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NATURE OF A CORTICAL INHIBITORY PROCESS

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SUMMARY

1. Since the inhibitory effect of direct or indirect cortical stimulation on cortical units can be overcome by excitation with even more L-glutamate, it is not likely to be due to an excessive depolarization.

2. Further evidence that surface stimulation has a hyperpolarizing action on cortical cells was obtained by intracellular recording from over 120 pericruciate cells. Inhibitory post-synaptic potentials (IPSPs) are seen in most cells, which are comparable in threshold and duration with the inhibitory effect observed extracellularly. The IPSPs are usually not preceded by a discharge of the same cells.

3. The extracellular slow wave corresponding to the inhibitory effect varies considerably with different preparations and different depths within the cortex. A predominantly positive wave is only seen occasionally. In general, the relevant wave recorded deep in the cortex tends to be mainly negative.

4. This negative slow wave can be much potentiated by tetanic stimulation, or, especially, by a large local release of L-glutamate; the last procedure is most effective either very near the surface, or below a depth of 1·0 mm. These observations suggest that inhibitory synapses occur more profusely in the superficial half of the grey matter.

5. Unlike L-glutamate, GABA tends to depress the 'inhibitory' slow wave.

6. The inhibitory effect must be produced by intracortical neurones, since it is fully preserved in isolated cortical slabs. In both acute and chronic slabs, the inhibition is particularly well marked and long lasting,

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partly because spontaneous activity and the usual post-inhibitory rebound of excitability are absent.

7. The intracortical pathways responsible for the spread of inhibition cannot be situated mainly in the superficial layers, as they are not readily blocked by surface cooling or the application of local anaesthetics.

8. One can record unit discharges immediately after a surface shock. Some of these discharges could be from inhibitory interneurones, but they do not last more than 10–20 msec.

9. We conclude from the observations described in this and a previous paper (Krnjević, Randić & Straughan, 1966*a*) that a widespread system of intracortical interneurones can be activated by direct or indirect stimulation of the cortex; these interneurones have a powerful and prolonged inhibitory action on most cortical cells.

10. The identity and distribution of the postulated inhibitory interneurones is discussed in the light of some relevant morphological evidence.

INTRODUCTION

The first paper of this series (Krnjević *et al.* 1966*a*) described a strong inhibitory effect elicited in the cortex by direct stimulation, as well as by various kinds of afferent activity. It was particularly evident against a background of unit firing evoked by the micro-iontophoretic application of excitatory agents. This inhibition was remarkable for its long and cumulative action, and its widespread distribution affecting neurones in all cortical layers. It was shown that the threshold for evoking inhibition was lowest when stimulating relatively deep within the cortex, but the intensity and polarity of effective surface shocks indicated that the neuronal elements responsible for the inhibitory effect were probably not deep pyramidal cells. This was confirmed by showing that antidromic pyramidal volleys have a comparatively weak inhibitory action on Betz cells and other cortical neurones.

The present paper describes experiments designed to throw further light on the mechanism of this inhibition. The possibility that afferent inhibitory pathways are responsible for this phenomenon was tested by examining neurally isolated cortical slabs. A correlation was also made between the inhibitory effect and slow changes of potential recorded both outside and inside cortical cells.

METHODS

Most of the methods have already been described in the first paprr (Krnjević *et al.* 1966*a*). For intracellular recording, we used conventional single-barrelled Pyrex glass micro-pipettes, having tip diameters of $< 0{\cdot}5\ \mu$ and filled with 2M-K citrate.

RESULTS

Mechanism of neuronal block

Several kinds of blocking mechanisms could produce a temporary reduction in neuronal discharge.

Presynaptic changes

If a neurone is excited by a synaptic bombardment, its discharge may cease because the exciting input is interrupted, for instance, by a distant inhibitory action, or by presynaptic inhibition. This type of mechanism cannot play a significant role here, because the inhibitory effect could be observed not only when cells were firing spontaneously, but also during discharges evoked directly, by an electric current, by ACh or by L-glutamate, applied from micropipettes (Fig. 2 in Krnjević *et al.* 1966*a*). It will be shown, moreover, that characteristic changes in membrane potentials are associated with the phase of depression.

Post-synaptic changes

Extracellular observations. Two kinds of change in the state of the membrane might give a block of discharge; either a hyperpolarization or an excessive depolarization associated with a process of inactivation. Several kinds of evidence are clearly against the second possibility.

For instance, one commonly observes somewhat larger spikes during the period of depression, especially if the spikes have tended to diminish during a prolonged application of L-glutamate (Fig. 17 in Krnjević *et al.* 1966*a*).

One would expect cells undergoing a cathodal type of depression to be particularly insensitive to depolarizing agents such as L-glutamate. Yet the inhibitory effect can usually be overcome by increasing the dose of glutamate. The unit illustrated in Fig. 1 was initially excited with a very small, continuous release of L-glutamate (2 nA), and the resulting discharge could be totally abolished by repetitive shocks at 60 V. At the first arrow (upper line), the rate of release of glutamate was raised to 3 nA; the stronger excitation cancelled out the later part of the inhibitory pause, but an increase in shock intensity to 80 V was sufficient to re-establish total inhibition. At the second arrow the stronger block was overcome by a further rise in the glutamate current (to 5 nA). Almost complete inhibition could still be obtained with shocks of 120 V; but when glutamate was applied still faster (8 nA, at third arrow), even the strongest shocks available produced only a relatively short-lasting silent period. This sort of interaction is exactly the opposite of what would be expected in the

presence of cathodal block; it suggests rather that a hyperpolarizing or stabilizing type of change is produced in the cell membrane by the inhibitory process.

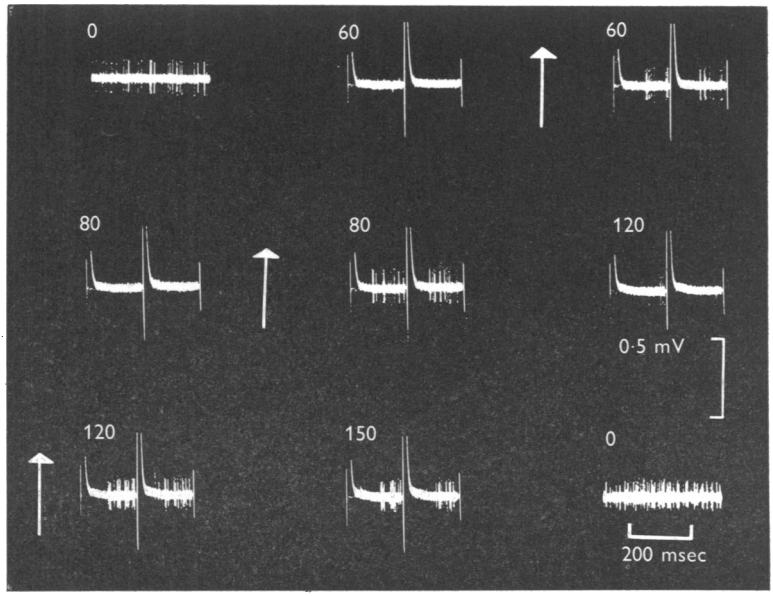

Fig. 1. The inhibitory effect of surface shocks can be overcome by increasing the rate of application of L-glutamate. A unit was excited by very slow iontophoretic release of glutamate (2 nA), as shown by first trace. Surface shocks (0·1 msec, 60 V, at 6/sec) inhibited this discharge completely, but the cell began firing again when the glutamate release was increased to 3 nA (first arrow). Raising the strength of the surface shock to 80 V abolished the discharge, but it started again when the glutamate release was raised once more, to 5 nA (at second arrow). 120 V shocks were now required to stop the discharge, but the inhibition was overcome by even more glutamate (8 nA from third arrow): after this, even the strongest shocks available (150 V) could not totally prevent the discharge. The last record is a final control showing the glutamate-evoked firing in the absence of surface shocks. In each record, several oscilloscope traces are superimposed.

Membrane potentials

Intracellular measurements of membrane potentials confirmed the above conclusion, since corresponding hyperpolarizing changes were seen consistently inside most of the cells examined in the pericruciate area in experiments on three cats.

Intracellular recording was usually only possible for very short periods, lasting a few seconds; but this was sufficient to detect definite depolarizing or hyperpolarizing changes evoked by surface shocks.

In the first experiment, twenty-one cells had an initial resting potential

of 50 mV or more; all these cells showed a prolonged hyperpolarization (lasting 100–200 msec) in response to cortical shocks which we know from other experiments to be adequate for evoking the inhibitory effect.

In the second experiment stable resting potentials were only found with great difficulty; in most cases the potentials disappeared almost immediately. Only eight cells out of sixteen were definitely hyperpolarized by surface shocks.

The recording conditions in the third experiment were much more satisfactory, so that sufficiently stable recording was possible from eighty cells. Cortical stimulation elicited a variable but definite hyperpolarization of all these cells, except one, which had a particularly stable resting potential of 75 mV, but showed no effect at all, either hyperpolarizing or depolarizing, even after the strongest shocks available.

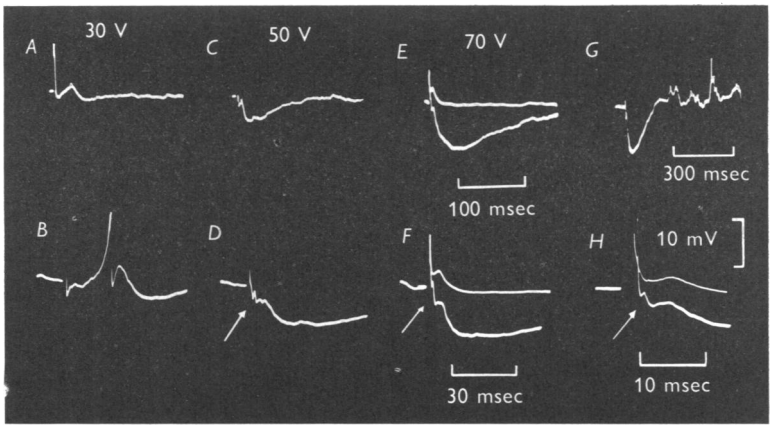

Fig. 2. Effects of surface shocks on membrane potentials recorded inside a post-cruciate cell. *A* and *B* show on relatively slow and fast time bases, respectively, the excitatory effect of a weak shock (0·1 msec, 30 V); in *A* there is a simple EPSP, while in *B* a spike was generated on top of the EPSP; only the lower part of this spike is visible. Stronger stimuli (50 and 70 V) initiated a hyperpolarization (*C–H*). The effects at 70 V are shown on four different time scales (*E–H*). In records *E*, *F* and *H* are also superimposed the traces obtained just outside the cells after withdrawing the electrode slightly (upper traces). Arrows point to a postulated, presynaptic spike. Note that in this figure and Fig. 3 a positive change or depolarization is shown by a deflexion upwards, instead of downwards as in most other figures; and that *A*, *C* and *E* are on the same time scale, indicated under *E*, while *B*, *D* and *F* are on a faster scale, indicated under *F*.

The exact changes produced depended on the intensity of stimulation. For instance, Fig. 2 illustrates a sequence of responses obtained with increasingly stronger shocks. At 15 V there was no marked effect, but at 30 V, a clear and relatively prolonged depolarizing potential (presumably an excitatory post-synaptic potential or EPSP) was elicited (*A*). It had a

relatively slow rising phase and lasted 20–30 msec, and sometimes there was a spike discharge associated (*B*) which did not erase the later phase of the EPSP.

A stronger volley completely abolished the initial EPSP, replacing it by a much more prolonged hyperpolarizing potential (presumably an inhibitory post-synaptic potential; Fig. 2*C*, *D*), which became even larger and longer when the stimulus was increased to 70 V (*E*). At this stage it reached its peak about 15 msec after the stimulus *F*, and lasted some 150 msec, being followed by a series of small depolarizing deflexions (*G*).

The traces recorded with a faster time base show in greater detail the initial phase of the IPSP (*F* and *H*). They reveal a very early and sharp positive deflexion, which appeared as soon as the intensity of stimulation was adequate to evoke the slow IPSP, which in fact followed immediately. This early deflexion did not change its latency but it became somewhat larger when stronger shocks were given. It was clearly not a spike produced by this cell (cf. Fig. 2*B*), nor was it due to a massed discharge of surrounding cells, since it is only just detectable in some of the control traces recorded from the immediate neighbourhood, after withdrawing the electrode from the cell (cf. upper trace in *H*). This may have been a presynaptic spike, recorded from certain nerve endings.

These control traces also show why the IPSP appeared to reach its maximum only comparatively slowly: the initial part of the intracellular trace is superimposed on a substantial positive field potential; if one compares the two relevant traces recorded inside and outside, it is clear that a marked hyperpolarization is already present at the end of the initial, spike-like deflexion.

We did not test the neuronal excitability under these conditions, but in some cases the cells were discharging spontaneously, as in Fig. 3, and it was then evident that the excitability was depressed during the phase of hyperpolarization. Several traces show other pauses in the discharge, which were associated with spontaneous hyperpolarizing potentials (presumably IPSPs).

Post-activation depression

It has already been pointed out (Krnjević *et al.* 1966 *a*) that the inhibitory effect being described did not require an initial excitation and discharge of the cell. The cells were sometimes fired by the stimulus, but, in the majority of cases, even the most careful examination of the initial phase failed to show any discharge. This is relatively difficult to illustrate convincingly when recording extracellular spikes, because of the relatively large stimulus artifact, but both Figs. 2 and 3 show quite clearly that the hyperpolarizing potentials were usually not preceded by a spike, and

therefore they cannot be considered analogous to the 'after-hyperpolarizations' which are seen, for instance, in spinal motoneurones (Eccles, Eccles & Lundberg, 1958).

The inhibitory effect of surface shocks is thus associated with a prolonged hyperpolarizing change in membrane potential, which is widespread among cortical cells in the vicinity of the stimulating electrode. This hyperpolarization bears no relation to the discharge of the cells and it is therefore

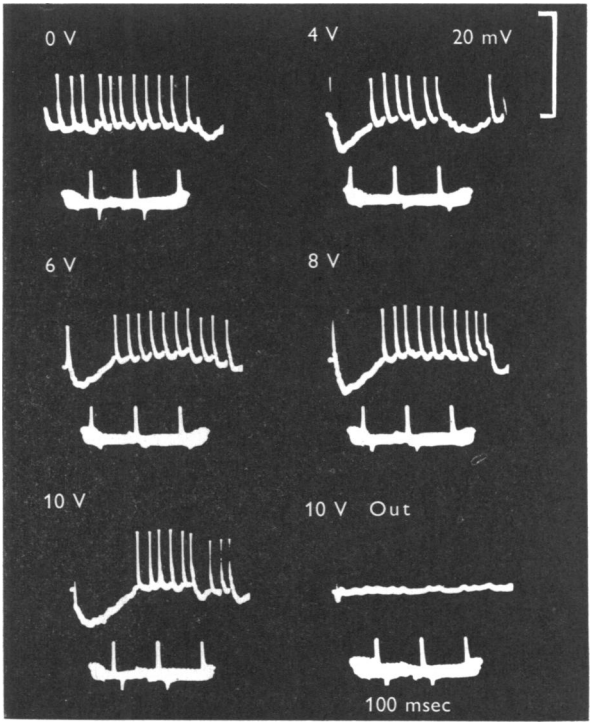

Fig. 3. Membrane potentials recorded inside a post-cruciate cell during cortical stimulation with 1·0 msec pulses, at the voltages indicated. The bipolar stimulating electrodes were about 3·0 mm away, on the pial surface. The recording electrode injured the cell, causing a maintained, high-frequency discharge. The last trace was recorded just outside the cell. Upward deflexions indicate depolarization.

comparable to an IPSP and not to an after-potential. The intracellular records therefore fully confirm in every respect the conclusions drawn from the changes in excitability demonstrated with extracellular applications of L-glutamate (see Krnjević *et al.* 1966*a*).

External slow potential changes corresponding to the IPSPs

We have already emphasized the widespread character of this inhibition, which synchronously affects cells throughout all cortical layers. This might

be expected to produce a very substantial and characteristic slow positive deflexion in the electrocorticogram, recorded either at the surface or within the cortex. In fact, one does not find regularly a characteristic and constant slow wave corresponding to the IPSPs, and the waves actually observed with surface stimulation are by no means always predominantly positive deflexions.

Surface shocks

It is almost impossible to describe a wholly typical sequence of waves, because of large variations in their appearance in different preparations (cf. Chang, 1951; Burns, 1951; Stohr, Goldring & O'Leary, 1963). The waves may be positive or negative, or biphasic; and even in the same animal their amplitude and shape may vary considerably with different intensities of stimulation. Sometimes they are relatively well defined, while at other times they can only be detected after careful examination.

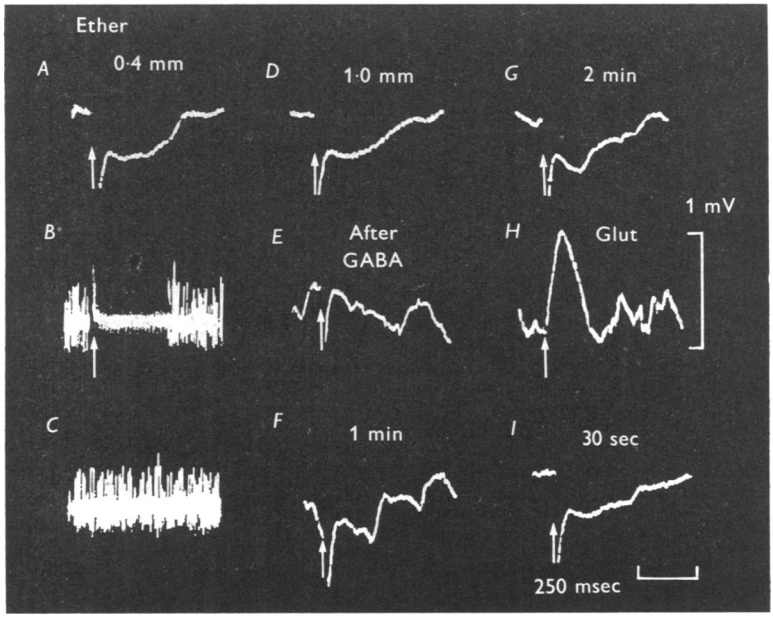

Fig. 4. Inhibition and slow waves in mid-suprasylvian gyrus of cat under ether. Cortical surface was stimulated with 20 V, 0·1 msec cathodal shocks, immediately above point of recording. *A*: slow positive wave recorded at depth of 0·4 mm. *B*: inhibition of glutamate (4 nA)-evoked unit discharge, at same depth. *C*: control showing discharge without cortical shocks. *D*: similar slow wave at depth of 1·0 mm. *E*: immediately after a large application of GABA (140 nA for 1 min) at point of recording. *F*: 1 min later. *G*: 2 min later. *H*: near end of a large application of L-glutamate (180 nA for 1 min). *I*: 30 sec later. Negative deflections upwards. In this and subsequent figures, arrows indicate stimulus artifacts.

Nevertheless, if the threshold and time course of the inhibition effect is used as a standard of reference, one can nearly always find some indication of slow waves related to the inhibition.

A particularly clear positive wave recorded in a cat under ether is illustrated in Fig. 4 (*A* and *D*). One can see from the pause in the glutamate-evoked discharge of Fig. 4*B* that the maximal inhibitory effect on cells

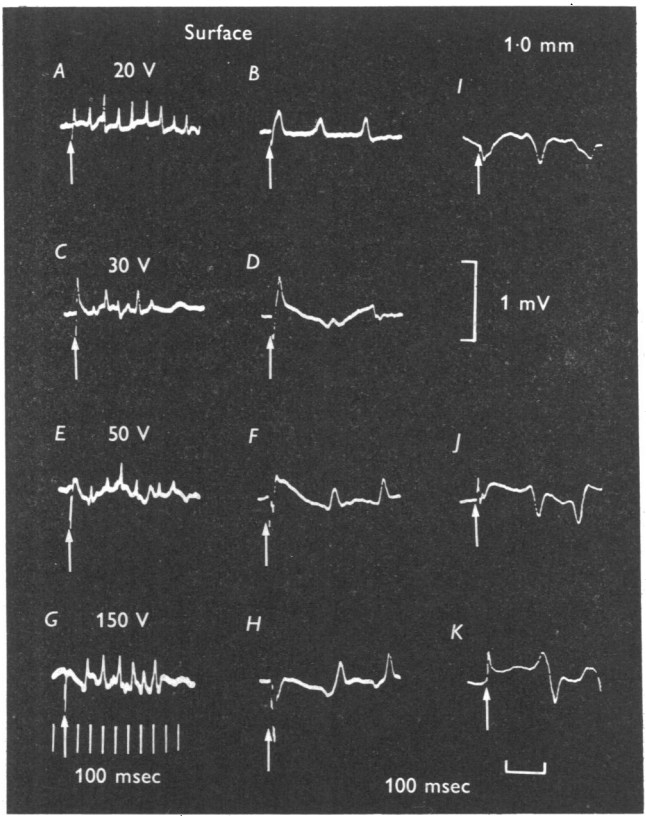

Fig. 5. Slow waves evoked in mid-suprasylvian gyrus by 0·1 msec surface shocks (intensity indicated). The waves recorded at the surface, 2·5 mm from point of stimulation, are shown on slow (*A*, *C*, *E*, *G*) and fast (*B*, *D*, *F*, *H*) sweeps, respectively. The corresponding waves recorded at a depth of 1·0 mm, are shown in *I*–*K* (on same fast time scale). The threshold for inhibition of units was at 25 V. Negative deflexions upwards.

at the same level agrees quite well with the peak of the slow wave. Such a definite positive wave, even relatively deep within the cortex, was very unusual. Another sequence of waves is illustrated in Fig. 5. In the first two vertical columns the waves recorded at the surface are shown on a very slow (*A*, *C*, *E*, *G*), and somewhat faster (*B*, *D*, *F*, *H*), time base,

respectively. Relatively weak shocks (20 V), below the threshold for inhibition, evoke a regular series of negative waves lasting altogether about 1 sec (Fig. 5*A*, *B*). Stronger shocks, above the threshold for inhibition, produced an interesting sequence of changes. At 30 V a slight slow positive tendency appeared, which was associated with a diminution of the first repetitive wave. At 50–150 V a new series of late waves appeared, starting somewhat later, and with a definitely biphasic character, each being at first positive and then negative; at the same time the slight positive wave became somewhat more pronounced after an initial negative component. The total duration of this wave was similar to that of the principal phase of inhibition.

The traces in the right-hand column of Fig. 5 show the corresponding waves recorded at a depth of 1·0 mm in the same area. The late repetitive waves, with their polarity reversed, are easily identified. The period of inhibition is here indicated by a slow negative wave.

It is evident from the traces shown so far, that cortical inhibition is not necessarily associated with very pronounced waves. In general, when the surface of the cortex is stimulated, under Dial anaesthesia, a relatively poorly defined positive or even a negative wave is likely to be recorded near the surface. The positive wave tends to disappear within 0·2–0·4 mm of the surface, often becoming a definite negative wave by a depth of 1·0 mm.

Potentiation of inhibitory waves

Ill-defined or latent 'inhibitory' waves can be made much more evident in at least two different ways. First, by tetanic stimulation and, secondly, by the release of large quantities of L-glutamate at certain levels within the cortex.

Post-tetanic potentiation (*PTP*)

After a 5 sec period of surface stimulation at 50–100/sec, above the threshold for inhibition, there is a marked increase, at certain depths, in the slow negative wave which corresponds to the inhibitory pause, as shown in Fig. 6. This increase is clearly visible only when recording either very near the surface (within 0·3–0·4 mm) or at or below about 1·0 mm. At the surface, this effect is maximal almost immediately after the end of the tetanus, and it disappears within about 20 sec. It is followed by a longer phase during which the first of the late negative repetitive waves is substantially potentiated.

By contrast, the PTP of the deep negative inhibitory wave reaches its maximum only after 5–10 sec, and it persists for at least another 20 sec. This PTP is quite evident even relatively deep (2·0–2·2 mm). In the intermediate zone (depth of 0·3–1·1 mm) there are only minimal changes.

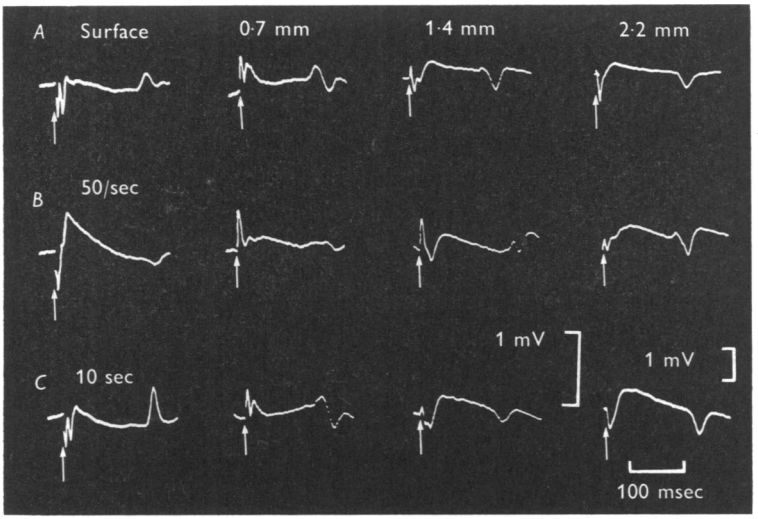

Fig. 6. Post-tetanic potentiation (PTP) of slow wave corresponding to phase of inhibition, recorded in same area as traces of Fig. 5, with surface shocks at 50 V. *A*: control traces at different depths. *B*: 2 sec after end of a 5 sec tetanus at 50/sec. *C*: 10 sec after end of tetanus. Note that PTP is immediate and short-lasting near surface, but delayed in deeper layers. Negative deflexions upwards.

L-*Glutamate effects*

One can induce an even more spectacular potentiation of a negative 'inhibitory' wave by a large micro-iontophoretic release of L-glutamate within the cortex (60–100 nA, for 20–30 sec); the best results are again obtained either close to the surface or below a depth of about 1·0 mm (Figs. 4 and 7). The superficial zone of maximal effect is quite narrow, within about 0·2 mm from the surface. In this region the action of glutamate tends to be very prolonged, a potentiated slow negative wave being evident for at least 30–40 sec after the end of the application of glutamate (Fig. 7).

This was not the case in the deeper zone of maximal effect, where glutamate produces an even greater change, which is extremely quickly reversible, as can also be seen in Fig. 7. This deep zone of maximal potentiation is fairly sharply limited to a depth of approximately 1·0–1·7 mm; below that, the effect rapidly disappears. In the intermediate zone (0·2–1·0 mm), one sees only a relatively small potentiation of the slow negative wave.

If the surface is stimulated tetanically during a prolonged application of glutamate within the cortex a phase of PTP is still clearly observed. The effects of glutamate and of tetanus are thus additive. It should

be noted that GABA has quite a different action, tending to abolish the slow wave; this effect may last for 1–2 min after the end of the application of GABA (cf. Fig. 4*D–G*).

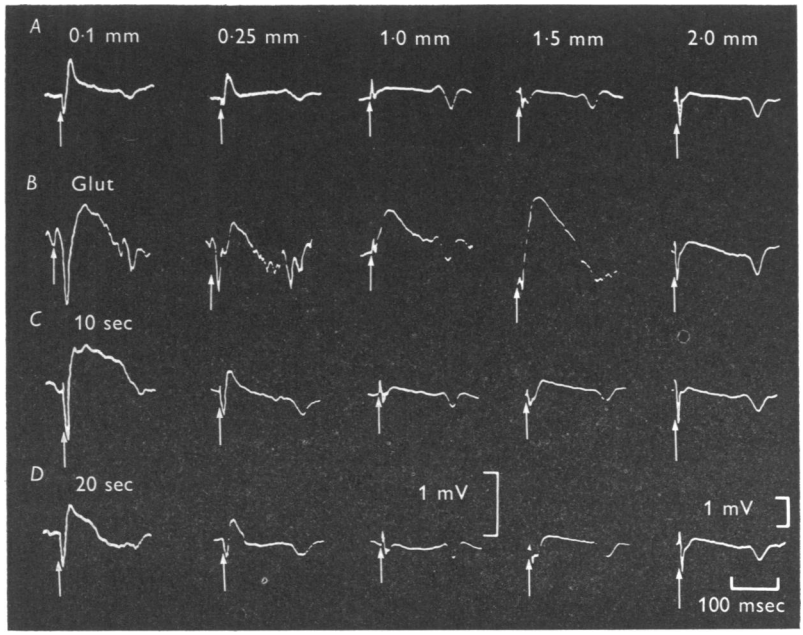

Fig. 7. Effect of large release of L-glutamate on slow waves evoked at different depths in mid-suprasylvian gyrus by 0·1 msec, 50 V surface shocks, *A*: control traces. *B*: traces recorded 30 sec after starting micro-iontophoretic application of glutamate at same depth; note especially large slow negative waves near surface and at 1·5 mm. *C*: 10 sec after end of glutamate release. *D*: 10 sec later. Note prolonged effect only near surface. Upward deflexions are negative.

Other slow changes in potential produced by repetitive surface stimulation

The cumulative depression caused by repetitive stimulation has already been described by Krnjević *et al.* (1966*a*). There is a similar cumulative increase in the associated slow waves, which leads to a marked change in local d.c. level, seen particularly well when the frequency of stimulation exceeds 10/sec. The principal effects observed are similar when stimulating the cortex locally, or indirectly by transcallosal activation. In both cases one can see a rapid negative shift during the tetanus (cf. Fig. 8*A*, *C*); this reaches a maximum at the end of the tetanus, and it tends to reverse rapidly towards the base line. However, if the tetanus is followed by a strong after-discharge, this appears to delay the return towards the base line. In most experiments one can detect a subsequent small positive shift, whose duration corresponds to that of the later portion of the inhibitory

pause (cf. Fig. 8). During this time, not only are cortical units less excitable, but there is also a marked reduction in spontaneous wave-activity.

Repetitive thalamic stimulation also produces a change in steady potential level in the somatic sensory cortex, but as can be seen in Fig. 8*B*, *D*, the negative shift is much less pronounced, or absent, while the post-tetanic positive shift is particularly clear.

Similar changes in steady potential level were produced in chronically isolated cortical slabs by surface stimulation; that is a negative shift during the tetanus, and a delayed positive shift afterwards.

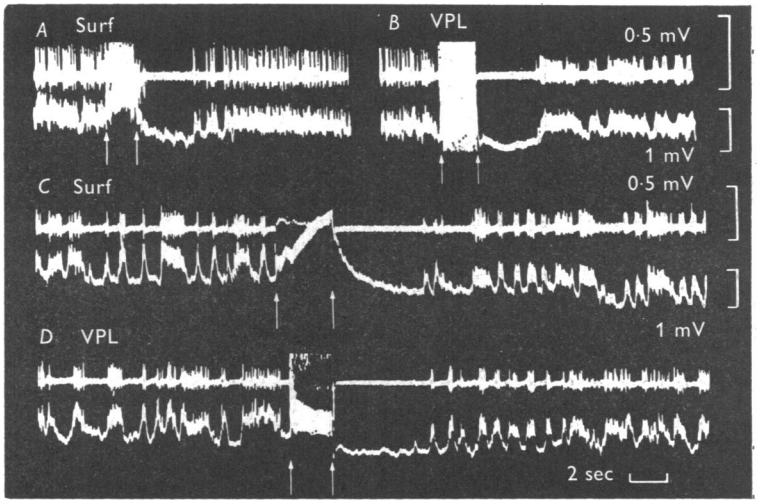

Fig. 8. Effects of tetanic stimulation of cortical surface (*A* and *C*) and specific thalamic nucleus (*B* and *D*) on unit firing (upper traces of each pair) and on the steady potential (lower traces) in posterior sigmoid gyrus. The tetani (between arrows) were at 50/sec in *A* and *B*, and at 20/sec in *C* and *D* (another unit). Negative deflexions are upwards; note greater negative tendency and after-discharge (*A*) with surface stimulation.

Deep stimulation

The slow waves evoked by stimulating with a micro-electrode inserted into the cortex differ somewhat from those produced by surface stimuli. A wave corresponding to the inhibition is elicited particularly well at a depth of 1·0–1·7 mm, where the inhibitory effect has its lowest threshold (cf. Krnjević *et al.* 1966*a*). This wave is seen either as a slow positive deflexion at all depths (Fig. 9), or as a positive wave in the superficial half of the cortex (down to 1·0 mm) and as a negative wave below. Slow negative waves are somewhat less obvious when stimulating deep than when stimulating the cortical surface.

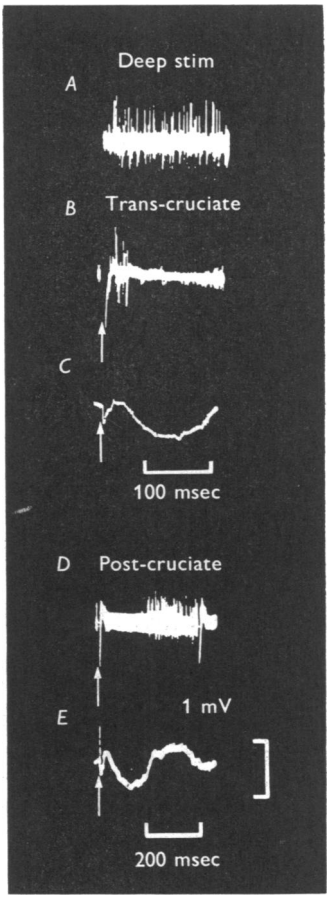

Fig. 9. Inhibition of unit discharge, and corresponding slow waves evoked by intracortical stimulation in a 'cerveau isolé'; 0·1 msec, 30 V, 2/sec cathodal pulses were applied at various depths through the monopolar stimulating micro-electrode. *A*: unit at depth of 0·7 mm in anterior sigmoid gyrus, excited by glutamate released from multi-barrelled recording micropipette; *B*: it was inhibited by stimulation 3 mm away, at depth of 1·0 mm within posterior sigmoid gyrus (several traces are superimposed in each record). *C* shows slow positive wave evoked at same point by identical stimulus. *D*: another unit, 2·0 mm deep in posterior sigmoid gyrus, which was inhibited by stimulating at depth of 1·5 mm in the same gyrus; *E*: the corresponding slow wave response. Note slower time scale in *D* and *E*.

Origin and course of inhibitory fibres

We have shown that the depression of cortical neurones induced by direct shocks is probably a post-synaptic inhibition. It was pointed out (Krnjević *et al.* 1966*a*) that stimulation of various other parts of the brain or even peripheral nerves produces a rather similar effect. It is therefore possible that the cortical inhibition is due to the activation of afferent

inhibitory pathways. The cortical shocks could excite such pathways either indirectly through corticofugal fibres, or directly by stimulating the terminal branches in the cortex. These two possibilities were tested by isolating small areas of the cortex from all parts of the brain, in both acute and chronic experiments.

Observations on isolated cortical slabs

An area of cortex was first undercut to sever its vertical connexions, and the isolation was completed by passing a blunt-tipped probe up to the pial surface, all round the isolated area, as in the procedure described by Burns (1951, 1958). One difference in the procedure, however, was that the undercutting was usually done through an adjacent gyrus, so that the isolated region included the full width of a gyrus. The technique was therefore in most cases similar to that used by Goldring, O'Leary, Holmes & Jerva (1961). In a few experiments, a more localized isolation was performed.

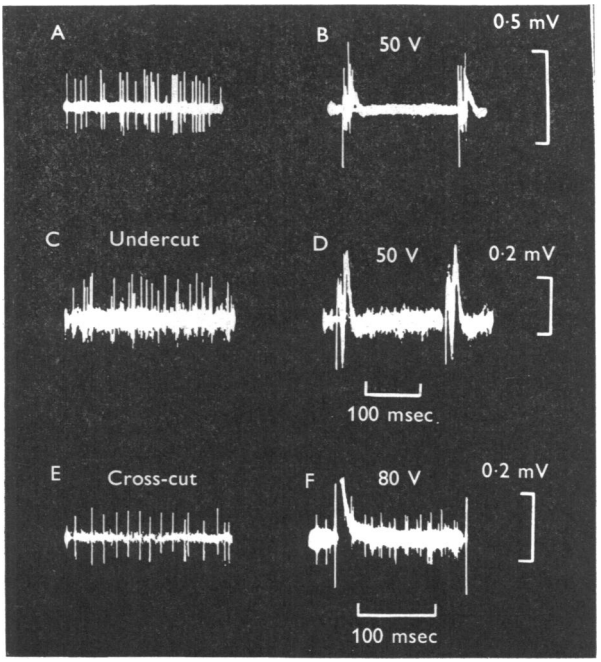

Fig. 10. Effect af acute undercutting on spread of cortical inhibition. A suprasylvian unit was made to discharge by a release of glutamate (*A*) and then inhibited by 50 V shocks (0·1 msec at 6/sec) applied to cortical surface, at a point about 5 mm away (*B*). *C* and *D* show comparable traces obtained 20 min after neural isolation of the same cortical area. *E* and *F*: cutting through the isolated cortex, half-way between points of recording and stimulation, abolished the inhibitory effect. In each record, several oscilloscope traces are superimposed.

Acute isolation. There is no systematic alteration in the inhibitory effect when re-examined some 20–60 min after isolation (Fig. 10*A*, *B*). If anything the inhibition is often more obvious than before, because of two striking changes in the electrical records. The testing, glutamate-evoked discharges are usually much more regular than in the intact cortex, because of the disappearance of spontaneous activity; and the later

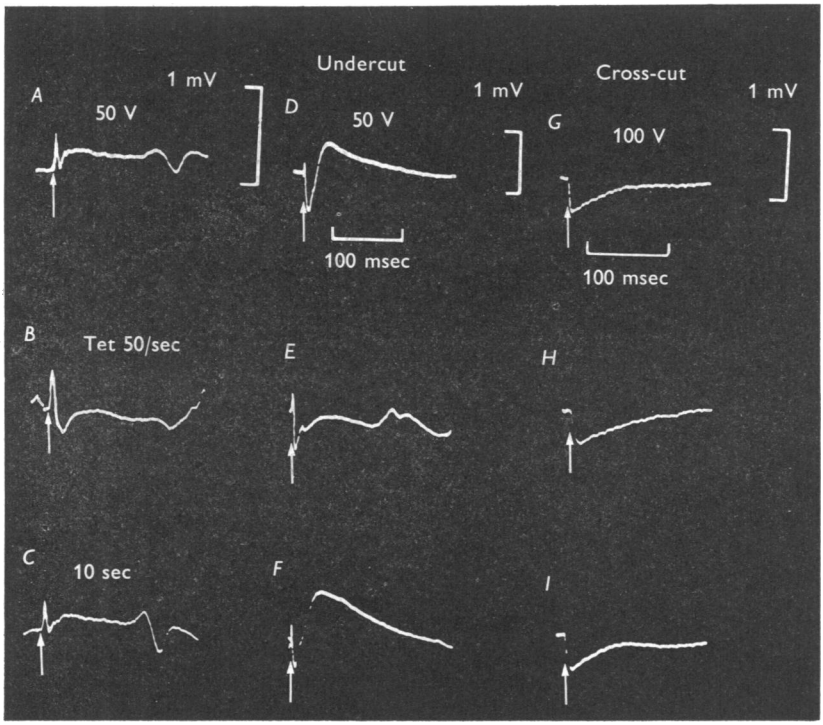

Fig. 11. Effect of acute neural isolation on the 'inhibitory' slow wave recorded at depth of 1·2 mm (same experiment as that of Fig. 10). *A–C* are the waves evoked by a 50 V surface shock, *A* before, *B* 2 sec after a 5 sec 50/sec tetanus, and *C* 10 sec after end of tetanus. Corresponding traces recorded 1 hr after neural isolation of cortical slab are shown in *D–F*, and further records were obtained after cutting across isolated slab, between stimulating and recording electrodes (*G–I*); only a large positive stimulus artifact now remains. Chloralose (50 mg/kg I.V.), given shortly before undercutting, may have contributed to enhancement of waves in *D–F*.

portion of the phase of inhibition is no longer cut short by the neuronal firing associated with the late repetitive waves (Fig. 11, cf. Creutzfeldt & Struck, 1962).

Immediately after undercutting, the cells appear to suffer from some injury. Units are difficult to find, the spikes tend to be positive, and a steady discharge is not readily elicited with glutamate. At this stage,

definite inhibition often cannot be evoked by direct stimulation. This condition is usually reversible, only lasting about ½–1 hr. But in two cases it persisted until the end of the experiment several hours later. This seemed to be associated with a particularly severe reduction in cortical circulation.

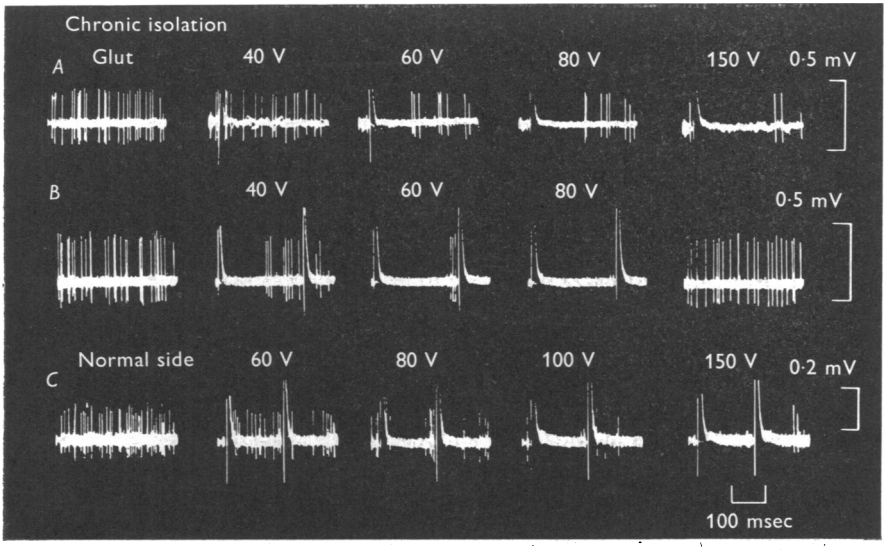

Fig. 12. Inhibition in a chronically (5 weeks) isolated slab of suprasylvian cortex. *A* and *B*: two units showing excellent inhibition by surface stimulation at 2 and 4/sec, with the shock intensities indicated on traces. *C*: unit in corresponding area of 'normal', contralateral hemisphere, much less readily inhibited even by strong shocks at 6/sec.

Like the inhibitory effect, the corresponding slow wave is usually readily detectable after cortical isolation (Fig. 11*D*), especially when it is potentiated by tetanic stimulation (Fig. 11*F*) or by a release of L-glutamate. The shape of the wave is not strikingly different from the normal, but it may stand out more clearly in the absence of spontaneous fluctuations and because the late repetitive waves evoked by direct shocks on the intact cortex are no longer visible.

Chronic isolation. Two cats were examined 5 and 10 weeks, respectively, after isolating a piece of suprasylvian cortex. In both cases, the inhibitory effect of cortical shocks was remarkably well developed, usually lasting well over 200 msec (Fig. 12*B*), so that a complete block could be produced by repetitive stimulation at an even lower frequency than usual (3–4/sec instead of 6–7/sec). A curious feature of the second experiment was unusually weak inhibition in the 'normal' hemisphere (Fig. 12*C*). The threshold was consistently very high, and even the strongest shocks

could not always produce the expected complete inhibition during stimulation at 6–7/sec.

In both experiments some unusual slow waves were recorded. In the first (at 5 weeks), there was a large positive wave at the surface, having a threshold and a time course very similar to those of the inhibition (Fig. 13*A–E*). This positive wave had a sharp initial component, and a slower later phase. Both were clearly visible in the superficial layers, but deeper the slower component became much smaller, and below a depth of 1·0 mm only the sharp positive wave remained (Fig. 14*A–D*). In both Figs. 13 and 14 the corresponding waves recorded on the opposite side, at the same depths, are also shown for comparison, on fast and slow sweeps.

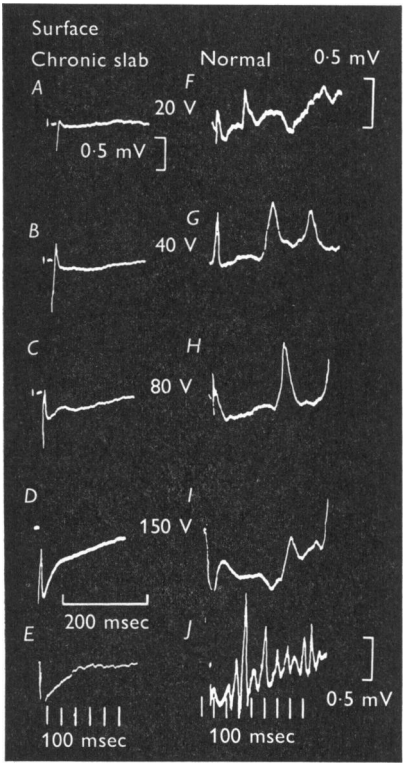

Fig. 13

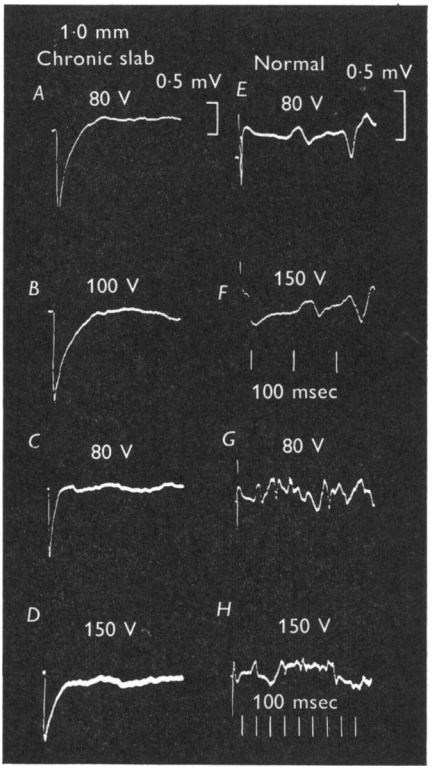

Fig. 14

Fig. 13. Slow waves elicited by surface shocks in a chronically (5 weeks) isolated suprasylvian slab (*A–E*), compared with corresponding waves recorded in symmetrical area of contralateral 'normal' hemisphere (*F–J*). All traces were recorded at the pial surface; *A–D* and *F–I* are on same time scale. Note absence of any late evoked waves in isolated slab (*E*), which is particularly striking when compared with activity on other side (*J*). Negative deflexions upwards.

Fig. 14. Slow waves recorded as in Fig. 13, but at depth of 1·0 mm. Traces are on relatively fast time scale in *A*, *B*, *E* and *F*, and on slower scale in *C*, *D*, *G* and *H*.

Intracortical spread of inhibition

The excellent inhibition observed in the chronically isolated cortical slabs shows that the inhibitory elements must be mainly intracortical. There is no doubt that the tangential spread of inhibition through the cortex must be mediated by nerve fibres, since in a cortical slab it is prevented by a vertical section through the whole thickness of the cortex (Fig. 10*F*). This procedure also abolishes the spread of the inhibitory slow waves (Fig. 11*G*).

We have not analysed the cortex systematically to find the depth at which the relevant fibres might be concentrated. These presumed tangential fibres are unlikely to be mainly in the most superficial layers, because the inhibition is not readily blocked either by surface applications of a local anaesthetic (procaine 2 %, w/v) or by cooling the pia.

The latter test was performed in several experiments by passing a solution at about 0° C through a copper tube resting lightly across the surface of an isolated slab, half way between the stimulating electrodes and the point of insertion of the recording micropipette (about 3 mm away). Cooling in this way for 5 min repeatedly failed to reduce the inhibitory effect (cf. Fig. 15). Longer periods of cooling obviously affected even deep cells within a radius of several mm. Units started discharging spontaneously; they later stopped giving regular responses to glutamate and finally all discharges ceased. Slow evoked waves also disappeared. These changes reversed within a few minutes when the temperature of the surface was again brought to its normal level. We could not block selectively the spread of inhibition by cooling the surface.

Effect of injury and ischaemia

Selective depression of cortical inhibition seems to occur sometimes as a result of local injury, poor blood supply or general anoxia. We have already mentioned this occurrence in some isolated cortical slabs.

For instance, inhibition may be difficult to elicit immediately after setting up an animal for electrical recording. At this stage the cortical circulation is frequently sluggish. However, if the cortex is kept well covered and the preparation allowed to settle down for $\frac{1}{2}$–1 hr, the circulation usually improves greatly and the inhibitory effect becomes much more prominent.

Local injury produced by prodding the cortex with a micropipette or a needle sometimes has a similar temporary effect, and systemic anoxia may also block inhibition selectively, but these effects are not very easily reproducible, partly because all these procedures readily cause an excessive depression of all neuronal responses (cf. Marshall, 1959).

Inhibitory interneurones

The simplest explanation for the observations described here and by Krnjević *et al.* (1966*a*), is that the cortex contains inhibitory cells, which can be activated by direct or indirect stimulation. One would therefore expect to find at least some units that are excited by cortical shocks after a short latency. In view of the prolonged inhibition, one might also expect to see a correspondingly prolonged interneuronal discharge.

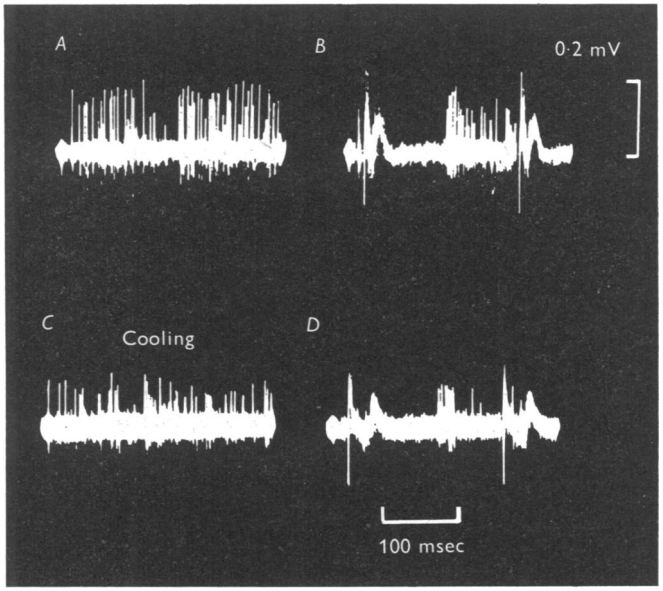

Fig. 15. Effect of surface cooling on inhibition by direct cortical shocks. *A* and *B*: glutamate-evoked discharge of suprasylvian unit, at depth of 1·3 mm, partly inhibited by stimulation at 5/sec (0·1 msec, 50 V); surface electrode was 3 mm away. *C* and *D*: similar inhibition of same unit by identical shocks 5 min after starting to cool surface, half-way between points of recording and stimulation. The cooling tube was perfused with a fluid at about 0° C.

One can often observe units discharging very early after a cortical shock, whose threshold is comparable with that of the inhibitory effect (Fig. 16). However, although such units often fire repetitively, the discharge only lasts 10–20 msec at the most. As already mentioned in Krnjević *et al.* (1966*a*), one practically never sees discharges having a duration comparable with that of the inhibitory effect.

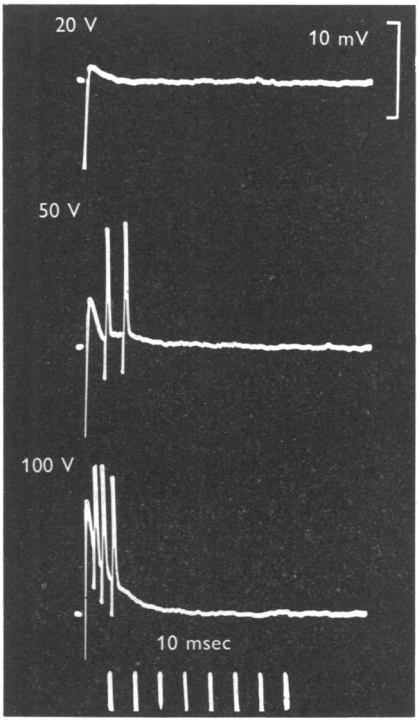

Fig. 16. Unit excited strongly after short latency by the 0·1 msec surface shocks indicated; it was found at depth of 2·5 mm in posterior wall of cruciate sulcus. The threshold for inhibition of cells in this region was at 40 V.

DISCUSSION

A true inhibition

The present results suggest that the inhibitory effect described in a previous paper (Krnjević *et al.* 1966*a*) is indeed a true inhibition. The fact that it can be overcome by a stronger dose of glutamate indicates a hyperpolarizing type of block, and it is in full agreement with the intracellular records which revealed widespread synchronous hyperpolarization of cortical cells. These results confirm some previous observations on the effects of surface stimulation on cortical membrane potentials (Phillips, 1956; Creutzfeldt, Baumgartner & Schoen, 1956; and especially Li & Chou, 1962). All these authors discussed the possible inhibitory nature of this phenomenon, which, however, could not be demonstrated conclusively without some ready means of testing neuronal excitability directly.

Our intracellular records also confirm our extracellular observations that the inhibitory phenomenon does not depend upon previous activation of the observed cell. The effect is therefore not an after-hyperpolarization What kind of effect is it?

Only two possibilities seem worth considering. The first and the most likely is that, as in the spinal cord, inhibitory fibres are present which can depress the excitability of other neurones, presumably by releasing a suitable inhibitory factor. The second possibility is that relatively strong stimuli can cause a non-specific reduction in neuronal excitability by changing the properties of the cell membrane: for instance, by altering its electrical characteristics, or by so increasing its permeability as to allow some inhibitory substances to leak out of the cells.

For several reasons the second alternative seems very unlikely: an apparently identical inhibitory effect can be produced indirectly by stimulating the thalamus or the contralateral cortex; and the current flow through the cortex caused by adequate surface stimuli is in fact much weaker than might appear at first sight, so that the possibility of causing any non-specific injury is very remote (Krnjević *et al.* 1966*a*). One can conclude that the observed phenomenon is probably a post-synaptic inhibition, evoked through the activation of inhibitory terminals.

An intracortical system

The full preservation of the inhibitory effect in the completely isolated cortical slab, even after several weeks (see also Creutzfeldt & Struck, 1962), shows that the postulated inhibitory system must be situated wholly within the cortex. In the chronically isolated slab, the inhibition is even more pronounced, and lasts longer than usual, presumably because it is no longer partly masked by the normal 'spontaneous' activity, which evidently requires a background of afferent input (cf. Burns, 1958; Creutzfeldt & Struck, 1962), nor is it cut short by the usual 'rebound' of excitability.

The inhibitory effect is thus likely to be produced by intracortical inhibitory interneurones, which can be brought into action by direct or indirect stimulation.

Inhibitory interneurones

It is not unusual to record neuronal discharges immediately after a direct cortical stimulus, just before or at the onset of the inhibitory effect; some cells therefore do fire at the required time, although, of course, we have no evidence that these particular cells have an inhibitory function. During intracellular recording, one can sometimes detect an early, small deflexion (cf. Fig. 2) which seems to be a presynaptic spike. Similar 'presynaptic' deflexions are recorded not infrequently at junctional sites in the central and peripheral nervous systems (Eccles, Schmidt & Willis, 1963; Katz & Miledi, 1965). Those recorded in the cortex may indicate the discharge of inhibitory terminals.

These interneurones and their principal branches are unlikely to be situated mainly in the most superficial cortical layers, since the spread of inhibition cannot be readily abolished by surface cooling or by the application of local anaesthetics. However, to account for the widespread distribution of inhibition, throughout the whole thickness of the cortex, the postulated interneurones must send at least some terminal branches into all the cortical layers.

The fact that inhibition is elicited most easily and effectively by stimulation at a relatively great depth suggests that the cell bodies of the inhibitory interneurones are mainly in the deeper layers, V and VI. An alternative explanation would be that the inhibitory interneurones are comparatively inexcitable electrically, and that their activity is triggered by other cells or pathways, which are themselves stimulated more readily at this depth.

This might happen, for instance, through the recurrent collaterals of deep pyramidal cells. This pathway appears to inhibit other pyramidal cells (Phillips, 1956; Suzuki & Tukahara, 1963; Stefanis & Jasper, 1964; Brooks & Asanuma, 1965*a*, *b*), but its action is very weak when compared with that of direct, transcallosal or thalamic volleys (Krnjević *et al.* 1966*a*). The inhibitory mechanism is therefore unlikely to depend upon this pathway.

The cells which must be excited to evoke inhibition probably differ from pyramidal cells in their shape and orientation, since inhibition is elicited more easily by a surface cathode, in contrast to the excitation of cortico-spinal neurones (Fritsch & Hitzig, 1870; Phillips, 1956; Hern, Landgren, Phillips & Porter, 1962). Surface cathodal shocks not only are less effective in evoking pyramidal firing, but they actually tend to inhibit pyramidal activity.

Identity of inhibitory interneurones

From our observations we conclude that inhibitory interneurones ought to be present in substantial numbers in all cortical areas, including the palaeocortex, where a similar kind of inhibition has been observed recently by Legge, Randić & Straughan (1965). The branches of these interneurones seem to reach cells in all the cortical layers, and in a tangential plane they could be spread over a distance of up to 1–2 cm, since this is the length of tissue in which the inhibitory effect of a single shock can be detected. However, the area of distribution of a single interneurone may be very much smaller, and the relatively large spread of inhibition may be due to the activation of many interneurones by tangential pathways (cf. Adrian, 1937; Burns, 1951, 1958). The deep region of lowest threshold corresponds to the depth where a horizontal spread of excitation of B-type cells takes place (Burns, 1958). It is of some interest that neither the inhibitory

interneurones, nor pathways which might excite them, appear to pass under most sulci.

The idea that the cortex contains a mixture of excitatory and inhibitory cells is by no means new. It was already proposed by some of the pioneers of cortical studies (Bubnoff & Heidenhain, 1881), but was largely ignored in the most extensive descriptions of cortical cytoarchitectonics (Campbell, 1905; Cajal, 1911; Brodmann, 1914; Vogt & Vogt, 1919; Lorente de Nó, 1949; Scholl, 1955). We suggested in an earlier article (Krnjević, Randić & Straughan, 1964*a*) that the cells whose features agree best with the characteristics of the postulated inhibitory interneurones are cells with long horizontal branches ending in pericellular nests, described by Cajal (1911, p. 556) as 'cellules à cylindre-axe résolu en nid péricellulaire'. Cajal pointed out the resemblance between these interneurones and the cerebellar basket cells. It is significant that the latter cells appear to play a comparable inhibitory role in the cerebellum (Anderson, Eccles & Voorhoeve, 1963).

A similar conclusion has been reached independently by Szentagothai (1964) (see also Colonnier, 1965); according to these three authors, the cell bodies of the cortical basket cell-like interneurones are mainly in layers III and IV, and their horizontal branches extend over several hundreds of micra. Their endings in pericellular nests form type-II synapses (Gray, 1959), on the soma and proximal dendritic segments of other cells. Since practically all pyramidal neurones, in the various cortical layers, are surrounded by such pericellular nests (J. Szentagothai, personal communication), these could well account for the widespread nature of the inhibitory effect. Furthermore, Szentagothai (1964) found that type-II synapses are particularly well preserved in chronically isolated cortical slabs. The cell bodies of these interneurones seem to be more superficial than one might expect from the inhibitory threshold at different depths but, as already mentioned, this may be because they are excited more effectively by indirect stimulation. These observations would therefore agree with a recent hypothesis (Andersen, Eccles & Løyning, 1963; Andersen, Eccles & Voorhoeve, 1963) which proposes that the inhibitory synapses responsible for the post-synaptic type of inhibition are generally axo-somatic.

However, some other observations suggest that the synapses responsible for the cortical inhibitory effect cannot be mainly axo-somatic: for example, the distribution and polarity of the inhibitory slow waves recorded extracellularly (see below) and also by the fact that a very marked cortical inhibition can be demonstrated in young kittens (Krnjević, Randić & Straughan, 1964*b*; Purpura, Shofer & Scarff, 1965), at a stage when the pyramidal cells have no axo-somatic synapses (Purpura, 1961).

Time course of inhibition

It is evident that surface stimulation brings into action excitatory as well as inhibitory synapses. The excitatory pathways appear to have a lower threshold and the resulting EPSPs are relatively short-lasting (cf. Fig. 2). Although the inhibitory effect ultimately blots out the early excitation, the initial EPSP probably masks the onset of the IPSP, at least when relatively weak shocks are given. This EPSP, and the interference by external fields, probably account for the fact that the IPSP only reaches a maximum after 10–20 msec. With strong volleys, the onset of the inhibition follows the supposed presynaptic spike without any detectable delay.

The inhibitory effect is too long to be accounted for by the probable membrane time constant of the cortical neurones (Nacimiento, Lux & Creutzfeldt, 1964). It cannot be due to a prolonged repetitive discharge of the inhibitory neurones, since such a discharge has never been observed in the course of many dozens of experiments. The most likely cause is a prolonged action of the inhibitory transmitter, which is presumably due to its relatively slow destruction or reabsorption. This would also explain the cumulative effect observed during repetitive stimulation even at quite low frequencies. The excitatory action of high-frequency stimulation is likely to develop by a similar accumulation of an excitatory factor, which probably accounts for the negative shift in the intracortical steady potential (see also Dusser de Barenne & McCulloch, 1939; Gerin, 1960; O'Leary & Goldring, 1964) and the after-discharge; these tend to mask the relatively small positive shift probably associated with the long inhibitory effect, which in any case may be clearly visible only at certain depths (see below).

Slow waves

A striking feature of the powerful inhibitory effect is that it is not associated with a marked extracellular slow wave. This no doubt partly accounts for the fact that the inhibition has been largely overlooked until recently. After the initial, surface-negative wave, during which cells tend to be excited (cf. Goldring *et al.* 1961; Li & Chou, 1962; Stohr *et al.* 1963), some kind of slow wave corresponding to the inhibition can often be seen, but its features are highly variable.

In general, at the surface, one can see a positive or a negative slow wave (cf. Chang, 1951; Goldring *et al.* 1961; Li & Chou, 1962; Stohr *et al.* 1963; O'Leary & Goldring, 1964); in the intermediate zone (0·2–1·0 mm), the wave is either positive or absent, while below 1·0 mm it tends to be more negative. The wave can usually be potentiated by tetanic stimulation or,

particularly, by a local release of L-glutamate, which has a strong depolarizing action. The potentiated wave is nearly always a relatively large negative wave, and it is seen best deep in the cortex (below 1·0 mm) or, somewhat less well, very superficially (within 0·2 mm of the surface).

The most likely explanation for these findings is that the inhibitory synapses are distributed most densely in the intermediate zone (0·2–1·0 mm), corresponding to layers II–IV. Activation of the inhibitory interneurones would therefore create sources of currents in this intermediate zone and electricals sinks both above and below, on the apical dendrites and many of the cell bodies, respectively. Since the IPSPs give only a relatively small hyperpolarization (cf. Figs. 2 and 3), the resulting flow of extracellular current would not produce large positive waves in the tissue; but somewhat greater waves might be expected after a tetanus, either because of post-tetanic potentiation of the inhibitory synapses, which would evoke larger IPSPs, or because the cells may be partly depolarized by a cumulation of excitatory effects. This interpretation is confirmed by the potentiating action of L-glutamate: by depolarizing the cell bodies or dendrites, glutamate would very much increase the current flow during the IPSPs and so give rise to much larger negative waves near the surface or below 1·0 mm.

The relatively prolonged action of L-glutamate in the superficial layers (when compared with its action in the deeper layers), suggests that glutamate is removed from the extracellular spaces only relatively slowly at this level, perhaps because apical dendrites cannot absorb glutamate as efficiently as cell bodies. Another possibility is that the exposed situation of the apical dendrites makes them more liable to mechanical injury or to drying, either of which may depress the required active transport. The absence of any clear effect of glutamate below a depth of 1·8 mm supports previous evidence that in the cortex glutamate acts mainly on cell bodies and dendrites (Krnjević & Phillis, 1963).

The marked variability of inhibitory slow waves may be accounted for by variations in the density of active inhibitory synapses at different levels and in different preparations. The preponderance of negative waves below 1·0 mm, even though deep cells always show a particularly clear inhibitory effect, suggests there are fewer inhibitory synapses on the cell bodies than on the dendrites.

In conclusion, although certain slow waves and steady potential shifts can be correlated with inhibitory and other cortical processes, the waves are usually too variable and they allow too many possible interpretations to be of great significance by themselves. One cannot safely draw definite conclusion about the underlying changes in neuronal excitability without testing single units directly.

Post-inhibitory 'rebound'

A marked apparent rebound of excitability is often seen at the end of inhibition, either as an increased tendency to firing or as a surface negative wave: this might seem analogous to the post-anodal rebound postulated by Andersen & Sears (1964) to explain repetitive activity in the thalamus, but the cortical rebound always disappears after undercutting the cortex (cf. Creutzfeldt & Struck, 1962). It is evidently due to an afferent, excitatory volley coming from subcortical or distant cortical regions which respond to the initial cortical stimulus only after a relatively long delay. This may well be the first volley of the thalamic repetitive after-discharge, first described by Adrian (1941) and Morison & Dempsey (1943).

Vulnerability of cortical inhibition

It appears that local injury, a poor blood supply, or anoxia may sometimes cause the inhibitory effect and the corresponding slow wave (cf. Chang, 1951) to disappear selectively. Cortical inhibitory interneurones and synapses may thus be especially sensitive to this kind of interference, but they are remarkably resistant to most pharmacological blocking agents, as is shown in Krnjević, Randić & Straughan (1966*b*).

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