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Altered neuronal excitability accompanying experimental prevention of supersensitivity in undercut cortex

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Cerebral cortex, partially neuronally isolated by undercutting, undergoes changes resulting in decreased threshold for, and increased duration of, afterdischarge elicited by surface electrical stimulation[3,14] This phenomenon has been called supersensitivity and can be prevented in most cases by daily sessions of brain stimulation begun shortly after the undercutting operation[1,2] The absence of supersensitivity to test stimuli persists after chronic stimulation has been discontinued[1,2] Neuronal excitability in undercut cortex can be evaluated by determining the effect of surface electrical stimulation upon postsynaptic or neuronal spike potentials[7,9] We report here a study of neuronal response patterns to surface stimulation in intact, undercut supersensitive and undercut non-supersensitive cortex and discuss possible mechanisms for the excitability changes observed

The methods are described in detail elsewhere[1,2] Briefly, the marginal gyrus of adult cat cortex was undercut on one side, 3–4 mm below the surface Animals which were to receive long-term electrical brain stimulation also had platinum wire electrodes implanted slightly within the undercut cortex and a pair of electrocorticogram electrodes placed on the dura Daily brain stimulation of twenty 2-sec trains was given for an average of 9 weeks The intensity of stimulation was below the threshold for elicitation of an afterdischarge At least one week following cessation of long-term stimulation, a terminal acute experiment was performed For this, most of the animals were anesthetized with chloralose, others were prepared under a short lasting barbiturate Cut edges and pressure points were locally anesthetized and immobilization was achieved with gallamine triethiodide (Flaxedil) Using bipolar surface electrical stimulation, each cat was tested for supersensitivity of the undercut cortex in comparison with contralateral cortex Extracellular unit studies were then made in undercut and contralateral cortex, comparing histograms generated by at least 50 surface stimulations at a frequency of less than 1/sec Cells without injury discharges were studied for 10–45 min and at least two poststimulus histograms and two spontaneous discharge

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histograms were taken in alternation If the poststimulus histograms were consistent over the period of study, averaged histograms were computed for cell classification Depth measurements were made using microdrive readings corrected for dimpling of the cortex

Results of our study are summarized in Table I Except for stimulated non-supersensitive undercut cortex (STIM NON-SS), the predominant effect was inhibition The somewhat decreased percentage of cells inhibited in supersensitive undercut cortex (UC) (60% compared with normal 79 2%) is consistent with the results of others, where intracellular recording revealed a decrease in IPSP amplitude[9] Also, in these cats there was a substantial increase in cells showing excitation followed by inhibition compared with intact cortex, and the initial excitation did not decrease with more intense stimulation as previously reported[9]

The most remarkable finding was that 60% of the cells studied in STIM NON-SS were unaffected by surface stimulation Even with intensities of stimulation exceeding 0 8 mA and 1 0 msec duration, and with the microelectrode only 1 0 mm from the stimulating pair, unaffected cells could be found. Repeated checks were made within minutes upon cells in the contralateral cortex where inhibition or excitation was observed at intensities of 0 8 mA or less and 0 2 msec duration

Although Flaxedil increases the duration of afterdischarge in both intact and isolated cortex[4], we found little difference attributable to Flaxedil in the cellular response patterns in either cortex opposite the undercut (OPP UC) or intact cortex

TABLE I

SPONTANEOUS RATES AND RESPONSIVENESS TO SURFACE STIMULATION OF NEURONS IN INTACT AND UNDERCUT NEOCORTEX

'Opposite undercut' includes 6 cats in supersensitive categories and 6 in non-supersensitive The undercut cortices of 5 cats were judged to be more or less supersensitive despite long-term brain stimulation 'Excit + inhib ' means an initially excited cell that later showed inhibition Spontaneous rates given in parentheses

	Number		*Percentage*			
	Animals	*Cells*	*Inhibited*	*Excit*	*Excit + inhib*	*No effect*
Normal	11	54	79 6 (6 8)	5 6 (7 1)*	3 7	11 1 (4 1)
Undercut, supersensitive	2	15	60 (6 9)	0 (5 2)*	40	0 —
Stimulated undercut, supersensitive	5	28	71 5 (5 7)	3 5 (4 9)*	21 5	3 5 —
Opposite undercut	12	44	86 4 (7 1)	0 (7 5)*	13 6	0 —
Stimulated undercut, non-supersensitive	9	42	35 7 (9 5)	4 7 (9 4)*	0	59 6 (6 0)

* 'Excit ' combined with 'Excit + inhib '

Further, all recordings for stimulated undercut cortex were obtained using Flaxedil, yet STIM NON-SS is markedly different from supersensitive stimulated undercut cortex (STIM SS) as well as from all other categories

Consideration of spontaneous firing rates may be helpful in interpreting any effects of test surface stimulation Average rates are given in Table I First, of all cells inhibited the rate for those in STIM NON-SS was the highest (9 5) Second, in the same preparation, those cells unaffected by surface stimulation had appreciably lower rates (6 0) This was also the case for intact cortex, where cells showing no effect had an

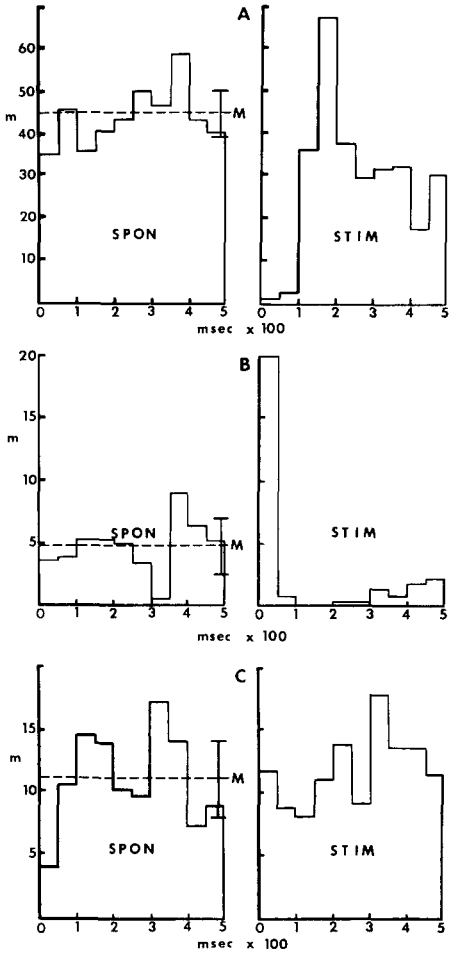

Fig 1 Unit spontaneous discharge (SPON) and poststimulus histograms (STIM) in three preparations M — mean of spike discharges in all 50 msec periods, vertical bar represents standard error of the mean, m — mean of spike discharge for each 50 msec period Stimulation occurred 15 msec prior to 0 A unit from intact cortex, 950 μm deep, 150 sweeps averaged, spontaneous frequency, f = 17 7/sec Stimulation was 0 8 mA, 0 2 msec duration Typical inhibition, as observed in all preparations, lasting up to 250 msec B unit in undercut, supersensitive cortex, 1700 μm deep, 100 sweeps averaged, f = 3 7/sec Stimulation was 2 0 mA, 0 2 msec duration Initial excitation for 50 msec followed by complete inhibition, C unit in stimulated undercut, non-supersensitive cortex, 1000 μm deep, 150 sweeps averaged, f = 4 4/sec No effect to surface stimulation of 1 0 mA, 0 2 msec duration

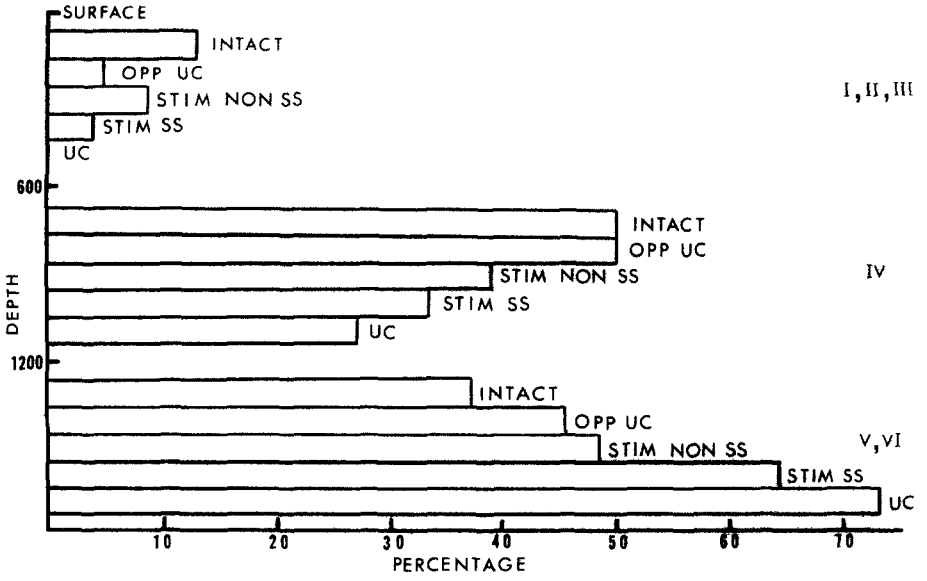

Fig 2 Distribution by depth in μm of cells studied Layer structure indicated on the right represents averaged values from published figures[1,11 13] and our own measurements, normalized to a cortical depth of 2 0 mm OPP UC = opposite undercut, STIM NON SS = stimulated undercut, non-supersensitive, STIM SS = stimulated undercut, supersensitive, UC = undercut, supersensitive

average rate of 4 1 compared with 6 8 for inhibited cells Finally, there was little difference between discharge rates of cells showing inhibition and those showing excitation or mixed effects

There was rarely any ambiguity in classifying a cell based upon the effect of surface stimulation The representative histograms in Fig 1 illustrate this point A given effect (inhibition, excitation or excitation followed by inhibition) of surface stimulation upon neuronal discharge had a typical histogram for all preparations

It is important to consider the possibility that different populations of cells were sampled in the various preparations Fig 2 shows that the distribution of cells by depth for STIM NON-SS seems to more closely resemble that of intact cortex and OPP UC than do the distributions of the other undercut preparations Also, cells in stimulated and non-stimulated supersensitive undercut cortex seem to have about the same distributions Since histology was not performed on all animals, assignment of cells to particular layers (the only meaningful comparison due to individual variability) is only approximate A further complicating factor may be seen in Table II, which gives the number of cells in 200 μm intervals about the cut-off points for Fig 2 Although this is a potential source of ambiguity in determining depth distribution as related to layer structure, depth was measured similarly for all cells Clearly the differences seen in Fig 2 suggest that in UC there is a relative lack of spontaneous activity of cells in layer IV

Our results and a consideration of the possible explanations of them will be helpful in understanding supersensitivity of undercut cortex and possible mechanisms

TABLE II

NUMBER OF CELLS WITHIN 200 μm INTERVALS CENTERED ABOUT 600 μm AND 1200 μm

Percentages of cells studied in each category are given in parentheses Abbreviations are as used in Fig 2

	Intact	*OPP UC*	*STIM NON-SS*	*STIM SS*	*UC*
500–700 μm	7 (13%)	2 (5%)	3 (7%)	1 (4%)	2 (13%)
1100–1300 μm	8 (15%)	10 (24%)	10 (24%)	2 (7%)	1 (7%)

for its prevention with long-term electrical stimulation Inhibition of neuronal activity by surface electrical stimulation, the predominant effect in intact cortex, is probably due in part to depolarization of axons, including recurrent collaterals of pyramidal cells, having excitatory synapses on inhibitory interneurons, and in part to depolarization of axons having inhibitory synapses on the cell being studied It has been shown that the inhibitory effect is a genuine postsynaptic inhibition rather than an injury phenomenon due to electrical stimulation[7]

Excitation, often followed by inhibition, was more frequently observed in UC than in intact cortex or OPP UC This may be understood in terms of two factors First, loss of recurrent collateral and afferent input may induce either loss or decreased efficiency of inhibitory interneurons Second, Krnjević has noted that large pyramidal cells are lost preferentially in undercut cortex and that the smaller pyramidal cells which remain are probably largely responsible for recurrent excitation[9] Thus, inhibition may become insufficient to overcome excitation in undercut cortex The period of inhibition which often follows excitation may be due to the generally longer duration of IPSPs than EPSPs[10] or to deficient recovery mechanisms, as seems to be the case for cells participating in the afterdischarge[9] This could be determined by intracellular recording and stimulation during the period of inhibition

In intact cortex the spontaneous neuronal activity of a small number of cells (11%) was unaffected by surface stimulation This may result from cancellation of excitatory and inhibitory input, inadequate current density to depolarize sufficient inputs to reach threshold or recording from a cell functionally isolated from observable effects of surface stimulation, *e g* , an afferent receptive cell Note that there were no unaffected cells found in cortex opposite the undercut This may be due to loss of interhemispheric input, with neurons therefore becoming more responsive to other inputs (either excitatory or inhibitory)

In STIM NON-SS there is a marked increase in the proportion of cells unaffected by surface electrical stimulation That the majority (59 6%) of the cells was unaffected by test stimuli is potentially the basis for the prevention of supersensitivity One explanation for this altered excitability would be the accommodation of axons to surface stimulation resulting in fewer and less powerful recurrent excitatory or inhibitory effects The persistence of non-supersensitivity without daily stimulation[12] argues against this explanation, but it could be tested using intracellular recording and

perhaps more intense test stimulation Another possible factor is that bipolar surface electrical stimulation of cortex causes a significant temporary increase (up to 1 h) in release of γ-aminobutyric acid (GABA) collected epicortically, and the release of GABA is associated with the inhibitory effect of surface stimulation[5] Daily, long-term electrical stimulation might promote a more prolonged presence of released GABA and thus tend to reduce spontaneous activity of some cells The differences in mean spontaneous rates suggest that unaffected cells may constitute a distinct population of neurons The increased sampling of this population in STIM NON-SS might result from lack of spontaneous activity of many of the cells which would otherwise have shown inhibition or excitation to test stimuli This could be tested using the Krnjević technique for eliciting background discharge with iontophoretic application of L-glutamate[6] Alternatively, long-term stimulation may preserve, through antidromic stimulation, those cortical neurons which normally function as afferent receptive cells Since many neurons are lost in undercut cortex[2], preferential preservation of these functionally isolated cells would make them more dense than in intact cortex The same cells would probably become inactive without stimulation in UC due to lack of input This is consistent with the decreased number of spontaneously active neurons in layer IV of UC and the near normal number in STIM NON-SS seen in Fig 2

In summary, it appears that supersensitivity is prevented by long-term daily electrical brain stimulation through alteration of the excitability of neurons to surface stimulation The mechanism for this alteration is uncertain, and three possible explanations are suggested First, an exceptionally long-lasting accommodation of axons to surface stimulation might result in fewer and less powerful recurrent effects Second, spontaneous activity of some cells may be reduced either through changes in cortical GABA or some other mechanism, leading to increased sampling of unaffected cells Third, and the explanation we prefer, is that a class of neurons whose spontaneous discharge is unaffected by test surface stimuli, *i e*, those functioning as afferent receptive neurons, is being preferentially preserved by long-term stimulation through antidromic activation

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