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# Effects of Time and Experience on Hippocampal Neurochemistry After Damage to the CA3 Subfield

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HANDELMANN, G. E., D. S. OLTON, T. L. O'DONOHUE, M. C. BEINFELD, D. M. JACOBOWITZ AND C. J. CUMMINS. *Effects of time and experience on hippocampal neurochemistry after damage to the CA3 subfield.* PHARMACOL BIOCHEM BEHAV 18(4) 551–561, 1983.—Bilateral injections of kainic acid into the hippocampal CA3 subfield destroyed the CA 3 pyramidal cells and produced a behavioral impairment, an inability to solve spatial maze problems. The behavior recovered, however, with daily experience in a maze task, and the rate of recovery was accelerated by additional daily experience. This recovery of function could be the result of compensatory changes in the distribution or function of the various hippocampal pathways. In the present experiment, this possibility was investigated neurochemically. Five putative neurotransmitters or their synthetic enzymes were measured in dissected regions of the hippocampal formation. Both the long-term effects of the lesions and the effects of behavioral training were determined. A number of alterations in hippocampal neurochemical systems were detected. Acute changes due to the lesions included a widespread loss of glutamate, and regionally specific decreases in glutamic acid decarboxylase (GAD) activity and cholecystokinin (CCK) and norepinephrine (NE) concentrations. Long-term changes included a decline in choline acetyltransferase (ChAT) activity throughout the hippocampal formation, and increases in NE in certain regions. Behavioral testing prevented the decline of ChAT activity, and increased the concentrations of GAD and CCK. The neurochemical conditions present at the time when trained rats recovered behavioral function may indicate the crucial conditions for the occurrence of the behavior.

Hippocampus Kainic acid Neurotransmitters Recovery of function Learning and memory

MICROINJECTIONS of kainic acid into the CA3 region of the hippocampus in rats destroy the CA3 pyramidal cells, but produce little damage to other hippocampal regions. Immediately following this lesion, rats are impaired in their ability to perform a spatial maze task [11]. This behavioral effect is similar to that produced by lesions of the fimbria-fornix [29,31], hippocampus, or entorhinal cortex [31], except that the impairment is not permanent. Rats with lesions of the CA3 subfield regain the ability to accurately perform the maze task after about 30 days of testing [11].

After brain lesions, a number of compensatory mechanisms may occur in the adult mammalian brain which may underlie recovery of behavioral function. These include compensatory alterations in neuronal activity in undamaged neural circuits and/or the establishment of new neuronal circuits through collateral sprouting (see [36] for reviews). These processes underlying recovery can take place within the neuroanatomical region containing the brain damage, or may occur in another brain region.

The present experiment was designed to investigate possible changes in either the distribution or function of the major hippocampal neural pathways after destruction of the CA 3 pyramidal cells. In addition, we investigated the influence of behavioral training on these changes. As mentioned previously, rats with damage to the CA 3 subfield were impaired in their ability to solve spatial maze tasks. With daily

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testing on the maze task, rats eventually recovered the ability, and increasing the amount of daily testing increased the rate of recovery [11,12]. Rats not tested on the maze did not show the recovery [12]. Behavioral training is therefore critical to the recovery, and the influence of training on the hippocampus is an important question. In addition, the neurochemical conditions present at the time when the rats recover the ability to perform the maze task may indicate what conditions are necessary for the behavior to occur.

This investigation was performed through measurement of putative neurotransmitters and enzymes which act as markers for the various pathways. Fortunately, the hippocampal pathways have been well-defined in terms of their anatomical distribution and their neurochemical identities. A number of these pathways demonstrate anatomical plasticity following partial damage to the hippocampus. One of the major extrinsic pathways projecting to the hippocampus is the fornix, which contains cholinergic nerves originating in the medial septum and the nucleus of the diagonal band [15,28]. Cholinergic terminals are located in the hippocampus, subiculum, dentate gyrus, and entorhinal cortex. The biosynthetic enzyme choline acetyl transferase (ChAT) serves as a neuronal marker for the cholinergic nerves. The activity of the fornix is crucial to normal hippocampal function, as indicated by both behavioral [29,31] and neurophysiological studies [32]. Nerve fibers derived from the fornix have been shown to sprout in the hippocampus after damage to the entorhinal cortex, making synapses on deafferented neurons [20,21]. A similar response may occur after deafferentation due to loss of the CA3 cells.

The second major extrinsic pathway projecting to the hippocampus is the perforant path, which originates in the entorhinal cortex and appears to use glutamate as a neurotransmitter [25,38]. The perforant path projects to both the CA3 and CA1 subfields and the dentate gyrus. This pathway also shows anatomical plasticity, sprouting in the hippocampus after destruction of the contralateral entorhinal cortex [37]. The time course of this sprouting correlates with recovery from the behavioral deficit caused by the unilateral lesion [34]. Perforant path fibers appear to sprout into the hippocampus following kainate-induced destruction of the CA3 cells (Handelmann, unpublished). If the perforant path were to innervate CA1 cell synaptic regions vacated by the lesions, they might reform the neural circuit interrupted by the loss of the CA3 neurons.

A third extrinsic pathway is the noradrenergic projection from the locus coeruleus. This projection is diffusely distributed in the hippocampus [16,33]. Central noradrenergic fibers are capable of collateral sprouting in the brain. For example, the normal noradrenergic innervation of the septum sprouts following fornix transection to replace degenerated hippocampal afferent terminals [24]. The proliferation of noradrenergic axons is preceded by an increase in norepinephrine in the original fibers [24]. Similar changes may be detectable in the hippocampal noradrenergic innervation after loss of the CA3 cells.

The intrinsic pyramidal and granule cells of the hippocampus are probably glutamatergic [5,38]. Little evidence exists concerning the plasticity of these neurons in response to hippocampal damage. The CA4 pyramidal cells, however, appear to form new synapses in the dentate gyrus following loss of the afferent fibers from the perforant path [26]. Other intrinsic neurons in the hippocampus are interneurons. The inhibitory basket cells use GABA as a neurotransmitter [38], for which the biosynthetic enzyme glutamic acid decarboxylase (GAD) is a marker. An additional set of intrinsic neurons contains cholecystokinin (CCK) [10], which is excitatory to hippocampal cells [6].

Sprouting of afferent nerve fibers into denervated regions is apparently common after lesions in the hippocampal formation. Another type of plasticity which may occur after lesions is an increase in activity in afferent nerves. Both of these physiological events would lead to a change in neurotransmitter content in the pathways involved. The neurochemical analysis undertaken in the present experiment indicates changes in neurotransmitter content in discrete regions of the hippocampus. The advantage of this analysis is that it measures functional changes in a number of hippocampal pathways in each brain. Identifying dynamic changes in the various hippocampal pathways at different times after the lesions and the influence of behavioral experience on these changes will be useful in determining the mechanisms underlying behavioral recovery following lesions of the CA3 pyramidal cells.

## METHOD

### *Subjects*

Fifty-six male Sprague-Dawley rats, weighing 250–350 g at the start of testing, were housed in individual cages with free access to water. They were kept in a colony room with a 10 hr dark/14 hr light cycle. Behavioral tests were conducted during the light portion of the cycle. Prior to beginning training on the maze task, each rat was gradually deprived of food until it reached 85–90% of its ad lib body weight, and was maintained at this level plus 5 g for each week for the duration of the experiment.

### *Surgery*

Rats received either multiple bilateral injections of kainic acid directly into the CA3 subfield, as previously described [11] or sham injections. The injections were performed stereotactically while the rats were anesthetized with Chloropent (3.0 cc/kg; Fort Dodge Laboratories, Fort Dodge, IA) and Valium (200 $\mu$g; Hoffman-La Roche, Nutley, NJ). Rats also received intramuscular injections of 0.05 cc of Bicillin (Wyeth, Philadelphia, PA). Rats receiving kainic acid injections (experimental rats) had approximately 0.8 nmoles of kainic acid (Sigma, St. Louis, MO) dissolved in 0.4 $\mu$l of phosphate buffered saline (pH 7.4) injected at each of four placements during a five minute interval at each placement. The injection was delivered through a 31 ga cannula. Rats receiving sham injections (control rats) had the cannula lowered at each of the four placements but did not receive injections.

### *Procedure*

*General.* Prior to surgery, each rat was trained to perform the spatial alternation task. After surgery, and a five day recovery interval, each rat was assigned to one of eight treatment groups.

The acute effects on hippocampal neurochemistry of the kainic acid injections were determined by measuring neuronal markers in the hippocampal formation in experimental and control rats which were killed five days after surgery. The effects of training were determined by measuring the markers in experimental rats which were tested either once daily or twice daily on the maze until they reached a postoperative criterion of stable, accurate performance, as described below. For each experimental rat, a matched con-

trol rat was given the same number of days of test experience and killed the same number of days after surgery as the tested experimental rat. To distinguish between the effects of training and the effects of the passage of time after surgery, the neuronal markers were also measured in matched untested experimental rats killed the same number of days after surgery as the tested rats.

*Behavioral task.* The behavioral task was discrete trial rewarded spatial alternation in a T-maze, as previously described [12]. Each trial of the alternation task had two components: a forced run and a free run. For the *forced run*, the rat was directed down one of the arms of the T-maze to obtain a food pellet. For the *free run*, immediately following, the rat was allowed to choose between the arm it just visited, which no longer contained a food pellet, and the opposite arm, which contained a pellet. A *correct choice* was choosing the arm containing the food pellet. An *incorrect choice* was choosing the arm previously visited during that trial.

Each *test session* consisted of eight trials, given within a period of 30 to 45 minutes, with intertrial intervals of about 5 min. Within a test session, the left and right arms of the T-maze were each used four times for the forced run although the order was varied daily. Rats were given either one daily test session or two daily test sessions separated by an interval of at least three hours.

Preoperatively, each rat was tested for at least six sessions, and until a criterion of two consecutive sessions with at least seven correct choices per test session was reached. Postoperatively, the rats receiving kainic acid injections were tested until they reached a criterion of five consecutive sessions with at least seven correct choices per session. Each control rat was matched with one of the experimental rats and given the same number of test sessions.

### *Tissue Preparation*

Each rat was decapitated. The brain was rapidly removed and frozen on powdered dry ice. The frozen brains were sectioned into alternate 20 um and 300 um coronal sections. The thin sections were mounted on slides and stained with cresyl violet for verification of the lesions.

The frozen thick sections were placed in serial order on glass slides and stored frozen at −70°C for dissection. They were dissected on the slides, which were placed on a cold plate to prevent thawing. Ten brain regions were removed with a cold scalpel blade from each section in which they appeared. For each brain region, half of the sample was placed in 500 μl of ice cold 0.1 N HCL and half was placed in 250 μl of ice cold 10 mM EDTA (pH 7.4) containing 0.2% Triton-X 100. Samples were homogenized by sonication and aliquots of each were taken for measurement of the protein content [18]. The samples were then stored frozen at −70°C.

The dissected brain regions included the septum, entorhinal cortex, and hippocampus. The hippocampal formation was divided into eight regions, including separate anterior and posterior dissections of the CA1 subfield, CA3 subfield, subiculum, and dentate gyrus. Anterior samples were obtained from the portion of the hippocampus extending approximately from levels A 4620 to A 2970 of König and Klippel [14]. Posterior samples were obtained from the portion of the hippocampus extending approximately from level A 2790 to A 1950.

### *Neurochemical Analysis*

Two 2 μl aliquots were removed from the EDTA Triton-X buffer and were used for the radiometric analysis of ChAT and GAD, which were performed by the methods of Fonnum [7] and Fonnum *et al.* [8], respectively.

Three 50 μl aliquots were removed from the 0.1 N HCl and were used to determine norepinephrine by the radioenzymatic method of Coyle and Henry [3], CCK by radioimmunoassay [1], and glutamate by the enzymatic fluorometric method of Lowry and Passonneau [17].

All neurochemical results were analysed in terms of the ratio of neural marker to protein content in each sample.

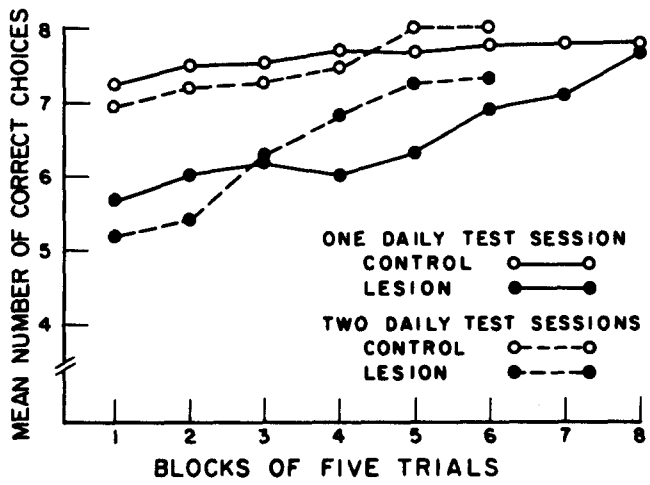



FIG. 1. Postoperative performance on the rewarded spatial alternation task. Each point is the group mean. In the first three blocks of five trials, the performance of the experimental rats is significantly different from controls ($p<0.05$).

### *Statistical Analysis*

The behavioral results were analysed in terms of the number of trials required to reach criterion. A *t*-test was used to compare the behavioral results of the two tested experimental groups.

The neurochemical results were analysed by analysis of variance. For each neuronal marker, a one-way ANOVA was performed for each brain region across all treatment groups. Duncan's multiple range test was used to determine how the treatment groups differed at the level of $\alpha=0.05$. Only results significant at this level are discussed below. In the figures, lesion group means are presented as percentage of control group means.

## RESULTS

### *Behavior*

Preoperatively, the rats completed the criterion in a mean of 6.2 days. Immediately after surgery, the control rats performed at the criterion level of accuracy and continued to do so throughout the test period. At the start of testing, the experimental rats showed impaired choice accuracy compared to the controls (Fig. 1). All of the experimental rats, however, eventually reached the criterion. The rats tested once a day required a mean of 39.7±2.2 days to complete the criterion, while the rats tested twice a day required a mean of 27.8±3.1 days to complete the criterion.

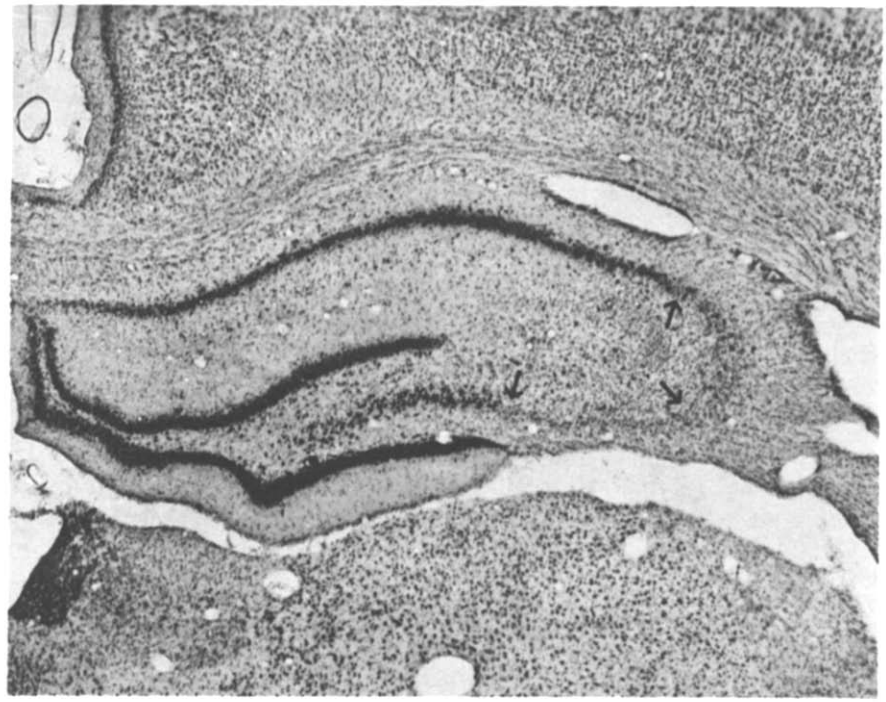


FIG. 2. Coronal section of the hippocampus after destruction of the CA3 pyramidal cells with kainic acid. The arrows indicate the region of degenerated pyramidal cells.

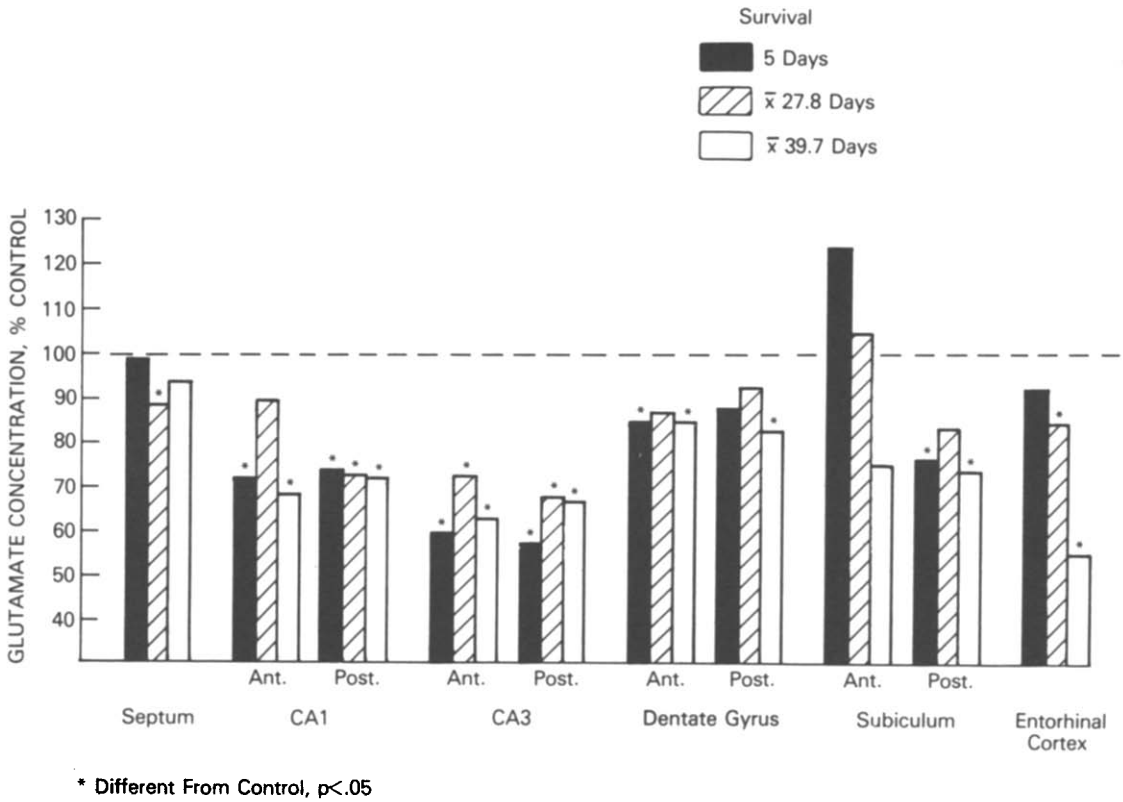



FIG. 3. Glutamate concentrations in the hippocampal formation in untested rats after receiving kainic acid injections.

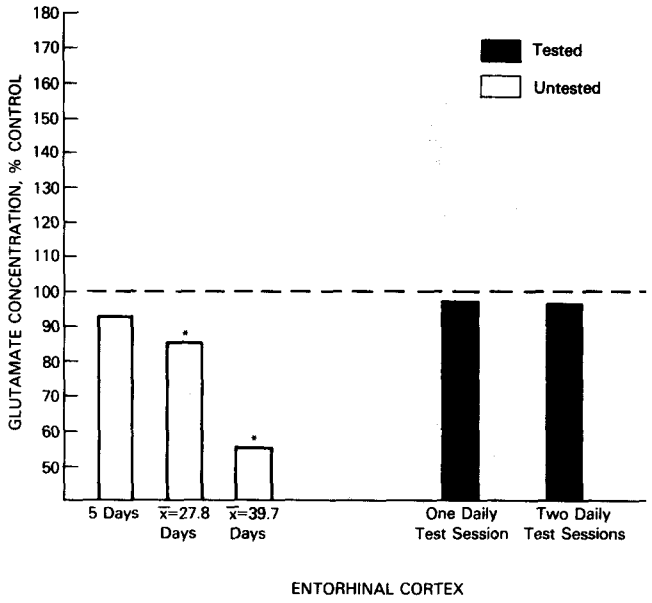



FIG. 4. Glutamate concentrations in the entorhinal cortex in tested and untested rats after receiving kainic acid injections. The white bars indicate concentrations in untested rats and their mean survival times after surgery. The black bars indicate concentrations in tested rats killed after reaching the criterion of accurate performance in the maze task.

*Histology*

A detailed histological analysis of the results of the intra-hippocampal injections of kainic acid as performed in this experiment has been presented previously [11]. In the present experiment, however, the addition of intraperitoneal injections of Valium to the surgical procedure reduced the amount of extrahippocampal neuronal damage, particularly in the amygdala and claustrum. The injections destroyed about 80% of the CA3 pyramidal cells through the length of the hippocampus. Other cell groups in the hippocampal formation remained relatively intact, as did the mossy fibers which pass through the CA3 region. The amount of hippocampal destruction appeared to be equivalent among the groups of rats. Figure 2 illustrates the extent of the lesion in a coronal section of the hippocampus.

*Neurochemistry*

*Control rats.* No differences were observed among the three groups of control rats in concentrations of any neurotransmitter or enzyme in any of the brain regions. These groups were therefore pooled for statistical analysis.

*Protein.* There were no appreciable changes in protein content of any region due to the kainic acid injections or as a result of time or training.

*Glutamate.* In the control rats, glutamate concentrations were comparable to those found in the hippocampus by Young and Lowry [40]. Five days after kainic acid injections, glutamate concentrations were significantly lower in a number of regions. The regions included anterior CA1, CA3, and dentate gyrus, and posterior CA1, CA3, and subiculum; glutamate levels remained low in these regions (Fig. 3). Training had no effect on the levels in any of these regions. In entorhinal cortex, the kainic acid injections had no initial effect of glutamate, but levels were significantly lowered over time. Training prevented this decline (Fig. 4).

*ChAT.* Five days after surgery, ChAT activity was unaltered in any region except the anterior subiculum, where ChAT activity was significantly increased (Fig. 5). ChAT activity declined over time. ChAT values were lowered considerably in every region in the untested group killed a mean of 39.7 days after surgery when compared to every other group. Training prevented the decline in ChAT activity, however, as the tested group killed at the same time had normal levels. In the posterior CA3 and subiculum, ChAT activity was elevated above control values in rats tested once and twice daily (Fig. 6). ChAT activity was also elevated above control levels in anterior CA1 and subiculum in the group tested once daily.

*Norepinephrine.* Five days after surgery, NE levels were significantly lowered in posterior CA1, CA3, and dentate gyrus, and the entorhinal cortex (Fig. 7).

NE values increased with time in a number of regions, including anterior and posterior CA1, CA3, and dentate gyrus, posterior subiculum, and entorhinal cortex. Rats killed a mean of 27.8 days after lesions had higher levels in these regions than rats killed 5 days after, while rats killed 39.7 days after had the highest levels. The latter were higher than the control values. Although there were no consistent effects of training on NE concentrations, anterior CA1 and CA3 had higher levels after two daily test sessions, and anterior CA1, posterior subiculum, and entorhinal cortex had lower levels after one daily test session.

*GAD.* Five days after surgery, anterior CA1, CA3, and dentate gyrus, and posterior CA3 showed decreases in GAD activity, while anterior subiculum showed an increase (Fig. 8).

Some regions showed a transient increase in GAD activity at around 27.8 days after surgery. These included septum, CA3, and posterior dentate gyrus. GAD activity in the anterior subiculum decreased dramatically over time.

Training once daily markedly increased GAD activity in a number of regions. These included CA3, dentate gyrus, subiculum, and posterior CA1. GAD activity was elevated above control levels in the dentate gyrus, subiculum, and posterior CA1 and CA3.

*CCK.* Five days after surgery, CCK concentrations were lowered in septum posterior CA3, and anterior dentate gyrus. CCK levels were increased in anterior subiculum (Fig. 10).

Over time, CCK levels increased in CA1, CA3, dentate gyrus, and posterior subiculum. CCK decreased to normal levels in anterior subiculum. Training increased CCK levels in CA1, CA3, and entorhinal cortex. Training twice daily increased CCK levels in all three regions, and increased CCK to above control values in posterior CA1, and entorhinal cortex. Training once daily increased CCK in the entorhinal cortex, but not to the same extent as training twice daily (Fig. 11).

## DISCUSSION

A neurochemical analysis was undertaken of the hippocampal formation in rats who received kainic acid injections to destroy the CA3 subfield. The analysis revealed a number of changes in various neural systems afferent or intrinsic to the hippocampus. These changes fall into three

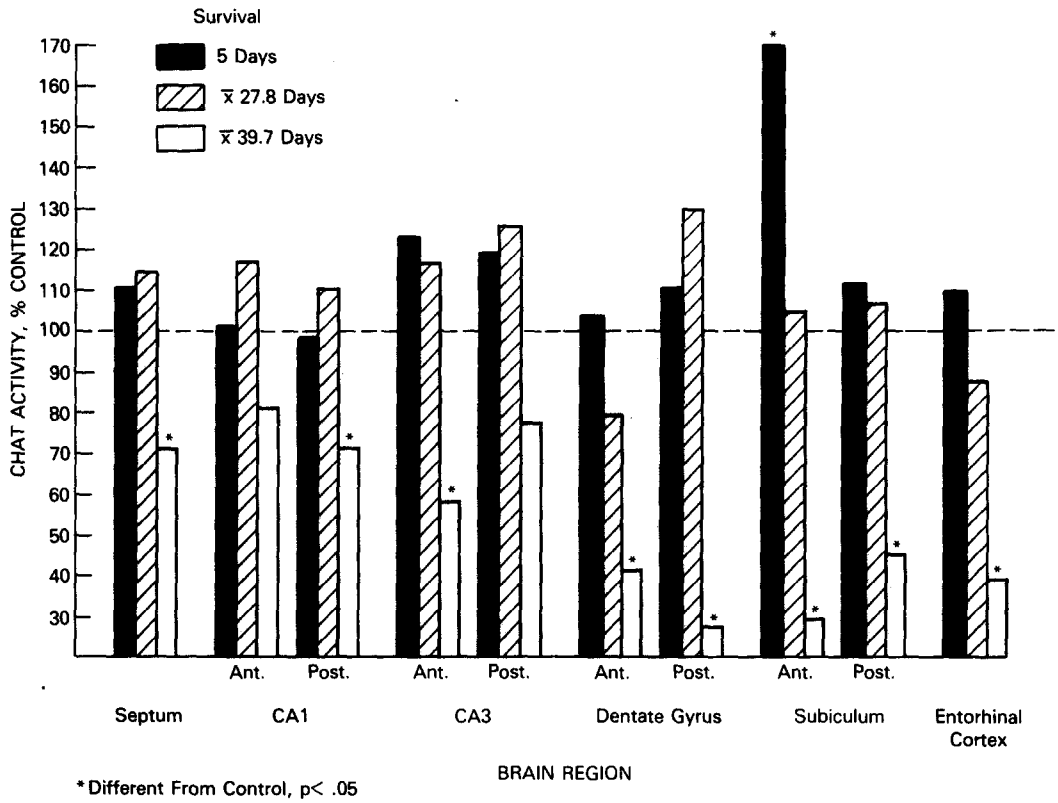



FIG. 5. ChAT activity in the hippocampal formation in untested rats after receiving kainic acid injections.

categories: (1) acute changes due to destruction of the CA3 pyramidal cells; (2) long-term changes due to the destruction; and (3) changes due to the addition of behavioral training on a maze-task. These three types of effects will be discussed in turn for each neural system investigated.

*Fornix: Afferent Cholinergic Projection*

The injections of kainic acid had no acute deleterious effect on the cholinergic projection to the hippocampus, a result which supports those of previous experiments showing that kainic acid does not destroy fibers of passage [4,30], and spares cholinergic afferents in the hippocampus [9, 11, 35].

At longer survival intervals, ChAT activity was dramatically decreased in untrained rats. The loss of ChAT activity possibly reflects hypoactivity of the septohippocampal projection and could result from the loss of the excitatory CA3 input to the septum [22]. Consistent with these results, injections of kainic acid which destroyed both the CA3 and CA1 subfields caused an acute decrease in choline uptake and rise in acetylcholine concentrations in the hippocampus, indicative of a decrease in cholinergic activity [4, 9, 35]. The decline of ChAT activity in this experiment was more gradual, as has been found in other experiments, probably because ChAT is normally present in excess of physiological requirement [2].

As indicated by ChAT activity, the septohippocampal hypoactivity was counteracted by training in the maze task, as trained rats showed normal or slightly elevated ChAT activity at long survival intervals. Behavioral experience may therefore be important in preventing neural hypoactivity following brain damage in adult rats.

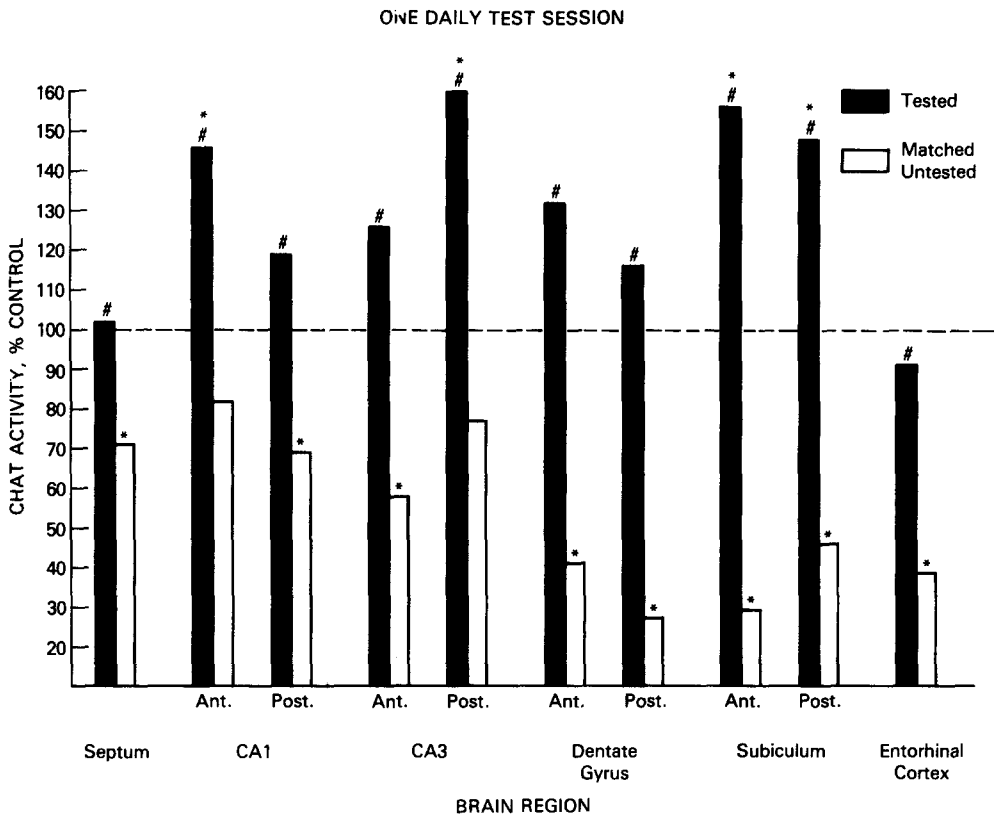
*Intrinsic Glutamatergic Systems and Perforant Path*

Glutamate is an amino acid which serves multiple roles in nerve cells. For one, it appears to be a neurotransmitter used by the CA3 pyramidal cells, the dentate granule cells, and the entorhinal perforant path [25,38]. After destruction of the CA3 cells, decreases in glutamate concentrations were found in the CA3 region and also in the projection areas of the CA3 cells: the CA1 subfield and the dentate gyrus. No decrease, however, was found in septum. Glutamate concentrations in the hippocampus remained low over time, even in the trained rats. This suggests that there was no sprouting or increased activity of the remaining glutamatergic nerves in the hippocampus after the lesions. In addition, lesion-induced gliosis probably does not contribute significantly to the glutamate content of the hippocampus following damage.

In the entorhinal cortex there was a progressive decline in glutamate levels in the untrained rats. Trained rats, on the other hand, had normal levels. The decline may therefore have been due to secondary degeneration or hypoactivity of entorhinal cortical cells as a result of the loss of input from the CA3 pyramidal cells. In this case, training may sustain the cells' normal activity through input from other brain regions.

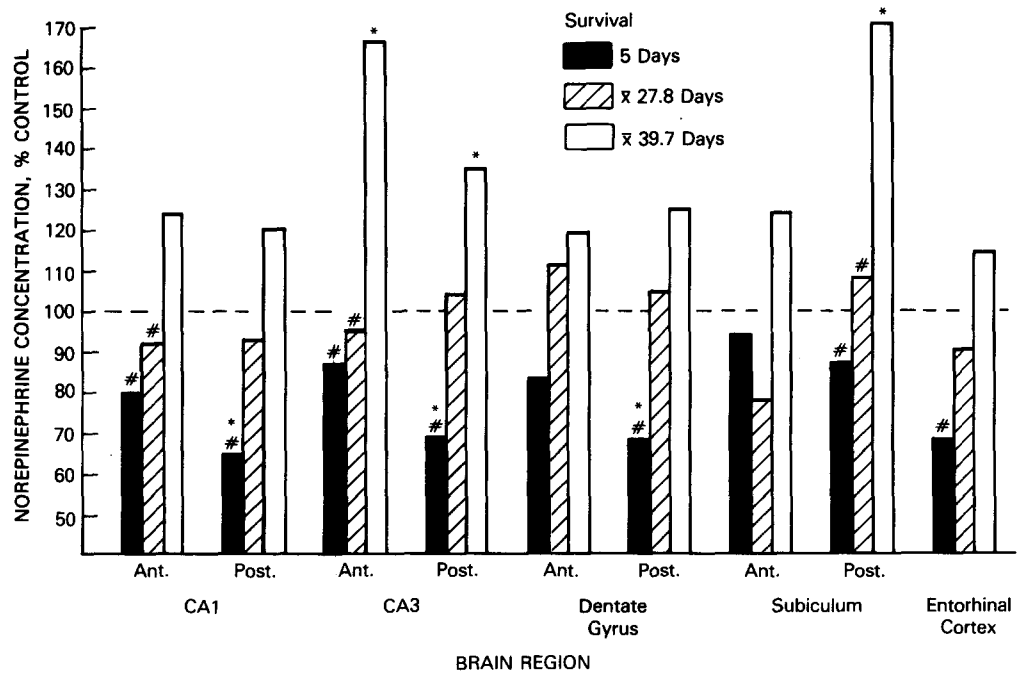
*Afferent Noradrenergic Projections*

The hippocampus contains relatively few noradrenergic




*Different From Control, $p < .05$
#Different From Unmatched Untested, $p < .05$

FIG. 6. ChAT activity in the hippocampal formation in rats with lesions tested once daily on the maze task, compared to their matched untested rats with lesions.




* Different From Control, $p<.05$
# Different From 39.7 Day Group, $p<.05$

FIG. 7. NE concentrations in the hippocampal formation in untested rats after receiving kainic acid injections.

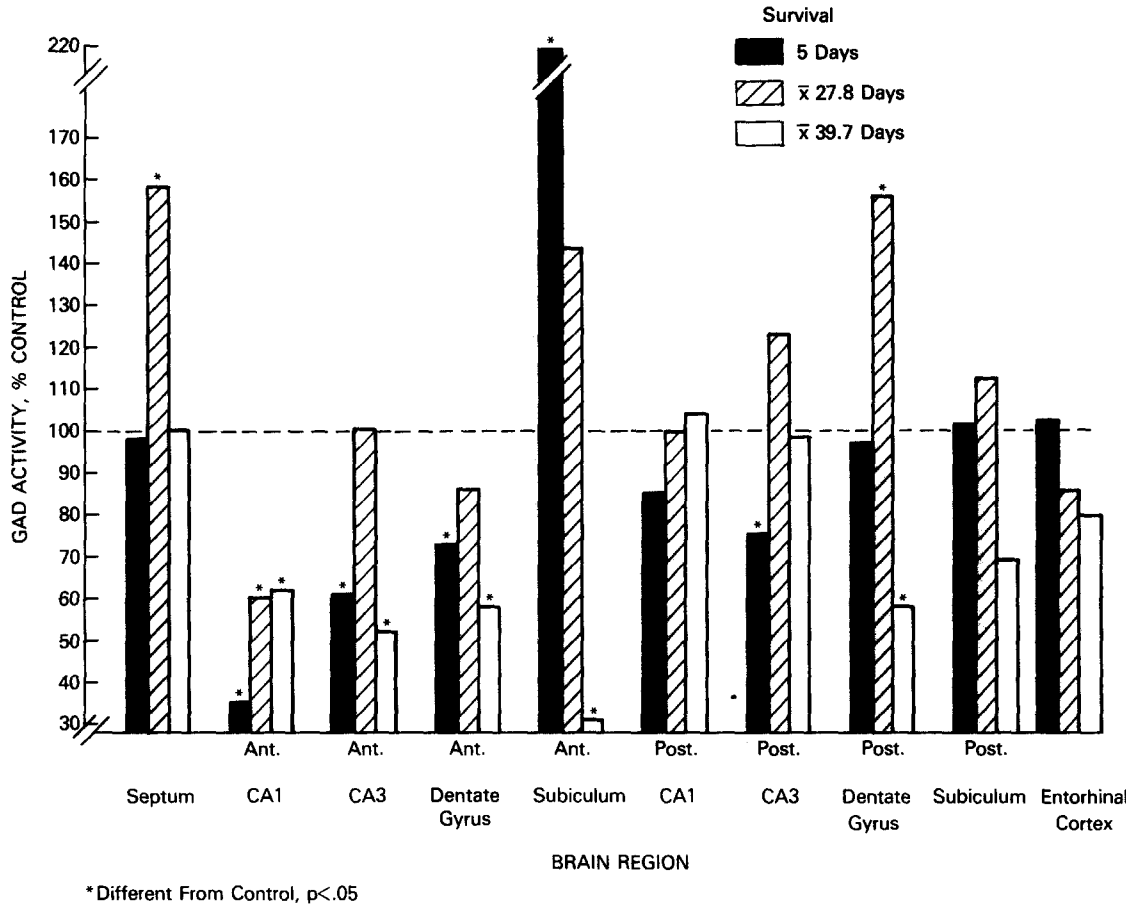



FIG. 8. GAD activity in the hippocampal formation in untested rats after receiving kainic acid injections.

fibers. These fibers are derived from the locus coeruleus [16,39]. The kainic acid injections produced a decrease in posterior CA1, CA3, and dentate gyrus, and in entorhinal cortex five days after surgery. Similarly, Schwarcz *et al.* [35] found a large decrease in NE in whole hippocampus after the injection of 10 nmoles of kainic acid. Because prevention of seizure activity through prolonged anesthesia prevented this depletion [27], the initial decrease in NE may be due to hyperactivity of the system resulting from the neuroexcitatory action of kainic acid [13]. Alternatively, seizure activity may damage noradrenergic terminals in the hippocampus.

In this experiment, NE levels steadily increased to above control values about 40 days after surgery in a number of regions, including CA1, CA3, dentate gyrus, posterior subiculum, and entorhinal cortex. Cessation of seizure activity in the hippocampus may account for some of this rise. On the other hand, the rise may be due to sprouting of either central or peripheral noradrenergic neurons. The peripheral noradrenergic innervation of the vasculature derived from the superior cervical ganglion sprouts in the hippocampus following damage to the fornix [19]. In this experiment, the fornix was not damaged. Hypoactivity of the fornix, however, might be hypothesized to act as a "functional lesion" and induce peripheral sprouting.

*Intrinsic GABAergic cells.* GAD activity is an indicator of the function of GABA-containing cells. In the hippocampus, the GABAergic cells are inhibitory interneurons situated in the major cell layers [38]. The injections of kainic acid caused a decline in GAD activity in a number of regions, particularly in the anterior hippocampus. These included anterior CA1, CA3, dentate gyrus, and posterior CA3. In the control rats, GAD activity was higher in the anterior than the posterior CA1 and CA3, which may have made these areas more vulnerable to destruction of GABAergic cells.

Training elevated GAD activity considerably in all regions of the hippocampus compared to controls and to the untrained rats. GAD activity was elevated both in rats tested once and twice daily, although the effect was evident in more regions in the rats tested once daily. This suggests that a certain amount of time is required for the change in activity to occur.

*Intrinsic CCK cells.* Although septum, hippocampus, and entorhinal cortex all contain considerable amounts of CCK [1], all of the CCK in the hippocampus is derived from intrinsic neurons [10]. Five days after kainic acid injections, CCK levels declined in septum, anterior dentate gyrus, and posterior CA3, indicating a loss or change in activity of CCK-containing cells.

At longer survival times, all of the brain regions showed CCK levels equal to or greater than those of the controls, indicating either a restoration of normal activity or sprouting of CCK cells in the depleted areas. Training twice daily ele-

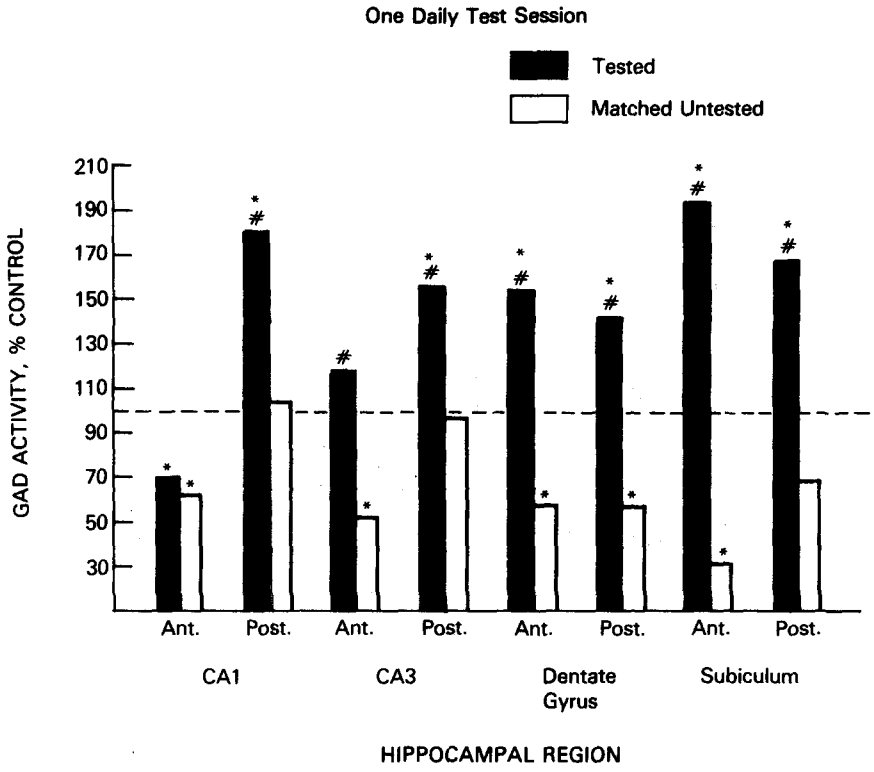



FIG. 9. GAD activity in the hippocampal formation in rats with lesions tested once daily on the maze task, compared to their matched untested rats with lesions.

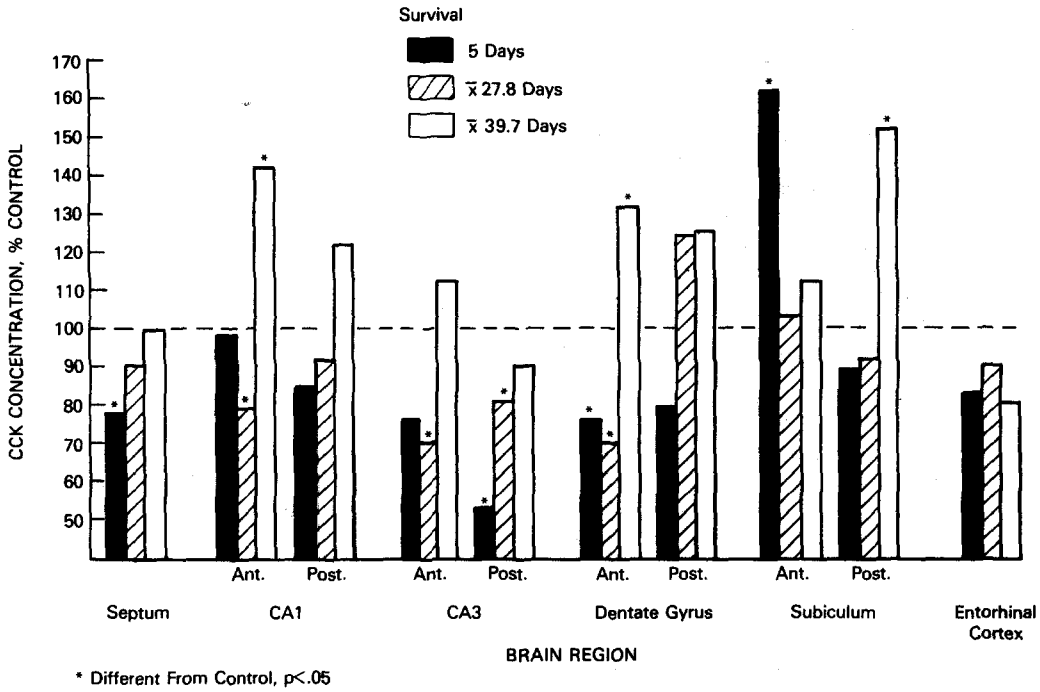



FIG. 10. CCK concentrations in the hippocampal formation in untested rats after receiving kainic acid injections.

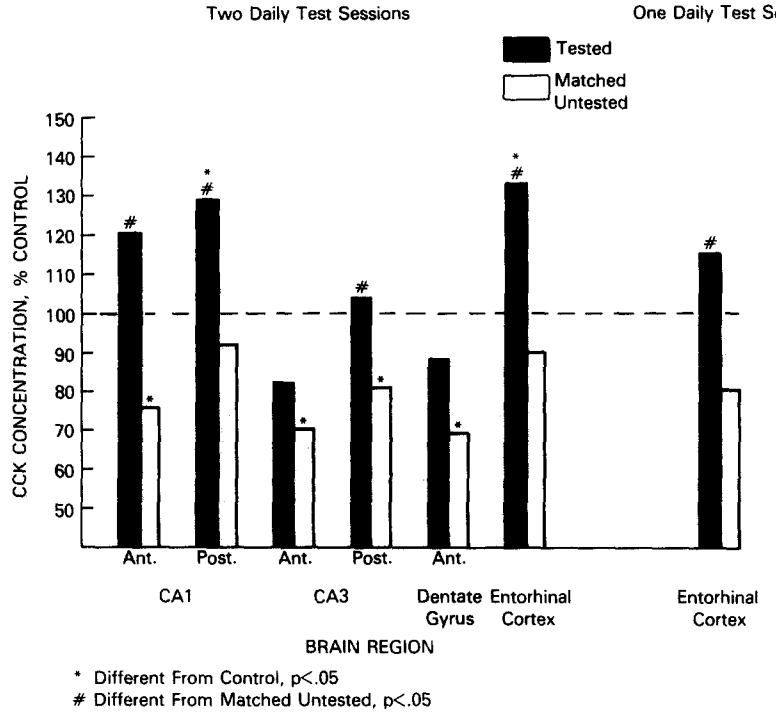



FIG. 11. CCK concentrations in some regions of the hippocampal formation in rats tested once or twice daily after receiving kainic acid injections. The values are compared to those of the matched untested rats with lesions.

vated CCK values in CA1, posterior CA3, while training either once or twice daily elevated CCK levels in entorhinal cortex.

Previous anatomical evidence indicated that microinjection of kainic acid into the hippocampal CA3 subfield produced a preferential lesion of the CA3 pyramidal cells, with no visible loss of other hippocampal cells or afferent fibers. The presence of degenerating fibers in the postcommissural fornix, however, suggested that the kainic acid might also damage cells in the subiculum, although no cell damage was observed [11]. The results of the present study, in conjunction with the anatomical evidence, indicate that the subiculum remains intact, as do the CA1 pyramidal cells, dentate granule cells, and fibers of passage. Interneurons in the pyramidal layers, however, are apparently damaged.

Although there was no evidence of damage to the subiculum, the anterior subiculum showed high ChAT and GAD activity and a high concentration of CCK five days after surgery. As the subiculum appears to be an important relay point in the communication between the hippocampus and septum [23], changes in activity due to the lesions might be expected to be particularly salient there.

Behavioral training had a number of effects on hippocampal neurochemistry after CA3 damage, including increasing concentrations of CCK, preservation of ChAT activity, and increasing GAD activity. There were no measurable effects of testing, however, on hippocampal neurochemistry in the normal control rats. Because behavioral training, or at least activation, is important to the recovery [12], the testing effects on hippocampal neurochemistry in rats with lesions may indicate which alterations in the hippocampus are important in behavioral recovery. Activation of the septohippocampal projection is obviously necessary for the occurrence of normal performance of the maze task. Restoration of the normal inhibitory influence of adrenergic and GABAergic fibers may also be important. The function of CCK-containing cells in the hippocampus is unknown, but they are situated in such a way that they may modulate the activity of each of the major hippocampal cells groups [10]. The concentration of CCK-containing terminals is particularly high in CA1. Because testing selectively influenced activity or sprouting of CCK cells in CA1 and entorhinal cortex, the activity of these two groups of cells may be of particular importance in the recovery of the behavior. An important issue which requires investigation is whether the effect of training is to accelerate the rate of physiological changes which would eventually occur, or to bring about changes which would not occur in the absence of training. These experiments do not provide information on the specificity of training required to influence either the behavioral recovery or hippocampal neurochemistry. In further experiments, it will be of interest to determine whether these phenomena are dependent on behavioral training in a task requiring the integrity of the hippocampus.

This study provides further evidence of the high degree of anatomical plasticity of the adult hippocampal formation. It also indicates that behavioral experience is an important influence on the anatomical plasticity and restoration of function after damage to the system. The broad neurochemical

screening approach applied in this analysis is valuable in that it demonstrates the dynamic changes in a number of interrelated neural systems within a single brain region. Focusing on any single neural pathway would have underestimated the complexities and possible cooperative interactions occurring in the hippocampus after the lesions.

## REFERENCES


1. Beinfeld, M. C., D. K. Meyer, R. L. Eskay, R. T. Jensen and M. J. Brownstein. The distribution of cholecystokinin immunoreactivity in the central nervous system of the rat as determined by RIA. *Brain Res* **213:** 51–57, 1981.
2. Cheney, D. L., G. Racagni and E. Costa. Distribution of acetylcholine and choline acetyltransferase in specific nuclei and tracts of rat brain. In: *Biology of Cholinergic Function*, edited by A. M. Goldberg and I. Hanin. New York: Raven Press, 1976.
3. Coyle, J. and D. Henry. Catecholamines in fetal and newborn rat brain. *J Neurochem* **21:** 61–67, 1973.
4. Coyle, J. T., M. E. Molliver and M. J. Kuhar. In situ injections of kainic acid: A new method for selectively lesioning neuronal cell bodies while sparing axons of passage. *J Comp Neurol* **180:** 301–324, 1978.
5. Crawford, I. and J. D. Connor. Localization and release of glutamic acid in relation to hippocampal mossy fiber pathway. *Nature* **244:** 442–443, 1973.
6. Dodd, J. and J. S. Kelly. The actions of cholecystokinin and related peptides on pyramidal neurons of the mammalian hippocampus. *Brain Res* **205:** 337–350, 1981.
7. Fonnum, F. A rapid radiochemical method for the determination of choline acetyltransferase. *J Neurochem* **24:** 407–409, 1975.
8. Fonnum, F., I. Grofova, E. Rinvik, J. Storm-Mathisen and F. Walberg. Origin and distribution of glutamate decarboxylase in substantia nigra of the cat. *Brain Res* **71:** 77–92, 1974.
9. Fonnum, F. and I. Walaas. The effect of intrahippocampal kainic acid injections and surgical lesions on neurotransmitters in hippocampus and septum. *J Neurochem* **31:** 1173–1181, 1978.
10. Handelmann, G. E., D. K. Meyer, M. C. Beinfeld and W. H. Oertel. CCK-containing terminals in the hippocampus are derived from intrinsic neurons: An immunohistochemical and radioimmunological study. *Brain Res* **224:** 180–184, 1981.
11. Handelmann, G. E. and D. S. Olton. Spatial memory following damage to hippocampal CA3 pyramidal cells with kainic acid: Impairment and recovery with preoperative training. *Brain Res* **217:** 41–57, 1981.
12. Handelmann, G. E. and D. S. Olton. Recovery of function following neurotoxic damage to the hippocampus: Importance of recovery interval and task experience. *Behav Neural Biol* **33:** 453–464, 1981.
13. Johnston, G. A. R., D. R. Curtis, J. Davies and R. M. McCulloch. Spinal interneurone excitation by conformationally restricted analogues of L-glutamic acid. *Nature* **248:** 804–805, 1974.
14. König, J. F. R. and R. A. Klippel. *The Rat Brain*. Baltimore: Williams and Wilkins, 1967.
15. Lewis, P. R., C. C. D. Shute and A. Silver. Confirmation of brain choline acetylase analysis of a massive cholinergic innervation to the rat hippocampus. *J Physiol (Lond)* **191:** 215–224, 1967.
16. Lindvall, O. and A. Bjorklund. The organization of the ascending catecholamine neuron systems in rat brain. *Acta Physiol Scand [Suppl]* **412:** 1–48, 1974.
17. Lowry, O. H. and J. V. Passonneau. *A Flexible System of Enzymatic Analysis*. New York: Academic Press, 1972.
18. Lowry, O. H., N. Rosebrough, A. Farr and R. Randall. Protein measurement with the Folin phenol reagent. *J Biol Chem* **193:** 265–276, 1951.
19. Loy, R. and R. Y. Moore. Anomalous innervation of the hippocampal formation by peripheral sympathetic axons following mechanical injury. *Exp Neurol* **57:** 645–650, 1977.
20. Lynch, G. S., D. Mathews, S. Mosko, T. Parks and C. W. Cotman. Induced acetylcholinesterase rich layer in rat dentate gyrus following entorhinal lesions. *Brain Res* **42:** 311–318, 1972.
21. Lynch, G., B. Stanfield and C. Cotman. Developmental differences in post-lesion axonal growth in the hippocampus. *Brain Res* **59:** 155–168, 1973.
22. McLennen, H. and J. J. Miller. The hippocampal control of neuronal discharges in the septum of the rat. *J Physiol* **237:** 607–624, 1974.
23. Meibach, R. C. and A. Siegel. Efferent connections of the hippocampal formation in the rat. *Brain Res* **124:** 197–224, 1977.
24. Moore, R. Y., A. Bjorklund and U. Stenevi. Plastic changes in the adrenergic innervation of the rat septal area in response to denervation. *Brain Res* **33:** 13–35, 1971.
25. Nadler, J. V., K. W. Vaca, W. F. White, G. S. Lynch and C. W. Cotman. Aspartate and glutamate as possible transmitters of hippocampal afferents. *Nature* **260:** 538–540, 1976.
26. Nadler, J. V., B. W. Perry and C. W. Cotman. Interaction with CA4-derived fibers accounts for distribution of septohippocampal fibers in rat fascia dentata after entorhinal lesions. *Exp Neurol* **68:** 185–194, 1980.
27. Nelson, M. F., R. Zacsek and J. T. Coyle. Effects of sustained seizures produced by intra-hippocampal injection of kainic acid on noradrenergic neurons: Evidence for local control of norepinephrine release. *J Pharmacol Exp Ther* **214:** 694–702, 1980.
28. Odenfeld-Nowak, B., O. Narkiewicz, J. Bialowas, J. Dabrowska, A. Wierscko and M. Gradkowska. The influence of septal nuclei lesions on activity of acetylcholinesterase and choline acetyltransferase in the hippocampus of the rat. *Acta Neurobiol Exp* **34:** 583–601, 1974.
29. O'Keefe, J., L. Nadel, S. Keightley and D. Kill. Fornix lesions selectively abolish place learning in the rat. *Exp Neurol* **48:** 152–166, 1975.
30. Olney, S. W., V. Rhee and O. L. Ho. Kainic acid: A powerful neurotoxic analogue of glutamate. *Brain Res* **77:** 507–512, 1974.
31. Olton, D. S., J. A. Walker and F. H. Gage. Hippocampal connections and spatial discrimination. *Brain Res* **139:** 295–308, 1978.
32. Rawlins, J. N. P., J. Feldon and J. A. Gray. Septo-hippocampal connections and the hippocampal theta rhythm. *Exp Brain Res* **37:** 49–63, 1979.
33. Ross, R. A. and D. J. Reis. Effects of lesions of locus coeruleus on regional distribution of dopamine-beta-hydroxylase activity in rat brain. *Brain Res* **73:** 161–166, 1974.
34. Scheff, S. W. and C. W. Cotman. Recovery of spontaneous alternation following lesions of the entorhinal cortex in adult rats: possible correlation to axon sprouting. *Behav Biol* **21:** 286–293, 1972.
35. Schwarcz, R., R. Zacsek and J. Y. Coyle. Microinjection of kainic acid into the rat hippocampus. *Eur J Pharmacol* **50:** 209–220, 1978.
36. Stein, D. G., J. J. Rosen and N. Butters. *Plasticity and Recovery of Function in the Nervous System*, New York: Academic Press, 1974.
37. Steward, O., C. W. Cotman and G. S. Lynch. Re-establishment of electrophysiologically functional entorhinal cortical input to the dentate gyrus deafferented by ipsilateral entorhinal lesions: Innervation by the contralateral entorhinal cortex. *Exp Brain Res* **18:** 396–414, 1973.
38. Storm-Mathisen, J. Localization of transmitter candidates in the brain: The hippocampal formation as a model. *Prog Neurobiol* **8:** 119–181, 1977.
39. Ungerstedt, U. Ascending monoamine pathways. *Acta Physiol Scand [Suppl]* **367:** 1971.
40. Young, R. L. and O. H. Lowry. Quantitative methods for measuring the histochemical distribution of alanine, glutamate, and glutamine in brain. *J Neurochem* **13:** 785–793, 1966.