like that of *R. rubrum* are nonlinearity of the time course of substrate reduction and enhancement of activity by 0.5 m*M* Mn^{2+}.

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9. The MoFe protein was purified by elution from a DEAE column with 150 m*M* malate at *p*H 7.5 in 50 m*M* triethanolamine (TEA) buffer followed by another DEAE column from which the MoFe protein was eluted by malate in a gradient from 0 to 400 m*M*. The Fe protein was eluted from the first DEAE column with 450 m*M* NaCl in 50 m*M* TEA buffer and then was diluted with buffer before being concentrated on a second DEAE column. The concentrated Fe protein was purified further by preparative gel electrophoresis.
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Reinnervation of Dentate Gyrus by Homologous Afferents Following Entorhinal Cortical Lesions in Adult Rats

Abstract. *Granule cells of the rat dentate gyrus which are denervated by unilateral destruction of the entorhinal cortex are reinnervated in part by proliferation of surviving pathways from the contralateral entorhinal cortex. The cells of origin of these lesion-induced projections were identified by retrograde labeling with horseradish peroxidase and were the same cell type which normally project to the ipsilateral dentate gyrus.*

Cells of the mature brain that are deprived of their normal synaptic input as a consequence of lesions can, in some cases, be reinnervated by nearby afferent systems which sprout new synaptic connections (*1*). The functional significance of such reinnervation has been difficult to ascertain, often because the relationship between the cells which were destroyed by the lesion and those providing the new synaptic connections is unclear. The hippocampal formation of the rat, however, provides a situation in which comparisons between normal and lesion-induced circuitry are possible.

Unilateral lesions of the entorhinal area (Broadman's area 28), which gives rise to the major extrinsic afferent nerves to the dentate granule cells of the ipsilateral hippocampal formation (*2*), apparently induce proliferation of several surviving afferents in the dentate gyrus, resulting in extensive reinnervation of the dentate granule cells (*3, 4*). Among the areas that give rise to fibers which reinnervate the granule cells is the surviving contralateral entorhinal cortex (*4*). While this area normally sends only a very sparse crossed projection to the contralateral dentate gyrus (*5, 6*), these crossed temporo-dentate projections proliferate by 500 to 600 percent in response to unilateral entorhinal lesions (*6*).

The reinnervation of the dentate granule cells by afferents of contralateral entorhinal origin clearly results in the reestablishment of a pathway which is similar in many respects to the ipsilateral circuitry that was destroyed by the lesion. First, the reinnervating fibers terminate in a topographically organized fashion which resembles the normal pattern of ipsilateral entorhinal innervation (*7*). Second, the crossed projections exhibit many of the physiological properties of the normal ipsilateral circuitry (*6*). Third, since the surviving entorhinal area is the contralateral homolog of the damaged region, the reinnervating afferents might well be anatomically homologous with the normal ipsilateral circuitry. Yet the most critical issue in evaluating homology is not whether the lesion-induced circuitry arises from a homologous cortical area, but rather whether it arises from the same cell types within the cortical area as the normal circuitry. To investigate this question, use was made of the retrograde transport of horseradish peroxidase (HRP) to identify the cells of origin of the normal ipsilateral and the lesion-induced crossed temporo-dentate circuitry.

A total of 14 male Sprague-Dawley rats served as experimental animals. The entorhinal cortical region was unilaterally ablated as described previously (*3, 4*). After the operation, the animals were allowed to recover for 9 to 12 months before being injected with HRP. A 30 to 50 percent solution of HRP (Sigma type VI) in distilled water was injected into the dentate gyrus which had been deprived of ipsilateral entorhinal input and subsequently reinnervated by the contralateral entorhinal cortex. Injection volumes ranged from 0.3 to 0.4 μl and were delivered over a period of 30 to 45 minutes. Forty-eight hours after injection, the animals were deeply anesthetized with sodium pentobarbital and perfused transcardially with 10 percent formalin-saline. The brains were removed, fixed for 2 to 3 hours in the perfusion solution at 5°C, and then sectioned in the horizontal plane. Sections were incubated in tris buffer (*p*H 7.4) containing 3,3′-diaminobenzidine and hydrogen peroxide, according to histochemical methods described elsewhere (*8*). The brown product that results from this histochemical reaction can best be viewed with dark-field illumination where HRP-positive granules appear greenish yellow (see Fig. 1). For the purpose of comparison with normal animals, illustrative material was drawn from 15 animals which had previously served to define the cells of origin of the normal entorhinal cortical projections (*8*).

We have recently described two subsystems of entorhinal cortical efferent nerves which differ on the basis of their topographic organization (*9*) and their cells of origin (*8*). One subsystem projects predominantly ipsilaterally to the dentate gyrus and regio inferior of the hippocampus proper (*9*), and originates from cells in layer II (probably stellate cells) of the entorhinal cortex (*8*). The second subsystem projects bilaterally to the regio superior of the hippocampus proper, and originates from cells (medium-sized pyramids) in layer III of the entorhinal region (*8*). Thus, injections of HRP which label the terminal fields of both subsystems result in bilateral labeling of cells in layer III, and predominantly ipsilateral labeling of cells in layer II (see Figs. 1 and 2). Rare, lightly labeled cells may be found in layer II contralateral to the injection, particularly in the medial entorhinal area (see Fig. 2, A and C), and these probably represent the cells of origin of the sparse, normal, crossed temporo-dentate projection (*8*), but in normal animals the major population of labeled cells in the entorhinal region contralateral to an injection are localized in layer III (see Fig. 2A).

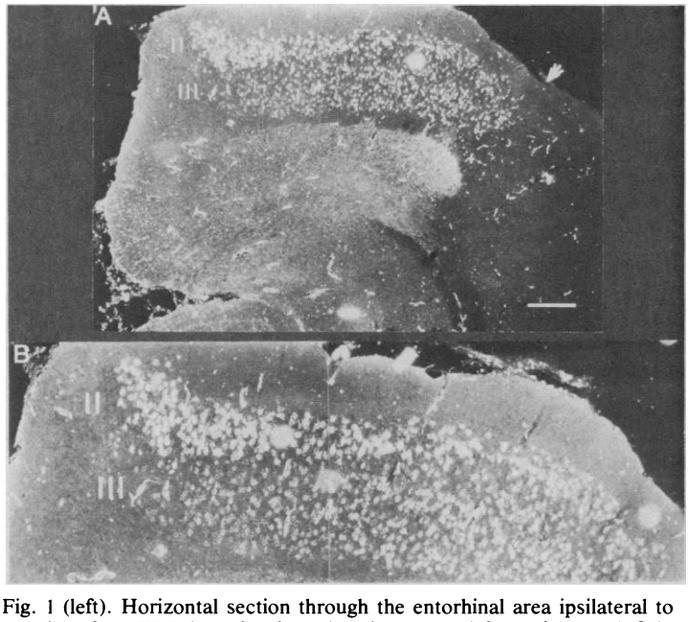
Injections of HRP into the reinner-

vated dentate gyrus in animals with long-standing unilateral entorhinal lesions result in a quite different pattern of retrograde labeling in the surviving contralateral entorhinal area. (For a comparison of the injection sites in the normal and operated animals, see Fig. 2.) In contrast to normal animals, where the labeled cells are confined almost exclusively to layer III, a conspicuous population of heavily labeled cells may be seen throughout layer II in the operated animals (see Fig. 2B), particularly in the medial entorhinal area (see Fig. 2D). This conspicuous population of cells in the operated animals is largely restricted to the dorsal-most portion of the entorhinal area. Since labeled cells in layer II contralateral to an injection are rare and only very lightly labeled in normal animals, but are conspicuous in animals with long-standing unilateral entorhinal lesions, this suggests that it is this population which gives rise to the fibers which reinnervate the dentate granule cells. The fact that this labeled cell population in layer II is restricted to the dorsal portion of the entorhinal area accords well with the observations that the lesion-induced crossed temporo-dentate projections originate predominantly from the dorsal portion of the surviving entorhinal area (*10*).

Since the cells in layer II are the same type of cells (stellate) which normally give rise to the ipsilateral projections to the dentate gyrus (*8*), the results suggest that the crossed afferents which proliferate in response to the unilateral lesions are anatomically homologous (except with regard to laterality) with the normal ipsilateral entorhinal projections to the dentate gyrus. If this is the case, two important issues remain unresolved: (i) Is the proliferation restricted to those cells which give rise to the sparse, normal, crossed projection to the dentate gyrus? (ii) Are the granule cells selectively reinnervated by cells of layer II, or do other cell types also give rise to lesion-induced projections?

With the present observations it is impossible to determine whether these are also the same individual cells which give rise to the sparse, normal, crossed projections to the dentate gyrus. The number of cells in layer II which can be filled by retrograde transport of HRP visibly increases in the operated animals, suggesting that some cells which normally do not project contralaterally may be induced to do so in response to the lesions. It is possible, however, that the very sparse, normal, crossed pathway does not transport quantities of HRP sufficient for detectable retrograde labeling of all cells which project contralaterally, but that the proliferated pathway transports additional HRP, bringing a greater number of cells above the threshold for detection.

In addition, cell groups other than layer II cells cannot be excluded as possible sources of some of the reinnervating fibers. In all animals of the present study, there was at least some spread of HRP from the fascia dentata into the nearby synaptic field of axons from the entorhi-



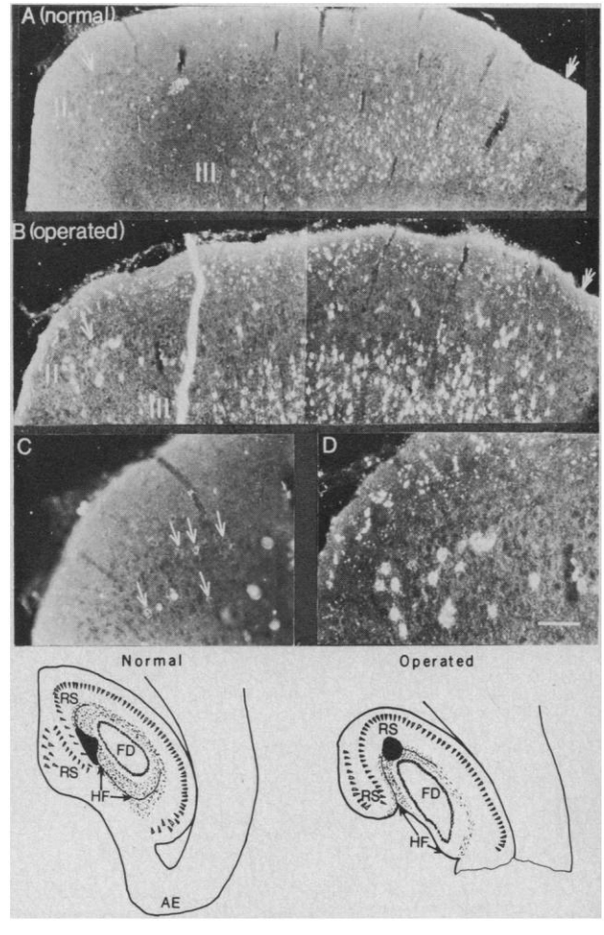


Fig. 1 (left). Horizontal section through the entorhinal area ipsilateral to the site of an HRP injection into the hippocampal formation (dark-field illumination). The HRP-filled cells are visible in layers II and III. The lateral border of the entorhinal region with the temporal neocortex is illustrated by the three-tailed arrow. Calibration bar: 250 μm for (A); 75 μm for (B). Fig. 2 (right). Horizontal sections through the entorhinal area contralateral to the site of an HRP injection into the hippocampal formation in normal (A and C) and operated (B and D) animals. In normal animals, the HRP reaction product is found predominantly in cells of layer III (except for the very lightly labeled population near the border with the parasubiculum) (see C), while in animals with long-standing unilateral entorhinal lesions, in which the surviving entorhinal area has reinnervated the contralateral dentate gyrus, a heavily labeled population of cells is evident in layer II. This population is particularly dense in layer II near the junction with the parasubiculum (see D). The diagrams illustrate the configuration of the hippocampal formation and entorhinal cortex in relatively dorsal horizontal sections. The injection sites are indicated in black. The dots schematically illustrate the distribution of the terminal fields of the normal ipsilateral afferent system and the lesion-induced crossed system, respectively. The extent of the lesion in a typical preparation is also illustrated. Abbreviations: *FD*, fascia dentata; *AE*, area entorhinalis; *RS*, regio superior of the hippocampus proper; and *HF*, hippocampal fissure.

nal cortex in the regio superior of the hippocampus proper. For this reason, cells were also found in layer III (the site of the cells of origin of the normal entorhinal afferents to the regio superior). Therefore, the possibility that some of the reinnervating fibers might also originate from this group of cells in layer III (which give rise to the normal crossed projections to the regio superior) cannot be excluded.

If the reinnervating contralateral entorhinal afferents did originate exclusively from cells in layer II, it would still be clear that some fibers which reinnervate the granule cells also originate from other sources. For example, acetylcholinesterase-containing fibers from the septal nucleus (*3*) and dentate commissural fibers from the contralateral hippocampus (*3*) also appear to proliferate in response to the lesion, reoccupying synaptic space which is normally the territory of ipsilateral entorhinal afferents. Thus, input is restored to denervated granule cells by one system which appears to be homologous with the normal circuitry (except with regard to laterality), and by other fiber systems which bear no obvious relation to the circuitry that was destroyed by the lesion. The functional consequences of this preservation of some aspects of normal specificity of neuronal interconnection, with concomitant loss of other specific features, must still be ascertained.

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Activation of Histone Gene Transcription by Nonhistone Chromosomal Phosphoproteins

Abstract. *Hybridization analysis of RNA transcripts from HeLa S3 cell chromatin to histone complementary DNA indicates that a chromosomal phosphoprotein fraction activates transcription of histone messenger RNA sequences in vitro with chromatin from a phase in the cell cycle when histone genes are normally silent.*

Although the factors involved in genetic regulation are beginning to be understood in prokaryotes, the mechanisms and specific macromolecules involved in the control of gene readout in eukaryotes remain elusive. However, the discovery of the enzyme reverse transcriptase (*1*) has enabled researchers to synthesize sensitive DNA probes for specific messenger RNA's (*2, 3*), and has led to the development of experimental systems that offer promise for elucidating the kinds of macromolecules involved in eukaryotic gene regulation. One system under study is that of histone gene transcription during the growth cycle of HeLa cells, where, by the technique of hybridization with a complementary DNA (cDNA) probe in which reverse transcriptase and polyadenylated histone messenger RNA (mRNA) are used (*4*), it has been shown that histone gene transcription from chromatin takes place only during the S phase of the cell cycle (*5*). Such phase-specific transcription of histone genes is consistent (i) with the restriction of histone synthesis to the S phase of the cell cycle (*6, 7*) and (ii) with in vitro translation (*7, 8*) and hybridization studies (*9*) which indicate that histone mRNA's are associated with polyribosomes only during S phase. Thus, synchronized populations of HeLa cells offer a viable model system for the study of factors affecting regulation of the transcription of a specific set of genes by allowing the fairly easy attainment of two populations in which it is known that histone genes are transcribed in one (S phase cells) and not in the other population (G1 cells) (*5*). By transcribing isolated chromatin from S and G1 cells in vitro and analyzing the RNA products for their abilities to form hybrids with histone cDNA, it is apparent that the factors responsible for the differences in transcriptional capabilities of S phase and G1 cells are endogenous to the isolated chromatin material (*5*). By dissociating chromatin from G1 and S phase HeLa cells in a mixture of NaCl (3*M*) and urea (5*M*) and reconstituting various components of the two chromatins, it has also been possible to determine that a component of the S phase nonhistone chromosomal proteins is responsible for the transient activation of histone gene transcription during the period of the cell cycle when DNA replication occurs (*5*).

Nuclear phosphoproteins have been implicated in the control of gene expression (*10–12*), and recent studies have shown that HeLa cell chromatin reconstituted with nonhistone chromosomal proteins which were partially dephosphorylated enzymatically has a decreased ability to serve as a template for histone gene transcription (*11*). We report experiments directed toward determining whether or not the component of S phase chromatin responsible for activation of histone gene transcription can be found in the phosphoprotein fraction of the nonhistone chromosomal proteins.

Phosphoproteins were isolated from chromatin obtained from HeLa cells (*5*)

Table 1. Characteristics of chromosomal protein fractions. The percentage of nuclear proteins and specific activities of the various protein fractions isolated from HeLa cell chromatin. Exponentially growing HeLa cells (1500 ml) were centrifuged at 1500 rev/min for 5 minutes and resuspended in 50 ml of phosphate-free Eagle's minimum essential medium containing 2 percent fetal calf serum and 10 mc of ^{32}P (obtained from ICN in water) for 1 hour. The protein fractions were isolated as described in the legend to Fig. 1. Protein concentrations were determined by the spectrophotometric method (*17*), and radioactivity was measured by counting 10-μl portions of the protein fractions in 10 ml of Triton-toluene scintillation fluid (*18*).

	Percent of total chromosomal protein	Recovery (mg)	Specific activity (count/min per mg)
Total nuclear protein	100.0	25.5	8.25×10^5
80,000*g* supernatant	33.0	8.4	7.36×10^5
80,000*g* pellet proteins	66.7	17.0	9.80×10^5
$CaPO_4$ nonbinding proteins	16.2	4.13	2.30×10^5
Phosphoproteins	16.8	4.28	3.20×10^6