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Morphological Changes in Pyramidal Cells of Mammalian Neocortex Associated with Increased Use

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After long-term electrical stimulation of the brain, which presumably produced increased neuronal use, histological studies were made of neocortical neurons involved in transcallosal and extracallosal systems. Adult cats with implanted electrodes received 20 trains (2 sec each) of electrical stimulation to the suprasylvian gyrus daily for several weeks. In four cats, brain stimulation was paired with foreleg shock (trained), in two, it was not (untrained). Cortical tissues ipsilateral and contralateral to the stimulated side were prepared with a modified Golgi–Cox method. In cortex contralateral to the stimulated side, apical dendrites of layers II and III pyramidal cells had significantly more branchings in terminal regions, greater lengths, and terminated nearer the pia than they did on the stimulated side. There were also more spines on oblique, vertical, and terminal portions of apical dendrites. Increases in oblique and vertical spine counts appeared to be more related to training than to just brain stimulation. Qualitatively, apical dendritic terminals in contralateral cortex had fine branchings, filamentous bare twigs, especially long spines, convolutions with close packing of spines, acute angles of terminals reflecting from the pia, and a general appearance of increased density of apical dendrites near the pia. The observed changes in neuronal structure described in these experiments are interpreted as evidence that increased use of specific pathways to the cerebral cortex produces postsynaptic growth in some cortical neurons.

INTRODUCTION

A "growth theory" of learning and memory that postulates structural synaptic changes, presynaptic or postsynaptic, or both, as a result of increased "use" is an old concept. In 1893 Tanzi (41) suggested that passage of nerve impulses results in increased nutritive activity within a neuron, increased cell volume, and lengthening of processes to effect better contact with neighboring neurons. Among more recent proponents of the growth theory the work and discussions by Eccles and his colleagues is noteworthy

(8, 9). They investigated spinal cord systems for possible electrophysiological and functional effects of increased neuronal activity. Unfortunately, their findings were perplexing since enhanced reflexes measured electrically were sometimes related to disuse as well as in other instances to excessive use.[1]

To our knowledge, a quantitative morphological study of neuronal elements after increased use has not been made on an adult mammal. An acceptable model for such study may be found in the experiments of Rutledge (31) on an extracallosal system in which an enhanced evoked response in cortex contralateral to conditional cortical stimulation could be interpreted as the cortical expression of the result of increased use of a labile, multisynaptic interhemispheric pathway. Because of the results of successful investigations on the cerebral cortex with the Golgi method in the past few years, a study of cortical neuronal structure in the Rutledge "facilitation experiments" (31) is important.

When the multisynaptic interhemispheric pathway (34) is activated by electrical stimulation of cat suprasylvian cortex, activation of the contralateral cortex occurs via this system and through the corpus callosum. The corpus callosum projection is in part to dendritic spines of oblique branches of pyramidal cell apical dendrites (rabbit) (13). The extracallosal system relays through the rostral mesencephalon, and the cortical response configuration includes a large positive potential likely representing underlying synchronous EPSPs (35). The system may project in part with excitatory synapses to the upper regions of cortical pyramidal cells' apical dendrites. This suggestion has no anatomical support, and even the distribution of specific afferent projections is still in question (40). If both interhemispheric systems were electrically stimulated for a long time, it would be possible, with the Golgi method, to look for possible effects on dendritic spines of cortical pyramidal neurons receiving input via the two systems. Furthermore, the larger postsynaptic structures, the apical dendrites proper, might show structural alterations after the assumed increased synaptic input during the long-term brain stimulation. The apical dendritic field of pyramidal cells, therefore, is another subject for morphological study. Additionally, since long-term electrical stimulation of denervated cortex prevents the expected loss of pyramidal cell axonal elements (33), and repetitive, long-term conditional cortical stimulation produces depolarizations of axons resulting in action potentials in pyramidal cell axons, axonal activity (increased use) might lead to changes in axon collateral lengths

[1] A precise definition of "use" cannot be made. For this paper it involves neural events including, but not necessarily limited to, sensory input and elaboration, electrical activity inferred as a result of direct electrical stimulation, synaptic functions, and metabolic activity.

or branchings. Therefore, histological study of pyramidal cell axons is desirable in these experiments.

Results of studies on immature brains are encouraging. There are now definitive data showing specific kinds of axonal and dendritic growth in developing brains (21, 24). More unspecific growth may even continue in cortex, well beyond the postnatal period, when animals are exposed to an enriched environment (7, 30).

The experiments described in this paper were designed to test the hypothesis that (a) axon terminals might sprout and grow in the adult cortex as a result of increased use, and (b) postsynaptic structures (dendrites) might reflect the increased amount of synaptic traffic by growing new lengths and synaptic sites (spines). In this primarily quantitative study most attention was directed to dendritic fields, apical dendritic terminals, and spines, that is, postsynaptic elements of pyramidal neurons in the upper layers of the adult cat cerebral cortex. The two major interhemispheric systems, the transcallosal and the extracallosal, were electrically stimulated to produce increased use in trained and untrained animals. A summary of the findings from these experiments has been given (36).

METHODS

Data were obtained from histological study of tissue removed from cat suprasylvian gyri. Modifications of the Golgi–Cox method as we have described it were used (33). This procedure works sufficiently well for quantitative study of adult cortical neurons, although not all tissue preparations are equally good.

Six adult cats were trained to remain quiet in a restraining device in which they sat comfortably on their haunches with their heads thrust through a hole in the cover. All six received unilateral suprasylvian gyrus electrode implants during sterile surgery. Four were trained with electrical brain stimulation as the conditional stimulus (CS) and foreleg shock (nonavoidance) as the unconditional stimulus (US). These animals learned to make foot flexions (CR) and formed the "trained group."[2] All cats except one of the untrained had the bipolar electrodes implanted on the left, the exception had electrodes on the right. The 27-gauge platinum–iridium electrodes were about 1.25 mm in length and smoothly sharpened to a needle tip. The electrodes, 6–8 mm apart, penetrated through the pia mater (dura removed) and into the cortex about 0.5 mm. The location in anterior midsuprasylvian gyrus was optimal for producing transcallosal or interhemispheric delayed responses on the contralateral cortex (31, 34). The CS

[2] Previous work using this paradigm indicated that larger and less equivocal CRs were established when each CS was reinforced. Later in training the intensity of the US was reduced considerably.

was a 2-sec train of 50 Hz, 1.0 msec pulses, initially at 0.4 ma and gradually increased over several weeks to about 1.0 ma. The 0.2-sec foreleg shock overlapped the CS by 0.1 sec. Twenty trials, spaced 1 min apart, were given at the same time daily. All four trained cats learned to a mean criterion of 77% within a mean of 535 trials. Average total trials was 1200 (about 100% overtraining) in 89 days. The other two cats received similar daily brain stimulation, on the minute, and foreleg shock on the half-minute (untrained group). Mean number of stimulations was 1070 (9% fewer than the trained group) in 77 days (13% fewer days). These two cats never gave any indication of learning that brain stimulation was to be followed by foreleg shock in 30 sec. Procedures were as similar as possible to those used previously in the "facilitation experiments" (31), but evoked potentials were not measured in this study.

At the cessation of training or stimulation, each cat was anesthetized with sodium pentobarbital, placed in a head holder with the head elevated, and the implanted electrode unit removed. An overdose of sodium pentobarbital was then used to produce cardiac arrest. When the cortex blanched, two blocks of tissue, each about 2 mm wide, were removed from midway between the stimulating electrodes and from the contralateral homotopic region. Tissues from the two sides of the brain for each animal were treated alike and processed together. The celloidin-embedded material was sectioned transversely on a sliding microtome at 100 and 125 μm, but all quantitation except for a small portion of the dendritic spine study (3%) and axon study (26%) was made on sections cut at 100 μm. Blocks, sections, and slides were coded so that the only identity possible was by block code and a code indicating different sides of a particular brain. At the conclusion of the dendritic spine study an unsuccessful attempt was made to identify the sections studied. It is believed that the selection of neuronal elements, the quantitation, and selection of material for artistic rendition was adequately controlled for possible experimenter bias. In only one aspect, that of the gross appearance at low microscopic power of what seemed to be apical dendritic packing (discussed below), was there any suspicion that material on some slides seemed to be different from that on others.

Appropriateness of Golgi Method. With experience it is possible to use the Golgi method successfully for quantitative studies on adult cat neocortex. In our work about 75% of tissue sections processed were acceptable for detailed examination. Criteria for selection of sections included uniform staining of neuronal processes including pyramidal cell, apical and basal dendrites and spines, but not necessarily axons, absence of debris such as precipitate on cellular membranes or background, good contrast between cells and background, and relatively uniform tissue thickness. Rigorous

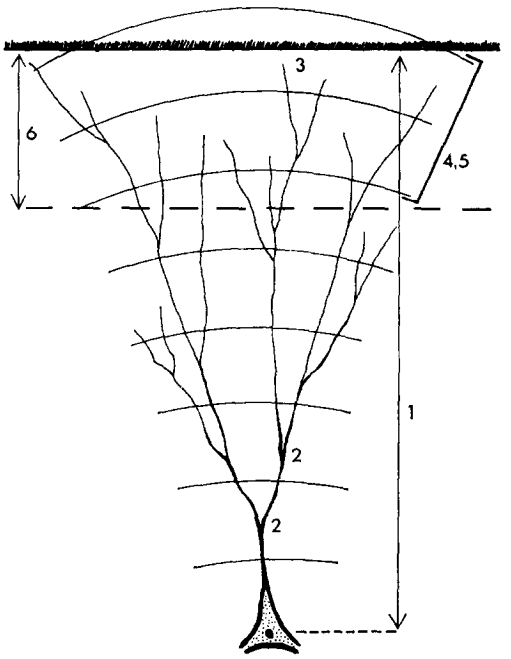

FIG. 1. Measurements made on pyramidal cells other than spine counts. 1 = soma–pia distance; 2 = apical dendritic branches, two marked; 3 = distance of closest terminal to pia; 4 = number of branches in last two concentric circles (2); 5 = number of segments in last two concentric circles (10); 6 = number of segments in 100-μm band parallel to pia (11).

controls are mandatory in quantitative studies, and they are best achieved if possible in each experimental animal, since tissue staining may vary considerably even between normal animals. In each of the animals used here tissue samples were taken from homotopic areas on the two cerebral hemispheres, with only one side of the brain having received chronic electrical stimulation.

Dendritic Study. In preparation for cell selection, the gyrus midpoint of each histological cross section was marked near the pial surface. Each section was divided into six parts 0.9- to 1.0-mm wide, and the two central parts of these subdivided into about 0.5-mm-wide portions. Only the two parts totaling about 2 mm in width, on either side of the midpoint, on the crown of the gyrus, were studied. Small to medium-sized pyramidal cells (384) with cell body centers located 300–500 μm from the pia were selected (Fig. 2). As far as possible, two neurons were selected from each of the parts closest to the midpoint and one each from the second part making six cells all within the crown of the gyrus in a total surface width of 3.6–4 mm. For each cat and on each side of the brain, approximately an equal

number of cells were studied from the separate subdivisions or portions. Selection of cells from each side of a brain were exactly matched on the basis of soma distance from pia.

Figure 1 shows the concentric circle method of Sholl (39) as applied to our measures on apical dendrites. Pyramidal cell somas were relatively uniform in width, 10–15 μm perpendicular to the long axis, but shape varied considerably. We did not use a correction for soma size, as has been suggested for pyramidal cells of layer V in rat cortex (2). With an eyepiece graticule of 50-μm concentric circles and using $\times$ 450 magnification, measurements were made of pyramidal cell soma distance from pia, soma distance from the gyrus midline and distance of the apical dendritic tip closest to the pia. Sketches were made of each neuron on a concentric circle pattern spaced at 1 cm. From these drawings summary profiles were constructed to show total number of dendritic branches, number of branches and segments in the last two concentric circles contacting the pia, and number of dendritic segments in a 100-μm band running parallel to the pia. The 100-μm band and the last two concentric circle areas are not necessarily the same since for large-diameter dendritic fields all of the branches and segments would not be counted in the last two concentric circles, but would appear in the 100-μm band area (Fig. 1). No corrections were made for changes in focal planes in the 100-μm sections.

Data were processed by computer to obtain means, standard errors of the means, standard deviations, Student t ratios, and probability levels (Table 1).

Dendritic Spines. Sections and slides were coded to reveal only an animal number. Sides of a brain, stimulated or contralateral, were not identifiable. Counts of spines were made in 10-μm lengths in four locations on pyramidal cells using a calibrated ocular and $\times$675 magnification. Locations were on basal dendrites and vertical, oblique, and terminal portions of apical dendrites. Pooled data sample sizes varied from 54 to 84 (Figs. 3 and 4). Not every cell yielded counts in all locations, but whenever possible cells were selected to give spine counts in at least two locations. No more than two 10-μm portions were counted in any of the four locations on a particular cell. On the average, less that four 10-μm counts were obtained from each cell out of a possible eight (2×4 locations). The 1658, 10-μm spine counts (816 stimulated, 842 contralateral) were made from 438 cells, 176 (40%) of which were from the population of 384 cells used in the dendritic study, the remainder (262) were of the same kind and configuration and at the same general location.

Since many apical dendritic terminals were sparsely populated or devoid of spines near their ends, terminal spine counts were started 35–45 μm from the tips. Measurements were made of the shortest distance from the

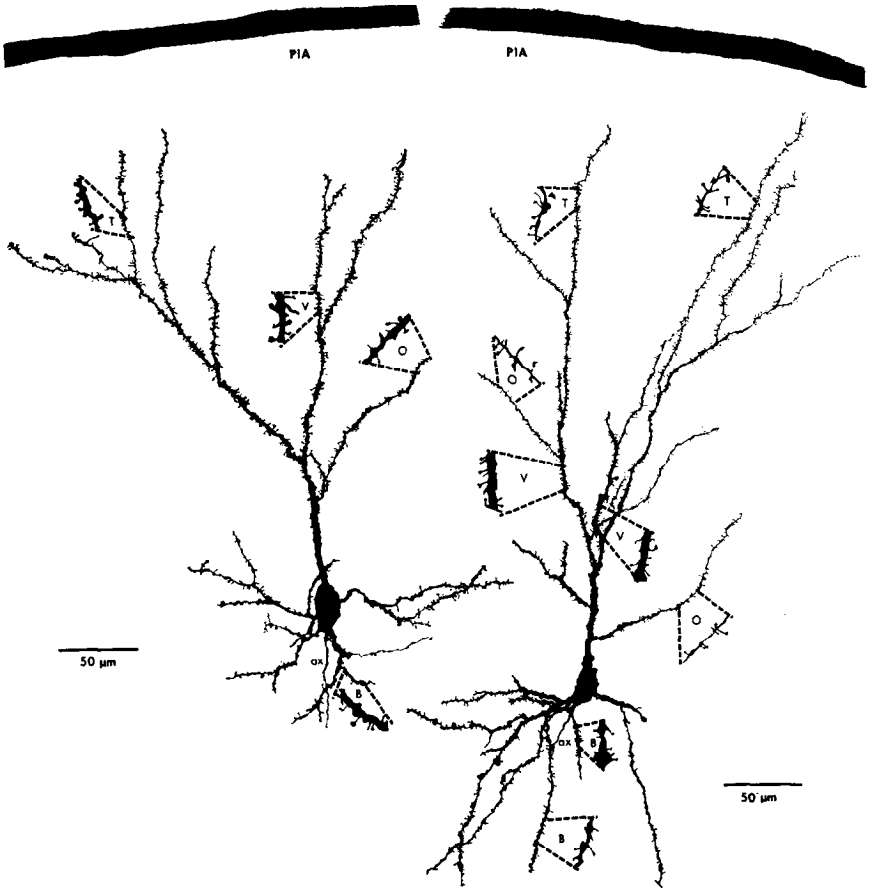

FIG. 2. Scale drawings of upper cortex pyramidal neurons. Left from stimulated cortex, right from contralateral cortex. Spine count 10-μm portions: B—basal, O—oblique, V—vertical, T—terminal. ax = axon. Note that spine density is generally least on basal dendrites.

pia to the terminal 10-μm counted portion in order to have a further evaluation of the terminal–pia relationship. Some terminals were close to and parallel to the pia for some distance, including the 10-μm portion; other terminals ended orthogonal to and a considerable distance from the pia. Fine, short branching terminals could not be used for spine counts since they did not have sufficient lengths distal to the branch. Because of the great variability in apical dendritic tips, from spineless twigs to thick, heavily spined endings, no quantitative means for study of spine density of the tips seemed possible.

As with the dendritic study, data were computer processed.

TABLE 1

MEASURES ON APICAL DENDRITES FROM STIMULATED (S) AND CONTRALATERAL (C) CORTEX SHOWING MEANS, STANDARD ERROR OF MEANS, *t* RATIOS, AND PROBABILITY LEVELS FOR MEAN DIFFERENCES BETWEEN S, C SAMPLES 126, 66

	Mean		SEM			
	C(126)	S(126)	C	S	*t*	*p*
Trained						
Total branches	12.69	11.05	0.30	0.30	3.86	0.0001
Branches, 2 circles	1.38	0.57	0.14	0.97	4.82	0.0000
Segments, 2 circles	8.94	4.17	0.49	0.36	7.81	0.0000
Segments, 100 μm	10.75	4.01	0.63	0.34	9.45	0.0000
Terminal to pia (μm)	24.56	67.41	2.82	3.32	9.83	0.0000
	C(66)	S(66)	C	S	*t*	*p*
Untrained						
Total branches	13.46	12.11	0.53	0.46	1.94	0.0547
Branches, 2 circles	1.55	0.71	0.24	0.12	3.10	0.0024
Segments, 2 circles	8.71	4.97	0.88	0.49	3.72	0.0003
Segments, 100 μm	11.89	4.64	1.29	0.53	5.21	0.0000
Terminal to pia (μm)	28.18	58.11	4.07	3.70	5.44	0.0000

Axon Measurements. All available tissue sections were searched for axons of small and medium-sized pyramidal cells of the same type and at the same general location as in the other samples. Somas were centered 350–425 μm from the pia. For selection each neuron had to have a "downward coursing" axon at least 200 μm long, (average length 260μm), and one or more collaterals of which one had to be at least 100 μm long. Because of inadequately stained axons in most pyramidal cells it was not possible to restrict this sample to those cells used in the other studies. Eighty of the 308 cells studied came from tissues sectioned at 125 μm. Drawings were made of each cell selected, but measurements were made through the microscope using a calibrated ocular and ×450 magnification. Data were computer processed.

Qualitative Aspects. Although all data collecting was from coded material, there were pronounced differences among the histological sections. Apical dendrites in some sections seemed to be heavily concentrated and packed close to the pia. At higher power these dendrites had terminals with unusual configurations and branches. A systematic study was made of terminals, noting general configuration, relationship to pia, unusual course, branches, spine presence, and location. Several descriptive categories were

identified and tissue sections were searched for good examples fitting the descriptive categories. Scale drawings were then made of nearly 60 terminals representing the various categories. When the sections were uncoded the drawings were assigned to the proper brain sides, stimulated or contralateral. It was now necessary to search for more "examples" from the stimulated cortex since this was the tissue showing few of the apparent apical dendritic terminal changes. Difficulty was encountered in finding comparable examples since the revealed code indicated that the descriptive categories had actually been made on terminals of contralateral cortical tissue. The final selection of terminals for illustration (Fig. 6) was done conservatively, i.e., differences in terminal types between stimulated and contralateral cortex were probably greater than the selected ones illustrate.

Scale drawings were made of two representative pyramidal cells, one from stimulated cortex, one from contralateral (Fig. 2).

RESULTS

In the two drawings of the pyramidal cells (Fig. 2) note the relatively small soma size, the oval soma shape, and the widely branching apical dendrites, characteristics of pyramidal cells in layers II and III. The main apical dendrite of nearly all the cells studied extended at least 40 μm from the soma before branching, a few (about 5%) branched close to the soma usually into two major divisions. Compared with the apical dendrites the basal dendritic fields were relatively small.

Dendritic Study. Data are summarized in Table 1 and since they were all in the same direction for trained and untrained animals the groups will be considered together. All measures except for total branches, untrained, showed significantly larger values contralateral to the stimulated side. There were more apical dendritic branches per neuron contralaterally (15% for trained, 11% for untrained), although in the untrained the difference reached only the .055 probability level.

When the number of branches in the last two concentric circles (Fig. 1) were compared the differences in both groups were significant ($P = 0.0000$ and 0.0024, Table 1). Although on the average there was less than one branch per cell on the stimulated side in the last two concentric circles, contralaterally there was more than one. When 192 cells were compared with 192 (trained and untrained) there was about 130% more apical dendritic branching of terminals near the pia in the contralateral cortex.

Another and better measure of field coverage by apical dendrites is indicated by the number of segments within a given area. Two measures were obtained, segments in the last two concentric circles and in a 100-μm band parallel to the pia. There were significantly more segments contralaterally in both measures (Table 1). When trained and untrained were

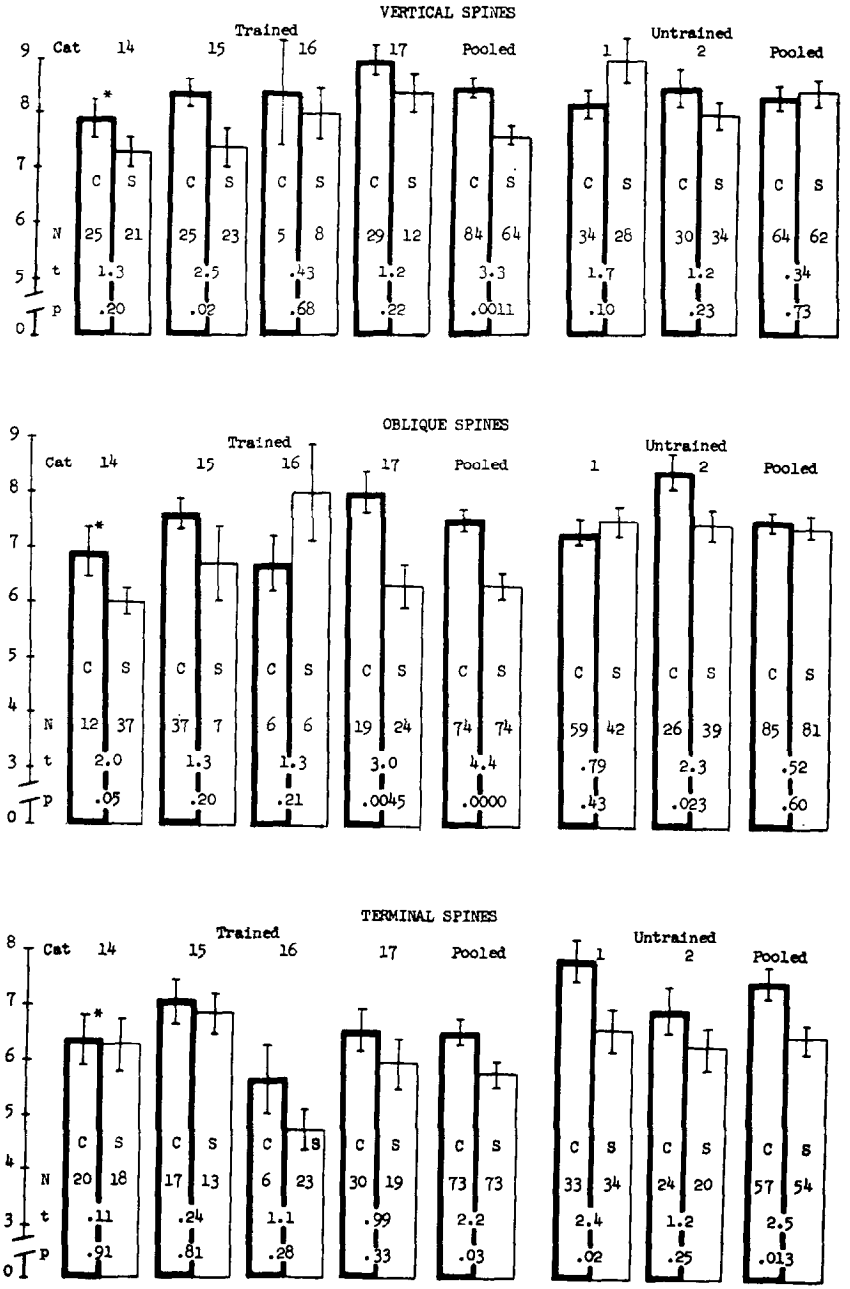

FIG. 3. Apical dendritic spine counts (ordinate) at three locations. Bars are means of the samples (N), and the standard error of the means (*), for individual animals and groups (pooled), trained and untrained. The *t* ratios (t) and probability levels (p) are for mean comparisons between contralateral (C, dark bars) and stimulated (S) cortex.

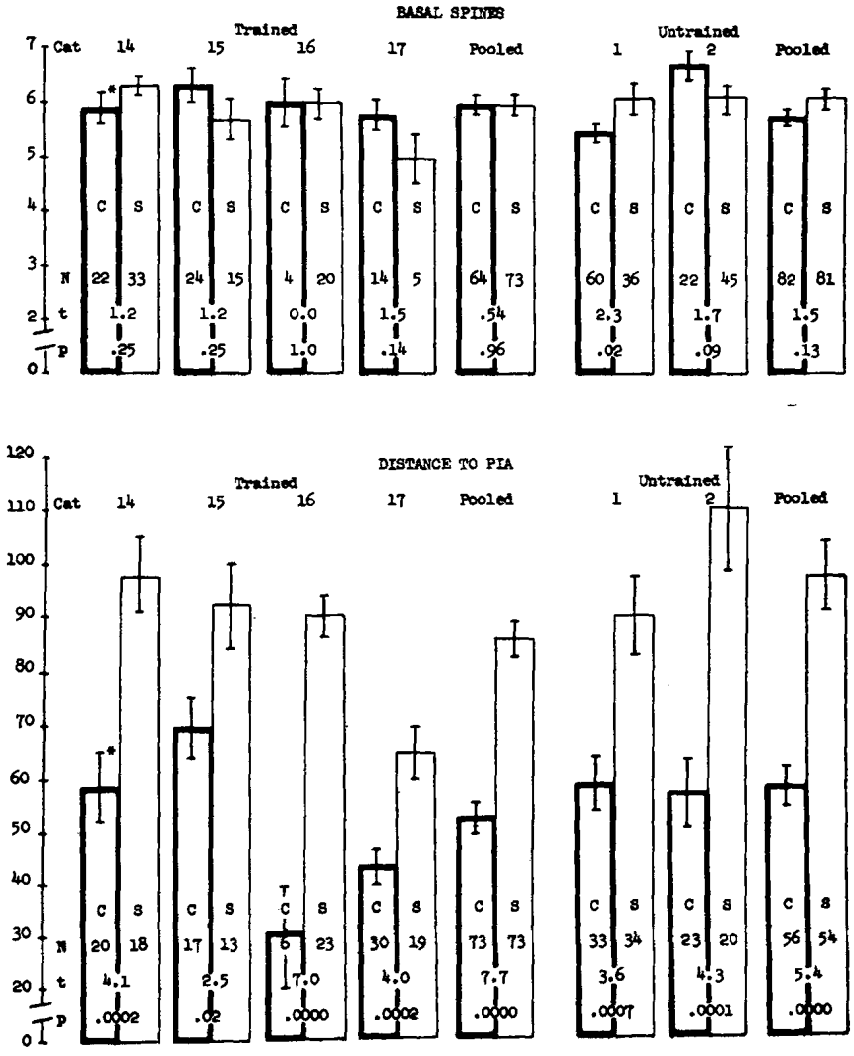

FIG. 4. Basal dendritic spine counts and distance in μm from the pia to the terminal portion counted. Notations as in Fig. 3.

combined these differences averaged out to be 95% for concentric circles and 160% for the 100-μm band near the pia.

Finally, contralateral to stimulation in both trained and untrained groups apical dendrites of pyramidal cells terminated closer to the pia (Table 1). Terminals were about twice as far from the pia in stimulated suprasylvian gyri than in contralateral gyri, or 35-μm closer contralaterally.

These results are clear and unambiguous. The apical dendrites of layers

TABLE 2

PYRAMIDAL CELL AXON COLLATERALS AND THEIR BRANCHES, WITH NOTATIONS AS TABLE 1

	Mean		SEM			
	C(129)	S(101)	C	S	*t*	*p*
Trained						
Collaterals	4.25	4.71	0.14	0.23	1.82	0.07
Branches	8.24	8.44	0.41	0.47	0.31	0.75
	C(57)	S(56)				
Untrained						
Collaterals	3.68	4.05	0.24	0.20	1.18	0.24
Branches	5.86	7.55	0.53	0.47	2.38	0.02

II and III pyramidal cells have more extensive branchings and longer lengths, contralateral to the cortex which received electrical stimulation as a conditional stimulus or a comparable amount of stimulation unpaired with foot shock.

Dendritic Spines. Figures 3 and 4 are data summaries for apical and basal dendritic spine counts and for a related measurement, the distance of apical dendritic terminals from the pia. To show that there were not large individual variations the means, standard error of the means, Student *t* ratios, and probability levels for each animal, as well as pooled data, are included in the figures. The number of portions counted (N) varied considerably as all tissue sections were not equally good. No attempts were made to increase sample sizes from those cases yielding small numbers after the sections were decoded. Since the absolute difference in spine counts between the two brain sides were relatively small, differences within an animal were sometimes not statistically significant. Certainly at least for the four animals in the trained group pooling the data in all three measures seemed justified.

For the counts made on vertical portions of apical dendrites contralateral cortex in the trained group had significantly more spines (pooled, Fig. 3). This 11% increase in spines was not seen in the two untrained animals since the differences were in opposite directions, although not significant. It seems reasonable to suggest that only with training were spines increased on vertical components of apical dendrites in cortex contralateral to that stimulated.

Counts made on oblique branches showed a difference between the two sides for both trained and untrained similar to that for the vertical counts

(Fig. 3). There were 19% more spines contralaterally on oblique branches in the trained group. For the untrained however, one animal showed significantly more spines contralaterally but the difference was not significant when the data from the two were pooled.

For the terminal counts on apical dendrites the trained and untrained groups showed small, but significant differences between the two sides (pooled, Fig. 3). The 12% difference for the trained group and the 15% for the untrained were actually reflected in only one of the six animals (Cat 1). If the individual differences among the animals had not been consistently in the same direction the difference in the pooled data most likely would not have been significant.

Over-all the data for the trained group (Fig. 3) seemed clear as to direction and statistical significance, although not all individual animal differences were significant. Importantly in only one instance out of 12 animal comparisons (Cat 16, oblique) was the absolute difference (though insignificant) in the opposite direction. In this case the number of portions counted on the two sides was small (6) and thus considerable reservation must be attached to this individual's data. For the untrained group, counts on terminals were in the same direction as for the trained, and significantly different between the two sides. Individually, there was a significant difference between the two sides (untrained) in only one case, Cat 2, oblique. When data were pooled no difference was seen. It may be concluded that the untrained group, in contrast with the trained, did not show convincing and significant differences in counts on vertical and oblique components between the stimulated and contralateral cortices. Noteworthy in the data in Fig. 3 is the fact that in no case was there a significantly larger value for the measures on stimulated cortex.

Counts made on basal dendrites turned out to be control measures for the other spine counts. They were, for the trained animals, the same on both sides of the brain in both individual and pooled data (Fig. 4). This finding lends greater credibility and validity to the other statistically significant differences (Fig. 3). Less weight can be attached to the basal counts made on tissue from the two untrained cats (Fig. 4). Although the pooled data show no difference, one animal's data indicated a significant difference between the sides, this being the one exception among the six cats. Note in Fig. 4 that the pooled data are nearly identical for the two groups.

Finally, more spines were counted on terminals contralateral to stimulated cortex (Fig. 3, terminal) and the counted portions were located significantly nearer (about 35 μm) the pia on the contralateral side (Fig. 4).

Axon Measurements. Pooled data on numbers of axon collaterals and collateral branches on 343 pyramidal cells are summarized in Table 2. Although all four values are somewhat greater on the stimulated side, only

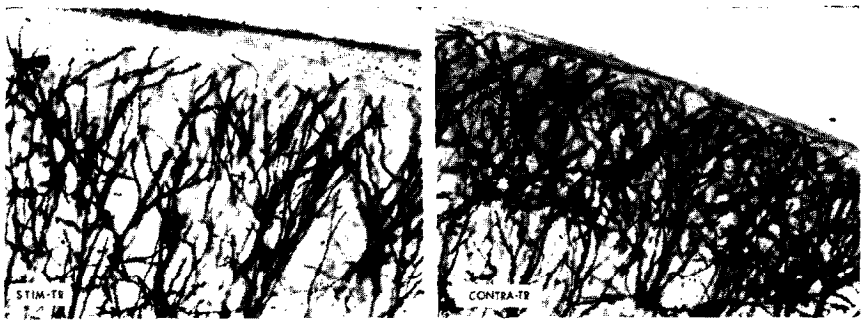

FIG. 5. Photomicrographs of cat suprasylvian cortex near surface. Left, stimulated side, right, contralateral. Both are from a trained animal. Sections cut at 100 μm. $\times$ 160.

one of these reached an acceptable level of significance (branches, untrained). In number of collaterals (trained) a probability level of 0.07 suggests a possible real difference in favor of the stimulated side. The data are suggestive but offer only equivocal support for the hypothesis that pyramidal cell axon collaterals in stimulated cortex become modified as a result of direct long-term electrical stimulation.

Qualitative Aspects. Upon surveying the histological material it became apparent that in some sections apical dendrites of layer II and III pyramidal cells were vastly different especially in terms of greater density. These differences were finally identified, in the decoded sections, with the contralateral cortex. Representative photomicrographs of stimulated and contralateral cortex from one of the six cats are shown in Fig. 5. In distinct contrast to the stimulated side the contralateral apical dendritic field showed a relatively heavy packing of dendritic terminals near the pia in layer I in both trained and untrained animals. In some areas a whorl-like pattern of the contralateral apical dendrites could be seen when the microscope focal plane was changed. This pattern came from groups of terminals appearing sometimes as bundles, that approached close to the pia and then reflected back, in a semicircular manner. Other terminals were seen reflecting sharply or lying laterally tightly parallel to the pia. Infrequently some of these features could be found in tissue from the stimulated side. The gross differences were not dependent upon whether an animal had been trained, and they were seen in tissue from all six animals.

As described in Methods, scale drawings of apical dendrite terminal types were made. Examples illustrating some of the types ars shown in Fig. 6. These unusual endings were found in contralateral cortex in all six animals. Some examples from trained animals appear in the figure. Two types were prominent, one consisted of terminals apparently firmly touching the pia (second row, Fig. 6); in the other type, terminals sharply reflected from

the pia or near the pia in an acute angle or circular manner. There were also more fine terminal branchings on the contralateral side. Some terminals, frequently with tips touching the pia and aligned perpendicular to it had contortions and twistings as if the terminal had actually lengthened but could do so only by folding. Note a good example of this in the second terminal from the left in the lower row of Fig. 6. Other terminals could be classified as being very small in diameter and relatively spine free or as having especially thin or small spines, or both. This is seen especially in the lower right terminal of Fig. 6, but note that this feature is found in an example from the stimulated side as well, upper row, second from left. It must be emphasized that, although some of the features prominently

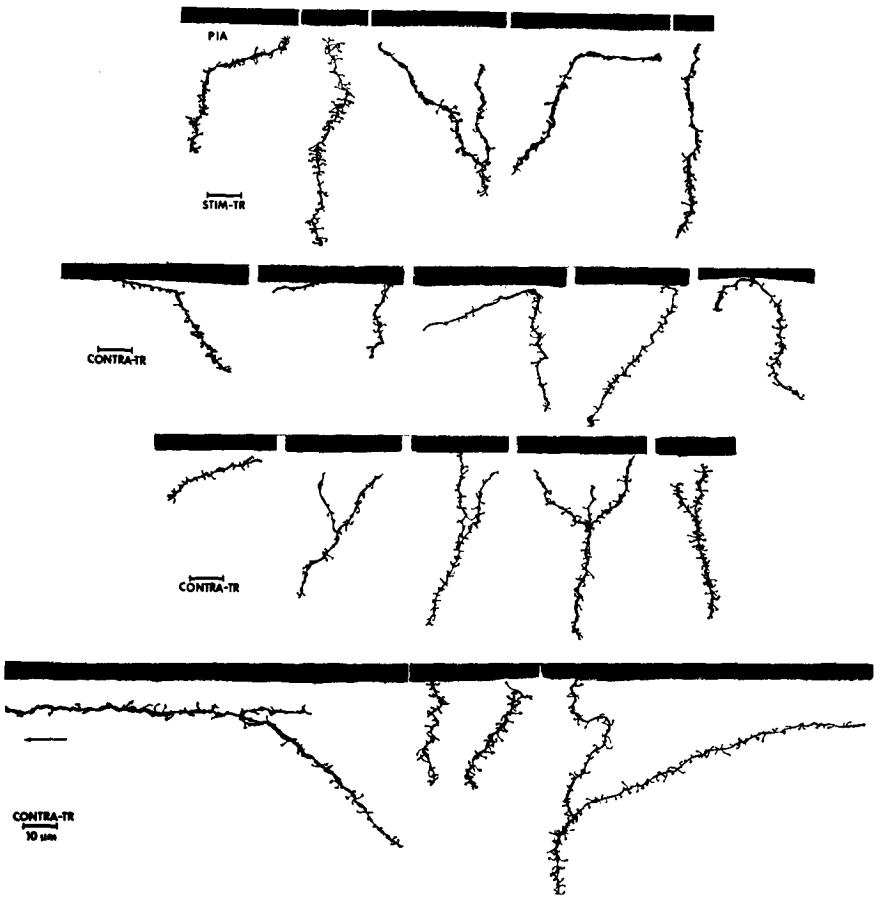

FIG. 6. Scale drawings of pyramidal cell apical dendritic terminals from stimulated cortex (STIM) and contralateral cortex (CONTRA), in trained animals. Arrow indicates terminal extends an additional 50 µm.

distinguishing contralateral terminals from those in the stimulated cortex could be found occasionally in the latter, it was exceedingly difficult to find there comparable examples although all histological material was thoroughly searched. A deliberate, though mostly unsuccessful attempt was made to find more comparable terminal types in stimulated cortex after the code was revealed.

DISCUSSION

The results of these experiments support the theory that increased use of a neural system produces structural change in the system components Apical dendrites of layer II and III pyramidal cells apparently developed significantly more branches and segments, longer terminals, and more dendritic spines as a consequence of long-term electrical activation of presynaptic pathways. Results showing increases in vertical and oblique spine counts suggested that the changes in dendritic spines were specifically related to the training the animals had received, that is, to the pairing of brain stimulation with foot shock. Because of the theoretical importance of these observations, it is essential to look carefully for possible errors in experimental design and in histological preparation. Two major questions may be asked.

First, is it possible that histological staining differences dependent upon stimulation, or lack thereof, can explain the observed changes in dendrites and spines? Perhaps neuronal elements stain better after a period of use and increased activity. There is a suggestion in recent work that more cells are stained with the Golgi method immediately after intensive sensory stimulation (37). However, if this is a valid relationship, it hardly applies in our experiments since animals were not killed for histological study before 48–192 hr (average 110) after the last brain stimulation. Although no comparable study with the Golgi method can be found, using the methylene blue procedure there appear to be no evident differences in staining properties of neurons in the cervical intumescence whether they were used for short periods or for long periods (10). Chronic training (exercise) did result in an increased volume of the nucleoli and better staining of nuclei, reflecting a likely increase in protein synthesis, but these changes were not seen throughout the neuron (10). A more recent study of the effect of exercise or stimulation of spinal motoneurons revealed a decrease of cytoplasmic and nuclear volume (11). An increase in motoneuron size was seen only very early after activation. It is unlikely that our results can be explained on the basis of differenial staining properties of neurons.

Second, is it possible that the presence of the indwelling electrodes on the stimulated side but not on the contralateral resulted in a loss or decrease of neuronal elements, especially apical dendritic terminals, stained on the stimulated side? From the photomicrographs of the apical dendritic areas

(Fig. 5) it appears that the stimulated side does not show dendritic terminals richly invading layer I. However, the quality of this Golgi-prepared material and the appearance of apical dendrites are equal to that of normal adult cat cortex studied in this laboratory. It is also comparable with O'Leary's work on cat visual cortex (25), Sholl's photomicrographs of cat sensorimotor cortex (39), and Colonnier's more recent photomicrographs (4). Brain tissue for our study was taken from only about the central 4-mm-wide portion located between the stimulating electrodes spaced 6–8 mm apart. No abnormal signs of gliosis or cell thinning could be identified in material on the stimulated side. For these reasons the presence of electrodes on the stimulated side seem to have had no adverse effects upon the cortical tissue used for study.

If the present observations are interpreted as growth of apical dendrites, including spines, it is decidely different from growth of the same structures in the maturing cat cortex (24) or the olfactory bulb (17). Morest (24) described "growth cones" at the ends of immature dendrites from which filopodia or appendages emerged. No such growth cones were observed in a careful study of our material although exceedingly thin and long spines were frequently seen on dendritic terminals in cortex contralateral to that stimulated. If a terminal reached the pia and reflected back in an abnormal acute angle it may be that the terminal and the spines appearing on it is still in a formative stage (Fig. 6). These changes in apical dendrites apparently take a somewhat different form from those seen during maturation and from those observed in the proximal spinal cord after hemisection and interpreted as reversion to an earlier maturational stage (1).

The observations in the present experiments may help us understand some of the results of "enriched environment" studies, including those concerning alterations in rate of brain development. It is assumed that there is increased sensory stimulation in these studies. Increasing afferent input to the central nervous system during the immediate postnatal period, when spines and dendrites of cortical neurons in the rat are growing rapidly, may enhance or increase the rate of spine and dendritic development (26, 37). Similar results may be observed even after the rapid postnatal maturation period (12, 16). In the young or adult brain enriched environments seem also to promote an increase in cortical weight (28–30) cortical depth and number of glia (7), increased depth (43), and large synaptic contacts (22).

Our findings of apparent neuronal growth as a result of increased use in the mature, adult cat cortex are somewhat similar to presynpatic growth observed in the mammalian brain after injury. It is certain that in the adult animal axonal sprouting after injury occurs in the spinal cord (18), in the hippocampus (20), superior colliculus (19), optic tract in the brain

stem (15), and septal area (23, 27). Results are even more pronounced in the developing brain (19, 20). In some of these reports new synaptic connections, whether functional or not, seem to have been formed after presynaptic damage and, therefore, the evidence points to at least presynaptic growth. This is not necessarily the case postsynaptically where disuse or denervation produces profound loss in the cerebral cortex (32, 42). The effects of restricting sensory input can involve a decrease in cortical dendritic fields (3), an apparent decrease in density of synapses in the lateral geniculate body (6), or fewer synapses in the visual cortex (5). These synaptic and postsynaptic effects of disuse or denervation are in most respects just the opposite of what we have apparently achieved by increasing the use or synaptic drive of a neural system.

After reviewing data related to use and disuse, Sharpless (38) convincingly argued against an "excessive use theory" as an explanation for the increased synaptic efficacy seen in learning. He drew heavily upon data from studies on the peripheral nervous system, especially the consequences of disuse, and from behavioral observations such as spontaneous recovery after disuse and extinction in the absence of reinforcement. Our data offer physiological and anatomical support for a "growth theory" and despite the fact that direct electrical stimulation produced the increased use, they suggest that the excessive-use concept should not be abandoned.

There may be a relationship between the facilitated electrical response found in our earlier experiments (31), the presently observed morphological changes and the associated learned behavior. Our interpretation of the potential evoked at the surface of the cortex, the extracallosal response, is that it represents in large part postsynaptic activity. Pairing brain simulation with foreleg shock enhanced this postsynaptic expression of activity (31) (the callosal system has not been comparably studied). In the present experiments the same kind of training seemed to produce an increase in apical dendritic spines specific to oblique and vertical components. Since the increase in spines and the enhanced evoked potential are most pronounced in trained animals, then it could be the result of afferent activity from foreleg shock converging with the input from the conditional brain stimulation upon the same cortical pyramidal cells, increasing their excitability levels. This suggestion needs careful experimental study since our design may represent a testable neural model for the frequently illustrated classical conditioning paradigm.

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