

CORRELATION BETWEEN MASS AND UNITARY POTENTIALS
EVOKED IN THE RAT CEREBRAL CORTEX
BY PERIPHERAL STIMULATION

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ABSTRACT

Rats were anaesthetized with urethane or trilene. Electrical stimuli were applied to the forepaw and responses recorded from the primary somatosensory cortex.

The cortical response and its variability were studied firstly in an attempt to identify and control variability in subsequent experiments. Depth and type of anaesthesia were relatively unimportant, but recording position was critical when results from different experiments were compared. The main variability was that due to interaction of evoked responses with ongoing spontaneous activity. It was found useful to divide all responses into two groups according to the presence or absence of ECoG activity at the time of stimulation.

The variability of cortical evoked potentials in these experiments apparently reflected a cortical stabilizing mechanism. The suppression by ongoing cortical activity of a widespread surface positive/depth negative component in the response was associated with the suppression of unitary responses in the deep layers of the cortex. A simple post-synaptic modulation of evoked IPSPs may be largely responsible for the suppression of these action potential responses. Postsynaptic modulation of PSPs is also considered in the context of another cortical

gating mechanism - direction specificity in responses to moving stimuli.

A striking correlation was found between unitary action potentials, local negative waves and surface positive waves in both the stable and variable components of the response to forepaw stimulation, thus supporting the hypothesis that evoked potentials could, at least in part, be due to summation of soma action potentials.

The expected contribution of soma action currents in pyramidal cells, to cortical surface potentials was estimated and found to be significant.

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I N T R O D U C T I O N

GENERAL INTRODUCTION

Localization of evoked potentials

Caton (1875) was the first to report the existence of small fluctuations in electrical potential at the surface of the cerebral cortex. He described spontaneous waves of electrical activity as well as electrical responses to sensory stimulation and reported that all these potentials were affected by anoxia and anaesthesia, and abolished by death, suggesting that they reflected biological events. Caton recognised the usefulness of the evoked waves for recording localized cerebral events, and he later studied them in monkeys as well as rabbits. He had already demonstrated cerebral localization of function when he had shown that localized electrical stimulation of certain points of the cerebral cortex produced localized muscular contractions.

Berger (1929) repeated these experiments and extended the study of spontaneous potentials by recording from the scalp of humans. He found that sleep, wakefulness, and certain brain diseases were associated with distinctive waveforms and he suggested that certain features of these waveforms could be helpful in diagnosis.

Detailed study of cortical potentials in experimental animals became possible about 50 years after Caton's first publication, when suitable amplifiers and display equipment became available. When Gerrard, Marshall and Saul (1933) recorded spontaneous and evoked activity from the cortex of

anaesthetized cats, they found that gentle displacement of a few hairs on the body surface gave rise to a localized evoked potential which was clearly distinguishable from ongoing spontaneous activity.

Marshall, Woolsey and Bard (1937) studied the cortical potentials evoked by peripheral stimulation in the anaesthetized cat and monkey. They reported that discrete tactile stimuli gave localized surface positive waves of sufficient magnitude and stability that they could be used to map a cortical representation of the body surface. The stability was such that potentials recorded from a single point were reproducible for up to 24 hours. At the points of maximal response the amplitude of the wave was $100\mu\text{V} - 1\text{mV}$, the latent period of onset was about 8 msec and the rise time 3 - 6 msec. The localization of the evoked waves was such that these authors considered that, if the amplitude of a wave was more than 10% of the maximal value at a position 1mm distant from the point of maximal response, then the wave was physiologically significant and not due to passive electrotonic spread from the region of maximal response. (In 1935, Adrian and Yamagiva had shown that even when recording through the thickness of the human skull, an active region could be localized to within one inch).

Topography of evoked potentials

In a later paper Woolsey, Marshall and Bard (1942) described in detail the spatial distribution of the cortical

responses to stimulation of each part of the body surface. Records were taken at 0.5 mm steps and for each small skin area the results showed that each active region consisted of a point of maximal response surrounded by a region 0.5 - 4.0 mm wide yielding a smaller response. Thus, there was a considerable amount of overlap of potentials evoked from stimulation of two neighbouring points on the body surface. If two such stimuli were given a few msec apart, the response to the second stimulus was often attenuated when compared with the response to the same stimulus given alone. Thus the overlap was apparently reflecting a genuine convergence of afferent activity, rather than a passive spread of electrical potential. Adrian (1941) had observed a similar interaction in records taken from the cortical grey matter. This interaction usually decreased as the distance between the stimulating points increased; however Malcolm and Darian Smith (1958) sometimes found interaction when the stimuli were applied to such widely separate areas as the contralateral hindpaw and forepaw. Woolsey et al (1942) also described a "masking effect"; the response to a single discrete stimulus could be attenuated or obliterated if there was continuous stimulation of nearby skin.

In their cortical mapping experiments on monkeys they used single stimuli and found (like Adrian, 1941) that the contralateral body surface was represented in an orderly sequence over the cortex in two distinct regions. Woolsey

(1947) later referred to these as somatic sensory areas I and II (or SI and SII).

Adrian (1941) recorded mass and low voltage unitary action potentials within the cortical grey matter to map the somatosensory receiving areas in the cat, dog and rabbit as well as the monkey. In each animal studied, the representation appeared as a continuous but distorted image of the body surface. In the different species the relative sizes of the cortical areas associated with different parts of the periphery varied according to their specialization of function. For example, the whisker area was relatively large in the cat, the hand area in the monkey, and the snout area in the pig. (Adrian 1943).

The present investigation is concerned with activity evoked in the primary sensory cortex (SI) of the albino rat in response to electrical stimulation of the contralateral forepaw. The rat has a very large whisker area but a small forepaw area (Woolsey 1958). Presumably the tactile sensibility of the forepaw is of little interest to the rat itself. However, in this investigation, the forepaw area was found to be particularly suitable for a study of functional organization; clear spatial and temporal patterns of spreading cortical activity can be recognised, even from gross surface electrodes, because the area of initial response is so small. Evoked potentials in the rat (like those of the rabbit, Bishop 1936) are comparatively simple to interpret, because of the lissencephalic cortex. In the present investigation the rats were anaesthetized with urethane or trilene.

Stability of evoked potentials

Adrian (1941) found that the extent of the receiving area was not significantly affected by depth or type of anaesthesia (chloralose or barbiturate) and also that there was good agreement with the areas already determined by histological evidence (Brodmann, 1909 and Poliak, 1932). Gerrard et al (1933) found that although ether depressed spontaneous waves, it had relatively little effect on potentials evoked by auditory and visual stimuli. Similarly, Derbyshire, Rempe, Forbes and Lambert (1936), Marshall (1938), and Marshall, Woolsey and Bard (1941) found that in the somatosensory cortex the initial response to peripheral stimulation was little affected by depth of anaesthesia (nembutal, dial, ether or chloralose) although the events which followed, including the recovery cycle, were markedly altered.

Adrian (1941) also reported that the initial surface positivity was a relatively stable event; it was not affected by ongoing spontaneous activity of the cortex and was dependent mainly on the size of the afferent volley. In contrast, the later waves of the evoked response were much more variable (Adrian, 1941; Marshall et al, 1941). The wave immediately following the initial positivity could either be a positive or a negative deflection. The form of this later response which was more widespread, was dependent on the preceding cortical activity (Forbes and Morison, 1939; Adrian, 1941; Dempsey and Morison, 1942; Bindman, Lippold

and Redfearn, 1964a). This interaction between the local evoked excitation and the background of spontaneous activity was considered by Adrian (1939) to be "an essential feature of the cortical response", and it is discussed in more detail below.

Microelectrode recordings

The early microelectrode studies in the somatosensory cortex (Amassian, 1953; Perl and Whitlock, 1955; Towe and Amassian, 1958) all revealed that the initial surface positive response to electrical stimulation of the skin was associated with a reversed negative wave and early unitary discharges (all-or-none action potentials) in the middle layers of the cortex. This early activity was relatively localized compared with later evoked activity (Angel and Holmes, 1967) which also spread into deeper and more superficial layers of the cortex (Amassian, Patton, Woodbury and Towe, 1955 and Li, Cullen and Jasper, 1956). Responding units could be found in all layers from II to VI inclusive (Mountcastle, Davies and Berman, 1957).

Towe and Amassian (1958) observed that electrical stimulation tended to evoke a single unitary action potential, whereas mechanical stimulation could give a burst of impulses. However Amassian (1953) had pointed out that because multiple discharges were sometimes associated with injury from the electrode, they required careful evaluation.

Jung, Baumgartner and Baumgartner (1952) observed both excitatory and inhibitory responses (signalled by increases

and decreases in firing frequency) in the visual cortex after switching on and off spots of light or after a large flash stimulus. Similar inhibitory and excitatory responses were also observed in the somatosensory cortex after electrical or mechanical stimulation at the periphery (Amassian, 1953; Towe and Amassian, 1958; Mountcastle and Powell, 1959b; Powell and Mountcastle, 1959).

Mountcastle (1957) found that closely adjacent penetrations revealed overlapping receptive fields. He suggested that this would operate against the attainment of tactile acuity as do the large receptive fields of many afferent fibres, and that there is likely to be a focussing mechanism such as afferent inhibition responsible for fine tactile acuity..

Mountcastle, Davies and Berman (1957) found that most responding cells in the primary somatosensory cortex of anaesthetized cats discharged spontaneously (i.e. in the absence of any overt stimulus). However there was a small proportion of units which showed no spontaneous activity but which showed clear responses to appropriate physiological stimuli.

In both the cat cortex (Mountcastle, 1957) and the monkey cortex (Powell and Mountcastle, 1959^a) it was found that each neurone was activated by only one mode of stimulation, for example hair movement, or pressure on the skin, or mechanical deformation of deep tissues. Cells in the same radial 'columns' were all activated by the same mode of stimulation at roughly the same peripheral position. The three types of modality columns were intermingled in a mosaic-like way,

but not completely randomly for more 'hair' and 'cutaneous' columns were seen posteriorly, and more 'deep' columns anteriorly.

Excitatory interaction of two different submodalities was not seen, whereas inhibitory interaction between skin and deep stimuli was sometimes found. Inhibitory interaction between skin-skin stimuli was often found. The modality of stimulation to which a cell was sensitive remained constant despite changing anaesthetic conditions, and was considered by Powell and Mountcastle (1959) to be a constant functional attribute of that neurone.

The columnar functional organisation observed by them fitted with the earlier anatomical studies of Lorente de Nó (1949) which revealed a structural cylindrical unit consisting of an ascending specific thalamo-cortical fibre and surrounding cortical cells. More recently Globus and Scheibel (1966) described similar ascending fibres forming cylindrical "arborisations" within the cortex.

Powell and Mountcastle (1959) concluded that the specificity of a cortical response (i.e. whether it was a response to tactile or deep stimuli) was not signalled by different temporal patterns of activity of a cortical cell but by activity in different neurones, i.e. that it was "anatomically determined". Marshall, Woolsey and Bard (1937) had also concluded that "... whatever functional variations may characterise the total cortical response to a tactile stimulus the response is based on a highly stable substratum..."

VARIABILITY OF EVOKED POTENTIALS: METHODOLOGICAL PROBLEMS

In spite of the constant anatomical, topographic and columnar organization in the somatosensory cortex and the stability of the first potentials evoked by peripheral stimulation, the later potentials evoked at the surface are extremely variable; Adrian (1941) said, "The fixed distribution of fibres to the receiving area contrasts with the great variability in the distribution of activity in the cortex after the primary afferent discharge has reached it".

Variability is seen even in animals anaesthetized with a long lasting anaesthetic agent like urethane. For example, Angel and Dawson (1963) recorded cortical surface responses in the primary receiving area of the rat anaesthetized with urethane and showed that after gross electrical stimulation of the contralateral forepaw, the initial stable positive wave was followed either by a positive or by a negative wave. These authors emphasized the variability in the recorded evoked potentials and pointed out that before any conclusions are drawn about the effect on such a cortical response of conditioning or other procedures, a number of responses should be superimposed or averaged.

Dawson had introduced both the photographic superposition technique (1947) and the averaging technique (1954) to neurophysiology. This enabled extraction of very small evoked potentials from the larger spontaneous waves in records taken from the human scalp. However, as Brazier pointed out in

1961, valuable information about the variability of the response can be hidden in an averaged response. It is a difficult problem to distinguish between the intrinsic variability of evoked potentials (which might be of physiological significance) and the contribution to variability of the background activity (Regan, 1972). In an extreme case of intrinsic variability, a simple average might be quite unrepresentative of individual responses (Mackay, 1969). Brazier (1964) and Werre and Smith (1964) emphasized that variations should be minimised and at least measured before interpretation of evoked responses. Werre and Smith (1964) suggested that variability might depend on recording position, existing spontaneous cortical activity, psychological state, the time interval between stimuli, potentiation induced by a previous stimulus quite apart from changes in the stimulus parameters.

The first aim

The first aim of the present investigation was to identify some of the sources of variability of cortical responses to forepaw stimuli (in the anaesthetized rat) so that they might be easily recognized and perhaps controlled in subsequent experiments concerned with the functional organization of the rat somatosensory cortex. The forepaw response and its variability were studied mainly as functions of three variables, 1) recording position, 2) type and depth of anaesthetic (urethane or trilene), 3) preceding spontaneous cortical activity.

VARIABILITY OF EVOKED POTENTIALS: UNDERLYING MECHANISMS

Cortical interaction

Adrian (1941) suggested that since the later evoked activity was much more dependent on the state of the cortex than the initial response and since it was influenced by local application of strychnine, it was probably due to activity in neurones whose cell bodies were in the cortical grey matter and were separated by one or more synapses from the afferent fibres.

Bishop and O'Leary (1950), Bindman et al (1964b) and Landau, Bishop and Clare (1964) all showed that surface positive polarizing currents increased evoked negative waves and/or decreased surface positive waves and that, conversely, surface negative polarizing currents decreased evoked negative waves and/or increased evoked positive waves. Creutzfeldt, Fromm and Kapp (1962) showed that surface positive currents can induce activation of cortical neurones. They found that transcortical currents below $200\mu\text{A}$ had no effect, and suggested that the spontaneously occurring DC potentials recorded from the cortical surface were probably only secondary phenomena, resulting from, but not modulating, cortical neuronal activity. The interaction of evoked potentials with artificially induced cortical potentials supports the possibility of a cortical mechanism.

Grafstein (1959) was able to isolate large areas of cat cortex from thalamic influences by severing afferent radiations whilst keeping the callosal connections intact.

In this decerebrate and unanaesthetized preparation, the surface of the deafferented cortex showed intermittent bursts of spontaneous activity separated by periods of quiescence (very like those of the urethane anaesthetized rat, e.g. Bindman et al, 1964a). The interaction of the spontaneous waves and the transcallosal evoked responses was such that the surface responses were more positive and/or less negative in the absence than in the presence of spontaneous activity. These results also support the possibility of a cortical mechanism for the variability of evoked potentials. Bindman et al (1964a) showed that in the rat anaesthetized with urethane, a spontaneous positive swing of the potential level at the cortical surface was associated with a predominantly negative surface evoked potential, whereas a negative swing was associated with a positive going evoked potential. Intra cortical stimulation gave similar variations of the surface evoked potential, suggesting that in the rat too, a major part of the variability can occur in the cortex. They found that the spontaneously occurring surface positive waves (plateaux) were associated with bursts of unitary activity in the depths of the cortex, and suggested that the associated increased surface negativity in the response to forepaw stimulation was reflecting an increased superficial response due to a lowered threshold of cortical cells.

However, they (Bindman et al, 1962) had previously shown that the amplitude of the surface positive wave was roughly proportional to the amplitude of the simultaneously recorded

negativity at 500 μ depth. (In the present investigation, depth profiles and unit recordings in even deeper layers of the cortex indicate that the increase in the evoked negative wave is largely due to the reduction in amplitude of a superimposed positive wave, and the suppression of a deep cortical response.)

The interpretation of surface evoked potentials can be complicated not only by the opposite effects of superficial and deep activity (as above), but also by the opposite effects on surface potentials of hyperpolarizing and depolarizing currents (Purpura, 1959).

Jasper (1963) suggested that non-specific activity might have more than one effect on specific cortical responses, and that inhibition might play at least as important a role as facilitation. He suggested that unit processes might underlie the changes in gross potential waves, and that such unitary phenomena might be 1) facilitation of unitary responses by a conditioning stimulus, 2) inhibition of spontaneous activity to give an "increased contrast", 3) inhibition of both unitary responses and spontaneous activity, or 4) increase of spontaneous unitary activity to give blurring or occlusion of responses.

Subcortical interaction

Although the variability of cortical responses discussed so far appears to be largely due to a cortical mechanism, there is also the possibility of interaction between

spontaneous and evoked activity occurring at a subcortical level. Dawson (1958) gave many examples of variability of cortical responses which were related to variability of afferent inflow. He suggested that such variability could in part be due to inhibitory influences from the cortex, in the thalamic or cuneate nuclei. Andersen and Eccles (1962) showed by recording intracellularly from the specific thalamic relay nuclei, that inhibition was abundant at that level. They showed that large and long-lasting inhibitory postsynaptic potentials (IPSPs), with or without preceding action potentials, could be evoked from stimulation of both the contralateral forepaw and the ipsilateral cortex. They suggested that a widespread collateral inhibition was present in the thalamus.

In rats anaesthetized with urethane the cortical surface negative response to a test forepaw stimulus can be increased by strong iterative stimuli anywhere on the body (Angel and Dawson, 1963). In this situation the increase in surface negativity was accompanied by an increase in the evoked thalamic response, both mass and unitary. Subthalamic-evoked activity was not influenced by the conditioning stimulus and three possible mechanisms for the increased cortical response were suggested: 1) increased reticular excitation of thalamus and cortex, 2) cortical inhibition of thalamus might be decreased, or 3) the responsiveness of the cortex itself might be increased.

Dawson, Podachin and Schatz (1963) showed in rats anaesthetized with trilene that the cuneate nucleus response

to forepaw stimuli was reduced by contralateral cortical stimulation, and concluded that since the cortical influence on the cuneate nucleus was mainly inhibitory, it could not play a part in the facilitation of cortical responses.

The possibility of reticular influence during strong peripheral stimulation or during cortical activity was supported by experiments of Angel (1964). He showed, again with rats anaesthetized with urethane or trilene, that strong peripheral stimulation increased the firing frequency of some neurones in the reticular nuclei, while other units showed a decrease in firing rate. The latter type of unit showed an increase in firing rate after cortical stimulation. Thus cortical activity and the activity following a strong peripheral stimulus can have different effects on different reticular units.

Variability of cortical responses to repetitive stimuli in the monkey (Poggio and Mountcastle, 1963) and reduction of the response to the second of a pair of stimuli in the rat and coypu (Angel, 1967a) apparently both occurred at the thalamic level, because similar variations were seen in both the cortical and the thalamic responses.

The similarity of effects of pinching the tail and of spontaneous cortical activity on the cortical surface response was less marked with cortical than with forepaw stimulation (Bindman et al 1964a). These authors suggested that at least part of the interaction caused by pinching the tail had occurred at the thalamic relay. Thus the interaction between competing peripheral stimuli

and the interaction between spontaneous and evoked cortical activity were probably due to different mechanisms, even though both were reflected by similar changes in the evoked potentials recorded at the cortical surface.

Physiological nature of variability

As mentioned earlier, interaction between spontaneous cortical activity and the later components of evoked responses is seen under widely differing experimental conditions and with different anaesthetics. It can also be seen in unanaesthetized animals (Grafstein, 1959) or in unanaesthetized humans (Rémond and Lesèvre, 1967). This type of variability is therefore unlikely to be an artifact of anaesthesia.

In particular, Bindman et al (1964a) showed that although the precise forms of spontaneous waves were different under urethane and tribromethanol, the relation between spontaneous and evoked potentials was the same for both anaesthetics. They also reported that the greatest variability was obtained when the animal was in good condition, under light anaesthesia and when the stimulus was inadequate to produce maximal responses.

It is perhaps significant that in spite of the widely differing experimental conditions and procedures described above (Bishop et al, 1950; Grafstein, 1959 and Bindman et al 1964a) the increases of evoked negative waves and/or decreases of evoked positive waves were always associated with an increase in background spontaneous cortical activity and/or positive potentials at the cortical surface. As Adrian

wrote in 1936, "The activity aroused by electrical stimulation in an anaesthetized animal is no doubt abnormal but demonstrates reactions which probably occur in the normal brain", and in 1939, "From the point of view of cerebral organization the most interesting point about the local electrical response (to sensory stimulation) is not its close dependence on the stimulus but the cyclical variations in size ... it is the interaction between the local excitation and the background of spontaneous activity which is an essential feature of the cortical response".

There is no doubt that in the present investigation variability was studied under very unphysiological conditions, since electrical stimulation of peripheral nerves must result in abnormally synchronised afferent volleys and these volleys were interacting with spontaneous cortical activity which was abnormally synchronised by the urethane anaesthetic. However it was hoped that such variability might reflect gating mechanisms (probably cortical) which modify responses to more physiological stimuli, for example the mechanisms responsible for direction specificity in cortical unit responses to moving joint stimuli (Mountcastle and Powell, 1959a) or moving skin stimuli (Whitsel, Roppolo and Werner, 1972).

The second aim.

The second aim of this investigation was to find out more about the mechanism behind the observed interaction of spontaneous and evoked potentials, in particular to find out whether the variability of the surface potentials was

matched by variability in the responses of individual cortical neurones, and if so whether the mechanism was likely to be cortical or subcortical, due to inhibition or fatigue, etc. With these problems in mind a rough picture of the related cortical synaptic organization was built up by studying the spatial and temporal patterns of both mass and unitary evoked activity as it spread through the different cortical layers and tangentially away from the localized area of initial response.

VARIABILITY OF EVOKED POTENTIALS: AN EXPERIMENTAL TOOL

Although in most studies the variability of evoked responses detracts from their use, this variability can be turned to good advantage if it can be controlled and/or correlated with certain changes in experimental conditions or other variable phenomena which might be related. In particular it is useful to test any particular hypothesis concerning the origin of evoked potentials.

The origin of evoked potentials

It would be of particular interest to know whether there was a causal relationship between unitary discharges and certain components of mass responses. If unitary discharges are responsible for certain mass potentials then simultaneous recording of the two types of potential would provide the experimenter with precise information on the activity of units together with an estimate, derived from mass response, of the size of the population of units exhibiting this particular type of activity.

Depth profiles are necessary to determine regions of current sources and sinks. Chang (1959) said, "Although as Helmholtz pointed out, even detailed knowledge of the electrical potentials appearing on the surface of the cortex is not adequate to ascertain the location of the internal electromotive force, it can indicate which parts of the cortex should be studied further if the source is to be found. Clues to the location of sources and sinks can be checked by a penetrating micro-electrode. If a detailed picture of the

potential changes with time at a set of points near such a source is obtained by multiple recordings, the distribution of current spread may be calculated and the largest sources located more finely. Only at these small locations will the comparisons between unitary and mass activity be significant, since cortical activity is highly complex and certainly not homogeneous". Since the cerebral cortex is the most superficial brain structure, activity in cortical structures is likely to contribute to potentials recorded from its surface. Indeed, most spontaneous (e.g. Bindman et al, 1964a) and evoked potentials (see below) reverse polarity within the cortical grey matter, indicating that they are cortical in origin.

These potentials reflect the flow of extracellular current; e.g. that resulting from non-uniform changes in membrane resistance or voltage of cortical cells. Possible generators (Landau, 1967) of such current include:-

- a) all-or-none action currents in axons
(afferent or axons arising from cortical neurones),
- b) graded postsynaptic currents in cortical neurones, and
- c) all-or-none action currents in the somata
of cortical neurons.

The relative contribution of a particular group of generators (Bishop, 1936) to cortical surface potentials depends on:-

- 1) the number and location of active generators.
- 2) the magnitude and time course of each event.
- 3) the membrane properties of the cells.
- 4) the geometry and orientation of each cell.
- 5) the spatial organization of cells within the group.
- 6) the temporal organization or synchrony of events.

These parameters can vary considerably from one experimental situation to another, and are difficult if not impossible to measure. Evidence in the literature concerning the comparative influence of the above types of generator has been reviewed (Eccles, 1951; Purpura, 1959; Amassian et al, 1964; Ajmone Marsan, 1965; Landau, 1967; Mountcastle, 1969; Regan, 1972).

However since the relative importance of each of the above factors varies and is often unknown, comparisons are difficult and the results, interpretations, and generalizations emerging from different investigations differ widely. Some of the points which have been made in the literature are outlined below.

Interpretation of surface evoked potentials

Caton had already demonstrated the biological nature of evoked potentials in 1875. Adrian (1936) showed that weak electrical stimulation of the cortical surface gave a local negative response with a positive surround. Repeated or

stronger stimuli lead to a large and widespread surface positive response. Adrian suggested that the surface negative wave was due to activity in neurones in the superficial layers of the cortex and that the positive response reflected activity in deeply situated cortical cells. This interpretation was supported by the finding that when the motor area was stimulated, appearance of movement in the limbs was correlated with the "deep" cortical response.

Before microelectrode techniques became available, the all-or-none action potentials of nerve fibres were the best known form of neural activity, and as a result potentials recorded from the cortical surface were then considered to be the summation envelopes of many action potentials, those in vertically oriented cortical cells being the most important. (Bishop, 1936). This theory was supported by the findings of Adrian and Moruzzi (1939) who showed that there was a strong correlation between the rhythmical EEG recorded from the cortical surface and the unitary impulses recorded in the pyramidal tract.

As mentioned above, Adrian, using gross electrical recording techniques in 1941, suggested that since the later variable components of the cortical response to peripheral stimulation were sensitive both to ongoing spontaneous cortical activity and the local application of strychnine, that they were probably due to activity in cortical neurones which were separated by one or more synapses from the afferent fibres. In contrast he suggested that the

initial surface positive wave, which was much more stable, was due to the specific afferent axon volley, because a similar positive wave was recorded from the white matter when the cortical grey matter had been removed. However as Eccles (1951) and Amassian, Waller and Macy (1964) pointed out, this phenomenon could be due to "killed end" recording from the damaged axons. Their view is supported by more recent evidence; microelectrode techniques have since enabled the recording of mass and unitary potentials from all depths within an intact cortex. With these techniques it was found that the early surface positive waves of the cortical response to skin stimulation were routinely associated with mirror image negative waves and unitary discharges in the deep layers of the cortex (Amassian, Patton, Woodbury and Towe, 1955; Perl and Whitlock, 1955; Li, Cullen and Jasper, 1956; Mountcastle, Davies and Bermann, 1957). The reversal of the surface positivity to a negativity within the cortical grey matter indicated that the potentials were generated within the cortex. Since some evoked action potentials (presumably from cortical neurones) were always seen before or during the initial surface positivity it was suggested by these authors that cortical neurones contributed to the initial surface positive response. Perl and Whitlock (1955) compared the early positive responses to thalamic and skin stimulation. They were very similar; both reversed at the same cortical depth, both were associated with early unitary discharges.

On occasion, a unit responding to skin stimulation could also be fired by a stimulus to the contralateral cortex, thus emphasizing the postsynaptic nature of the primary positive responses.

Amassian et al (1955) described how the later components of the evoked potential reversed at successively more superficial levels, and suggested that this might reflect activity travelling towards the cortical surface, leading finally to the surface negative wave. However they also pointed out that the surface negative wave might also be due to positive after-potentials in the deeper layers of the cortex.

Before intracellular, and even extracellular records were made from cortical neurones, Eccles had put forward in 1951 the possibility that graded postsynaptic potentials (PSPs) might contribute to cortical evoked potentials. He emphasized the need for depth profiles with monopolar recording to reveal active regions of sources or sinks of electric current, as well as the need for intracellular recording. The early intracellular study of Albe-Fessard and Buser (1955) as well as more recent ones (Creutzfeldt, Watanabe and Lux, 1966; Purpura, 1967; Humphrey, 1968a) revealed many correlations between cortical surface potentials and subthreshold postsynaptic potentials of cortical neurones, thus supporting Eccles' hypothesis.

However, some of these correlations were found to be negative; i.e. membrane depolarization can sometimes be associated with surface negative potentials (e.g. Creutzfeldt

et al, 1966). This should perhaps be expected, for as Purpura (1959) and Amassian (1961) pointed out, if PSP activity summates to produce potentials at the cortical surface, then interpretation of evoked potentials is extremely hazardous, even if the distribution of sources and sinks is known. Hyperpolarizing currents of inhibitory IPSPs would tend to oppose the effects of nearby excitatory EPSPs, but "superficial" depolarization might look similar to "deep" hyperpolarization. Also, negative correlations might be expected in a convoluted cortex, such as that of the cat, where unitary activity deep in a sulcus can be associated with both positive and negative surface potentials (Holmes and Howard, 1971).

Action potentials versus postsynaptic potentials

Eccles' (1951) view that "it would appear that the synaptic potential generated in neurones by synaptic bombardment provides the basis of most of the cortical (evoked) potentials" is still the view held by most authors. While the 'postsynaptic potential' theory gathered popularity during the 1960's, the early 'action potential' theory lost popularity and was sometimes ignored. This apparent backlash has been encouraged not only by the wealth of data from intracellular recordings but also by emphasis on negative correlations between unitary action potentials and cortical surface potentials (Amassian et al, 1964). These authors referred to anaesthesia effects from Li and Jasper (1953) and anoxia effects from Chang and Kaada (1950), but these

negative observations remain inconclusive because entirely different populations of neurons could have been involved. A similar point was made by Ajmone Marsan in 1965 when reviewing the literature, "The various reports on the subject are somewhat contradictory. Yet in most instances in which the two phenomena are seemingly independent, critical evaluation would indicate that such "negative" findings cannot be taken as evidence that the phenomena are truly unrelated. Rather they can be rationally accounted for in different ways". He also stressed that although evoked potentials were relatively slow, graded potentials, this should not automatically exclude the possibility of their resulting from summation of faster, all-or-none action potentials.

In contrast, Amassian et al (1964) had suggested that it was unlikely, on theoretical grounds, that the surface primary response was due to the summation of action potentials because "action potentials are either diphasic or triphasic in sign" and asynchronous discharge "will promote cancellation because of summation of opposite phases". However diphasic extracellular potentials can in theory arise from monophasic generating currents (if they are brief and localized) because of the capacitance of the surrounding membrane. Similarly, extracellularly recorded action potentials can reflect either the first derivative, or even the second derivative if nearby membrane becomes active, of the intracellularly recorded potential (Murakami, Watanabe and Tomita, 1961, and Burns, 1961). Also, even if (hypothetically) t

inward and outward currents of a diphasic action potential were of equal amplitude and duration, the result of summation would not be a zero potential as suggested by Amassian et al (1964) it would be a slow di- or multiphasic potential of integral zero which was proportional to increase in frequency of unit firing (Bishop, 1936): i.e. a potential proportional to the first derivative of the relevant post-stimulus histogram. Similarly, triphasic action currents might sum to give the second derivative of the post stimulus histogram. In practice the after hyperpolarization of an action potential is much longer lasting and smaller in amplitude than the preceding active depolarization.

The after hyperpolarization of cortical pyramidal cells is particularly small in amplitude, sometimes negligible (Eccles, 1966), and summation is therefore particularly effective. One should also expect from a theoretical point of view (Eccles, Llinas and Sasaki, 1966) that the field at the tip of the apical dendrite (i.e. the surface of the cortex) would be (as in the case of the end of a nerve segment) either diphasic positive/negative with dendritic propagation, or monophasic positive if spread is only passive as is probably the case in the cortex (Rosenthal, 1967). Thus, the fields associated with action potentials in the somata of cortical pyramidal cells will tend to sum, (not cancel) even if they occur asynchronously.

Landau (1967) and Mountcastle (1969) suggested that action potentials in axons are so small in amplitude and

duration (by comparison with other sources of current), that they do not contribute significantly to surface potentials unless they are evoked in strict synchrony by electrical stimulation. Landau (1967) did include soma action potentials in his list of possible generators but they were omitted from Mountcastle's (1969) list. The literature thus seems to be overwhelmingly in support of synaptic currents being the principal generators of evoked potentials. However, there still seems to the author to be too much emphasis on the correlations between intracellularly recorded synaptic potentials and simultaneously recorded mass potentials; summed postsynaptic potentials are inevitably related to action potentials in the same neurone (a positive correlation) and to action potentials in presynaptic fibres (a positive or negative correlation). and the observed correlations do not necessarily imply cause and effect.

The third aim

In early exploratory experiments in this investigation a striking correlation was found between unitary action potentials, local negative slow waves and positive surface waves in the responses to forepaw stimulation. My third aim has been to study such experimental correlations in more detail with the help of depth profiles and with particular reference to the variable components of the evoked response. It was hoped that the results might support the theory that evoked potentials could, in part, be due to summation of soma action potentials.

A related problem has been to make an absolute estimate of the expected contribution of soma action currents in pyramidal cells to cortical surface potentials. At the time there was no quantitative theory available which was simple to apply to this problem, although more recently the work of Humphrey (1968b) and Nicholson and Llinas (1971) has appeared. Their models are very similar to mine. Humphrey compares fields related to synaptic currents with those due to action currents in a typical pyramidal cell. However the boundary conditions are not strictly comparable so his interpretation was again biased towards the 'post-synaptic potential' theory. This problem of interpretation will be left to the discussion section.

SUMMARY

The experiments of this investigation have been performed on rats anaesthetized with urethane or trilene. Records of electrically activity have been taken from the forepaw area of the primary somatosensory cortex. The main topic, the variability of responses evoked by electrical stimulation of the contralateral forepaw, was studied a) from a methodological point of view, in order to recognize and control variability in subsequent experiments, b) in an attempt to understand cortical gating mechanisms and c) as a tool to test for a correlation between unitary and gross potentials.

METHODS

Anaesthesia

Forty three Wistar and C.F.E. albino rats weighing 150-300g were used. They were anaesthetized with urethane or trilene.

Urethane was given as a 25% solution in 0.9% NaCl solution intraperitoneally after induction with ethyl chloride. After an initial dose of 1cc, further small doses were given until the required level of anaesthesia was reached (a total of 1.5-2.0cc). The level of anaesthesia was tested by squeezing a hind paw and watching for a withdrawal reflex. In most experiments anaesthesia was maintained at a level such that the reflex withdrawal was feeble. This necessitated the administration of urethane at the rate of about 0.1-0.2cc/hr. In the other experiments the depth of anaesthesia was deliberately varied.

Trilene anaesthesia was induced with a trilene-soaked mask and maintained by means of a flow of trilene vapour into a tracheal cannula. The level of trilene anaesthesia was judged mainly by observing spontaneous whisker movements which vanished when anaesthesia was light or very deep; the respiration rate and the withdrawal reflex were also useful. In these experiments there was no direct measurement of trilene concentration in the inhaled air, but any particular state of anaesthesia could be achieved and maintained by controlling the flow of trilene vapour.

In a few experiments, deep trilene anaesthesia was changed to urethane anaesthesia while cortical responses were being recorded. This was achieved by discontinuing the trilene and giving three 0.5cc doses of urethane at 4 min intervals for a total of 1.5cc of urethane.

Preparation

After the tracheotomy, the skull was exposed and a craniotomy performed. In early exploratory experiments a large craniotomy was performed and a large area of dura removed so that the distribution of surface potentials over the primary somatosensory area could be studied in detail. In later experiments, when the location of particular events had been established, smaller exposures were made to give greater stability for unitary recording. The bone was removed with a fine dental burr, and the dura was reflected with a surgeon's needle and watchmaker's forceps. A high power binocular dissecting microscope was used for the whole preparation.

The animal's head was immobilized in a home made rat head holder, with the incisor bar placed 5 mm above the ear bars to conform with the de Groot orientation (de Groot, 1959). This orientation was the most suitable for these experiments because the coronal plane in the region of the primary somatosensory area of the cortex intersects the cortical surface at right angles (see fig. 1a) & b)). A pool was formed over the exposed cortex with liquid paraffin at 37°C which had been equilibrated with 0.9% NaCl solution. The rat lay on a polystyrene mat and was kept warm with an overhead anglepoise lamp. (The lamp was well screened to prevent 50 c/s

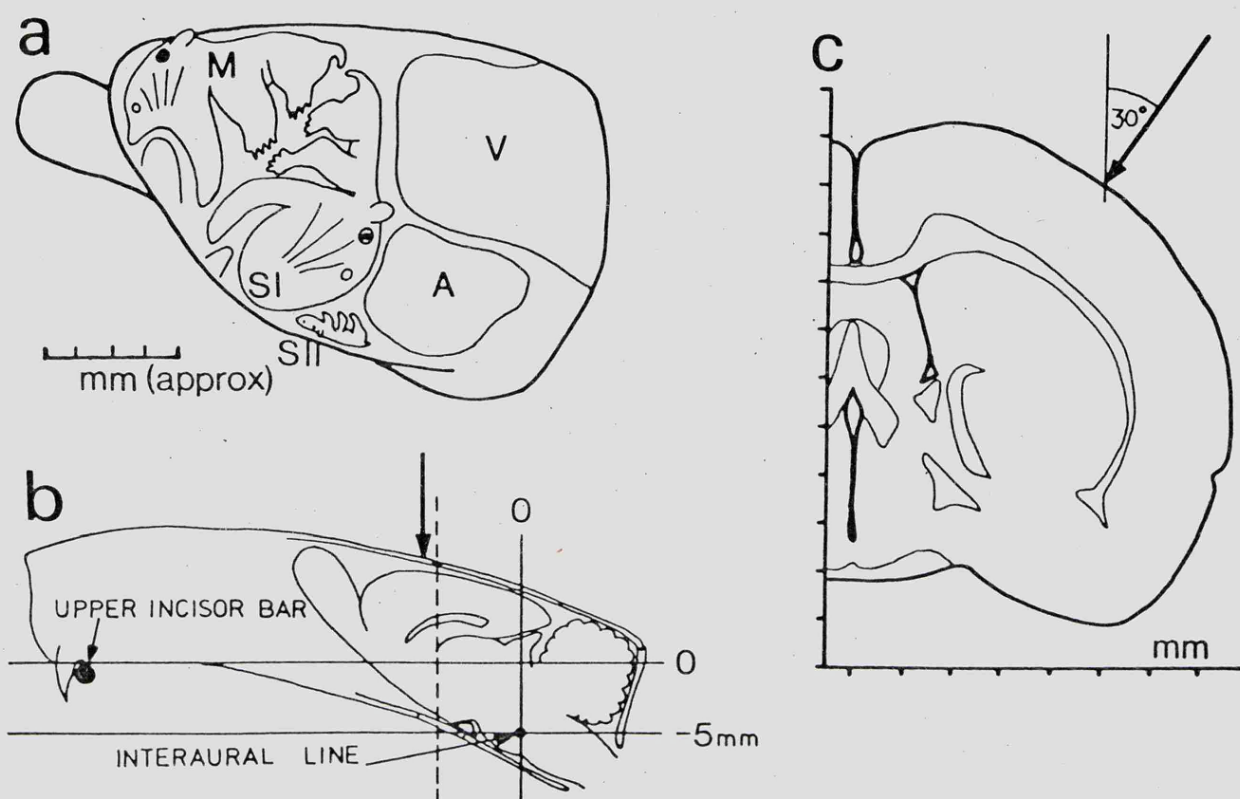


Figure 1. The position of the forepaw receiving area in the cortex of the rat.

a) Redrawn from Woolsey (1958) showing the position of the primary somatic sensory area (SI) in relation to the other sensory areas (V = visual, A = Auditory, SII = secondary sensory) as well as to the motor area (M).

b) Redrawn from de Groot (1959) showing the orientation of the skull used in these experiments (with the upper incisor bar 5mm above the ear bars). The vertical dashed line indicates the position of the bregma.

c) Also redrawn from de Groot (1959) showing a section through the brain about 1.5 mm anterior to the bregma.

The heavy arrows in (b) and (c) indicate the approximate position and orientation of microelectrode penetrations used in this investigation.

interference). Rectal temperature was maintained near 36°C .

Stimulation

Electrical stimuli were applied to the fore paw via saline moistened wicks touching the 1st and 4th toes of the animal's paw. The stimuli were rectangular, 0.1-0.5 msec in duration. The amplitude (about 70v) was arranged to be just insufficient to cause a visible muscular contraction of the paw. Triggering pulses for the stimulator were generated by a Devices Digitimer which was either allowed to cycle automatically or was triggered manually. (see fig. 2 for layout)

Recording

Ball-ended chlorided silver wire electrodes of tip diameter of about 0.3 mm were used to record potentials from the cortical surface. Fine shanked microelectrodes were used for recording potentials within the cortex. The shank was less than 50μ in diameter for at least 1mm from the tip which was itself less than 1μ in diameter. The microelectrodes were usually oriented at 30° to the vertical in the coronal plane so that they would be perpendicular to the cortical surface. (See fig. 1c). Non-polarizable glass micropipettes filled with 3M NaCl solution were usually used so that the longer-lasting mass potentials could be observed at the same time as unitary potentials. Sometimes etched stainless steel microelectrodes (insulated with 'Insl-X') were used for unitary recording. The resistances of all microelectrodes were in the range 3-10 megohms, when tested with a sine wave whose frequency was in the range 100 c/s to 5 Kc/s.

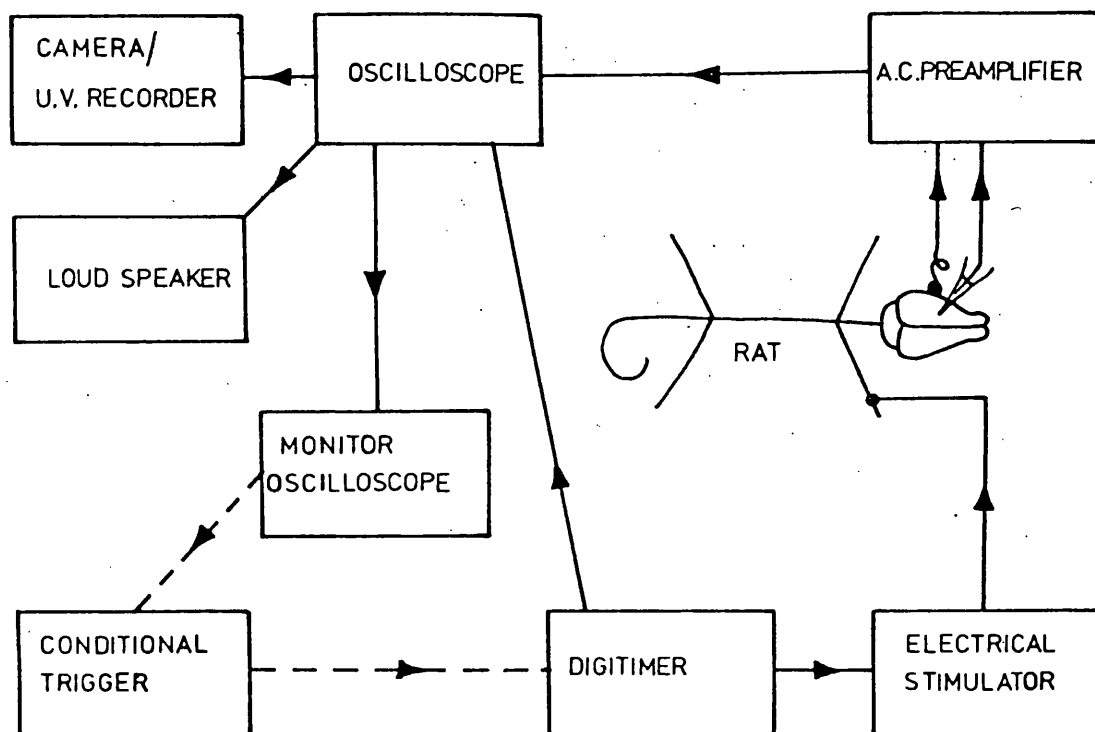


Figure 2. Layout of recording and stimulating equipment.

Amplification and Display

The recording electrodes were connected by 10 cm long screened leads to a four channel capacitance coupled source follower placed close to the animal's head. The indifferent electrode was a chlorided silver wire placed on the skull in the region of the lambdoid suture. The voltages of the signals from the source follower were amplified $\times 1000$ by an A.C. pre-amplifier. Each signal was directly coupled to the input of a Tektronix 502A oscilloscope; the decay time constant of the complete recording system was then about one second. The frequency response curves of the complete recording system were flat in the ranges 100 c/s-5 Kc/s (steel electrodes) and 10 c/s-5 Kc/s (glass micropipettes). Thus, although the absolute potential level was lost, the glass electrodes gave no significant distortion of the first 20 msec of each response. Occasionally the signals from the low resistance silver electrodes were fed directly into the Tektronix (bypassing the source follower and pre-amplifier) so that D.C. potentials could be observed from the cortical surface.

Filming

A Cossor camera was attached to the oscilloscope to take film records of all the experiments. The signals from the X and Y deflection plates were directly coupled to a two channel slave oscilloscope to check filming and to make on line observations. The spot brightness was not suppressed between sweeps on the slave scope; the slow changes in

spontaneous cortical activity (stationary X plate between sweeps) could then be monitored on the same instrument as the cortical responses (X deflection driven by the first oscilloscope). Thus, if the spontaneous changes were not too frequent, the experimenter could use the manual trigger on the Digitimer conditionally. In this way, separate groups of responses were obtained corresponding to different initial conditions. Occasionally, to test the success of the 'conditional trigger' technique, a continuous record of both evoked and spontaneous activity was made with an ultra-violet oscillograph recorder. A 1 msec time scale from the Digitimer was used to calibrate records. (The layout of apparatus is shown diagrammatically in fig. 2).

Histology

After each physiological experiment the animal's brain was fixed, sectioned and stained for the checking of positions of electrode tracks. The animal was killed with an overdose of anaesthetic. Formalin (5% in physiological saline) was poured into the paraffin pool (leaving a layer of paraffin to prevent evaporation). The electrode was left in situ for about twelve hours. After removal of the electrode, the craniotomy was enlarged and the dura reflected. A block was cut from the brain with a steel microelectrode which was held in the micromanipulator used for recording so that the edges of the block would all be parallel to the track. The vernier on the manipulator was used to position each cut 2mm away

from the small hole left by the microelectrode. After further fixation in formalin for at least 3 days, the block was cut with a Leitz 230K freezing microtome into 20 μ sections. The position of the track could be predicted within 100 μ and was easily seen under a high power binocular dissection microscope while the block was being sectioned. The relevant sections were then stained with haematoxylin and eosin; an aqueous method was used to minimize shrinkage of tissue. The section containing the deepest part of the track was then identified microscopically. The microscope incorporated a camera lucida. Drawings were made of consecutive sections, small blood vessels were traced and matched up so that some of the other tracks (made by electrodes which had been removed before the end of the experiment) were also identifiable, but their outlines were not so clear.

RESULTS

RECORDING FROM A SINGLE POINT ON THE CORTICAL SURFACE

Form and variability of evoked potentials

In this investigation rats were anaesthetized with urethane or trilene. Brief electrical stimuli were given to the forepaw. Stimulus strength was just insufficient to cause a muscular contraction. Records were taken from the surface of the primary receiving area of the contralateral cerebral cortex.

The first component of the surface cortical response to electrical forepaw stimulation was always positive. The first deflections of the response were seen 4 to 6 msec after the stimulus. This initial positivity was relatively stable with an amplitude of up to 0.4 mV. The peak of the positivity occurred about 7 msec after the stimulus and was followed by a complex wave which was very variable; sometimes this wave was positive and at other times predominantly negative.

Angel and Dawson (1963) emphasized this variability and fig. 3a is taken from their paper. Fig. 3b shows a similar group of superimposed responses, but these were from my experiments and were recorded with a much longer decay time constant. In fig. 3a the variability of the evoked potential appears to be much greater than that of the initial background potential. In fig. 3b the variability at the time of the stimulus is approximately the same as that at about 12 msec after the stimulus, but at about 9 msec after the stimulus the variability is momentarily reduced. This is

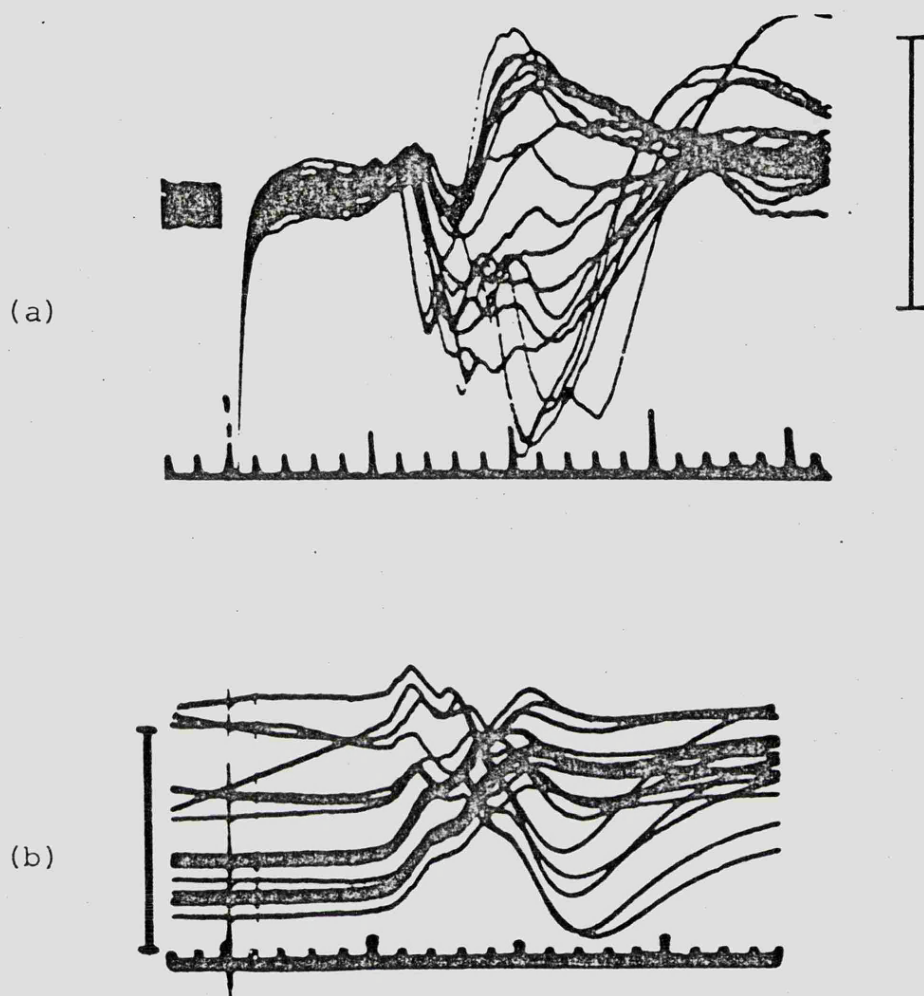


Figure 3. Variability of potentials evoked at the cortical surface.

a) Reproduced from Angel & Dawson (1963). This figure shows 20 consecutive, superimposed responses recorded from the primary receiving area of the left cortex in response to an electrical stimulus applied to the right forepaw in a rat anaesthetized with urethane. (Time constant of recording approximately 5 msec).

b) A similar group of responses from my own experiments (also with urethane). A.C. recording 1 sec time constant. Stimuli were given 4 secs apart, 16 consecutive responses are superimposed.

Each vertical bar is a 1 mV calibration. In this and all other records positivity is upward. A msec time marker is shown.

apparently because the initially positive potentials tended to be followed by an evoked negative wave, and the initially negative potentials by an evoked positive wave. It is clear from both these figures that the variability in the response was different from that which would be expected from the superposition of background spontaneous activity alone. The interaction between spontaneous and evoked activity evident in fig. 3b and 4 was described by Bindman et al (1964a).

The relationship between spontaneous and evoked potentials

In a rat moderately anaesthetized with urethane the electrocorticogram (ECoG) showed bursts of activity separated by periods of quiescence. If the low resistance surface electrode was directly coupled to the oscilloscope it could be seen that these waves of activity were associated with a steady positive potential of about 1 mV. A clear relationship between spontaneous and evoked activity could be seen. In fig. 4, the top trace shows spontaneous ECoG activity. Six responses to forepaw stimuli given once every four seconds are shown below. Each stimulus was given 120 msec after the beginning of the sweep. If the stimulus was given during a burst of ECoG activity, then the evoked potential was largely negative going (figs. 4d to f) whereas a stimulus given during a period of quiescence resulted in a positive evoked potential (figs. 4 a to c).

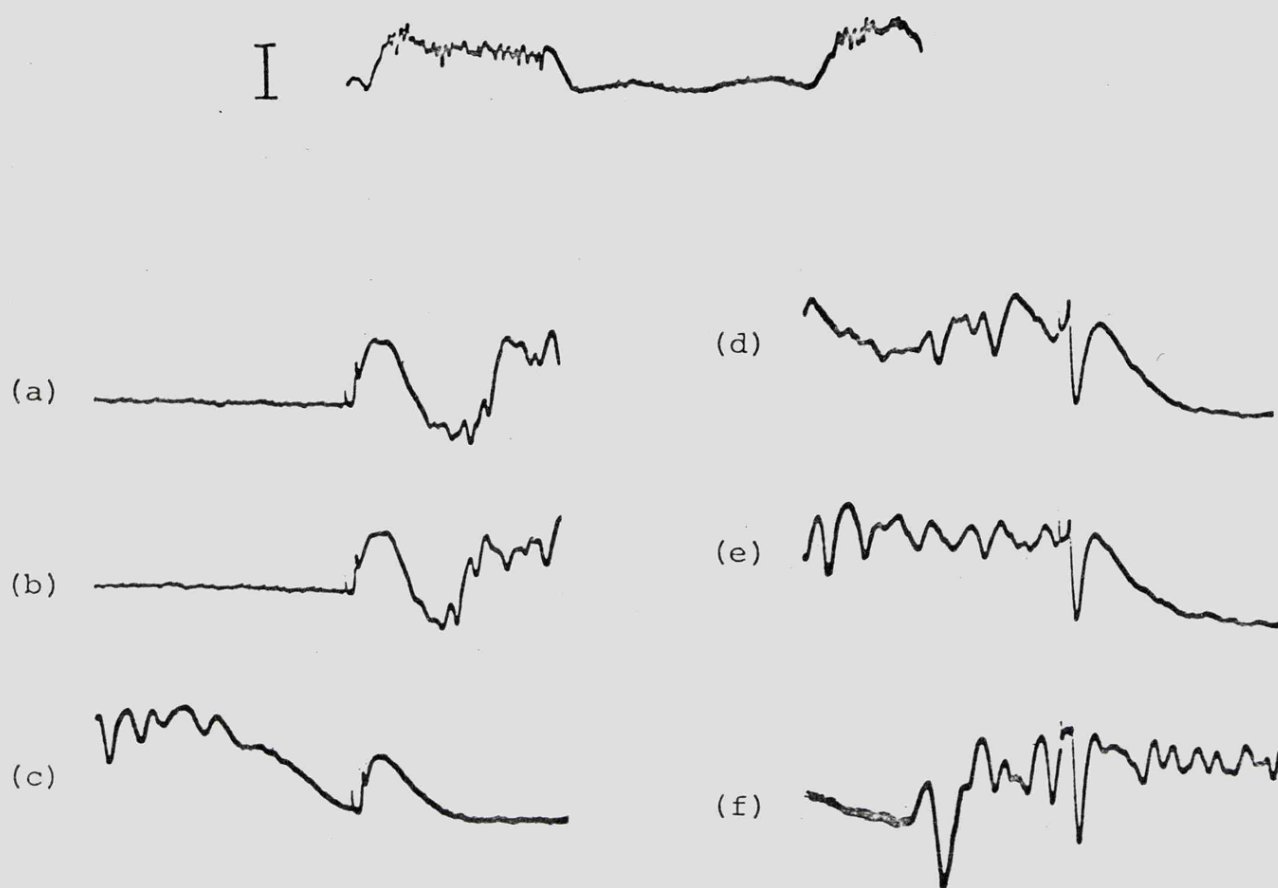


Figure 4. D.C. records of cortical surface potentials

Above: A record of spontaneous ECoG (Total sweep 2 seconds).

(a) to (f): Responses to forepaw stimuli given 240 msec after the beginning of each sweep.

Voltage calibration: 1 mV for top record and 0.5 mV for (a) to (f).

In rats moderately anaesthetized with urethane, periods of spontaneous activity of about 0.5-1.0 second duration alternated with periods of quiescence. In this anaesthetic state, the ECoG could often be switched from an inactive state to an active one (and vice versa) by peripheral stimulation. This switch occurred if the stimulus was given in a period of activity or quiescence when it had been established for 300 msec or more (see figs. 4a, b, d and e); a switch was not usually seen if the stimulus was given during the first 300 msec of a period of activity or quiescence (see figs. 4c and f).

Responses to trains of stimuli

If a group of stimuli was given at a rate of 10 per second, the responses to the second or third stimulus of such a group depended on the state of induced background activity of the ECoG; negative responses were associated with background activity and positive responses with initial quiescence, as with responses to single stimuli. Fig. 5 shows a sequence of consecutive responses recorded with a directly coupled amplifier. Four seconds elapsed between each group of three stimuli. The background activity was 'switched' on or off by the first stimulus in most groups of responses (e.g. in fig. 5 all those except the fourth and fifth, where the stimulus was given at the beginning of a quiescent and active period respectively). Thus the second response of each group of three was usually different from the first.

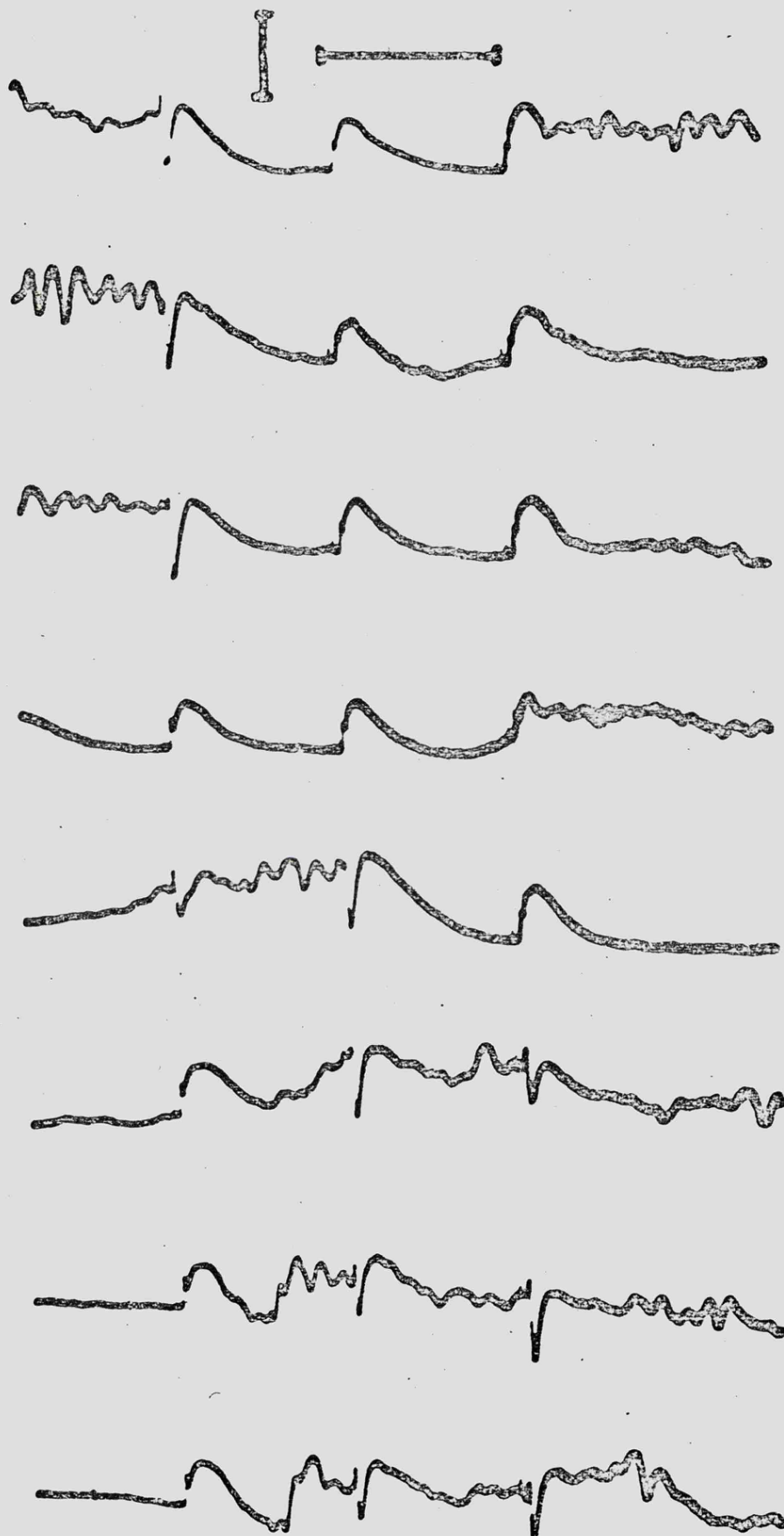


Figure 5. Responses to repeated stimuli

D. C. records were taken from the cortical surface. Groups of three stimuli (100 msec separation) were given to the contralateral forepaw every 4 seconds. Eight consecutive responses are shown. The rat was anaesthetized with urethane. Calibration: 1 mV and 100 msec.

The form of the responses to the third stimulus of each group of three was less predictable but was always related to the background activity in the same way as those to the first two. Thus, if the rat was anaesthetized with urethane and a train of stimuli was given, there was a dependence of evoked potentials on induced ECoG activity similar to the dependence of responses on spontaneous ECoG activity.

The directly coupled records of figs. 4 and 5 both suggest that the variability in evoked potentials (absolute rather than relative potentials) is much less than that which would be expected from superposition of the spontaneous background potentials. Thus the variability in the form of the evoked potential could perhaps reflect a stabilizing or resetting phenomenon.

Control of variability

The quiet periods in the ECoG of moderate urethane anaesthesia usually lasted half a second or more as did the active periods. It was therefore possible for the experimenter to watch a monitor scope and trigger the stimulus cycle manually so that stimuli were given only during quiet or only during active periods. This conditional triggering was used to superimpose groups of positive going responses of quiescent cortex on film separately from groups of negative going responses of active cortex. These two types of response will be referred to as 'Q' responses and 'A' responses respectively.

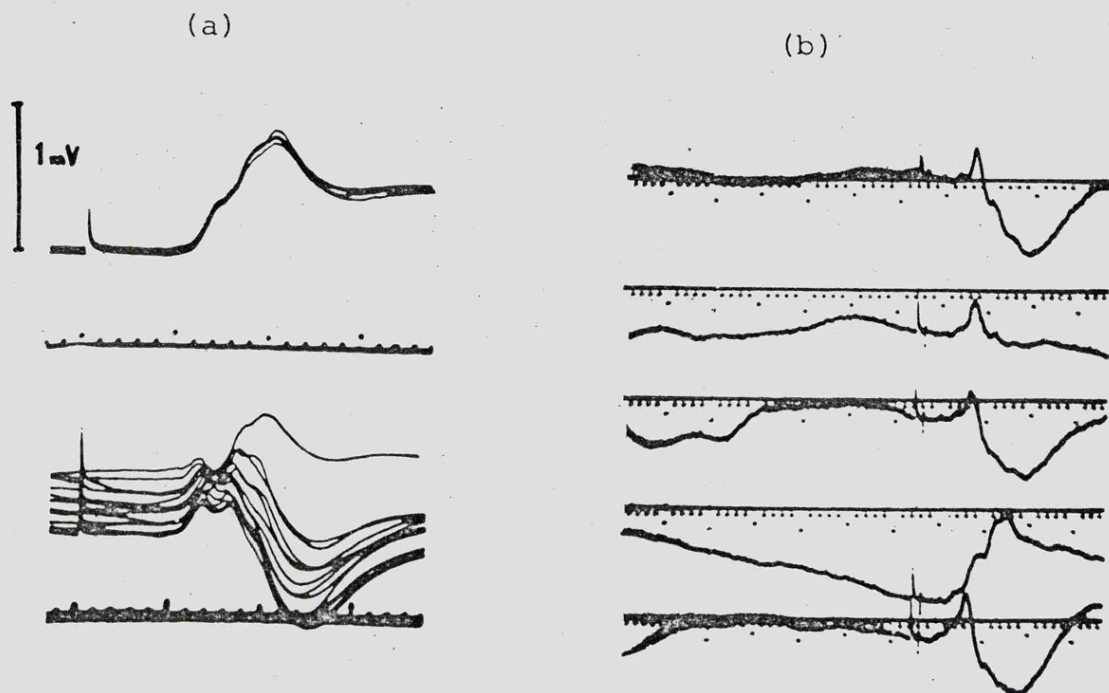


Figure 6a. Separation of responses into two groups by conditional triggering (urethane anaesthesia).

Above, six consecutive superimposed responses to forepaw stimuli given when the cortex was quiescent ('Q' responses). Below, twelve consecutive superimposed responses to stimuli given when the cortex was spontaneously active ('A' responses).

The spontaneous activity was observed continuously on a monitor scope and conditional triggering done by hand. Stimuli were 4 or more seconds apart. Time marker, 1 sec. Voltage calibration 1 mV. A.C. recording with 1 second time constant.

Figure 6b. Relationship between spontaneous and evoked potentials with trilene anaesthesia.

Five consecutive responses to stimuli given every 4 seconds. A. C. recording with 1 second time constant. Time marker, 1 msec. Voltage calibration 2 mV.

Fig. 6a shows a separation of 'A' and 'Q' responses. The experimenter's reaction time was about 250 msec and if the spontaneous activity changed within that time, occasional 'non typical' responses were recorded (for example the positive response of the lower group in fig. 6a). Whenever activity was continuously monitored on the U.V. recorder, it was found that such non typical responses were always associated with changes of background activity within the previous 300 msec. However the number of such mistakes was small, and the occasional non typical responses were ignored. The two basic types of response could always be identified.

This method of studying the two response types separately with the use of a conditional trigger was used in most of the experiments under urethane. As well as retaining information about the two distinct types of response, this method seemed more satisfactory than using an average of responses such as those of fig. 3; responses were clearly of two distinct types and the significance of such averages would be far less than that of a 'normally' varying response.

Effects of anaesthesia

All the experiments described above were performed on rats under moderate urethane anaesthesia. However the form and variability of the evoked potentials in animals anaesthetized with trilene or different levels of urethane were very similar (See fig. 6). The only significant differences were seen with trilene or light urethane anaesthesia. In these conditions

the latent periods of the various waves were 0.5-1.0 msec less and also the 'switching' of background activity was not seen. The shorter latent periods might have been partly due to increased spontaneous activity in these conditions. Even with moderate urethane anaesthesia it was often possible to see a reduction of about 0.5 msec in latency during a burst of activity, i.e. in an 'A' response.

Although the form of spontaneous activity was greatly altered by the type and depth of anaesthesia, the relationship between spontaneous and evoked potentials was always the same; positive responses were associated with a negative or quiescent ECoG and negative responses with an active or positive ECoG.

In the case of light urethane anaesthesia (or of trilene anaesthesia) the ECoG was active most of the time and there were only brief spells of quiescence. Thus most of the responses were negative going, but if the stimulus was given by chance during one of the brief quiescent periods, the response evoked was a positive one. Unfortunately the manual conditional triggering technique (used to separate 'A' and 'Q' responses during moderate urethane anaesthesia) could not be used with these trilene or light urethane experiments because the quiet periods were so brief. In these experiments the stimuli were given at regular intervals and each response was recorded separately on a moving film.

Fig. 6b shows a sequence of consecutive responses which included one associated with a brief quiet period (a short-lasting smooth drop in voltage level). As mentioned above all such 'Q' responses of trilene anaesthesia were positive ones. In most experiments under trilene, responses were recorded individually on film with a trigger to stimulus delay similar to that of fig. 6b and any classifications or observations concerning background spontaneous activity were made in retrospect. The short quiet periods of trilene anaesthesia sometimes occurred in groups at a frequency of 6 per second but usually they were so infrequent and so short (20-40 msec) that the probability of stimulating during a quiet period was only about 1:20. It was thus necessary to record long sequences of responses in experiments under trilene, if the positive going 'Q' responses were to be studied. For example there would only be about 9 stimuli given during quiet periods if stimuli were given once every 4 seconds for twelve minutes. For this and other technical reasons most of the experiments of this investigation were performed with urethane anaesthesia. Unless otherwise indicated all the following figures will be from experiments on animals anaesthetized with urethane.

At moderate and deep levels of anaesthesia, spontaneous cortical unitary activity (action potentials recorded from single cortical cells) usually increased with increasing trilene concentration, whereas it decreased in urethane

experiments when supplementary doses of urethane were given. It was therefore hoped that features of the evoked potentials which were sensitive to anaesthesia might be revealed when evoked potentials from experiments with the two types of anaesthetic were compared. A few 'control' experiments using trilene anaesthesia were performed in an attempt to see whether particular results might have to be considered as specific artifacts of urethane anaesthesia.

SUMMARY

The form and variability of evoked potentials recorded from the cortical surface were similar in all anaesthetic conditions used (various levels of trilene and urethane anaesthesia).

Much of the variability of evoked potentials recorded at one point on the cortical surface of the primary receiving area in response to peripheral stimulation appeared to be closely related to background spontaneous activity of the ECoG. Surface positive responses were associated with absence of spontaneous ECoG activity at all depths of urethane and trilene anaesthesia used. In experiments under urethane the two main types of responses could be separated by conditional triggering of the stimulus, but with trilene experiments the responses had to be classified in retrospect.

SPATIAL DISTRIBUTION OF SURFACE EVOKED POTENTIALS

Another type of variability of surface responses was that seen when comparing the results from different experiments. This variability was not so large as that associated with spontaneous cortical activity. It consisted of a difference in the shape of the waveform, and sometimes in latent period rather than a change in polarity. The first positive wave and the large negative wave were apparently localized to a small area whereas the later positivity (when it occurred) was found over a larger area. It therefore seemed likely that the variability from one experiment to another might in part be a function of recording position, since the area yielding responses of shortest latent period was usually near or under a large blood vessel. This idea was tested by systematically recording from a number of points on the cortical surface in each of 17 experiments. A fine grid (0.5 mm) was used to determine the spatial distribution of the evoked potentials. The results from different experiments were in good general agreement and supported the idea that much of the variability from one animal to another was due to different relative recording positions rather than real differences in the cortical response.

There was no consistent relationship between the positions of large surface veins and the position of the area of initial response. This area was very localized and in 17 experiments, it was covered by a large blood vessel 5 times, it was near one 4 times, and well clear of the major blood vessels only 8 times.

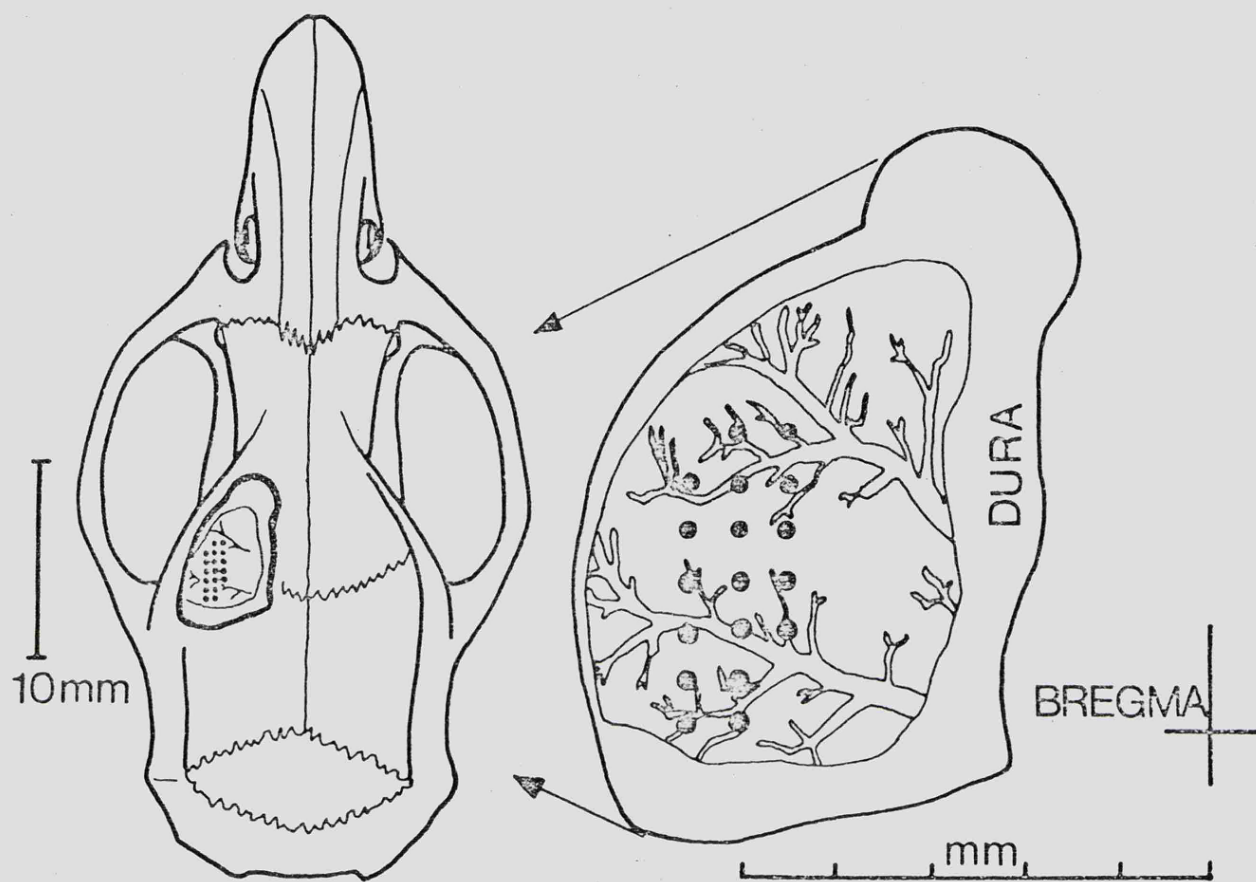


Figure 7. To show recording positions of a mapping experiment.

The records taken from these positions are shown in figures 8 and 9. A 0.5 mm grid was used.

The blood vessels usually prevented the completion of a 0.5mm recording grid, but one mapping experiment was successful in this respect.

A mapping experiment

Fig. 7 shows some of the recording positions used in this mapping experiment. The dura had been removed and a small chlorided silver ball electrode was placed at each recording point in turn. At each position a group of 'Q' responses was superimposed on film with the conditional triggering described above (fig. 8a). A group of 'A' responses was next recorded (fig. 8b). The electrode was then moved on to the next recording position, the positioning being determined by the two horizontal verniers on the micro-manipulator. It was found in later experiments when recording with pairs of electrodes up to 2mm apart that the spontaneous bursts of ECoG activity were synchronized, and that the two simultaneously recorded responses were of the same type. It is therefore suggested that each of figs. 8a and 8b show the types of responses which would have been recorded simultaneously from an ideal 18 channel recording system. To check that the waveform was not changing slowly with time as well as (or instead of) with recording position, records were taken again about 1.5 hours later in a different sequence. The responses of the right hand side of fig. 9 are from this control set; it is clear that each recording position was associated with characteristic response waveforms. These waveforms remained stable for many hours

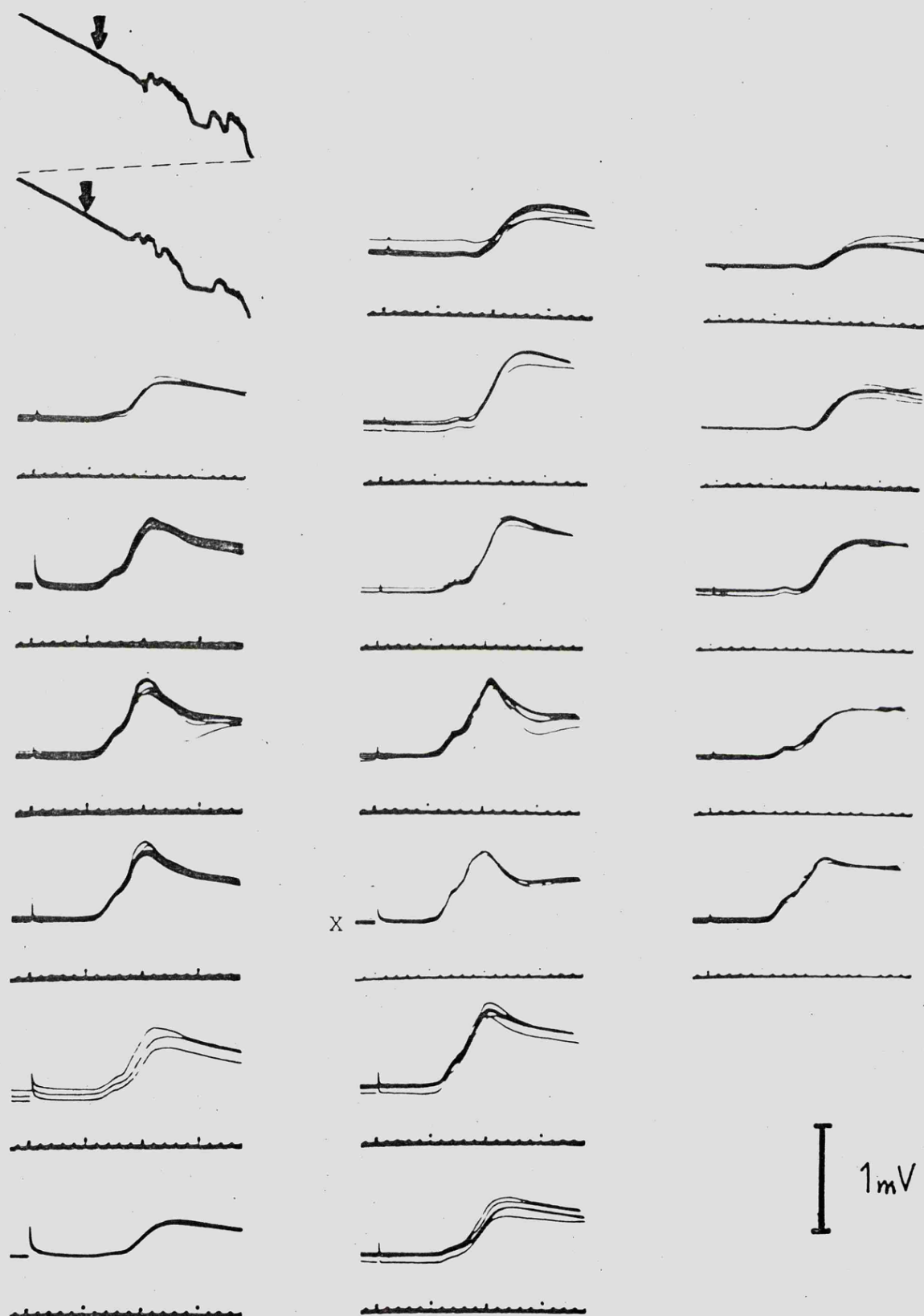


Figure 8a. Cortical surface responses to stimuli given when there was no spontaneous ECoG activity ('Q' responses).

The 18 recording positions are those shown in fig. 7, i.e. anterior upwards and medial to the right. At each recording position a group of 'Q' responses was recorded superimposed on film, then a group of 'A' responses (see fig. 8b). The electrode was then moved to the next recording position and the process repeated. (Urethane anaesthesia).

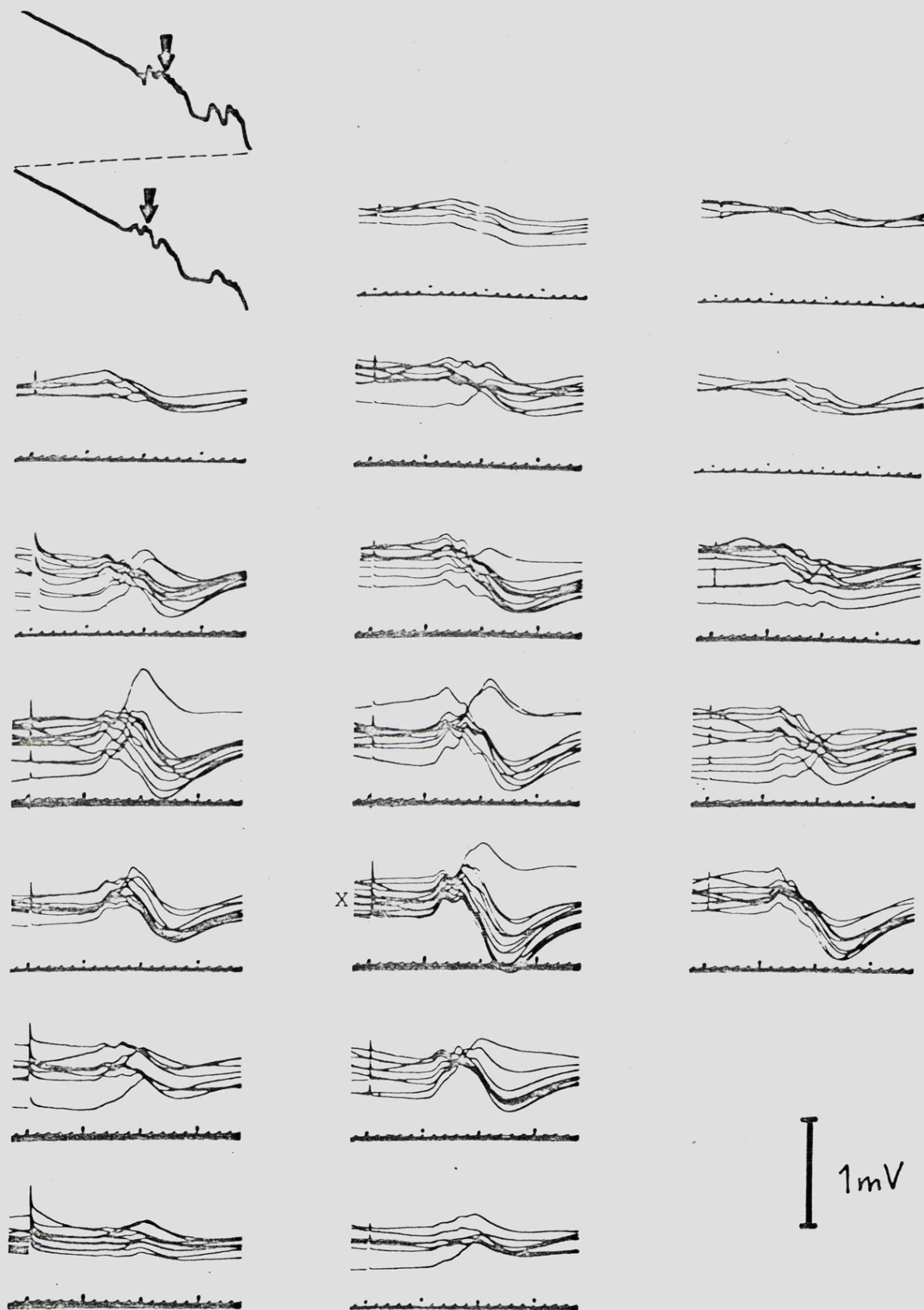


Figure 8b. Cortical surface responses to stimuli given when there was already spontaneous activity ('A' responses).

These are from the same experiment and recording positions as those of fig.8a. X marks the apparent centre of the receiving area.

unless a pool of C.S.F. formed around the electrode; in this case removal of the C.S.F. resulted in the return of the original waveform. The association of surface positive responses with a quiescent ECoG was found in all recording positions. This positive wave occurred over a wide area of cortex (see fig. 8a), whereas the negative wave of the 'A' response was more localized (fig. 8b). In the group marked with a cross, large negative waves were seen, but only 0.5 mm away in each direction the amplitude of the negative wave had dropped to about half. The first positive wave appeared to be localized to the same areas as the negative wave in the 'A' responses of fig. 8b; the smaller deflections recorded in the surrounding area appeared to have the same time course and were probably reflecting passive electrotonic spread from a very localized active region under the position of maximum response.

The first positive deflection in the 'Q' responses of fig. 8a was localized in the same way. Although there was no absolute negativity there appeared to be a negative going notch in the otherwise rounded later positivity. This negative going notch in the 'Q' responses of fig. 8a appeared to be localized to the central area like the negative wave of the 'A' responses of fig. 8b.

Thus, the surface evoked potential appears to consist of a number of components, of different polarities and of different spatial distributions. To clarify the features of this complex response, the responses from the central columns of figs. 8a and 8b were averaged separately and combined to form contour plots.

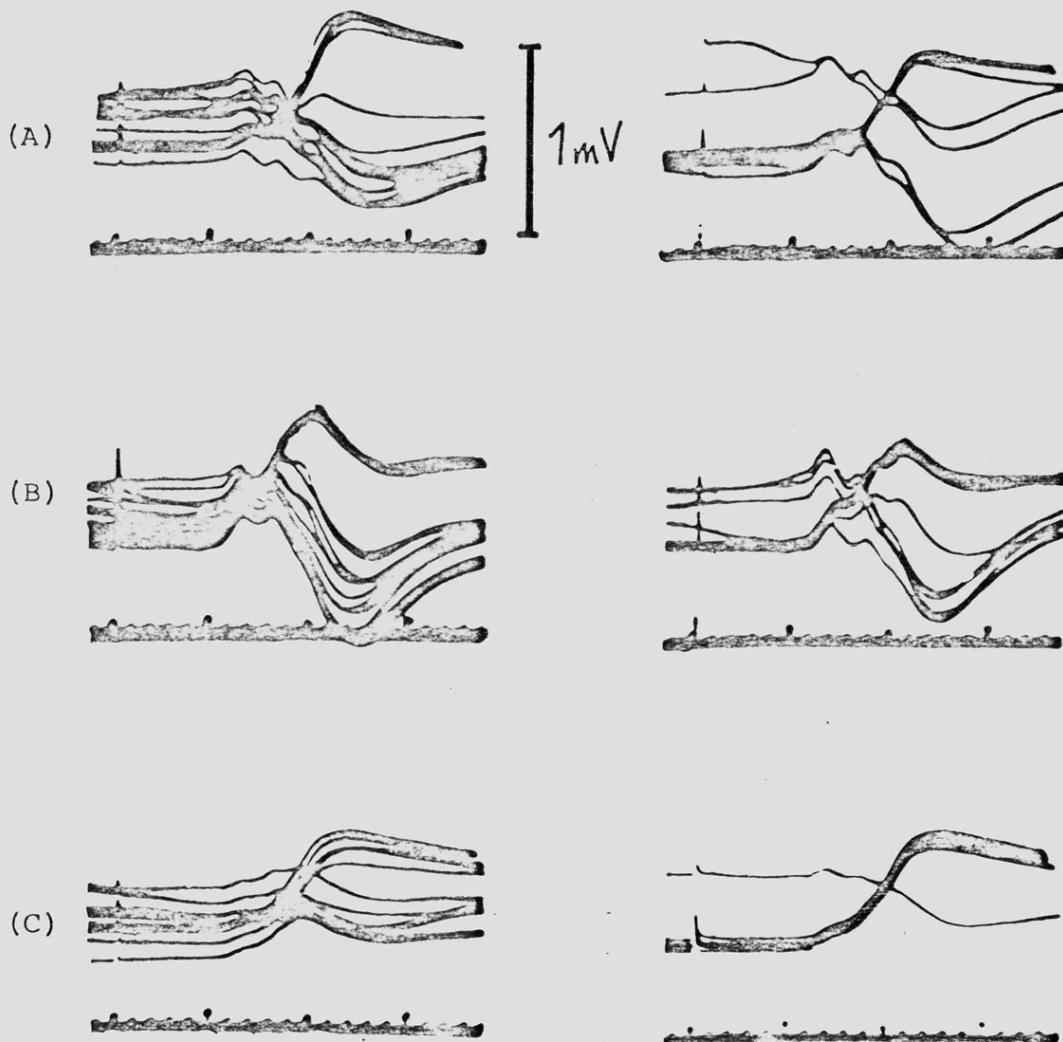


Figure 9. Stability of waveforms over long periods of time.

The same experiment as figs. 8a and 8b. Records were taken from the position of shortest latency (B) as well as 1 mm anterior (A) and 1 mm posterior to this position (C). Records taken at the beginning of the experiment are shown on the left and those taken 1.5 hours later on the right.

Contour plots of surface responses

Each averaged response contributed to the contour plot in the way shown in fig.10. Equipotential points were then joined up to form the contour lines. Seven 'Q' responses of fig.8a were used to construct the left hand contour plot of fig.11. The seven corresponding 'A' responses of fig.8b were used to construct the middle contour plot. The difference between the 'A' and 'Q' responses was measured and plotted in the right hand diagram.

The 'A' response was complex and appeared to 'spread' outward from the early response areas (i.e. the maximum positivity at X occurred 2-3 msec earlier than at points 1-1.5 mm away). The 'A' response was localized and did not 'spread' outward, supporting the idea that the attenuated waves observed around the central area of maximum response might result from electrotonic spread of potentials. Although the 'A' response consisted of several distinct components, they all appeared to be restricted to the same small area of cortex. It can be seen that the 'difference' component had a simple rounded shape and was almost uniform over 2 or more millimetres. Although the polarity of the difference was arbitrary it has been indicated as positive (i.e. equal to the 'Q' response minus the 'A' response) to suggest that the 'Q' response consists of an 'A' type response plus a surface positive 'difference' response (as opposed to the 'A' response being similar to a 'Q' response plus a surface negative response). The former alternative seemed likely from the surface records; the 'A' response was

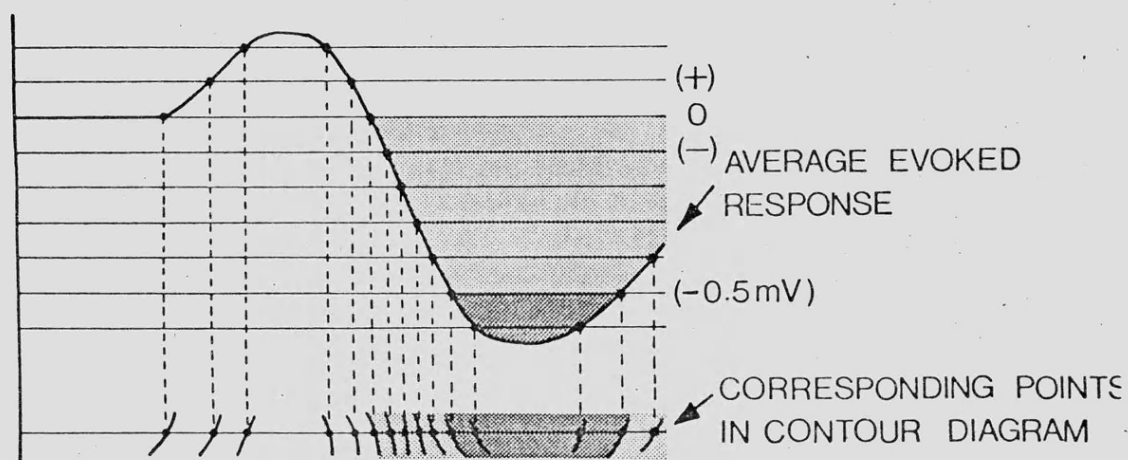


Figure 10. Construction of a contour diagram.

The crossing points of the average evoked response across either 0.125 mV (or 0.1 mV as here) voltage levels were plotted along a line as shown. Neighbouring (equally spaced) recording positions were represented by neighbouring (equally spaced) lines of such points. Equipotential points were then joined to give the contour diagram. Half millivolt levels are indicated in the next and following contour diagrams by thicker contour lines (and in the case of negative potentials by different tones of grey).

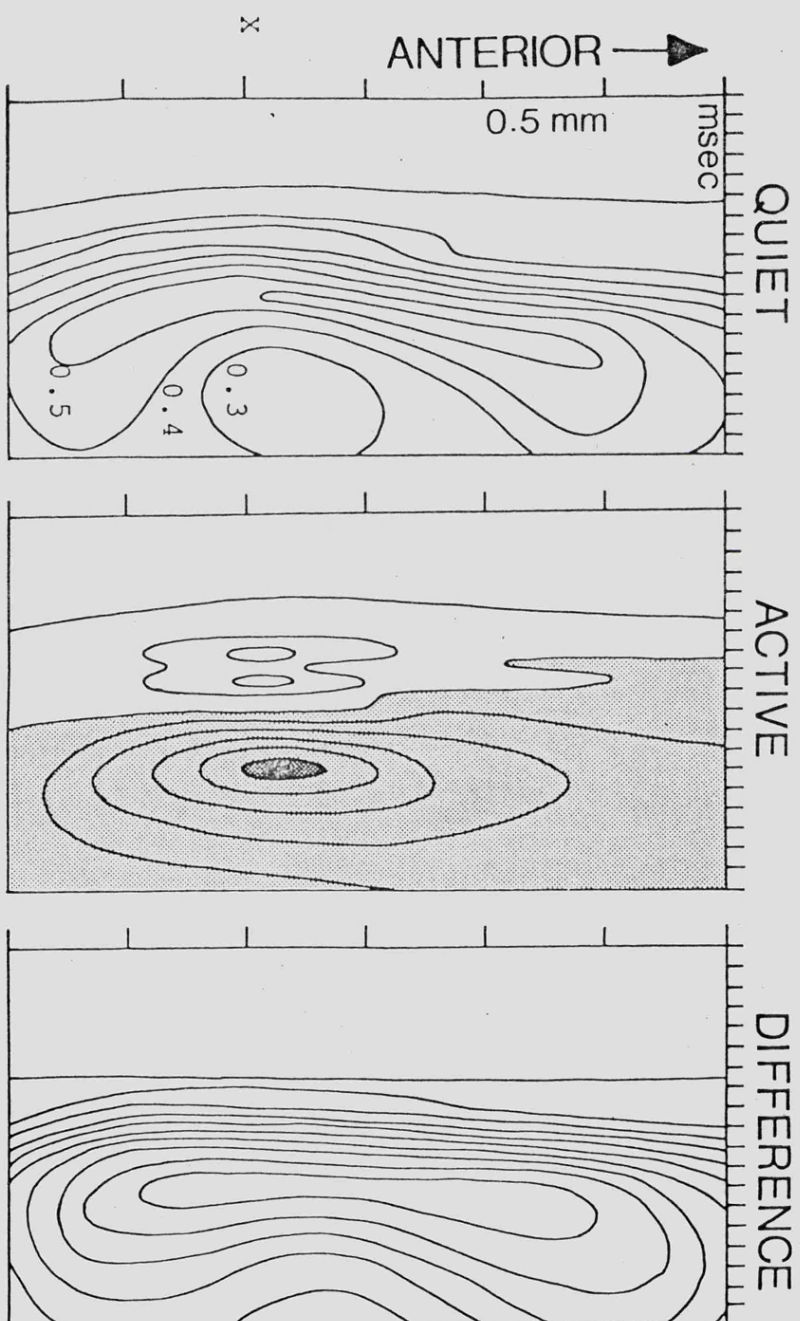


Figure 11. A summary of the responses recorded along an anterior posterior line on the surface of the cortex.

The responses are those from the middle column of figs. 8a and 8b. The 7 recording positions are marked at the left hand side and msec time intervals after the stimulus along the top. Each group of responses was averaged and represented on the contour diagrams by a series of points as shown in fig. 10. Contours at 0.1 mV intervals. Grey tone represents negativity. On the left the 'Q' responses of initially quiescent cortex are represented (from fig. 8a). In the centre the 'A' responses of initially active cortex are represented (from fig. 8b). The right hand 'difference' contour map was calculated by subtracting the averaged 'A' responses from averaged 'Q' responses.

very small in amplitude at the outer edge of the area considered and the necessary balance of equal and opposite potentials at all these positions (in the alternative situation) seemed extremely unlikely. (It will be seen later that unit and mass recordings within the cortical grey matter support this interpretation). Assuming that the 'Q' response is a sum of the 'A' response plus the surface positive 'difference' response, the apparently continuous spread of activity is seen to be misleading. The impression is apparently created by the superposition of distinct (non-spreading) responses because they have different spatial and temporal features.

Nomenclature

The early deflections recorded at any point of the receiving area all had peak latent periods identical to those of waves recorded simultaneously at the centre of the region. It was thus possible to use the following nomenclature: (see for example, fig. 13, averaged surface responses)

R ... early rise, latent period about 4.5 msec;

P1, P2 and P3 ... positive waves with peak at about 7, 8.5 and 10.5 msec respectively after stimulus;

N ... negative wave, peak 12 msec after stimulus;

Pq ... positive wave found only in the response of quiescent cortex, peak at 10.13 msec after stimulus.

With trilene anaesthesia too, all the above components could be recognised, although the responses were approximately 1 msec less than with urethane. The P1, P2, P3 and N waves were localized in the same way as with urethane anaesthetic, i.e. to a small area of diameter approximately 0.5 mm which was usually about 1.0 mm anterior and 5 mm lateral to the bregma.

The P1 component was little changed by different anaesthetic conditions or by background spontaneous activity, whereas the Pq component was suppressed by background activity. The small P2 and P3 waves diminished with increasing depth of anaesthesia but increased slightly with background activity. P1, P2 and P3 waves were up to 0.5 msec earlier during spontaneous activity by comparison with the components of 'Q' responses. These differing effects of anaesthesia and spontaneous activity on the various components as well as the different spatial and temporal features suggest that each of the above labelled components of the surface response reflected different and often independent cortical events.

SUMMARY

These mapping experiments showed that there was a small area of cortex, just anterior to the bregma, where the initial positivity (P1) and the negative wave (N) of the response evoked by forepaw stimulation had maximum amplitude. This area (diameter approximately 0.5 mm) was often obscured by large surface veins. At surrounding points these waves were considerably reduced in amplitude; it is

probable that these smaller responses reflect passive spread of potentials from the central responding area. In contrast, the amplitude of the variable surface positive (Pq) response (seen only in initially quiescent cortex) was maintained up to 1 mm away. At points a further 1 mm away, the early components were not recorded whereas the Pq component remained prominent. It is probable from the results described so far that the Pq component reflects a large response of a widespread area of cortex, i.e. that the surface positive response of initially quiescent cortex reflects not only a localized 'A' type of response like that seen in initially active cortex, but an additional and more widespread cortical response. Thus the response of initially quiescent cortex appears to be a larger and more widespread response than that of initially active cortex. As different components of the evoked response had different spatial distributions, it is probable that recording position, the presence of excess CSF and the presence of large surface veins can all contribute to the variability in the form of surface evoked potentials when one compares results from different experiments.

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POTENTIALS EVOKED FROM DIFFERENT CORTICAL DEPTHS

Microelectrode recordings

Records were made with fine micropipettes filled with 3M NaCl solution. The penetrations were made perpendicular to the cortical surface and records were taken from various depths (down to about 1.7 mm) within the cortical grey matter. At each recording position, about 10 'Q' responses were superimposed on film; the film was then advanced and a group of 'A' responses superimposed in a similar way (see for example figs. 12a and b). Average responses were estimated from the filmed responses. Surface responses were recorded simultaneously to ensure that each group of depth recordings was associated with a typical group of surface responses. The test rectangular voltage pulses of fig. 12c shows that the first 20 msec of the response was undistorted by the capacitance coupled recording. The decay time constant was relatively long (about 1 second).

The main features of gross potentials recorded with microelectrodes in this investigation are illustrated in fig. 13. It shows averages of potentials evoked at various depths in the cortex during two microelectrode penetrations. The two penetrations were made simultaneously; one was in the area of the shortest latent period and the other 1.0 mm anterior to this. At 1.2 mm depth in the cortex, there were negative waves associated with the surface positive waves and a weak depth positivity associated with the surface negative wave. The changes of the surface response associated with spontaneous ECoG activity were reflected by large changes

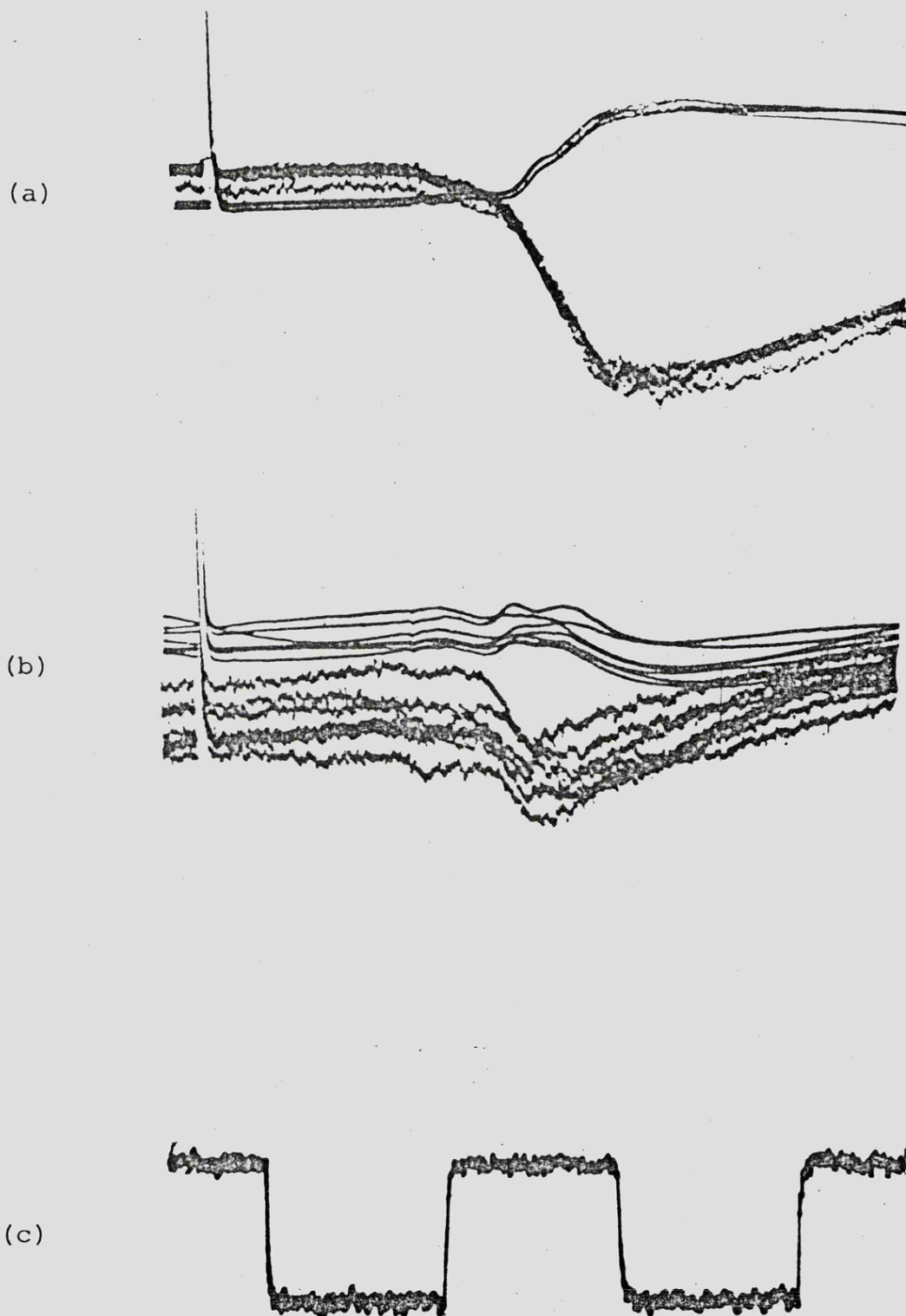


Figure 12. Examples of microelectrode recordings

- a) Superimposed records taken at a depth of 800μ , together with simultaneously recorded surface responses. Stimuli given during quiescent periods in the ECoG.
- b) The same as (a) except that stimuli were given during ECoG activity.
- c) Test square wave: 0.5 mV pulses, 5 msec duration.

in the response recorded deep in the cortex. However in the superficial layers at about 0.3 mm, these changes had almost vanished. The variability of responses recorded deep in the cortex was such that large depth negative waves (about 1 mV in amplitude) were always associated with the 'Pq' surface positive wave; i.e. they only appeared in the response of initially quiescent cortex.

The surface records show the same features as those of figs. 8a and b; i.e. the R, P1, P2, P3 and N components were localized and the Pq component was more widespread. The intense negativity at depths of 0.3-0.4 mm in the area of the shortest latent period appeared to be localised in a similar way to the surface negative wave. This wave at 0.3 mm depth did not appear to be influenced by background spontaneous activity. Small negative waves which mirrored the surface P1, P2 and P3 waves were seen in the deeper layers, and were localized in the same way as those surface waves. The P1 waves were little affected by spontaneous activity, but the P2 and P3 waves were often difficult to identify in the response of quiescent cortex. This may have reflected a real suppression or may have been due partly to distortion caused by the superposition of the large negative wave. The deep negative wave which was seen only in the response of quiescent cortex was much more widespread than any of the other components of the response; it could be found (like the associated surface positive Pq wave) up to about 2mm away from the region of the shortest latent period response.

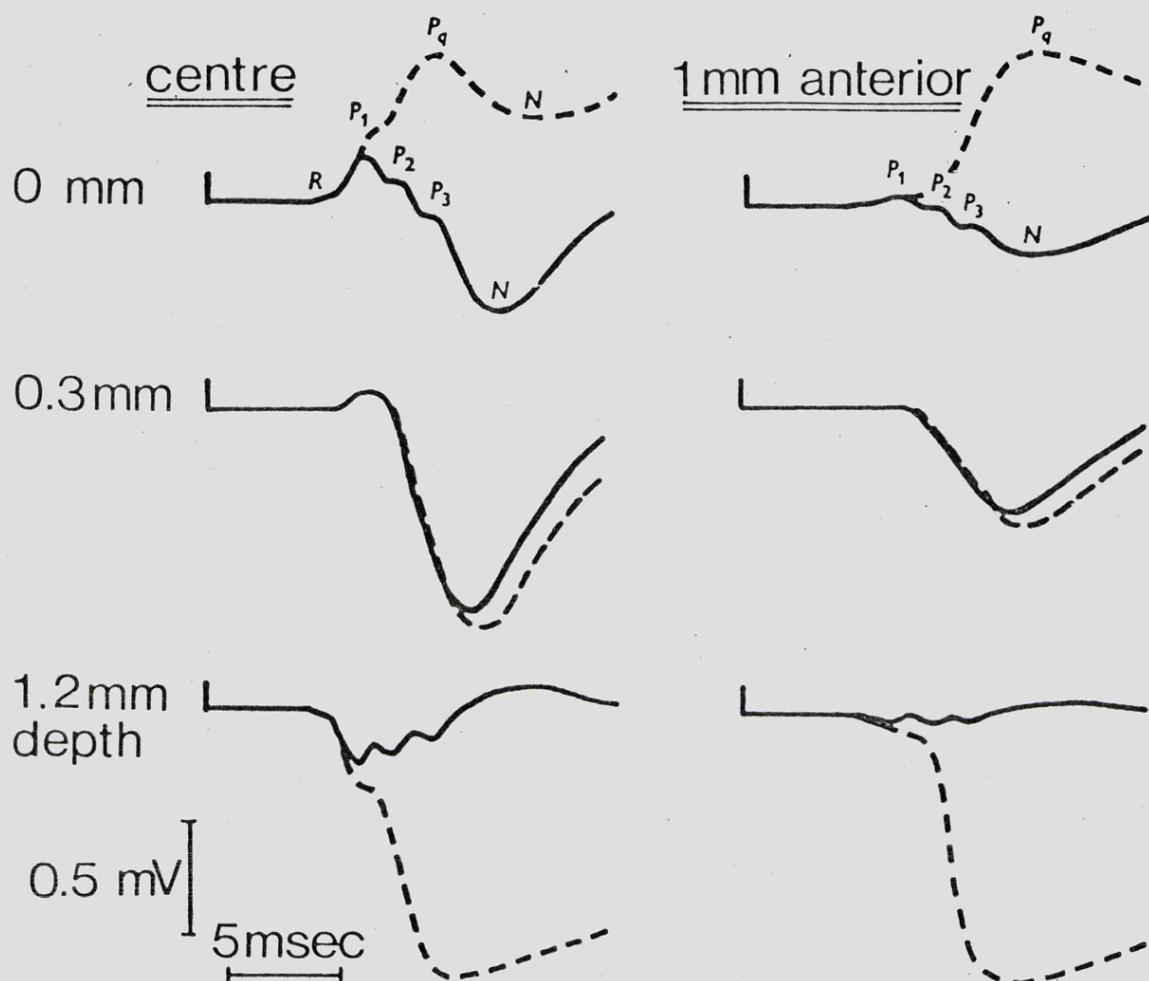


Figure 13. Averaged responses.

Two simultaneous microelectrode penetrations: one in the area of shortest latency and one 1.0 mm anterior. Averages of responses from the surface, 0.3 mm deep and 1.2 mm deep are shown. The continuous lines show 'A' responses of initially active cortex and the broken lines show 'Q' responses of initially quiescent cortex. A.C. recording with one second time constant. (Urethane anaesthesia).

Depth profiles

It was found that every component of the surface response reversed polarity within the cortical grey matter. However the actual depth of reversal was different for the different components.

The largest amplitude waves of the variable component were seen at about 1.2 mm depth. To examine the depth profile and reversal pattern of this component as well as the other more stable components, single penetrations were examined in more detail. Responses were recorded with a penetrating microelectrode at intervals of 50 μ . Simultaneous records were taken from a surface electrode nearby so that the stability of the response could be checked. The 'A' responses of initially active cortex were recorded and averaged separately from the 'Q' responses of initially quiescent cortex. Contour plots (fig. 14) were then constructed in the same way as those in fig. 11. A 'difference' diagram shows the calculated difference Q-A. When triene anaesthesia was used, as in the experiment of fig. 15, each response was recorded separately on a moving film, and the classification of 'A' and 'Q' responses done in retrospect according to the presence or absence of background activity.

The main features of the profiles of figs. 14 and 15 were very similar, suggesting that the first 20 msec of the cortical response to forepaw stimulation was little affected by anaesthesia, although latent periods were

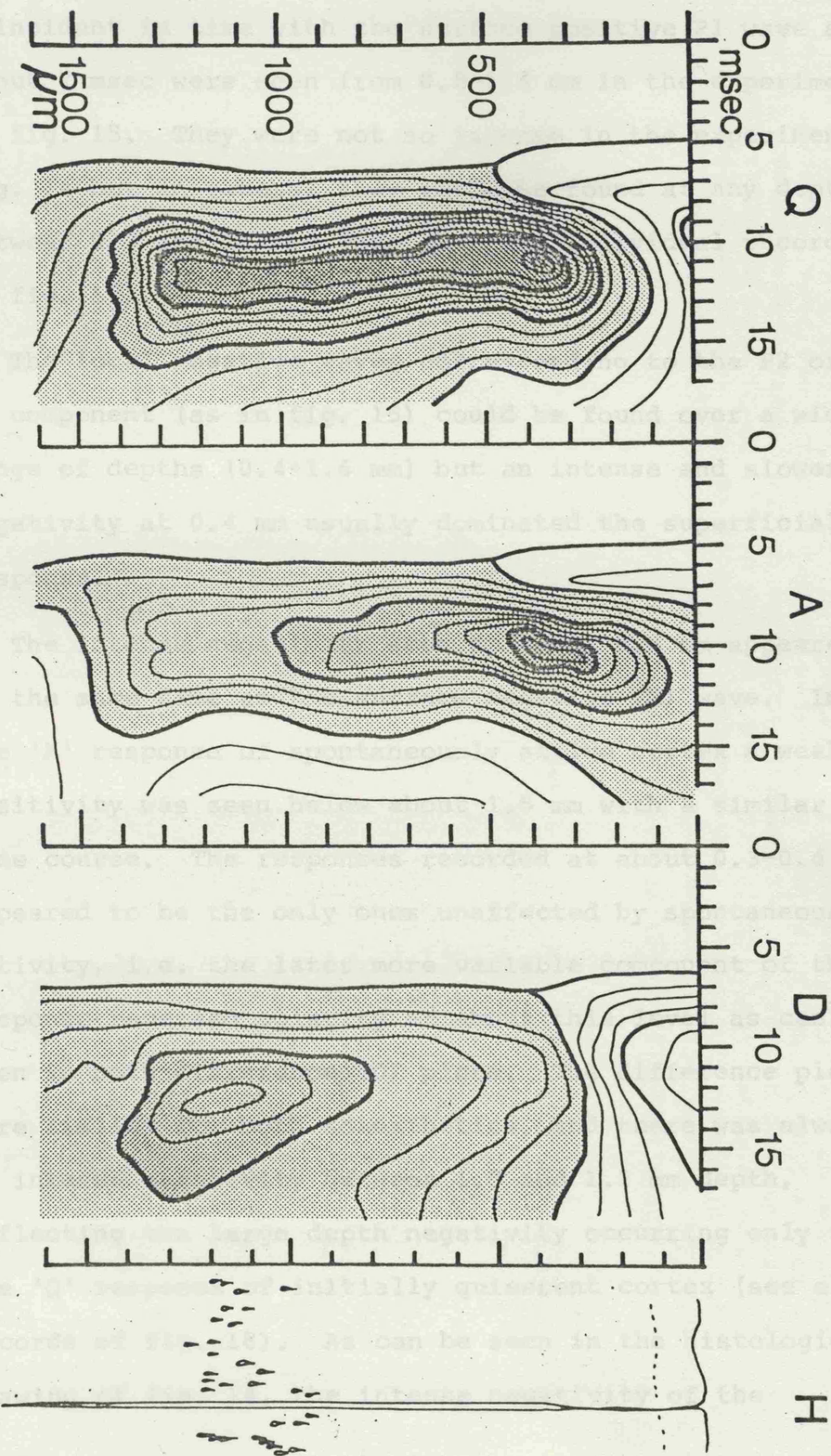


Figure 14. Depth profiles - urethaneanaesthesia.

Depth profiles of cortical responses near the region of shortest latency. Contour plot constructed as in fig. 11 from averaged depth responses. Successive recording positions were 50 μ apart: cortical depth is marked on the left hand side in intervals of 100 μ . The time in msec after the stimulus is marked along the top. Contours at 0.125 mV intervals. Thick contours were drawn at 0.5 mV intervals and grey indicates negativity. A.C. recording with 1 second time constant. Q = 'Q' responses; A = 'A' responses; D = Difference ('Q' responses - 'A' responses); H shows track and position of large pyramidal cells in this experiment (see fig. 39a for histology).

about 1 msec shorter with trilene. The initial surface positivity (P1) reversed polarity at about 0.5 mm. Local negative potentials (or negative potential gradients) coincident in time with the surface positive P1 wave at about 7 msec were seen from 0.8-1.4 mm in the experiment of fig. 15. They were not so intense in the experiment of fig. 14 but in general they could be found at any depth between 0.7 and 1.4 mm (see also the individual records in fig. 17).

The local negative waves corresponding to the P2 or P3 component (as in fig. 15) could be found over a wider range of depths (0.4-1.6 mm) but an intense and slower negativity at 0.4 mm usually dominated the superficial response.

The intense negativity seen at about 0.4 mm appeared at the same time as the surface negative (N) wave. In the 'A' response of spontaneously active cortex a weak positivity was seen below about 1.5 mm with a similar time course. The responses recorded at about 0.3-0.4 mm appeared to be the only ones unaffected by spontaneous activity, i.e. the later more variable component of the response reversed polarity at about this level as can be seen in both difference (D) plots. The difference plots were similar for both anaesthetics, and there was always an intense negativity between 1.0 and 1.2 mm depth, reflecting the large depth negativity occurring only in the 'Q' response of initially quiescent cortex (see also records of fig. 18). As can be seen in the histological drawing of fig. 14, the intense negativity of the

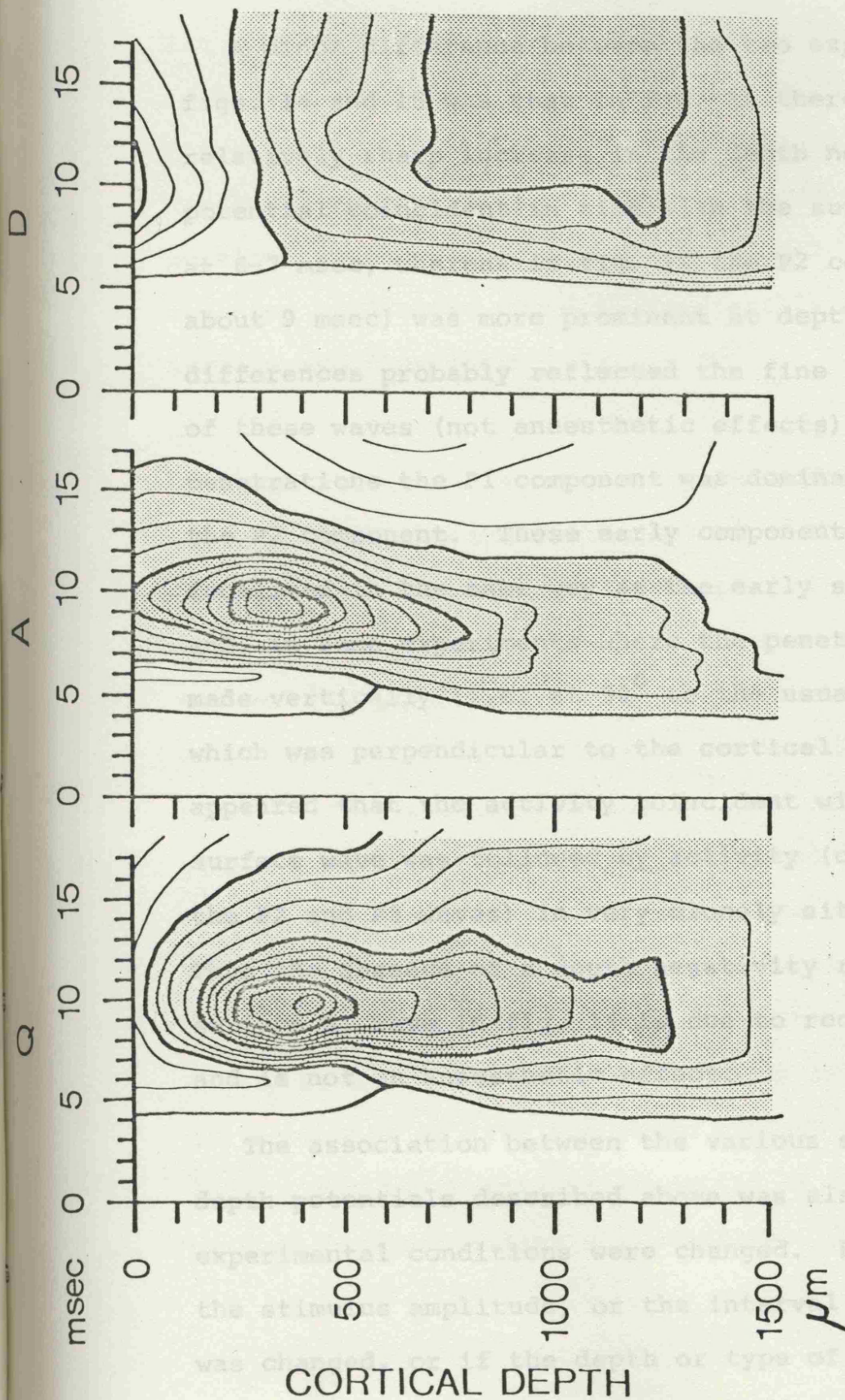


Figure 15. Depth profiles - trilene anaesthesia

Depth profiles of cortical responses in the region of shortest latency. Trilene anaesthesia. Each contour plot was constructed from averages of groups of 'A' and 'Q' type responses. The responses were recorded individually on a moving film with a 20-50 msec stimulus delay and then divided into the two groups 'in retrospect' according to the presence or absence of ECoG activity at the time of the stimulus. Successive recording positions were 50 μ apart: cortical depth is marked on the left hand side in intervals of 100 μ . The time in msec after the stimulus is marked along the top. Contours at 0.125 mV intervals, grey shading indicates negativity. D is the difference plot constructed as in figure 14.

difference (D) plot is found in layer V where large pyramidal cells are located.

A minor difference between the two experiments of figs. 14 and 15 was that in fig. 15 there was a relatively sharp increase in the depth negative potential coincident in time with the surface P1 wave at 6-7 msec, whereas in fig. 14 the P2 component (at about 9 msec) was more prominent at depth. These differences probably reflected the fine localization of these waves (not anaesthetic effects). In some penetrations the P1 component was dominant and in some the P2 component. These early components were always localized in the same way as the early surface waves and, in some experiments where the penetration had been made vertically (i.e. at 30^0 to the usual penetration which was perpendicular to the cortical surface), it appeared that the activity coincident with the P1 surface wave was followed by activity (coincident with the P2 and P3 waves) in very closely situated 'columns'. Thus the absence of a depth negativity reflecting the surface P1 wave in fig. 14 is due to recording position and is not an anaesthetic effect.

The association between the various surface and depth potentials described above was also seen when experimental conditions were changed. For example, if the stimulus amplitude, or the interval between stimuli was changed, or if the depth or type of anaesthetic was changed, then the amplitude and latent period of each

component recorded at depth varied in the same way as the corresponding component in the surface response. (In some experiments deep trilene anaesthesia was followed immediately by urethane anaesthesia). These close relationships between potentials recorded at depth and mirror image potentials recorded on the cortical surface could perhaps be direct relationships; the same generators could be responsible for both surface and deep potentials.

SUMMARY

These observations on gross potentials recorded at various depths in the cortex and at various distances from the area of shortest latent period are compatible with the following hypothesis. In spontaneously active cortex, the response to forepaw stimulation is confined to a small cylinder of elements responsible for the brief surface positive waves, (P1, P2 and P3), together with a localized superficially situated group of elements responsible for the surface negative wave (N). In initially quiescent cortex, these same elements respond to the stimulation in a similar way, but in addition, deeply situated elements over a wider area of cortex are activated to give the large surface positive wave (pq). This hypothesis is supported by evidence from unitary recordings (see next section).

POTENTIALS RECORDED FROM CORTICAL UNITSLocation and form of action potentials

As in the experiments of the previous section all records were made through a capacitance coupled preamplifier and the decay time constant of the complete recording system was about 1 msec. Thus the all-or-none extracellular action potentials were superimposed on local mass potentials in all the records presented here.

Action potentials were recorded at various cortical depths between 200 μ and 1600 μ . The occurrence of such unitary potentials was highly correlated, both in position and timing, with local negative going mass potentials. This was true for spontaneous as well as evoked potentials.

The slower components of the spontaneous ECoG reversed near 400 μ depth and were particularly conspicuous near 1200 μ depth (see fig. 16). Many spontaneously active units were found in the deeper layers of the cortex (see part S of fig. 20a). If they were not injured by the recording electrode they only fired spontaneously during ECoG activity, i.e. only during local negative waves or during surface positive plateaux.

The action potentials recorded in this investigation (with microelectrode resistance in the range 3-10 megohms) were usually initially negative and diphasic.

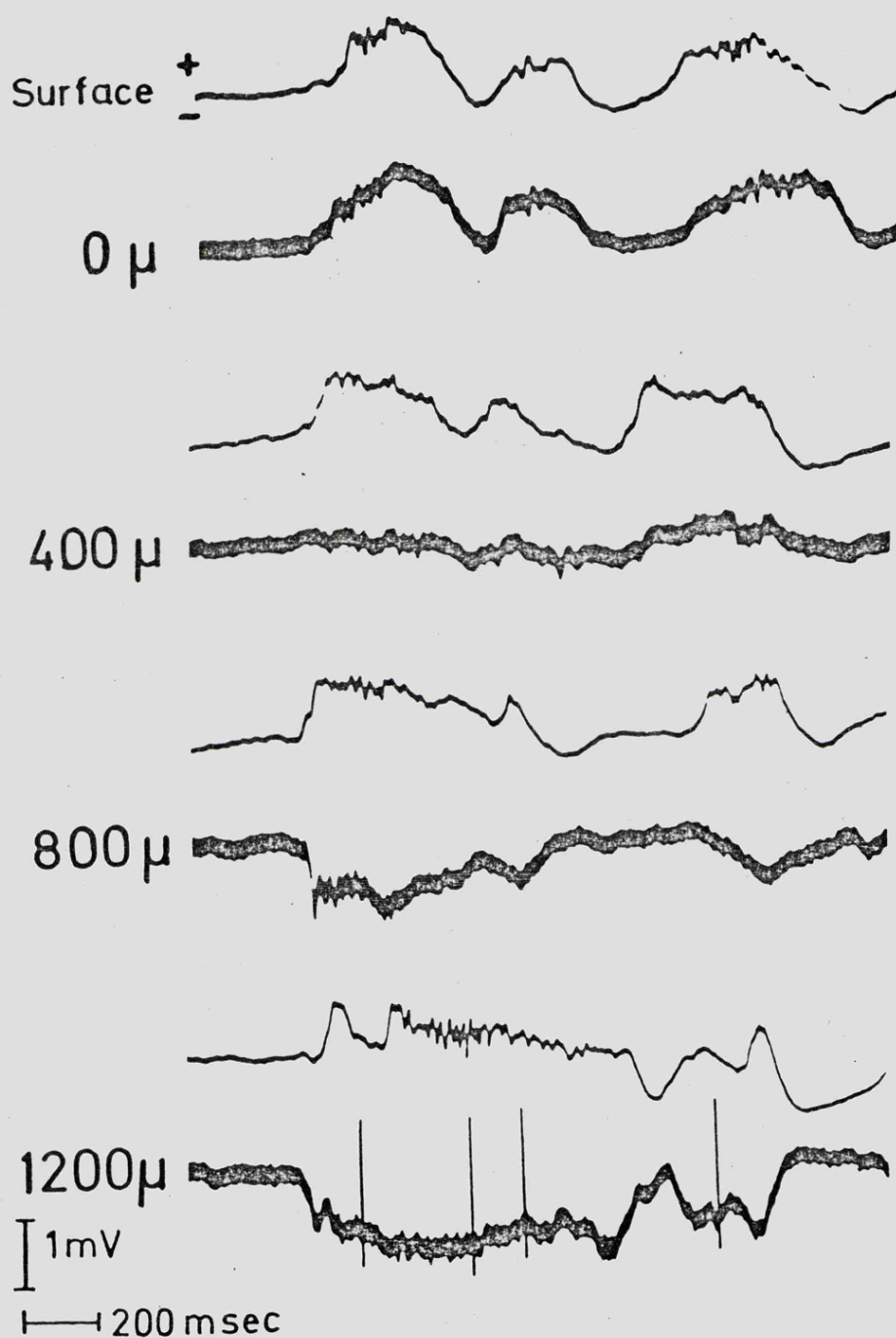


Figure 16. Depth records of spontaneous activity.

Records were taken from the somatosensory cortex of a rat anaesthetized with urethane. Directly-coupled monopolar recordings taken simultaneously from a penetrating microelectrode and a chlorided silver ball surface electrode. Positivity upwards.
(From Creutzfeldt and Houchin (1974)).

The initial negative component had an amplitude of up to 2 mV and a duration of 0.5-1 msec. The after-positivity was slower than the initial negativity and of small amplitude. It was difficult to measure because it was usually superimposed on a large (usually negative going) mass potential. With some units it was often completely hidden and even when it was recognisable it did not have an amplitude of more than one quarter of the initial negative component (see fig. 17b). This slow after-positivity seemed to last for 3 msec or more.

The initially negative diphasic action potentials were usually unaffected by small movements of the micro-electrode and could often be recorded in an attenuated form over a distance of 100-200 μ . In contrast, the other type of action potential which was found less frequently (initially positive, di- or tri-phasic) was less stable. It seemed to be sensitive to small movements of the microelectrode; action potential amplitude (up to 5 mV) and spontaneous firing rate could change considerably. Recordings of initially positive action potentials often ended with an injury discharge (e.g. those of figs. 17a and 19f and g).

Thus, the more stable initially negative type of action potential was considered to be due to the intense currents associated with a soma action potential. The largest amplitude initially negative action potentials (2 mV) were presumably recorded very close to large

pyramidal cells. Since the larger pyramidal cells in rat cortex have a diameter of about 20μ , these 2 mV initially negative action potentials could have been recorded at about 10μ from the centre of such cells. This idea has been used in the theoretical estimation of field potentials around a model neuron in the next section. The initially positive action potentials were presumably recorded when the electrode was touching the soma or proximal dendrites of a neurone, and they were very localized so they were not used in the field potential calculations.

The typical response to electrical stimulation of the contralateral forepaw consisted of a single action potential. However the units which gave initially positive action potentials sometimes gave multiple discharges in response to stimulation. If a unit responded to the stimulus within 17 msec, its latent period of discharge did not vary by more than 1 or 2 msec. All units gave loosely synchronized discharges 50 or more msec after the stimulus but these discharges were apparently reflecting stimulus locked changes in spontaneous activity, which are excluded from consideration here.

The cortical units which responded within the first 17 msec after the stimulus fell into two groups. One group showed stable responses which were little affected by ongoing spontaneous activity; the other group gave

responses which were suppressed by ongoing spontaneous activity.

Stable unitary responses

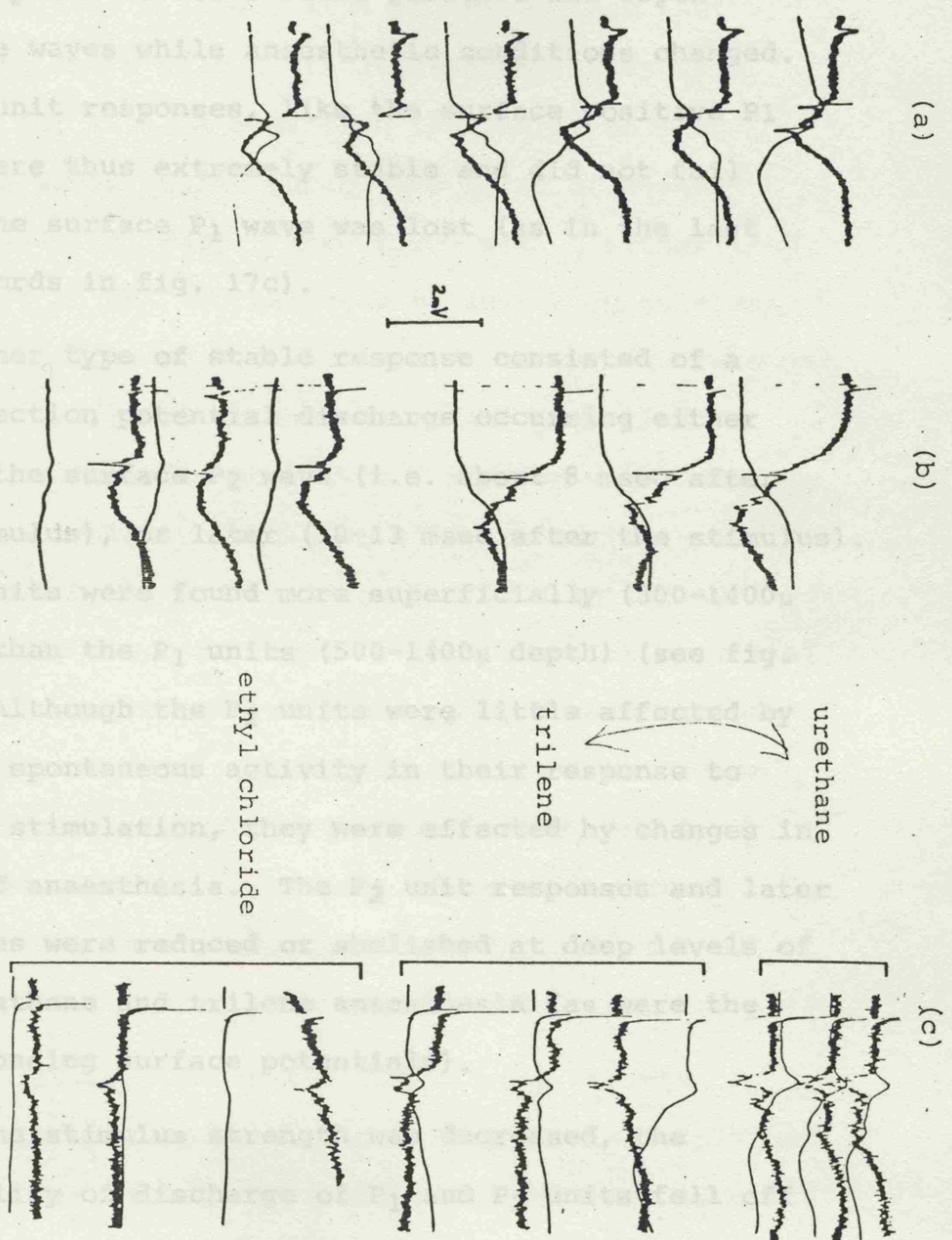
The stable unitary responses were found in the region yielding the earliest evoked surface response. They were apparently localized to an area less than 0.5 mm in extent. They could be found between 300 and 1400 μ deep, but most were found between 500 and 1000 μ .

Many of these units gave their single action potential response during the initial positivity (P_1) of the surface response, i.e. with a delay of 6-7.5 msec (see figs. 17 and 20a). The stability of these " P_1 " unit responses was such that the probability of response to a stimulus was 80-100%. These P_1 cells fired about 0.5 msec earlier if the stimulus was given during ECoG activity than if it was given during ECoG quiescence. Thus the timing of the P_1 cell discharge varied in a similar way to that of the simultaneously recorded surface P_1 wave (see fig. 17a). In some cells the action potential responses seemed to have smaller amplitude during 'A' responses than during 'Q' responses (compare upper and lower groups of records in fig. 17b).

In a few experiments it was possible to change from trilene anaesthesia to urethane anaesthesia while recording a particular type of response. It was found that the P_1 waves and P_1 unit responses were little

Figure 17. P₁ unit responses and simultaneously recorded surface responses.

- a) A unit at 0.6 mm depth, stimuli 1 per 4 seconds (urethane anaesthesia).
- b) A unit at 1.2 mm. Above, stimuli given only during cortical quiescence, below only during cortical activity (urethane anaesthesia).
- c) A unit at 1.3 mm depth, stimuli 1 per 4 seconds (anaesthesia changed from trilene to urethane to an overdose of ethyl chloride; gaps of 30 & 10 mins. Voltage calibration 2 mv for (a) and (b) and 1 mv for (c). Sweep duration 20, 18 and 21 msec in (a), (b) and (c).



affected by the change of anaesthetic conditions. However the latent period of the P_1 unit response was always increased by about 1 msec with urethane. Fig. 17c shows the constant relationship of a P_1 unit response to the surface positive and depth negative waves while anaesthetic conditions changed. The P_1 unit responses, like the surface positive P_1 wave, were thus extremely stable and did not fail until the surface P_1 wave was lost (as in the last two records in fig. 17c).

Another type of stable response consisted of a single action potential discharge occurring either during the surface P_2 wave (i.e. about 8 msec after the stimulus), or later (10-13 msec after the stimulus). These units were found more superficially (300-1400 μ depth) than the P_1 units (500-1400 μ depth) (see fig. 20a). Although the P_2 units were little affected by ongoing spontaneous activity in their response to forepaw stimulation, they were affected by changes in depth of anaesthesia. The P_2 unit responses and later responses were reduced or abolished at deep levels of both urethane and trilene anaesthesia (as were the corresponding surface potentials).

If the stimulus strength was decreased, the probability of discharge of P_1 and P_2 units fell off with the amplitude of the surface P_1 and P_2 waves. The threshold of P_1 units was about one third of that

of the later responding units.

Unit responses suppressed by background activity

These responses (again single action potentials) were found over a larger area of cortex than that containing units with a stable response; they were found as far as 1 mm away from the centre of the receiving area. These units were found at depths between 400 and 1600 μ . They fired with a probability of 65-100% in the 'Q' response of initially quiescent cortex at delays of 8.5-16.5 msec. In the 'A' response of initially active cortex the action potential response was usually abolished. However with some units (particularly at higher stimulus strengths) there was a 5-20% probability of discharge during the 'A' response. In these cells the action potential response (when it occurred) was up to 2 msec earlier in the 'A' response than in the 'Q' response. Thus the occurrence of these unit responses was highly correlated with the occurrence of local negative waves as well as that of the surface positive P_q wave (see fig. 18a). They will be referred to as ' P_q ' units.

The quiet periods in the ECoG during trilene anaesthesia were so brief and infrequent (as explained earlier) that it was not possible to identify P_q units during these trilene experiments. (For example if stimuli were given once every 4 seconds for 12 minutes,

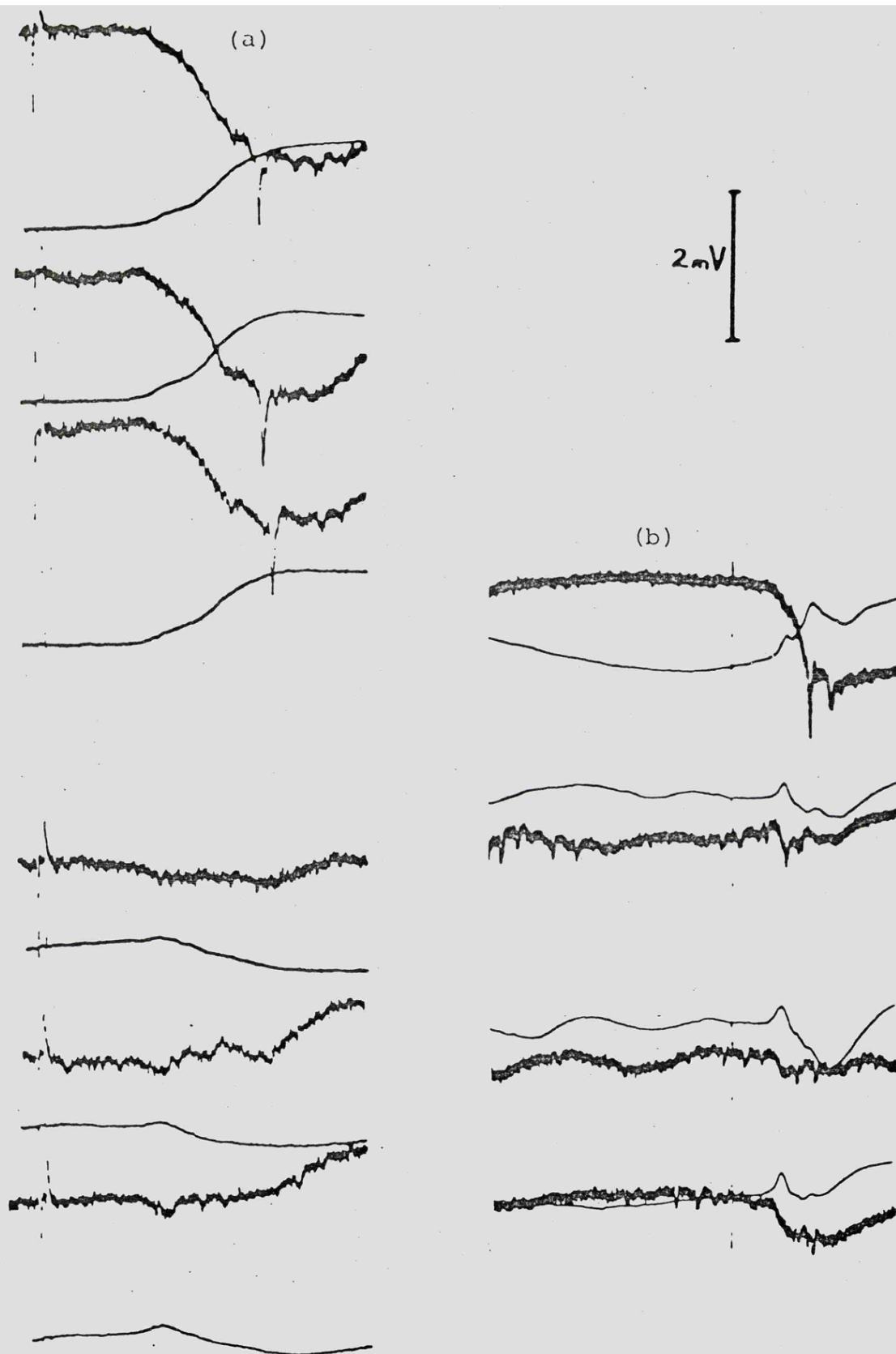


Figure 18. Mass and unitary responses (P_q units)

- a) A unit at 1.3 mm depth, the top 3 records show responses to stimuli given during cortical quiescence, and in the bottom 3 stimuli were given during cortical activity. (Urethane anaesthesia).
- b) A unit at 1.25 mm depth, consecutive responses to stimuli given 4 seconds apart. (Trilene anaesthesia).

there would be only about 9 stimuli given during quiescent periods in the ECoG). It was therefore not possible to follow P_Q unit behaviour while changing from trilene to urethane anaesthesia.

However a few P_Q units were found by chance after inspection of the films from trilene anaesthesia experiments. Such a unit is shown in fig. 18b.

The large action potential in the top record was found to occur each time the stimulus was given during a brief quiescent period (i.e. each time there was a surface positive response). In this experiment the stimulus was given at 4 second intervals for 15 minutes. During this time there were only 10 'Q' responses (each with the unit discharge), whereas there were about 200 'A' responses (without the unit discharge) during the same period.

Most P_Q units were found between 950 and 1350 μ depth. It is therefore probable that many were pyramidal cells. It was occasionally possible to identify them histologically if they had been badly injured by the recording electrode. The electrode was withdrawn a small distance and the cell allowed to degenerate for a few hours before the animal was finally killed. Recording was continued to check that there were no further injury discharges. Three P_Q cells were identified in this way; all were

pyramidal cells. (See for example fig. 39b in Appendix IV(a) which shows the punctured cell after the injury of the unit shown in fig. 19f and g).

Responses to paired stimuli

If paired stimuli were given with a separation of 100 msec or more, little or no refractoriness was seen. The P_1 and P_2 unit responses to the second stimulus (like the corresponding components of the evoked potential) were like those to the first stimulus. However the form of the later part of the response to the second stimulus was often different from that to the first because it was related to the induced presence or absence of cortical activity. The P_q unit responses to both the first and second stimuli of each pair were abolished by ongoing cortical activity, spontaneous or induced, and were again associated with a local negative wave and a simultaneously recorded surface positive wave (see fig. 19b to e).

In fig. 19f and g the two stimuli of each pair were only 80 msec apart. Although the second stimulus was apparently given during a relatively refractory period (slower time course of mass potentials), the P_q unit response to the second response in (g) (although delayed) was still associated with local negativity and surface positivity like that to the first stimulus in (f).

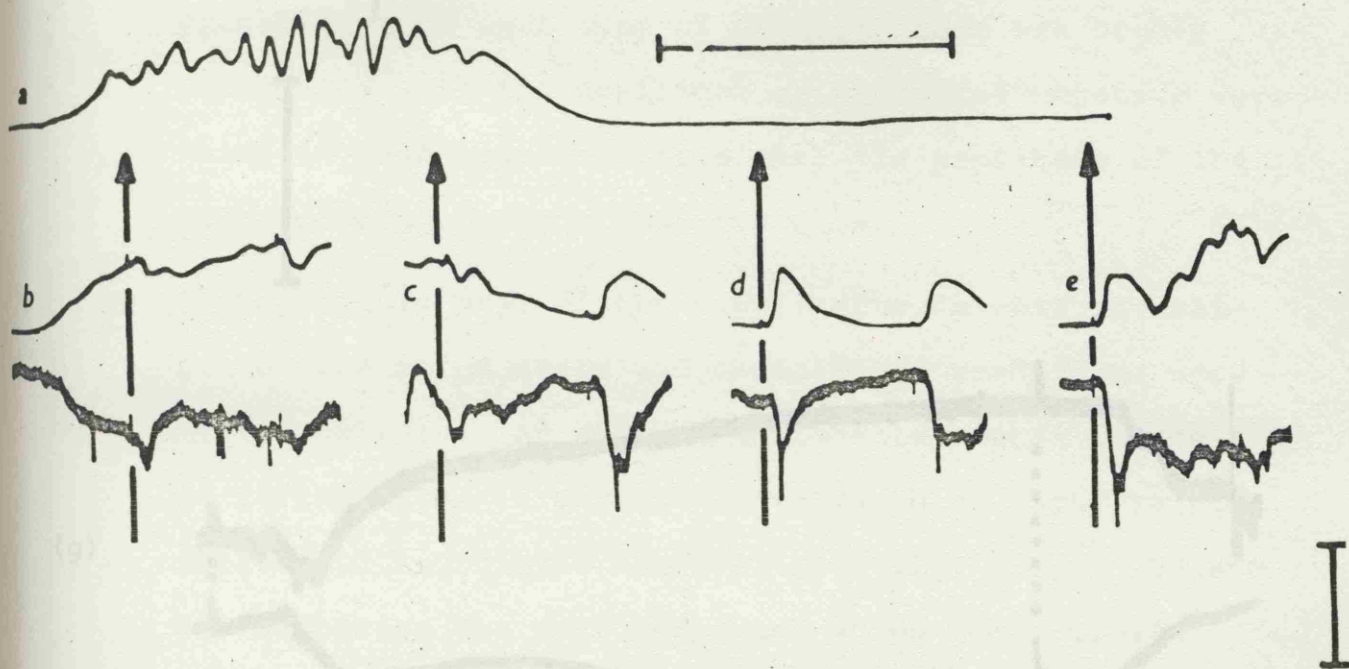


Figure 19. P_Q unit responses to paired stimuli

a) Directly coupled surface record of a burst of spontaneous activity.

b) to e) Simultaneous records from the cortical surface (upper trace directly coupled recording) and at a cortical depth of 1.2 mm (lower trace capacitance coupled, time constant 2 sec).

The pairs of stimuli were given 100 msec apart at various times during active and quiescent periods. Time calibration 200 msec. Voltage calibration 2mV. (From Houchin, 1969).

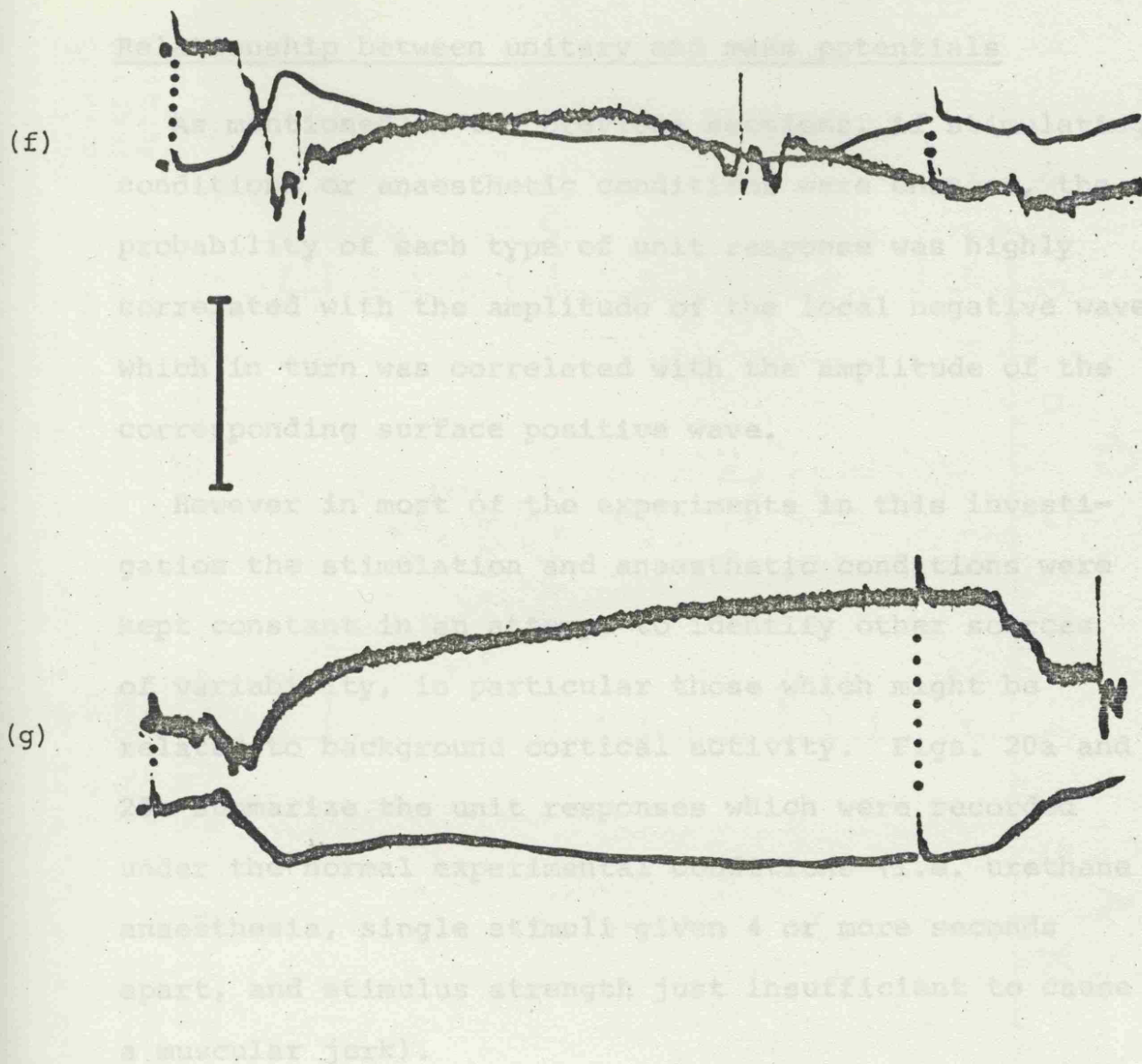


Figure 19 (continued). P_q unit responses to paired stimuli.

(f) and (g) records taken at 1.25 mm depth. Pairs of stimuli given 80 msec apart. This unit recording ended with an injury discharge and subsequent histology revealed a punctured pyramidal cell (see fig. 39b). (Urethane anaesthesia). Voltage calibration, 1 mV.

Relationship between unitary and mass potentials

As mentioned in the previous sections, if stimulating conditions or anaesthetic conditions were changed, the probability of each type of unit response was highly correlated with the amplitude of the local negative wave which in turn was correlated with the amplitude of the corresponding surface positive wave.

However in most of the experiments in this investigation the stimulation and anaesthetic conditions were kept constant in an attempt to identify other sources of variability, in particular those which might be related to background cortical activity. Figs. 20a and 20b summarize the unit responses which were recorded under the normal experimental conditions (i.e. urethane anaesthesia, single stimuli given 4 or more seconds apart, and stimulus strength just insufficient to cause a muscular jerk).

The stable responses (dots in fig.20) of P_1 units (approx. 6.5 msec delay) and of P_2 units (approx. 8 msec delay) are seen in both the 'A' response of initially active cortex and the 'Q' response of initially quiescent cortex. These stable responses are found predominantly in the middle layers of the cortex. Most of the unitary responses which follow are abolished or reduced by spontaneous cortical activity and are found in the deeper layers of the cortex. These P_q unit responses are represented by crosses in the 'Q' response diagram and in the 'D' difference diagram.

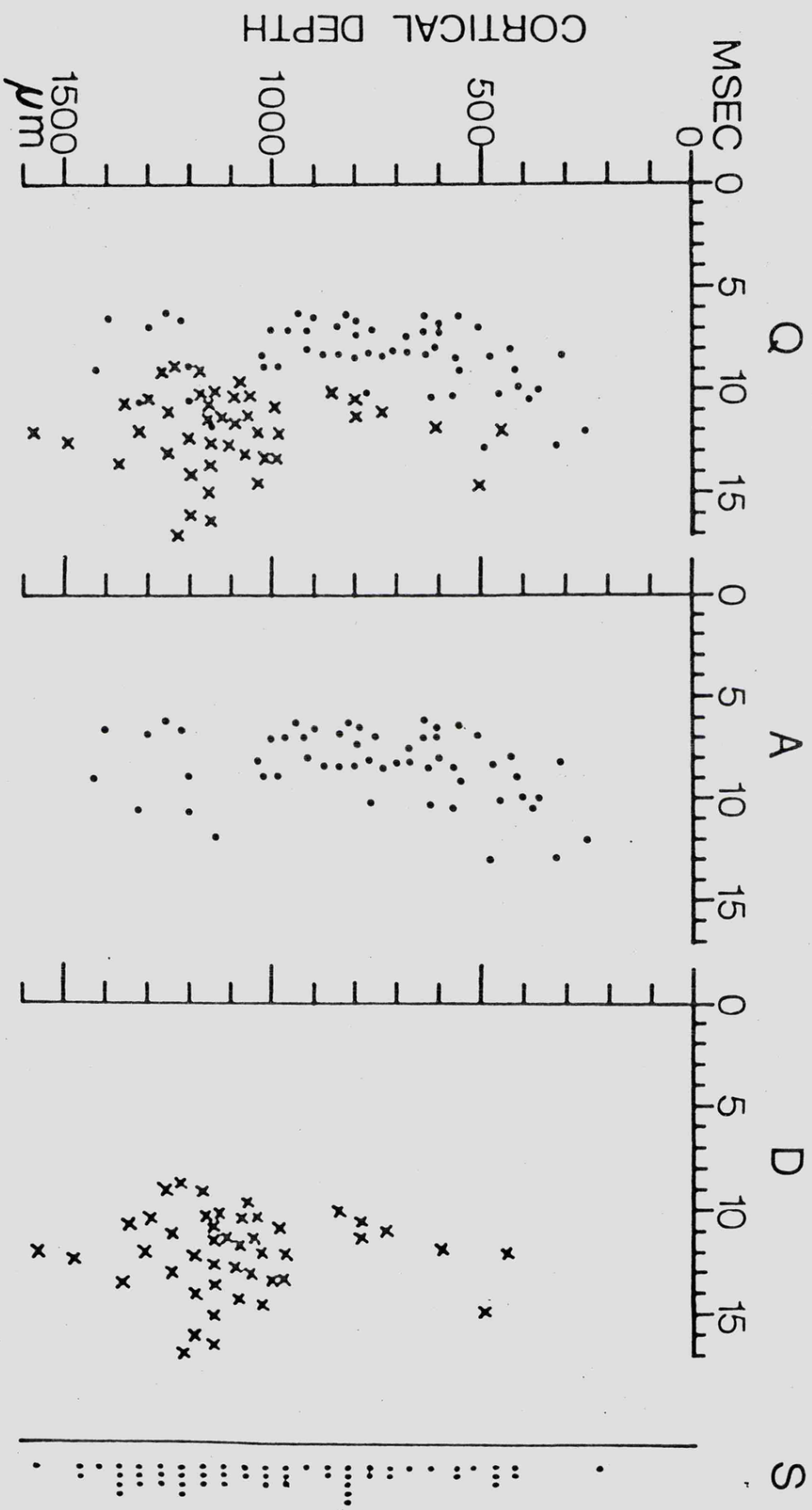


Figure 20a. Summary of unitary responses. Q = Q response, A = A response, D = difference (Q-A). The mean latency of discharge for each unit is plotted against cortical depth. Dots indicate stable unitary responses which were relatively unaffected by ECoG activity. Crosses indicate units whose discharge was suppressed by ongoing ECoG activity. The main difference (D) between the responses of quiescent (Q) and active (A) cortex is the presence in 'Q' of these later discharges. The depth distribution of spontaneously active units which were not excited within 20 msec of the stimulus is indicated under (S).

The P_1 (and P_2) unit responses were seen either just before or with the peak of the P_1 (and P_2) surface waves and were apparently well synchronized. The scatter of latent period for each group was not more than 1 msec. Some of the scatter may be due to variations in the timing of the P_1 wave (i.e. the whole P_1 component) from one experiment to another. A scatter of 0.5-1 msec might be expected with changes of anaesthetic or stimulating conditions from one experiment to another.

Fig. 20b shows the relationship between the occurrence of unit responses in fig. 20a and the main features of the depth profile of mass responses shown in fig. 14. It can be seen how both the stable unitary responses (in A) and the P_Q unit responses (in D) are highly correlated with local negative potentials in the corresponding component of the mass response. The unit responses were often superimposed on the leading edge of the local negative wave. However some negative potentials always appeared before (or even without) unit discharges. (See for example the first 2 msec of the 'D' component and the most superficial part of the 'A' response. Such potentials are therefore more likely to reflect subthreshold synaptic currents than currents of all-or-none action potentials.)

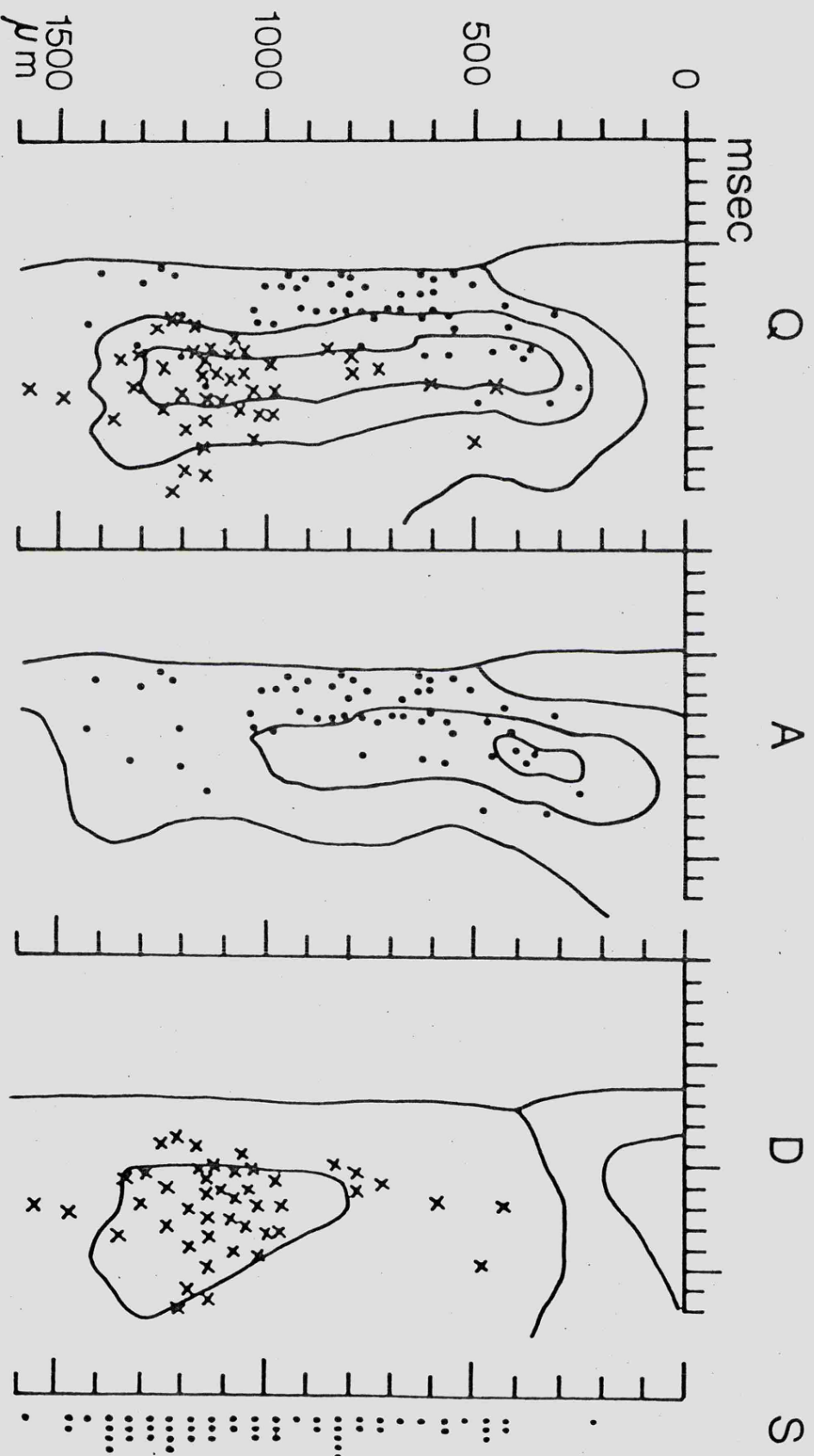


Figure 20b. Relationship between unitary responses and mass responses.

The 0.5 mV contour lines* are drawn onto the plot of figure 20a. Note that the unitary discharges are highly correlated with local negativity, particularly the leading edge of local negative waves.

Summary

The probability^{of} occurrence of each type of unit response (in cortical depth, in time and in tangential position) was highly correlated with the occurrence of local negative waves, which in turn was correlated with the occurrence of surface positive waves. This correlation was seen even if stimulating conditions or anaesthetic conditions were changed and even if there was interaction of spontaneous and evoked activity. It is probable that many of the units whose response was suppressed by spontaneous activity were pyramidal cells.

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A MODEL PYRAMIDAL CELL IN A VOLUME CONDUCTOR:

THEORETICAL FIELD POTENTIALS

Details of the theory are given in Appendix I.
The main results are outlined here.

Spatial distribution of electric fields

In fig. 21~~7~~, theoretical extracellular fields generated by depolarization of different parts of a model pyramidal cell are shown. It was assumed that spread into the apical dendrite was electrotonic (non-propagated) and that there was an insulating oil boundary at the surface.* The extracellular potential field was calculated using volume conductor theory (Plonsey, 1964), and the current field was constructed from "Stokes' stream function" of fluid mechanics (see Rutherford, 1959). (The flow of electric current in a 3D volume conductor is mathematically equivalent to the 3D flow of incompressible fluid.) The length constant of the apical dendrite was 200μ , and that of the axon was $\sqrt{2} \times 100\mu$. In this figure the spatial distribution of the electric field was calculated for exponentially distributed current sources. The fields shown can therefore ^(see page 117) be considered as the average over a time t of the field generated by a short pulse of depolarizing current starting at time zero, where t is longer than the time constant of the membrane (say 8 msec for a cortical neurone).

* The volume conductor was taken to be uniform.

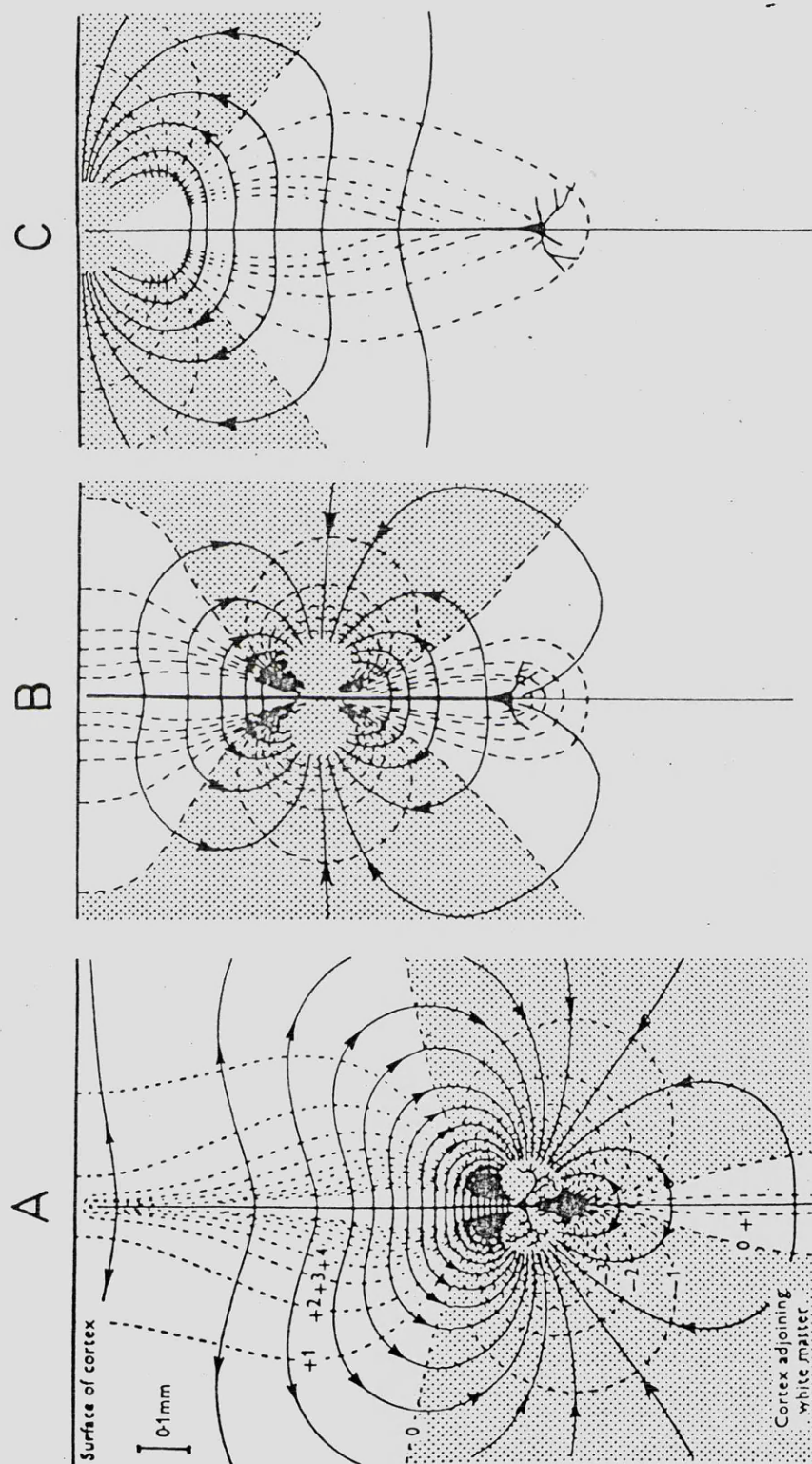


Figure 21a. Theoretical extracellular fields generated by depolarization of different parts of a pyramidal cell

- A. Depolarization of somatic region
- B. Depolarization of middle region of the apical dendrite
- C. Depolarization of the distal end of the apical dendrite

A from Holmes and Houchin (1967), B and C from Creutzfeldt and Houchin (1974).

Solid lines are current flow lines; arrows indicate the direction of flow of positive charges. The density of flow lines indicates the density of current. Broken lines are voltage contours numbered on an arbitrary but linear scale with reference to the potential at a distant point. For clarity the most intense parts of the electric field have been omitted. Shading indicates negative potential. See Appendix I for details.

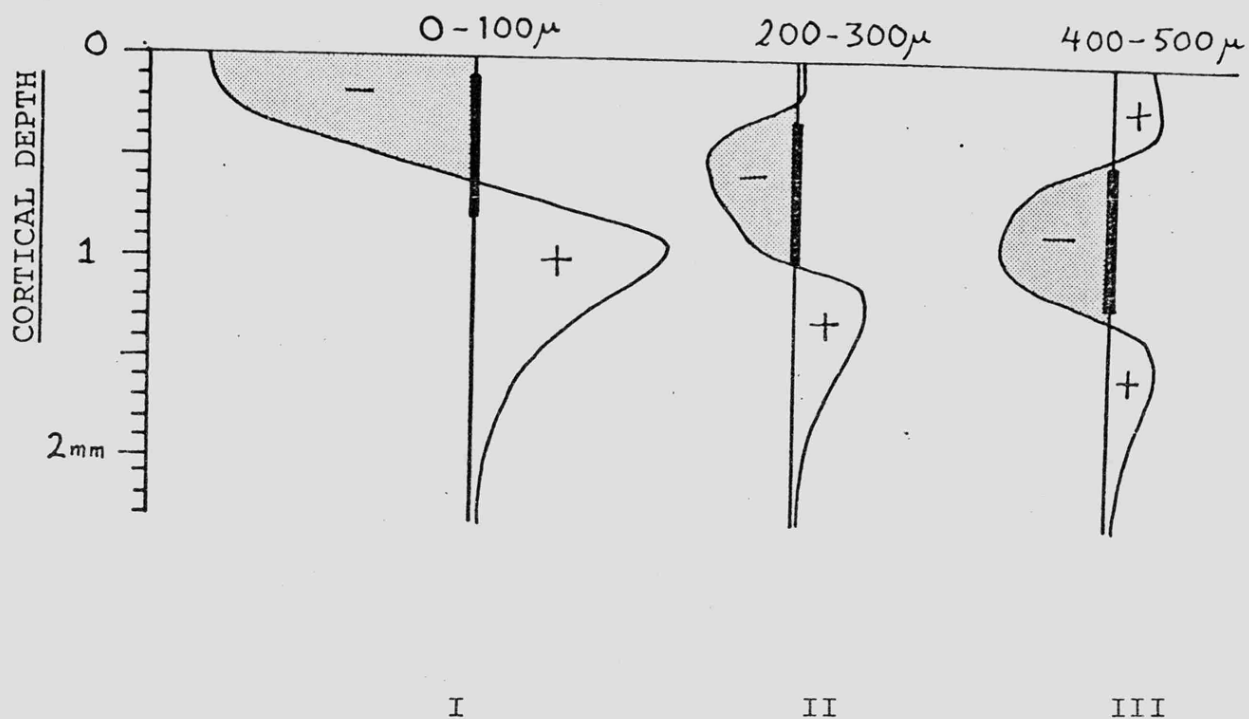


Figure 21b. Theoretical depth profiles associated with synaptic depolarisation of upper regions of apical dendrites.

A total of 120 apical dendrites were evenly distributed across a cylinder of 1000 μ diameter. The tips of the apical dendrites were evenly distributed between depths of 100 μ and 700 μ . Each apical dendrite was assumed to be a long closed cylinder of length constant 200 μ . Voltages are plotted on an arbitrary linear scale.

In (I) the synaptic input was assumed to arrive on the first 100 μ at the end of each apical dendrite. The active current sinks were evenly distributed over the 700 μ range of depths shown by the black bar.

In (II) the synaptic input was located 200-300 μ from the tip of the apical dendrite and in (III) 400-500 μ from the tip.

Intermediate cases (between those of B and C in figure 21a) are considered next. Figure 21b shows depth profiles of population potentials (again averaged over time) generated by depolarising synaptic currents in superficial parts of 120 apical dendrites. These dendrites were evenly spaced across a 1000μ diameter cylinder. The central core of cortical tissue, of 150μ diameter which surrounded the hypothetical electrode track, was free of active neurones so that local effects were eliminated. The closed tips of the dendrites were evenly spaced between 100μ and 800μ depths. The dendritic length constant was again 0.2 mm . In (I) only the first 100μ of each dendrite was depolarised, in (II) and (III) the regions between 200 and 300μ and between 400 and 500μ from the tip were depolarised. These profiles were found to be closely related to the three different membrane current distributions along the individual dendrites. Depolarisation within one length constant of the tip of the dendrite gives large surface negative and depth positive potentials (see I). Depolarisation well beyond one length constant gives a trimodal, surface positive, profile (see III). Depolarisation just more than one length constant from the tip has very little effect on surface potentials; with activity at this critical distance the membrane current distribution resembles that of an infinite cable which is folded back on itself (see equation (16) on page 126) to produce maximum cancellation effects in distant field potentials, particularly those at the cortical surface (see II).

The spatial distribution of the potential field changes continuously with time. Conversely the polarity and time course of potential changes depend critically on the position in the volume conductor.

Magnitude and time course of potential changes

The magnitude of a potential change at any particular point in the uniform volume conductor is directly proportional to the magnitude of the generating current producing that change. In particular, the potential change at a distant point has a definite relationship to the potential change near the active site (even if the specific resistance of the conducting medium is unknown). It is therefore possible to estimate absolute magnitudes of potential changes at distant points using the potential change at the active site as a boundary condition. The only information that is required is the position of the two points relative to the neurone in terms of length constant of the apical dendrite, and the time course of current flow (or the time course of the extracellular potential change near the active site as used here) in terms of the membrane time constant.

The specific resistance of intracellular fluid and of extracellular space, and the membrane resistance and capacitance are not required directly although rough estimates are needed to find appropriate length constants or time constants. In Figs. 22, 23 and 24 the length constant of the apical dendrite and axon were taken to be

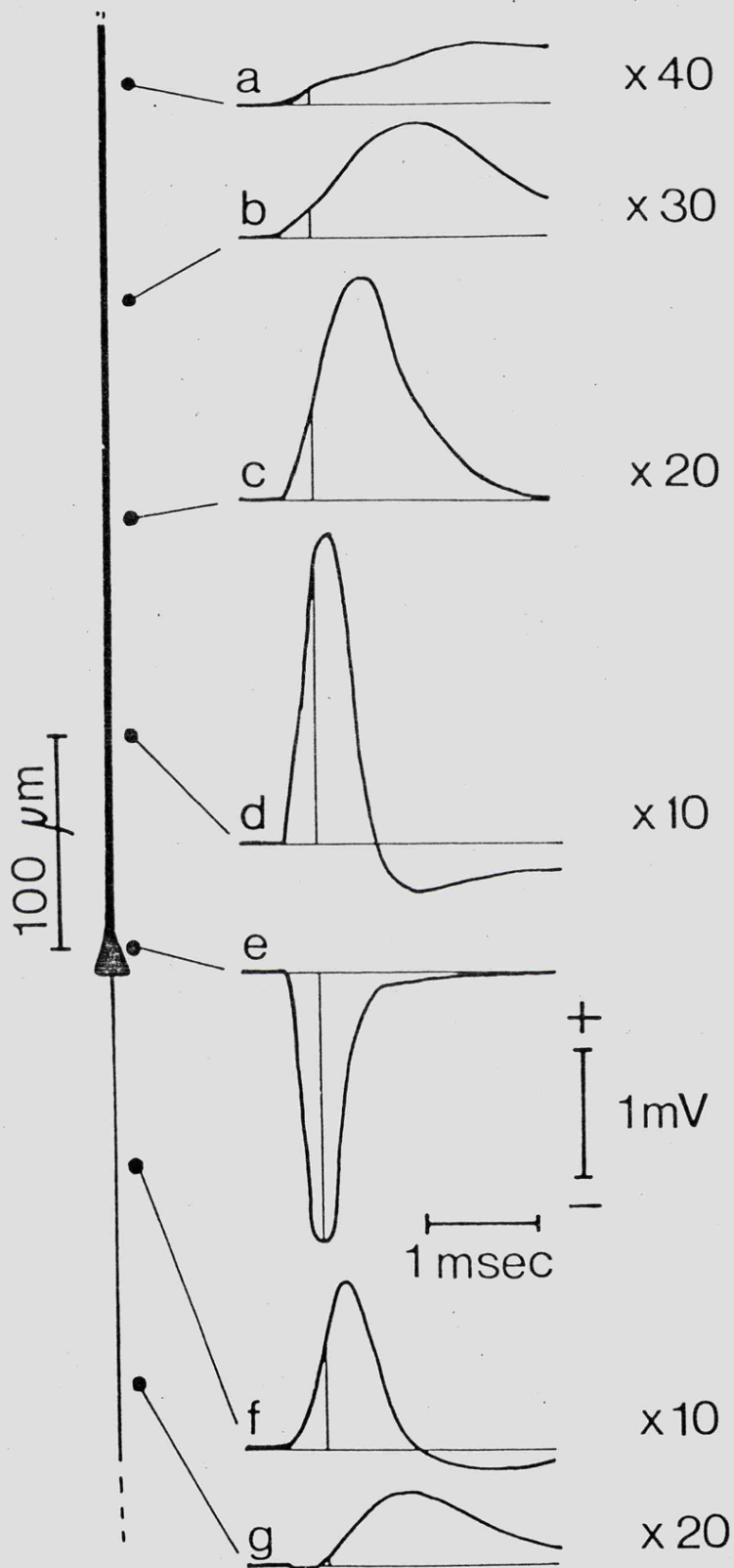


Figure 22. Calculated extracellular potentials associated with a monophasic current across the soma membrane of a pyramidal cell. Length and time constants of the apical dendrite 200μ and 8 msec respectively. All points considered here were 10μ distant from the axis of symmetry. The thin vertical line indicates the time of peak current flow at the soma. Voltage calibration applies directly to (e), the waveform near the active site, and the other waveforms were amplified by the factors indicated on the right. (See Appendix I for details).

200 μ and $\sqrt{2} \times 100\mu$ respectively and the membrane time constant was taken to be 8 msec (see Appendix I for details). It was assumed that current spread away from the soma was electrotonic (non-propagated); the axon action potential was ignored. The membrane current resulting from a pulse of current was calculated from the Hodgkin Rushton (1946) cable equations. Only the apical dendrite and axon were considered in this model. It was assumed that the external resistance was effectively zero ^{in the calculation of membrane current}. The mean extracellular potential field was then calculated for successive 0.2 msec time intervals. The resulting waveform was smoothed by hand as shown in fig. 24a.

Fig. 22 shows extracellular potentials associated with a monophasic generating current across the soma membrane of a pyramidal cell. The generating current (depolarizing) lasted 0.6 msec and had a time course as the initial component of I_m shown in Fig. 24a. The amplitude of the generating current was such that there was a 2 mV change close to the source. Fig. 22 illustrates that although the generating current was monophasic, diphasic potentials could be recorded at neighbouring points. It also shows how the potentials at distant points reached their maximum amplitude considerably later than the time of peak current flow (see the thin vertical line).

Fig. 23 is similar to fig. 22 but here the depolarizing current was followed immediately by a hyper-

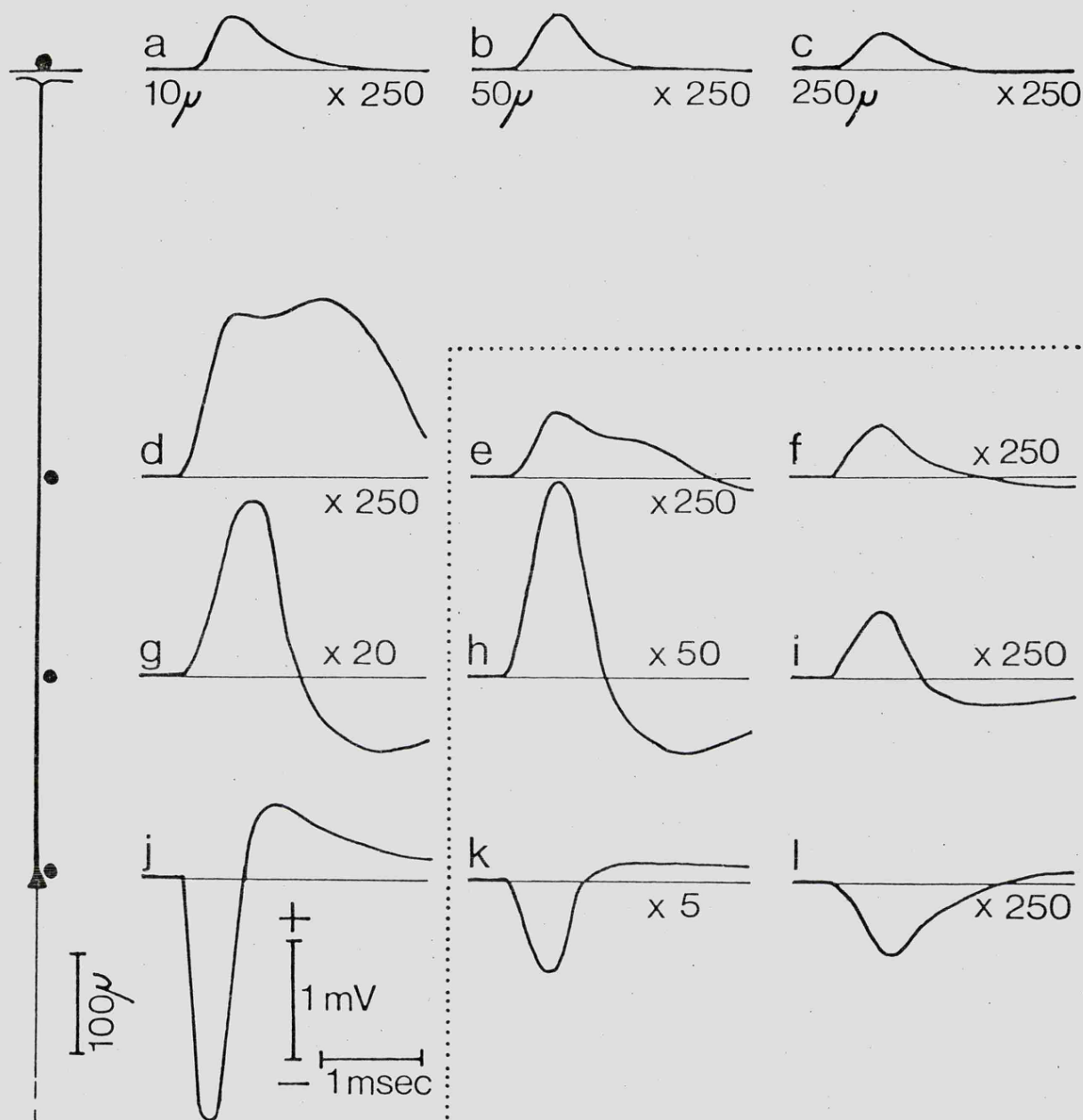


Figure 23. Calculated field potentials - distant from the active site, at which a biphasic current flowed.

Length constant and time constant as in fig.22. Points a, b and c are on the surface of the cortex distances 10μ , 50μ and 250μ away from the axis of symmetry. Points d, e and f are also 10μ , 50μ 250μ distant but are only 400μ above the level of the soma. The other points are spaced likewise as indicated. The area enclosed by the dotted line is compared later with similar plots of Humphrey (1968). The nett active generating current across the soma was zero during the time considered here.

polarizing current so that the nett generating current, during the 2.4 msec period considered, was zero.

The hyperpolarizing current tended to oppose the effects of the initial depolarizing current, i.e. to decrease the amplitude of positive potentials 400μ or more above the soma. However, because it was later and of slower time course than the depolarizing current, the effects of the depolarizing current dominated the first 2.4 msec of the potentials seen at 400μ or more away. Thus in spite of the biphasic generating current, the surface potentials were practically monophasic positive in the time considered. Since the nett generating current was zero and summation was linear, there would have been a long and slow negative component later in the surface potential to bring its integral over time to zero. However, the scatter of time of the experimentally recorded responses was only 1 msec for each of the P_1 and P_2 components and only about 5 msec for the P_q response. Therefore one might expect good summation effects from these surface potentials to give population or mass potentials.

Summation to give population potentials

Potentials such as those of fig. 23 were added together linearly to make estimates of certain population potentials.

First, discs of cells of various diameters from 150μ to 1000μ were considered. The cells were uniformly

distributed over each disc so that they were separated by 50μ or more. The potentials were calculated at 50μ intervals along the vertical axis of symmetry and then added together in space to represent potentials due to columns of cells. The waveforms were then averaged over 1.6 to 2 msec to simulate scatter of discharge times.

Examples of population potentials which might be seen at the cortical surface are shown in fig. 24b. The 'disc' response corresponds roughly to part of the experimentally observed P_Q responses and the 'column' to the observed P_1 unit responses (see fig. 20a).

The effect of halving the hyperpolarizing current, on the local action potentials, is indicated by the dotted line in fig. 24a. It is probable from the experimentally observed shape of extracellularly recorded action potentials in this investigation (and of intracellular action potentials in other studies) that the best estimate for the hyperpolarizing current would be about $3/4$ of that used here. Thus the cancellation effect of the negative component in the surface potentials would be smaller and the resulting surface potentials would be even more positive (i.e. larger) than those shown ^{by the solid lines} in fig. 24b.

Figure 24. Single action potentials (a) and their summation to give population potentials at the cortical surface (b).

a) Extracellular voltage (V_o) 10μ away from the centre of a pyramidal cell soma. Inward trans-membrane current flow (I_m) and intracellular voltage (V_i) during a hypothetical action potential. Current pulses of 0.2 msec duration (thin line in I_m) were combined to form a biphasic 'action' current. The thick line shows the same waveform smoothed by hand. During the time considered total inward and outward active currents were equal. (The dotted line indicates current flow if the later hyperpolarizing current was halved).

The thin lines in V_o and V_i show mean voltages over 0.2 msec periods due to the current flow of I_m . The thick lines are again smoothed waveforms drawn by hand and represent the extracellular and intracellular potentials close to the active site. (The dotted lines show the potential which would correspond to the current flow indicated by the dotted line in I_m). The scales of I_m and V_i are arbitrary. The scale of V_o was chosen to give an amplitude of 2 mV (like the largest recorded action potentials).

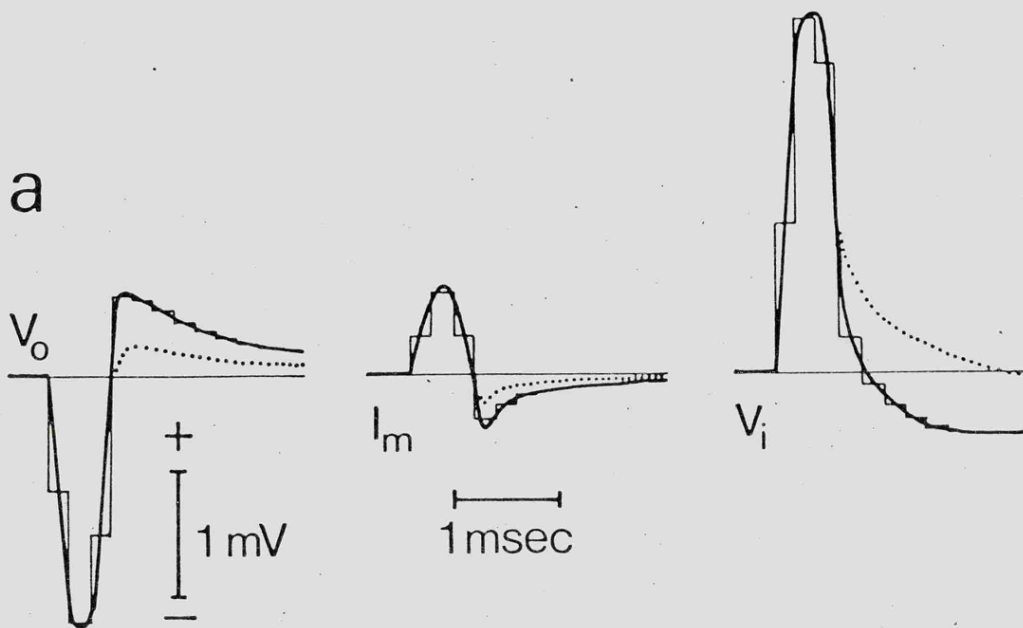
b) Population potentials which would be recorded at the cortical surface if a disc of 2700 cells or a column of 450 cells was active during the times indicated in 'PSTH'. The disc was 1000μ across and situated 950-1250 μ deep in the cortex. Each cell was 50 μ or more from its neighbours and fired only once during the 2 msec period considered. The column had a 300 μ diameter and was situated 550-1050 μ deep in the cortex. The cells were again 50 μ or more apart and each discharged only once during the 1.6 msec period considered.

PSTH - post stimulus time histogram, shows time distribution of model action potential discharges.

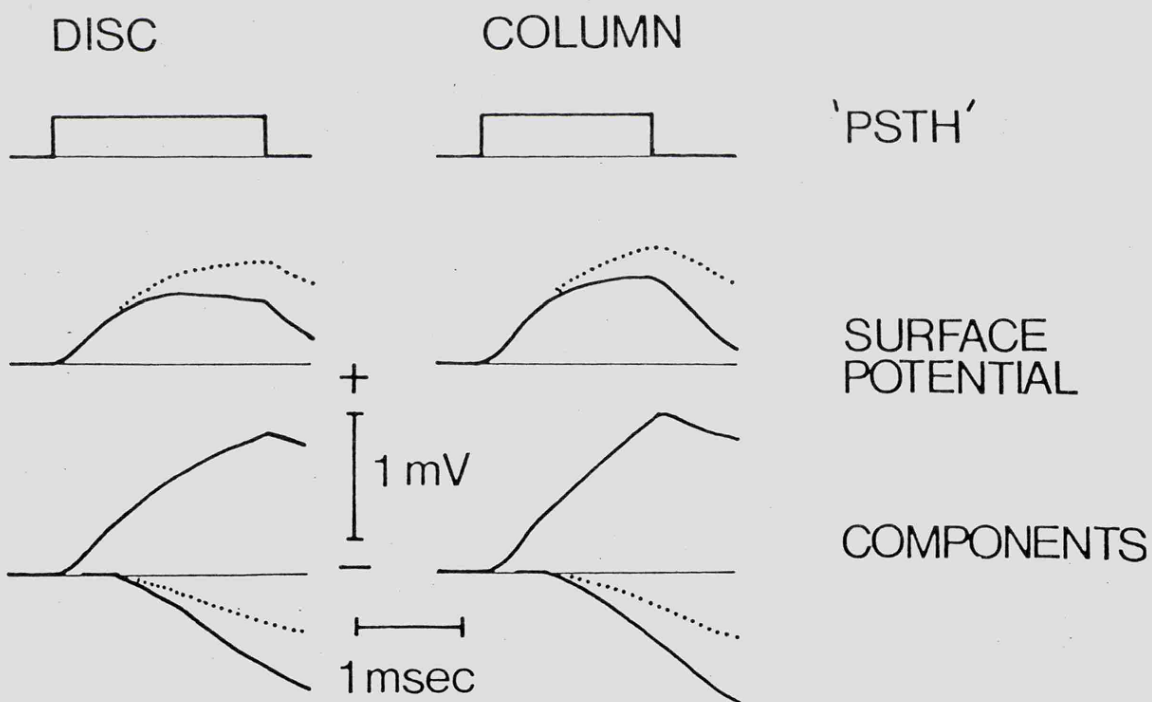
SURFACE POTENTIAL - the summation of field potentials was linear. The waveform V_o in (a) was the boundary condition used to calculate the absolute magnitudes of potential changes (as in the calculations of figs. 22 and 23).

COMPONENTS - the components of the surface potentials which arose from the depolarizing and hyperpolarizing components of I_m taken separately are shown at the bottom of the figure.

a



b



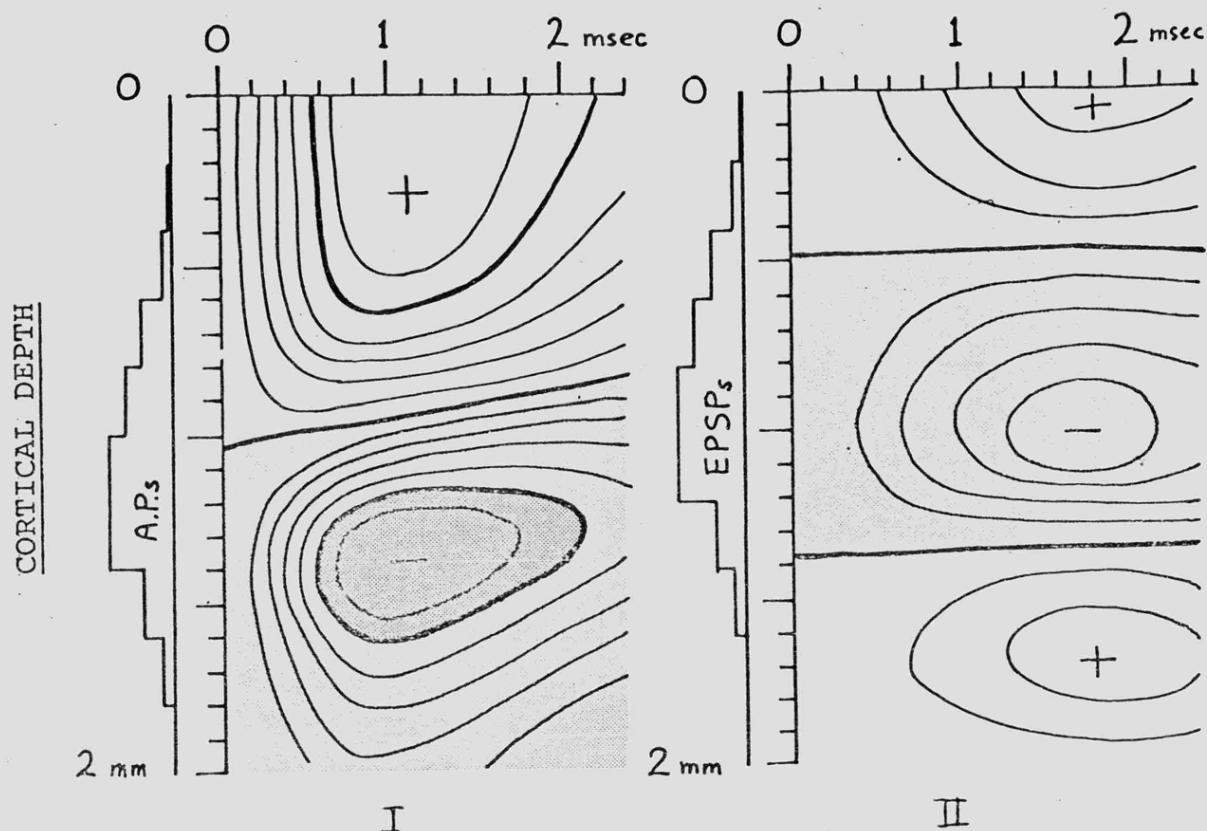


Figure 24c. Theoretical depth profiles during the first 2.4 msec of a response.

I. The potentials expected from action potentials (A.P.s) occurring in the somata of 2500 pyramidal cells distributed from 300 to 1500 μ depth as shown on the left. The cells were evenly distributed across a cylinder of 1000 μ diameter. Each cell fired once during the 2.4 msec considered and the resulting PSTH was assumed to be step shaped. The current flowing during the action potential was obtained by using the waveform (V_0) in figure 24a as a boundary condition.

II. The potentials expected from EPSPs occurring 200 μ above the somata of the same pyramidal cells. The depth distribution of synaptically active sites is shown on the left. For comparison with (I), the total synaptic current in each cell was assumed to be equal to that occurring during the depolarising phase of the soma action potential.

Voltage contours are drawn in 0.1 mV steps.

A more physiological depth distribution of action potentials is considered in the theoretical profiles of figure 24c. This corresponds roughly to the distribution of P_q units found in the experiments (see figure 20a part D). However, more superficial units were included here because there was probably a bias against recording from superficial units. The somata of superficial neurones tend to be smaller and in these experiments (particularly expts. with larger craniotomies) there appeared to be more mechanical stability in the deeper layers. Again, the neurones were considered to be evenly distributed across a 1000μ diameter cylinder (apart from the central inactive core of 150μ diameter).

The theoretical profile corresponding to soma action potentials in a total of 2500 model neurones is shown in (I). Each cell fired an action potential once during the 2.4 msec period considered, and the corresponding PSTH for the whole population was a simple step function. A profile resulting from EPSPs in the same neurone population is shown in (II). The magnitude and time course of the synaptic current was the same as that of the depolarising component of the action potential. In each cell the synaptic site was assumed to be 200μ above the soma. Thus, during the 2.4 msec considered the membrane current was almost equally distributed above and below the active site. This resulted in an almost symmetrical trimodal potential/^{depth} distribution of field potentials surrounding individual neurones. Thus, there are more cancellation effects and smaller potentials in (II) than in (I) where the component profiles are mostly bimodal. This means that although the same depolarising currents are involved, although the EPSPs are situated

200 μ nearer the cortical surface than the action potentials, and although cancellation due to hyperpolarising currents is only present in (I), the surface positivity in (I) is considerably larger than that in (II). Thus action potentials in the somata of pyramidal cells can contribute significantly to both surface positive waves and deep negative waves. However, negative waves in the middle or upper layers and relatively superficial reversal of depth negative waves (as those in figures 14, 15 and 16) must reflect more superficial depolarising currents such as those associated with synaptic excitation of the middle regions of apical dendrites (see figures 21a/B, 21b/II and 24c/II & III).

Summary

The magnitude and form of field potentials resulting from soma action potentials in a model pyramidal cell were estimated using the experimentally recorded extracellular action potential as a boundary condition. Linear summation of such potentials (simulation of population potentials) indicates that soma action potentials can contribute significantly to surface positive waves in cortical evoked potentials.

DISCUSSION

IDENTIFICATION AND CONTROL OF VARIABILITY OF EVOKED POTENTIALS

The first aim of this investigation was to identify some of the sources of variability of the cortical response to forepaw stimulation, so that they might be easily recognised, and perhaps controlled, in subsequent experiments concerned with the functional organization of the rat somatosensory cortex. The forepaw response and its variability were studied mainly as functions of three variables - 1) recording position, 2) type and depth of anaesthesia and 3) preceding spontaneous cortical activity. It was found that information about recording position was important for the comparison of results from different experiments. Changes in type and depth of anaesthesia could result in changes in the response either in the comparison of records from different experiments or of those in one experiment. However, these changes were usually small (consisting of up to 1 msec alterations in latent period). The form of the evoked response was apparently not directly affected by anaesthesia, only indirectly (when there was a change in background spontaneous activity). The most important source of variability in records from a single experiment was the interaction of spontaneous and evoked activity like that described by Bindman et al (1964a).

Recording position

In these experiments, responses at the centre of the receiving area had a characteristic waveform. There was a relatively large initial positivity/^(P₁) followed by a large negative (N) wave or by a large positive wave (P_q) with a superimposed negative inflection. (The P_q wave was only seen in the response of initially quiescent cortex). At points 0.5 mm or more away, the P₁ and N waves were greatly attenuated although the P_q component remained large up to 1 or 2 mm away. In other words, the responses recorded 0.5 mm or more away from the centre had quite a different shaped waveform; they were not simply attenuated versions of the response at the centre (unless the stimulus was given during ECoG activity). In this surrounding region the response of initially quiescent cortex was dominated by the surface positive P_q wave and was more rounded in shape. Similarly, the shape and magnitude of mass responses and the occurrence of unitary responses within the cortical grey matter depended critically on recording position; the early components were much more localized than the later ones.

This difference in spatial and temporal distribution of the various components could lead to considerable variability in evoked potential waveforms or types of unit response when comparing results from different experiments. To make such comparisons more meaningful it would be useful to locate the central area (of earliest

response) in each experiment by mapping the surface responses. However this is sometimes difficult. Firstly, large veins can obscure this area, and secondly in experiments where subsequent unitary recordings (particularly intracellular recordings) are required, it is preferable to make a small craniotomy (like that in fig. 29) for mechanical stability.

The difference in spatial and temporal distribution of the individual components in the surface response could also lead to variability in evoked waveform if the amount of CSF fluid, dura or bone near the recording electrode varied from one experiment to another. The more short-circuiting there was across the surface of the cortex, the more the surface electrode would tend to pick up an integrated waveform (i.e. a weighted mean response from a wide area). This could make the localization of the initial response area even more difficult.

As one might expect from theoretical considerations (see for example fig. 23a, b and c), the population of P_1 units could be even more localized than the corresponding P_1 wave. So far I have been unable to find P_1 units which were more than 250μ apart tangentially (unpublished results, 1975). These units are therefore relatively difficult to find, but once a P_1 unit is found it can serve as a useful reference point for defining all the other recording positions in that experiment.

Anaesthetic effects

Most experiments in this investigation were performed under urethane. Trilene was used in some control experiments, and trilene anaesthesia was followed by urethane anaesthesia in other control experiments.

Changes in depth or type of anaesthesia had little effect on the first 20 msec of the cortical response to forepaw stimulation, although there were significant changes in spontaneous cortical activity. However the various components of the mass (or unit) responses occurred up to 1 msec earlier with trilene or with moderate-light urethane anaesthesia than with moderate-deep urethane anaesthesia.

It should be mentioned here that the ranges in depth of anaesthesia used in these experiments were relatively small; they were similar to those which might be expected in a normal run of experiments where a useful moderate level of surgical anaesthesia is aimed at. In experiments where anaesthetic effects are the main interest and much larger ranges are used, clear anaesthetic effects can be seen. Angel and Unwin (1970) showed (with rats) that there was a clear suppression of the responses to forepaw stimulation at the thalamic level with 40%-100% increases in the dose of urethane. Angel (1967b) also showed (with rabbits) how both the P_1 and the N wave were affected by 'attention' as well as by induction of nembutal trilene, or urethane anaesthesia.

In the experiments of the present investigation the later components of the response sometimes varied with different anaesthetic conditions but this appeared to be an indirect relationship; the depth and type of anaesthetic affected the type of spontaneous activity which in turn affected the response to forepaw stimulation.

Interaction with spontaneous activity

Even when all the main experimental parameters were kept constant (recording position, anaesthesia, and stimulus parameters) the response to forepaw stimulation was extremely variable. This variability was related to changes in spontaneous ECoG activity but was clearly different from the superposition of spontaneous waves and appeared to reflect a cortical stabilizing mechanism (see next section). In the surface response of initially active cortex ('A' response) the initial positivity (P_1) was followed by a negative wave (N), whereas in the response of initially quiescent cortex ('Q' response) there was a large surface positive wave (P_Q) superimposed on the N wave. This relationship was the same for all anaesthetic conditions used here and was the same as that observed previously by other authors (in particular Bindman et al, 1964a).

These authors recorded responses separately on moving film and made observations concerning the interaction of

spontaneous and evoked potentials in retrospect. This method was used in the early experiments of this investigation. However as the alternating periods of quiescent and active periods in the spontaneous ECoG (of the rat under moderate to deep urethane anaesthesia) usually lasted 0.5 sec or more, a conditional triggering method was used to superimpose groups of 'A' responses on film separately from groups of 'Q' responses. This grouping of responses helped in making observations about the intrinsic variability of the response which appeared to be physiologically significant. In control experiments with lighter urethane anaesthesia or with trilene anaesthesia, the quiescent periods in the spontaneous ECoG were too brief for the conditional triggering procedure, so individual responses were made on a moving film, and the grouping done in retrospect.

The interaction between spontaneous and evoked activity at the cortical surface was reflected by similar interaction in the deeper layers of the cortex. The suppression of the surface positive P_q wave by spontaneous activity was associated with a suppression of a widespread mirror image negative wave as well as the suppression of many unit responses deep in the cortex. Thus depth mass potentials and unit responses can also depend critically on the background activity of the cortex. The early P_1 wave was relatively little affected by spontaneous activity, but like the associated depth responses (including unit

responses) it was slightly earlier in the response of initially active cortex than in the response of initially quiescent cortex.

The stimulus parameters were usually kept constant in these experiments but it was noted that if pairs or groups of stimuli were given (with 100 msec or more separation) then the form of depth or surface mass response and the occurrence of unit responses of the later response depended on the induced presence or absence of background activity in the same way that the response to the first stimulus depended on the presence or absence of spontaneous activity. In particular, the absence of a purely negative wave in the later responses was associated with (and probably due to) the presence of a large depth negative/surface positive response and unitary discharges just as it was in the response to the first stimulus.

Angel (1967a) observed an absolute refractory period of about 30 msec and a relatively refractory period of up to 500 msec at the thalamic level when he gave paired stimuli to various sites along the somatosensory pathway. It is therefore possible that the large variability in responses to grouped stimuli (seen in the present investigation) could hide a fatigue effect. However, the recovery cycle is known to be significantly altered by changes in type and depth of anaesthetic (Marshall et al, 1941). The present results suggest that the

apparent absence of a particular component (such as the N wave) in the surface response to the second of a pair of stimuli does not necessarily imply that the second response is smaller.

Summary

Depth and type of anaesthesia had mainly an indirect effect on the variability of evoked potentials; they influenced spontaneous cortical activity which in turn interacted with evoked activity. The interaction of spontaneous and evoked potentials was the main form of variability seen in these experiments. Recording position was important in the comparison of results from different experiments.

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MECHANISMS UNDERLYING THE VARIABILITY OF EVOKED POTENTIALS

The second aim of this investigation was to find out more about the mechanism behind the observed interaction of spontaneous and evoked potentials, in particular to find out whether the variability of the surface responses was matched by variability in the responses of individual neurones.

The variable component of the evoked response (i.e. the part of the response which was affected by spontaneous cortical activity) was found to be surface positive and depth negative. The deep negativity of this variable component was associated in time, cortical depth and tangential position with unitary responses (P_Q units) in the deep layers of the cortex. Both the P_Q unit responses and the variable component of the mass response were suppressed during background ECoG activity. It is probable that many of the P_Q units were pyramidal cells and therefore that evoked synaptic potentials (EPSPs) and evoked action potentials in these units contributed to the variable P_Q component of the mass response (see 'D' in fig. 20b and discussion below). Information about the suppression of action potential responses in P_Q units might therefore help to explain the variability in the evoked potential.

As mentioned earlier, the interaction between competing stimuli (Angel et al, 1963) and the variability of cortical

responses to high frequency repetitive stimuli (Poggio et al, 1963), or paired stimuli (Angel, 1967) can occur subcortically. However it is probable that the interaction of spontaneous and evoked activity seen in the present investigation was largely a cortical phenomenon since direct stimulation of the cortex (Bindman et al, 1964a), or of the afferent fibres in the underlying white matter (see Appendix IIb) can give very similar results. Procedures such as application of strychnine to the cortex (Adrian, 1941), local application of electric currents (Bishop et al, 1950), and deafferentation of cortex (Grafstein, 1959) had already given results which supported this idea (see Introduction). It appeared from the directly coupled surface evoked potentials and depth responses in this investigation that this interaction might reflect a stabilizing mechanism; this could perhaps be due to inhibition or fatigue of cortical neurones.

Synaptic organization of cortical cells

Since the P_Q units fired 2 or more msec after the P_1 units, and since stimulation of the afferent fibres gave similar patterns of both mass and unitary responses as did forepaw stimulation (Appendix IIb), it is probable that there is a synaptic delay between the P_1 units and the P_Q units. The P_1 units respond to white matter stimuli at about 2 msec delay, suggesting that they are

first order cortical neurones (not afferent fibres) and that P_q units are second order neurones.

On the basis of available physiological and anatomical evidence Eccles (1969) proposed that the afferent fibres make excitatory connections with cortical neurones (both pyramidal and stellate cells) and that these in turn can make intrinsic cortical connections (excitatory or inhibitory) with neighbouring neurones. In particular he suggested that many of the monosynaptically excited cells were inhibitory neurones, and that there were also second order inhibitory neurones. He also suggested from anatomical evidence (see below) that some of the stellate cells could be inhibitory to pyramidal cells.

Most investigators who recorded patterns of cortical excitation and inhibition intracellularly stressed the importance of inhibition of second order neurones by first order neurones; they found, after synchronous activation of the cortex, that the onset of inhibition was about 1 msec after the onset of excitation. This was found after electrical stimulation of the pyramidal tract (Phillips, 1959; Stefanis and Jasper, 1964; and Rosenthal, Waller and Amassian, 1967) of the lateral geniculate body (Li, Ortiz-Galvin, Chou and Howard, 1960), of the cortex itself (Li and Chou, 1962), of the dorsal column nuclei or spino cervical tract (Andersson, 1965), of group I muscle afferents (Oscarsson, Rosen and Sulg, 1966; and Grampp and

Oscarsson, 1968) and of the optic tract and optic radiation (Watanabe, Konishi and Creutzfeldt, 1966).

Mountcastle et al (1959) had already observed a surround inhibition effect in neurones of the somatosensory cortex, and that an inhibitory stimulus was most effective if it was given 3 - 4 msec before an excitatory one. They suggested that the apparent delay in the inhibitory pathway was due to inhibitory interneurones but they pointed out that inhibition could occur cortically or subcortically.

It was suggested by Li et al (1960) that the delay in the inhibitory pathway might be due to different conduction velocities. Whitehorn and Towe (1968) also favoured the idea of direct inhibition by afferent fibres because they found a few cells which showed evoked inhibition without any previous excitation and because they observed long lasting IPSPs while most cortical cells were silent. However, if the spatial distribution of inhibitory connections is wider than that of excitatory ones, as seems probable (Asanuma and Rosen, 1973; Houchin, 1975b- see Appendix IIa), then pure inhibition should be expected. Also, the long lasting IPSPs could reflect a fall-off of excitation; artificial depolarisation of the otherwise resting membrane of an inhibited cell reveals a much shorter IPSP than that seen during spontaneous depolarisations (Houchin, unpublished - but see the two middle traces in figure 33). Finally, Watanabe et al (1966) had stimulated the visual pathway at various levels and shown that the delay in the inhibitory pathway was independent of

conduction distance. It is therefore more likely that inhibition of cortical cells is mediated via first or second order cortical inhibitory interneurons.

Toyama, Matsunami, Ohno and Tokashiki (1974) made a detailed study of patterns of synaptic activity in the visual cortex. They suggested that layers III to V receive monosynaptic excitation and disynaptic inhibition from lateral geniculate afferents and that layers II and VI receive disynaptic excitation and trisynaptic inhibition. Innocenti and Manzoni (1972) suggested that trisynaptic inhibition was more important than disynaptic inhibition because the earliest responding cells never showed bursts of action potentials in their response to afferent stimulation; they had only seen a few of the burst responses in later responding cells. By analogy with burst activity of Renshaw cells, whose inhibitory action on spinal motor neurones is known (Renshaw, 1941; Eccles, Fatt and Koketzu, 1954; and Eccles, 1955), they suggested that inhibitory neurones in the cortex should also show bursts of action potentials. However, although inhibition is abundant in the cortex, burst responses are rare. As mentioned above, the apparently long duration of evoked IPSPs in the cortex may be due to a fall-off of excitation, the IPSP itself being quite short, so that it is possible that cortical inhibitory neurones, like excitatory ones, respond to afferent stimulation with a single action potential.

To return to the P_1 and P_q unit responses of this investigation, it appears that the afferent fibres excite

P_1 units which in turn excite or inhibit P_q units (directly or through interneurons). The P_1 unit responses are relatively unaffected by spontaneous cortical activity, therefore the suppression of P_q unit responses during ECoG activity must occur either (1) presynaptically (at the terminals of P_1 fibres or interneurone fibres) or (2) at the level of an interneurone or (3) postsynaptically in the P_q cell itself.

(1) There is no anatomical evidence for the existence of presynaptic inhibition in the cortex (Jones and Powell, 1973). However, after Steriade, Wyzinski and Apostol (1973) found (like Evarts in 1960) that pyramidal tract cells showed less clear responses to stimulation of specific afferents at times when their spontaneous discharge rate was high, they suggested that presynaptic inhibition might be responsible for this "paradoxical depressed responsiveness". A recent intracellular study suggests that a presynaptic mechanism is unlikely in the present context (see Appendix IIb and page 93 below).

(2) The response of the earliest P_q units is too soon after the discharge of P_1 units to allow for an interneurone (fig. 20), so there must be another mechanism responsible at least for the suppression of the earliest P_q unit responses. If the behaviour of the later responding P_q units was determined by interaction at the level of an interneurone then this could involve (a) the non-specific excitation of inhibitory interneurons (Richardson, 1970) or (b) the non-specific inhibition or (c) fatigue of excitatory interneurons.

Assumption (a) would imply the existence of cells whose response was greatly facilitated by spontaneous cortical activity. As the only facilitation observed in this investigation was a small decrease in latent period this hypothesis seems unhelpful in the present context.

Assumption (b) would imply the existence of interneurons (which were themselves P_q units) which showed no spontaneous activity during ECoG activity and this was not the case; P_q cells fired spontaneously during ECoG activity and were otherwise silent. More recently it has been shown that their membranes are depolarised during ECoG activity and return to a resting level during cortical quiescence (Appendix II).

Assumption (c) would imply a clear relationship (again in a P_q cell) between the probability of an action potential response and the timing of the most recently occurring spontaneous action potential. This was never observed; the probability of a response was much more closely related to the presence or absence of ECoG activity at the time of the stimulus (Appendix IIb).

Thus interaction of evoked and spontaneous activity of P_q units does not appear to occur at the level of an interneurone in the ways suggested in (a), (b) or (c). The remaining possibility (3) is that the interaction takes place at the level of the P_q cell itself. As discussed above, fatigue and non-specific postsynaptic inhibition do not appear to play a role, thus either presynaptic inhibition or evoked postsynaptic inhibition must be responsible. Recent

intracellular recordings from P_q cells (Houchin, 1974, 1975b — see Appendix II) support the hypothesis that the suppression of P_q action potential responses is largely due to post-synaptic modulation of evoked IPSPs.

Postsynaptic inhibition

Sherrington (1906) developed the concept of synaptic inhibition (but not that of separate inhibitory pathways). In 1925 he also introduced the idea of algebraic summation of excitatory and inhibitory action on the central portion of spinal neurones. Renshaw (1941) demonstrated that refractoriness was not sufficient and that inhibition must be necessary for the inhibitory influence of spinal motor neurones on their neighbours. Brock, Coombs and Eccles (1952) were the first to observe the hyperpolarising action of inhibitory synapses using intracellular recording techniques (also in spinal neurones). Coombs, Eccles and Fatt (1955b) showed that the inhibitory postsynaptic potential (IPSP) had an equilibrium potential just below the resting membrane potential and that the hyperpolarising action was due to increases in membrane permeability to Cl^- and K^+ ions.

Eccles (1955) suggested that neurones obeyed Dale's principle; neurones were either purely excitatory or purely inhibitory. He also suggested that all inhibitory cells in the CNS were short axon neurones lying within the grey matter while transmission pathways were formed by excitatory cells.

Adrian (1939) stressed the importance of inhibition for

the organisation and control of cortical responses. He said that factors which check the tendency for the level of cortical activity to rise might include fatigue but that inhibition was far more important; "In the normal working of the cerebrum the spread of activity is directed into particular channels which vary from moment to moment and this canalisation must involve inhibition as much as excitation. Indeed the development of any organised response would be quite impossible without some means of controlling the tendency for activity to spread in all directions. But inhibition must be studied by other methods...".

Jung et al (1952) often observed suppression of cortical cell discharge rates in response to visual stimulation, and they suggested that inhibition was present. Jung and Baumgartner (1955), like Adrian, emphasised the importance of inhibition in the cortex; (my own literal translation) "One difficult problem in neurophysiology is to understand the maintenance of an excitatory equilibrium in the brain through inhibitory and stabilising mechanisms. Without such inhibitory mechanisms an orderly brain function would be impossible and the synaptic powder keg of the brain would explode with the massive discharge of millions of active cells. Even with strong sensory or with single electrical stimuli, the healthy brain gives an orderly response with specific and localised impulses. In spite of the countless connections of cerebral neurones, no snowballing effect occurs. Therefore there must be a regulating mechanism

which guarantees a limit to excitation and a stabilisation of cortical discharge."

Towe et al (1958) and Mountcastle et al (1959b) observed 'surround inhibition' in many cortical neurones when they stimulated the digits or skin of anaesthetised monkeys. Mountcastle et al (1959b) suggested that a surround inhibition at the cortical level could play a role in two point discrimination as well as in pattern and contour recognition.

Phillips (1959), recording intracellularly from the motor cortex, showed that there was a delay of IPSP onset relative to EPSP onset after antidromic invasion of pyramidal tract cells and wrote; "If the pyramidal tract collaterals are indeed responsible for the depolarising as well as the hyperpolarising effects in neighbouring cells, it would be expected according to Dales principle that one of the effects, presumably the inhibitory one, would require an interneurone and the other not.". He too stressed the importance of cortical inhibition; "A healthy system of this kind (cortical inhibitory apparatus) would obviously tend to prevent the degeneration of natural high frequency firing into epileptiform discharge.".

Early intracellular recordings from the sensory cortices (where the pyramidal cells are smaller than those in the motor cortex) appeared to be less stable (Albe-Fessard et al, 1955; Li, 1955; and Li, 1961). However, during the 1960s many intracellular studies were made. Thus, it was possible to observe cortical inhibition directly, after stimulation of

the sensory pathways (see previous section). Andersson (1965) found powerful inhibition in most somatosensory cortical neurones after stimulation of the skin or ascending pathways. He commented that excitatory phenomena had dominated findings from extracellular studies in the somatosensory cortex, although inhibitory action had been reported (Mountcastle et al, 1959b; and Towe et al, 1958). Even when surround inhibition had been observed it had been thought to reflect inhibition at a thalamic level, as inhibition had been observed in the thalamus (Andersen et al, 1962).

Post-excitatory suppression of action potentials, or cessation of a burst response, could be due to a fatigue phenomenon rather than inhibition. Burns (1953) proposed a cortical model consisting of interconnected neurones which did not repolarise uniformly and which finally stopped firing because the refractory period exceeded the circuit time. In 1968 he wrote that any general theory concerning the cortex regarded as a network of interconnected neurones must take the stability of discharge frequency of cortical neurones into account; there must be some sort of feedback to explain this stability. However, he referred to cortical IPSPs as 'occult phenomena' and suggested that the prolonged hyperpolarising potentials seen after direct cortical stimulation could be due to interstitial current flow.

Presumably the IPSPs discussed in the next section must reflect 'true' inhibition because they often have large amplitudes and the amplitude can vary independently of

extracellular potentials, either spontaneously (compare the extracellular potentials of figure 14 with the data of figure 31 or because of intracellularly injected current (see figure 32a).

Postsynaptic modulation of synaptic potentials

One mechanism for postsynaptic attenuation of synaptic potentials during ECoG activity might be ^{an}increase in membrane conductance associated with spontaneous synaptic activity. However, this is unlikely to be an important factor in these experiments. Firstly, there has been no observable, i.e. large, deviation from linearity in the superposition of spontaneously occurring synaptic potentials and artificially induced membrane potentials (with membrane currents up to about 1nA - unpublished results from experiments such as that of figure 32b). That is, there are no clear differences in membrane resistance between the active and resting states. (There may be small resistance changes which are hidden by the small but apparently inevitable polarising potentials at the electrode tip.) ^{Secondly,} The modulation of synaptic responses by spontaneous background activity is so strong that the responses can reverse polarity. Evoked IPSPs must therefore be involved (see figure 30 c & d).

Both EPSPs and IPSPs change their amplitude if the background membrane potential is changed. The IPSP equilibrium potential (reversal potential) in both spinal and cortical neurones is near the resting membrane potential, and the EPSP equilibrium near zero membrane potential (Coombs, Eccles and Fatt, 1955 b & c; and Creutzfeldt, Lux and Watanabe, 1966).

For small intracellular injection currents the relationship between applied current and resultant membrane voltage change is linear (Coombs et al, 1955a; Lux, Creutzfeldt and

Watanabe, 1964; and Nelson and Frank, 1967). Therefore, linear modulation of synaptic potentials with small injection currents (Lux et al, 1964; and fig 32 here) should reflect a linear relationship between the amplitude of a synaptic potential and the background membrane potential (like fig.31).

Potentiation of evoked IPSPs by spontaneous depolarisations (such as that of fig. 31) is apparent in figure 4b of Lux and Klee (1962) which shows inhibitory responses of a cortical neurone to thalamic stimulation. Potentiation of evoked IPSPs (and diminution of evoked EPSPs) by evoked EPSPs has often been demonstrated (Curtis and Eccles, 1959; Lux, Nacimientos and Creutzfeldt, 1964; and Rall, Burke, Smith, Nelson and Frank, 1967). It has been discussed from a theoretical point of view by Rall (1967).

The linear modulation of PSP amplitude by changes in the background membrane potential corresponds precisely to the changes that would be expected if the current generating the PSP was due to ions/^{passively} moving down their electrochemical gradients (Coombs et al, 1955b). Inhibitory synapses tend to bring the membrane potential in mammalian preparations to about -80 mV (Eccles, 1964). The apparent resting membrane potential in cortical neurones is not usually more than -70mV (Phillips, 1956; and Creutzfeldt et al, 1966). This may be due to the high frequency of spontaneous depolarisations in cortical neurones; Phillips (1956) suggested that the true membrane potential could perhaps only be measured under deep anaesthesia. Thus the small threshold depolarisations (4-10 mV) and large displacements of background membrane

potential from IPSP equilibrium (10-20 mV) seen in many cortical cells (Creutzfeldt, Lux and Nacimientto, 1964; and Creutzfeldt et al, 1966) could have been due to a continuous barrage of background depolarisations. Also, 'true' equilibrium potentials tend to be nearer to resting membrane potential than those estimated experimentally because the current injection has less effect at a distance than at the recording site (Calvin, 1969). It is perhaps not surprising that the IPSP equilibrium in my experiments (figs. 31 and 32) was much closer to resting membrane potential than that of Creutzfeldt et al. (1964 and 1966); with urethane anaesthesia the membrane potential of cortical neurones really did seem to return to a resting level during ECoG quiescence (fig. 29).

The evoked synaptic responses of P_q cells appeared to have an equilibrium near spike threshold, and the absolute potential level reached was always near this equilibrium; i.e. the appropriate XPSP line (see figure 34a) had a slope very near -1. This behaviour would be expected if the evoked synaptic potential was generated by passive current flow induced by a brief but large conductance change following a strong and synchronised mixture of excitatory and inhibitory inputs. A model similar to that of figure 34a was considered (1969) by Calvin/as an alternative way to explain suppression effects which might otherwise have to be attributed to presynaptic inhibition.

If the membrane potential of a P_q cell is artificially depolarised (or hyperpolarised) by means of intracellularly injected current, the variations in amplitude of the evoked

compound PSP are similar to those seen during spontaneous changes of membrane potential as would be expected from the model (see figure 32b). Thus, suppression of Pq cell responses seems to be largely if not wholly postsynaptic.

The spike threshold is probably not constant; it should increase as the rate of rise of current or rate of rise of voltage decreases (Hodgkin and Huxley, 1952; and Adams and Brown, 1975). The sharply rising EPSPs evoked from resting membrane should therefore be more likely to initiate an action potential than the slowly rising ones of initially depolarised membrane. Also, the long lasting background depolarisations occurring during ECoG activity would tend to reinforce these accommodation effects. Figure 33 is an attempt to test for accommodation in a Pq neurone; there is apparently a shift in threshold of about 2 or 3 mV if the cell is depolarised for more than about 50 msec. The model of 34a requires just such an accommodation effect if it is to explain the suppression of action potential responses. This is because, in this case of passive postsynaptic modulation of the evoked XPSP, the slope of the XPSP line can never be greater than -1, even if the excitatory and inhibitory regions are separated from each other. ^(see next paragraph) As mentioned in Appendix II, measurement of thresholds and slopes of XPSP lines is difficult because of the instability of recordings. However, some XPSP lines seemed to be slightly steeper than -1. Such cells could perhaps have received excitatory inputs from earlier discharging Pq units, in these cases a presynaptic variation would be responsible for the steeper gradient.

(If PSPs spread electrotonically over a passive membrane which shows normal capacitative and resistive properties, then a recorded XPSP can be considered, at least at all times after the cessation of the brief and synchronised subsynaptic conductance changes, as a linear sum of component potentials which arise from the different active sites. Each of these component potentials is a monotonic increasing function when considered in terms of the initial potential - after synaptic activation - (a boundary condition) at the distant site in question. When various background depolarisations are considered (as in fig. 34a) there can be no overshoot effects at any of the individual synaptic sites since synaptic current is assumed to be passive. Thus there can be no overshoot effects in the component potentials or in their sum, the XPSP. In other words, the slope of the XPSP line in fig. 34a cannot be > -1 .)

The model has an inherent stability. The negative slopes of the PSP lines in fig. 34A imply a strong negative feedback effect; any particular input to the cell would result in larger IPSPs and smaller EPSPs if the postsynaptic membrane was initially more depolarised. This stability could also be useful in a network of such cells; the parameters concerning the number and strength of connections in Malsburg's (1973) model of the visual cortex can take wider values and the model is more stable if such 'modulated' PSPs are used instead of constant amplitude PSPs (Malsburg, 1975, personal communication). The stability of the single neurone model allows it to detect small asymmetries in a temporal input pattern of mixed excitation and inhibition; ie. it can behave as a gradient detector or give direction specific responses to moving stimuli (see Appendix III and below). Gradient emphasis and the stabilisation of activity level are just two of the possible functions of inhibition which are always opposed by excitation (Mackay, 1968).

To return to the experiments of this investigation, it was already apparent from the D.C. surface records that

the variability of evoked potentials reflected some kind of stabilising mechanism; the variability in absolute potential level 15 - 20 msec after the stimulus was much less than that seen just before the stimulus (see figs. 4 and 5). A very similar and simultaneously occurring phenomenon can now be recognised at the intracellular level i.e. as postsynaptic modulation of mixed evoked EPSPs & IPSPs in the P_q cells and as postsynaptic modulation of evoked IPSPs in the 'non-responding' cells (see group II and group III responses in Appendix II). Smaller factors in the interaction of spontaneous and evoked potentials might be the slight suppression of evoked EPSPs in P_1 cells during ECoG activity (see group I responses in fig. 30) and the modulation of the amplitudes of evoked action potentials by changes in the postsynaptic membrane potential (Adams et al, 1975, and see fig. 17b).

Direction specific responses to moving stimuli

Adrian wrote in 1936 "The activity aroused by electrical stimulation in an anaesthetised animal is no doubt abnormal but demonstrates reactions which probably occur in the normal brain.", and in 1939 "...it is the interaction between the local excitation and the background of spontaneous activity which is an essential feature of the cortical response.". Thus since the model neurone discussed in the previous section is sufficient to explain the main features of the observed interaction between spontaneous and evoked potentials it might be interesting to consider its behaviour when it is

subjected to more 'physiological' inputs, ie. to temporal sequences of excitation and/or inhibition instead of artificially synchronised inputs. The interaction of spontaneous and evoked activity in P_q cells could be thought of as a gating phenomenon; the occurrence of an action potential response depends on the recent 'past history' of the cell or of neighbouring cells. I therefore hoped (while planning the intracellular studies of Appendix II) that this gating might be functionally related to other more physiological cortical gating mechanisms. In particular, the direction specific responses of cortical neurones to stimuli moving backwards and forwards across the receptive field could be described as gated responses which depend on the recent 'past history' of the cell or of neighbouring cortical cells.

Direction specificity has been observed in many cortical neurones. It was found in the ^{anaesthetised} visual cortex (Hubel and Wiesel, 1959), in the auditory cortex (Whitfield and Evans, 1965) and in the somatosensory cortex (Whitsel, Roppolo and Werner, 1972; and Werner and Whitsel, 1973). It has also been observed in the visual cortex of unanaesthetised monkeys (Wurtz, 1969) and unanaesthetised cats (Noda, Freeman, Gies and Creutzfeldt, 1971).

Barlow, Hill and Levick (1965) observed direction specificity in retinal ganglion cell of rabbits. They later suggested that active inhibition in the null direction was an important feature (Barlow and Levick, 1965). Some cells

were able to detect direction of motion irrespective of the position of the stimulus in the receptive field. Direction specificity is rare in the retinae of higher animals and at the thalamic level (Grüsser and Grüsser-Cornehls, 1972), therefore that seen in the cortex is probably cortical in origin.

Direction specificity of cortical neurones is relatively insensitive to the velocity of stimulus movement; a five-fold change in velocity can often be tolerated (Pettigrew, Nikara and Bishop, 1968; and Grüsser et al, 1972). Creutzfeldt and Ito (1968) recorded intracellularly from visual cortical neurones and found that pure inhibitory patches in the receptive fields were rare. However, Bishop, Coombs and Henry (1973) used extracellular recording techniques in the visual cortex and demonstrated (after first increasing the background firing rate of the cell) that there were inhibitory side bands in the receptive fields; these were usually to one side of the excitatory discharge centre in cells which showed direction specific responses to moving stimuli. Even with the increase in background activity the direction specificity was preserved. Sillito (1974) showed (with local application of bicuculline) that most aspects of receptive field organisation in simple cells of the visual cortex (including direction specificity) originated from intracortical mechanisms involving GABA mediated inhibition.

Models for direction specificity

Special wiring diagrams such as that of Bishop, Coombs and Henry (1971) could help to explain direction specificity. However, the velocity of stimulus movement can be critical in such models; the model of Bishop et al requires disinhibition of strategically placed geniculo-cortical afferents and an optimal velocity of movement. Asymmetrically arrayed inputs along dendrites could also give direction specific responses (Fernald, 1971), but again a special wiring diagram is required and the responses are sensitive to stimulus velocity. Poggio and Reichardt (1973) attempted to produce a general theory "which would provide a set of constraints concerning the nervous mechanisms responsible for motion detection.", but they acknowledged that although the theory provided a functional description of the system properties, it "could not specify in any way the structural realization of a given system.". Their theory assumes interacting inputs and linear summation of fixed amplitude PSPs. In contrast, my model (see Appendix III) assumes postsynaptic modulation of PSP amplitudes and does not require interacting inputs; this independence of inputs is seen as a complete lack of direction specificity in the inputs. My model does not require any special wiring diagram and for simplicity all the synapses are close to the soma, ie. the trigger zone.

The model here is effectively the same as that considered in the previous sections (see fig. 34A); the only difference is that a sequence of small amplitude PSPs is considered instead of a large synchronised PSP. The same equilibrium

potentials are used again, but a 'unit' PSP now has an amplitude of 0.8 - 1 mV near threshold (like the spontaneously occurring PSPs observed by Watanabe and Creutzfeldt, 1966). The rise time of each PSP is less than 0.5 msec (for simplicity) and the decay time of both EPSPs and IPSPs is 10 msec (similar to those observed by Watanabe et al, 1966; and Creutzfeldt and Ito, 1968). Summation of PSPs is again linear.

Direction specificity in a unit response implies some asymmetry in time of the input pattern (if, as here, there is no direction specificity in the inputs themselves). It should be expected, from the integrative properties of the membrane capacitance, that grossly asymmetrical patterns such as that in fig. 35a would give direction specific responses; a depolarisation following a hyperpolarisation is less likely to reach spike threshold than one preceding a hyperpolarisation. More interestingly, the inherent stability of the model allows it to detect much smaller asymmetries, even those in a random pattern (fig. 38). The model almost 'prefers' mixtures of excitation and inhibition to pure excitation in the input pattern (compare figs. 36 b and c); the direction specificity is preserved with superposition of background activity (even suprathreshold excitation as in the experiments of Bishop et al, 1973 - Houchin, unpublished). The model's sensitivity to fast changes in, and relative insensitivity to the absolute intensity of, the input sequence (fig. 34B) result in a relative

insensitivity to stimulus velocity across the receptive field (compare figs. 36 and 37).

The importance of the assumption of postsynaptic modulation of PSPs in this model is illustrated by figures 35(CONTROL) to 38(CONTROL) in which modulated PSPs were replaced by constant amplitude PSPs. In the steady state (see fig. 34B) a (+4,-4) input once every 0.5 msec gives the same membrane depolarisation in the first model and in the control model. However, the control model only gives direction specific responses if there is a gross asymmetry in the input pattern or if the membrane potential is already near threshold. The control model is more sensitive to the velocity of movement than to the direction of movement.

Many of the properties of this model are those of direct specific neurones (see previous sections). Firstly, it can detect direction of stimulus movement irrespective of position in the receptive field. Secondly, it is insensitive to the velocity of the stimulus. Thirdly, it does not require pure excitatory or pure inhibitory patches in its receptive field. Fourthly, direction specificity is preserved with superposition of background activity. Fifthly, it does not require presynaptic gating of (direction specificity in) either its excitatory or its inhibitory inputs. Lastly, it depends on postsynaptic cortical inhibition. Also the main assumptions (concerning the postsynaptic modulation of synaptic potentials) are very general and could apply to any neurone which receives both excitatory and inhibitory inputs.

In particular the model could be applied to retinal ganglion cells as well as neurones in the other sensory cortices.

Summary

The variability of surface evoked potentials reflects a cortical stabilising mechanism and is matched by variability in both mass responses and action potential responses of Pq units in the deep layers of the cortex. The localised group of P1 units is excited by the afferent fibers. The P1 units (some excitatory and some inhibitory) then appear to act on units in the surrounding cortex. The suppression of Pq action potential responses to forepaw stimulation during spontaneous ECoG activity is apparently postsynaptic. Postsynaptic modulation of evoked PSPs by, and accommodation of spike threshold during, spontaneous depolarisations are together sufficient to explain this phenomenon. Thus, a simple single neurone model is sufficient to explain the variability of Pq unit responses. Consideration of less synchronised, i.e. more physiological inputs reveals that the same model is also sufficient to explain the direction specificity in response to moving stimuli seen in many cortical neurones.

CORRELATIONS BETWEEN UNITARY AND MASS POTENTIALS

The third aim of this investigation was to study correlations between unitary and mass potentials with the help of depth profiles and with particular reference to the variable component of the evoked response. It was hoped that the results might support the theory that evoked potentials could in part be due to summation of soma action potentials. A related problem has been to make an absolute estimate of the expected contribution of soma action currents in pyramidal cells to cortical surface potentials.

Since the spatial distribution of membrane current and the geometry of an active cell both influence the field potential which it generates, the morphological identification of cells and the structural organisation of their interconnections are both important.

Structural organisation of cortical cells

There are two main types of neurone in the cerebral cortex, pyramidal cells and stellate cells (Sholl, 1955; Globus and Scheibel, 1966; and Jones and Powell, 1973).
 each
 Pyramidal cells/have a thick apical dendrite which ascends to the upper layers of the cortex. They always send their axons outside the cortex (some down the pyramidal tract), some give off intracortical collaterals too. Stellate cells have short dendrites which ramify in all directions, their axons ramify in the vicinity of the soma but they

can extend horizontally for a few millimetres or vertically through several layers.

Specific afferents end mainly in or near layer IV (Globus and Scheibel, 1967; Jones and Powell, 1970b; and Szentagothai, 1973). Some afferents end directly on pyramidal cells and others on stellate cells (Globus et al, 1967). It is evident from electron microscopy that most specific afferents end on dendritic spines of pyramidal cells (probably those on the middle portion of the apical dendrite), but some end on stellate cells (Jones et al, 1970b and Szentagothai, 1973). Non-specific afferents terminate on distal side branches of the apical dendrite. Eccles (1969) suggested that the longer duration of non specific EPSPs compared with specific EPSPs (Nacimient, Lux and Creutzfeldt, 1964) should be expected if the non specific synapses were further from the soma (the presumed recording site) than the specific ones (Burke, 1967; and Rall, Burke, Smith, Nelson and Frank, 1967).

The horizontal spread of specific afferents is about 500μ (Sholl, 1955; and Szentagothai, 1973). Some cortical cells have horizontal axons in layer V spreading up to 2 mm/. There are a few deep lying cells whose axons ascend to the superficial layers and then branch with a tangential spread of up to 8 mm (Szentagothai, 1973). Also surface stimulation of cat's the/isolated cortex seems to excite superficially located axons which spread up to 10 mm tangentially (Burns, 1950). However in experiments very similar to those in the present investigation, Ennever (1975) was unable to induce any

increase in the normal range of tangential transmission; even in the presence of polarising currents or strychnine the evoked activity following forepaw stimulation was not transmitted laterally across the cortex for more than 2 mm and it was not influenced by spontaneous activity beyond a range of about 2 mm.

Biscoe and Curtis (1967) used iontophoretic techniques to demonstrate the intrinsic cortical nature of inhibition of pyramidal tract cells; local excitation of cortical neurones resulted in the inhibition of pyramidal tract cells lying a few hundred microns away and usually deeper in the cortex. Kubota, Sakata, Takahashi and Uno (1965) used depth profiles to localise the site of inhibition in these cells; it was the same as that generating the large antidromic spike after pyramidal tract stimulation, presumably at the somata of the cells. The sharp onset, large amplitude and sensitivity to current injection seen in evoked IPSPs of cortical neurones (figs. 31 and 32) also support the idea that there are many inhibitory synapses on the somata of pyramidal cells. (In appendix IIa strongly inhibited cells were identified as pyramidal cells after injection of Procion Yellow dye.)

(1911)
Ramon y Cajal described stellate cells which branched extensively to form dense pericellular nests around pyramidal cell somata (like the basket cells of the hippocampus). Szentagothai (1965 and 1967) and Colonnier (1966) suggested that these neocortical basket cells were inhibitory by analogy with the basket cells of the hippocampus (Andersen,

Eccles and Loyning, 1963) and cerebellum (Andersen, Eccles and Voorhoeve, 1963) whose inhibitory function is well known. Marin Padilla (1969) found basket cells with horizontal axons up to 1mm in length in human/^{motor}cortex and suggested that they too might be inhibitory.

In electron microscope studies, Westrum (1966) and Colonnier (1966) observed Gray type II synapses (those which were symmetrical - Gray, 1959) on pyramidal cell somata, and again by analogy with the cerebellum they and Eccles (1964) suggested that these type II synapses were inhibitory.

Bodian (1966) and Uchizono (1967) classified synapses by the shape of synaptic vesicles (after gluteraldehyde fixation) and suggested that the synapses with ellipsoid vesicles were inhibitory and that those with spherical vesicles were excitatory. Jones and Powell (1970a) found in the somato-sensory cortex that most synapses containing spherical vesicles made contact with the dendritic spines of pyramidal cells. Each spine received at least one such synapse but could also receive one with ellipsoid vesicles. Jones et al (1973) suggested that the dendritic spine was not simply a specialisation for increasing surface area, but was part of a special mechanism for synaptic interaction between afferents from different sources. The apical dendritic shaft received synapses mainly with ellipsoid vesicles, and the soma only those with ellipsoid vesicles. Stellate cells received both types of synapse all over. Colonnier (1968) had already found the same organisation in the visual cortex and had

shown that the Gray type I and II synapses corresponded to the synapses with spherical and ellipsoid vesicles respectively.

Fisken, Garey and Powell (1973) observed patterns of degeneration under the electron microscope after they had made small intrinsic lesions in the visual cortex. Using the Gray type I and II classification they were able to identify the degenerating synapses; they suggested that intrinsic inhibitory connections were less numerous but more widespread than excitatory ones. This fits well with the physiological results of Asanuma et al (1973) and Houchin (1975b - see Appendix IIa).

Brooks and Jung (1973) suggested (like Eccles in 1955) that inhibition was an intrinsic cortical function and that afferent input to the cortex was probably only excitatory, but they commented that Anatomy and Physiology of the cortex were still too far apart in both methods and results. However, in recent years intracellular dye injection techniques have helped many investigators to make direct comparisons of anatomical and physiological data.

Rayport (1957) used Prussian blue to identify some neurones which responded at short latency to the specific afferent volley; they were pyramidal cells. However, these pyramidal cells and those giving early responses in the experiments of Houchin (Appendix IIa) were probably not pyramidal tract cells; McComas and Wilson (1968) showed that neurones in the somatosensory cortex of the rat which

responded between 7 and 9 msec after a forepaw stimulus were all non-pyramidal tract cells (those which responded later were a mixture of PT and non-PT cells). Holubar, Hanke and Malik (1967) showed by staining with methyl or analine blue, that cells whose spontaneous firing rate was decreased by penicillin were stellates found in all cortical layers, whereas those whose firing rate was increased were large pyramidal cells in or near layer V.

Kelly and Van Essen (1974) used Procion Yellow dye in the visual cortex and showed that the majority of simple cells were stellates and the majority of complex cells were pyramidal. The P_1 units in my experiments were similar in several ways to the simple cells of the visual cortex; they included stellates and were apparently first order neurones (Houchin, 1975b - See Appendix IIa) and had a low discharge rate in the absence of stimulation (Houchin, unpublished).

Procion Yellow injection after intracellular recording in the rat somatosensory cortex (Houchin, 1973 and 1975b) indicated that all stellate cells were P_1 or P_2 cells, ie first or second order neurones whose response was relatively little affected by spontaneous cortical activity. The other P_1 and P_2 cells were small pyramidal cells. Practically all P_q cells and all inhibited cells were pyramidal cells. As in the work of Van Keulen (Procion Yellow injection into Renshaw cells, 1971) it was not possible to stain more than a few hundred microns of the axon or its branches, it was therefore not possible to identify any basket like processes.

However, some of the stellate cells looked very similar to the basket cells described by Ramon y Cajal (1911) (Houchin, unpublished) and they might have been inhibitory neurones.

There is thus good agreement between Anatomists and Physiologists on the probable organisation of the neocortex. The specific afferents end mostly in or near layer IV. They excite pyramidal cells (mainly synaptic contacts with spines on the middle portion of the apical dendrite) as well as stellate cells. Non-specific afferents make contacts with the more distal parts of the apical dendrites of pyramidal cells. The first order neurones are stellate cells and small pyramidal cells but they do not include pyramidal tract cells and are rare in layer V. The intrinsic connections can be excitatory or inhibitory. Inhibitory connections are more widespread than excitatory ones. Dendritic spines receive mixed but mainly excitatory inputs, the dendritic shafts receive mixed but mainly inhibitory inputs and the somata of pyramidal cells receive purely inhibitory inputs (probably from basket cells).

Thus, since P_1 , P_2 and P_q unit populations all include pyramidal cells, action potentials and/or synaptic potentials in all three groups could contribute to surface evoked responses. It is probable from the above anatomical evidence that synaptic excitation (depolarisation) of the apical dendrites and action potentials in the somata of these pyramidal cells both contribute to surface/^{positive}potentials, and that synaptic inhibition of the somata contributes to

surface negative potentials.

Correlations between unitary and mass potentials in experimental data

In this investigation the probability of occurrence of each type of unit response (in cortical depth, in time and in tangential position) was highly correlated with the occurrence of local negative waves which in turn was correlated with the occurrence of surface positive waves (see figs. 14 and 20(a & b)). This correlation was seen even when the stimulating conditions or anaesthetic conditions were changed and even if there was interaction of spontaneous and evoked activity.

In particular, the stability and localisation of the initial surface positivity (P_1) was matched by stability and localisation of the corresponding unitary responses and local negative waves in the middle layers of the cortex. Even more striking was the finding that the suppression of the later and more widespread surface positivity (P_q) during background ECoG activity was closely matched by suppression of unitary responses and local negative waves in the deep layers of the cortex. It is probable that most of the P_q units were large pyramidal cells and that the P_1 units were a mixture of small pyramidal cells and stellate cells (see Appendix IIa).

It is generally agreed (Eccles, 1951) that surface potentials are almost exclusively generated in pyramidal cells, and that depolarisation of the somata and/or of the middle or proximal regions of the apical dendrites would explain the above type of correlation (see fig 21 a & b). The association of surface positive spontaneous ECoG waves with spontaneous/unitary discharges and

large amplitude negative waves in the deep layers of the cortex (Bindman et al, 1964a; and figs 16 and 20a-S) could be explained in the same way.

The surface negative (N) wave reversed in the cortex too; there was an intense negativity at about 0.4 mm depth and a weak positivity in the deep layers (Figs 13 and 14). Thus, the N wave is probably due to depolarisation of the tips of apical dendrites like the 'superficial response' following direct cortical stimulation (Eccles, 1951) (see fig. 21c and next section). It is possible that the after-hyperpolarisation of P_1 and P_2 unit action potentials also contribute. The N wave does not resemble the surface negative wave following antidromic stimulation of pyramidal tract cells (which is clearly associated with strong hyperpolarisation of the somata of P.T. cells and large amplitude depth positive waves (Kubota et al, 1965; and Humphrey, 1968a); ^{here} the inhibitory responses of pyramidal cells were observed over a wide area of cortex (Appendix IIa) whereas the intense negativity at about 0.4 mm depth like the surface N wave was localised (fig 13). Relatively few action potential responses were found at 0.4 mm depth/^(fig. 20) so it is probable that many of the depolarisations responsible for the intense negativity at that depth were sub-threshold EPSPs.

It is usually difficult to determine the relative importance of subthreshold currents and of those associated with action potentials (see next section) but in a few special cases the distinction can be made. Monophasic (non reversing) potentials such as the positive plateaux in the surface ECoG of urethane anaesthesia) are presumably due to summation of monophasic

(subthreshold) generating currents. It is therefore not surprising that intracellular recordings reveal a strong positive correlation between these surface positive plateaux and subthreshold membrane depolarisations (see fig 29). In the cortical response to pyramidal tract stimulation, the extremely short latency (Porter and Sanderson, 1964) and the depth profile (Kubota et al, 1965) of the initial surface positive ^{response} / indicate that it is due to antidromic action potentials in the somata of pyramidal cells. As mentioned above, the surface negative wave which follows is apparently due to the large amplitude IPSPs (Phillips, 1959) evoked in the somata of pyramidal tract cells. Thus, synchronised action potentials and synchronised synaptic potentials, in or near the somata of pyramidal cells, can both contribute significantly to cortical surface potentials (in practise as well as in theory).

Like the components of most evoked cortical potentials, the P_1 and P_q waves in the present experiments were only part of a complex response and they could have been either monophasic or diphasic (initially positive). Thus, the possibility that they arose from the summation of diphasic generating currents cannot be excluded. A biphasic generating current could equally well arise from an action potential or from an excitatory/-inhibitory sequence of synaptic currents (see next section).

Even if there is a striking correlation between different phenomena (here the surface positive/ depth negative potentials and the unitary discharges), the phenomena are not necessarily causally related. In the context of evoked activity it is probable that many correlations are artifacts of synchrony.

Conversely, negative correlations are not necessarily significant; they can often be explained in other ways (Ajmone Marsan, 1965; and Elul, 1972). For example, failure to find stable unitary responses (e.g. Amassian et al, 1964) should perhaps be expected if like the P_1 unit responses here, the stable responses are very localised (Angel et al, 1967).

Many correlations could be functional rather than causal; any increase in amplitude of evoked EPSPs (or decrease in evoked IPSPs) would tend to increase the probability of action potential responses. Thus, however well (or badly) the recorded PSPs or action potentials are correlated with evoked potentials, it is inevitable that correlations (or lack of correlations) between action potentials and evoked potentials are associated with correlations (or lack of correlations) between PSPs and evoked potentials, and vice versa. Thus it is not surprising that the stable action potential responses of P_1 units are associated with relatively stable evoked EPSPs and that the suppression of action potential responses in P_q units (during ECoG activity) is associated with suppression of the evoked EPSP and/or enhancement of a superimposed evoked IPSP (see fig. 30 and Appendix IIb).

The recording of intracellular potentials does not necessarily help in deciding whether subthreshold PSPs or suprathreshold action potentials contribute most to a particular evoked potential; the relative contributions of the two types of generator depend on their positions and strengths and on the morphology of the cell in question. These factors vary from one type of response to another and from one cell to another, and in the neo-cortex they are difficult to determine/.

(Regan, 1972)

In contrast to popular belief, it is suggested in the next section that action potentials and synaptic potentials are (from a theoretical point of view) equally likely to generate evoked potentials at the cortical surface.

It is perhaps more fruitful to think in terms of absolute potentials rather than relative strengths of generating currents. Raabe and Lux (1972) showed by manipulating the postsynaptic membrane potential of inhibited pyramidal cells (with intracellular current injection) that a 5 mV IPSP was directly associated with a 0.1 mV positive potential about 50 microns away. Similarly, it should be possible (as suggested by Holmes—personal communication) to measure the direct contribution of single cell action potentials to surface potentials by triggering an averager from the action potential itself. However, even spontaneous discharges might be correlated with synaptic currents in neighbouring cells, so the resulting waveform might be difficult to interpret. Ideally one would only average during quiescent periods of the ECoG when the other cortical cell membranes would be resting. Multiple discharges could then be induced by injury or (better) by intracellular current injection, but the intracellular technique would be difficult as long and stable recordings would be required.

An alternative but less accurate way to estimate the absolute contribution of an action potential to field or surface potentials is to consider the form and amplitude of the extracellularly recorded action potentials; they can be used as boundary conditions, the membrane time constant and probable dendritic length constant can then be used to estimate the temporal and spatial distributions of the associated field

potentials (see figs. 21 - 24, and next section).

Evidence for correlations between mass and unitary potentials is abundant (Ajmone Marsan, 1965) both during spontaneous activity (Calvet, Calvet and Scherrer, 1964; and Bindman et al, 1964a) and during evoked activity (see introduction; Holmes and Short, 1969; and Holmes and Howard, 1971). These correlations are so strong, particularly during evoked activity, that mass potentials can be useful in predicting the location, strength and timing of unit responses even if, as is usual, their origin is unknown (Fox and O'Brien, 1965; and Andersen, Bliss and Skrede, 1971). In addition, it appears from the results of this investigation, that the spontaneously occurring variability (or stability) of unit responses can be predicted (see D in fig. 20b). However, since neo-cortical evoked potentials are complex and cancellation effects can be misleading (Purpura, 1959; and Mackay and Jeffries, 1973), depth profiles (like figs 14 and 15) and surface maps (like figs. 8 - 11) are needed before accurate predictions can be made.

The striking correlation between unitary action potentials and mass potentials seen in this investigation is clearly useful as an experimental tool. However, although it supports the idea that action potentials might contribute to ^{surface positive} evoked potentials, it does not imply such a direct relationship.

Theoretical correlations between unitary and mass potentials

If the activity of a neurone results in non uniform changes in membrane potential, then electric currents appear in the surrounding tissue. The resulting extracellular potentials are obtained by solving Laplace's equation (by using equation 20 in

Appendix I); positive potentials are seen near sources of current and negative potentials near current sinks (Lorente de No, 1939 and 1947a, and Hubbard, Llinas and Quastel, 1969). Thus, depolarisation of the middle or deep portions of a pyramidal cell gives a surface positive potential and depolarisation of the tip of the apical dendrite gives a surface negative potential (see fig. 21).

Surface negative potentials can also arise from depolarisation of slightly deeper regions of membrane, if (as in the neo-cortex) the apical dendrites terminate well below the cortical surface, because a large proportion of the current still is/forced to leave the dendrite below the active site.

(After a return to the resting state, the nett capacitative membrane current at any point is zero. Thus, the mean membrane is given by the mean resistive current, i.e. by the mean membrane potential which is proportional to the steady state potential during a continuous current injection (see derivation of equation 8 in Appendix I). Thus, the mean membrane current following localised activity at $X=0$ (normalised coordinates) is proportional to (e^{-X}) in the case of an infinite cable or to the 'folded exponential' $(e^{-X} - e^{-(2d-X)})$ for a finite dendrite (from equation 16))

In particular, depolarisation anywhere within 0.2 mm^* of the 0.4 mm deep terminations of a layer V cell (Ramon Moliner, 1961) would give a surface negative potential as well as an intense local negativity (like the cortical responses to forepaw stimulation in figs. 14A and 15A).

The magnitude and (because of the membrane capacitance) the

*if the dendritic length constant is 0.2 mm , as in the model.

time course of changes in the membrane current depend on the distance of the point in question from the active site. The time to peak or reversal of membrane current increases with distance (see equations 12, 14 and 15). Extracellular potentials therefore reach their maxima, or reverse, at times which also increase with distance (see figs 22 and 23, and Bogdanov and Golovchinskii, 1970 ; and NicholSEN and Llinas, 1971).

the spatial
Consideration of/distribution of membrane current (see fig. 42 here which is taken from Humphrey, 1968b) or extracellular potential (Bogdanov and Golovchinskii, 1969⁴) only at the time of peak generating current can therefore be very misleading. In such diagrams the spread of current or potential from a long duration generating current always appears (misleadingly) to be greater than that from a brief current pulse (potentials at points a, b and c in fig. 22 are less than half their peak values). Both Humphrey and Bogdanov et al were lead to suggest (wrongly) that the apical dendrite acts as a low pass filter, and then (wrongly) that brief duration currents like those of action potentials are much less likely to contribute to surface potentials than long duration synaptic currents.

The spatial distribution of mean (or nett) membrane current is independent of the duration of the generating current since it is proportional to $(e^{-X} - e^{-(2d-X)})$ - see above. It follows that short duration currents give larger amplitude surface ^{of the same nett current flow} potentials than do long duration ones // since the potential waveform at any extracellular point following any particular pattern of activity is (by superposition) a weighted mean of the potential waveform following a brief pulse of generating current. In particular, the initial depolarising component

of an action potential would contribute more to surface positive potentials than a nearby, long duration synaptic current of the same nett strength. The small cancellation effects of the afterhyperpolarisation (see fig. 24) would tend to reduce this difference. It therefore appears that for any given nett depolarising current, PSPs and action potentials are equally likely to contribute to evoked potentials such as those seen in this investigation. Differences would only arise if the nett generating currents were significantly different.

If activity is widely distributed (e.g. up to 20 msec and 2 mm in the evoked responses of Towe (1966)) it is particularly difficult to distinguish between the summation of action potential currents and the summation of EPSP/IPSP sequences. In Towe's model of the evoked potential, 98% of the neurones were given biphasic synaptic currents/sited only 50 micron above the soma (EPSP onset 1 msec before the first recorded spike and IPSP 3 msec after it) which were. Towe suggested that the good fit between recorded and theoretical potentials indicated that the evoked potential was in large part due to synaptic currents. He wrote "Although action potential currents may make a minor contribution it would not be detectable in such a crude model as presented in this paper.". However, since by his own definition, the synaptic currents and action potential currents are very closely related in both space and time, and since they are both biphasic it would have been more correct to say "Action potential currents and synaptic currents are equally likely to make such a contribution; it would not be possible in the present (Towe's) model to distinguish between

these two possibilities."

It should perhaps be mentioned here that although the mathematical assumptions in Humphrey's (1968b) and my model are the same, there are small discrepancies between his figure 11 (see fig. 42 here) and my figure 23 (with length constant, i.e. distances doubled). These discrepancies must have arisen from differences in numerical methods. (1) Reversal of action potentials occurs earlier at the cortical surface than near the soma, in his figure. Here, reversal is later at the surface (as would be expected from equation 15). (2) Although his action potential has approximately equal positive and negative components near the soma, the after-negativity dominates the surface potentials. Here the surface negativity is delayed as expected (and would by definition lead to an integral zero potential eventually). (3) The points g, h and i in figure 23 here should correspond to the surface in Humphrey's figure (He did not include an insulating boundary and the length constant was approximately double that used here). In my figure, the ratio of the positivities at g, h and i to the negativities at j, k and l (when multiplied by two times the distance ^{from the dendrite} in mm) give 'normalised' values of 1.1, 1, and 1 respectively. However, the 'normalised' ratios in Humphrey's figure are only 0.5 and 0.25. In other words, the surface positivities in Humphrey's figure are much smaller than those which would be expected from figure 23 here. These three discrepancies could perhaps have arisen if the membrane current associated with the initial depolarising component of the action potential was ignored, for all points ^{from the soma} more than one length constant λ in Humphrey's numerical methods; he did suggest (misleadingly) from his figures 13 and 14 that this current was "negligable".

Extracellular potential amplitudes are proportional to the specific resistance of the cortex ($222 \Omega \text{ cm}$ - Freygang and Lindau, 1955). This parameter was not needed in the present calculations as the extracellular action potential was used as a boundary condition. The internal resistance of mammalian motor neurones has been estimated as $70 \Omega \text{ cm}$ (Barrett and Crill, 1973). If, as is probable, the internal resistance of cortical neurones is more than $22 \Omega \text{ cm}$, then the external resistance can be taken (as here) to be zero in the calculation of dendritic length constant; errors are less than 1% (see page 132). The 0.2 mm length constant used for the apical dendrite here was deliberately underestimated; it corresponds to a small radius of 1 micron, a (high) internal resistivity of $200 \Omega \text{ cm}$ (as in Humphrey, 1968b) and a (low) membrane resistance of $1600 \Omega \text{ cm}^2$ (Humphrey used $2000 \Omega \text{ cm}^2$). The membrane resistance of cortical neurones ranges from 1000 to $6000 \Omega \text{ cm}^2$ (Lux and Pollen, 1966) and is significantly higher than that of motor neurones (mean $600 \Omega \text{ cm}^2$ - Coombs, Curtis and Eccles, 1958). Eccles (1966) pointed out that this could account for the long time constant of cortical neurones (near 8 msec - Creutzfeldt et al, 1964; Lux et al 1966; and Takahashi, 1965) compared with that of motor neurones (2 to 5 msec - Coombs et al, 1958). An increase in capacitance per unit length of dendrite would also give a longer time constant. The parameters used here and in the model of Humphrey (1968) imply membrane capacitances of $5 \mu\text{F}/\text{cm}^2$ and $4 \mu\text{F}/\text{cm}^2$ respectively. These values are apparently high when compared with the $1.3 \mu\text{F}/\text{cm}^2$ of giant axons (Hodgkin et al, 1946). However Eccles (1955) and Lux et al (1966) suggested that cat motoneurones and cat cortical neurones had capacitances of $3 \mu\text{F}/\text{cm}^2$ and $1.5\text{--}5 \mu\text{F}/\text{cm}^2$ respectively. An apparent increase in membrane capacitance might be expected in the presence of thin dendritic spines; the membrane capacitance would tend to increase more than the membrane conductance per unit length of dendrite. If there is no such apparent increase in membrane capacitance to explain the long time constant, then perhaps a higher membrane resistance would be more realistic, e.g. $R_m = 4000 \Omega \text{ cm}^2$ gives $C_m = 2 \mu\text{F}/\text{cm}^2$ and $\lambda = 320 \mu$.

Although Chang (1951) suggested that action potentials might be conducted up the dendrites, it is now generally agreed that neo-cortical cell dendrites do not normally exhibit spikes (Purpura, 1967; Nelson and Frank, 1964 a&b; Rosenthal, 1970). The effectiveness of localised stimulating currents and the amplitude of recorded spikes have similar localisation (Stoney, Thoson and

Asanuma, 1968) supporting this idea. Changes in spike (like those seen around motoneurones-Fatt, 1957) amplitude and latency/should however still be expected from passive electrotonic spread (Nelson et al, 1964a&b; Bogdanov and Golovchinskii, 1970; and fig. 23 here); action potentials recorded from different parts of a neurone have different durations and sometimes different polarities. Also it has been shown by Cooper and Robson (1969) ^{that} damaged axons can give much longer duration action potentials than undamaged ones. Choice of a 'typical' soma action potential is therefore difficult.

Initially negative action potentials are presumably recorded near the trigger zone; Li and Jasper (1953) suggested that they represented soma action potentials. The maximum amplitude of initially negative action potentials in this and other investigations was 2 mV (Fatt, 1957; Li, 1955; and Amassian, 1953). They were found to be more stable (i.e. less sensitive to electrode movement) than initially positive action potentials in this and other investigations (Li, 1955; Mountcastle, 1957; and Mountcastle et al, 1957) thus supporting the idea that they reflected a relatively strong and widespread generating current. The initially negative action potential reverses after about 0.6 msec (Amassian, 1953; and Li, 1955) but as with the intracellularly recorded action potentials (Stefanis and Jasper, 1964; and Eccles, 1966) the after potential is small in amplitude and long in duration. The potential waveform 'Vo' in fig. 24a is intended to represent the extracellular action potential immediately outside the soma (during a complete soma action potential).

In most respects, the surface potentials in figures 23 and 24 were deliberately underestimated. Firstly the dendritic length constant was underestimated (see above). Secondly, the after-

potential was exaggerated (see solid lines in fig, 24) to emphasise how small the cancellation effects are. Thirdly, in the calculations of fig. 24b it was assumed that the cell bodies were 50 microns or more apart, but in practise the cells could be more closely packed (e.g. see fig 39a). However, if basal dendrites had been included in a more realistic model, with say an axon of 1 micron diameter, four basal dendrites of 2 microns diameter, and an apical dendrite of 3 microns diameter, then the surface potentials would have been about half of those shown in figures 23 and 24 (since the current which escapes down a dendrite of diameter 'a' is proportional to $(a/\bar{a})$).

With this latter error in mind, it can be concluded from figure 24b, that summation of loosely synchronised action potentials in a reasonable number of cells could (in theory) contribute at least 0.5 mV to the surface positive potentials in the cortical response to forepaw stimulation.

Comparing the theoretical profiles (figs. 21b and 24c) with the experimental depth profiles (Figs 14 & 15), it can be seen that although soma action potentials in pyramidal cells could play an important role in the generation of surface positive and depth negative waves, they are not sufficient to produce the more superficial negativities or the superficial reversal pattern seen in the experiments. Synaptic depolarisation of the middle regions of apical dendrites are probably responsible for the superficial negativity and reversal, while surface negative waves probably reflect depolarisation of the most superficial regions of apical dendrites. The nett current crossing each cell membrane is zero/and, as each model cell was assumed to lie in a vertical axis, the complete integral over depth of any

of these profiles would therefore be zero by definition. If, as is probable, horizontally - or non vertically - oriented dendrites act as additional passive sources or sinks, then a non-zero depth integral would be expected. For example, penetration of a region of active depolarisation would reveal a bias towards negative potentials, as in the experimental profiles here

Summary

The strong correlation between action potentials and mass potentials, seen in this investigation, suggests, but does not imply, that surface positive evoked potentials are in part generated by summation of soma action potentials. The surface positive P_1 , P_2 and P_q waves could be due to depolarisation of the somata (action potentials) and/or proximal dendrites (EPSPs) of pyramidal cells. (The P_q wave, the evoked EPSPs and action potentials of P_q units are all suppressed during ECoG activity.) The surface negative N wave is probably due to depolarisation (EPSPs) of the superficial parts of apical dendrites (the after-polarisations of P_1 and P_2 cell action potential responses may also contribute). Evoked IPSPs, although abundant, do not contribute significantly to the surface N wave in these experiments.

Comparison of experimental and theoretical profiles suggests that in these particular experiments soma action potentials could play an important role in the generation of surface positive and deep negative waves, and that depolarising synaptic currents in the middle regions of apical dendrites are probably responsible for negative potentials in the middle and superficial layers, i.e. for the relatively superficial reversal of potentials. It also suggests that the recorded surface negative wave reflected depolarisation

of the most superficial regions of apical dendrites.

Consideration of the temporal and spatial features of theoretical field potentials indicates that, in general, action potential and synaptic currents are equally likely to contribute to evoked potentials. The average spatial distribution of electrotonic currents or potentials is independent of the time course of the generating current. Thus, brief current pulses produce larger amplitude field potentials than do long lasting currents of the same nett strength. Although afterpolarisations tend to oppose the summation of action potential depolarisations, they have small amplitude so that cancellation effects are not serious during evoked activity. Surface positive potentials such as P_1 and P_q could in theory be generated by loosely synchronised action potentials in a reasonable number of cells. In practice, action potential currents and PSP currents probably both contribute to evoked potentials. However, their relative importance can only be estimated if the relative strengths of the generating currents are known.

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THE END!

APPENDIX I : THE CALCULATION OF FIELD POTENTIALS AROUND A MODEL CORTICAL NEURONE

The model neurone considered here consisted of a spherical soma with cylindrical, cable like processes representing the axon and the apical dendrite of a pyramidal cell. The electrotonic (i.e. non propagated) effects of localised active current flow (on either soma or dendritic membrane) were calculated. This required detailed information of transmembranal current flow over the whole cell membrane. The results of injecting single steps or short square pulses of current at the 'active' site were calculated first. The effects of action currents with more physiological time courses were then calculated by a superposition technique.

A single cable

This problem is mathematically the same as that of Hodgkin and Rushton (1946), but the main points are repeated here for completeness. The cable had ^a surface membrane with resistance and capacitance and an internal conducting core. It lay in an infinite volume conductor with a low uniform resistance. All resistances were assumed to be ohmic and to remain constant. A current pulse or step was injected into the cable at a localised point.

Definitions

x distance along cable in cm
t time in seconds

a the radius of the cable in cm
 i the internal longitudinal current in amps
 i_m outward membrane current in amps cm^{-1}
 R_i specific resistance of internal core in Ωcm
 R_m specific membrane resistance in Ωcm^2
 C_m specific membrane capacitance in F cm^{-2}
 $r_i (= R_i/\pi a^2)$ internal resistance/unit length in Ωcm^{-1}
 $r_m (= R_m/2\pi a)$ membrane resistance/unit length in Ωcm
 $C (= C_m \times 2\pi a)$.. capacitance / unit length in F cm^{-1}
 $\lambda (= \sqrt{(r_m/r_i)})$.. length constant of cable in cm

(See below for validity of this approximation - p.129)

$\tau (= r_m C = R_m C_m)$.. time constant in seconds
 $x (= x/\lambda)$ distance in normalised coordinates
 $T (= t/\tau)$ time in normalised coordinates
 V_m transmembranal voltage in volts
 V_i internal voltage in volts

The membrane current can be written as resistive plus capacitative flow:

$$i_m = \frac{V_m}{r_m} + C \cdot \frac{\partial V_m}{\partial t} \quad \dots\dots\dots (1)$$

Also, from Ohms law,

$$\frac{\partial V_i}{\partial x} = r_i \cdot i = \frac{\partial V_m}{\partial x} \quad \dots\dots\dots (2)$$

if we assume that $V_m = V_i$ (that the external resistance through the volume conductor is negligible). Also,

$$i_m = \frac{\partial i}{\partial x} \quad \dots\dots\dots (3)$$

since there is no current loss. Thus, (1), (2) and (3) give:

$$-\lambda^2 \frac{\partial^2 V_m}{\partial x^2} + \tau \cdot \frac{\partial V_m}{\partial t} + V_m = 0 \quad \dots\dots\dots (4)$$

This equation must be solved with the boundary conditions

$$V_m = 0 \quad (-\infty < t < 0) \quad \text{and} \quad V_m = 0 \quad (x = \pm\infty)$$

with i and V_m continuous at all points.

The case of a step of current is considered first. The solution ($0 < x < \infty$ here) was given by Hodgkin and Rushton (1946) and can be written as:

$$V_m = K \cdot \left[e^{-X} (1 - \operatorname{erf}(X/2\sqrt{T-\sqrt{T}})) - e^X (1 - \operatorname{erf}(X/2\sqrt{T+\sqrt{T}})) \right] \quad (5)$$

where K is a constant proportional to the amplitude of the current step and

$$\operatorname{erf}(z) = \frac{2}{\sqrt{\pi}} \cdot \int_0^z e^{-w^2} dw$$

The resistive membrane current is given by

$$V_m/r_m \quad \dots\dots\dots (6)$$

which can be calculated from (5).

The capacitative current is given by

$$\frac{1}{r_m} \cdot \frac{\partial V_m}{\partial T} = \frac{1}{r_m} \cdot \frac{2K}{\sqrt{\pi T}} \cdot e^{-\left(\frac{X^2}{4T} + T\right)} \quad \dots\dots\dots (7)$$

A current pulse may be considered as a positive step superimposed by a later negative step of the same amplitude.

If V_s is any voltage associated with a unit step of current injection and V_p is the corresponding voltage associated with an infinitely short pulse, then (for $T > 0$)

$$V_p = \frac{\partial V_s}{\partial T} = \frac{2K'}{\sqrt{\pi T}} \cdot e^{-\left(\frac{X^2}{4T} + T\right)} \quad \dots\dots\dots (8)$$

where K' is a constant proportional to the total current flow.

The derivative of V_p with respect to time is thus

$$\begin{aligned}\frac{\partial V_p}{\partial T} &= \frac{2K'}{\sqrt{KT}} \cdot e^{-\left(\frac{X^2}{4T} + T\right)} \cdot \left(\frac{X^2}{4T^2} - \frac{1}{2T} - 1\right) \\ &= V_p \cdot \left(\frac{X^2}{4T^2} - \frac{1}{2T} - 1\right) \dots\dots\dots(9)\end{aligned}$$

and since $\frac{\partial V_p}{\partial T} = 0$ at the peak of the potential, then X and T are related (at the time of the peak) by the equation

$$X^2 = 4T^2 + 2T \dots\dots\dots(10)$$

(This identity was derived by A.L.Hodgkin for the endplate potential calculations of Fatt and Katz, 1951).

From (1) the membrane current following the pulse is

$$\begin{aligned}i_m &= \frac{1}{r_m} \cdot (V_p + \frac{\partial V_p}{\partial T}) \\ &= \frac{V_p}{r_m} \cdot \left(\frac{X^2}{4T^2} - \frac{1}{2T}\right) \dots\dots\dots(11)\end{aligned}$$

Thus the membrane current reverses at time T_0 , where

$$T_0 = \frac{X^2}{2} \dots\dots\dots(12)$$

Thus T_0 increases with X and comparing (10) and (12) we see that current reversal occurs after the peak of the intracellular voltage. Now from (11),

$$\frac{\partial i_m}{\partial T} = \frac{V_p}{r_m} \cdot \left[\left(\frac{X^2}{4T^2} - \frac{1}{2T} - 1\right) \left(\frac{X^2}{4T^2} - \frac{1}{2T}\right) - \frac{X^2}{2T^3} + \frac{1}{2T^2} \right]$$

Thus, if there is a depolarising current at the origin, then T and X are related at the maximum positive, or negative,

current respectively by:

$$x^2 = 2T^2 + 2T(3 \pm \sqrt{T^2 + 4T + 6}) \dots\dots\dots(13)$$

In particular, for $1 \leq x \leq 2$, the time of maximum positive current (T_{pos}) is given approximately by:

$$T_{pos} = \frac{x - 0.66}{4} \quad (\pm 0.01) \dots\dots\dots(14)$$

Thus, for $\tau = 8$ msec and $\lambda = 200\mu$ (and $1 \leq x \leq 2$), this current peak travels with a velocity of 0.1 m/sec.

Now from (13), $T_{pos} < x^2/10$ for all x , i.e. $T_{pos} < T_0/5$ for all x . In other words, the rise time of the current to its maximum is less than one quarter of its decay time to zero for all x . Therefore if a depolarising pulse at ($T = 0$) is followed closely by an equal and opposite hyperpolarising current at time ($T = b$), then the resulting current reversal time $T_0(b)$ will be more than $(T_{pos} + b/2)$ so that,

$$T_{pos} + b/2 < T_0(b) < T_{pos} + b \dots\dots\dots(15)$$

In particular, if $\tau = 8$ msec and $b = 0.3$ msec, then the time of current reversal is more than 0.83 msec for $x \geq 1$, and at $x = 2$ it is more than 2.83 msec.

Lastly, in the case of a finite cable of length d the membrane current (i_m^*) is given by reflection at $x = d$, i.e.

$$i_m^* = (1 + e^{-2d}) (i_m(x) + i_m(2d-x)) \dots\dots\dots(16)$$

That is, at the distal end of the cable (or dendrite) the membrane current has about double the value it would have if the cable was infinite.

Although equations (8) and (9) would be useful for calculations of resistive and capacitative current away from the origin, they cannot be used near the origin, where singularities occur, so equation (5) and expressions (6) and (7) were used for the calculations of field potentials shown in figures 22 to 24.

Field potentials in a volume conductor

The membrane current was averaged over $\tau/40$ periods (i.e. 0.2 msec) and over distances of $\lambda/20$ (10μ) following a square pulse generating current lasting $\tau/40$ (0.2 msec).

The mean capacitative current from T to $T + \tau/40$ (obtained by integration of equation (7), i.e. from equation (5)) is:

$$40 \left[V_m(T + \tau/40) - V_m(T) \right] / r_m \quad \dots\dots\dots (17)$$

The resistive current is a smooth (initially increasing) function of time, and the mean value (away from the origin) was taken to be:

$$\left[V_m(T + \tau/40) + V_m(T) \right] / 2r_m \quad \dots\dots\dots (18)$$

Similarly the mean membrane current from X to $X + \lambda/20$ (away from the origin) was taken to be:

$$\left[i_m(X + \lambda/20) + i_m(X) \right] / 2 \quad \dots\dots\dots (19)$$

Expressions (18) and (19) are only approximations, so near the origin ($X \leq 0.2$ and $T \leq 0.05$) a fine grid was used to improve these estimates; T was considered in steps of 0.005τ (0.01 msec) and X in steps of 0.01λ (2μ). It was then found that the error in the total current flow over a length of 16λ (seen as a non-zero nett current) was always less than 0.1%. This error was compensated for by an appropriate correction at the origin.

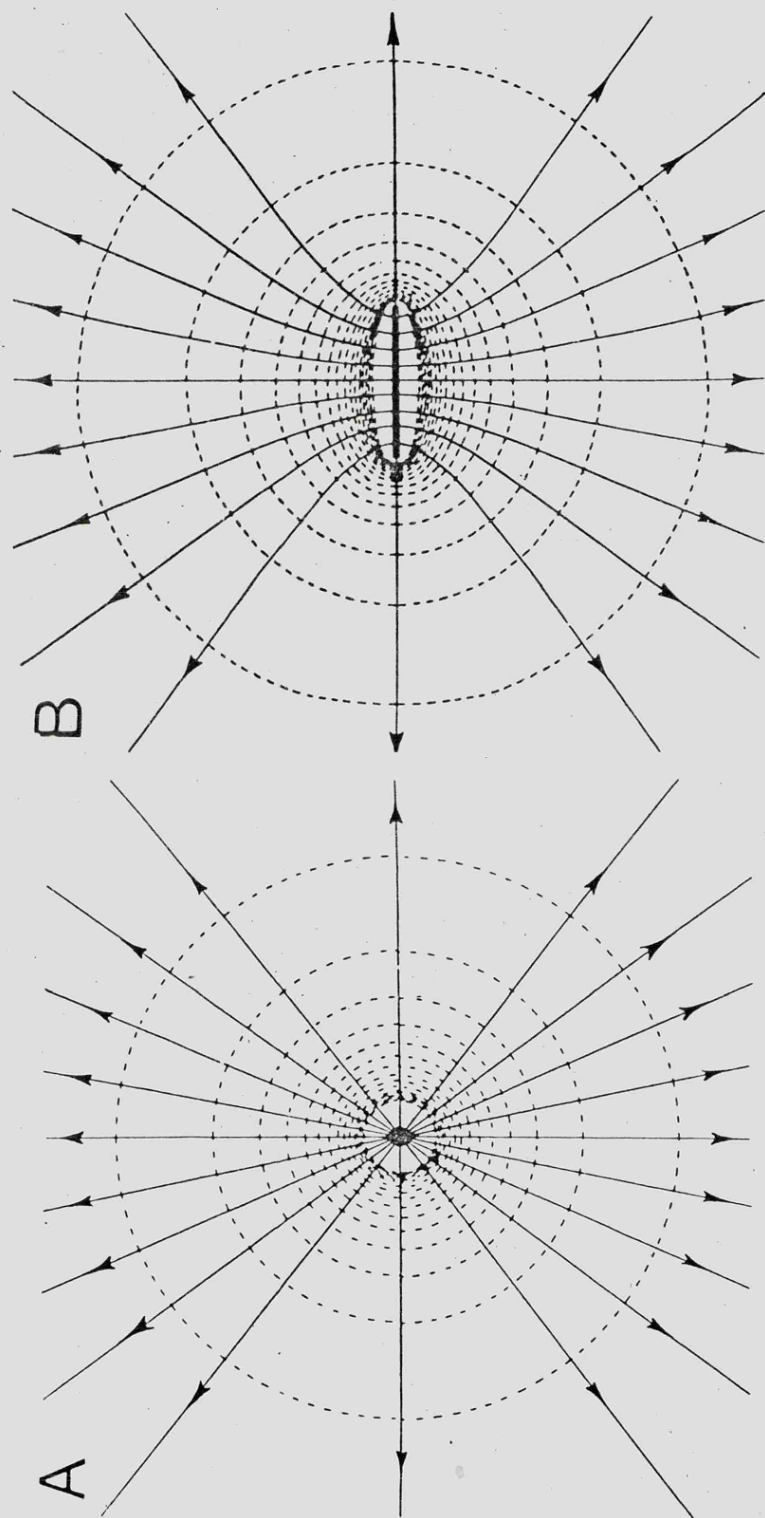


Figure 25. Potential fields and current flow lines surrounding localised sources in a 3D volume conductor.

A) a point source. B) a uniform line source. Dashed lines - equipotential lines (on a linear scale). Solid lines - current flow lines (also on a linear scale so that density of current is indicated by density of flow lines - see text for details).

Equation (16) was then used to allow for a finite dendritic length. The dendrite, spherical soma and axon were assumed to lie along a straight line (initially in an infinite volume conductor). The axis of symmetry and the centre of the soma were used to define the cylindrical coordinate system (R, z)

The electrical potential ϕ at any extracellular point (R, z) due to a current source of strength m at (O, X) is (Plonsey, 1964):

$$\phi = m / ((z - X)^2 + R^2)^{1/2} \dots\dots\dots (20)$$

The 3D irrotational flow of non-viscous incompressible fluid is analogous to the flow of electric currents through a volume conductor; since there are no vortices and no current lost from elements of volume outside regions of sources and sinks, both the electric potential (Hellerstein, 1968) and the velocity potential (Rutherford, 1959) must satisfy Laplace's equation. The description of 3D current flow is well developed in the field of fluid mechanics, so use was made of Stokes's Stream Function ψ (section 35 in Rutherford, 1959) in the calculation of current spread in figs. 21, 25 and 26. The stream function at (R, z) due to a current source of strength m at (O, X) is:

$$\psi = m \left[(z - X) / ((z - X)^2 + R^2)^{1/2} \right] - m \dots\dots (21)$$

The path of a stream line is then obtained by putting $(\psi = \text{constant})$; by definition, if streamlines are drawn on a linear scale (as in figs. 21, 25 and 26), then they divide the current flow into equal parts. Thus the density of streamlines indicates the density of current flow (e.g. the streamlines in fig 25A cut out equal conical shaped volumes when they are rotated about the horizontal axis of symmetry).

A linear superposition technique was used for the construction of more complex potential fields (and for the stream

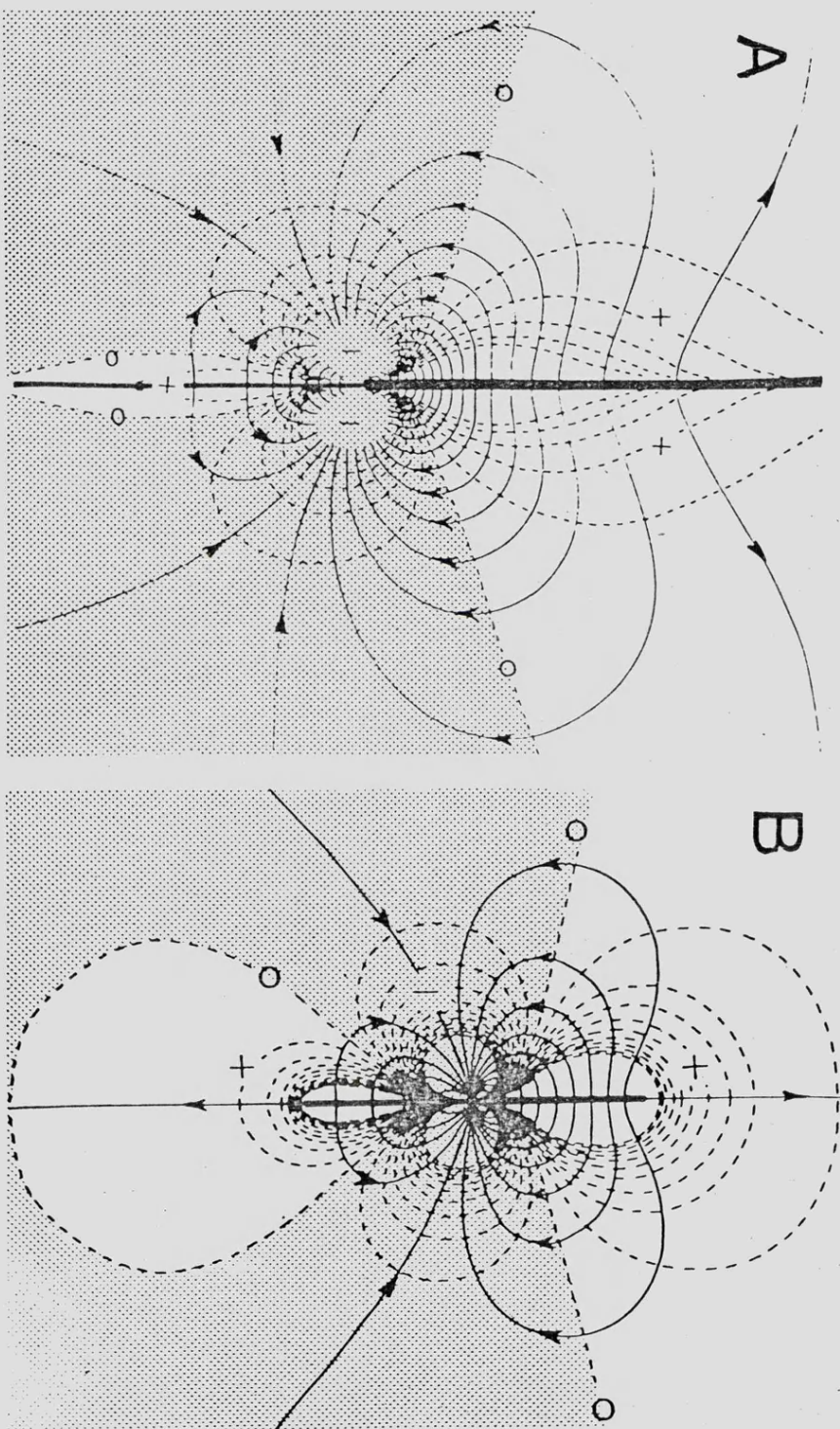


Figure 26. Combinations of sources and sinks along a straight line.

- A) two semi-infinite line sources and an equal and opposite point sink. The line sources were exponentially distributed and the upper was stronger than the lower by a factor of $2\sqrt{2}$.
- B) two finite uniform line sources (one twice the intensity of the other) and an equal and opposite point sink.

function, since sources and sinks were always along the axis of symmetry).

Asymmetrically distributed current sinks and sources give asymmetrically distributed field potentials (see fig. 26B where line sources, like that in fig. 25B, were combined with an equal and opposite point sink. The case of a leaky cable (fig. 26A) is similar to that of average electrotonic spread of current along dendrites; in both cases the distribution of current is exponential. Finally, to allow for a non-conducting cortical surface (as in figs. 21, 23 and 24), the usual method of images was used; the original potential or current field (infinite volume conductor) was added to its reflection in the flat cortical surface.

Estimation of effective external resistance

For large external volumes it is usual to avoid mathematical complications by setting the external resistance (σ_0) equal to zero in the estimation of membrane current or voltage distributions, or of dendritic length constants (Clarke and Plonsey, 1966; and Rall, 1969b), this gives a simple estimate of length constant ($\lambda = \sqrt{r_m/r_i}$ - Rall, 1969 a & b).

Rall (1969b) suggested that for typical values of the parameters the errors in this estimate would be less than 1%. However, the general theories concerning extracellular field potentials (e.g. Clarke et al, 1966; Hellerstein, 1968; and Rall, 1969b) are inherently complex and relatively difficult to apply to practical problems; it is often mathematically simpler to consider particular cases separately.

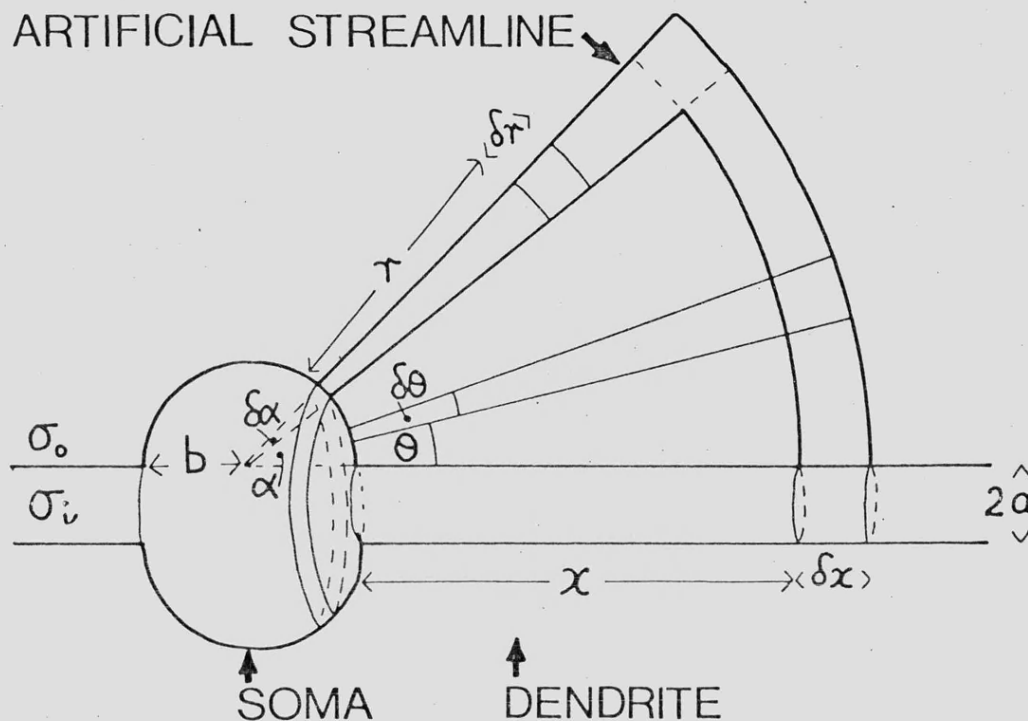


Figure 27. Geometry of Artificial streamline used in estimation of effective external resistance.

The soma of the model neurone is almost spherical: for convenience its surface is that swept out by a semi-circle of radius b rotated about the axis of symmetry, which is a distance a away. The width of the dendrite is $2a$. Current which flows out across the membrane at a distance x from the soma is constrained to flow outward along a spherical surface and then in along a conical one. The cone subtends an angle α with the axis of symmetry. The variables r and θ are used in integration of electrical resistance. The constants σ_o and σ_i are the external and internal specific resistivities.

The case considered here is that of a spherical soma and two equal semi-infinite dendrites lying in an infinite uniform volume conductor (see fig. 27). Current flows into the soma and out through the dendritic membrane. Extracellular current is constrained to flow along 'artificial' streamlines as shown. This enables direct estimation of external resistance using analytical rather than numerical methods. No streamlines cross each other and the whole extracellular space is used. The resulting external resistance is presumed to be higher in general than that during unconstrained flow and is used to estimate an upper limit for the 'real' external resistance.

The current entering the soma does so (almost) uniformly; the streamlines are distributed exactly as those in fig. 25A, (but they are displaced a small distance 'a' from the axis of symmetry). An equal amount of current leaves the dendrites and is distributed exponentially with length constant λ . Thus if a stream line leaves the dendrite at a distance x from the soma and enters the soma at an angle α with the axis of symmetry, there is a unique relation between x and α . Since the amount of current flowing into the soma between this streamline and the vertical one is proportional (by definition) to $\cos \alpha$ (from equation 21) and since the current flowing out of the dendrite beyond the distance x is proportional to $e^{-x/\lambda}$, then:

$$\cos \alpha = e^{-x/\lambda} \quad \text{and} \quad \sin \alpha \cdot \delta \alpha = \delta x \cdot e^{-x/\lambda} / \lambda \dots\dots (22)$$

The resistance along the spherical pathway (R_s) is given by:

$$R_s = \frac{\sigma_0}{2\pi \cdot \delta x} \int_0^\alpha \frac{d\theta}{y + \sin \theta}, \quad \text{where } y = \frac{a}{x + b}$$

$$\begin{aligned} \text{Thus, } R_s &= \frac{\sigma_o}{2\pi\delta x} \cdot \frac{1}{\sqrt{1-y^2}} \cdot \left[\log_e \left\{ \frac{y \cdot \tan \theta/2 + 1 - \sqrt{1-y^2}}{y \cdot \tan \theta/2 + 1 + \sqrt{1-y^2}} \right\} \right]_0^{\alpha} \\ &= \frac{\sigma_o}{2\pi\delta x} \cdot \frac{1}{\sqrt{1-y^2}} \cdot \log_e \left\{ \frac{y \cdot \tan \alpha/2 + 1 - \sqrt{1-y^2}}{y \cdot \tan \alpha/2 + 1 + \sqrt{1-y^2}} \cdot \frac{1 + \sqrt{1-y^2}}{1 - \sqrt{1-y^2}} \right\} \quad (23) \end{aligned}$$

$$< \frac{\sigma_o}{2\pi\delta x} \cdot \frac{1}{\sqrt{1-y^2}} \cdot \log_e \left(\frac{2}{y} \right) \quad \text{for all } 0 < \alpha < \pi/2.$$

$$\leq \frac{\sigma_o}{2\pi\delta x} \cdot \frac{1}{1 - \frac{a^2}{b^2}} \cdot \log_e \left[\frac{2(x+b)}{a} \right] \quad \text{for all } x \gg 0. \dots (24)$$

The resistance along the cone (R_c) is given by:

$$R_c = \frac{\sigma_o}{2\pi\delta x} \cdot \int_0^x \frac{dr}{(r+b)^2 \cdot \sin \alpha + (r+b)a} \dots (25)$$

$$< \frac{\sigma_o}{2\pi \sin \alpha \cdot \delta x} \cdot \int_0^x \frac{dr}{(r+b)^2} \quad (\sin \alpha, r, b, a \text{ are all +ve})$$

$$= \frac{\sigma_o \cdot \lambda \cdot e^{x/\lambda}}{2\pi\delta x} \cdot \left[\frac{1}{b} - \frac{1}{x+b} \right] \quad (\text{from (22)}) \dots (26)$$

Now we consider the almost one dimensional case where current flow from the dendrites is restricted along the inside of a concentric tube of diameter $2\sqrt{2}a$. Then the resistance per unit length is $\sigma_o/\pi a^2$ outside and $\sigma_i/\pi a^2$ inside the dendrite. The intensity of extracellular longitudinal current at a distance z ($0 \leq z \leq x$) is again proportional to $(\lambda e^{-z/\lambda})$. The small stream of current from the element δx has a relative strength of $(\delta x \cdot e^{-x/\lambda})$. Thus it encounters a resistance along a small length δz equal to $(\sigma_o \cdot \lambda \cdot e^{-z/\lambda} \cdot \delta z / \pi a^2 \cdot e^{-x/\lambda} \cdot \delta x)$. Thus the total resistance (R_t) from $z=0$ to $z=x$ is:

$$R_t = \frac{\sigma_0 \lambda e^{x/\lambda}}{\pi a^2 \delta x} \cdot \int_0^x e^{-z/\lambda} \cdot dz = \frac{\sigma_0 \lambda^2}{\pi a^2 \delta x} \left[e^{x/\lambda} - 1 \right] \dots (27)$$

Putting the values $a=1\mu$, $b=10\mu$ and $\lambda=200\mu$ into (24), (26) and (27),

$$\frac{R_s + R_c}{R_t} < \frac{1}{1000} \cdot \left\{ \frac{0.5 \log_e (400x/\lambda + 20) + e^{x/\lambda} \cdot \frac{(100 x/\lambda)}{(20x/\lambda + 1)}}{40(e^{x/\lambda} - 1)} \right\}$$

so that for $x/\lambda = (1/16, 1/8, 1/4, 1/2, 1, 2, \text{ and } 3)$,

$$\frac{R_s + R_c}{R_t} < \frac{1}{10000} (18.8, 11.6, 5.7, 3.9, 2.3, 1.5 \text{ and } 1.3).$$

Thus for all $x \gg 12.5\mu$, the external resistance in the 3D case is less than 2/1000 of that in the 1D case discussed.

Thus if the effective (3D) length constant is λ' and $\lambda_0 = \sqrt{r_m/r_i}$,

then:

$$\frac{\lambda'}{\lambda_0} > \sqrt{\frac{\sigma_i}{\sigma_i + 2\sigma_0/1000}}$$

Therefore:

$$\frac{\lambda'}{\lambda_0} > \frac{99}{100} \text{ if } \sigma_0 \leq 10 \sigma_i$$

That is, setting $\sigma_0=0$ gives an error in the length constant estimate which is less than 1% if σ_0 is less than about $10\sigma_i$.

Although the calculations here are concerned with limits rather than absolute values of external resistance, it is clear from (23) and (25) that R_s and R_c are dominated by the resistance near the membrane. Therefore, as the current flow through and near the membrane is distributed realistically, it is probable that (24) and (26) are close approximations to the true external resistance (with no artificial constraints).

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APPENDIX II: RECENT INTRACELLUR STUDIES

a) Synaptic organization in the rat somatosensory cortex.*

Rats were anaesthetized with urethane. Intracellular recordings were used to study spatial and temporal patterns of excitation and inhibition in cortical neurones following electrical stimulation of the contralateral forepaw. Procion Yellow dye was injected into some of the cells. (See fig.29 in next section and also Appendix IVb for technique).

The initial response was stable and localized (area diameter $< 0.5\text{mm}$): EPSPs and action potentials were seen in 17 cells including 3 stellate, (e.g. fig 41a) and 2 small pyramidal cells, $400\text{--}900\mu$ deep. This was followed by inhibition up to 2mm away (18 cells incl. 7 pyramidal cells, $700\text{--}1400\mu$ deep). The IPSP amplitude varied linearly with spontaneous or artificial membrane depolarization: slope -0.7 to -1.0 , reversal 2mV below resting potential. A slower spread of excitation across the same area led to a fine balance of excitation and inhibition in 16 other cells (incl. 1 fusiform - see fig.41b, and 3 pyramidal cells, $1200\text{--}1600\mu$ deep - e.g. fig 40a and b). Inhibition was enhanced by spontaneous or artificial depolarizations in these cells too. The variability of the resulting compound PSP was so great that the same stimulus could evoke suprathreshold responses from resting membrane and subthreshold responses from depolarized membrane. This modulation (apparently postsynaptic) can be simulated in a simple model neurone using the observed behaviour of evoked

* (From the Proceedings of the German Physiological Society September meeting in Vienna, Houchin 1975b. This work was done at the Max-Planck-Institute for biophysical chemistry in Göttingen, West Germany, with the support of the Wellcome Trust.

IPSPs. Thus, after the initial stable response, the action potential responses of neurones in the surrounding cortex could be 'turned off' if their postsynaptic membranes were already spontaneously depolarized.

b) A study with intracellular micro-electrodes of the interaction of spontaneous and evoked activity in the primary somatosensory cortex of the anaesthetized rat.*

In the rat anaesthetized with urethane, the form of cortical potentials evoked by electrical stimulation of the contralateral forepaw depends on the spontaneous background cortical activity (Bindman, Lippold and Redfearn, 1964a), as does the spread of evoked potentials across the cortex (Carter, Holmes and Houchin, 1969). The evoked discharge of some cortical units can be suppressed by spontaneous or induced increases in cortical activity, (Houchin, 1969b). I have been seeking an explanation of this suppression by making intracellular recordings.

Bindman et al (1964a) had already shown that stimulation of the cortex and stimulation of the forepaw gave similar variation of evoked potentials (see also fig. 28) and they suggested that a major part of the variability occurred within the cortex. In similar experiments at NIMR (unpublished results), I found that stimulation of the appropriate area of cortical white matter and stimulation of the forepaw gave very similar surface responses (as well as similar interaction with

* Largely from the Proceedings of the Physiological Society, Houchin, 1974, enlarged and illustrated. This work was done with a Wellcome Trust Fellowship at the Max Planck Institute for biophysical chemistry while on leave of absence from the National Institute for Medical Research, London.

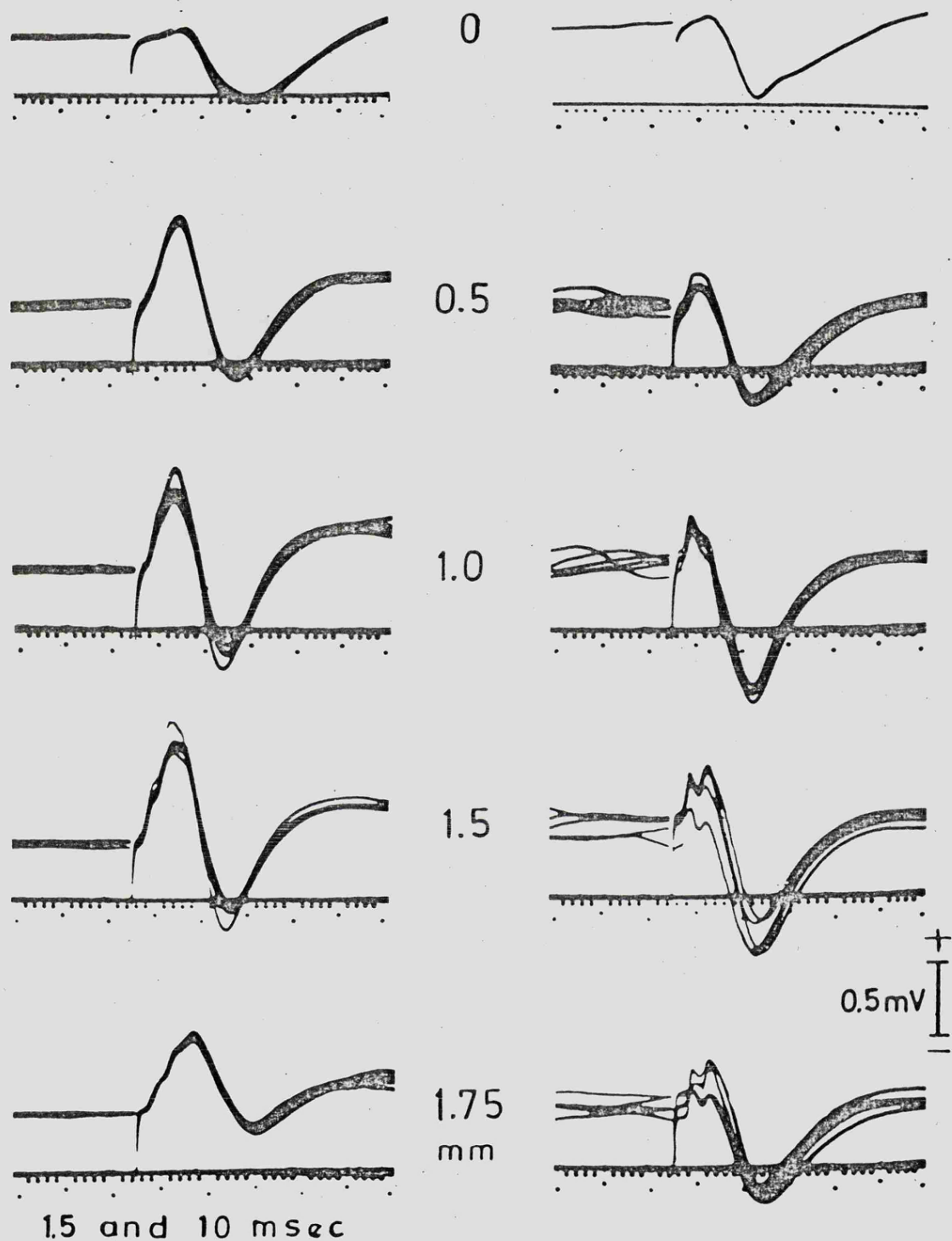


Figure 28. Surface potentials after stimulation of the cortex at different depths.

Laminar stimulation of the somato-sensory cortex of the rat 0.5 mm behind the forepaw receiving area with a steel micro-electrode of 3 meg ohms tip resistance. Records were taken from the surface above the forepaw area. Column (1) shows responses evoked when the ECoG was flat, in (2) those evoked by the same stimulus when the ECoG was active. A.C. recording with 0.5 sec decay time. Stimulus strength at 1.75 mm depth was 1.5 V and at all positions above, 2.0 V. Calibrations 1 msec and 0.5 mV. Upward deflections are positive.

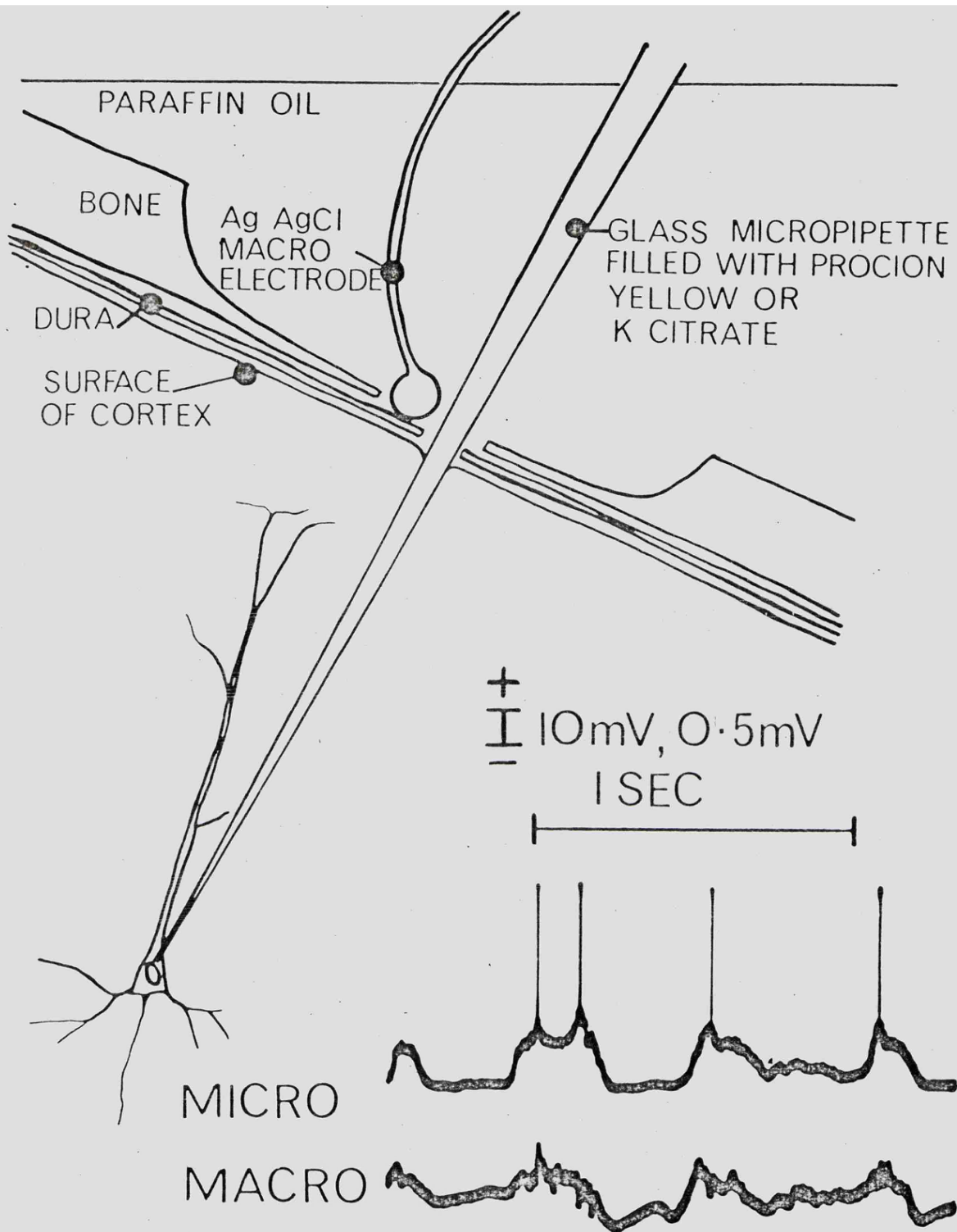


Figure 29. Cross section of thin bone window and small craniotomy used for intracellular studies.

Below is a record of intracellular recorded spontaneous activity together with simultaneously recorded surface ECoG. The intracellular record was taken at a depth of 1.6 mm from the fusiform cell shown in fig. 41B. (Urethane anaesthesia).

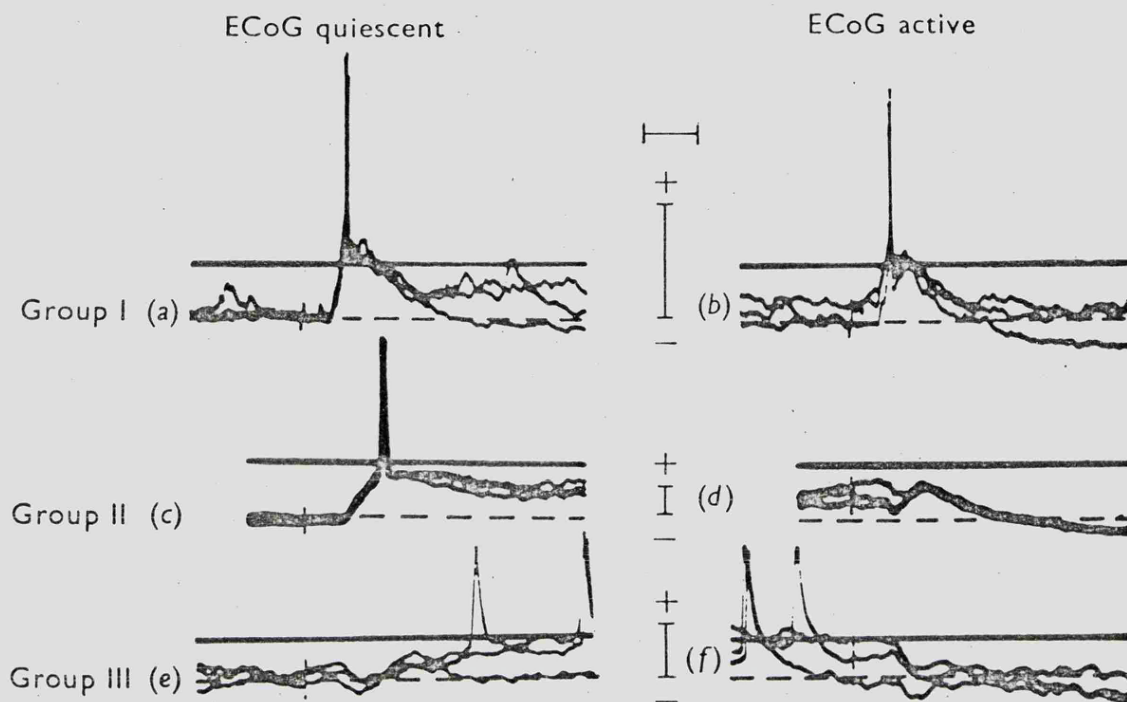


Figure 30. Intracellular records from the somatosensory cortex of the rat anaesthetized with urethane; postsynaptic responses after electrical stimulation of the contralateral forepaw.

a) and b): Superimposed records from a 'group I' (or P_1) cell responses to stimuli given when ECoG activity was absent and present respectively. This was a stellate cell at 900μ depth (see figure 41a); resting membrane potential -30 mV.

c) and d): Similar comparison of responses in a 'group II' (P_2) cell. This was a fusiform cell at 1600μ depth (see figure 41b); resting membrane potential -60 mV.

e) and f): A similar comparison in an (unexcited) 'group III' cell, 1200μ depth; resting membrane potential -35 mV (K citrate electrode).

Voltage calibration 10 mV. The resting level and approximate threshold level are indicated by broken and solid lines respectively. Time calibration 10 msec.

with spontaneous activity). (Compare lower traces of fig. 27 with those of fig. 6a). In these exploratory experiments, I also found similar surface and depth profiles as well as similar spatial and temporal patterns of unitary activity. These results suggested that the spread of excitation through the cortex and the variability of the evoked responses were both cortical phenomenon, and that there was a good chance of revealing useful information about the mechanism of variability by making intracellular recordings within the cortical grey matter.

Data were obtained from 51 cortical cells with glass micropipettes filled with either 2m K citrate solution or 4% Procion Yellow (see Appendix IVb for procedure). There was a net depolarization of the membrane potential in all cells during bursts of ECoG activity*. In quiescent periods the membrane potential returned to a steady resting level.

When electrical stimuli (just insufficient to cause a reflex contraction) were applied to the contralateral forepaw, seventeen cells (group I) were found showing stable evoked excitatory postsynaptic potentials (EPSPs) which always reached threshold for spike initiation (see fig. 30 a and b).

Sixteen other cells (group II) showed evoked EPSPs but they only gave evoked spikes when the stimuli were applied during times of cortical quiescence. This absence of evoked spikes during spontaneous activity was due to a reduction of the evoked synaptic response to subthreshold levels (compare fig. 30 c and d).

* See fig. 29.

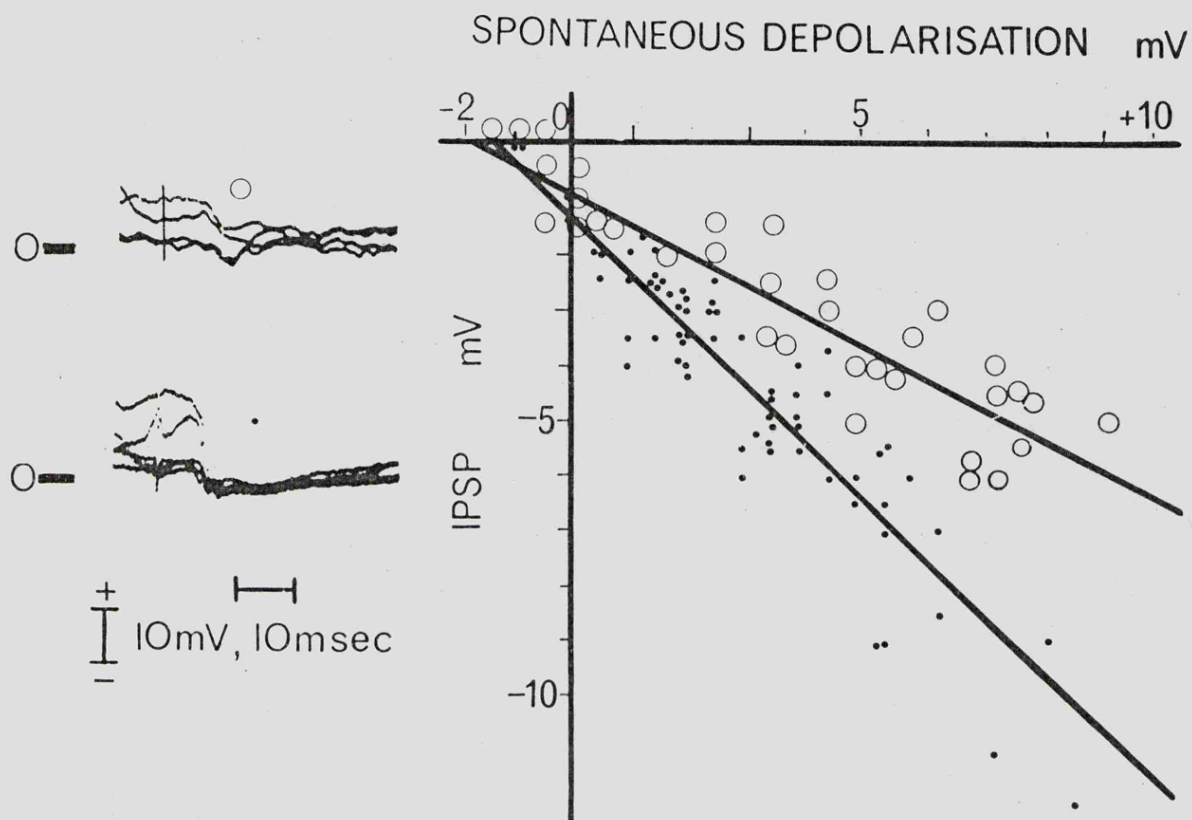


Figure 31. Linear dependence of evoked IPSP amplitude on spontaneous membrane depolarisation.

Records of IPSPs from two different cells are shown on the left. Individual IPSPs from these two cells are represented by individual dots or circles on the diagram. The IPSP amplitude was measured relative to initial membrane potential, and the initial spontaneous depolarisation (immediately before response) was measured relative to the apparent resting membrane potential. Stimuli to contralateral forepaw. Urethane anaesthesia.

The remaining eighteen cells (group III) responded to forepaw stimulation with evoked hyperpolarizations (IPSPs) (see fig. 30 e and f). The amplitude of the evoked IPSP increased linearly if there were spontaneous increases of background depolarizations of the cell membrane (see fig. 31). Artificial depolarization by means of intracellular current injection, produced similar variations of evoked IPSP amplitude, suggesting a postsynaptic mechanism (see fig. 32^a_b).

The decrease in amplitude of the evoked EPSPs in group II cells was similar to the increase in amplitude of evoked IPSPs in group III cells for any given increase in background depolarization, and could perhaps result from the superimposition of an enlarged IPSP like group III on a stable EPSP (like group I). This idea is supported by the results of a few experiments in which the membrane potential of group II cells was artificially depolarized and hyperpolarized by means of intracellular current injection (see fig. 32^b). In such experiments the effects of spontaneously occurring potentials could be reversed by artificial changes in potential of the same or very similar amplitude. The relationship between injection current and resulting changes in membrane potential were linear in the range considered (up to about 2 nA).

Fig. 34^a shows how the variability of the response of the group II cells might arise postsynaptically. If such a cell received excitatory and inhibitory inputs simultaneously,

POLARISATION OF A GROUP III CELL (EC₆G QUIESCENT)

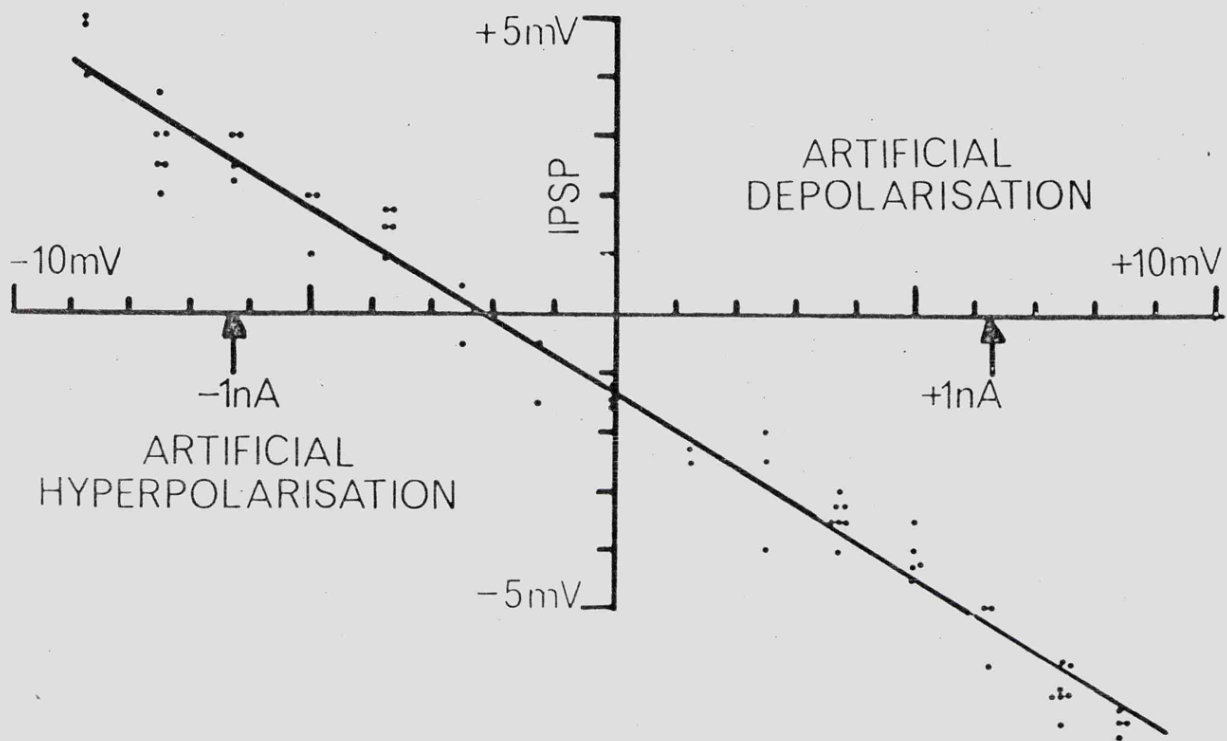


Figure 32a. Linear dependence of evoked IPSP amplitude on artificial depolarisation or hyperpolarisation of membrane.

Data from another cell plotted in a similar way to that of fig. 31. Stimuli to contralateral forepaw. Urethane anaesthesia.

POLARISATION OF A GROUP II CELL

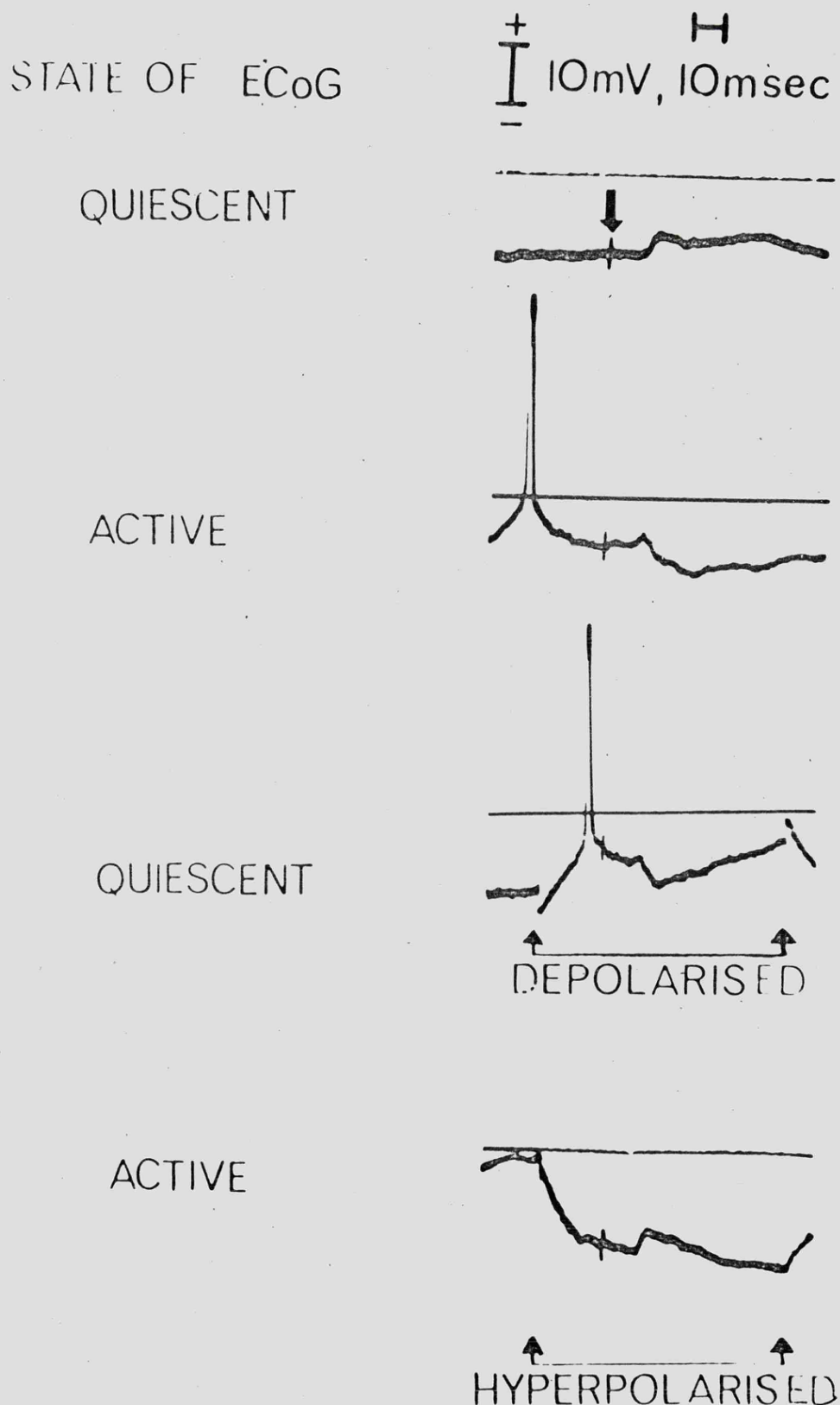


Figure 32b. Postsynaptic modulation of a compound evoked PSP in a group II cell (P_q unit).

To show similar effects of spontaneous changes in background membrane potential and the artificial changes induced by brief injection of 1.3 nA through the recording electrode. Solid line - a reference level near threshold. (Urethane anaesthesia).

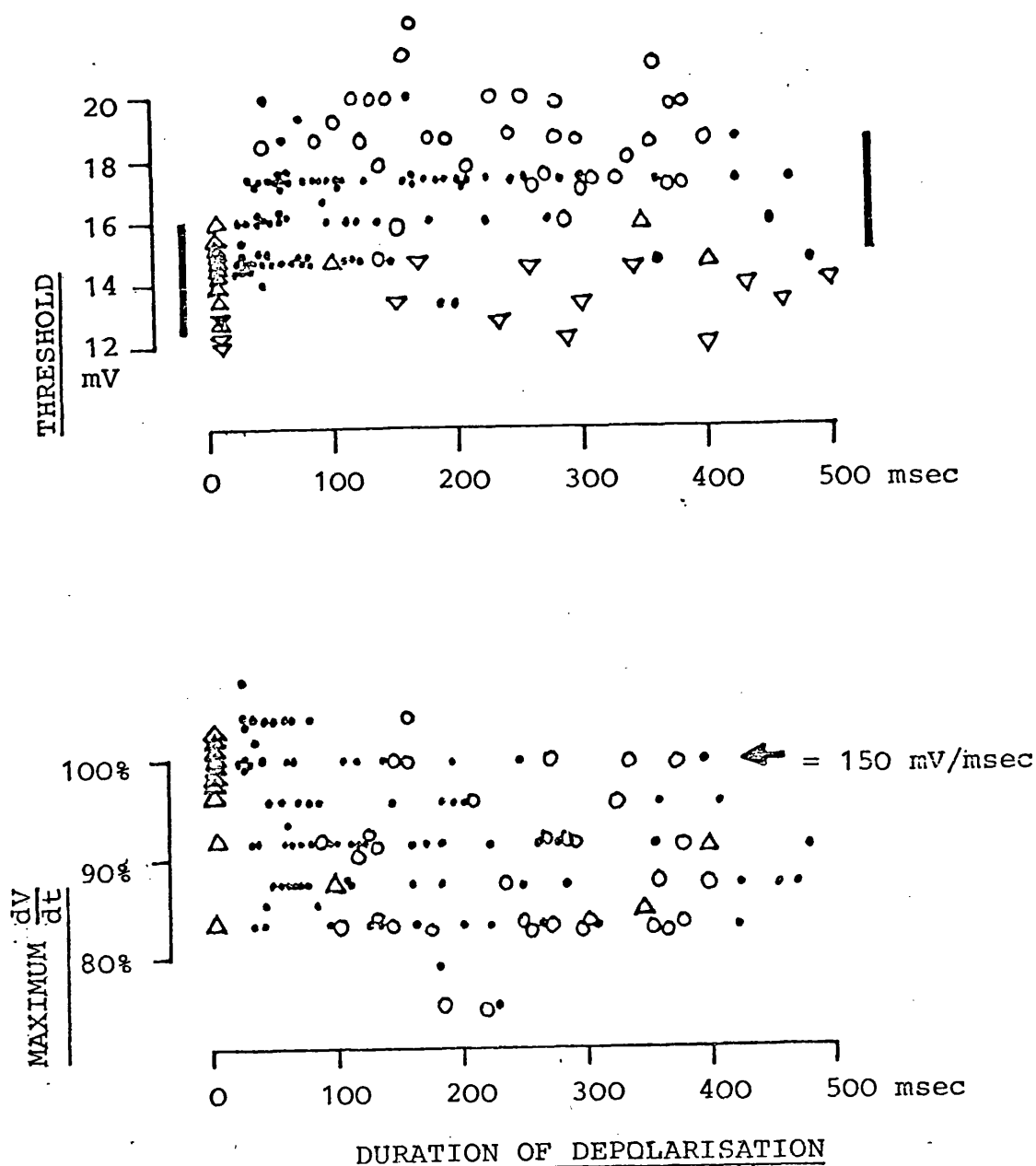


Figure 33. Attempt to test for accommodation in a P_q unit.

Analysis of a 2 minute record from the cell of Figs. 29, 30c&d and 41b.

I. Thresholds of evoked action potentials (Δ), of first ($\bullet$) and subsequent ($\circ$) spontaneous action potentials plotted against time after onset of a spontaneous depolarisation (or, on l.h.s., of an evoked depolarisation). The threshold of initially resting membrane was very near the peak of the synaptic response (see Fig. 30c). Downward pointing triangles (∇) indicate the size of synaptic responses which failed to reach threshold. Potentials are given relative to the resting membrane potential.

II. The recording of (I) was replayed through a high pass filter (> 10 kc/s). The height of the first (+ve) component of the resulting 'differentiated' action potential was used as an estimate of the maximum rate of rise of the action potential (maximum dV/dt). These estimates are plotted as percentages of that which corresponded to a 150 mV/msec slope. Symbols and time scale as in (I).

then the resulting compound 'XPSP' would appear to have an equilibrium potential somewhere between the EPSP equilibrium and the IPSP equilibrium. If these inputs were strong and synchronised enough, the peak of this XPSP could be very near this equilibrium, regardless of the initial membrane potential. That is, the slope of the XPSP line could be very near -1 (as in figure 34a). If, as in the experiments, the mixture of excitation and inhibition was such that this equilibrium was near spike threshold, then only a small change in this threshold would be needed to explain the suppression of action potential responses.

I have not yet been able to test for accommodation systematically. The instability of recordings, the polarising potentials which appear at the electrode tip during artificially induced depolarisations, and the tendency for cells to fire more rapidly (i.e. abnormally) after impailment are some of the problems which make such tests difficult. Extracellular negative waves and intracellular depolarisations were so closely related during both spontaneous and evoked activity, that even if absolute membrane depolarisations were 5-10% underestimated, it is unlikely that relative changes in membrane potential, or threshold, were distorted significantly. However, the record from the cell of figures 29, 30c&d and 41b was relatively stable, so a 2 minute section of this record was analysed in detail (see figure 33). There appeared to be small accommodation effects (increase in spike threshold and decrease in dV/dt), but these effects were almost hidden by the overall variability in the data. The 2 to 3 mV

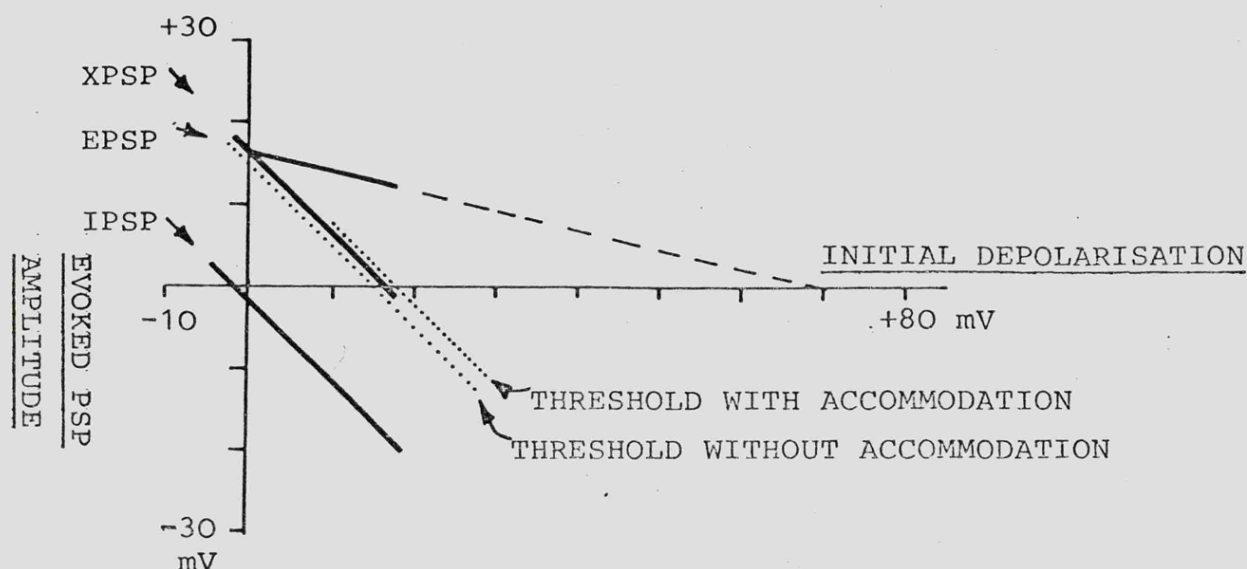


Figure 34a. Modulation of evoked PSPs by changes in initial membrane potential in a model neurone.

Relative PSP amplitude is plotted against initial membrane depolarisation (as in Figs. 31 & 32a). The lower thickened line represents a large evoked IPSP such as that seen in Fig. 31. The upper thickened line represents a relatively weak but supra-threshold EPSP. A constant threshold, such as that seen in the absence of accommodation might be represented by the lower dotted line. Points above and below this dotted line therefore represent supra- and sub-threshold PSPs respectively. If the cell membrane has been depolarised for say 50msec or more (to near threshold levels) then the threshold level might increase say 2-3mV. This new threshold could then be represented by the upper dotted line.

If there was a mixture of strong and synchronised inhibitory and excitatory inputs, then the resulting compound evoked synaptic potential (XPSP) might be represented by the middle thickened line. In this case the XPSP would initiate an action potential if the membrane was initially resting, but it would fail to do so if the membrane had been depolarised to near threshold levels for more than about 50msec.

increase in threshold which is apparent after 50 or more msec depolarisation would be sufficient to complete the model in figure 34a. Thus, the evoked XPSP can reach suprathreshold levels when evoked from a resting membrane, even if it fails to reach threshold from an initially depolarised membrane.

Although this model is very simple and requires no elaborate wiring diagram or presynaptic gating, it has also been useful in the context of direction specificity as discussed in the next section.

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APPENDIX III : A MODEL NEURONE

Direction specificity in cortical responses to moving stimuli - a simple model.*

Many neurones in the auditory (Whitfield and Evans, 1965), somto-sensory (Whitsel, Roppolo & Werner, 1972) and visual cortex (Hubel & Wiesel, 1959) exhibit direction specificity in their responses to moving stimuli. Special wiring diagrams (Whitsel et al) or inhibition of presynaptic elements (Bishop, Coombs & Henry, 1971) could explain direction specificity, but a simpler model is considered here. Malsburg (1973) showed that, in theory, even the patterned mosaic of columns of orientation detectors across the visual cortex could arise from randomly connected afferent fibres. This fact, together with the disorganized anatomical appearance of the cerebral cortex, prompted the construction of this simple single neurone model.

It is simulated on a DIGICO MICRO-16 P computer. The hypothetical neurone is effectively bombarded with synaptic inputs by entering a sequence of number-pairs on the teletype. Each number-pair represents the strength of excitation (E) and inhibition (I) per unit time. A MATHCHAT programme instructs the computer to calculate the resulting changes in postsynaptic membrane potential, using the assumptions below which are essentially the same as those used in the previous section (see fig. 34A): the main difference here is that the PSP amplitudes are smaller.

* From the Proceedings of the Physiological Society, Houchin 1975a, enlarged and illustrated. This work was done at the National Institute for Medical Research, London.

- 1) The amplitude of each inhibitory postsynaptic potential (IPSP) increases with background membrane depolarization, reversing near the resting membrane potential. This, the only important assumption, reflects the observed behaviour of cortical and other IPSPs (Watanabe, Konishi & Creutzfeldt, 1966; Eccles, 1964 respectively). For simplicity the increase is linear.
- 2) In a similar way, the amplitude of each excitatory postsynaptic potential (EPSP) decreases linearly with background membrane depolarization, reversing near zero membrane potential.
- 3) For simplicity of calculations, PSPs decay exponentially, spatial organization and attenuation of PSPs are ignored, all potentials are added linearly and spike threshold is constant.

(The unitary components are sufficiently small (about 1 mV at spike threshold) and the inputs so asynchronous that this linear summation of potentials does not deviate significantly from the potentials obtained from a more realistic summation of conductance changes. Recent trials with the improved conductance change model indicate that all the results presented here are valid, in particular the clear distinction between the sub- and suprathreshold responses remains unchanged)

APPROACH TO STEADY STATE WITH UNIFORM
INPUTS OF (+4,-4) AND (+8,-8) UNITS / HALF MSEC

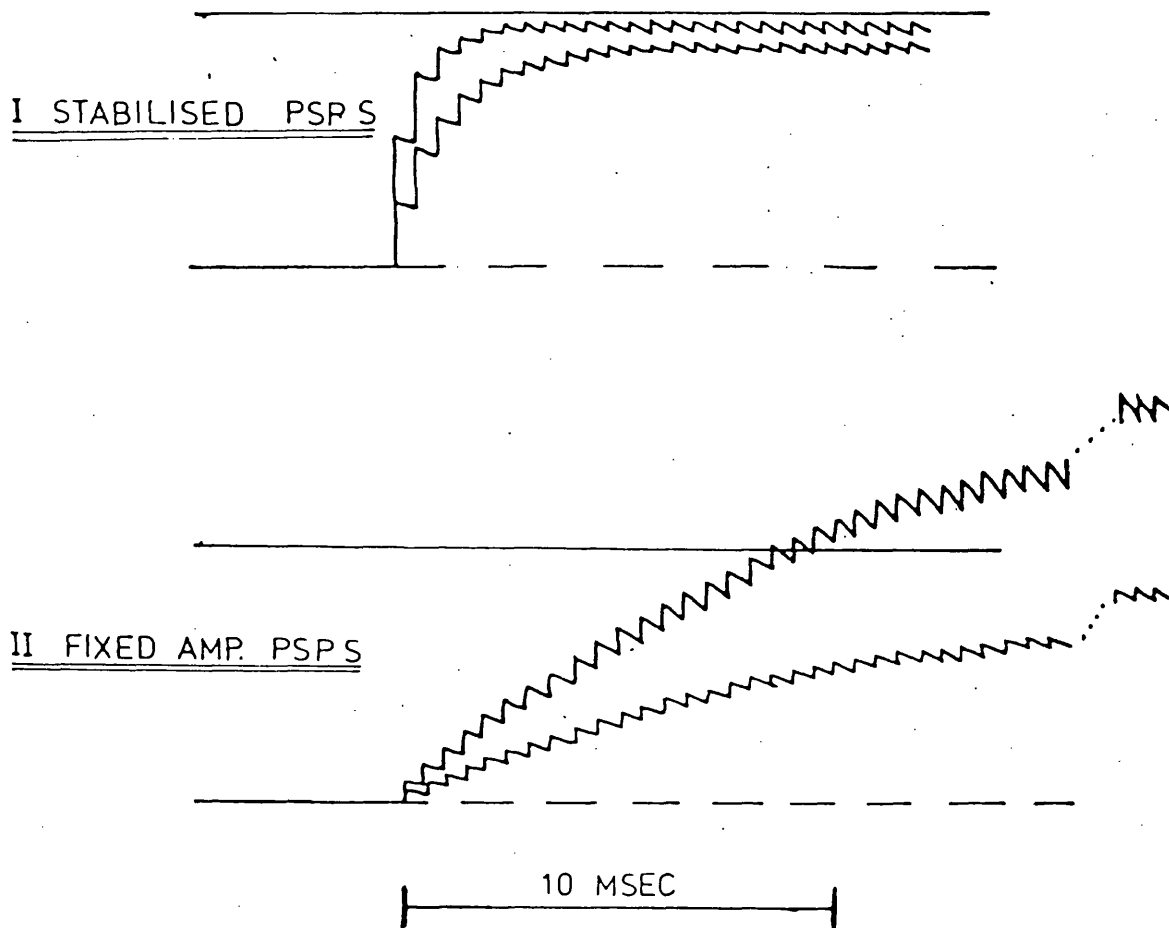


Figure 34b. Response to a step change of input of mixed excitation and inhibition.

General Conditions (for this and subsequent figures).

Dashed line indicates resting potential, and solid line at +20 mV depolarisation, indicates threshold.

Stabilised IPSPs and EPSPs have equilibrium potentials near resting and zero membrane potentials as in fig. 34a. Unit PSPs have an amplitude of 1 mV at threshold level. Constant amplitude IPSPs and EPSPs have unit values of -0.8 mV and +1 mV respectively. Time constant of membrane taken as 10 msec for EPSPs and IPSPs.

- I Stabilised PSPs give sharp rise compared with membrane time constant, and doubling the amplitude of input does not greatly change the final membrane potential.
- II Fixed amplitude PSPs give a slow exponential rise reflecting the membrane time constant. Doubling the input amplitude (or frequency) gives a double amplitude depolarisation.

General behaviour of the model

The first assumption implies a strong negative feedback, giving the model an inherent stability which counteracts the integrative action of the membrane capacitance. Rapid changes of input (represented by the difference E minus I) are followed accurately by changes in the membrane potential. The membrane potential is relatively insensitive to slow changes of input (or to the intensity of a steady input). The change in membrane potential following a step change in input has a rise time which is considerably less than the time constant of the

Responses to moving stimuli

Stimulus movement across a receptive field is represented by an appropriate sequence of synaptic inputs and reversed motion by a reversed sequence.

Grossly asymmetrical patterns of mixed excitation and inhibition can give direction specific action potential responses (see fig. 35a). However, more interestingly, so can small asymmetries (fig. 35b and 36), even those occurring in a random pattern (fig. 38). The inherent stability of the model is reflected by a relative insensitivity to both velocity of movement (fig. 37) and superposition of steady (fig. 35c) or slowly changing background activity. The model only reacts to significant changes in the E/I ratio, and the preferred

* See fig. 34B.

** See Fig. 34A.

direction is one in which there is an increase in E/I immediately before a decrease in E/I. The stability and reliability of responses is lost if assumptions (1) and (2) are omitted, i.e. if modulated PSPs are replaced by constant amplitude PSPs (see CONTROL, figs. 35 to 38). The model can also act as an expansion or contraction detector, analogous to a neurone which gives direction specific visual responses to movement of objects towards or away from the animal in a sagittal plane. This occurs if the region(s) of asymmetry in the receptive field are curved (convex outwards) with inhibitory regions outermost or innermost respectively. The direction specificity of the model responses can take a general form, independent of size or position of the stimulus within the receptive field. This is because the asymmetries can be very small and numerous.

Thus, if a neurone has small asymmetries in its receptive field, simple postsynaptic modulation of potentials is sufficient for reliable and sophisticated direction specific responses; complicated wiring diagrams involving gross anatomical or functional asymmetries, presynaptic inhibition, etc. are not necessary. The application of this theory to cortical responses is supported by recent findings of Sillito (1974); blocking of GABA mediated inhibitory inputs to simple cells in the visual cortex abolished the direction specificity in their responses to movement.

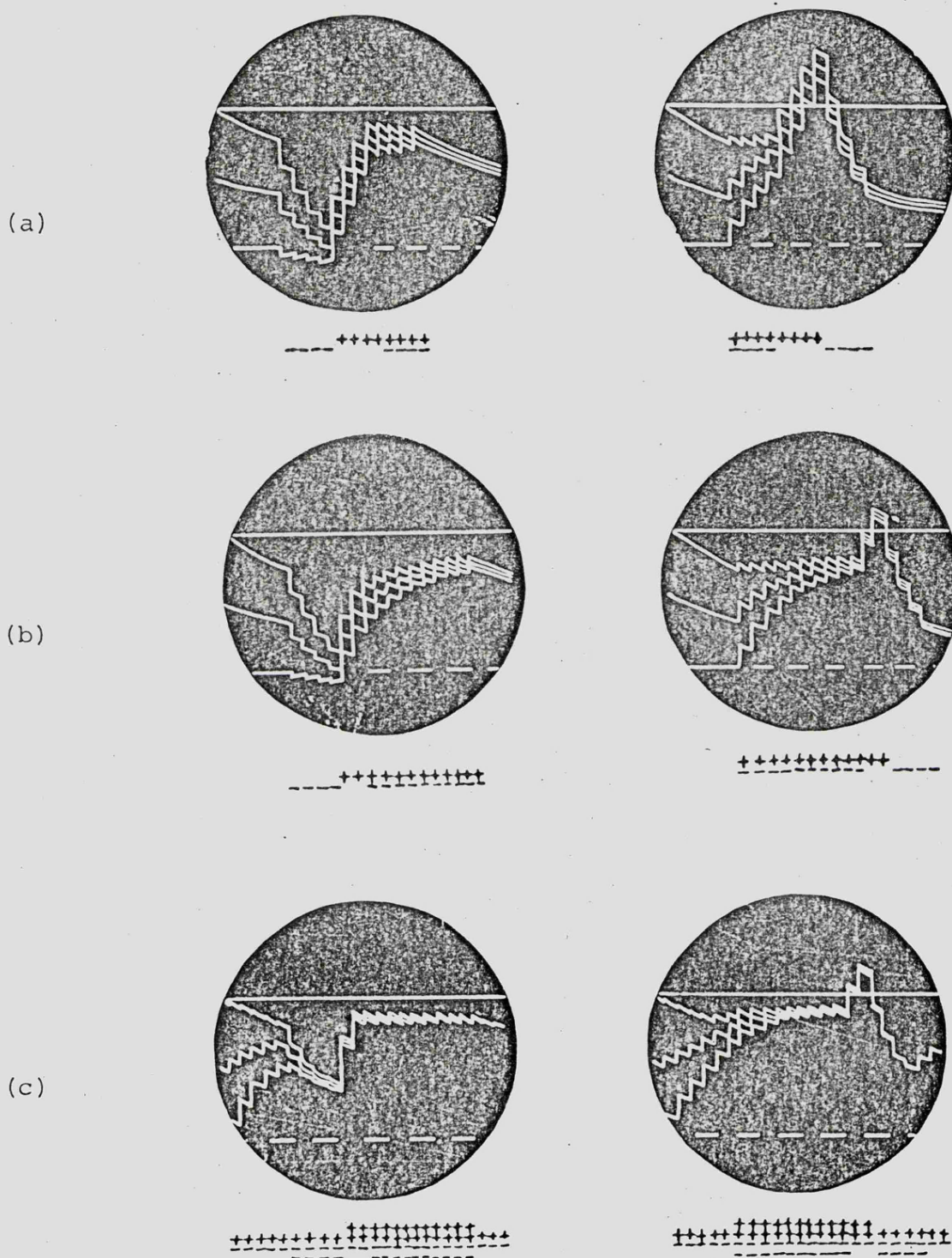


Figure 35. Model responses to various input patterns (stabilised PSPs).

- Input patterns - as indicated below each group of superimposed responses. Each +/- indicates 2 units of excitation/inhibition per half msec. Three different starting conditions are considered with each input pattern.
- a) a grossly asymmetrical input pattern and its reverse in time.
 - b) a smaller asymmetry.
 - c) same as (b) but with a uniform mixture of excitation and inhibition superimposed.

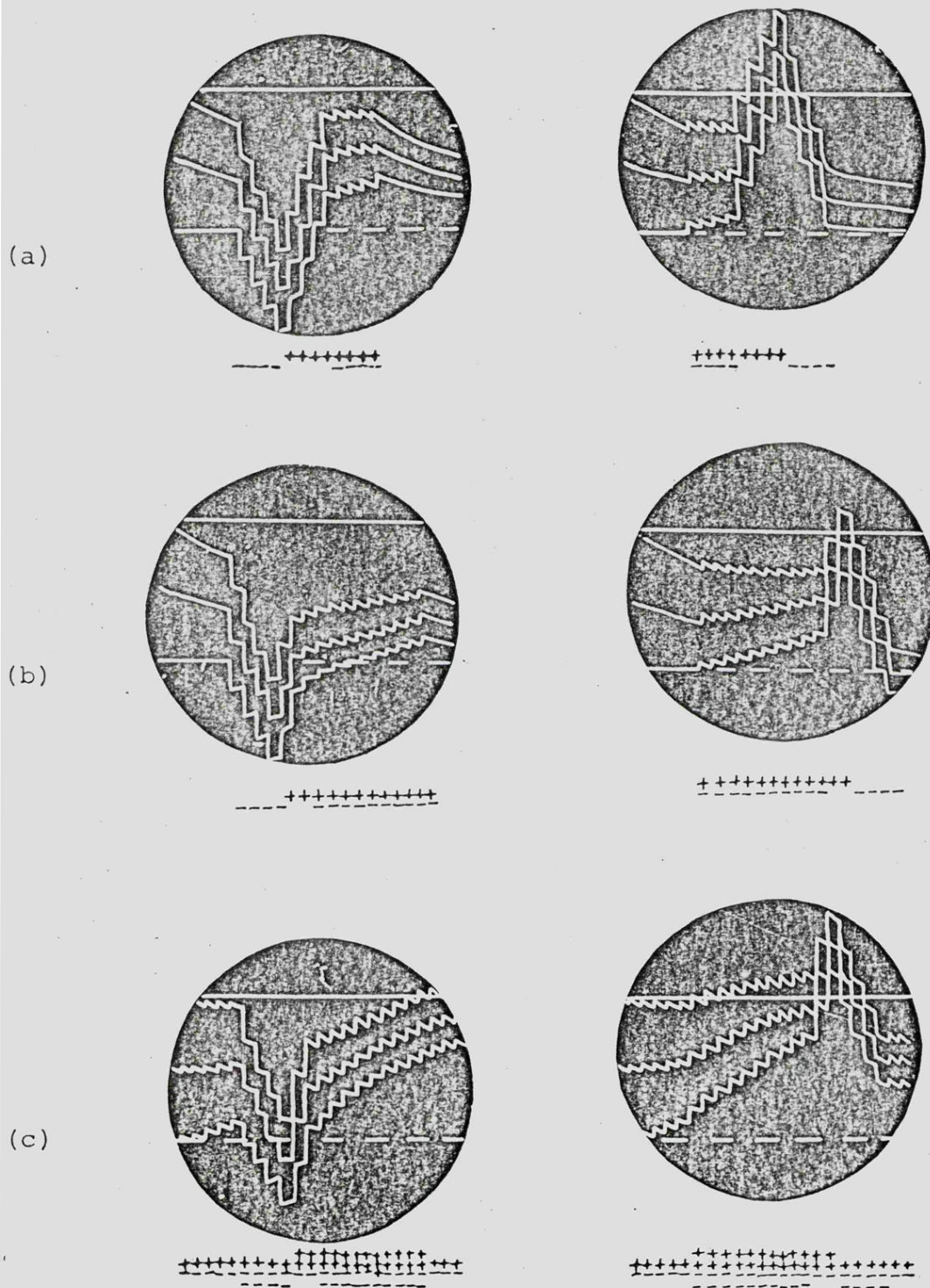


Figure 35. (CONTROL) Responses to various input patterns (constant amplitude PSPs).

As fig.35 but with constant amplitude PSPs instead of stabilised ones - to show importance of PSP modulation for stability and reliability of response to any particular input pattern..

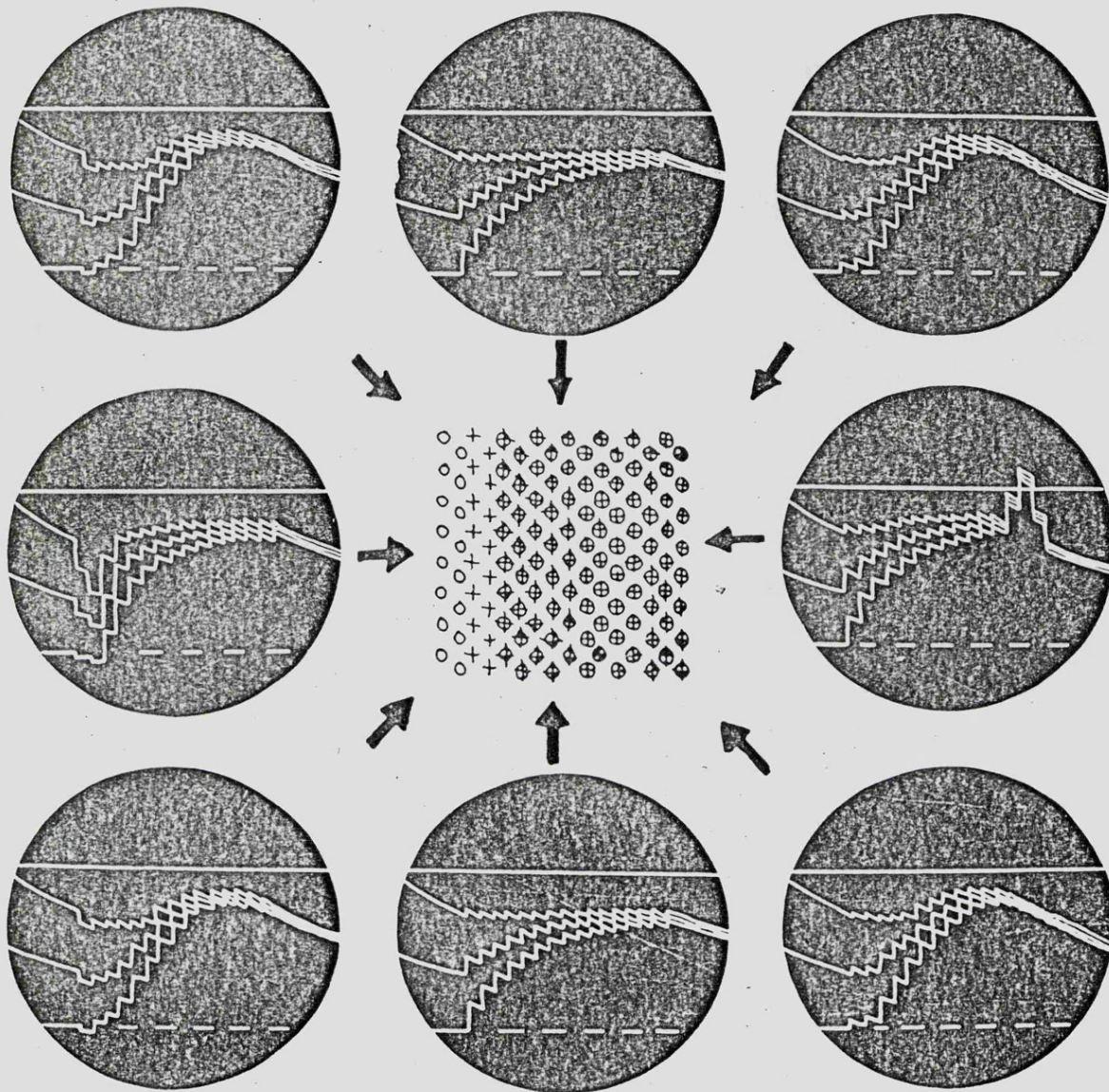


Figure 36. Direction specific responses of model to a bar stimulus moving across a 2D receptive field. (Stabilised PSPs).

Excitatory and inhibitory points in the receptive field are indicated by (+) and (o) respectively.

Velocity of movement is same in each direction; 2 rows per msec in horizontal and vertical directions and $\sqrt{2}$ rows in $\sqrt{2}$ msec for diagonal movements.

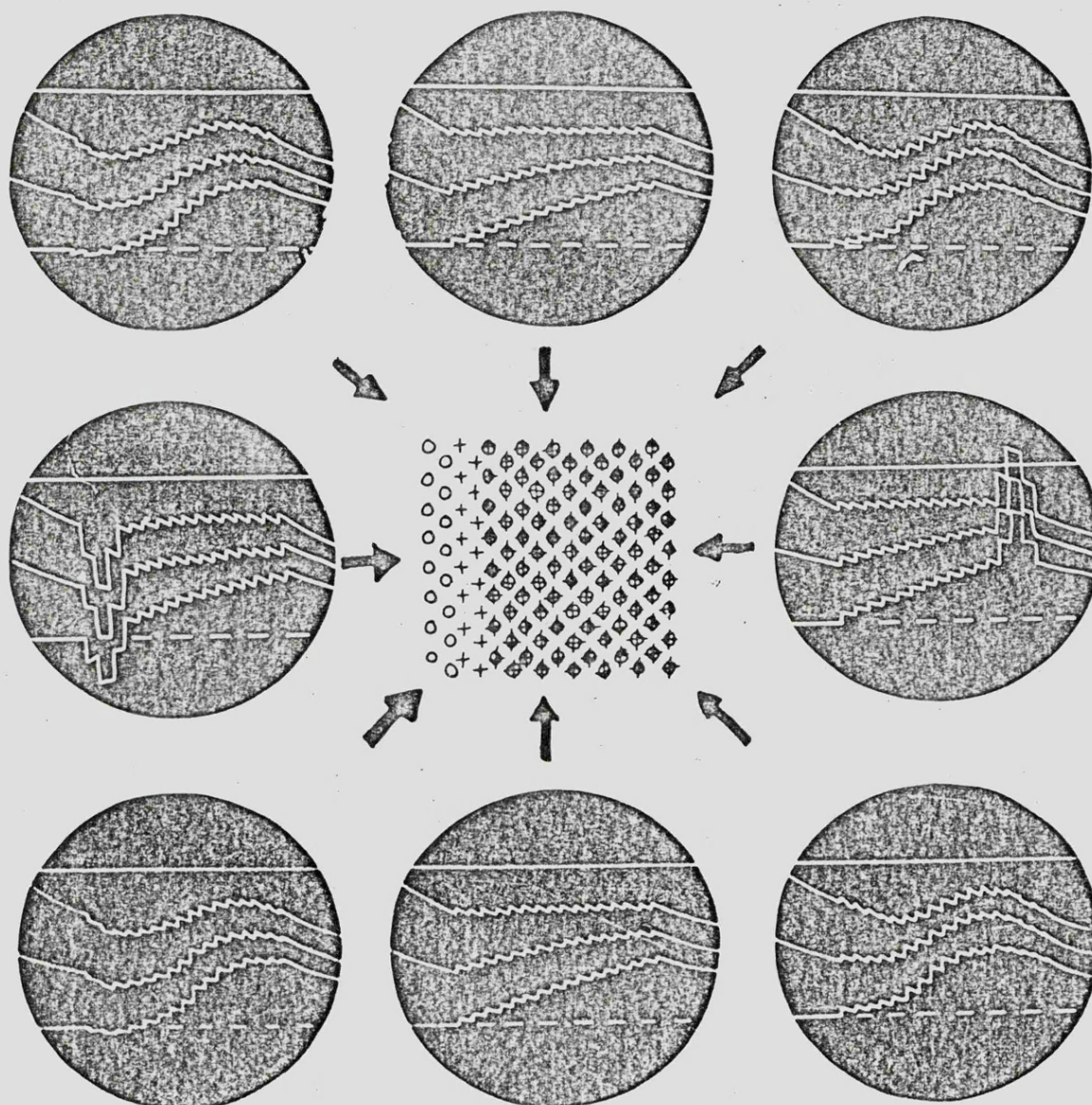


Figure 36. (CONTROL) Responses to a bar stimulus moving across a 2D receptive field (constant amplitude PSPs).

As fig.36 but with constant amplitude PSPs instead of stabilised ones.

Suprathreshold response depends on initial conditions.

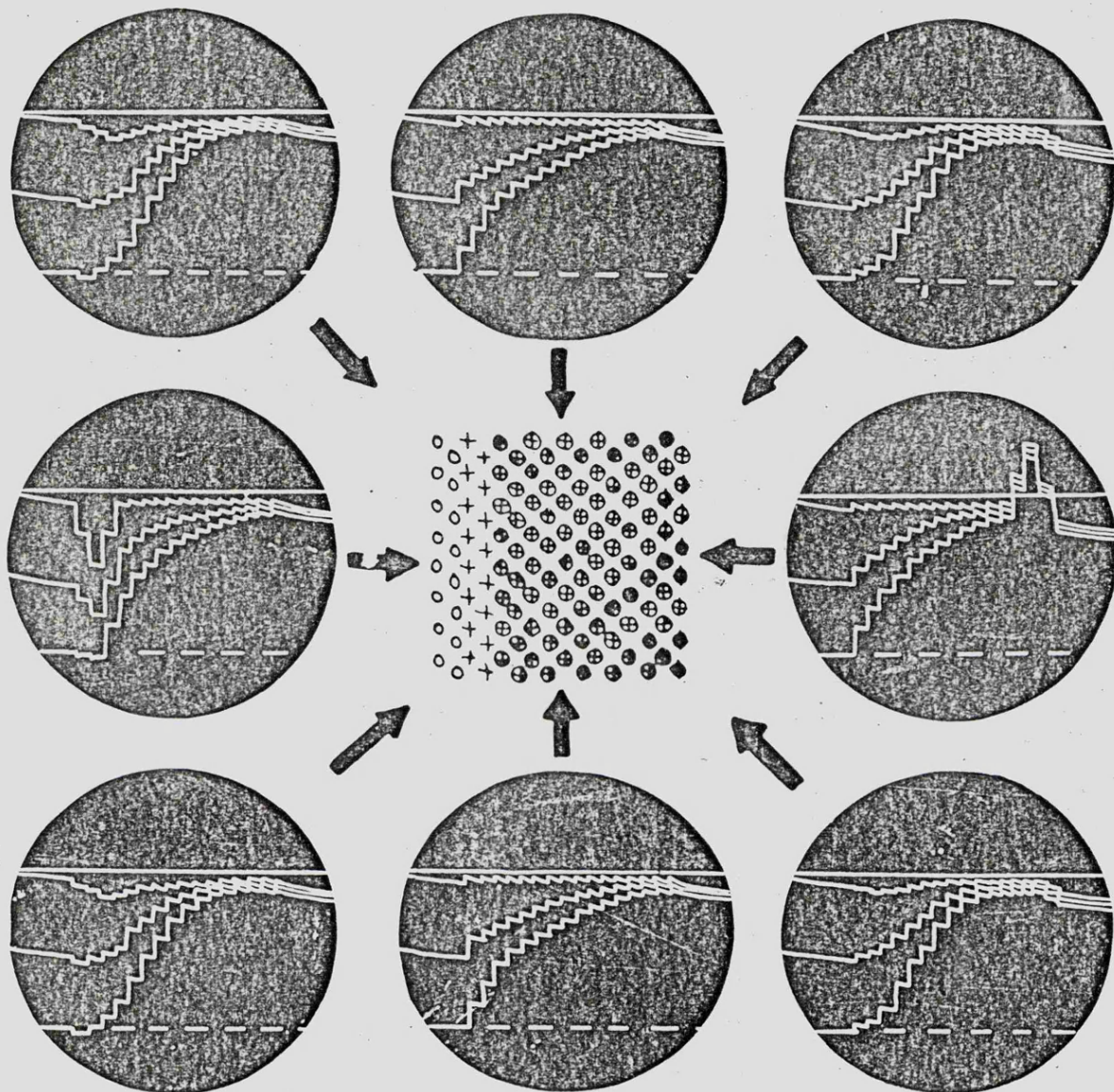


Figure 37. Relative insensitivity of model responses to velocity of moving bar (stabilised PSPs).

Same conditions as in fig. 36 but here velocity of stimulus is increased five-fold.

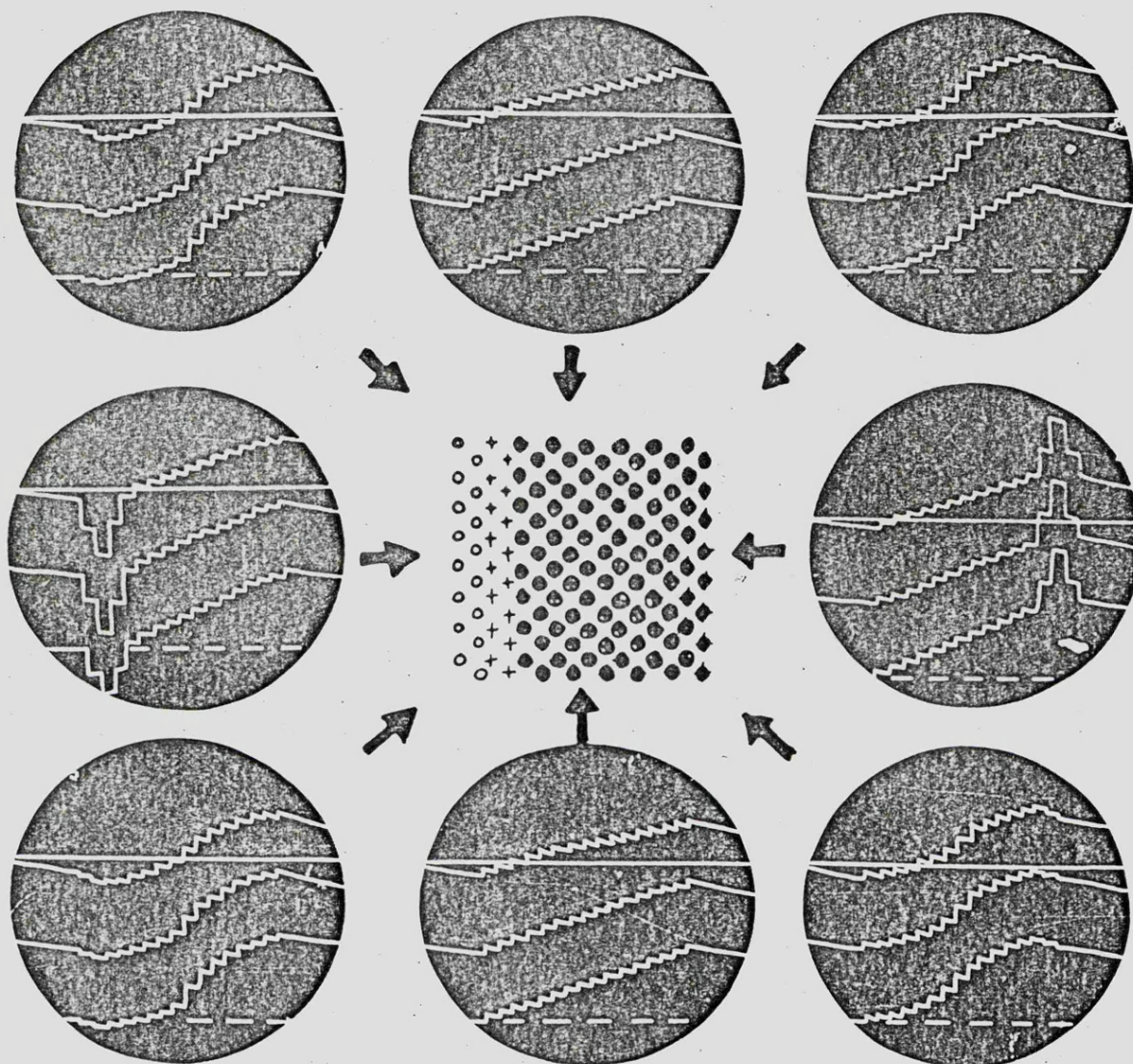


Figure 37. (CONTROL) Instability of control responses when velocity of movement is increased (constant amplitude PSPs).

Same conditions as in fig.36 (CONTROL) but here velocity of stimulus is increased five-fold. Suprathreshold responses depend more on velocity of movement and initial conditions than on direction of movement.

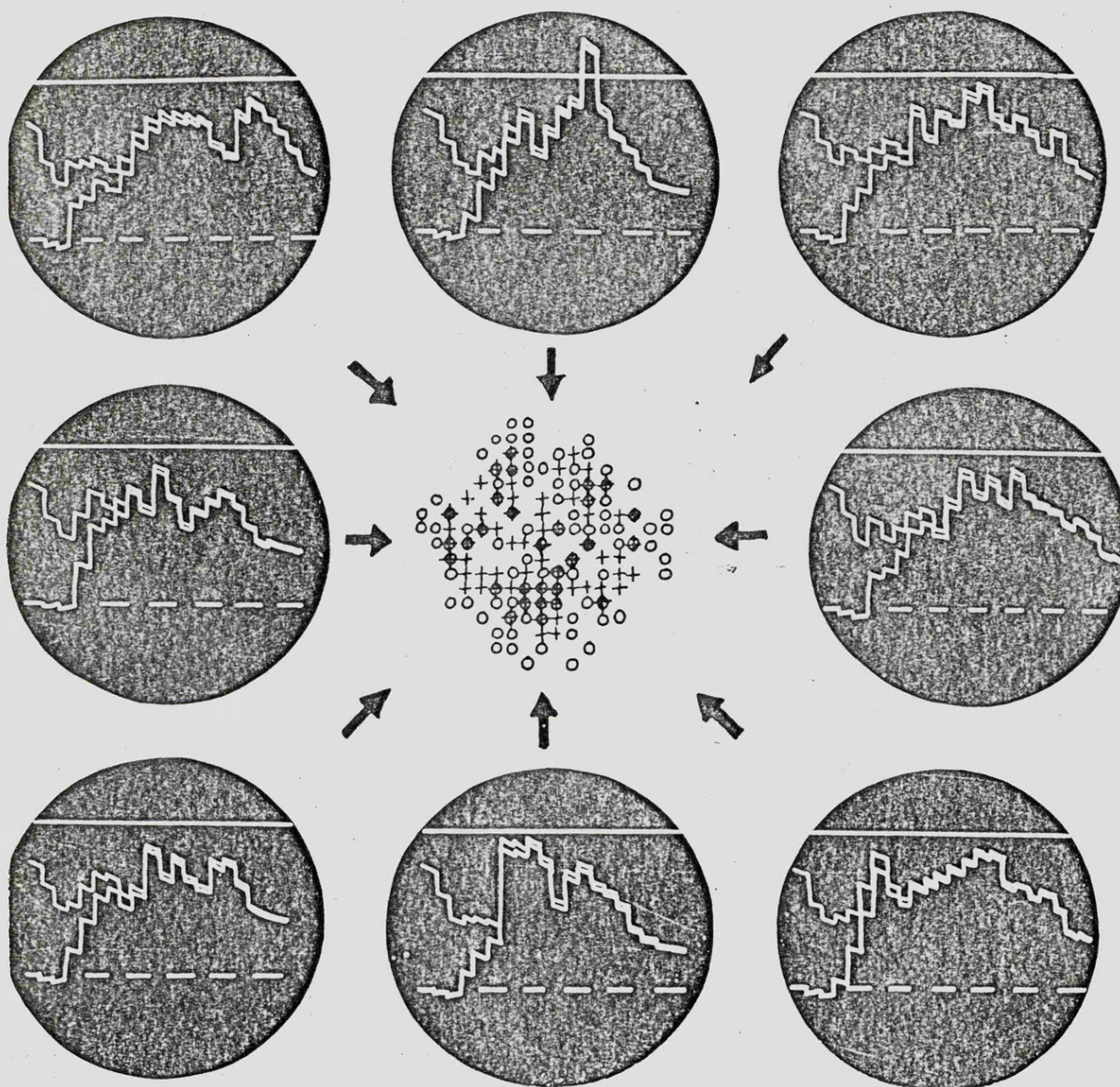


Figure 38. Direction specific responses of model to movement of a bar stimulus across a randomly connected receptive field (stabilised PSPs).

Velocity of movement is same in each direction (i.e. 2 rows in 1 msec or 2 diagonal rows in $1/(\sqrt{2})$ msec).

Small asymmetries in the randomly occurring clusters are sufficient for good direction specific responses.

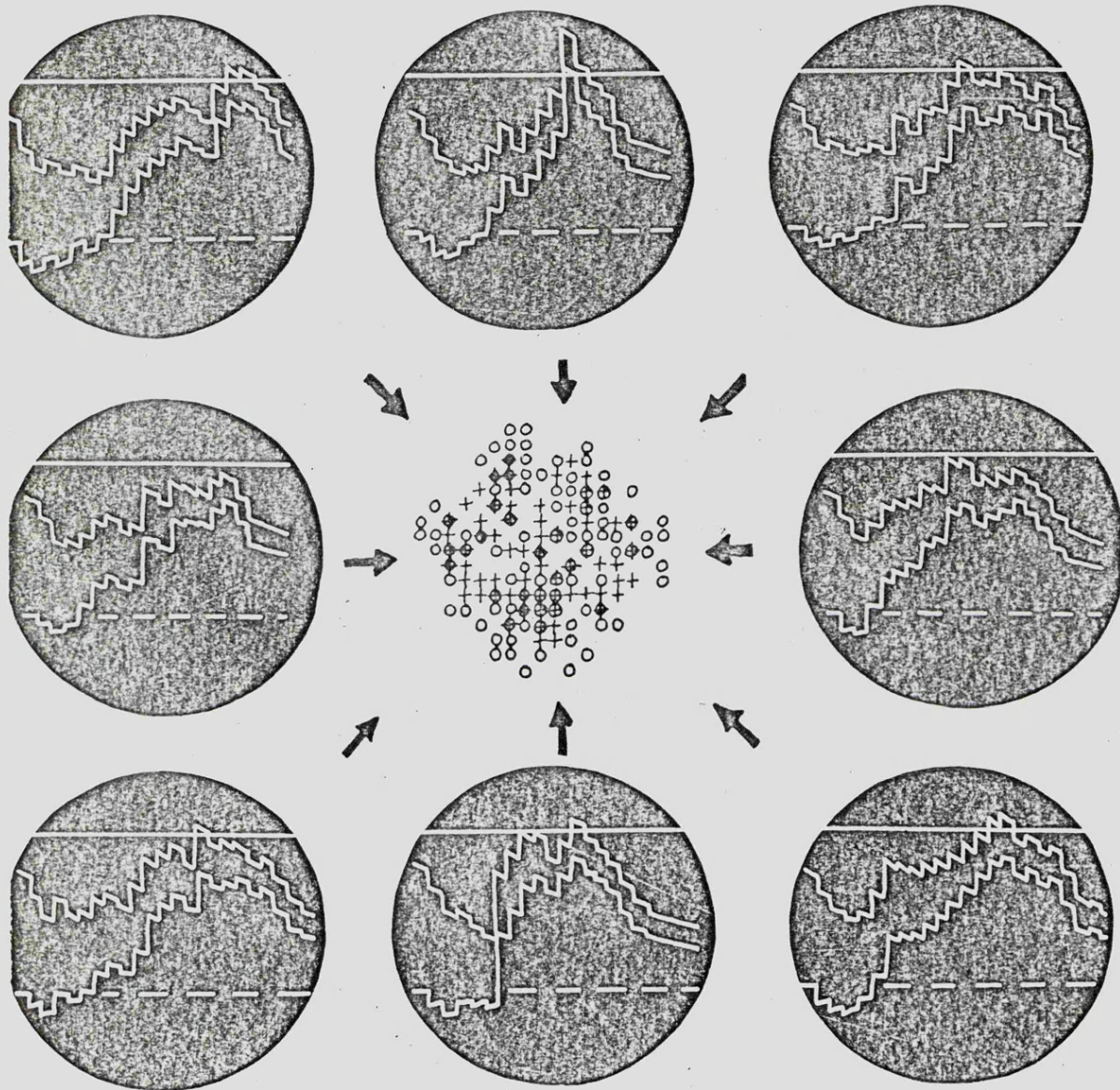


Figure 38. (CONTROL) Instability of responses to movement of a bar stimulus across a randomly connected receptive field (with constant amplitude PSPs).

In this control situation suprathreshold responses are more closely related to initial conditions than to input pattern, so that direction specificity is almost lost.

(Same receptive field pattern as in fig.38).

APPENDIX IV : HISTOLOGY

a) Electrode tracks

The physiological experiments were routinely followed by histological identification of electrode tracks. The technique (Houchin, 1969a) is described in the methods section.

Fig. 39a shows the electrode track of the experiment of fig. 14.

Fig. 39b shows the punctured pyramidal cell which was found after the experiment of fig. 19f and g. This cell was about 50 μ below the last visible part of the electrode track, and exactly in line with it.

b) Procion Yellow electrodes for intracellular recording and staining of neurones in the somatosensory cortex of the rat.*

Stretton & Kravitz (1968) have described a technique for intracellular injection of the fluorescent dye Procion Yellow (M4RS from ICI) to determine the neuronal connexions in lobster abdominal ganglia. In a study of alligator Purkinje cells, Llinas & Nicholson (1971) modified this technique so that they could identify the parts of cells from which records had been taken. These methods have been adapted for the identification of neurones, and parts of neurones, in the somatosensory cortex of the rat, from which intracellular records have been taken.

* From the Proceedings of the Physiological Society, Houchin 1973, enlarged and illustrated. This work was done at the Max Planck Institute for biophysical chemistry, Göttingen, West Germany. I am indebted to the Wellcome Trust for the fellowship which enabled me to do this work, and to the M.R.C. for leave of absence from N.I.M.R. I would also like to thank Prof. O. Creutzfeldt for his help and encouragement, and Dr. E. Lang from the Institute of Anatomy, Budapest, who guided me in the initial stages of this Procion

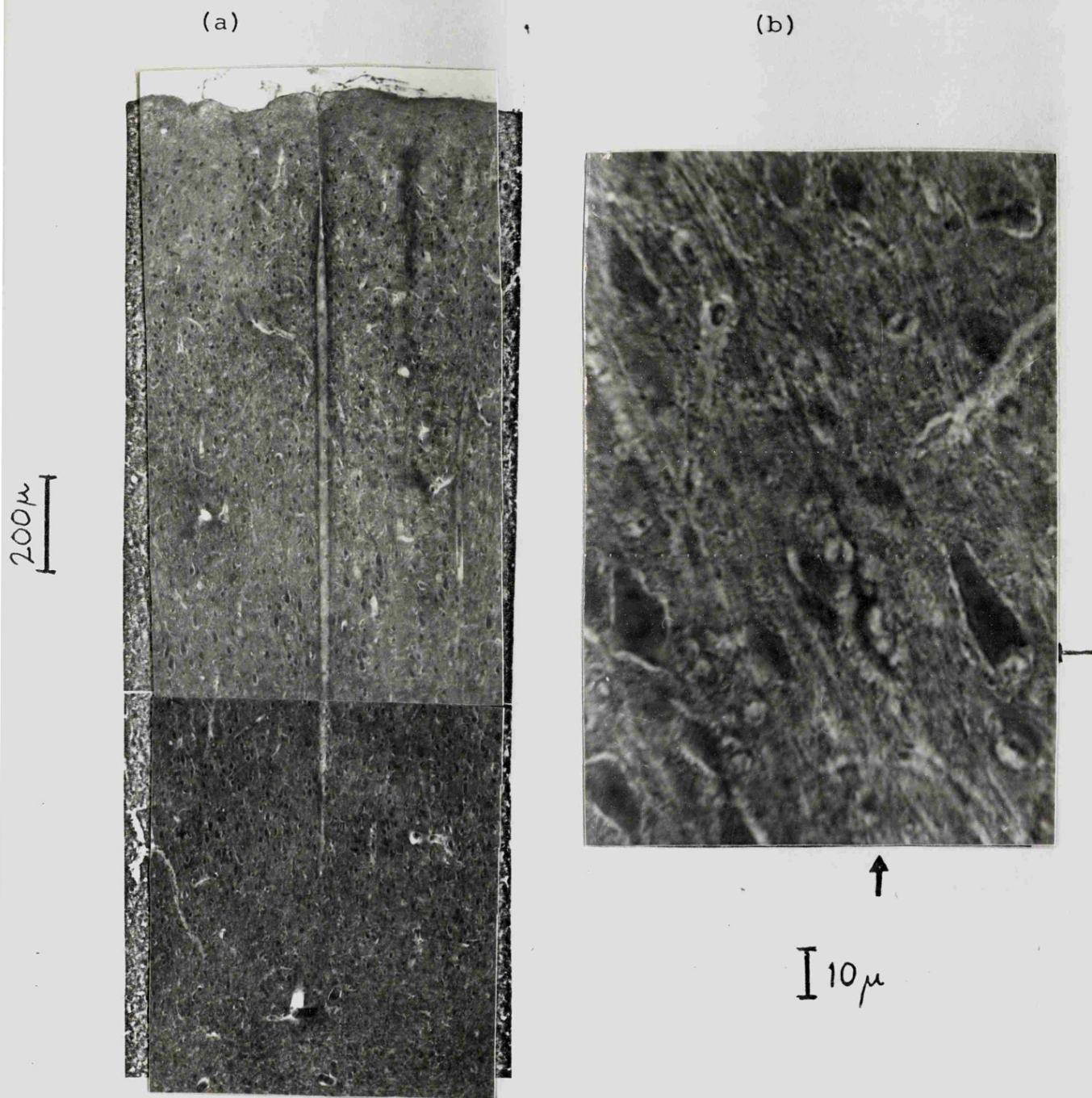


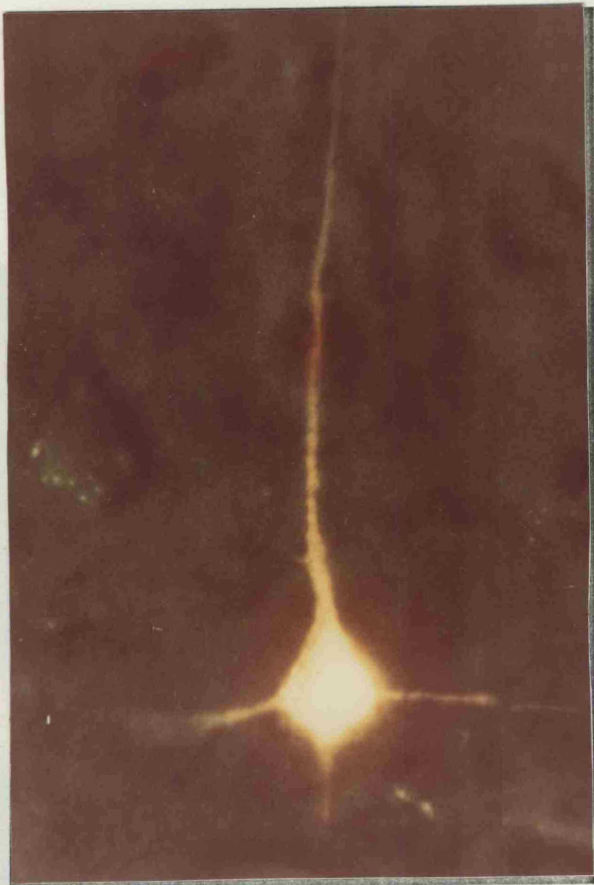
Figure 39. Histology.

- a) The electrode track of the experiment of fig.14.
- b) The punctured pyramidal cell which was found after the experiment of fig.19 (f) and (g).

Fine glass micropipettes (external tip diameter less than $1\mu\text{m}$) were filled with a 4% solution of Procion Yellow in distilled water. Best results were obtained with electrodes whose resistances were between 100 and 200 $\text{M}\Omega$. The rat was anaesthetized intraperitoneally with urethane. Successive layers of bone were removed from an area of about 6mm^2 overlying the somatosensory cortex, until only a transparent 'window' of bone remained. This bone was pierced with a needle and a small flap lifted away to expose an area free of large blood vessels. The whole area was covered with a paraffin pool. A hydraulic drive was used to advance the electrode through a slit which had been made in the dura.

A drop of potential of 30-50mV was seen on penetration of a cell, also synaptic activity of 5-20mV and action potentials of 15-60 mV were recorded. If such a penetration was successful, i.e. if there was no immediate and obvious injury discharge, the cell was usually held for 10-60 min. When the necessary physiological data had been collected, a tip-negative current of 1-4nA (in the form of a square wave at 10-100 Hz) was applied for a period of about 2-5 min, after which the action potentials were usually of smaller amplitude. However, if as was usual, the synaptic and resting membrane potentials were little changed, then it was assumed that the electrode tip was still inside the cell. If larger injection currents were used the membrane potentials were rapidly and permanently lost, so these small currents were continued for as long as possible: the injection and subsequent

(a)



(b)



(c)



10μ

Figure 41. Identification of recording site with Procion Yellow.

Figure 40. Identification of recording site with Procion Yellow.

- a) Soma and proximal dendrites of a pyramidal cell stained by Procion yellow injection.
- b) Dendrite of large pyramidal cell penetrated and stained 300μ above the soma which was unstained (see c)). (More than 400μ of this apical dendrite was lying inside the 20μ section so identification of soma was unmistakable.

(a)

(b)



10 μ

Figure 41. Identification of two cells whose responses are shown in fig.30.

- a) The stellate cell from which the records of fig.30(a) and (b) were taken.
- b) The fusiform cell from which the records of figs. 29 and 30(c) and (d) were taken.

monitoring were repeated until the membrane potentials were lost (usually rapidly and within 30 min of the initial injection). The micro-electrode was withdrawn after the injection. If the dye was required to remain close to the recording site, the rat was killed soon after the injection, otherwise the rat was kept alive for 3-6 hours to allow spread of the dye along the cell processes.

A marker electrode was inserted nearby, parallel to the tracks used for recording, after the craniotomy and opening in the dura had been enlarged. The cortex was covered by a pool of 10% formalin for 12 hours. The marker electrode was removed (leaving a small hole at the top of its tracks). A block was cut with sides parallel to the tracks and further fixed for 4 days. Frozen sections (20 μ m thick) were cut and mounted under a 'water mounting medium' (Edward Gurr Ltd). Many cells in the cortex showed autofluorescence but the colour and appearance of the fluorescence of the injected cells was quite distinguishable when viewed with a high power objective. The fluorescence faded slowly with time and rapidly with exposure to the U.V. light of the fluorescence microscope, so colour photographs were taken immediately after identification of a cell.

After twenty successfully monitored injections, sixteen neurones were identified histologically. They comprised two apical dendrites of large pyramidal cells; two small pyramidal somata; one fusiform soma; eight small pyramidal cells

with their main dendritic processes; three stellate cells with processes.* The larger amplitude and faster action potentials were apparently recorded from somata, e.g. that in fig. 40a, whereas the two apical dendrites, e.g. that in fig. 40b, yielded smaller and longer-lasting action potentials.

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* See figs. 40 and 41.

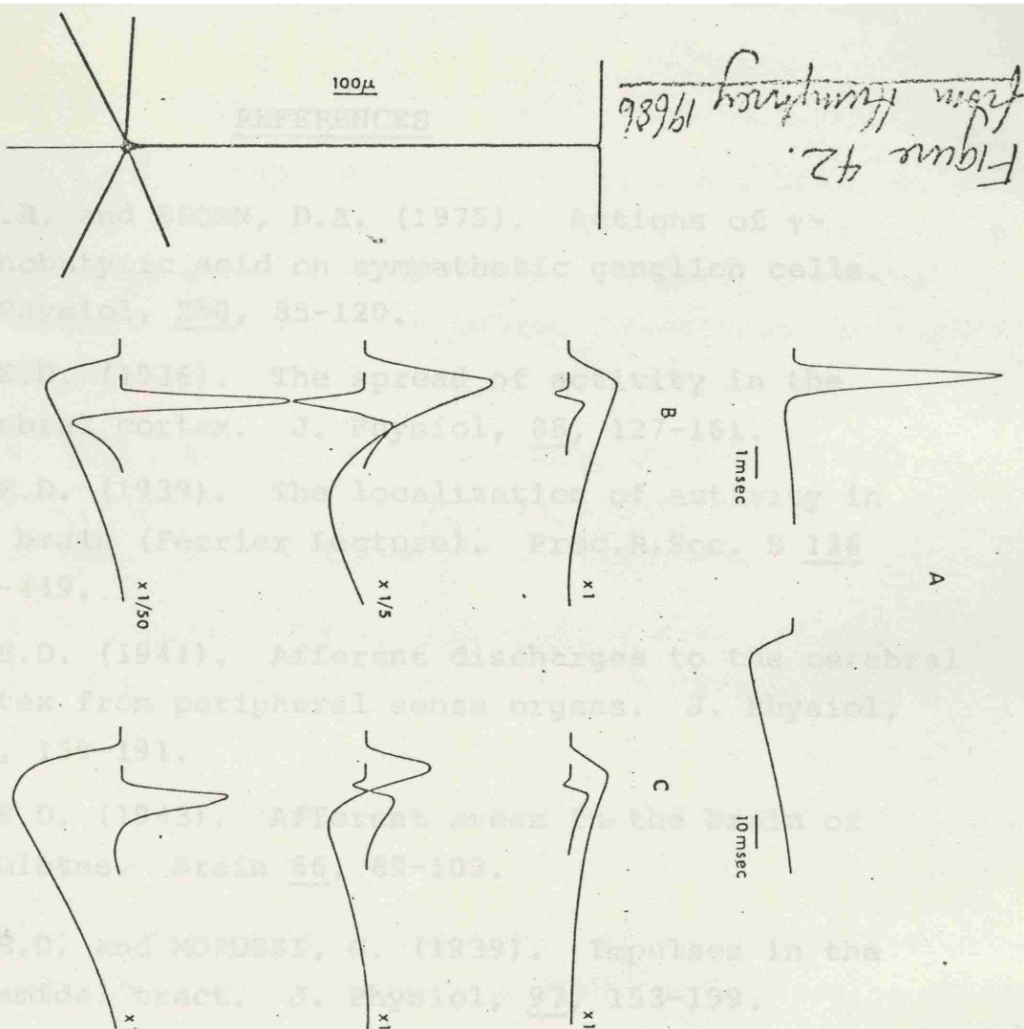


Fig. 11

Calculated extracellular potentials resulting from a spike and an IPSP in a pyramidal-shaped model neuron. A drawing of the model cell shows the relative diameters and orientation (but not the lengths) of the dendrites and axon. *A*: Intracellular wave forms at the soma; *note different time scales*. Depolarization (positivity) is indicated by an upward deflection; all potentials are defined with respect to an infinitely distant reference point. *B*: Extracellular potentials at various levels (indicated by the baseline of each pair of traces) above the soma, along a line parallel with but 50 μ lateral to the apical dendrite. *Negativity* is indicated by an upward deflection. In each pair, the longer trace is the potential generated by the IPSP and the shorter trace that resulting from the spike; time scales are as in *A*. The number to the right of each pair of traces represents the relative "amplification" or scaling factor necessary to plot all potentials in one figure. It is important to note that the scaling factor is the same for *both* members of each pair, e.g., if the potential generated by the spike at the level of the soma is 2 mV in absolute amplitude, that resulting from the IPSP is approximately 1 mV. *C*: Same as in *B*, but

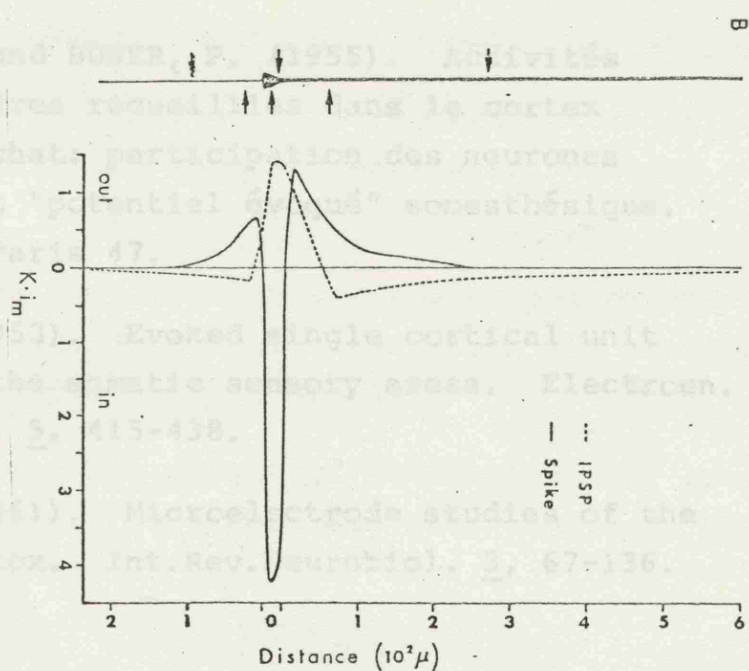


Fig. 13

B: Approximate distribution of membrane current (on an arbitrary but linear current scale) over the simplified model cell shown to the left, during the peaks of the intra-somatic spike (solid line) and IPSP (dashed line). See Fig. 11, *A* for the voltage wave forms at the soma, and text for further details.

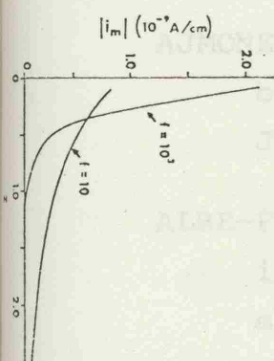


Fig. 14

Steady-state amplitude of the calculated membrane current along the apical dendrite of the model cell shown in Fig. 13, *B*, for a 1 mV sinusoidal voltage function within the soma. Distance from soma is shown on the abscissa in units of the characteristic or electrotonic length constant. The two curves correspond to two frequencies of input voltage (1000 and 10 c/sec).

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