

PREVENTION OF SUPERSENSITIVITY IN PARTIALLY ISOLATED CEREBRAL CORTEX¹

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INTRODUCTION

Partial or total neuronal isolation of the cerebral cortex leads to changes in electrical after-discharges, locally elicited by drug and electrical stimulation (Sharpless and Halpern 1962; Echlin and Battista 1963). Thresholds decrease and duration of after-discharges increases over weeks and months following partial or complete isolation of cerebral cortex. This phenomenon has been called supersensitivity.

In the present series of experiments we have successfully altered the expected course of development of this type of supersensitivity.

METHODS AND MATERIALS

Two groups of adult cats were studied. The cerebral cortex of the fifteen animals in the first group (control) was undercut unilaterally (Fig. 1) under aseptic conditions. These cats were kept for 2-18 weeks for supersensitivity to develop before a terminal acute experiment. Three of the fifteen had chronic cortical electrode units, but received no stimulation.

Undercutting was designed to sever all sub-cortical fibers 3-4 mm below the surface of mid-marginal gyrus. The midline length of the cut was 10-22 mm, and the lateral entrance site was 10-16 mm long.

In the seventeen animals of the second group the cortex was undercut and cortical stimulating electrodes were implanted (Rutledge and Doty 1962). These were pairs of platinum wires, 2-3 mm apart, usually oriented parallel to and 4-5 mm from the midline (but see Fig. 1). In six of the

seventeen animals an array of Ag ball dural recording electrodes was also implanted symmetrically 4 and 8 mm anterior and posterior to the nearest stimulating electrode of the pair (see Fig. 1). ECoG recordings were made during every brain stimulation on a given day and at least twice each week. A terminal acute experiment, 7-10 days after the last stimulation, completed the study.

Brain stimulation began 1 week (6 weeks in 2 cats) after surgery, and was applied 6-7 days per week; it consisted of 20 daily applications of a 2 sec train of 1.0 msec pulses at 50/sec spaced 1 min apart. Total stimulation was about 400 applications at an intensity of 0.6 mA, 400 at 0.8 mA and 200 at 1.0 mA (all constant current). These intensities were below the threshold for inducing electrical after-discharges.

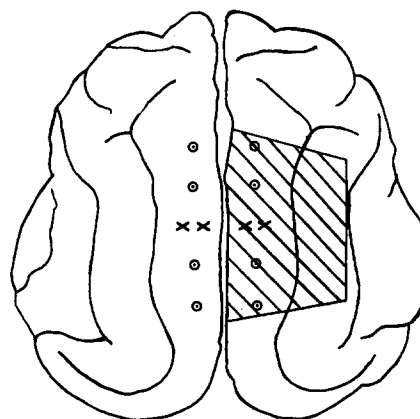


Fig. 1

Location of undercuts, stimulating acute (x x) and recording (o) electrodes. Chronic stimulating electrodes were usually nearly parallel with and 4-5 mm from the midline in order to avoid pain fiber stimulation near the sagittal sinus.

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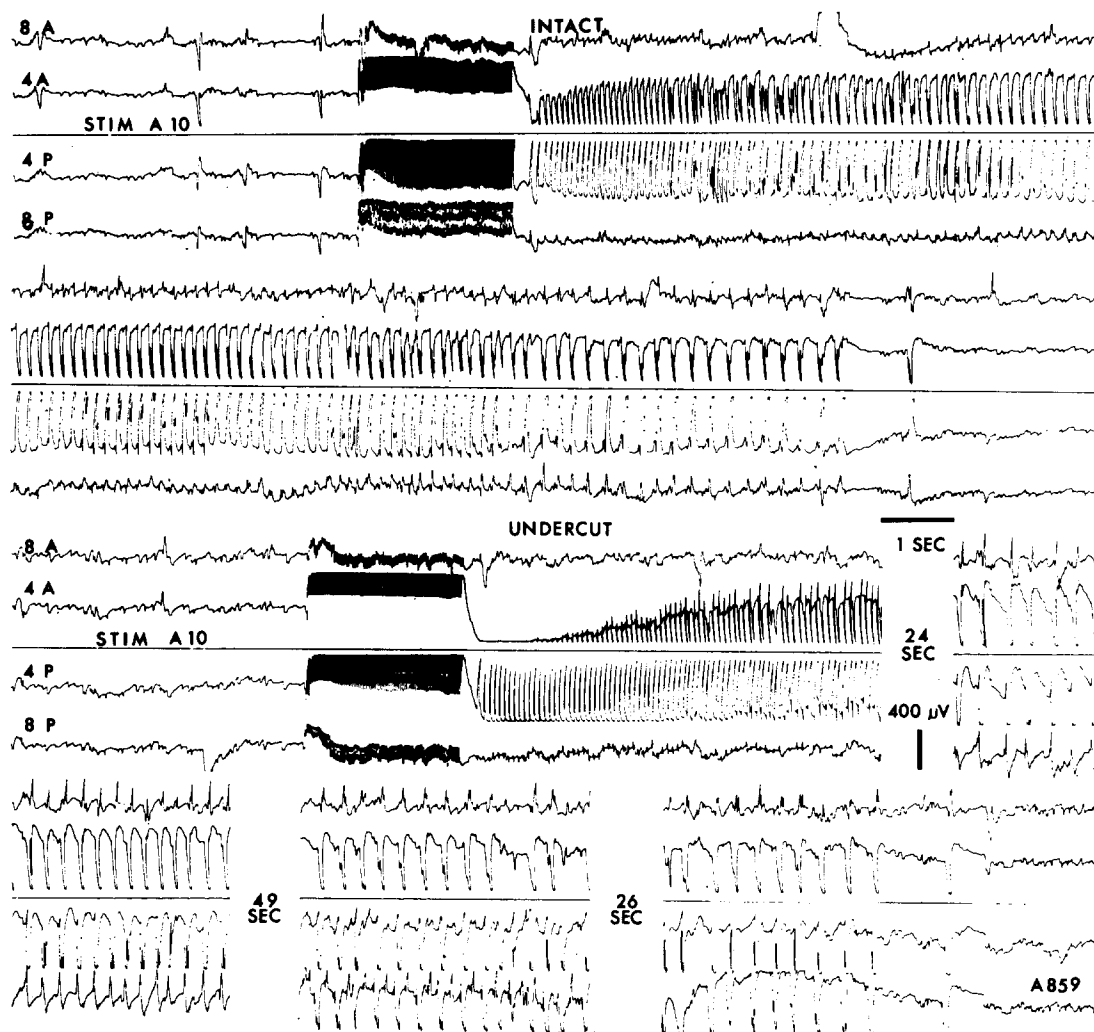


Fig. 2

ECoG from intact and undercut cortex in an unstimulated brain. Cortex undercut 12 weeks prior to acute experiment. Stimulation at frontal plane A 10 with recordings at 4 and 8 mm posterior and 4 and 8 mm anterior (4,8 P; 4,8 A). The four lines of recording are continuous except where a section was cut out (e.g., "24 sec"). Total duration of AD on undercut: 118 sec, on intact: 20 sec. Chloralose anesthesia 60 mg/kg plus 0.3 ml of 2.5% Surital initially. Records taken 4 h after chloralose injection. Stimulation both sides, 1.5 mA, 2 sec duration, 1.0 msec pulses at 50/sec.

All terminal acute experiments were performed on cats under chloralose anesthesia (60 mg/kg plus supplementary doses of 10–15 mg/kg if necessary) with the brain widely exposed bilaterally under mineral oil. Fig. 1 shows the electrode arrangement for stimulation and ECoG pick-up. The stimulation site was the same as it had been for the chronic stimulation, or, for non-stimulated undercut cortex, at frontal plane 10 (Jasper and Ajmone Marsan 1954). Homotopic positions

were used on the intact side. Electrical stimulation, usually 1.5 mA, 2 sec duration, 1.0 msec pulses at 50/sec, was applied first to the intact side in all but two cats. Ten minutes after the first stimulation, the procedure was repeated on the undercut side. In all experiments this was done in reverse order after a 2 h wait. The after-discharge (AD) was recorded on a Grass EEG machine with the filters usually set on a 5–70 c/sec frequency response band to minimize

amplifier blocking during the electrical stimulation. Animals were finally sacrificed and brains removed for histological study.

RESULTS

Control animals

Longer lasting ADs were obtained from the undercut side of twelve of the fifteen non-chronically stimulated cats. One example is shown in Fig. 2. The duration of AD for the twelve animals was, on the average, 5.5 times longer on the undercut side as compared with the intact side. There was a general tendency for lower voltage resting background activity in the undercut cortex. Although time lapse after undercutting was deliberately varied between 2 and 18 weeks, no differences that might be related to

temporal factors were apparent from our data. Of the three remaining cats, two showed longer ADs on the intact side and in one the results were equivocal.

Animals with undercut, chronically stimulated cortex

Examples of the ECoG and AD data on the second group of seventeen cats are shown in Fig. 3 and 4. Fig. 3, which is from one of the six cats with implanted electrodes, illustrates two important points, the subthreshold nature of the chronic stimulation and the reliability of AD durations in the acute experiment. The first line was recorded at the 4 mm posterior location on the first stimulation day (1 week after undercutting and implantation of recording electrodes) during which,

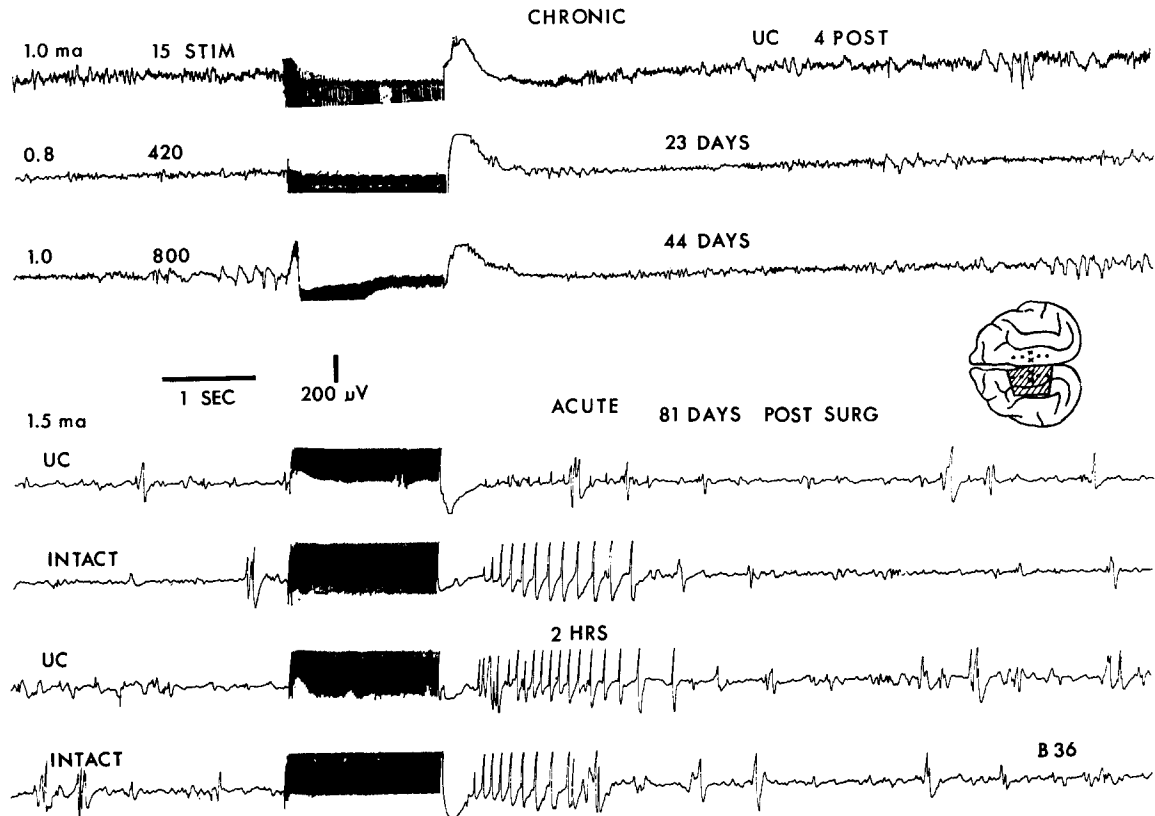


Fig. 3

Chronic and acute ECoG from chronically stimulated, undercut cortex. Records shown only for the 4 mm posterior location (4 POST). Records taken on the first stimulation day (after 15 stimulations), on days of current change at 420 (23rd day) and 800 (44th day) stimulations, and following 1000 stimulations (81st day), on the acute experiment day. Electrode and undercut (UC) locations on inset drawing. Chloralose anesthesia for acute, 60 mg/kg 6 h before these records. Chronic stimulation tests at 1.0, 0.8, 1.0 mA (see Methods). Acute stimulation as in Fig. 2.

for most cats, one or two stimulations at intensities up to 1.0 mA were applied. The first three lines illustrate that no abnormal activity was produced by the chronic electrical stimulation. During the terminal acute experiment at a current of 1.5 mA, AD threshold was barely reached on the chronically stimulated undercut side (4th line). The repeat after 2 h produced ADs about equal in duration on the two sides.

In one cat no supersensitivity was evident in the terminal experiment which was done 6 weeks after the last chronic stimulation.

Conditional stimulation of cortex

In a previous study (Rutledge and Doty 1962), it was found that in animals receiving chronic conditional electrical stimulation of the cortex, retraining was possible after much additional stimulation following undercutting of the stimulated cortical area. Undercutting had produced loss of conditioned foot flexion responses. Supersensitivity was thought to play a role in the reacquisition of the conditioned responses.

Three of the seventeen cats in the second group were actually handled as in the Rutledge

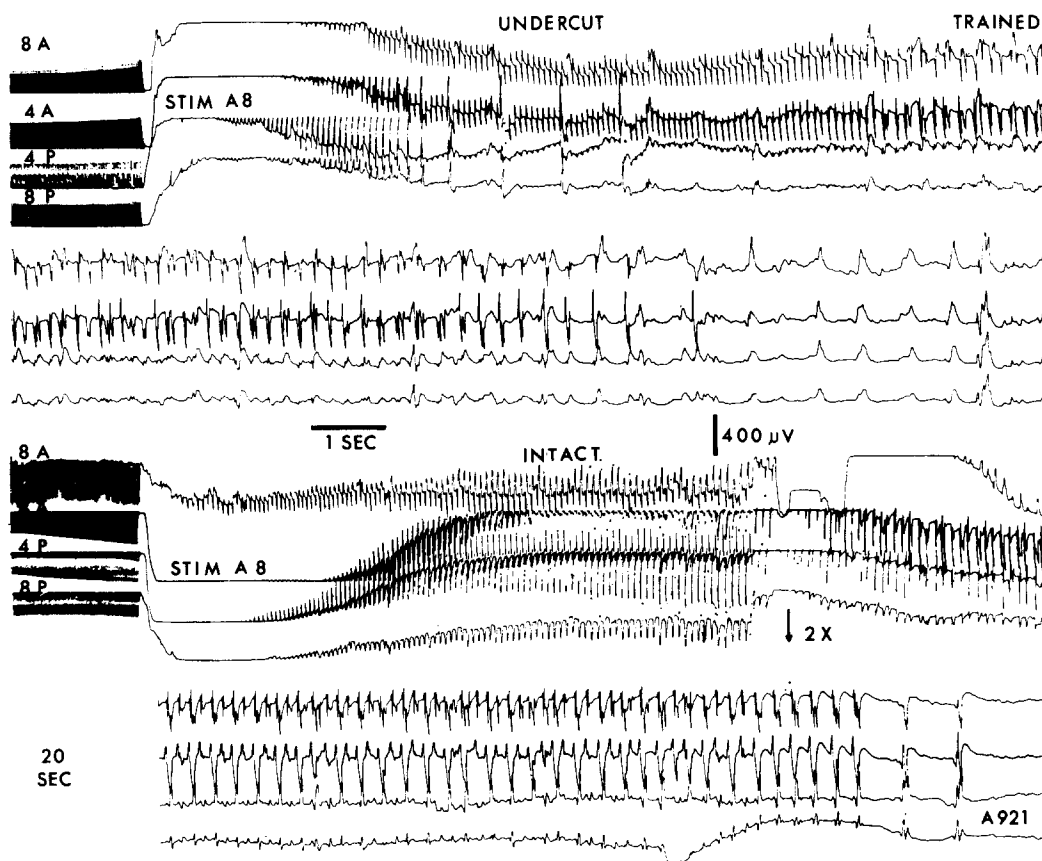


Fig. 4

ECoG from an acute, stimulated and "trained" undercut cortex compared with intact cortex. Stimulation at A 8, recordings as in Fig. 1. Continuous records except 20 sec cut out on lower set. Gain reduced twice for intact at 2X. To the same intensity of stimulation (1.5 mA) the duration of AD is 40 sec on the intact and 20 sec on the undercut cortex. Voltages of AD wave forms are much greater on the intact side. There is a slower spike frequency and waves are more irregular on the undercut side. This cat had received conditional excitation of the cortex before undercutting and was subsequently retrained to a 60% criterion within 400 trials in 4 weeks (6 weeks total post-surgery). Chloralose anesthesia 60 mg/kg 7 h before recording and 0.35 ml 2.5% Surital 4 h before recording. Acute stimulation as in Fig. 2.

and Doty study. ADs were studied, as with the others, in a terminal acute experiment. Two of these cats did not show supersensitivity. Records from one are shown in Fig. 4. In the remaining cat in which supersensitivity did develop in spite of the stimulation, the latter had not been resumed until 6 weeks after undercutting.

Fig. 4 also illustrates the observation that AD duration was often longer on the side which had not received the chronic stimulation. Further, as seen in this figure, it was frequently noted that AD amplitude was markedly less on the chronically stimulated cortex. There was also less tendency for the AD to spread in cortex that had been chronically stimulated than in undercut, supersensitive cortex.

Histology

A Nissl-stained section of an undercut cortical region in a cat in which stimulation and recording had been carried out chronically, is shown in Fig. 5. This illustrates the desired partial isolation preparation of this work. With our

present histological methods we are unable to detect any differences between undercut and chronically stimulated undercut cortex. There is some thinning of cells in both preparations. Some cellular loss by way of degenerating fibers in the corpus callosum would be expected in homotopic cortex and thus, although not histologically or electrically identifiable in our experiments, homotopic cortex is not strictly "intact".

Results summarized

Our findings are graphically summarized in Fig. 6. This bar graph compares the AD results for the non-stimulated and chronically stimulated "undercut" preparations with the latter group divided into those animals in which an array of recording electrodes had been chronically implanted and those with chronic stimulating electrodes but evaluated only during the acute experiment.

About one-fifth of our "undercut-only" cats did not show supersensitivity of the under-

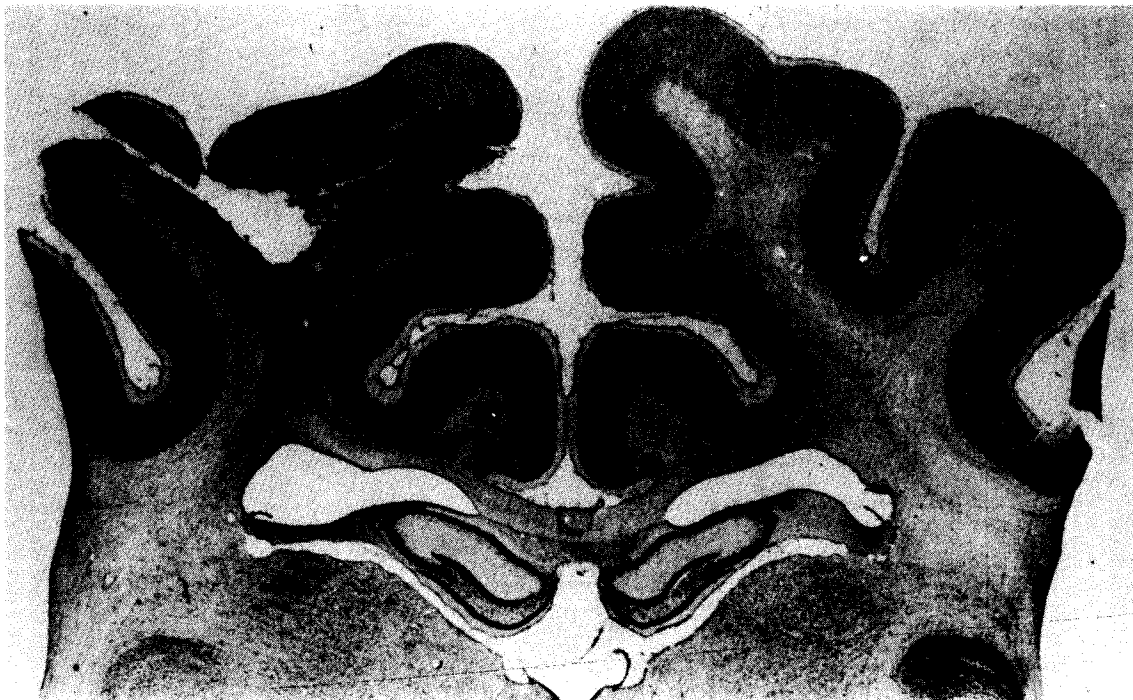


Fig. 5

Nissl-stained section of undercut cortex. Tissue from central portion of undercut area of animal whose records appear in Fig. 3.

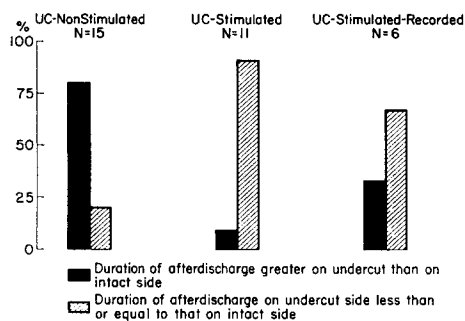


Fig. 6

Summary graph of after-discharge data on chronically stimulated and unstimulated cats. Percentages of animals in each group comparing AD duration for intact and undercut cortex as determined in terminal acute experiments.

cut side at the acute experiment (see also Echlin and MacDonald 1954; Sharpless and Halpern 1962).

More "failures" occurred in those cats with recording electrodes (Fig. 6.)

DISCUSSION

These experiments indicate that partially neuronally isolated cortex does *not* yield prolonged ADs if it has been chronically stimulated electrically. This holds even with 7–10 days intervening after cessation of chronic stimulation. The only way, that we are aware of, in which an AD is decreased in duration in partially isolated cortex, is after prior ADs (Sharpless and Halpern 1962). However, the effect we observed could occur when the chronic electrical stimulation was subliminal for the production of ADs. Also, the longest recorded duration of the effect of prior AD was only 36 h (Sharpless and Halpern 1962).

The previously mentioned experiments (Rutledge and Doty 1962) cannot be explained by developing supersensitivity nor can the conditioning or training situation have much apparent effect upon our present results. Chronic electrical stimulation of the brain is the critical feature.

The changes observed in chronically deafferented cortex have been considered to be an indication of "supersensitivity" or increased "excitability" (Echlin and MacDonald 1954; Sharpless and Halpern 1962). Two major mecha-

nisms for the development of supersensitivity (in denervated structures) have been observed; an increased sensitivity to transmitter substance and sprouting of neuronal terminals. Under certain conditions, both may appear in denervated cerebral cortex (Cajal 1959; Purpura and Housepian 1961; Echlin and Battista 1963). Either mechanism could be initiated by a decrease in the amount of synaptic transmitter substance reaching the cell (a disuse supersensitivity), or by the actual degeneration of afferent fibers (a denervation supersensitivity). Our results on the prevention of supersensitivity would seem to argue against an explanation based upon degeneration of afferent fibers. This does not imply that sprouting does not occur in undercut cortex without chronic electrical activation; however, in adult animals with cortex undercut similar to ours, Cajal (1959) did not see axon collateral proliferation or any other changes that had been observed in immature brains.

Since there has been some implication that partially deafferented cortex and related "supersensitivity" might play a role in certain forms of experimental epilepsy, our findings suggest that local chronic electrical stimulation might be effective therapy in some focal epilepsies. Of prime importance is, however, the necessity to determine to what extent chronic electrical stimulation can actually reverse an already established level of supersensitivity. Our results, from experiments started 1 week after undercutting, were probably preventing supersensitivity rather than reversing it.

In view of some alleged effects of chloralose upon the release of brain acetylcholine (Mitchell 1963; Beleslin *et al.* 1965) and the possible role of this latter compound in an isolated slab of cortex (see Sharpless 1964), it is conceivable that some of our results could be due to or modified by the use of chloralose. It should be kept in mind, however, that equal amounts of chloralose were used in the chronically stimulated and the unstimulated groups and, therefore, other basic conclusions should remain valid.

SUMMARY

1. A portion of the marginal gyrus of the cerebral cortex on each of fifteen cats was undercut 3–4 mm deep. In terminal experiments under chlor-

alose, 2–18 weeks later, local electrical stimulation produced after-discharges (in 12 cats) which had a longer duration on the undercut side than on the intact side.

2. Another group of seventeen cats, each with an undercut marginal gyrus, received daily electrical stimulation (subthreshold for after-discharges) of the undercut cortex starting 1 week after undercutting (6 weeks delay in two cats). Total stimulation was about 400 applications at 0.6 mA, 400 at 0.8 mA and 200 at 1.0 mA. In terminal experiments under chloralose 1 week after the end of stimulation (6 weeks for one cat), fourteen of these cats did not show supersensitivity of the undercut cortex.

3. These results suggest that chronic electrical stimulation can prevent the development of supersensitivity.

RÉSUMÉ

PRÉVENTION DE L'HYPERSENSIBILITÉ DU CORTEX CÉRÉBRAL PARTIELLEMENT ISOLÉ

1. Sur quinze chats, une partie du gyrus marginal du cortex cérébral est isolée par section sous-corticale à 3–4 mm de profondeur. Dans des expériences terminales sous chloralose, pratiquées 2–18 semaines plus tard, la stimulation électrique locale détermine des post-décharges (chez 12 chats) d'une durée plus longue du côté sectionné que du côté sain.

2. Un autre groupe de dix-sept chats, chacun avec un gyrus marginal isolé par section sous-corticale, reçoit des stimulations quotidiennes (sous-liminaire pour les post-décharges) sur le cortex sectionné à partir d'une semaine après la section (ce délai a été de 6 semaines pour deux chats). La stimulation totale est d'environ 400 applications de 0,6 mA, 400 de 0,8 mA et 200 de 1,0 mA. Des expériences terminales sous chloralose

une semaine après la fin de la stimulation (6 semaines pour un chat), montrent que quatorze chats ne présentent plus d'hypersensibilité du cortex sectionné.

3. Ces résultats suggèrent que la stimulation électrique chronique peut prévenir le développement de l'hypersensibilité.

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