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Synaptic Rearrangement in the Dentate Gyrus: Histochemical Evidence of Adjustments after Lesions in Immature and Adult Rats

(synapse/acetylcholinesterase/septum)

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ABSTRACT **In immature animals, ablation of the entorhinal cortex elicited a rapid intensification of acetylcholinesterase (EC 3.1.1.7) staining in the outer one-quarter of the molecular layer of the dentate gyrus. Subsequent lesions of the septum eliminated this acetylcholinesterase intensification. Electron-microscopic histochemical analysis demonstrated a 30-fold increase in the number of acetylcholinesterase-positive synaptic endings in the intensification zone. The acetylcholinesterase augmentation thus appears attributable, in part at least, to an increase in the number of acetylcholinesterase-rich synaptic endings established by septo-hippocampal fibers. Observations in a comparative study of immature and adult rats point to the animal's developmental state as a major determinant of differences in these lesion-induced neuronal adjustments.**

Both the anatomical and functional effects of brain injury are generally attributed solely to the loss of damaged or ablated neurons. Findings in recent experiments indicate that this view of the effects of brain lesions needs to be reexamined. Under some conditions, axons from intact neurons grow into areas vacated by the axons of damaged neurons and form new synapses that reoccupy the vacated synaptic territories (1–9).

If such capacity for neural reorganization is a general property of brain tissue, these findings have several important implications for understanding the consequences of brain damage. First, in efforts to explain the physiological and behavioral effects of brain lesions it may become necessary to consider not only the net loss of neurons caused by the lesion, but also a possible secondary effect: the formation of new connections by intact neurons. Second, the reorganization of neuronal circuitry after brain damage could explain the frequently observed, but poorly understood, phenomenon of partial recovery of function after brain damage. Third, these findings have demonstrated that neuronal organization is much more dynamic than was heretofore realized; in fact, it has already been suggested that synaptic rearrangements are continuously taking place in the brain even under normal conditions (10). Thus, the induction of reactive synaptogenesis by a brain lesion provides a method of investigating what might well be one of the bases of functional plasticity in normal animals.

We examined changes in the organization of the synapse population in the dentate gyrus of the rat's hippocampal formation after lesions that selectively destroy the massive afferent system originating in the entorhinal cortex. In studies of synaptic reorganization, the dentate gyrus offers several advantages: (*a*) its normal synaptic organization is simple and well defined; (*b*) it is suitable for electrophysiological recording (11) so that the functional significance of any known structural changes eventually may be assessed; (*c*) the afferents of the dentate gyrus are accessible to experimental manipulation (e.g., surgical lesion); and (*d*) the system to some extent lends itself to a histochemical study of synaptic reorganization because of the sharply localized distribution of the acetylcholinesterase-positive septo-hippocampal system (12).

The dentate gyrus contains a row of granule cells whose dendrites ramify in the molecular layer. As illustrated schematically in Fig. 1, its major afferents, originating in the ipsilateral entorhinal cortex and from pyramidal cells in both the ipsi- and contralateral Ammon's horn, contact the granule-cell dendrites in a strictly ordered fashion such that the entorhinal afferents synapse on the distal two-thirds of the dendrites (13, 14), while commissural (contralateral) and associational (ipsilateral) hippocampal fibers terminate on the proximal one-third (15–17). A third afferent fiber system originates in the septum and terminates in a stratum between the granule-cell bodies and the zone of termination of commissural afferents, as well as in a zone beneath the granule cell layer

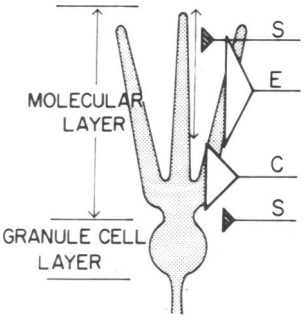

FIG. 1. Schematic diagram illustrating the normal arrangement of synapses on the granule cells of the anterior region of the rat's dentate gyrus. Ipsilateral entorhinal-fiber synapses (*E*) predominate in the outer two-thirds of the molecular layer, while the axon terminals of commissural and ipsilateral hippocampal fibers (*C*) occupy the inner one-third. AChE-positive septal-fiber terminals (*S*) are nested between the granule-cell soma and the zone of hippocampal-fiber synapses, as well as more sparsely in the outer two-thirds of the molecular layer.

Abbreviation: AChE, acetylcholinesterase.

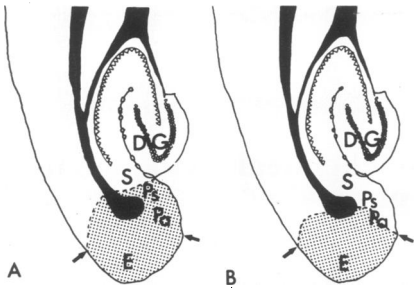

FIG. 2. Representative examples of entorhinal-cortex lesions in 11-day-old (*A*) and adult (*B*) rats. In both cases the lesion (*stippling*) has removed the entire entorhinal cortex (*E*) (*arrows*). In the animal operated on at 11 days, parasubiculum (P_a) and presubiculum (P_s) were also removed; in the adult rats a portion of the parasubiculum was usually included in the lesions. The relative position of the dentate gyrus (*DG*) and subiculum (*S*) is shown. The AChE patterns observed in these cases are illustrated in Fig. 3.

(13, 18); a sparser termination of septal fibers in the outer half of the molecular layer has also been suggested (19).

In order to follow changes in a synaptic system, we have made use of the selectively positive staining of the septo-hippocampal system for acetylcholinesterase (AChE; EC 3.1.1.7) (18–21). Fibers originating from the septum or passing through the septum represent the major, if not the only, cholinergic input into the molecular layer of the dentate gyrus. Both destruction of the septum or transection of the fimbria cause nearly complete disappearance of AChE and choline acetyltransferase (EC 2.3.1.6) from the molecular layer of the dentate gyrus (8, 20, 22), whereas lesions of other extrinsic afferents produce no such loss (26). Microchemical and histochemical studies have shown that AChE is organized in laminae (23) which closely correspond to patterns of terminal degeneration observed by degeneration methods (18). Microchemical analyses also have shown a corresponding localization of choline acetyltransferase (20, 24). The septal fibers have been traced from the septum by way of the fimbria to the hippocampus both experimentally by the aid of degeneration methods (18) and by following AChE-positive fibers in normal material (19, 25). The laminar distribution and specific histochemical properties of the septo-hippocampal system provide a convenient means of examining any changes in the system's organization that might result from lesions to other extrinsic afferents.

The experiments reported here examine the effect of lesions of the entorhinal cortex in developing rats on the localization of septal-fiber terminals in the dentate gyrus. Entorhinal lesions consistently were found to elicit a considerable increase in the number of AChE-positive synaptic terminals in the molecular layer. This increase affects a narrow band corresponding to the outer one-quarter of the molecular layer, i.e., the same zone in which optical microscopy reveals an increase in AChE staining after entorhinal lesions. The effects of such lesions in immature animals will be compared with those previously observed in adults (8).

MATERIALS AND METHODS

Male and female Sprague–Dawley rats (Simonsen Laboratories, Gilroy, Calif.) were used throughout this study. Thirty 11-day-old rats were allowed to survive extensive ablation of entorhinal cortex for 2–200 days. Examination of horizontal sections stained with cresyl violet typically showed the lesion to have removed both the medial and lateral subdivisions of the entorhinal cortex, and often portions of parasubiculum and presubiculum as well (Fig. 2). The distribution of AChE was determined light-microscopically in formalin-fixed sections stained for AChE (8).

Electron-microscopic localization of AChE by Lewis and Shute's (27) method was performed in an additional three rats, 70–100 days old. These animals were perfused with 1% glutaraldehyde–4% formalin buffered at pH 7.4 by 0.08 M cacodylate buffer containing 1 mM $CaCl_2$. After fixation, coronal sections through the anterior part of the dentate gyrus were cut at 150 μm with a Porter–Bloom tissue chopper, and from each single section both lesion-side and contralateral-control samples of the ventral leaf of the molecular layer were removed and stained for AChE at 4° for 2.5–4.5 hr (27). Upon completion of the staining, the specimens were embedded in epon-araldite. Thin sections were stained with potassium permanganate-lead (28) and lead citrate (29) before examination in a Zeiss electron microscope. The presence of non-specific staining was examined by inhibiting all cholinesterases with eserine (0.1 mM); under these conditions no positive reaction was obtained.

In two of the three cases studied electron microscopically the distribution and number of AChE-stained axon terminals was determined in a montage composed of 17–21 rows of 10 electron micrographs taken at a magnification of 6000, together covering the entire thickness of the molecular layer. Each such montage covered a total area of 106 μm by 180–

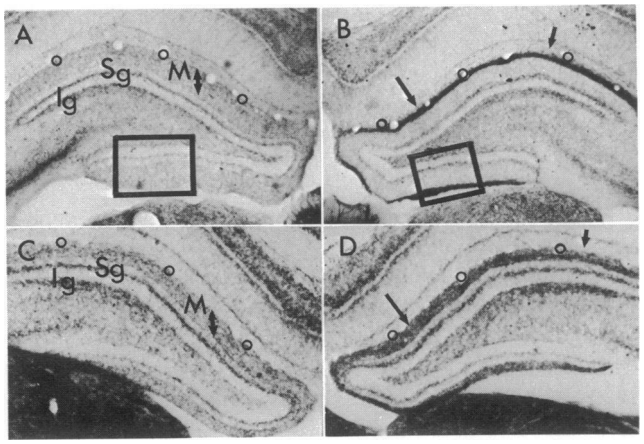

FIG. 3. AChE staining patterns in the dentate gyrus after unilateral entorhinal-cortex ablation in 11-day-old (*A* and *B*) and adult (*C* and *D*) rats. The respective normal staining pattern, shown by the control sides (*A* and *C*), shows stain deposits in the infra(I_g)- and supragranular (S_g) zones, with a more lightly stained region in the outer two-thirds of the molecular layer (*M*). The obliterated hippocampal fissure (○ ○ ○) marks the dorsal boundary of the dentate gyrus. The new band of intensified AChE staining induced by the lesion is shown by *arrows* on the lesion sides (*B* and *D*). In an animal operated on at 11 days and killed 200 days later, a narrow band of strongly positive AChE reaction appears in the outermost one-quarter of the molecular layer (*B*). A similar but wider zone corresponding to the outer two-thirds of the molecular layer is seen in the rat operated on as an adult and killed 40 days later (*D*). The location of tissue samples taken for electron-microscopic analysis is indicated by *framing* in *A and B*. Magnification, ×22.

212 μm, depending on the height of the molecular layer in that particular section. The variations in height among control and lesion samples appeared to be random, and probably represent slight differences in the plane of sectioning relative to the line of granule cells. In these montages only those synaptic profiles were counted that exhibited sufficient stain deposit to encompass one-half or more of their total circumference.

RESULTS

The pattern of AChE staining determined by light-microscopic histochemistry after entorhinal lesions in 11-day-old rats is shown in Fig. 3*A* and *B*. The normal pattern is illustrated by the control side (Fig. 3*A*). Two intensely staining zones: the infra- and supragranular layers, and one lightly staining zone in the upper two-thirds of the molecular layer are seen. On the side of the lesion a strongly AChE-positive band is found in the upper one-quarter of the molecular layer (Fig. 3*B*). The increase in AChE staining in this outer molecular layer band is first noticeable 2–4 days after ablation of entorhinal cortex, gradually becomes more accentuated, and persists for at least 200 days after the lesion, the longest survival time allowed in the present series of experiments.

Our electron-microscopic histochemical studies confirm the general distribution of AChE demonstrated by light-microscopic histochemistry. In addition, these studies provide evidence that (*a*) on both the normal and the operated side the stain is confined to neuronal processes and (*b*) on the operated side a larger number of synaptic endings are stained in the outer molecular layer. As illustrated in Fig. 4, the stain deposit is localized in the extracellular space surrounding small fibers and axon terminals. In the molecular layer of both sides the stained fibers and synaptic endings are found in small clusters, suggesting that the deposit has not extensively diffused from the source and that the enzyme sources are widely scattered.

A comparison of the molecular layer of the lesion and control sides did not reveal any major qualitative contrasts. The essential difference is in the larger number of synaptic endings that stained for AChE on the side of the lesion. To obtain a more quantitative impression of this difference, we counted all AChE-positive axon terminals appearing at various depths of the molecular layer in the montages of bilaterally matched samples from the ventral leaf of the dentate gyrus (framed areas in Fig. 3*A* and *B*). The result of these counts (Fig. 5) indicates that the number of AChE-positive terminals in the outer one-quarter of the molecular layer is about 30 times higher on the operated than on the normal side. This increase in the outer zone doubled the total number of stained synaptic endings in the molecular layer of the lesion side (207% ± 28 SEM, $n = 3$). In the inner one-half of the layer about equal numbers of synaptic endings stain on both sides (lesion side 110% of control). The difference in total number of AChE-positive synaptic endings counted cannot be attributed to a lesion-induced volume change, for in the analyzed samples the total areas on the three control and lesion sides agree to within 10%. These data support the contention that the higher count on the operated side reflects the formation of numerous new axon terminals of high AChE content or the induction of more enzyme in existing projections. However, these explanations need to be viewed with caution, because microchemical analyses of AChE reveal relatively little if any difference in the specific or total activity in the outer molecular layer of the two sides in 80-day-old experimental rats (30). One explanation which is compatible with both the histochemical and biochemical data is that a larger number of terminals are present on the lesion

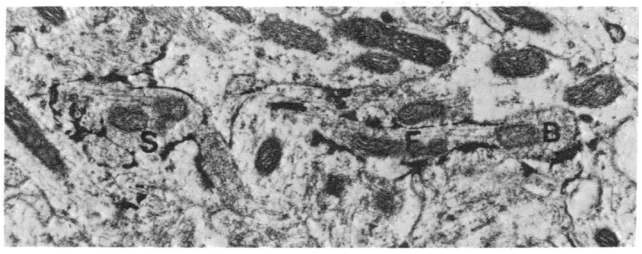

FIG. 4. Electron micrograph illustrating positive AChE reaction on the side of the lesion in the outer one-quarter of the molecular layer. The stain deposit is localized in the extracellular space surrounding an axon (*F*) which ends in a terminal bouton (*B*), and surrounding a synaptic ending (*S*). Magnification, ×11,640.

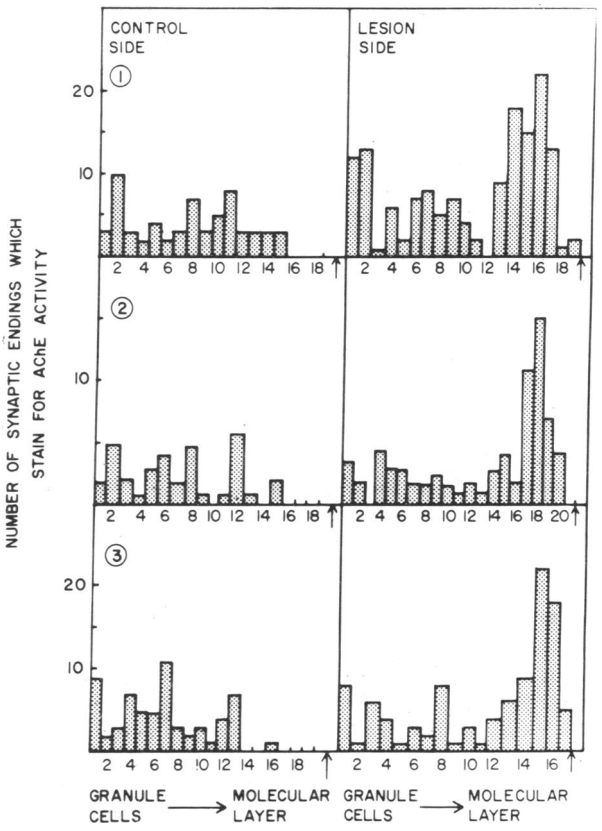

FIG. 5. Distribution of AChE-positive synaptic endings within an area of the ventral leaf of the molecular layer in a rat with unilateral entorhinal-cortex ablation 11 days after birth. The *abscissa* marks the location of narrow rectangles 10.6 × 106 μm that are oriented parallel to the pial surface and, stacked together, cover the entire width of the molecular layer, from immediately above the granule-cell layer (*left*) to the surface (*arrow* on right side of each graph). The number of AChE-positive synaptic endings within each area is shown on the *ordinate*. The outer one-quarter of the molecular layer contains a very much larger number of such axon terminals on the side of the lesion than on the contralateral side.

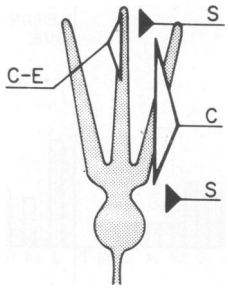

FIG. 6. Summary of synaptic rearrangements in the dentate gyrus induced by ipsilateral entorhinal-cortex ablation in the 11-day-old rat. An increased number of septal nerve terminals (*S*) are induced in the outer zone of the molecular layer. The zone of commissural-fiber terminals (*C*) spreads from its normal region in the inner one-third so as to occupy all but the outermost stratum of the molecular layer. Synapses from the contralateral entorhinal cortex (*C–E*), which normally do not form detectable synapses in the molecular layer, grow into this area and innervate the granule cells.

side, but the enzymatic activity per terminal is reduced relative to the control side. Yet other possibilities for the disparity can not be excluded. The histochemical procedure may preferentially demonstrate forms of the enzyme or involve other problems implicit with the copper-thiocholine technique (31).

In several rats previously subjected to unilateral ablation of the entorhinal cortex, the septo-hippocampal connection was destroyed either by placing large lesions in the septum or by transecting the fimbria fornicis bilaterally. Both interventions, in agreement with earlier observations (8, 20, 25), eliminated all AChE-positive structures from the molecular layer on the otherwise intact side. The significant feature of these experiments is that the ablations also removed all AChE staining in the molecular layer on the side on the entorhinal-cortex lesion. These findings strongly suggest that the great increase in the number of AChE-positive axon terminals induced in the outer zone of the molecular layer by entorhinal lesions represents a change in the septo-hippocampal connection and, therefore, is not due to a modification of intrinsic hippocampal circuitry.

DISCUSSION

The findings reported here indicate that (*a*) entorhinal-cortex lesions in immature rats result in a rapid intensification of AChE staining in the outer one-quarter of the molecular layer of the dentate gyrus, and (*b*) this intensification corresponds to an apparent increase in the total number of the AChE-positive synapses established by the septo-hippocampal pathway. Our initial goal was to compare the relative plasticity of synaptic arrangements in the dentate gyrus in immature and adult animals. Previous studies on neuronal adjustments after lesions have not afforded an opportunity for such a comparison.

In contrast to the narrow band of intensified AChE staining in the 11-day-old rat, a wide zone of increased AChE content has been observed in adult rats subjected to entorhinal-cortex ablation (8). The differences between the two sets of observations are illustrated in Fig. 3. The intensified zone in an 11-day-old rat corresponds to the outer one-quarter of the molecular layer of the dentate gyrus (Fig. 3*B*), whereas in the adult rat it occupies approximately the outer two-thirds of the layer (Fig. 3*D*). The intensification in adults requires 4–7 days to appear, whereas in the rat operated on at 11 days it becomes noticeable 2–3 days after the lesion.

One possible basis for this difference between adult and 11-day-old animals could be sought in a nonequivalence of the respective lesions. For example, the cortex might shift location during development so that at 11 days the entorhinal decortication is not complete. This possibility, however, can be excluded since the typical lesion in an 11-day-old animal is large and removes all of the entorhinal area as well as the parasubiculum and presubiculum (Fig. 2*A*). A comparable lesion in the adult rat (Fig. 2*B*) results in a typically adult AChE-intensification pattern. Finally, ablation of neighboring cortical areas that do not project to the molecular layer of the dentate gyrus does not elicit an AChE intensification in the dentate gyrus in either immature or adult animals. In light of all these findings, the crucial variable appears to be the developmental state of the dentate gyrus rather than a difference in the extent of the lesion.

The plasticity of synaptic organization in developing animals probably depends, in part at least, on the state of development of various afferent fiber systems in relation to granule-cell development at the time of the lesion. The dentate gyrus is immature at birth, and only 5% of its ultimate population of synapses appear to have formed at 11 days (32). Such marked developmental immaturity could be imagined to pose few constraints on synaptic reorganization and, hence, on functional compensation after trauma. The septo-hippocampal connection is not the only fiber system affected by an entorhinal lesion. We have found that such lesions in immature rats cause the terminal distribution zone of the hippocampal commissure to expand well beyond its normal location in the inner one-third of the molecular layer so as to occupy almost the entire height of the granule cell's dendritic tree (33). Additional changes appear in such cases in the projections from the intact contralateral entorhinal cortex. Normally the entorhinal cortex sends very few, if any, fibers to the molecular layer of the contralateral dentate gyrus. After lesion of the entorhinal cortex at 11 days we have observed that the dentate gyrus on the side of the lesion is invaded by fibers from the contralateral entorhinal cortex that establish synapses in the outer and middle stratum of the molecular layer (34). The presumptive "ipsilateral-entorhinal zone" of the molecular layer thus becomes populated with the axon terminals of contralateral-entorhinal and commissural afferents, in addition to the AChE-positive endings of the septo-hippocampal system. Fig. 6 summarizes the resultant reorganization in the dentate gyrus.

An outstanding feature of this reorganization is the complementarity of the final arrangement. Although different from normal, the synaptic population of the dentate gyrus largely maintains an orderly laminar organization. The intensification of septal inputs and in part the invasion of the crossed-entorhinal axon terminals occurs in the distal dendritic zone left largely unoccupied by the expanded commissural system (Fig. 6). This observation suggests that the dendritic zone occupied by the entorhinal and septal system is delimited by the commissural system. In normal development, as well as in development after lesion, the zone of commissural synapses is segregated from that occupied by entorhinal-fiber synapses, a fact suggesting that the developmental factors leading to the abnormal organization are similar to those governing normal development. The reorganization does not result from a

generalized proliferation of the septal system, since no AChE intensification is observed in existing AChE-positive zones that are not associated with projections from the entorhinal cortex (lower *S*, Fig. 1). The available data also argue against a solely random normal or abnormal development, since an orderly laminar organization could hardly be expected under such circumstances. The data are no easier to explain on the basis of chemical factors rigidly specifying the synaptic affinities of each zone of the granule-cell dendrite: the commissural system, for example, is capable of spreading beyond its normal restricted locus (33). The apparent complementarity of the commissural and AChE-positive septal inputs after lesion implies that temporal competition may be involved. According to this model, entorhinal-cortex ablation would trigger a rapid proliferation of commissural-fiber branches spreading distalward over the dendritic segment now denuded of ipsilateral-entorhinal axon terminals. This distal spread would continue until it is halted by the band of likewise proliferating crossed-entorhinal and septal afferents. Preliminary experiments in which more than one afferent have been removed suggest that factors other than temporal competition are involved. The pattern of AChE staining after a combined contralateral hippocampectomy and ipsilateral lesion of the entorhinal cortex is equivalent to that observed after an entorhinal lesion alone. In addition, the crossed-entorhinal projection grows contacts into the ventral as well as the dorsal leaf, and those synapsing in the ventral leaf probably need to grow over considerably longer distances than required for septal, commissural, or associational connections. Aside from growth guided by temporal competition, fibers may be able to capture existing synaptic space selectively or generate new sites.

The synaptic rearrangements after lesion of the entorhinal cortex in adults display a similar complementarity. The broad septal AChE intensification is juxtapositioned by the expanded commissural zone (35). The difference in AChE effects after lesion found when immature and mature rats are compared (broad AChE zone in adults, narrow in young rats) might reflect temporal competition or a reduced sprouting capacity of the commissural system during maturation. In the adult rat the zone of commissural-fiber terminals does not proliferate over as vast a dendritic area and thus may allow the septal fibers a better handicap. More information on the exact nature of the AChE effect and on the state of the initial and final synaptic shuffle on the recipient cells is required to develop a model for the regulation of synaptic proliferation over deafferented zones.

From our present findings, as well as earlier observations of synaptic rearrangements after lesion in other regions of the mammalian central nervous system (1–9), there is evidence that the resulting new synaptic pattern depends to some extent on the age of the animal at the time of the lesion. The dentate gyrus has proved to be a particularly useful model for studies of structural plasticity, because the sharp spatial segregation of its normal synapse populations has enabled us to demonstrate a high degree of order in the synaptic rearrangements that occur when one of these populations degenerates. It is an open question in what manner such shifts in synaptic relationships after lesion affect the function of the regions in which they take place.

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