concentration of 0.2 μg-atom/liter, is 0.17 μg-atom/cm². Consequently, a catastrophic overturn of the top 20 cm of sediment by storm surge, slumping, or dredging operations would liberate into the water column more than ten times its normal content of inorganic phosphate. In the upper quarter of the bay there appears to be sufficient soluble iron in the sediments to precipitate all of this phosphate. In the lower three quarters of the bay, however, this is not the case; there is sufficient soluble iron to remove only about 25 percent of the phosphate in this manner.

The rather large gradients in phosphate concentration in the upper 10 to 20 cm indicate a possible upward diffusive flux of phosphate into the overlying water. If we assume that the concentration gradient is linear with an average value of 15 μg-atom/liter per centimeter and estimate the diffusion coefficient to be 10^{-6} cm²/sec (*12*), we calculate an upward diffusive flux of 9×10^{-3} μg-atom/cm² per week. This would amount to an addition of slightly more than 5 percent of the total phosphate content of the water in a week. These few calculations indicate the amount and potential availability of phosphate in the most accessible portion of the sedimentary nutrient reservoir.

We have been interested in the mechanisms within the sediment–interstitial water system that control the interstitial phosphate concentrations. Approaching this problem from a thermodynamic point of view, we calculated from published inorganic stability constants (*13*) the speciation of the free and complexed forms of phosphate, iron (II), and carbonate. Appropriate activities for these species were determined from the product of the calculated free ion concentrations and Debye-Hückel activity coefficients (*14*). The logarithmic activities for carbonate, phosphate, and iron (II) calculated from our data on interstitial water for the entire length of the bay correlate well with those for the siderite-vivianite equilibrium phase boundary (Fig. 2B). These results and the presence of siderite and vivianite as mineral phases in these sediments, as suggested by our preliminary x-ray data, indicate an apparent equilibrium among the three soluble species and two solid phases. The results of Troup *et al.* (*4*), which describe the codependent distribution of carbonate, phosphate, and iron (II) in the interstitial water of the upper bay, are thus extended to include the interstitial water of the lower bay, in which the maximum iron (II) concentrations are an order of magnitude lower.

J. T. Bray
O. P. Bricker
B. N. Troup

Department of Earth and Planetary Sciences, Johns Hopkins University, Baltimore, Maryland 21218

References and Notes

1. J. E. Harwood, R. A. van Steenderen, A. L. Kuhn, *Water Res.* **3**, 417 (1969).
2. P. C. Mangelsdorf, T. R. S. Wilson, E. Daniell, *Science* **165**, 171 (1969); J. L. Bischoff, R. E. Greer, A. D. Luistro, *ibid.* **167**, 1245 (1970).
3. K. A. Fanning and M. E. Q. Pilson, *ibid.* **173**, 1228 (1971).
4. B. N. Troup, O. P. Bricker, J. T. Bray, in preparation.
5. B. J. Presley, R. R. Brooks, I. R. Kaplan, *Science* **158**, 106 (1967); R. R. Brooks, B. J. Presley, I. R. Kaplan, *Geochim. Cosmochim. Acta* **32**, 397 (1968); E. Callendar, in *Proceedings of the 12th Conference on Great Lakes Research* (International Association for Great Lakes Research, University of Michigan, Ann Arbor, 1969), pp. 124–160; J. L. Bischoff and T. Ku, *J. Sediment. Petrology* **41**, 1008 (1971); B. J. Presley, Y. Kolodny, A. Nissenbaum, I. R. Kaplan, *Geochim. Cosmochim. Acta* **36**, 1073 (1972).
6. W. S. Reeburgh, *Limnol. Oceanogr.* **12**, 163 (1967).
7. E. B. Sandell, *Colorimetric Determination of Traces of Metals* (Interscience, New York, 1959), pp. 537–542.
8. R. D. Cahn, *Science* **155**, 195 (1967).
9. H. P. Orland, Ed., *Standard Methods for the Examination of Water and Waste Water* (American Public Health Association, New York, ed. 12, 1965), pp. 56–58.
10. J. H. Carpenter, D. W. Pritchard, R. C. Whaley, in *Eutrophication: Causes, Consequences, Corrections* (National Academy of Sciences, Washington, D.C., 1969), pp. 210–221.
11. W. B. Cronin, *Volumetric, Areal, and Tidal Statistics of the Chesapeake Bay Estuary and Its Tributaries* (Special Report No. 20, Reference 71-2, Chesapeake Bay Institute, Johns Hopkins University, Baltimore, 1971), p. 1.
12. W. Stumm and J. O. Leckie, in *Proceedings of the 5th International Water Pollution Research Conference*, S. H. Jenkins, Ed. (Pergamon, New York, 1970), pp. III-26/1 to III-26/16.
13. R. M. Garrels and C. L. Christ, *Solutions, Minerals, and Equilibria* (Harper & Row, New York, 1965), p. 413; J. O. Nriagu, *Geochim. Cosmochim. Acta* **36**, 459 (1972); L. G. Sillen and A. E. Martell, *Stability Constants of Metal-Ion Complexes* (Special Publication No. 17, Chemical Society of London, London, 1964).
14. R. M. Garrels and M. E. Thompson, *Amer. J. Sci.* **260**, 57 (1962).
15. Supported by AEC contract AT(11-1)-3292. We thank M. Manella, R. Mervine, and G. Holdren for aid in analyses, and G. M. Lafon, J. Ferguson, J. Gavis, and H. Eugster for commenting on the manuscript.

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Postlesion Axonal Growth Produces Permanent Functional Connections

Abstract. *Lesions of the entorhinal cortex in neonatal rats cause the commissural projections to the dentate gyrus to spread from their normal location in the inner molecular layer into the outer molecular layer, a region normally occupied by afferents from the entorhinal cortex. These lesions also cause the short-latency response to commissural stimulation to spread into the outer molecular layer, a result suggesting that these abnormally located connections are operative.*

Neurons in the mammalian brain which normally interconnect to form intricate circuits are now known to form new connections following brain lesions. These effects are particularly dramatic when lesions are made in developing animals. The types of changes observed include (i) rerouting of a fiber path to a different cell population (*1*), (ii) spread of an afferent system into areas of a given dendritic field that it would not normally occupy (*2*), and (iii) sprouting of synaptic connections within an area normally innervated (*3*). Whether or not any of these forms of postlesion growth result in the formation of permanent functional synaptic contacts is unknown. Studies in the peripheral nervous system have suggested that abnormal innervation of muscle can produce synapses that appear structurally perfect but are rendered inactive when normal innervation is reestablished (*4*). Thus, it is possible that the abnormally located synaptic contacts that form in brains as a result of lesions are never operative or are suppressed or retracted, possibly as part of the recovery of function phenomenon. This study provides strong evidence that postlesion axonal growth results in the formation of permanent functional contacts.

We have reported anatomical evidence that removal of the entorhinal cortex in rats 11 days old causes the commissural projections to the granule cells in the dentate gyrus to greatly increase and occupy much of the space made available by the lesion (*2*). In the normal adult rat, entorhinal projections to the dentate gyrus occupy the outer molecular layer (Fig. 1a) (the molecular layer contains the granule cell dendrites of the dentate gyrus), while the commissural terminals innervate exclusively the inner molecular layer (Fig. 1b). The two projections thus are located in adjacent layers on

the dendrites of the granule cells, with little or no overlap between them. After removal of the entorhinal cortex in rats 11 days old, the commissural fibers hyperdevelop and spread into the zone normally occupied by the entorhinal projections (Fig. 1c). In this way they come to occupy both the inner and outer segments of the dendritic field.

This precise lamination of afferents within the molecular layer of normal animals is accompanied by a corresponding preciseness in the distribution of extracellular field potentials in response to stimulation of these afferent systems (*5, 6*). We reasoned that if the commissural axons that invade the outer molecular layer form funtional synapses, then the distribution of extracellular responses to commissural stimulation would be changed accordingly. To test this hypothesis we performed laminar analyses (*7*) of these field potentials within the molecular layer of the dentate gyrus. Stimulation of entorhinal and hippocampal commissural pathways was performed in normal adult rats. Only commissural stimulation was employed in a second group of adult rats in which unilateral lesions of the entorhinal cortex had been made at 11 days of age (*7*). Conventional neurophysiological techniques for stimulation and recording were utilized (*8*).

In normal rats, we found that electrical stimulation of both the entorhinal cortex and the CA3 field of the contralateral hippocampus (where the commissural afferents to the dentate gyrus originate) produced short-latency responses whose polarity and magnitude depended on the depth of the electrode in the molecular layer (Fig. 1, a and b). Laminar profiles demonstrated a relatively precise stimulus specificity, with maximal negativity situated in the area of the molecular layer innervated by the terminals of the particular afferent system. Stimulation of the entorhinal cortex produced a short-latency negative response localized in the outer molecular layer, and 100 μm above the cell layer this response abruptly became positive (Fig. 1a). Commissural stimulation also produced a similar short-latency negative response, but it was localized to the inner molecular layer and changed to a positive potential immediately above the granule cell layer (Fig. 1b). Similar results were obtained in experiments on eight normal rats without lesions. Therefore, stimulation of commissural or entorhinal fibers produces a maximum negative extracellular response restricted to those regions of the molecular layer of the dentate gyrus in which the terminals of the stimulated afferent system are located. This response either diminishes dramatically or becomes positive in dendritic zones not innervated by these terminals. These results are in full accord with those reported elsewhere (*5, 6*).

When rats with lesions made at age 11 days were studied as adults (a minimum of 100 days later), the laminar profile in the molecular layer in response

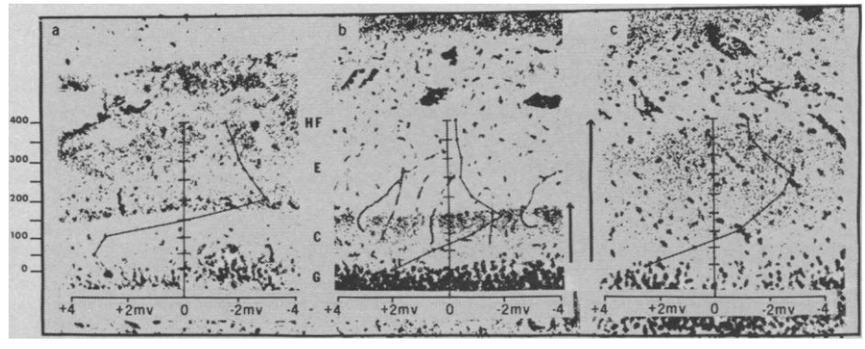

Fig. 1. The distribution of degeneration products in the dentate gyrus after lesions of the entorhinal cortex (a) or the contralateral anterodorsal hippocampus (b) in normal adult rats. The right-hand panel (c) shows the distribution of degeneration in the dentate gyrus after a lesion of the contralateral hippocampus in an adult rat that had received a lesion of the ipsilateral entorhinal cortex at 11 days of age [Fink-Heimer method; some of the material was drawn from an earlier study (*2*)]. The solid lines on each photomicrograph are plots of the voltage and polarity of the short-latency response (5 msec or less) recorded at various depths in the molecular layer in response to stimulation of the entorhinal cortex (a) or commissural system in a normal rat (b), and of the commissural system in an adult rat that had received a neonatal entorhinal lesion (c). The vertical scale gives the distance (in micrometers) above the granule cell layer. In all cases the negative responses correlate precisely with the location of the degeneration products from the stimulated afferent system. The vertical arrows between (b) and (c) emphasize the percentages of the molecular layer occupied by degeneration from a contralateral lesion in normal rats and in those with neonatal entorhinal lesions; also indicated are the granule cell layer (*G*); the inner molecular layer, which is occupied by commissural projections (*C*); the outer molecular layer, which is innervated by afferents from the entorhinal cortex (*E*); and the hippocampal fissure (*HF*).

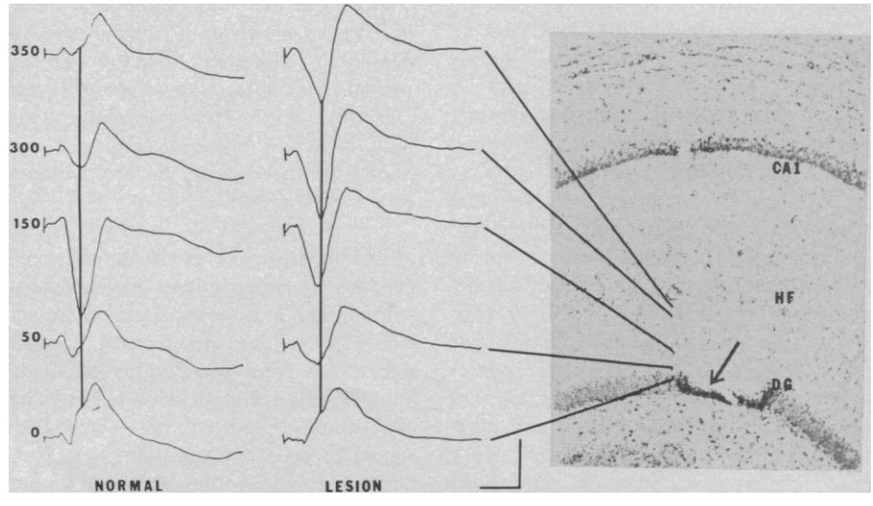

Fig. 2. Short-latency responses recorded at various depths in the molecular layer of the dentate gyrus in response to commissural stimulation in a normal adult rat (left) and in an adult rat that had received a lesion of the entorhinal cortex at 11 days of age (center). Each potential is the average of eight recordings; calibration marks are 5.0 msec (horizontal) and 1.0 mv (vertical). At right is a photomicrograph of a recording microelectrode tract. Numbers at left refer to the distance in micrometers above the termination of the recording tract (arrow). The dark coloration of the granule cells was caused by the ejection of a small quantity of dye from the recording electrode, at its most ventral excursion; *CA1*, the pyramidal cell layer; *HF*, hippocampal fissure; and *DG*, granule cell layer of the dentate gyrus.

to commissural stimulation was radically changed (Fig. 1c) (*9*). Figure 2 shows that animals with neonatal lesions generate short-latency, large negative responses to commissural stimulation throughout the entire extent of the molecular layer. The maximal response was obtained 200 to 250 μm above the granule cell layer. In terms of latency and wave form, the responses at all levels of the molecular layer were identical to those recorded in the inner molecular layer of normal rats (Fig. 2). These results were replicated in six rats with verified complete entorhinal lesions.

To summarize, the commissural response is found in both the inner and outer molecular layers of the dentate gyrus in adult rats that received a lesion of the entorhinal cortex at an early age (11 days). In normal rats the maximum response to commissural stimulation is found only in the inner molecular layer (Fig. 1, b and c, and Fig. 2).

The most plausible interpretation of these data is that the commissural axons that migrate into the outer molecular layer after neonatal entorhinal lesions do in fact form permanent functional synaptic connections in that region. The similarity of latency and wave form of the potentials in normal animals and those with lesions argues against the abnormally located responses being caused by postlesion pathology. Also relevant to this argument are our studies on changes in the entorhinal system after neonatal commissural lesions. Anatomical experiments have shown that the entorhinal projections are not changed by these lesions (*10*) and we have data showing that the laminar profile of the response to entorhinal stimulation is also unaffected.

We have provided data indicating that the acetylcholinesterase-containing septal projections to the dentate sprout after entorhinal lesions (*11*). The significance of these different forms of postlesion growth in the hippocampus with respect to the behavioral consequences of entorhinal lesions are not clear, but the results reported here, combined with earlier behavioral work (*1*), suggest that abnormal connections that form after brain damage may play a critical role in phenomena such as recovery from brain damage.

GARY LYNCH
SAM DEADWYLER
CARL COTMAN

Department of Psychobiology,
University of California,
Irvine 92664

References and Notes

1. G. E. Schneider, *Brain Behav. Evol.* **3**, 295 (1970).
2. G. Lynch, S. Mosko, T. Parks, C. Cotman, *Brain Res.* **50**, 174 (1973); G. Lynch, B. Stanfield, C. Cotman, *ibid.*, in press.
3. D. C. Goodman and J. A. Horel, *J. Comp. Neurol.* **127**, 71 (1966); G. Raisman, *Brain Res.* **14**, 25 (1969); R. D. Lund and J. S. Lund, *Science* **171**, 804 (1971); R. Y. Moore, A. Bjorklund, U. Steveni, *Brain Res.* **33**, 13 (1971).
4. L. R. Marotte and R. F. Mark, *Brain Res.* **19**, 41 (1970).
5. P. Anderson, T. W. Blackstad, T. Lomo, *Exp. Brain Res.* **1**, 236 (1966).
6. T. Lomo, *ibid.* **12**, 18 (1971).
7. Others (*5, 6*) have shown that as an electrode is moved through the molecular layer, the maximum negative extracellular current produced by stimulation of a given afferent system coincides exactly with the point of maximum innervation by that afferent. Thus, in precisely laminated structures, such negativity is the extracellular reflection of many dendritic excitatory postsynaptic potentials (EPSP's). A plot of the magnitude and polarity of these extracellular potentials versus the vertical position of the electrode is called a laminar profile and can be used to establish the locus of activated synaptic endings. In the present situation the recorded slow-wave potential met the following criteria for an EPSP: (i) It followed high-frequency (100-hz) stimulation. (ii) Fiber potentials corresponding to the "axonal" spikes described by Lomo (*6*) were commonly recorded before the onset of the negative slow potential. (iii) It is unlikely that a volley of incoming presynaptic potentials could generate the source-sink relations of the slow depth responses recorded by our microelectrodes; the extracellular current flow from axons would be perpendicular to that occurring within the dendrites postsynaptically (*5, 6*).
8. Glass micropipettes with a tip diameter of 1 μm and impedance values of 1 to 10 megohms were lowered into the hippocampal formation until the granule cell layer of the dentate gyrus was reached. Potentials were led through a preamplifier to an oscilloscope and averaging computer. Unit recordings were obtained with the use of a high-frequency (0.5 to 10 khz) filter. Stimulation of the CA3 field of the contralateral hippocampus or of the ipsilateral entorhinal cortex was accomplished with glass-coated tungsten microelectrodes (tip diameter of 10 μm). These stimulation electrodes were manipulated until the maximal cell discharges and associated evoked field potentials were obtained within the granule cell layer. The recording electrode was then raised dorsally in 50-μm steps, and eight responses at each successive step were averaged and recorded until the pyramidal cells of the hippocampus (CA1) were encountered by the electrode tip. (Comparable results were obtained when the profile was constructed by lowering the electrode in 50-μm steps from CA1 to the dentate.) The recording procedure was repeated three or four times per animal (all in the same anterior-posterior plane of the dorsal hippocampus). In earlier experiments the recording electrode was moved slightly in a mediolateral plane to aid in locating the ventral extent of the recording tract. At the conclusion of later experiments in this series, a small quantity of fast green dye was ejected from the tip of the microelectrode using negative current of 10 to 15 μa. This provided precise localization of a particular electrode trace. After these procedures animals were killed and the brains were removed and stained with cresyl violet. The position of stimulating and recording electrodes as well as lesion placements were carefully checked and plotted on reconstructions of relevant sections.
9. Recording immediately after surgery was vastly complicated in these experiments by the fact that the rat brains were far from mature in the immediate postlesion period, that is, at 11 days of age.
10. G. Lynch, T. Parks, B. Stanfield, C. Cotman, in preparation.
11. G. Lynch, D. Matthews, S. Mosko, T. Parks, C. Cotman, *Brain Res.* **42**, 508 (1972); C. Cotman, D. Taylor, D. Matthews, G. Lynch, in preparation.
12. Supported by NSF grant GB35315X and NIMH grants R-MH19793-01 and MH19691.

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Methionine Sulfoximine–Resistant Mutants of Tobacco

Abstract. *Selecting mutants from populations of haploid plant cells cultured in vitro may provide a rapid method for recovering agriculturally useful variants. Mutants of* Nicotiana tabacum *were recovered which were resistant to methionine sulfoximine, an analog structurally similar to methionine. Induction of chlorosis was prevented or less evident in mutant plants that were inoculated with* Pseudomonas tabaci, *a bacterial pathogen which produces a toxin that is a structural analog of methionine. Several mutants show a specific increase in the level of free methionine.*

Recent advances in the somatic cell genetics of higher plants have demonstrated that it is possible to utilize selective techniques to recover mutant individuals from populations of single haploid cells cultured in vitro (*1*). The experiments reported here were designed to pose two questions: (i) Is it possible to select mutants of a higher plant which have an altered response to a pathogen by recovering cells which are resistant to the toxin produced by that pathogen? (ii) Is it possible to increase selectively the level of a nutritionally important component in a plant by selecting mutants resistant to a toxic structural analog of that component? Both of these questions can be resolved by recovering and analyzing mutants of *Nicotiana tabacum* which are resistant to the methionine analog, methionine sulfoximine (MSO). It has been demonstrated by Braun (*2*) that toxin produced by *Pseudomonas tabaci*, the bacterial pathogen which causes the wildfire disease of tobacco, is a structural analog of methionine. Methionine sulfoximine, although not the true bacterial toxin, will elicit an identical response from tobacco leaves, and mutants of *Chlorella vulgaris* resistant to MSO are also resistant to the toxin. Methionine sulfoximine produces a chlorotic halo on tobacco leaves which is similar to the halo induced by the pathogen.