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AN INHIBITORY PROCESS IN THE CEREBRAL CORTEX

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SUMMARY

1. In cats, rabbits and monkeys, single cortical shocks can reduce the excitability of cortical neurones for 100–300 msec; the inhibitory effect is readily demonstrated, even in previously quiescent cells, against a background of activity evoked with small amounts of L-glutamate, released from an extracellular recording micropipette by iontophoresis.

2. Other forms of cortical activity are also inhibited in a similar way by direct or indirect cortical stimulation; they include single unit discharges produced by iontophoretic applications of ACh or by a cathodal current, spontaneous discharges, and slow wave activity, both spontaneous and evoked.

3. Most stimuli which elicit cortical activity also evoke some inhibition in the cortex, for instance, transcallosal volleys, and thalamic or peripheral shocks. In each case, a characteristic, prolonged depression is produced by single shocks.

4. The most effective stimuli are direct cortical shocks, especially when applied within the cortex, below a depth of 0·6 mm; surface cathodal shocks are more effective than anodal shocks. These stimuli do not first excite the cells which are inhibited and they are not strong enough to cause appreciable local injury.

5. Because of its long duration, the inhibition is often readily maintained by repetitive stimulation at frequencies of 5–7/sec. A cumulative effect leads to a further silent period after the end of stimulation; this increases with the strength, frequency and duration of the tetanus, so that after stimulation at 50–100/sec, the silent period may last for over 1 min. During this time, a stronger depolarizing stimulus can initiate firing.

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6. The inhibitory effect is often preceded and followed by phases of increased excitability; these may also show cumulative enhancement during repetitive stimulation, and a high frequency tetanus often leads to a short after-discharge, which is then followed by a long silent period, as above. Comparable changes take place in rabbits during spreading depression.

7. The inhibitory effect of a direct shock can spread over an area covering 1 cm of cortical surface, affecting the cells through all cortical layers; but the spread is uneven in different directions, being particularly poor under most sulci.

8. This type of inhibition can be elicited in all areas of the neocortex, and it is evident in kittens within a week of birth.

9. Antidromic pyramidal stimulation is very much less effective in evoking inhibition of Betz cells, and other cortical neurones, than direct cortical stimulation; the inhibition by direct shocks is therefore not likely to be mediated through pyramidal excitation.

INTRODUCTION

Like many other topics, cortical inhibition has received a widely fluctuating amount of attention from neurophysiologists. It was recognized very early as a probably essential feature of cortical function, in a remarkably clear-sighted paper by Bubnoff & Heidenhain (1881). However, in spite of an extensive and most fruitful use of the concept of cortical inhibition in the analysis of conditioned reflexes (Pavlov, 1927), until recent years it was largely ignored in studies of the cerebral cortex. Several possibly related phenomena, such as the mutual interference between the effects of simultaneous cortical stimulation at two different points (Graham Brown & Sherrington, 1912), or the 'suppression' of motor responses produced by stimulating certain cortical areas (Dusser de Barenne & McCulloch, 1941; Dusser de Barenne, Garol & McCulloch, 1941), were assumed to be essentially subcortical in nature. With a few exceptions (e.g. Rosenblueth & Cannon, 1942; Leão, 1944), most authors have considered various types of cortical depression, like extinction (Dusser de Barenne & McCulloch, 1937) and spreading depression (Marshall, 1959; Ochs, 1962) to be caused mainly by previous, more or less excessive activity, rather than by an inhibitory process.

Although, more recently, several observations of possible inhibitory effects were made when single unit recording became possible (Amassian, 1953; Jung, 1953), they could not convincingly be distinguished from occlusive phenomena. Stronger evidence of true cortical inhibition came from subsequent studies of the effects of direct cortical shocks (Creutzfeldt, Baumgartner & Schoen, 1956), and of transcallosal (Creutzfeldt *et al.* 1956;

Asanuma & Okamoto, 1959) and peripheral stimulation (Mountcastle, 1957; Mountcastle & Powell, 1959), as well as stimulation of the thalamus (Li, 1956), the caudate nucleus (Spehlmann, Creutzfeldt & Jung, 1960) and the pyramidal tract (Suzuki & Tukahara, 1963; Asanuma & Brooks, 1965, Brooks & Asanuma, 1965). However, even these observations cannot be considered wholly conclusive, since there was little or no evidence that the inhibitory mechanism was operating directly on the observed cortical cells.

More definite evidence has become available from intracellular studies in the cortex, which have revealed what appeared to be inhibitory post-synaptic potentials (IPSPs), evoked either by direct (Phillips, 1956*b*; Li & Chou, 1962), or by various forms of indirect stimulation (Phillips, 1956*a*, 1959; Branch & Martin, 1958; Lux & Klee, 1962; Li, 1963; Purpura & Shofer, 1964; Nacimiento, Lux & Creutzfeldt, 1964; Pollen, 1964; Stefanis & Jasper, 1964).

Neuronal inhibition can only be demonstrated conclusively by a procedure which directly tests the neurone's ability to fire impulses. Changes in membrane potential are of great interest in the further analysis of neuronal processes, but they are not necessarily simply correlated with changes in excitability. Cellular inhibition can be associated with an increase or a reduction in membrane potential, or no change at all (Kuffler, 1960). Moreover, the presence of an electrode inside the cell may itself radically alter synaptic potentials (Coombs, Eccles & Fatt, 1955).

Another disadvantage of intracellular recording is that conditions in the cerebral cortex are unfavourable for prolonged observations. Inevitably, this means that an adequate study can be made of only relatively few cells, whose properties may not be typical of cortical neurones in general.

Any method which allows one to test neuronal excitability from outside the cell is therefore particularly valuable for analysing inhibitory phenomena. Some neurones can be excited by small currents from extracellular micro-electrodes (Strumwasser & Rosenthal, 1960; Burns & Salmoiraghi, 1960; von Euler & Green, 1960), but many cells are not readily excited in this way. A more reliable and convenient technique is the release of small amounts of excitatory substances from a micropipette outside the cell. The most useful general excitant is probably L-glutamate, because of its sharp and quickly reversible action, which does not lead to desensitization during a prolonged application (Krnjević & Phillis, 1963*a*). If the rate of release is controlled by an iontophoretic current, cells can be made to fire at will, in a predictable way, and this background of activity can be used to demonstrate inhibition occurring 'spontaneously' or evoked by various forms of stimulation (Krnjević & Phillis, 1963*a*, *b*).

We have studied cortical inhibition by this method; since this does not require penetration of the cells by micro-electrodes, it is possible to

investigate in one experiment a relatively large number of cells, in many areas and for long periods of time. The results of these experiments are presented in a group of three papers: the present paper describes the main features of the inhibition, the second deals with its possible mechanism (Krnjević, Randić & Straughan, 1966*a*) and the third is a survey of its pharmacological properties (Krnjević, Randić & Straughan, 1966*b*). Many of these observations have already been summarized in two preliminary publications (Krnjević, Randić & Straughan, 1964*a*, *b*).

METHODS

Animals

The experiments were done mostly on cats, and in a few cases on rabbits and monkeys.

Anaesthesia

Most of the animals were anaesthetized with a diallylbarbituric acid-urethane mixture (Dial compound, Ciba Ltd.) given intraperitoneally (0·7 ml./kg). This was supplemented if necessary with small I.V. doses of pentobarbitone sodium (Abbott Ltd.).

Other anaesthetics used were ether (after induction with ethyl chloride) or chloralose (Hopkins & Williams Ltd.; 80 mg/kg I.V.). One experiment was also done in the absence of anaesthesia on a cat's 'cerveau isolé', prepared by cutting the brain stem just in front of the bony tentorium (under ether).

Preparation of cortex

The brain was always exposed widely. After opening the dura, most of the cortex was covered with strips of opaque polythene to prevent drying and reduce cooling. The area for recording and stimulation was kept moist by irrigation with a warm physiological solution, whose composition approximated that of C.S.F. Most of this area was covered by a transparent Perspex disk, pressing lightly on the cortical surface to reduce local pulsations; the disk had a small central hole through which the recording micropipette was inserted into the tissue.

Stimulation

We used several types of electrodes; for direct cortical stimulation, surface shocks were given through single or bipolar platinum or silver ball-tipped electrodes; deeper stimulation was through single- or double-barrelled glass pipettes containing fine platinum or tungsten wire (20–50 μ). When monopolar electrodes were used, the indifferent electrode was a large silver plate embedded deep in muscle in the back of the neck.

Subcortical areas were stimulated with concentric electrodes; the cats were fixed in a conventional stereotactic apparatus and the electrodes were guided according to the co-ordinates given in the atlas of Jasper & Ajmone-Marsan (1960). Electrode tracks were subsequently examined in frozen sections of the brain.

The stimulating pulses were usually 0·1 or 1·0 msec square waves, which were sometimes delivered through a radio-frequency isolation unit. In some experiments, the stimulating current was monitored across a suitable series resistance (about 10 Ω).

Recording micropipettes

Multi-barrelled. The double or five-barrelled Pyrex glass micropipettes were prepared as described by Krnjević & Phillis (1963*a*). One barrel, filled with 2·7 M-NaCl, was connected to the grid of a cathode follower and was used for recording extracellular unit spikes: its tip resistance was about 2–4 MΩ. Another barrel always contained 1 M-Na L-glutamate (British Drug Houses Ltd.), at a pH value of 7–8. Small amounts of glutamate were released

iontophoretically *in situ* by passing small inward currents through this barrel. Other barrels, when available, were filled with various pharmacological agents. The spontaneous outflow of glutamate and other substances was prevented by a small steady braking current. The over-all, outside tip diameters of multi-barrelled pipettes varied between 2 and 12 μ.

Single-barrelled. For intracellular recording, we used conventional Pyrex glass microelectrodes, with outside tip diameters of $< 0{\cdot}5\ \mu$; they were filled with 2 M–K citrate.

Cortical surgery

Isolated cortical slabs were usually prepared by cutting all deep connexions of a given area with a right-angled, blunt-tipped probe inserted through an adjacent gyrus. The tip of the probe was carried to the surface around the undercut area, thus completing the isolation. In some experiments this procedure was confined within a single gyrus, to avoid damaging the adjacent region.

To prepare chronically isolated slabs, undercutting was done under strictly aseptic conditions, and only small holes were made in the dura. The exposed dura was covered with a strip of polythene to prevent adhesion to the overlying tissue.

RESULTS

All aspects of cortical activity can be inhibited by direct stimulation of the cortical surface or by stimulating various pathways which evoke a response in the cortex. Typically the inhibition lasts for a relatively long period, of the order of 100 msec, but it is often preceded by a short phase during which the cells are somewhat more excitable than usual, but without necessarily discharging impulses. These effects can be observed in animals under several forms of anaesthesia, or without giving any anaesthetic (Krnjević *et al.* 1966*b*).

The various kinds of activity which can be inhibited will be described first.

Cortical activity showing inhibition

Unit responses evoked directly

Practically all types of unit activity provide a suitable background for demonstrating inhibitory effects. The most useful discharges are those produced by direct stimulation of a given cell, because they can give direct evidence of post-synaptic inhibition.

Responses elicited with L-*glutamate*

Most units are readily excited by small amounts of L-glutamate released from a multi-barrelled micropipette, and the unit discharge can be recorded extracellularly from another barrel of the same pipette. Inhibition is then manifested by a reduction in this glutamate-evoked discharge, and it is seen on the oscilloscope trace as a silent period following the stimulus artifact (cf. Figs. 6, 9, 10, 11, etc.). When the evoked discharge is comparatively slow and irregular, the significance of the silent period can be increased greatly by superimposing several traces photographically.

In this way, one can very quickly detect any inhibition which may be present. For a more complete quantitative analysis, one can estimate the probability that the unit will fire an impulse at various times after the stimulus. This can be done either by counting the responses on a photographic record or by using a suitable computer. A post-stimulus histogram obtained with such a computer (Burns, Ferch & Mandl, 1965) is illustrated in Fig. 1. The upper record is a control histogram showing random variations in the discharge during a steady application of L-glutamate over

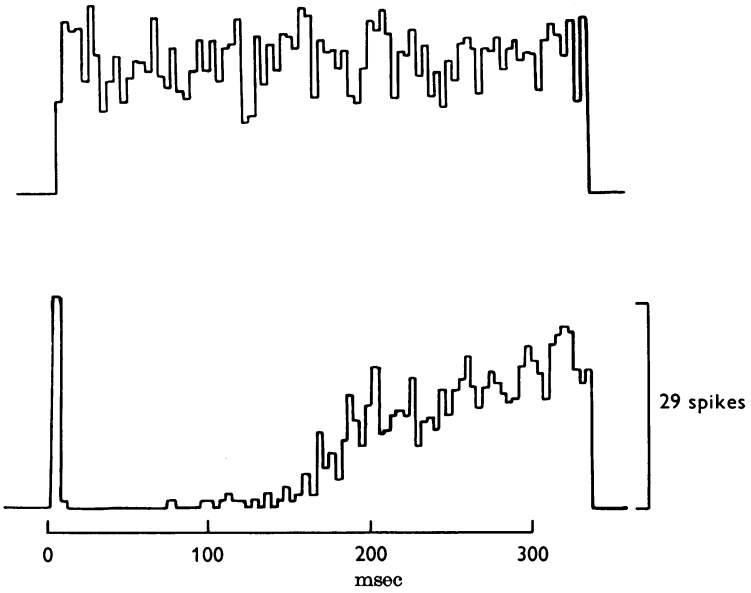

Fig. 1. Inhibition of unit found at depth of 0·9 mm in post-cruciate region, by monopolar stimulation of cortical surface (10 V, 0·1 msec pulses); the focal cathode was placed 3 mm further medially along gyrus. Unit was excited throughout by steady release of L-glutamate (10 nA) from another barrel of the multi-barrelled micropipette. *Below.* Probability of firing at various times after stimulus, shown as a 'post-stimulus histogram'; the computer counted the number of responses occurring in about eighty, 4 msec intervals following each stimulus, over a period of about 30 sec, during stimulation at 2·5/sec. Initial deflexion represents the stimulus artifacts. *Above.* Control histogram showing the glutamate-evoked discharge during a similar period of time, in the absence of cortical stimulation.

a period of about 30 sec, in the absence of electrical stimulation. Each column represents the number of spikes recorded in 750 intervals, each lasting 4 msec. When this procedure was repeated while stimulating the cortical surface at a rate of 2·5/sec, the initial stimulus artifacts were followed by a phase lasting nearly 100 msec during which there were no responses; in the subsequent period of reduced discharge, the mean firing

rate approached the initial level asymptotically. The inhibitory phase altogether lasted between 200 and 300 msec.

This phenomenon was demonstrated most often by using a continuous application of L-glutamate to produce a suitable background of excitation. Sometimes one can evoke a short burst of discharge by a transient release of glutamate (cf. Krnjević & Phillis, 1963*a*). This is possible only when the tip of the micropipette is particularly close to a sensitive cell. By this technique one can show that a continuous discharge is not essential for the demonstration of the inhibitory effect (cf. Fig. 15, and Fig. 5 in Krnjević *et al.* 1966*b*). Another advantage is that one can study even cells which are exceptionally easily depolarized by L-glutamate, and thus cannot be made to fire steadily, as in young kittens (Krnjević, Randić & Straughan, 1964*c*).

Unit responses elicited by other forms of direct activation

Although L-glutamate is the most convenient excitatory agent, other forms of direct stimulation can be used, for instance, a background of activity produced by releasing acetylcholine (ACh) near sensitive cells, or by a relatively large inward current through a barrel containing NaCl (Fig. 2).

The evidence presented so far proves that an inhibitory or depressant change is occurring in the cells actually under observation. One can thus exclude any presynaptic interference, either at the level of the synaptic terminals, or at some other point in the excitatory pathway. This cannot usually be done except when recording and stimulating inside the cells.

Other types of unit response

Other types of unit responses, which are evoked indirectly or which are caused by spontaneous activity, are also readily affected by the same presumed inhibitory mechanism. For instance, the lowest traces in Fig. 2 illustrate the abolition of a spontaneous discharge by the same form of stimulation.

Unit firing evoked indirectly in various ways, such as by thalamic or peripheral stimulation is blocked in a similar manner by direct cortical stimulation, as shown in Fig. 3 (inset traces). This type of interference between direct cortical stimulation and spontaneous or indirectly evoked activity has been described by previous authors (Jung, 1953; Creutzfeldt *et al.* 1956; Stohr, Goldring & O'Leary, 1963).

Only antidromic responses of Betz cells may not be blocked by even the strongest cortical shocks (cf. Fig. 13). This is in agreement with previous observations of a high safety factor of antidromic invasion of these cells (Phillips, 1956*a*; Krnjević & Phillis, 1963*a*).

Spontaneous and evoked slow waves

Figure 3 also shows that the primary slow wave response evoked in the cortex by peripheral stimulation is also markedly depressed by surface shocks. The recovery curves have a time course comparable with that of the inhibition of unit responses elicited directly with L-glutamate (cf. Figs. 1 and 5).

Spontaneous waves are also markedly depressed by cortical stimulation (Fig. 17*A*, and fig. 8 in Krnjević *et al.* 1966*a*), and so are fast waves which can sometimes be evoked locally by releasing L-glutamate in the most

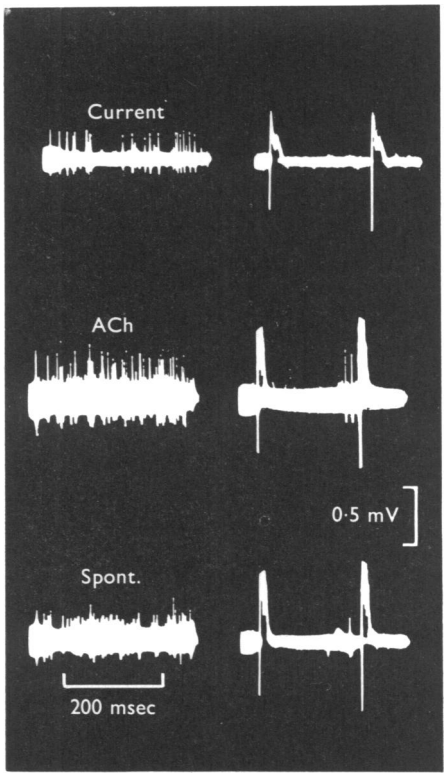

Fig. 2. Inhibition can be demonstrated when a unit is excited by other means than L-glutamate. In each line, traces at left are controls: above, unit was excited by a relatively strong inward current (250 nA) (through an NaCl-containing barrel of the multi-barrelled micropipette); middle, same unit was excited by ACh released iontophoretically (100 nA outward) from another barrel: below, spontaneous discharge. Traces at right show inhibitory effect of direct cortical stimulation with bipolar surface electrodes (60 V, 0·1 msec at 5/sec); these electrodes were 2·5 mm apart on suprasylvian gyrus and recording micropipette was inserted at a point 6·0 mm posterior to cathode, to a depth of 1·1 mm. Several traces have been superimposed in each record.

superficial layer of the cortex (Fig. 4), The exact significance of these superficial waves is not certain but they may represent local responses of apical dendrites.

Since slow cortical waves are not dependent on the firing of cells in the cortex, often being seen best when unit activity is minimal (Li & Jasper, 1953; Mountcastle, Davies & Berman, 1957), they are likely to be gene-

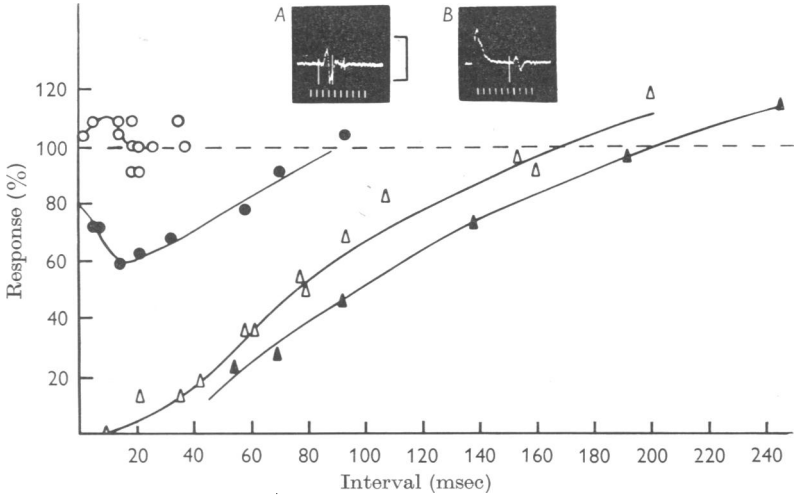

Fig. 3. Stimulation of posterior sigmoid gyrus inhibiting Betz cell firing, and slow wave response, evoked by single shocks to contralateral forepaw. Inset traces: *A*, stimulus artifact and control response produced by peripheral shock; slow wave is distorted by relatively short time constant of recording; *B*, a preceding surface shock blocks unit response and reduces evoked waves. Time marks: 10 msec. Graph shows effects of preceding surface shocks, at various intervals; peak-to-peak height of control wave (cf. *A*) was taken as 100 %. Intensity of 0·1 msec cortical shocks: ○ 15 V, ● 20 V, △ 40 V, ▲ 100 V.

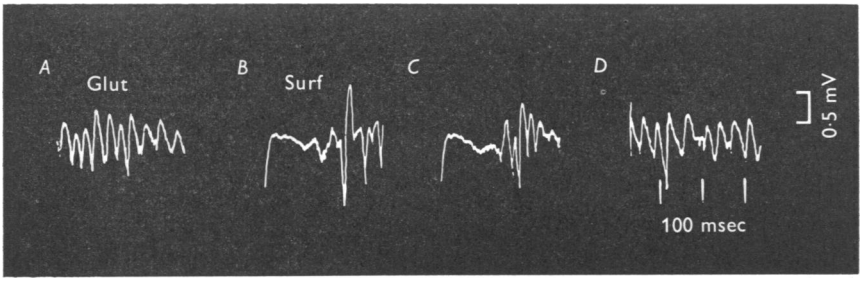

Fig. 4. *A*, *D*: fast wave activity evoked at depth of 0·1 mm in suprasylvian cortex by steady iontophoretic release of L-glutamate (60 nA). This type of response, not associated with distinct units, is often seen in superficial layer. *B*, *C*: surface shocks caused a prolonged block of this activity, followed by an apparent rebound, comparable with changes in excitability seen with unit responses.

rated by synchronized synaptic potentials. It therefore appears that the inhibitory effect not only changes the firing threshold of the cells, but it also reduces their synaptic potentials.

Stimuli which evoke cortical inhibition

Some cortical inhibition seems to result from all forms of stimulation capable of evoking activity in the deeper layers of the cortex.

Direct cortical stimulation

The simplest, and perhaps the most effective way of evoking inhibition is to stimulate the cortex electrically, with single or repetitive pulses. As might be expected the effectiveness of a given pulse varies directly with its duration.

Surface stimulation. One can use bipolar or monopolar electrodes. Although monopolar electrodes give a larger stimulus artifact, they require less current and make it easier to estimate directly the current needed for a given effect and to compare the effects produced by cathodal and anodal pulses.

When different electrodes or different kinds of electrical pulses are tested, a useful standard of reference is the local slow wave response elicited by stimulation. Relatively weak stimuli, which evoke a simple negative, and mainly superficial response (Adrian, 1937) have an excitatory effect (cf. Fig. 3, and fig. 2 in Krnjević *et al.* 1966*a*). Inhibition is only obtained regularly with stimuli strong enough to give rise to a more complex slow wave response (see Krnjević *et al.* 1966*a*). When stimulating near the motor area, the required intensity is very much below the threshold for muscle movement.

In absolute terms, the smallest effective stimuli are surprisingly similar in different areas and different animals. Thus, with bipolar electrodes, having a separation of 1–2 mm, the threshold voltage is about 20–30 V when using 0·1 msec square pulses, and about 3–4 V when using 1·0 msec square pulses. The threshold value for monopolar cathodal stimulation (with a large indifferent electrode embedded in muscle on the back of the neck) is about 4–5 V for 0·1 msec pulses.

The corresponding threshold *current* is in the range of 2–10 mA for 1 msec bipolar stimulation and 1–5 mA when using focal 0·1 msec pulses. The total movement of electric charge needed to evoke an inhibitory effect can thus be as little as 0·1 μC (cf. Fig. 7). This can be compared with a total of 0·25–2·5 μC needed to excite Betz cells when using 10 msec pulses (Phillips, 1956*b*).

Whereas Betz cells are more readily stimulated by surface positive pulses (Fritsch & Hitzig, 1870; Phillips, 1956*b*; Hern, Landgren, Phillips

& Porter, 1962), cortical inhibition is consistently obtained more effectively with surface negative pulses (Fig. 5); if bipolar electrodes are used, stronger inhibition is seen when the nearest lead is cathodal (Fig. 6).

When the intensity of stimulation is raised, the inhibitory effect becomes stronger and lasts longer, approaching a maximum at an intensity 3–4 times greater than the threshold (Fig. 7; cf. curves in Fig. 3).

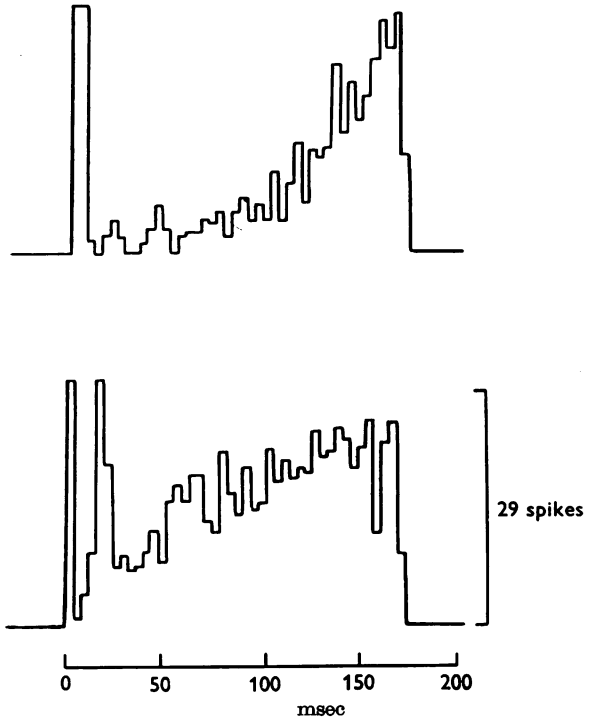

Fig. 5. Inhibition of unit, 0·9 mm deep in posterior sigmoid gyrus, by cathodal (above) and anodal (below) surface shocks (10 V, 0·1 msec); focal electrode was 4·5 mm from recording electrode. Post-stimulus histograms show probability of firing at various times after shocks, as in Fig. 1. Large initial deflexions are produced by stimulus artifacts.

Intracortical stimulation. If a fine, bipolar or monopolar focal electrode is inserted into the cortex, very localized stimulation is possible. It is then found that inhibition can be evoked in the surrounding tissue by much smaller stimulating currents; under optimal conditions, using 1·0 msec pulses, inhibition can be clearly elicited with currents of 30–50 μA, or a total of 0·03–0·05 μC.

A penetrating monopolar electrode is most effective when it is cathodal and is situated in the deeper half of the cortex, below a depth of 0·6 mm (Fig. 8).

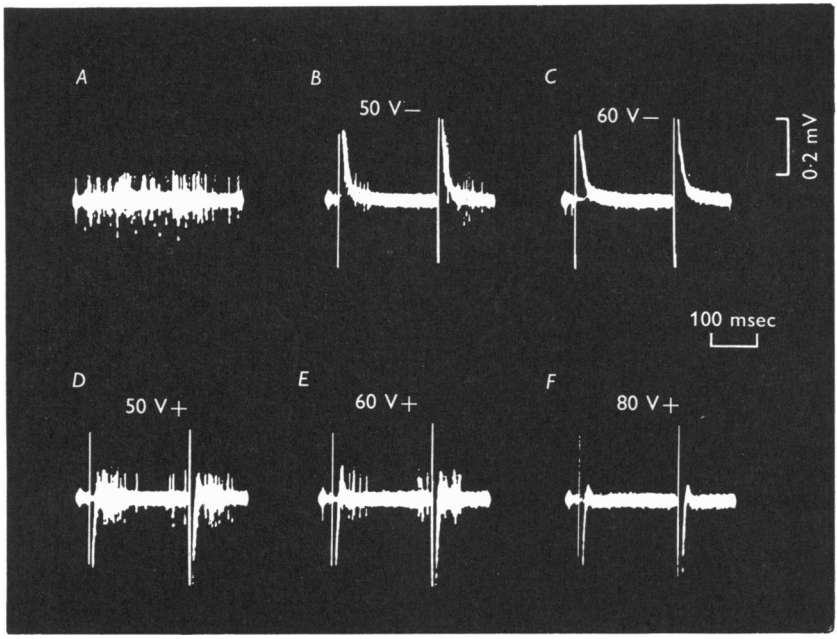

Fig. 6. Comparing effectiveness of cathodal and anodal shocks in causing inhibition of unit in posterior sigmoid gyrus, at depth of 1·4 mm and about 4 mm from nearest stimulating electrode (bipolar surface electrodes were 2 mm apart). Nearest electrode was relatively negative in *B* and *C*, and positive in *D–F*. *A* is a control trace, showing discharge evoked with glutamate. Cortical stimulation was at rate of 6/sec, and several oscilloscope traces have been superimposed on each record.

Extracortical stimulation

Apparently most forms of indirect stimulation which lead to a cortical discharge can be associated with some sign of cortical inhibition.

Thalamic stimulation. The effectiveness of thalamic shocks varies with the location of the stimulating and recording electrodes. Thus, if one is recording unit activity in the sensorimotor region in the cat, in the peri-cruciate area, inhibition is readily obtained by stimulating the specific nuclei in the thalamus (Figs. 9 and 10). The time course of inhibition obtained in this way is comparable with the effect of direct stimulation, although usually somewhat shorter. The thalamic stimulus sometimes triggers off a series of inhibitory pauses, alternating with phases of increased firing; the whole sequence may last as much as 1 sec (Fig. 9*C*).

Units in other cortical areas are affected much less by stimulation of the specific nuclei. For instance, systematic stimulation of various thalamic nuclei, while testing units in the anterior half of the suprasylvian gyrus,

gave the following results. The most effective points for eliciting inhibition in the cortex were in the nuclei lateralis dorsalis, lateralis posterior and anteroventralis and, to a lesser extent, medialis dorsalis. Some units showed weak inhibition during stimulation of the ventroposterolateral and medial nuclei, but there was no effect at all from the nuclei ventrolateralis, ventralis anterior, centrum medianum and the pulvinar.

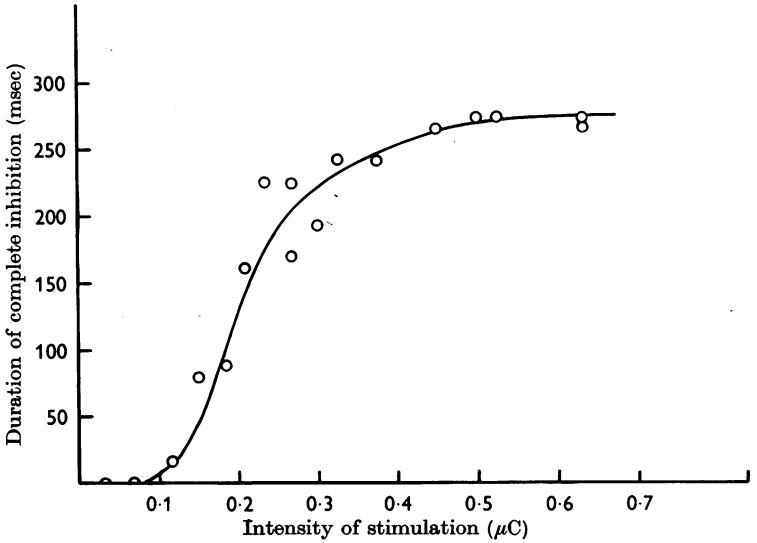

Fig. 7. Relation between intensity of 0·1 msec surface shocks and duration of resulting phase of total inhibition of a post-cruciate unit, excited by a steady iontophoretic release of glutamate. Unit was 0·85 mm deep, and focal stimulating cathode at a horizontal distance of 3 mm within same gyrus.

Since some of the most effective points were in nuclei that are said to project to the parietal cortex (Walker, 1938), it seems that the existence of a relatively direct thalamocortical link is an important determining factor.

Peripheral stimulation. Since different volleys from the periphery reach the cortex through the relay nuclei, one might expect to see inhibitory effects of peripheral stimulation in the corresponding cortical sensory areas. We have not studied the effects produced by various sensory modalities, using only electric shocks applied to each of the four paws, of sufficient intensity to induce a withdrawal reflex. Many units in the somatosensory areas were inhibited by such stimuli, particularly from the contralateral forepaw (Fig. 10). In general the larger the evoked cortical potential, the stronger the inhibitory effect; but this was never as marked as that produced by direct stimulation. Other cortical areas were not obviously affected by such stimuli.

Transcallosal stimulation. A similar kind of prolonged inhibition was also evoked by stimulating a symmetrical area in the contralateral cortex (Fig. 11), presumably by transcallosal activation, as previously shown by Asanuma & Okamoto (1959).

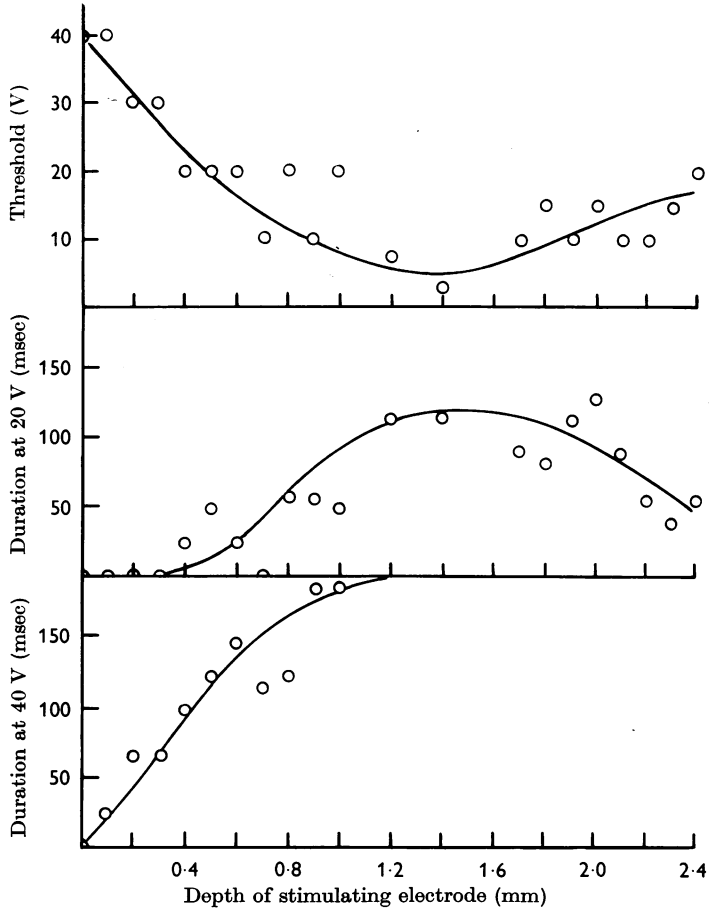

Fig. 8. Inhibition of cortical unit (0·5 mm deep in suprasylvian gyrus) by focal electrical stimulation at various depths within cortex. Focal electrode entered cortex at a point 3 mm distant from insertion of recording micropipette, and 1·0 msec cathodal pulses were applied at 5/sec. Graphs show for each depth of stimulation the threshold, and the duration of inhibition after 20 and 40 V pulses respectively. unit was studied throughout, being excited by continuous iontophoretic release of L-glutamate from recording, multi-barrelled micropipette.

Pyramidal stimulation. The effects of antidromic pyramidal volleys are of special interest, because several observers have described in recent years a recurrent inhibition of cortical Betz cells (see Introduction). It therefore

seemed possible that cortical inhibition is mediated by a purely recurrent mechanism, being elicited only through activity in the collateral branches of Betz cells and other deep pyramidal cells. To test this possibility, we compared in one experiment the inhibitory effects produced in the cortex by direct cortical shocks and by antidromic stimulation of the medullary pyramid.

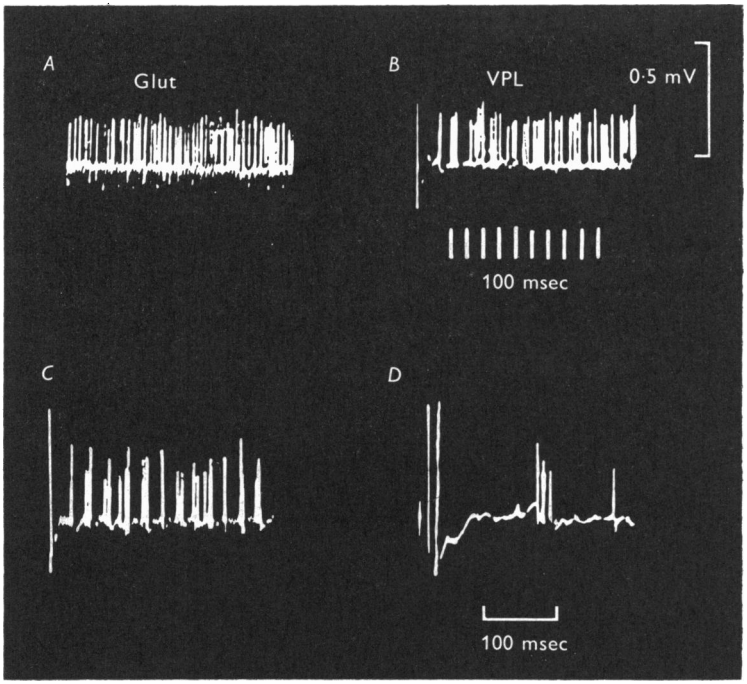

Fig. 9. Post-cruciate unit excited by steady release of L-glutamate (*A*), showing alternating phases of inhibition and facilitation after a weak (*B*) and a strong (*C*, *D*) thalamic stimulus, applied through stereotactic needle-electrode inserted into ventroposterolateral nucleus. Initial inhibition is better seen on expanded sweep in *D*. Each trace shows only one sweep.

Although some inhibition could undoubtedly be produced by pyramidal stimulation, it was very weak and far more localized than the effects of direct stimulation. For instance, Fig. 12 shows a Betz cell which could be excited after a short and constant latency by 9 V pulses applied to the medullary pyramid (*A*); and which followed repetitive antidromic stimulation at 200/sec (*E*). Its identity as a Betz cell was proved conclusively by exciting the cell body strongly for several seconds with a local application of glutamate (*B*); this caused a temporary increase in the pyramidal threshold for antidromic activation (*C* and *D*). Inhibition of this cell was tested as usual against a background of activity induced by a steady small

release of glutamate (*F* and *I*). Pyramidal stimulation had no inhibitory effect with volleys less than about 20 V (more than twice the threshold of its own axon), and 40 V was needed for a strong inhibition. In this case, the antidromic inhibition was unusually effective, being even comparable with the inhibition obtained by surface stimulation *J*.

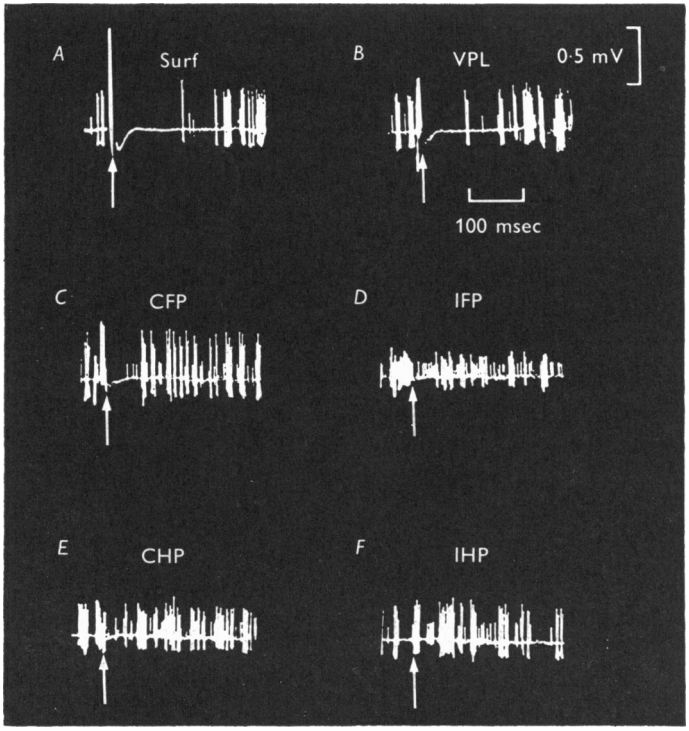

Fig. 10. Inhibition of two cortical units by several kinds of stimulation. Units were at depth of 0·3 mm in posterior sigmoid area, and were excited by steady iontophoretic release of glutamate. The discharge was inhibited by surface shocks (*A*), by stereotactic stimulation in ventroposterolateral nucleus of thalamus (*B*), and by shocks to contralateral forepaw (*C*). Shocks to ipsilateral forepaw, and contralateral and ipsilateral hindpaws were much less effective (*D–F*). Arrows indicate stimulus artifacts.

In every instance very strong pyramidal volleys were required before any inhibition was seen at all. Thus the Betz cell of Fig. 13, whose axon in the medulla was excited by 10 V pulses (*A*), showed no antidromic inhibitory effect except with shocks stronger than 80 V (*C* and *D*); and even 100 V pulses were far less effective than surface shocks (*E*). However, even the strongest surface shocks available could not prevent antidromic invasion (*F*).

In this experiment, five Betz cells were identified, of which three showed some degree of antidromic inhibition, though only with pyramidal shocks that were much greater than the threshold of their own axons. The other two Betz cells showed no antidromic inhibition at all (Fig. 14*C*). Moreover, twenty-one other cells were examined in the same area, which could not be activated after a short and constant latency by pyramidal stimulation, and therefore were probably not Betz cells. All these cells were readily inhibited by surface stimulation, like the Betz cells, but most of them showed no inhibitory effect of strong pyramidal

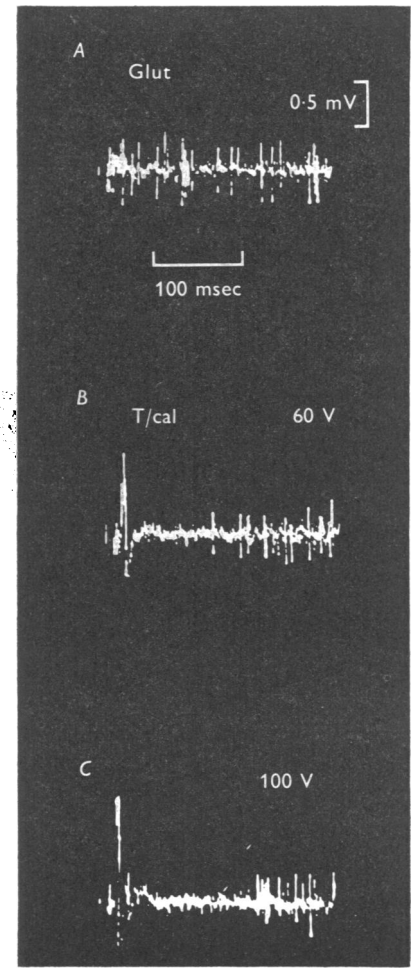

Fig. 11. Unit in suprasylvian gyrus inhibited by 60 V and 100 V 0·1 msec shocks applied to symmetrical area of contralateral hemisphere. Discharge was caused throughout by steady release of glutamate (*A*).

stimulation (Fig. 14*F*, *I*); only in one case was there some inhibition, after a relatively long delay.

We conclude from these observations that antidromic pyramidal activity does not trigger the cortical inhibitory mechanism particularly effectively. In spite of some impressive evidence of recurrent pyramidal

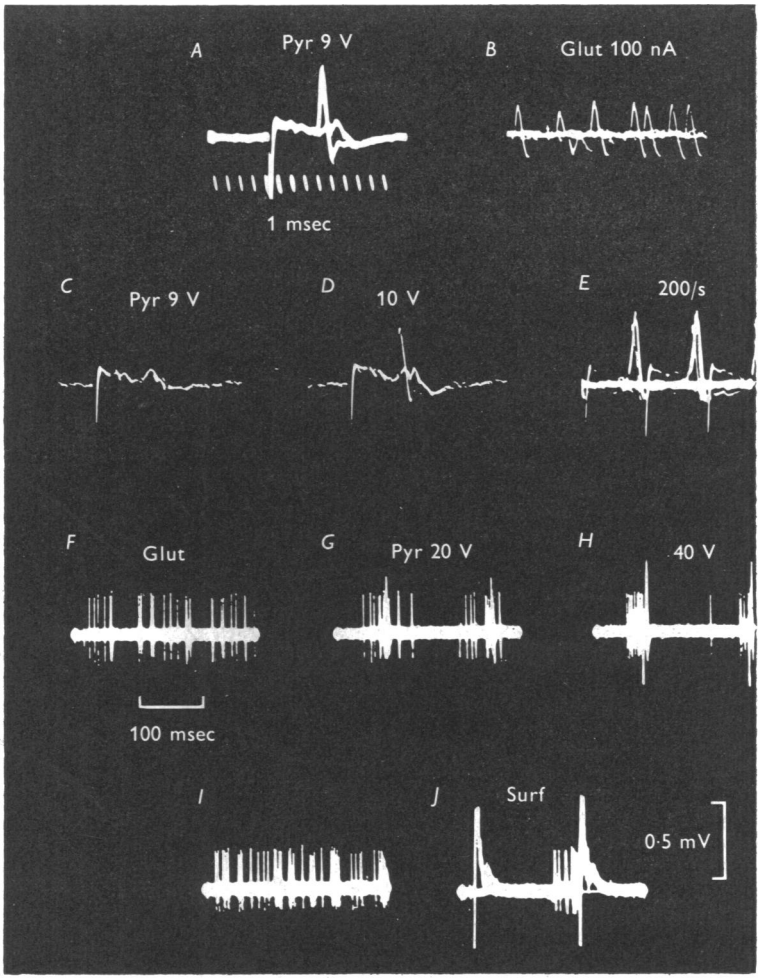

Fig. 12. Inhibition of Betz cell by surface shocks and by antidromic pyramidal volleys. Unit was identified by fixed, short latency response to electrical stimulation (9 V) of medullary pyramid (*A*), even at 200/sec (*E*). Identity was confirmed by demonstrating a temporary increase in medullary threshold for antidromic activation (*C* and *D*) after strong excitation of cell in cortex by copious application of glutamate (100 nA for 10 sec, *B*). To show inhibition, a smaller amount of glutamate was then released to give a steady discharge (*F* and *I*); definite inhibition could only be obtained with strong pyramidal volley (at 7/sec) (*G* and *H*) or by direct stimulation of cortical surface (*J*). Note different time scale for *A*–*E* and *F*–*J*.

inhibition (Phillips, 1959; Suzuki & Tukahara, 1963, Stefanis & Jasper, 1964; Asanuma & Brooks, 1965), the very strong pyramidal stimuli required to obtain inhibition suggest that some of the effects we observed may have been due to excitation of other tracts, by current spread in the medulla.

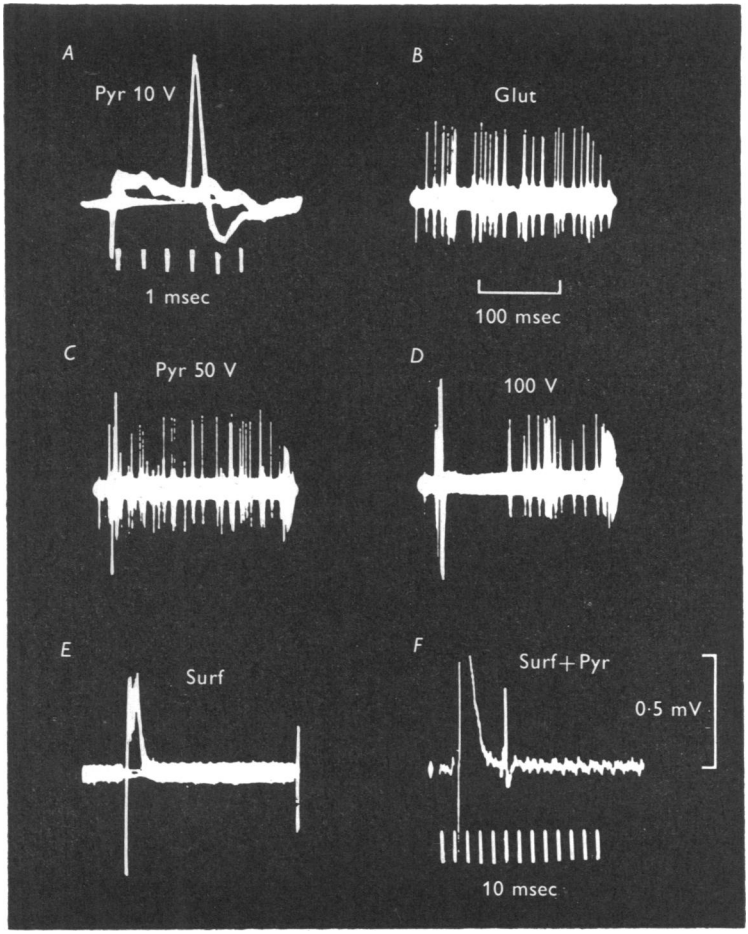

Fig. 13. Another Betz cell inhibited far more effectively by 30 V surface shocks (*E*) than by antidromic pyramidal volleys (*C* and *D*). The cell was identified by its short latency response to stimulation of medullary pyramid (*A*) and it was excited by steady release of glutamate (*B*). Last trace (*F*) shows that even a 100 V surface shock could not prevent antidromic invasion. Note same time scale for *B–E*.

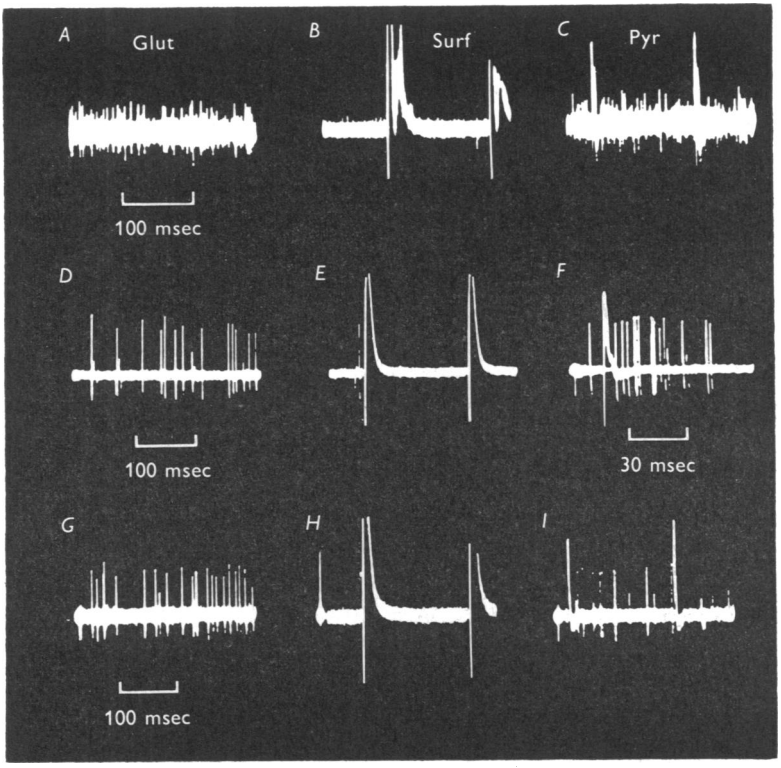

Fig. 14. Further comparison of inhibitory effects produced by surface (*B*, *E*, *H*) and pyramidal (*C*, *F*, *I*) shocks. In each case, the strongest pyramidal volleys available were used (150 V). Unit shown in *A–C* was a Betz cell; the other two (*D–F* and *G–I*) were not regularly excited by pyramidal stimulation. All were found in pericruciate region, and were excited throughout with glutamate. (Control traces in *A*, *D*, *G*.)

Inhibitory effect in different species and at different stages of development

Cortical inhibition was readily elicited by direct stimulation in the three different mammalian species studied; cats, rabbits and monkeys. There was no major qualitative difference between the effects seen in the various animals. Certain peculiarities of the effects produced by repetitive stimulation of the post-central region in the monkey (cf. Fig. 18) will be described later.

Inhibition can be seen in young kittens very soon after birth (Krnjević *et al.* 1964*c*; Purpura, Shofer & Scarff, 1965). Unit responses are not easily obtained during the first week and they cannot be studied conveniently, because the cells are very quickly depolarized or inactivated by applica-

tions of L-glutamate. Towards the end of the first week, when recording conditions improve, inhibition is not only clearly evident, but it seems even more potent and prolonged than in the adult, so that a complete block of activity may be produced for over 0·5 sec by a single direct cortical stimulus. This is illustrated in Fig. 15 where a single unit response, elicited by a short pulse of L-glutamate (*A*), was inhibited by a preceding direct stimulus to the cortex. The inhibitory effect is shown either by failure of response (as in *B* and *D*) or by a longer latency of discharge (*E*).

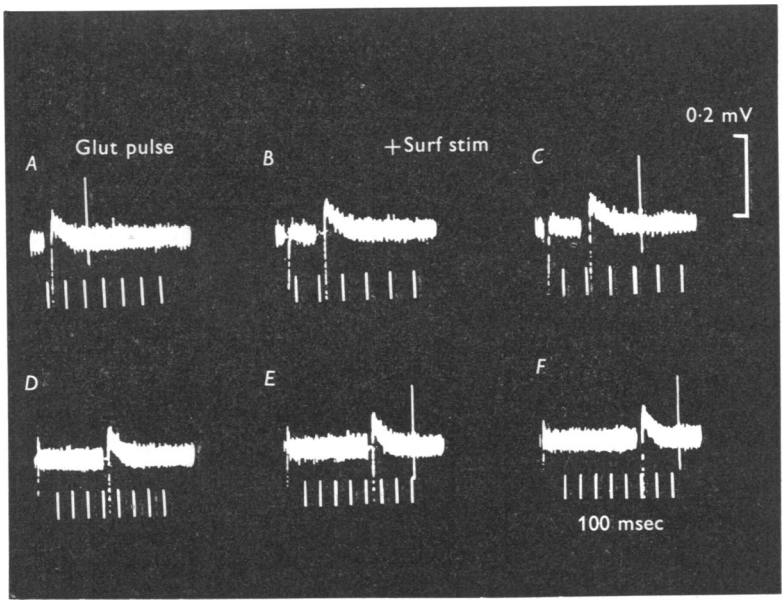

Fig. 15. Prolonged cortical inhibition in twelve day old kitten. Control record (*A*) shows single response of post-cruciate unit evoked by a 10 msec pulse of glutamate. Preceding surface shocks (60 V, 0·1 msec) at two intervals (*B–C*) inhibited the unit, either preventing a response or increasing its latency. Surface shock was then raised to 100 V; this increased duration and intensity of inhibition (*D*, *E*, *F*).

When a stronger cortical shock was given (*D–F*) an approximately normal response was only seen if the interval between the testing and the inhibiting pulses exceeded 0·6 sec (*F*). Since the pulse of glutamate only excited the unit after a latency of about 200 msec, presumably because of a relatively long diffusion distance, the total period of inhibition must have been at least 0·8 sec.

Spatial distribution of inhibitory process

In different areas of the cortex

The usual prolonged inhibitory effect was observed in all cortical areas tested in cats: the anterior and posterior sigmoid gyri, the whole suprasylvian gyrus, including the region in front of the ansate sulcus; the marginal gyrus, the cortex below the suprasylvian sulcus (in the upper portions of the sylvian and ectosylvian gyri), as well as gyri on the medial aspect of the hemisphere, such as the splenial and cingulate.

Spread of inhibition within a given cortical area

Horizontal spread. Perhaps the most remarkable feature of this inhibition is that it affects an astonishingly large number of neurones. Stimulation of a given point on the surface, by a single suprathreshold shock, can have an inhibitory effect on every detectable unit throughout all layers, for a distance of 10 mm along the same gyrus (Fig. 16). Some inhibitory

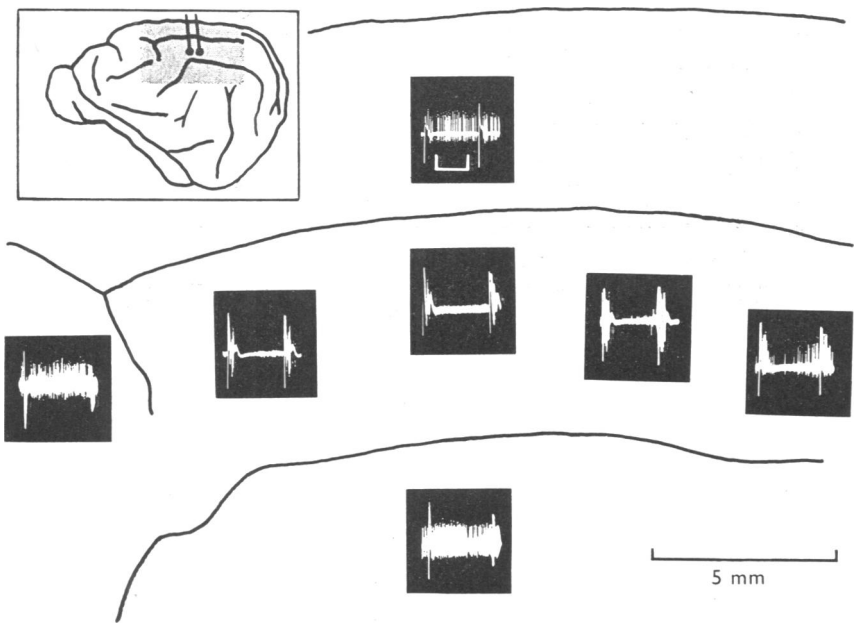

Fig. 16. Diagram of parietal cortex of a cat, showing spread of inhibitory effect caused by bipolar surface stimulation in suprasylvian gyrus at about 6/sec. Within area indicated by inset traces, practically all 88 units tested at various depths showed clear inhibition but effect was somewhat greater behind stimulating electrodes (up to a distance of 11 mm) than in front. Most of 33 units tested outside suprasylvian, in marginal and ectosylvian gyri, or in front of ansate sulcus, showed no clear effect (only 1/15 was definitely inhibited in ectosylvian and 0/10 in marginal). Time scale, 100 msec.

effect extends even beyond 10 mm but it becomes much less pronounced, and the required stimulus intensity, which is approximately constant for units within about 10 mm, now rises substantially.

The spread of inhibition may not be the same in all directions. For instance, in the experiment illustrated in Fig. 16, the inhibition was more pronounced behind the stimulating electrodes than in front, and the threshold was consistently somewhat higher for units in the rostral part of the gyrus. In most areas, inhibition does not spread across sulci to adjacent gyri (cf. Fig. 16). The only exceptions that we have found are in the region of the cruciate sulcus in the cat, and the central sulcus in the monkey; in both cases a substantial inhibitory effect overlaps both pre- and post-sulcal areas (cf. Fig. 9 Krnjević *et al.* 1966*a*).

Local variations in the magnitude and threshold of the inhibitory effect are seen even better if, instead of stimulating at one point and testing various units, one analyses the inhibitory effect produced on one particular cell by stimulating the cortex at different points. The effectiveness of stimulation is then found to vary substantially with position and direction. It is difficult at this stage to make any general statements with great confidence, but from our limited observations it seems that the inhibitory mechanism is elicited more readily and widely by surface stimulation near the apex of a gyrus than in the walls of sulci.

Vertical spread. As already mentioned, the inhibitory effect is evident when recording from units in all the cortical layers, whether stimulating the surface or at various depths. The efficacy of a cortical shock in causing inhibition is approximately the same for superficial and for deep units, the lowest threshold being always found relatively deep in the cortex.

Time course of inhibition

After single shocks

It is evident from the results already described that the inhibitory effect varies with the site, the intensity and the polarity of stimulation. The longest, as well as strongest, inhibition is obtained with direct cathodal shocks. All forms of indirect stimulation tend to have a shorter action, and the further the point of stimulation, the shorter and less effective the inhibition (cf. Fig. 10). Nevertheless, even peripheral volleys can produce an effect lasting as much as 100 msec or more. Much of the variation no doubt depends upon the general excitatory inflow into the cortex and the balance between intracortical excitation and inhibition.

Direct stimulation

The full time course of inhibition is studied best in post-stimulus histograms which reveal even a small reduction in excitability. The typical

duration after a relatively strong volley is in the order of 150–300 msec (cf. Figs. 1 and 5). The time course changes with the intensity of stimulation, as shown in Fig. 7 (cf. also Fig. 3).

As already mentioned the weakest effective surface stimuli only have an excitatory action, lasting some 10–20 msec (Fig. 3). Inhibition appears only with stronger volleys. At first, the peak of inhibition comes relatively late, but stronger stimulation enhances the inhibitory effect, which starts and reaches its peak much earlier. There is a phase of increased excitability immediately after the inhibition, giving it a sharper end. The time course of the inhibitory effect is thus partly distorted by early and late excitation (see Krnjević *et al.* 1966*a*).

Latency of onset

This circumstance makes it difficult to estimate exactly the latency of onset of the inhibition, particularly when recording extracellularly. When early excitation is minimal, definite inhibition can easily be detected within 10 msec of the stimulus; and when spikes are especially large and the stimulus artifact small, the inhibition can sometimes be shown to occur within 4 msec of a shock applied at a point not more than 1–2 mm away.

During and after repetitive stimulation

One of the most striking features of cortical inhibition is its cumulative effect during repetitive activity, showing no tendency to fatigue. Over a certain range of frequency, the maintained inhibitory effect can often prevent cells from discharging for as long as the stimulation is continued. The end of stimulation is usually followed by a further silent period, whose duration increases with the strength and the frequency of the previous repetitive shocks.

The traces in Fig. 17 illustrate some of these effects. In *A*, the spontaneous wave activity in the cortex was markedly depressed by 6/sec direct stimulation, while in *B*, unit firing, evoked with L-glutamate, stopped completely during similar stimulation. When this was repeated during a faster release of glutamate (*C*), the inhibition was less complete, and the following silent period much reduced. The spikes, which became rather small owing to an excess of glutamate, grew significantly larger during the period of stimulation; this is of some interest, as it points to a hyperpolarizing rather than a cathodal type of block (see Krnjević *et al.* 1966*a*).

As might be expected, the minimal frequency at which more or less complete inhibition occurs depends on the duration of the effect of a single shock. In most cases, the period of strong inhibition lasts 150–200 msec and the corresponding minimal blocking frequency is 5–7/sec. In kittens,

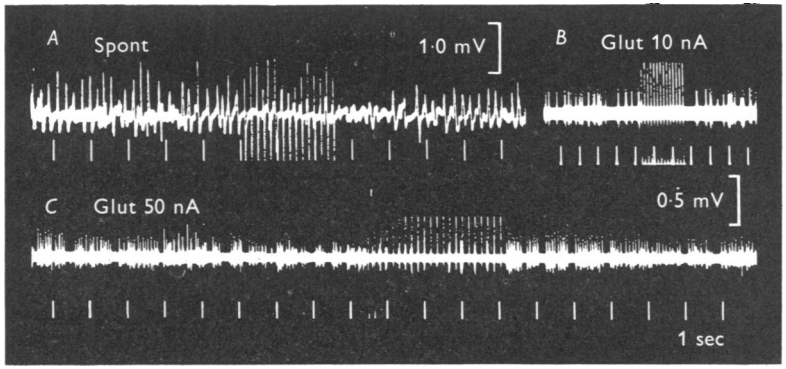

Fig. 17. *A*: spontaneous waves recorded in suprasylvian gyrus at depth of 0·6 mm and inhibited by repetitive surface shocks at 6/sec (60 V, 0·1 msec pulses) *B*: unit discharge evoked with L-glutamate (at 0·4 mm in suprasylvian area) also blocked by similar surface stimulation at 6/sec. *C*: when same unit was excited more strongly by faster release of glutamate, it could no longer be inhibited completely by identical surface stimulation. Note increase in spike heights during and just after tetanus.

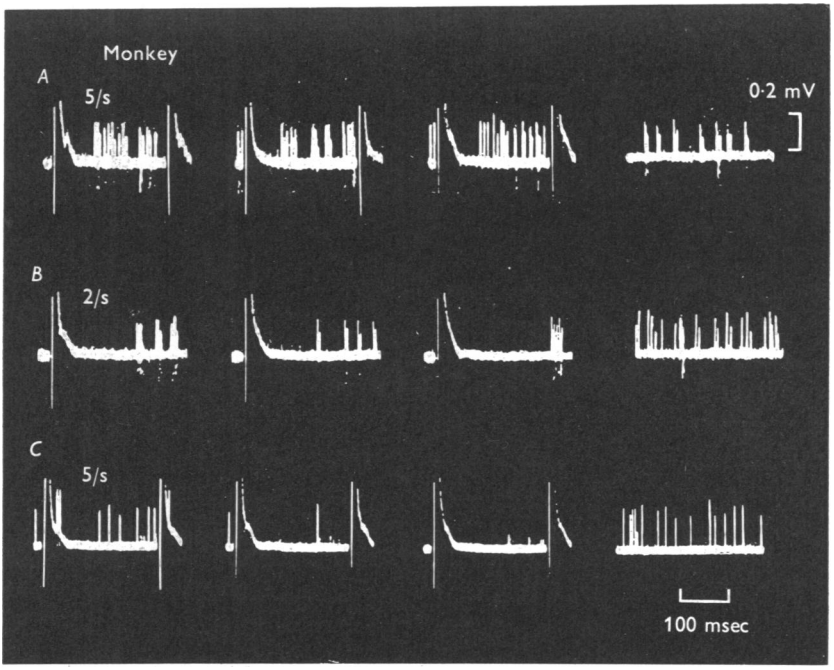

Fig. 18. Two units recorded in post-central gyrus of rhesus monkey (*Macaca mulatta*); both were excited with L-glutamate released iontophoretically (control traces at right). Direct surface stimulation caused usual inhibitory effect, increasing in duration with intensity of surface shocks (all 0·1 msec pulses, at 60, 100 and 120 V, from left to right in each row). When first unit (*A* and *B*) was tested with surface shocks at 5/sec (*A*), the inhibition was much less effective than at 2/sec (*B*): second unit was strongly inhibited even at 5/sec (*C*).

where the effect may last over 0·5 sec, frequencies as low as 2/sec may be sufficient.

Another limitation arises from the early and late excitatory effects of direct shocks, mentioned above. When these are very marked, total inhibition cannot be maintained at any frequency; in fact, the excitation may itself be cumulative, in which case inhibition is best seen at relatively low rates of stimulation. This factor is especially prominent in the sensori-motor area, and it was particularly clear in the post-central gyrus of a monkey (Fig. 18). The unit in *A* and *B* was inhibited far more effectively

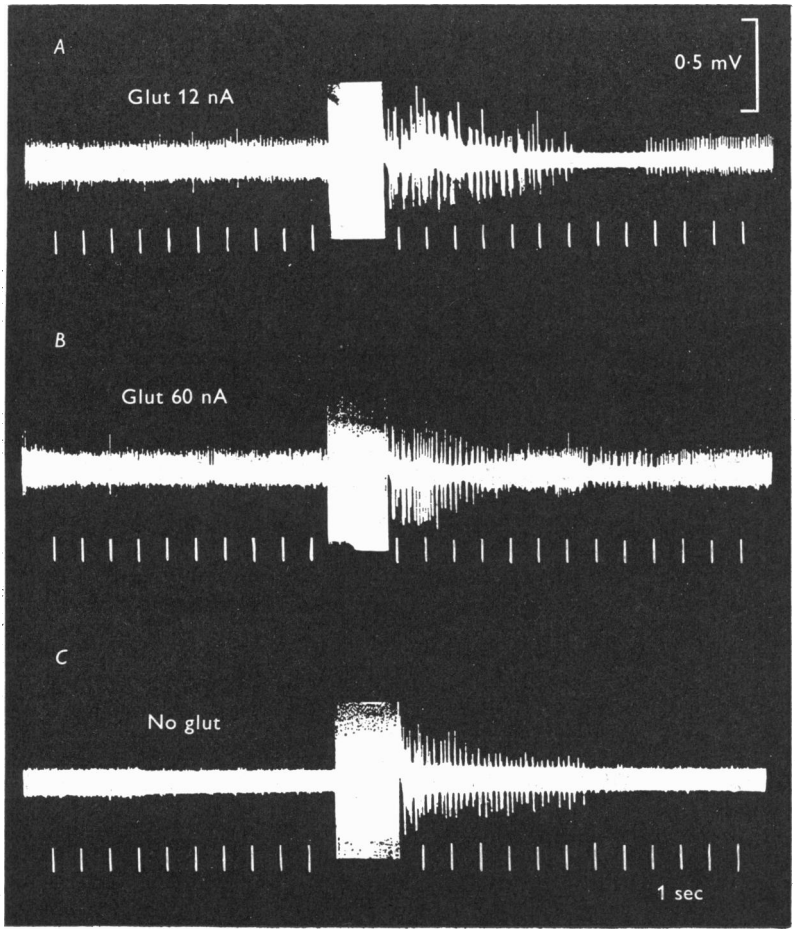

Fig. 19. Effect of short 100/sec surface tetani on a unit in an acutely isolated slab of suprasylvian cortex. *A*, unit was excited throughout by a slow release of glutamate (12 nA). *B*, glutamate release was now increased to 60 nA; this very much shortened silent period after tetanus. *C*, without glutamate, to show more clearly strong after-discharge.

at 2/sec than at 5/sec. Some other cells, however, like that in *C*, were readily blocked even at the higher frequency.

In all areas, the potentiation of the excitatory effect may become predominant if the frequency of direct stimulation great y exceeds 8–10/sec. A short (5–15 sec) tetanus at 20–100/sec is often followed by a strong neuronal discharge, which may last a few seconds (Fig. 19). The strong inhibitory effect of the tetanus only becomes evident at the end

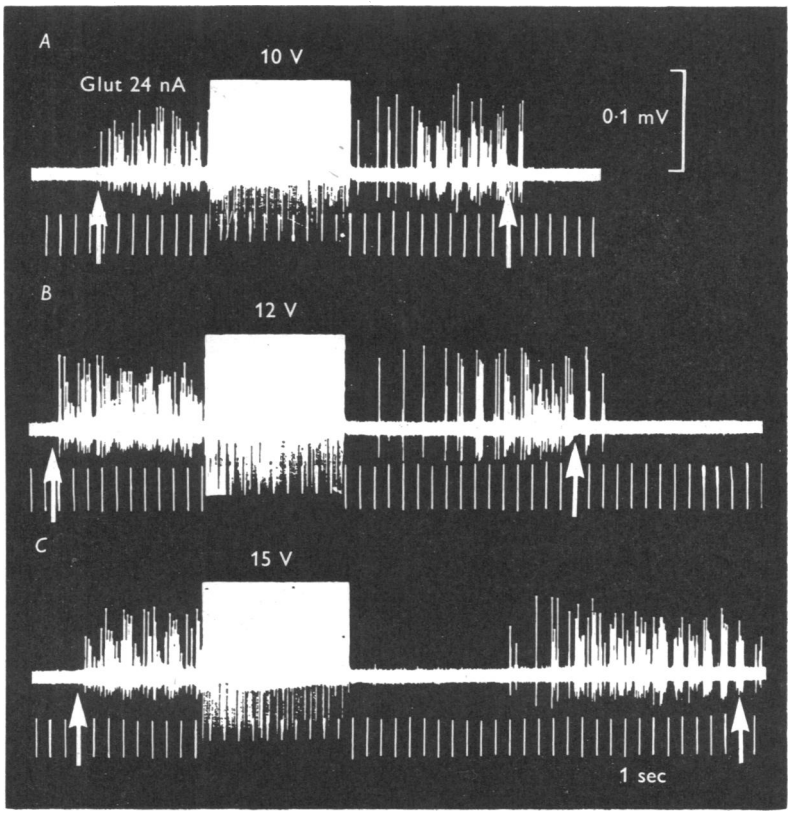

Fig. 20. Prolonged inhibition of unit by surface tetani in 5-week-old kitten. Shock intensity (at 25/sec) was increased from above down. Arrows indicate onset and end of glutamate release.

of this after-discharge, as a subsequent silent period whose duration varies with the strength (cf. Fig. 20), the frequency and the duration of the tetanus (cf. Fig. 21). After a particularly powerful tetanus, the phase of inhibition may last for as much as 1 min (Fig. 21). During the silent period the cells are not blocked by an excess of depolarization, since the silent period can be cut short by releasing a larger quantity of glutamate (Figs.

19*B* and 21). The after-discharge is often absent after a relatively long tetanus (Fig. 21).

This kind of double effect, an after-discharge followed by a prolonged inhibition, can be elicited by tetanic stimulation applied directly or transcallosally. On the other hand, thalamic stimulation, which is also effective in producing inhibition, does not lead to a cortical after-discharge, so that after a tetanus one only sees a prolonged inhibition (Fig. 8 in Krnjević *et al.* 1966*b*).

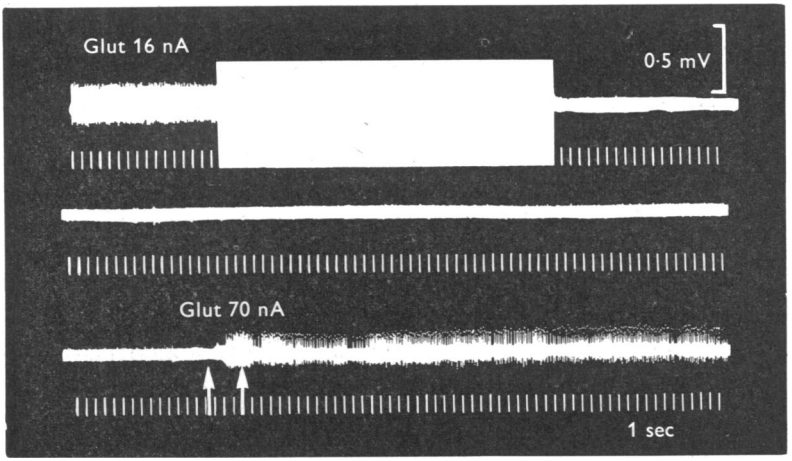

Fig. 21. Long-lasting blocking action of prolonged surface tetanus (100 V, 0·1 msec, at 50/sec). Glutamate was released at a rate of 16 nA throughout, except for short period, between arrows, when iontophoretic current of glutamate was raised to 70 nA. Traces are continuous.

The typical sequence of changes produced in the cat by a cortical tetanus, that is, an after-discharge followed by a long silent period, is very much like the phenomenon of spreading depression (Leão, 1944; Grafstein, 1956). Tests on rabbits, where spreading depression is elicited much more readily, have shown great similarity between the behaviour of units after tetanic stimulation in the cat and during spreading depression in the rabbit (Krnjević & Randić, 1965). It is very likely that the same inhibitory mechanism is active in both cases, and that the long period of depression is at least partly due to a similar hyperpolarizing type of inhibition, and not merely to an excess of depolarization as has been suggested previously Grafstein, 1956; Marshall, 1959).

DISCUSSION

Only a partial discussion is possible at this stage, since other relevant observations are presented in the next paper. Nevertheless, certain features of the experiments require some comment.

Justification of the method

There is little reason to doubt that L-glutamate depolarized the cortical cells by a direct effect on the membrane. Responses are obtained regularly only in the grey matter; under optimal conditions, when glutamate can be applied in pulses, its effect occurs after a minimal latency, which leaves hardly any time for an indirect mechanism of activation (Krnjević & Phillis, 1963*a*; Krnjević, 1964). Similar observations could also be made on spontaneously active cells when exciting the cells with ACh or by a cathodal focus. Any presynaptic effect of glutamate (Schmidt, 1963) is therefore unlikely to be here of much significance (cf. also Curtis, Phillis & Watkins, 1960). The most reasonable conclusion is that by this method we were indeed testing the excitability of the cells and that the observed inhibitory effects were probably post-synaptic.

Possible injury by the stimulating current

Although the voltages used may seem rather large, suggesting that some kind of non-specific injury could have resulted, the actual intensity of direct stimulation needed for an inhibitory effect was relatively low when compared with the amounts of current required to excite Betz cells (Phillips, 1956*b*; Hern *et al.* 1962).

Bipolar surface shocks are very inefficient, since much of the current is shunted along the surface of the cortex. With monopolar stimulation, less current is necessary but even this method is relatively inefficient, because the region of lowest threshold is deep within the cortex (Fig. 8). When the shocks are given through a deep focal electrode, the threshold for inhibition (measured several mm away) corresponds to a total flow of electric charge of only about 30 nC.

The focal stimulating electrode used here had a 20 μ radius at the tip and the threshold current was 30 μA. If the specific resistance of the cat's cortex is 222 Ω cm (Freygang & Landau, 1955), the total power dissipation in the tissue would be about 16 μW, and since 1 msec pulses were used, at the rate of about 1/sec, the energy expenditure per second would be only 16 nJ. Within a radius of 100 μ from the point of stimulation (where 90% of the power is dissipated) the rate of heating would not exceed 1 mcal $cm^{-3}sec^{-1}$.

The total flow of electric charge at threshold (30 nC) corresponds to a displacement of only 0·3 p-equiv of ions, which is minute compared with the total amount of cations and anions already present in the tissue.

For surface stimulation, a platinum sphere with a radius of 120 μ was pressed lightly against the pia. At a threshold current of 1 mA (cf. Fig. 7), the total power dissipation would be 5·6 mW, and the work done 0·56 μJ; which is negligible, especially since it is spread over a much larger volume of cortex (90% within a radius of 1·2 mm).

A significant local change is only likely to occur during a prolonged maximal tetanus (e.g. 100/sec). The work done on the tissue by stimulation at this frequency would be about 1·5 mJ sec^{-1}; the rate of heat production within a radius of 0·25 mm of the point of stimula-

tion could be as much as 11 cal $cm^{-3}sec^{-1}$. However, the volume of tissue directly affected under these extreme conditions is still very small when compared with the distribution of the inhibitory effect.

Inhibition by various inputs

A similar kind of prolonged inhibition could be elicited by stimulating the periphery and certain thalamic nuclei, or by transcallosal volleys, in agreement with the observations of many previous authors (see Introduction). The most likely explanation is that most afferent inputs can bring into action the same inhibitory system.

One way in which this might happen could be through a very powerful recurrent inhibitory mechanism, activated solely by the discharge of corticofugal elements. Effective thalamic and transcallosal volleys would always first activate corticofugal fibres.

Our observations do not support this hypothesis. Pyramidal antidromic volleys produce little cortical inhibition, except possibly when repetitive shocks at a high frequency are used (cf. Stefanis & Jasper, 1964; Brooks & Asanuma, 1965). Moreover, the greater effectiveness of surface cathodal than anodal shocks, and the relatively weak currents required, suggest that the population of neurones excited by direct cortical stimulation differs from the Betz cells studied by Phillips and his collaborators (Phillips, 1956*b*; Hern *et al.* 1962). The fact that a surface cathode is more effective does not necessarily mean that the cells concerned are relatively superficial (cf. Hern *et al.* 1962); but rather suggests that they do not have a predominantly vertical orientation.

The inhibitory mechanism is therefore probably not dependent on a recurrent pathway, but antidromic volleys may well act on the same inhibitory system as various kinds of afferent activity or shocks applied directly to the cortex.

Intracortical distribution of inhibitory effect

The neural elements responsible for the spread of inhibition are mainly situated relatively deep in the cortex, but they can exert their influence throughout all layers. The wide distribution of inhibition is consistent either with inhibitory fibres stretching over a horizontal distance of at least 5 mm, or with much more localized inhibitory elements which can be brought into action by tangential fibres.

Variations in local threshold suggest that the inhibitory system, though widespread, is not uniformly distributed. For instance, it is only poorly developed in the walls of sulci. This adds to previous evidence that the cortex buried in sulci functionally differs from the cortex exposed at the surface of the gyri (von Economo, 1926; Kreiner, 1961; Krnjević & Silver, 1965).

Duration of inhibition

The prolonged effect of a single volley is of interest in several respects. It could play a significant role in the genesis of the electrocorticogram, because of the widespread, synchronous inhibition of a large number of cells. The fact that frequencies in the order of 10/sec are a prominent feature of the electrocorticogram thus seems rather suggestive. The long duration and cumulative effect would make it possible to maintain a state of inhibition almost indefinitely, with only a very low rate of activity in the inhibitory system.

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