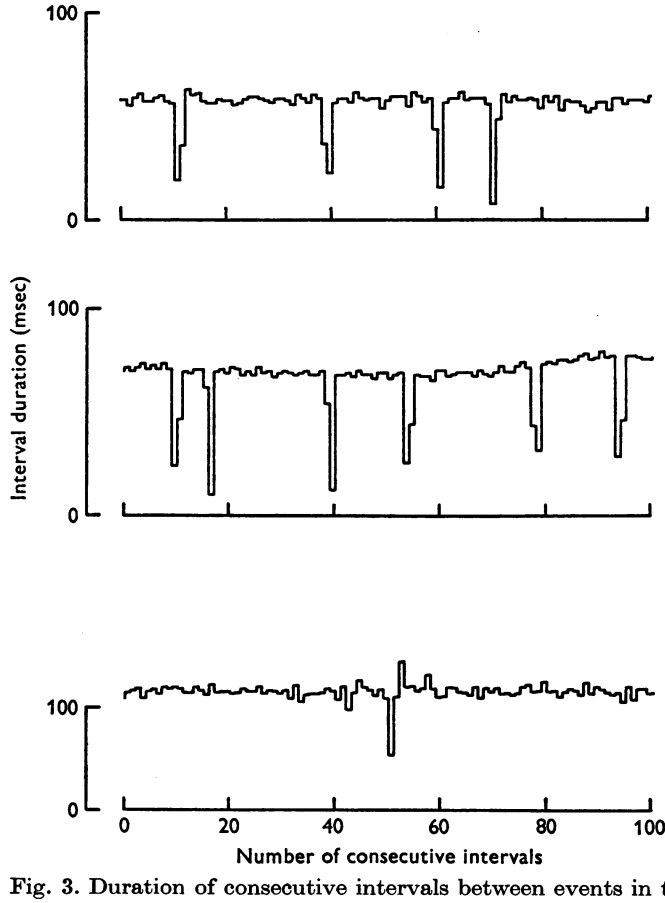

Fig. 3. Duration of consecutive intervals between events in three cuneate neurones. Top and bottom neurones had touch fields, middle neurone had touch and hairfields.

example of 'retiming', rarely encountered in periodically discharging cuneate neurones, is shown in Fig. 3, bottom. Other intervals in the same run exhibited splitting.

In the bottom row of Fig. 2, the discharge pattern of a touch field neurone may be explained by the splitting of most of the intervals between

periodic events by an aperiodic discharge; the ID shows a gradually decrementing incidence with increasing duration. In this run, a periodic component is not evident in the ID and is revealed only by computing the ED function. However, we have found that rather abrupt truncation of the ID at the long interval end usually indicates the presence of a periodic process which determines the longest interval.

A more complex version of the one period type of discharge results in a multimodal ID in which successive modes are multiples of the period of the first mode (Fig. 4). The data are from a proprioceptive cuneate neurone under two conditions (*A*, *B*) of steady-state excitation. Under

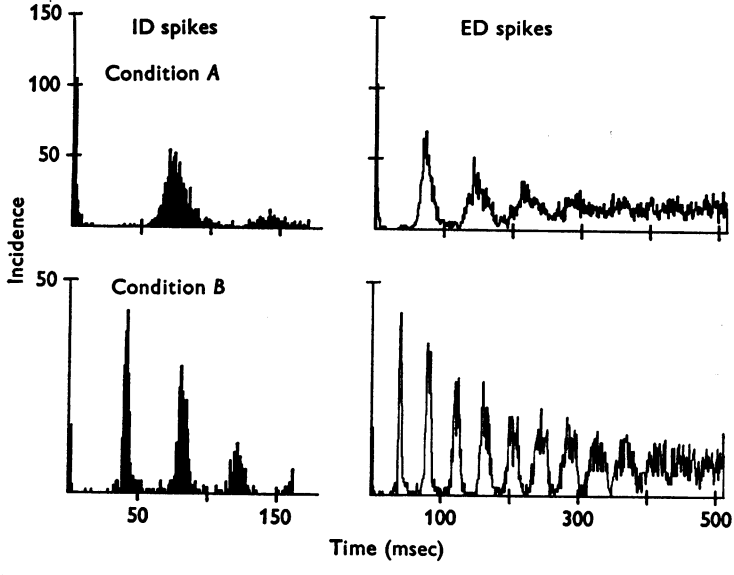

Fig. 4. Interval distributions and expectation density functions of spikes derived from a cuneate proprioceptive neurone under two conditions of stimulation (*A*, *B*).

condition *A*, the basic period was 72 msec, but intervals were also grouped around 144 msec. The area under the second mode is markedly smaller than that under the first. Under condition *B*, the basic period decreased to 41 msec and its dispersion also diminished. Three subsequent modes which progressively decrease in amplitude are seen at multiples of the basic period. It may be noted that although the duration of the second modal interval in *B* exceeds slightly that of the first in *A*, its dispersion is much less. Presumably, the more numerous peaks in the ED of *B* as compared with *A* reflect the reduced dispersion of the interval modes of *B*.

A still higher level of complexity results from a mixture of two periodic components (Fig. 5, top; Fig. 8, Stim-pre block). When two periodic components were identified, they might differ markedly (e.g. 56 and 76 msec in Fig. 1*B* of Amassian & Giblin, 1968) or they might be so close (e.g. 72 and 79 msec in Fig. 5, top) as to be distinguishable only in later modes of the ED; for example, the separation of the β mode is barely evident in the second peak of the ED, but is more apparent in the third. The fourth peak is *less* dispersed than the third, presumably because it contains only the α component. In Fig. 8, pre block, although the periods differ markedly

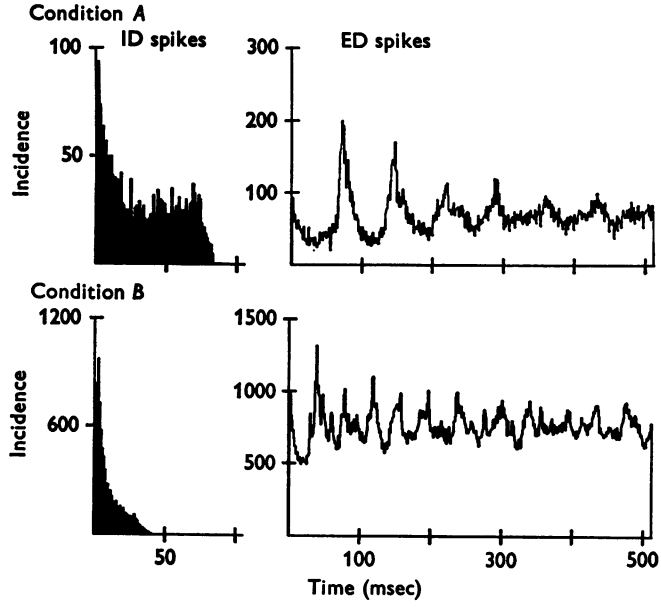

Fig. 5. Interval distributions and expectation density functions of spikes derived from a proprioceptive cuneate neurone under two conditions of stimulation (*A*, *B*).

(32 and 70 msec), the second mode of the shorter period is close enough to the longer period to result in addition of some modes in the ED.

Except at brief intervals, the ID is usually equiprobable out to the briefer of the two periods, where a mode may be evident if the two periods differ significantly. The equiprobable portion of the ID is attributable to the splitting of periodic intervals by discharges due to the other periodic component and vice versa; usually, the periodic components are mutually prime, leading to splitting of the intervals at all possible positions. The accumulations of short intervals both in Fig. 5, top, ID and Fig. 8, Stim-pre block, ID are due to additional subdivisions of intervals by *aperiodic* discharges.

Exceptionally, more than two periodic components were identified in the ED. Fig. 5, bottom, shows, under altered stimulus conditions, that at least three and probably four periodic components are present in the ED; the periods are 30, 37, 39 and 48 msec. The striking feature of the ID is the absence of an equiprobable portion of the distribution; instead, with increasing duration, the intervals rapidly decrement in incidence. A previous model predicted a linear decrement with *three* periodic inputs (Amassian *et al.* 1964).

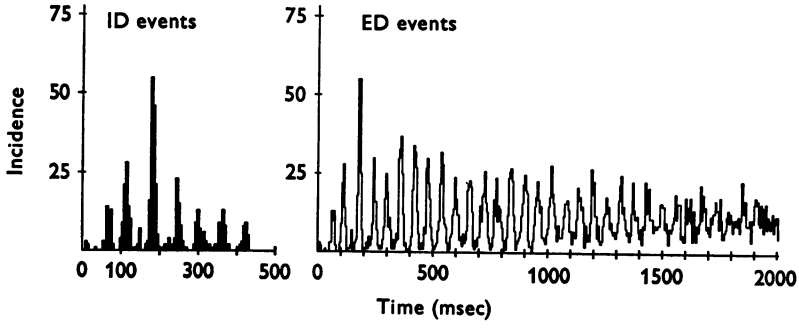

Fig. 6. Interval distribution and expectation density function of events in resting discharge of a touch field neurone. Forelimb was denervated except for a single nerve trunk.

It should be noted that if a single periodic component changes its period *discontinuously* during a run, additional modes corresponding to such periods might occur in the ED; this could lead to the erroneous conclusion that several periodic components were *simultaneously* present. However, such changes in mean period could not account for an equiprobable portion of the ID: they were explicitly excluded by demonstrating that the two periodic components in Fig. 5, top, were present in both halves of the run.

In a single touch field cuneate neurone, the ID of the resting discharge showed a series of modes which were not exact multiples of the first mode (Fig. 6). The ID of events had modes at 68, 113, 180, 245, 298 and 360 msec (measured on an ID with a 1 msec binwidth). The areas under the modes increased up to the third mode and subsequently diminished. Each mode may be accounted for by the 'beating' between two periods α and β, 16·4 and 22·5 msec in duration, respectively, near synchrony occurring with 4α and 3β, 7α and 5β, 11α and 8β (optimum), 15α and 11β, 18α and 13β, 22α and 16β cycles. Further comment on this unusual neurone is postponed till later.

The description of the dispersion of the intervals about the mean has thus far been qualitative. In Fig. 7, the standard deviation is plotted as

a function of the mean period for post-synaptic touch and touch-hair field cuneate neurones with periodic components in the expectation density function. For touch neurones, the coefficient of variation (CV) ranged between 0·024–0·182; for touch-hair neurones, the CV ranged between 0·04 and 0·23.

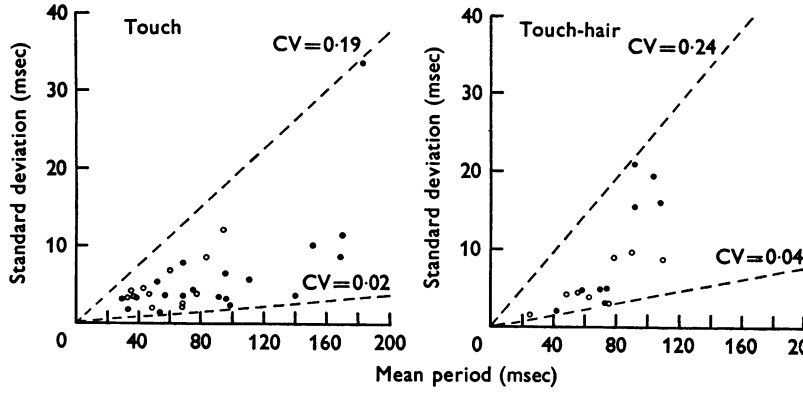

Fig. 7. In periodically discharging touch and touch-hair neurones, S.D. of first mode intervals between events is plotted as a function of the basic period. Under different stimulus conditions, a neurone may exhibit either a single periodic drive with different values of the mean interval of the first mode or multiple periodic drives simultaneously. When such multiple states were observed, only the highest and lowest values of the modal interval were plotted. Data are derived from event interval distribution or expectation density function. ●, Single state; ○, highest and lowest periods of multiple states.

Afferent source of periodic components

Periodic components are much more frequently present in the resting discharges of touch and proprioceptive field cuneate neurones than in those of neurones which can be driven only by movement of hairs; this difference presumably reflects the periodicity of afferent discharges in type II touch afferent and proprioceptive afferent fibres and its absence in hair afferent fibres. As might be expected, periodic components were not seen in resting discharges by acutely deafferented cuneate neurones. None of fifty-four cuneate neurones previously deafferented by peripheral nerve ligation, and none of twenty-four neurones deafferented by brachial plexus ligation discharged periodically.

A disadvantage in comparing populations of cuneate neurones with and without peripheral innervation is that the modality type of the deafferented neurone is unknown. Significantly, when recording from the *same* cuneate neurone, peripheral stimulation usually alters the period of

pre-existing discharge (Fig. 4) or adds one or more periodic components (Fig. 8). Exceptionally, we saw the reverse; in two touch-hair neurones, periodic components in the resting discharge with mean durations of 70 and 107 msec and CV's of 0·17 and 0·15 respectively, were lost during the peripheral stimulation.

Periodic components were lost following either ligation or anodic block of the sole remaining nerve in the forelimb. For example, in Fig. 8, with the limb in one position, the pattern of discharge by a cuneate proprioceptive field neurone prior to anodic block had two periodic components:

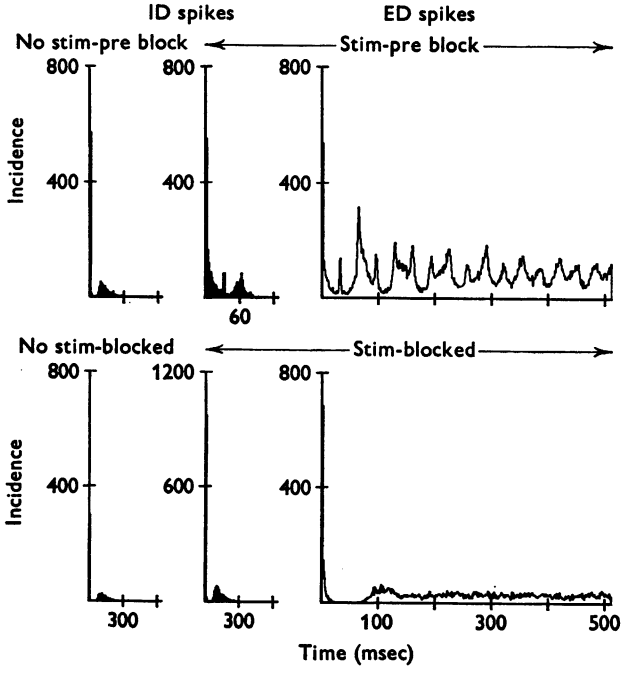

Fig. 8. Effect of anodic block of afferent input on cuneate proprioceptive neurone under different loading conditions. Upper records show, before block, the interspike interval distribution with the limb in the minimum firing position (left) and interval distribution and expectation density function of spikes with limb midway between flexion and extension (right). Lower records obtained under same stimulus conditions during block.

these were lost during anodic block and the pattern of aperiodic discharge then resembled that recorded with the limb in the minimum firing position for this neurone. In the latter position, the pattern of discharge was unaffected by anodic block. The periodic components reappeared after recovery from the block and this effect of anodic block was reproducible.

In the touch field neurone of Fig. 6, the resting discharge was abolished by ligation of the sole remaining nerve.

The finding of an aperiodic resting discharge in touch and touch-hair cuneate neurones need not imply that such discharges result from resting activity in skin afferents, because central drive may also contribute to resting discharge (Schwartz *et al.* 1964). However, steady peripheral stimulation failed to add periodic components to the discharges of six touch-hair and one touch neurone. In six of these neurones, the evoked activity had a mean event period of 22·4–114·5 msec with a CV of 0·64–0·77; in the different neurones, a semilog plot revealed an exponentially decrementing incidence of intervals longer than 2, 22, 35, 54, 80, 112 msec in duration. (In the seventh neurone, the event ED had a first mode with a mean period of 110 msec and a CV of 0·16, but only one subsequent mode.) Thus, peripheral drive may contribute not only periodic but also aperiodic components in the evoked discharges. Both contributions may occur together (Fig. 2, middle).

Relationship between pre- and post-synaptic periodic components

Although afferent input often contributes one or more periods to the discharges by a cuneate neurone, it does not necessarily follow that a period in the post-synaptic discharge is identical with the period of discharge by any individual afferent fibre synapsing with the neurone. Thus, discharges by a motoneurone stimulated by maintained stretch of its muscle are markedly periodic, but the period is probably determined by the duration of the afterhyperpolarization of the motoneurone (Eccles, 1953), and is not identical with that of discharge by any particular Group Ia fibre impinging on it.

If periodic discharge by a cuneate post-synaptic neurone reflects a response to all or most impulses in a periodically discharging presynaptic fibre impinging upon it, then intracellular recording should reveal periodic e.p.s.p. (or potentials due to electrotonic coupling) large enough to elicit post-synaptic spikes. Following stimulation of peripheral nerves, large prepotentials, which were identified by their time course as e.p.s.p.s, were recorded from cuneate post-synaptic neurones (Andersen *et al.* 1964*b*). The subthreshold prepotentials in their Fig. 8*A*, *E*, *F* and *G* had rise times of 0·77, 1·2, 1·2 and 0·88 msec, respectively; in all but *F*, the membrane potential returned with a remarkably rapid time course to resting level. Although the possibility of some contamination by i.p.s.p.s cannot be excluded, a more likely explanation for the rapidity of the decay of the prepotential is that the membrane time constant was shortened by an increase in membrane conductance due to damage to the neurone (see also Fig. 7 in Kuno & Miyahara, 1968).

Fig. 9 shows prepotentials evoked in two cuneate post-synaptic neurones by stimulation of the dorsal columns a few millimetres from the recorded neurones (one of the neurones was identified as a projection neurone). When a spike failed to occur, the rise times of the prepotentials were 1·2 msec (*B*) and 1·5 msec (*E*), i.e. they equalled or slightly exceeded those recorded by Andersen *et al.* (1964*b*). Although neither in our experiments nor in those of Andersen *et al.* was reversal of the prepotentials

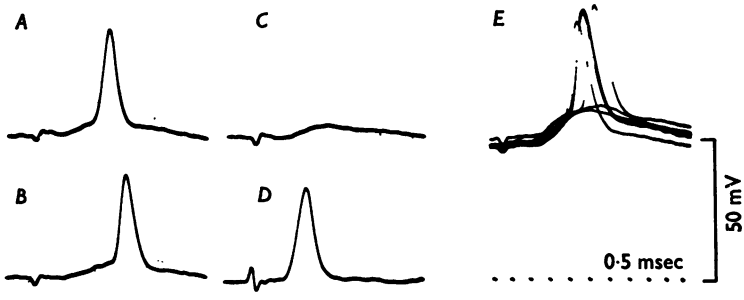

Fig. 9. Intracellular recordings of evoked activity in two cuneate neurones. A–C show supra- and subthreshold e.p.s.p.s evoked by identical stimuli to the dorsal columns a few mm caudal to the neurone. *D* shows antidromic invasion following a stimulus to the contralateral medial lemniscus. *E* shows, in another neurone, superimposed supra- and subthreshold e.p.s.p.s to dorsal column stimulation. Originally recorded on FM tape with d.c. coupling throughout; RC coupling used for above records. Calibrations apply to all records.

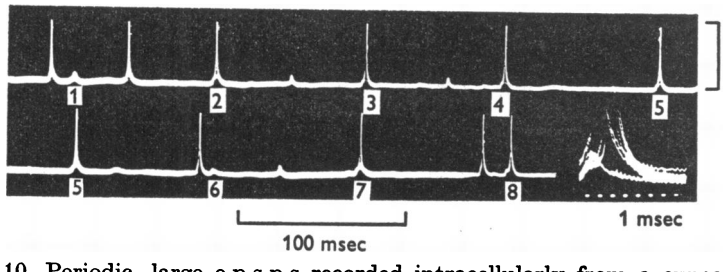

Fig. 10. Periodic, large e.p.s.p.s recorded intracellularly from a cuneate neurone. E.p.s.p.s attributed to a single periodic series are numbered 1–8. (note that 5 is same in top and bottom records). D.c. coupling throughout. Inset shows at higher, RC amplification, superimposed sweeps triggered during rising phases of supra- and subthreshold e.p.s.p.s. Records obtained during injection of inward current.

during injection of large depolarizing pulses tested, the prepotentials resemble more closely e.p.s.p.s than hippocampal dendritic fast prepotentials which have a mean time-to-peak of 0·7 msec with a range of 0·3–1·0 msec (Spencer & Kandel, 1961).

Figure 10 shows an intracellular recording of the discharges by a cuneate

neurone in which some of the e.p.s.p.s (numbered 1–8) belong to a single, uninterrupted periodic series and range in amplitude between 3·5–5·0 mV. Except when closely preceded by a spike not belonging to this series (numbers 1 and 6), each periodic e.p.s.p. elicits a spike. The periodicity of the e.p.s.p.s suggests that they are caused by a *single*, periodically discharging, afferent fibre and the relatively narrow range of the e.p.s.p. amplitudes is consistent with this inference. The other e.p.s.p.s present are not part of another uninterrupted, periodic series; surprisingly, although not recently preceded by a discharge, some of these large e.p.s.p.s, e.g. between 3 and 4, failed to elicit a spike. This suggests that the recording electrode was not intrasomatic, but perhaps lay in a large dendrite further from the source of the periodic e.p.s.p.s than that of the others.

The intracellular recordings of Fig. 10 are unusual in showing a pattern of spike discharge comparable to that encountered in extracellular recordings from other neurones: usually the pattern of discharge is disturbed by impalement. Clearly, it would be advantageous if in the long runs of activity of uninjured cuneate neurones, obtained with extracellular recording, the timing of afferent activity could be specified. In extracellular records from a few units, periodically occurring, monophasic, positive 'prepotentials' have been observed. In Fig. 11, top right records, the prepotentials either precede positive-negative spikes or, rarely, they occur alone at the time predicted from the period of the antecedent prepotentials. Aperiodic spikes also occur, but are not preceded by prepotentials. The onset of the prepotential precedes that of the positive-negative spike by an interval varying usually between 0·50 and 0·95 msec. Presumably, the prepotential reflects the external field potential associated with an e.p.s.p. set up by a periodic impulse in a single afferent fibre; by contrast, the field associated with the afferent impulse would not be monophasic. In either case, because of the brevity and invariance of the functional delay for setting up an e.p.s.p. (0·3 msec; Eccles, 1964), each interval between prepotentials would equal that between corresponding afferent impulses.

The neurone was maximally driven by wrist extension; with the wrist in mid-position (condition *A*), the intervals between spikes preceded by prepotentials are either narrowly dispersed about the mean period or are split by non-prepotential preceded spikes, yielding the equiprobable portion of the ID. Under stimulus condition *B*, both the period in the ID and its dispersion increased and intervals that were multiples of the first mode were present. Aperiodic discharges were absent. In this run, spikes were not preceded by propotentials, suggesting that a different afferent fibre, synapsing at greater distance from the recording electrode, was responsible for this periodic discharge.

Under condition *A*, the dispersion about the periodic mode in the ID of spikes is indistinguishable from that of prepotentials, implying that the post-synaptic variability is determined largely by dispersion of the afferent period rather than by variability in the delay for initiation of the post-synaptic spike. In previous experiments (Amassian & De Vito, 1957), the latency of cuneate neuronal discharge following strong electrical

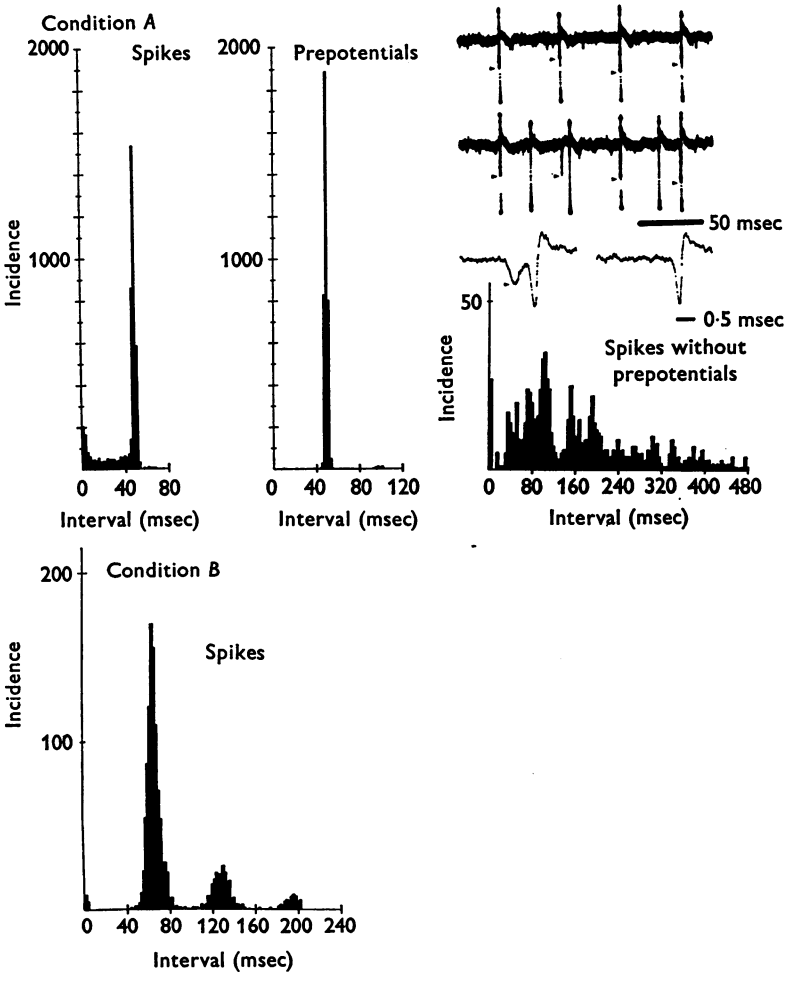

Fig. 11. Interval distributions derived from a cuneate proprioceptive neurone under two conditions of stimulation. Under condition *A* (top), interval distributions shown between all spikes, between prepotentials and between spikes not preceded by prepotentials. Inset shows samples from the run at two sweep speeds, with prepotentials indicated by arrows. Under condition *B* (bottom), prepotentials were absent. Further details in text.

stimulation of peripheral nerves showed very little variation (e.g. ± 20 μsec), but the post-synaptic response to an impulse in a single afferent fibre might be expected to show greater variability. In later experiments (Giblin & Amassian, 1966), stimulation of the dorsal columns 1–2 mm away from the recorded neurone at a critical, weak intensity yielded a discontinuous distribution in latency of discharge by ten out of twenty cuneate projection neurones tested. For example, in Fig. 12, the early discharges are narrowly dispersed (approximately 200 μsec) about the median latency of 1·24 msec while the later discharges are more widely dispersed about a median of 2·06 msec. The delicacy of adjustment of stimulus intensity

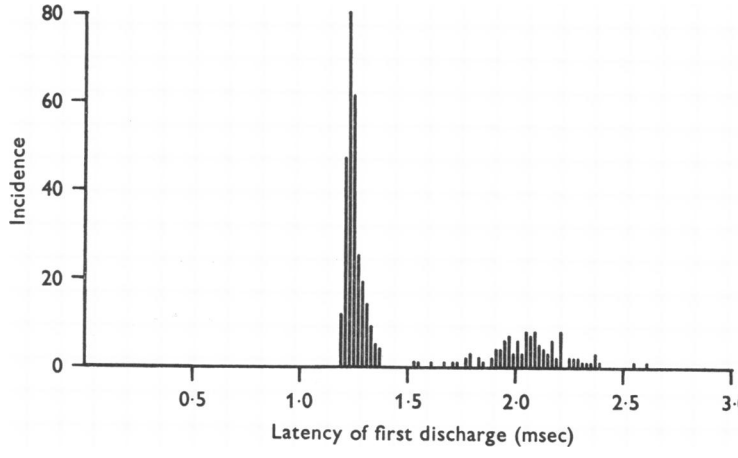

Fig. 12. Post-stimulus histogram of latency of first discharge by cuneate neurone to threshold dorsal column stimulation. Class interval 20 μsec. Latencies measured to nearest 10 μsec with crystal controlled interval timer. Number of stimuli = 391 and probability of a response = 0·997.

required to elicit such 'jumping' behaviour is comparable to that required for threshold play of individual axons, e.g. when antidromic invasion of a neurone is just elicited by stimulating its projection axon. Therefore, we believe that the early discharges are due to monosynaptic excitation of the neurone by one, or at most a very few afferent fibres ascending in the dorsal columns. (The later discharges may be due to excitation by an interpolated neurone in the nucleus.) Regardless of its origin, the total latency dispersion with such weak stimuli is much smaller than that of periodic modes of post-synaptic discharge (e.g. Figs. 2 and 3) a finding which supports the conclusion that the variability of intervals in periodic post-synaptic discharge is largely determined by that of the afferent input.

If, as inferred above, periodic discharges by a cuneate neurone may

reflect the period of impulses in an individual afferent fibre, then artificially reducing the excitability of the neurone after a periodic discharge might result in the failure of the next expected periodic discharge, but should not result in a continuously variable waiting time for this, or subsequent discharges. An example of such a test (Fig. 13) shows several typical effects on a positive-negative unit of injecting a large pulse ($2{\cdot}2 \times 10^{-8}$ A for 130 msec), with the electrode tip negative and just external to the membrane. These include (1) the emergence of a prepotential with, or without, a spike at the expected time of the arrival of the periodic afferent impulse; the duration of the prepotential is many times that of the spike, a finding implying that it reflects the external field of the e.p.s.p.; (2) an increase in amplitude of the positive component of the spike; and (3) a reduction in amplitude of the negative component.

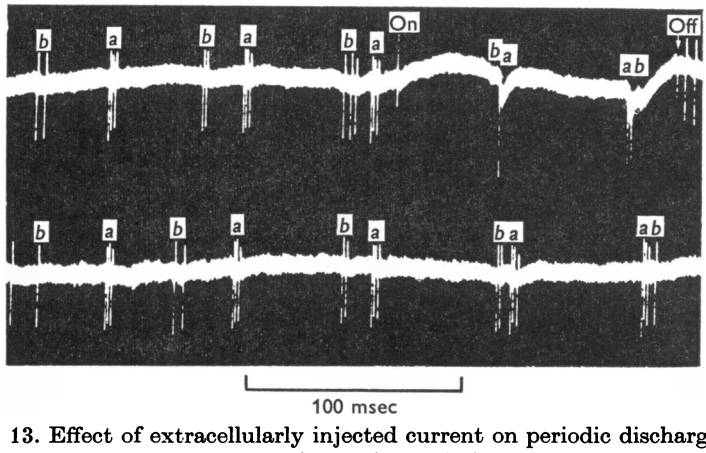

Fig. 13. Effect of extracellularly injected current on periodic discharge by a cuneate neurone. Portions of run shown before (top-left record), and after (bottom record) the current pulse was injected (on and off in top record). The records were selected and aligned to show as nearly as possible similar phase relationships of periodic drives *a* and *b*. The *a* drive had the shorter period and the larger number of discharges per event and was incompletely blocked by the current pulse. The expected times of occurrence of *a* and *b* drives during current injection may be extrapolated from the preblock portion of the run.

Mathematical model of cuneate periodic discharges

The data in the above sections suggest a model for periodic postsynaptic discharge in which, under steady-state conditions, either a discharge or a high frequency burst (event) represents a successful outcome of a 'trial' by a periodic impulse in a single afferent fibre. Why the response probability may range from one downwards need not be detailed now. In the simplest version of the model, each trial is independent and

intervals are generated which equal, or are multiples of the basic period. An interval equal to the basic period (first mode) results when two successive afferent impulses each produce a post-synaptic response; an interval of twice the basic period (second mode) results when a failure intervenes between two successes. Assuming independent trials, the areas under first (N_1), second (N_2), third (N_3), fourth (N_4)...modes in the ID are given by Np^2, Np^2 $(1\text{-}p)$, Np^2 $(1\text{-}p)^2$, Np^2 $(1\text{-}p)^3$...etc, where p, the probability of success of a trial = N_e/N, N_e = the number of periodic events in the run, N = the number of afferent trials. N is most accurately computed by summing N_1+2 N_2+3 N_3+4 N_4...where N_1, N_2, N_3 N_4 are the numbers of first, second, third and fourth mode intervals, respectively, but is closely approximated by dividing the duration of the run (T) by the period of the first mode (t_α). Four neurones in the sample yielded runs that fitted this model, that is, the observed numbers of intervals in successive modes, did not differ significantly by the χ-square test (5 % level) from those predicted by the model. The p values in this sample ranged from 0·68 to 0·86 and the basic periods ranged from 35 to 73 msec. For other runs of one of the above neurones and a run from an additional neurone, the multi-modal ID was not fitted by this model; in each run, the *first* mode of the ID had *fewer* than the predicted number of intervals. This suggests a model in which the outcome of a periodic trial depends on that of the immediately preceding trial, a success being followed by a decreased probability of a success on the next trial. According to the 'conditioning' model previously described (Amassian & Giblin, 1968), the areas under the (N_1), (N_2), (N_3), (N_4)...modes in the ID are given by Np_0p_s, Np_0 $(1\text{-}p_s)p_t$, Np_0 $(1\text{-}p_s)$ $(1\text{-}p_t)p_t$, Np_0 $(1\text{-}p_s)$ $(1\text{-}p_t)^2$ p_t..., etc., where N is defined above, p_0 = over-all probability and is computed identically with p above, and p_s and p_t are the probabilities of success conditioned by an immediately preceding success and failure, respectively (clearly, the 'conditioning' model reverts to the simple one when $p_s = p_t = p_0$). p_s and p_t are computed from equations

$$p_s = \frac{N_1}{Np_0} = \frac{N_1}{N_e}, \quad p_t = \frac{N_2 p_s}{N_1 (1\text{-}p_s)}$$

(obtained from the N_1 and N_2 terms in the 'conditioning' equation) and are used to predict the areas under third and later modes; Table 1 shows only small differences between observed (o) and expected (e) values. Alternatively, p_t is computed from equation $p_t = 1\text{-}N_3/N_2$ and is then used to predict the area under the first mode $N_1 = N_e - N_2/p_t$, both equations being derived from the 'conditioning' equation. The differences between observed and expected values were small; for example, two runs obtained from the same neurone under different stimulating conditions

yielded ratios of observed to predicted values for the first mode of 1082/1071 and 524/520, respectively. It should be noted that while the observed value of the first mode could be much *less* than that predicted, it could not exceed the predicted value by more than the sum of fourth and subsequent modes, which in the examples cited were 29 and 28, respectively.

TABLE 1. In a single neurone, p_o, p_s, p_i and the ratio p_s/p_t (all defined in text) are shown for runs with increasing basic periods. Numbers of observed intervals (*o*) in third and fourth modes are contrasted with those expected (*e*)

Period (msec)	p_o	p_s	p_i	p_s/p_t	Third mode		Fourth mode	
					o	*e*	*o*	*e*
39·5	0·40	0·22	0·50	0·44	19	16	5	8
40·3	0·47	0·39	0·46	0·86	19	15	9	8
40·5	0·50	0·36	0·56	0·64	18	16	10	7
41·2	0·54	0·39	0·77	0·51	10	12	2	3
41·7	0·53	0·39	0·70	0·55	13	13	3	4
42·5	0·51	0·42	0·60	0·70	14	14	6	6
43·5	0·62	0·56	0·73	0·77	85	82	19	22
46·3	0·74	0·71	0·83	0·86	78	73	10	13
46·3	0·69	0·65	0·76	0·86	111	106	25	26

With one exception, the ratio of p_s/p_t in the series, ranged continuously from 0·44 to the unconditioned value of one (in one proprioceptive field neurone, $p_s = 0$ and $p_o = 0{\cdot}35$).

For the unit in Table (1), p_s was highly correlated ($R = 0{\cdot}945$) with the mean period; p_t was less highly correlated ($R = 0{\cdot}787$). If a *single* variable, operationally defined as 'post firing' depression, accounts for the finding that p_t exceeds p_s, then an increase in p_s from 0·22 to 0·50 would be expected only if the modal period doubled; in fact, when the modal period increased slightly from 39·5 to 43·5 msec, p_s increased from 0·22 to 0·56. This implicates at least one additional factor, presumably related to the basic period in the afferent fibre.

A more complex model results from the addition of aperiodic discharges to those resulting from a single periodic drive. In this model, the probability of success of the periodic drive is calculated from the *event* ED (see Appendix); subsequently, the mean rates of periodic and aperiodic events are calculated. When the event ED reveals two periods, the probability of success of each periodic drive may be separately calculated, using the same equation. The presence of a second periodic drive splits periodic intervals due to the first, yielding an equiprobable portion of the ID; the presence of aperiodic discharges cannot then be recognized by simple inspection of either the ID or the ED. However, when the over-all mean

rate exceeds the sum of the mean rates of the periodic drives, the difference represents the magnitude of the aperiodic drive. Such estimates permit any effect of a change in peripheral stimulation to be evaluated on periodic and aperiodic components separately (see Table 2). Thus, in the

TABLE 2. Composition of complex discharges of 6 individual neurones exhibiting two or more mean rates of discharge. Runs are arranged in order of over-all mean rate of events. Mean rates are shown of events (Ev) produced by the α periodic drive, by the β periodic drive (if present) and by the aperiodic drives. p_α, T_α and σ_α are the over-all probability of response to the α drive, the basic mean period of the α drive and its standard deviation, respectively. p_β, T_β and σ_β give corresponding values for the β drive. In the 52-series, two different periodic drives are inferred from the differing relationships between the mean period and its S.D. In 48–10, * indicates that the values had to be derived from the second α and β modes in the expectation density function because of the degree of overlap of the first modes. In the 36-series, ** indicates that the basic α and β periods were too close to be resolved and were arbitrarily given equal values

Run	Total Ev/sec	α Ev/sec	β Ev/sec	Aperiodic Ev/sec	p_α	T_α	σ_α	p_β	T_β	σ_β
53–21	23·2	14·5	—	8·8	0·97	67·2	2·2	—	—	—
–22	43·3	11·2	20·9	11·2	0·91	80·6	2·5	0·89	42·1	4·4
52–23	1·8	—	—	1·8	—	—	—	—	—	—
–15	12·4	12·4	—	—	1·0	82·1	8·6	—	—	—
–16	12·9	12·9	—	—	0·76	58·5	6·8	—	—	—
–19	13·1	13·1	—	—	1·0	76·5	8·3	—	—	—
–20	14·1	—	14·1	—	—	—	—	1·00	73·6	11·7
–21	14·7	—	14·7	—	—	—	—	0·98	67·5	13·1
–24	18·8	10·5	—	8·3	0·84	79·8	8·4	—	—	—
48–6	15·2	15·0	—	0·3	1·00	67·6	2·5	—	—	—
–7	17·7	17·6	—	0·1	1·00	57·1	2·1	—	—	—
–11	20·8	19·1	—	1·7	0·99	51·7	2·0	—	—	—
–12	23·2	20·3	—	2·9	1·00	48·7	2·0	—	—	—
–9	30·5	18·2	—	12·3	0·98	53·7	2·0	—	—	—
–10	37·7	16·1	17·8	3·8	0·81	49·5	2·5*	1·00	56·3	4·5*
36–9	28·0	14·9	10·3	2·7	0·95	63·2	2·2	0·79	76·6	3·9
–10	33·5	16·9	13·9	2·8	0·84	49·6	5·5	0·95	67·8	5·3
–12	34·2	15·7	15·7	2·8	0·87	54·9	2·1**	0·87	54·9	2·1**
-11_{3rd}	40·7	19·3	19·3	2·1	0·90	46·2	2·6**	0·90	46·2	2·6**
-11_{2nd}	46·8	21·8	21·8	3·2	0·85	40·2	3·7**	0·85	40·2	3·7**
-11_{1st}	52·6	23·7	23·7	5·2	0·82	34·6	4·1**	0·82	34·6	4·1**
30–16	12·9	7·5	—	5·4	0·69	92·3	12·1	—	—	—
–14	13·8	13·8	—	—	0·61	45·6	3·9	—	—	—
–9	15·8	15·5	—	0·3	0·99	63·8	3·3	—	—	—
–8	18·1	15·6	—	2·5	1·00	64·1	4·1	—	—	—
–11	18·5	15·0	—	3·5	0·95	63·2	4·4	—	—	—
–15	20·5	17·9	—	2·6	0·97	54·3	3·8	—	—	—
28–9	11·7	6·0	—	5·7	0·66	108·5	8·9	—	—	—
–7	17·9	9·1	—	8·7	0·57	61·2	3·9	—	—	—

53-series, the total mean rate was nearly doubled by the addition of a second periodic component, the other drives showing only small changes. Comparing 52–15 and –24, the mean rate increased by one-half because of the addition of a large aperiodic component. Similarly, the addition of an aperiodic drive accounts for most of the doubling of the mean rate in 48–9 compared with –6, but a still greater increase in –10 resulted instead from an additional periodic drive. In the 36-series, the increases in mean rate resulted from marked increases in both periodic drives, the aperiodic drive changing only by a small amount. In the 30-series, the increases in the single periodic drive account for the increases in the mean rate. Finally, in the 28-series, an approximately equal increase in periodic and aperiodic drives accounts for the increase in the mean rate.

An increase in a given periodic drive could result from an increased probability of response (cf. 48–10 and –11), or from a reduction in modal period (cf. the 36-series, 28–9 and –7) or from both (cf. 30–16 and –9). Often, modal period and probability of response are inversely related, resulting in a smaller increase in mean rate than would otherwise be expected from the reduction in modal period.

The probability of response in touch, touch-hair and proprioceptive cuneate neurones ranged continuously from 1 to 0·3; it did not differ significantly among these modality types.

DISCUSSION

Several lines of evidence including, the intracellular recording of a periodic, 'giant', unitary e.p.s.p. and the occasional extracellular recording of a periodically occurring, unitary prepotential preceding the spike imply that some somatic afferent fibres exert an extraordinarily powerful excitatory action on cuneate post-synaptic neurones. The morphological correlate of such excitation is most likely a large presynaptic knob located on a dendrite (Walberg, 1966). The presence of two, and, exceptionally, as many as four differing periodic drives implies that *several* large knobs may be present on the same cuneate neurone, a prediction as yet unconfirmed morphologically. Large knobs located elsewhere in the central nervous system, for example on Clarke's column neurones, were shown to release a large number of quanta of transmitter (Eide *et al.* 1969*b*; Kuno & Miyahara, 1968). Cuneate neurones, being smaller than Clarke's column neurones would be expected to have a higher input resistance and therefore to exhibit still larger e.p.s.p.s.

In the limiting case, each impulse in a single touch or proprioceptive afferent fibre may with a probability of one, cause either one or a burst of discharges by the cuneate neurone, with only small variations in delay.

However, most cuneate neurones do not respond to every periodic impulse in an afferent fibre. The responses are probabilistic, often fitting simple models in which successive periodic afferent trials are either independent or a successful outcome (discharge) diminishes the likelihood of success at the next trial. The finding that the over-all probabilities of success (p_o) in both touch and proprioceptive cuneate neurones ranged continuously from one to 0·3 suggests an artificial truncation at the lower limit, perhaps due to difficulty in identifying the period in the ED and in measuring its area. In one neurone (Fig. 6), responses occurred only when harmonics of two different basic periods became nearly coincident; this implies that the probability of response to one input may approach zero.

Several mechanisms operating pre- or post-synaptically could account for a failure to respond. These include:

(1) intermittent conduction in dorsal column afferent fibres, which was reported by Barron & Matthews (1938). Under the conditions of our experiments, such intermittence was not seen in fibres recorded within a few millimetres of the cuneate nucleus, making it unlikely that this is a factor;

(2) a random variation in number of transmitter quanta released by the individual afferent impulse would be predicted by the findings elsewhere, e.g. the neuromuscular junction (del Castillo & Katz, 1954) and the Group Ia synapse on the motoneurone (Kuno, 1964*a*). More pertinent to the cuneate nucleus, the quantal analysis of transmission at the Clarke's column relay (Eide *et al.* 1969*b*) revealed large unitary e.p.s.p.s and a range of 25–121 mean quanta/impulse. With such high mean values, a vanishingly small probability of failure of the e.p.s.p. would be predicted, and no failures were observed. However, the e.p.s.p. varied in amplitude with standard deviations ranging from 0·12 to 0·43 mV. If present in cuneate neurones, such variations might randomly cause a reduction in e.p.s.p. below firing level. Our finding that p_o is usually less than one would then imply a remarkable specialization in which the amount of transmitter released by most endings is precisely that required to depolarize the cell to a level very close to firing. If this hypothesis is correct, any factor that alters quantal release should significantly change p_o. Quantal release at Group Ia endings on the motoneurone initially was reduced when the interval between presynaptic impulses was reduced, e.g. to 100 msec, but was increased when the interval was 5–10 msec (Kuno, 1964*b*). Although in the cuneate neurone of Table 1 a reduction in afferent period was positively correlated with a reduced p_s and p_f, other neurones (Table 2) showed a similar change, or no change, or a reversed change in p_o. Nevertheless, quantal variability cannot be excluded as a factor in probabilistic firing of cuneate neurones, because the relationship of quantal release to

frequency of discharge is not monotonic and the time relations may not be the same at all endings;

(3) a fluctuating level of inhibition has been invoked to account for multimodal patterns of discharge by retinal ganglion cells (Bishop, Levick & Williams, 1964; Sanderson, Kozak & Calvert, 1973). Such inhibition occurring at either pre- or post-synaptic levels might similarly account for our findings, except that afferent inhibition was not demonstrated in touch neurones (Perl *et al.* 1962; Gordon & Jukes, 1964*a*);

(4) a probabilistic aspect might be introduced in the generation of a 'dendritic' spike (Spencer & Kandel, 1961) or in the effect of such a spike upon the IS segment. Periodic prepotentials were recorded both intra- and extracellularly from cuneate neurones, but were considered to be e.p.s.p.s rather than dendritic spikes (see Results);

(5) random fluctuations in membrane potential were shown to cause probabilistic firing of frog axon (Siebenga & Verveen, 1972) and most likely occur in the membrane of the cuneate neuron at the site of spike generation.

Usually, the discharge pattern is not restricted to a single periodic component. Discharges either from an aperiodic drive or from an additional periodic drive are interpolated between responses to a given periodic drive, yielding an 'equiprobable' portion of the ID. Such interpolation would be expected to result in (1) powerful inverse conditioning, that is, a long interval would tend to be followed by a short and vice versa and (2), the sum of pairs of successive intervals would equal one of the periods. Both phenomena were previously demonstrated in cuneate proprioceptive field neurones (Amassian *et al.* 1964); as was previously emphasized (*ibid*, Walloe, 1968) inverse conditioning may reflect the temporal characteristics of the *presynaptic* input, rather than *post-synaptic* depression. However, when the ratio of $p_s/p_t < 1$, post-synaptic depression may be presumed.

A generally applicable method of investigating the mechanism of steady state discharge is to inject current through an *extracellular* micro-electrode impinging on, or dimpling the cell membrane, in an attempt to modify the waiting time for the next discharge (Fig. 13). When the micro-electrode tip is very close to the cell membrane, is negative with respect to the reference electrode and passes large current pulses (e.g. 2×10^{-8} A), the recorded response resembles an attenuated but non-differentiated, intracellularly recorded e.p.s.p. with or without a spike. Given that the area of contact of a micro-electrode of tip diameter 2 μm, or less, with the cell membrane is of the order of 3 μm^2, the maximum density of current flowing *outward* into the micro-electrode is of the order of 7×10^{-9} A μm^{-2}. A current density of such magnitude is much larger than that associated

with *intracellular* injection of current. Thus, outward currents up to 6×10^{-8} A injected intracellularly may be required to drive a motoneurone close to its maximal rate (Kernell, 1966); assuming that approximately 5% of the injected current flows through the soma membrane (Rall, 1959), the density of current flowing outward through this region of a motoneurone, treated as a sphere 60 μm in diameter, may reach 3×10^{-13} A μm^{-2}, i.e. $2{\cdot}5 \times 10^4$ times less than in our experiments. If the micro-electrode tip lay a few microns away from the membrane, then some current would enter it without traversing the cell membrane, but the current density at the membrane would still be much greater than during intracellular injection. The quasi-intracellular recording associated with such high current densities most likely results from a temporary break-down in capacitative and resistive properties of the membrane under the micro-electrode. Although much lower than under the extracellular micro-electrode, the density of inward current at the distant spike generator evidently was adequate to reduce the probability of discharge.

The limits of variability in timing of post-synaptic discharge by a cuneate neurone following even threshold stimulation of the dorsal columns are quite small (e.g. Fig. 12). This finding is consistent with the large amplitude and fast rise time of some e.p.s.p.s. The mean duration of the basic period of post-synaptic discharge and its standard deviation might, therefore, be expected to approximate those in the related afferent fibre. The periodic discharges of cuneate touch neurones usually have a coefficient of variation (CV) of the mean period $< 0{\cdot}19$, resembling discharges by Type II touch fibres which have a CV usually $< 0{\cdot}2$ and differing from the static discharge of Type I fibres which had a CV of 0·30–0·35 (Iggo & Muir, 1969). Recently, Chambers, Andres, Duering & Iggo (1972) gave the CV of the resting discharge of Type II touch afferent fibres as 0·06–0·29 and that of the static driven discharge as $< 0{\cdot}25$. The Type II touch receptor was identified as the Ruffini end organ. An increased value of $> 0{\cdot}5$ was given for the CV of Type I discharge.

Our findings are consistent with the demonstration that Type II touch afferent fibres ascend uninterruptedly in the dorsal columns (Petit & Burgess, 1968). The much larger CVs encountered in responses by other cuneate neurones driven by touch neurones may represent activation by second order dorsal column fibres in the touch projection system (Uddenberg, 1968), or by the dorsolateral funiculus (Dart & Gordon, 1970) or they may be accounted for if some Type I fibres also ascend uninterruptedly in the dorsal columns as reported by Brown (1968).

That both the *period* of discharge in each afferent fibre and the *number* of such fibres activating a cuneate neurone are preserved in its discharge pattern may have important implications for the coding of stimulus

parameters. Thus, a rough object pressed against the skin would tend to activate Type II end organs non-uniformly and would thus lead to excitation of the cuneate neurone by afferent fibres with widely differing periods of discharge. By contrast, a smooth object would tend to activate the same end organs more uniformly and thus lead to excitation of the cuneate neurone by afferent fibres with more similar periods of discharge. *Even if*

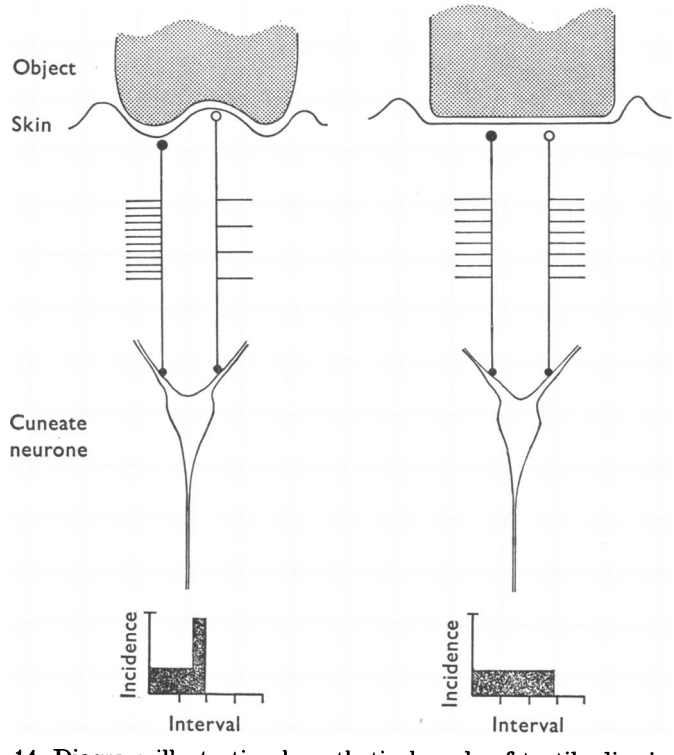

Fig. 14. Diagram illustrating hypothetical mode of tactile discrimination. A single tactile ending is shown connected to each of two Type II afferent fibres, which converge on a single cuneate neurone (the number of receptors connected to each afferent fibre is unknown). A rough (left) stimulus causes a more marked difference in frequency of discharge in the two afferent fibres compared with a smooth (right) stimulus, resulting in a different pattern of post-synaptic discharge. Interspike interval histograms are shown below. The periods at right are assumed to be close to one another and mutually prime.

the mean rates of postsynaptic discharge were identical, the temporal patterns of discharge would markedly differ (compare the left and right IDs in Fig. 14). We suggest that such differences in pattern could contribute to tactile discrimination. (In fact, *both* drives need not be periodic; given that one drive is periodic, admixture with an aperiodic drive might be decoded with

a similar implication for discrimination provided that, at a higher level, the basic period could be extracted and compared with the over-all mean rate of discharge.) This hypothesis does not exclude the participation of somatosensory inhibition (Amassian, 1952, 1953) in tactile discrimination (Mountcastle, 1957). However, the earliest data showed that inhibition at thalamocortical levels was greater the deeper the level of barbiturate anaesthesia. Furthermore, surround inhibition could not be elicited in individual neurones of thalamic nucleus ventralis posterior of the conscious human (Bates, 1971). At the level of the dorsal column nuclei, neither Perl *et al.* (1962) nor Gordon & Jukes (1964*a*) could demonstrate afferent inhibition of touch neurones, although hairfield neurones displayed prominent inhibition. A hypothesis such as we propose seems necessary to resolve the paradox that the type of neurone presumably subserving tactile discrimination, i.e. the touch neurone, is the least affected by afferent inhibition.

It may be objected that the response of a cuneate neurone to a periodically discharging afferent fibre is usually probabilistic, but this does not necessarily prevent the signalling of the basic period which might be computed from the harmonic intervals, resulting in an economy in neural discharge in the somatosensory relays. A more serious criticism is that our observations were made in the steady state; with naturally occurring tactile stimuli, the discharge rate in each afferent fibre probably changes markedly due to a varying contact. However, cross-correlation of activities of a number of equivalent but independent channels might extract the periods within a few cycles and thus obviate the need for autocorrelation during a prolonged steady state.

It is a pleasure to acknowledge the collaboration of Dr Susan Schwartz in many of the experiments in this paper.

APPENDIX

A first mode interval in the ED function implies a discharge at the origin and another at the first mode. Given a uniform probability of response p_α to N_α independent trials of a periodic generating process, then the area under the first mode in the ED, $N_{1(\alpha)} = N_\alpha p_\alpha^2$. A second mode interval in the ED is generated by a discharge (success) at the origin and a discharge at the second mode, with either an intervening success or a failure at the first mode; the area under the second mode $N_{2(\alpha)} = N_\alpha p_\alpha^3 + N_\alpha p_\alpha^2(1-p_\alpha) = N_\alpha p_\alpha^2$. Subsequent modes in the ED also have areas equal to $N_\alpha p_\alpha^2$ (despite the equality of areas of successive modes they show increasing dispersion about their means).

Two aspects of complex cuneate discharges invalidate a simple computation from the ED function of the probability of response by using $N_{1(\alpha)} = N_\alpha p_\alpha^2$. The first is the occurrence of 2–3 spike bursts in response to periodic afferent impulses; bursts multiply the areas under the periodic modes of the ED. However, the bursts may be treated as single physiological events timed from the initial spike (see Methods) and the ED computed on such events. The second error derives from the presence of additional aperiodic or periodic drives, which then contribute to bins of the first periodic mode; such contribution may be estimated from the 'equiprobable' portion of the ED. The contribution by these additional drives to the area of the first mode should be approximated by the product of the number of bins over which the first mode is dispersed (N_d) and the mean content of each equiprobable bin (N_q). However, implicit in the definition of an event is an operational dead time resulting from the inclusion in the same event of spikes due to other drives. The number eliminated during the first mode is a function of the interval duration (t_d) chosen to define the event, the number of periodic events in the first mode (approximated by $N_\alpha p_\alpha^2$) and the mean rate of the other drives $N(e\text{-}N_\alpha p_\alpha)/T$, where N_e is the number of events in the run and T is its duration. Thus, the number of other drive events that are eliminated by the α periodic drive, or vice versa, approximates $N_\alpha p_\alpha^2 . t_d\ (N_e\text{-}N_\alpha p_\alpha)/T$; this must be doubled because elimination of an event results in a deficit of two counts in the first mode, as the eliminated event is not subsequently translated to the origin and therefore does not begin a new first mode interval. The number of events eliminated in the first mode might be underestimated by the above, because although the dead time associated with an isolated discharge is t_d, that associated with a high frequency burst is the burst duration plus t_d. The bursts that are associated with periodic drives by touch afferent fibres are usually brief, often containing two to three discharges. Furthermore, discharges due to another drive are unlikely to be interpolated during high frequency discharges caused by the periodic drive in which the interspike intervals are usually 1–2 msec. The usual occurrence of an equiprobable portion of the ED at intervals of more than a few milliseconds implies that the 'physiological dead time' does not long outlast the burst.

The equation for the number of events eliminated at the first mode implies that the number eliminated and t_d are linearly related. This relationship was tested on a run (53–21 of Table 2) in which all the burst intervals were less than 2 msec. When $t_d = 2$ msec, $N_e = 1479$ and $N_{1(\alpha)} = 953$. The decrements in $N_{1(\alpha)}$ for successive increments in t_d of 2 msec were 24 ($t_d = 4$), 23 ($t_d = 6$) and 25 ($t_d = 8$), that is, the predicted linearity was confirmed.

From the above considerations, the area under the first mode of the ED is the sum of the periodic component and the other components, less twice the number eliminated. That is,

$$N_{1(\alpha)} = N_\alpha p_\alpha{}^2 + N_d . N_q - 2 N_\alpha p_\alpha{}^2 . t_d (N_e - N_\alpha p_\alpha)/T.$$

Rearranging,

$$p^3 [2 N_\alpha{}^2 t_d] + p^2 [N_\alpha T - 2 N_\alpha t_d . N_e] + T [N_d . N_q - N_{1(\alpha)}] = 0.$$

The values of 'p' were obtained on the CDC 160A computer. Subsequently, an estimate of the number of periodic events eliminated was obtained which, when added to $N_\alpha p_\alpha$, yielded a corrected value for the number of periodic events, and hence yielded a corrected value for 'p'. This process was repeated a second time yielding the values in Table 2.

Because of the many approximations used in deriving this equation for 'p', it was tested on an 'artificial' run made by mixing two known, mutually prime, periodic drives, each of which was derived from an afferent fibre and was uncomplicated by other drives. The original runs had 2,023 and 953 discharges and periods of 20·5 and 42·5 msec, respectively. The mixed run had 2,953 discharges, that is 23 (0·8 %) discharges were lost in the mixing. With t_d = 4 msec, the uncorrected values of p_α and p_β yielded by the equation were 0·889 and 0·773, respectively. The first correction yielded values of 0·957 and 0·910; the second yielded the final values of 0·975 and 0·947. Given that the mixing reduced the theoretical 'p' from 1 to approximately 0·99, the observed 'p' was underestimated by up to 5 %: while we have no exact estimate of the magnitude of the error in 'naturally' mixed runs, it is believed to be similar in size.

It will be noted that the equation used to estimate 'p' from the ED makes no allowance for an inequality between p_o, p_s and p_t, that is, for 'conditioning' (see Results). Neglecting the correction for event dead time, the effect of conditioning on the ED can be estimated from the equation

$$\frac{N_{1(\alpha)} - (N_d N_q)_1}{N_{2(\alpha)} - (M_d N_q)_2} = \frac{N p_o p_s}{N[p_o(1-p_s) p_t + p_o p_s{}^2]} = \frac{1}{\frac{p_t}{p_s} + p_s - p_t}$$

if the left-hand term < 1, then $p_t > p_s$, that is, depressive conditioning is present. Seven runs were selected using the following criteria: (1) the second mode was clearly measurable, (2) only one period was present, (3) a significant amount of aperiodic drive was present, (4) 'p' estimated from the first mode was 0·8 or less. The values for the periodic component in the first ED mode divided by that in the second mode equalled 0·98, 0·99, 1·18, 1·23, 1·28, 1·86 in these runs, which makes it unlikely that depressive conditioning was present. (The ratio may exceed one

probably because the area of the second mode is underestimated due to its dispersion.) Presumably, in complex runs, any 'conditioning' effects in a periodic drive are obscured by intervening discharges from other drives.

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