Title

Spatial learning in the rat: impairment induced by the thiol-proteinase inhibitor, leupeptin, and an analysis of [3H]glutamate receptor binding in relation to learning.

Journal

Behavioral and neural biology, 47(3)

ISSN

0163-1047

Authors

Morris, R G
Hagan, J J
Nadel, L
et al.

Publication Date

1987-05-01

Peer reviewed

BEHAVIORAL AND NEURAL BIOLOGY 47, 333–345 (1987)

Spatial Learning in the Rat: Impairment Induced by the Thiol-Proteinase Inhibitor, Leupeptin, and an Analysis of [^{3}H]Glutamate Receptor Binding in Relation to Learning

R. G. M. MORRIS AND J. J. HAGAN

MRC Cognitive Neuroscience Research Group, University of St. Andrews, St. Andrews, Fife KY16 9JU, Scotland

L. NADEL

Department of Psychology, University of Arizona, Tucson, Arizona 85721

AND

J. JENSEN, M. BAUDRY, AND G. S. LYNCH[1]

Center for the Neurobiology of Learning and Memory, University of California, Irvine, California 92717

Rats were given continuous intraventricular infusion of saline or the thiol-proteinase inhibitor leupeptin, via subcutaneously implanted osmotic minipumps, while being trained on a spatial learning water task using spaced trials. Leupeptin caused overnight forgetting during training, but performance eventually reached asymptote in both groups. A retention test conducted 48 h later to assess spatial memory revealed no significant group differences, but did cause, in saline-treated rats only, a disruption of subsequent retraining back to the correct spatial location. The groups showed no differences in Cl-dependent [^{3}H]glutamate receptor binding to hippocampal or entorhinal cortex membranes subsequent to training. In a second experiment, normal rats trained on the same task also showed no differences in Cl-dependent [^{3}H]glutamate binding relative to rats exposed to the water task but given random spatial position training and handled controls. The results are discussed in relation to the hypothesis of Lynch and Baudry (*Science* (1984) **224,**

[1] The work was supported by MRC Fellowship Award G83/14974N to R. G. M. Morris, SERC Grant Gr/C/39071, and by NIH/NSF grants to Michel Baudry and Gary Lynch. We are indebted to Bill Dewar, Mary English, and Chris Barman for technical assistance, and to Marjorie Anderson for help in preparation of the manuscript. Correspondence and reprint requests should be addressed to R. G. M. Morris, Department of Pharmacology, University of Edinburgh, 1 George Square, Edinburgh EH8 9JZ, Scotland.

0163-1047/87 $3.00

1057–1063) that a calcium-dependent thiol proteinase is involved in memory formation through its ability to modify glutamate receptor distribution and dendritic spine shape.

Recently, a new and specific hypothesis concerning the biochemical mechanism underlying the storage of certain types of information in the central nervous system, has been proposed (9). The postulated mechanism involves the activation by calcium of a calcium-dependent protease (calpain), the resulting degradation of the cytoskeletal protein fodrin, and the uncovering of receptors for the excitatory neurotransmitter glutamate. The hypothesis is based upon biochemical findings indicating that the calpain-induced degradation of fodrin results in an irreversible increase in [^{3}H]glutamate receptor binding in telencephalic membranes (1,25).

The theory is also supported by recent behavioural experiments showing that continuous infusion of the calpain inhibitor, leupeptin, via osmotic minipumps (30), can cause impairments of a spatial radial maze task (28)—without effect upon feeding, motor activity, or avoidance learning. In the radial maze study, the performance impairment was restricted to the pattern of choices made on arm choices 5–8 of the eight-arm maze when choices 1–4 were separated from choices 5–8 by a long time interval. Leupeptin-treated rats were unable to remember which arms they had visited earlier, while saline-treated rats successfully avoided reentry into arms 1–4.

Radial maze learning was selected because it is known to be disrupted by hippocampal lesions, and by lesions of its extrinsic and intrinsic circuitry (6,20,21). Another spatial task severely impaired by hippocampal lesions is "place navigation," in which rats must learn to find a hidden platform submerged 1 cm below the surface of opaque water (15,18,29). The present study examined the effect of leupeptin on the performance of rats in this task. In addition, we investigated whether changes in [^{3}H]glutamate receptor binding would occur as a result of learning, and whether such changes would be blocked by leupeptin.

Two experiments were conducted. Experiment 1 compared learning by rats implanted with osmotic minipumps containing leupeptin or saline. In Experiment 2, unoperated (normal) rats given the same training as in Experiment 1 were compared with random spatial position and "handling only" control groups with respect to [^{3}H]glutamate receptor binding. To test for effects of leupeptin upon binding, the brains of the rats in Experiment 1 were also assayed in the same way.

MATERIALS AND METHODS

Subjects

Adult male rats (300–350 g) bred at St. Andrews were used. They were kept in individual cages, maintained on ad libitum food and water, and housed in a room on a 12-h light/dark cycle (lights on: 7 AM).

Behavioral Procedures

The rats were trained to escape from opaque water (i.e., water to which powdered milk had been added) by swimming to a submerged hidden escape platform (9-cm diam; 1 cm below water surface) in a large pool (2.14-m diam × 0.4-m height) (16). The swimming paths taken by the rats were tracked using an overhead video system connected, via an image analyzer (HVS, Ltd.) to a BBC microcomputer.

The *training procedure* consisted of three daily trials spaced at 4-h intervals, starting at 9 AM. A trial consisted of taking each rat from the vivarium to the testing room, placing it into the pool at any one of four randomly selected starting points (N, E, S, or W) and timing its escape onto the hidden platform, which was placed at a predetermined position at the center of either the NE or the SW quadrant over successive trials (counterbalanced across groups). If the rat had not escaped within 120 s, it was placed manually onto the platform. A trial ended after the rat had spent 30 s on the platform. It was then returned to its homecage in the vivarium immediately. Training continued for 5 successive days (total = 15 trials).

A post-training *retention test* was conducted 40 h after the last training trial. The rats were placed in the pool for 60 s with no escape platform, and the paths they took in searching for the (now absent) platform tracked automatically. From the digitized array of X, Y coordinates obtained, we calculated the time spent in any quadrant and the number of occasions that a rat swam through an annulus, and marked the exact previous location of the platform.

Prior to sacrifice, a series of four *reinstatement trials* were given in which the platform was returned to the same location as it had occupied in earlier training. Starting 30 s after the retention test, these four trials were given in rapid succession with a 30-s intertrial interval (which the rats spent on the escape platform). At the end of these trials, all rats were escaping from the water rapidly. The rats were sacrificed 3–5 min after this final reinstatement trial, and the brain was rapidly removed (1–2 min) and placed in ice-cold saline. Dorsal and ventral hippocampus and dorsomedial and ventrolateral entorhinal cortex were dissected (left and right sides pooled together); these tissue samples were stored frozen for transport from St. Andrews to Irvine.

Surgery and Other Treatments

Experiment 1 involved two groups of rats: *Leupeptin* and *Saline*. The animals were anesthetized with Avertin and given stereotaxic implantations of a stainless steel cannula (25 gauge) into the right lateral ventricle (coordinates relative to Bregma: −0.9 AD, 1.3 L, −4.5 V; incisor bar at +5 mm). An osmotic minipump (Alzet 2002, pumping rate = 0.5 μl/h)

containing either leupeptin (20 mg/ml in 0.9% sterile saline, $N = 11$) or saline ($N = 9$, dropping to $N = 8$ as one rat was excluded at sacrifice due to disconnection between pump and cannula) was attached via a catheter and implanted beneath the dorsal skin surface. The wound was dressed with Acramide and the animals were injected with Duplocillin (0.25 ml per rat im). They were allowed 4 days to recover from the effects of surgery before training commenced and to allow the csf concentration of leupeptin to stabilize at 50–100 μM (35).

Experiment 2 involved three groups of rats: *Trained, Random,* and *Homecage*. None of the animals received any surgery. The first group ($N = 11$) was trained in the water task as described above. The random-position group ($N = 8$) also swam in the pool but no platform was present. Individual rats were matched to members of the trained group with respect to the time spent in the pool on each trial, and were removed from the water at whatever place they occupied when the latency of its "yoked" partner (run previously) had elapsed for that trial. The homecage group remained in the vivarium throughout the duration of the study, excepting that they were handled three times per day at the same times as other rats received their behavioral training.

Biochemical Assays

Crude synaptic membrane preparation. Tissue samples were allowed to thaw 15 min in 2 ml of 0.32 *M* sucrose with 1 m*M* EGTA and 4 m*M* Tris before homogenization in a 2-ml glass–Teflon homogenizer (700 rpm, 10 strokes). The homogenate was centrifuged at 1000*g* for 10 min and the supernatant, with an additional 8 ml of 0.32 *M* sucrose (w/1 m*M* EGTA, 4 m*M* Tris) was recentrifuged at 22,400*g* for 20 min. The P_2 fraction was resuspended in 4 ml of cold distilled water (w/1 m*M* EGTA, 4 m*M* Tris) and after 40 min the suspension was centrifuged at 7310*g* for 20 min. The supernatant and the upper layer of the pellet were collected, diluted with 3.5 ml of cold 1 m*M* EGTA, 4 m*M* Tris solution, and centrifuged at 41,200*g* rpm for 30 min. The supernatant was discarded and the pellet was first resuspended with a Pasteur pipette in 1 ml of cold 1 m*M* Tris solution, and then sonicated for 5 s at low intensity. An additional 7 ml of cold 1 m*M* EGTA, 4 m*M* Tris was added and the suspension was recentrifuged at 41,200*g*. The final pellet was again first resuspended by Pasteur pipette in 1 ml of cold 50 m*M* Tris–HCl buffer, pH 7.4, followed by sonication for 5 s at low intensity.

L[^{3}*H*]Glutamate binding assay. Duplicate aliquots of each membrane preparation (0.09 ml) and 0.01 ml of Tris–Cl buffer, pH 7.4 (B°), were preincubated at 30°C for 10 min. L-[^{3}H]Glutamate (0.1 ml, 50 Ci/mmole (ICN, Irvine) was added and the incubation was continued for 15 min. Incubation was terminated by addition of 3.5 ml of cold Tris–HCl buffer and filtration under vacuum on Millipore cellulose filters (0.45 pore size).

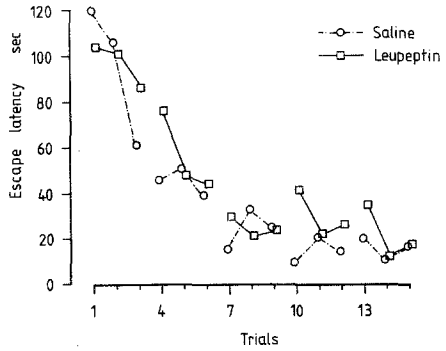

FIG. 1. Mean escape latencies of saline (○) and leupeptin (□) rats over trials 1–15. The ITI was 4 h between the three daily trials. Note that the leupeptin-treated rats were slower on the first trial of each day, excepting Day 1.

The tubes were rinsed with 3.5 ml of the cold buffer and the filters were washed with 3.5 ml of cold Tris–HCl buffer. Scintillation cocktail (ACS, Amersham, 3.0 ml) was added and radioactivity was counted in a Beckman scintillation counter with an efficiency of 35%. Ca^{2+}-stimulated binding was determined in the same manner, except that 0.1 ml of 0.005 *M* Ca^{2+} solution (final concentration 250 μM) was substituted for the 0.01 ml of buffer in the preincubation step. Nonspecific binding was assessed in the presence of an excess of cold glutamate (100 μM). The mean nonspecific binding was subtracted from its corresponding baseline and Ca^{2+}-stimulated binding values in the calculation of B° and $B^{C}a^{2+}$.

Protein assay. Protein concentration of the membrane preparation was measured using a modified Bradford protein assay (4). Commassie blue protein reagent (Bio-Rad) diluted 1:5 was added to 20 μl of each sample and absorbance values were read at 595 nm on a Beckman spectrophotometer using disposable plastic microcuvettes. Standards were prepared from bovine serum albumin (Sigma) diluted with Tris buffer.

RESULTS

Experiment 1

Effect of Leupeptin Treatment on Spatial Learning

Saline- and leupeptin-treated rats swam effectively using the normal adult swimming posture. Chronic infusion of leupeptin did not affect swimming ability nor the tendency to escape onto the hidden platform.

The escape latencies over trials 1–15 revealed efficient acquisition by both groups (Fig. 1). An unequal *N* analysis of variance showed that the improvement over trials was highly significant ($F(14/252) = 22.4$, $p < .0001$), but there was no difference between groups ($F(1/18) = 1.32$, $p > .10$). However, inspection of Fig. 1 suggests that, once training had

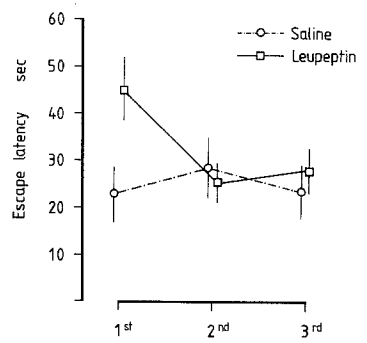

FIG. 2. Mean escape latency (± 1 SE) saline (○) and leupeptin (□) rats on the first, second, and third trials of the day averaged over Days 2–5.

begun, leupeptin rats were slower on the first trial of each day. A separate analysis was therefore conducted of performance on each first daily trial, excepting trial 1 of training (i.e., trials 4, 7, 10, and 13). As shown in Fig. 2, the groups differed significantly on this trial ($F(1/18) = 6.18$, $p < .025$), with leupeptin rats taking over twice as long to find the platform as saline rats. Moreover, the deficit was specific to the first trial of the day: an analysis of trials 4–15 in which Days and Trials within each day were treated as separate factors showed a significant Groups × Trials interaction ($F(2/36) = 3.97$, $p < .03$). These findings imply that while both groups improved their performance across days, leupeptin rats caused overnight forgetting.

The retention test provided measures of swimming speed and memory for the platform location. Both groups swam at the same mean speeds (saline = 24.2, leupeptin = 25.0 cm/s; $F < 1$). They both spent more time in the training quadrant than elsewhere ($F(3/54) = 19.10$, $p < .0001$), and also crossed the former platform location more often than the equivalent positions in other quadrants ($F(3/81) = 5.13$, $p < .005$). However, neither the spatial distribution of quadrant times nor that of Annulus crossings differed across groups (Fs < 1). Although nonsignificant, an interesting trend emerged in the quadrant times data shown in Table 1. Overall, saline and leupeptin rats spent 40.0 and 46.0% of the total 60-s transfer

TABLE 1

	Full 60 s of retention test (Time (sec) spent in each quadrant)				% Time in training quadrant		
Group	ADJ/L	TRAIN	ADJ/R	OPP	Full 60 s	First 15 s	Last 15 s
Sal	13.4	24.3	11.4	11.4	40.0 ± 5.8%	55.1%	29.2%
Leu	11.7	27.6	12.6	8.0	46.0 ± 4.4%	54.2%	42.2%

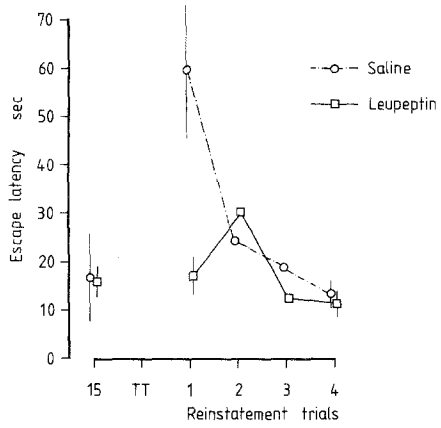

FIG. 3. Mean escape latency (± 1 SE) of saline (○) and leupeptin (□) rats on trial 15 of training and the four trials of reinstatement conducted immediately after the retention test. The ITI was 30 s. Note disruption of performance in the saline group.

test in the training quadrant respectively (chance = 25%). But, when the first and last 15 s of this 60-s period were considered separately, the leupeptin group showed a trend towards more persistent swimming in the training quadrant towards the end of the 60-s period. This trend approached, but did not quite reach significance ($.10 > p > .05$).

The four reinstatement trials following the retention test (Fig. 3) revealed the reverse pattern to that shown during acquisition: the saline group was more disrupted by the intervening retention test than the leupeptin group. Analysis of escape latencies across the four trials revealed a significant Groups × Trials interaction ($F(3/54) = 4.71$, $p < .01$). The impairment in the saline group was restricted to the first trial of reinstatement ($F(1/18) = 12.86$, $p < .005$), reflecting search for the escape platform in other quadrants of the pool during this trial.

Effects of Leupeptin Treatment on [^{3}H]Glutamate Binding

Tissue samples of hippocampus and entorhinal cortex from saline- and leupeptin-treated rats were analyzed for chloride-dependent binding, and the stimulation of this binding by calcium (Table 2). No differences in baseline or Ca^{2+}-stimulated binding were found in any area (all ps $>$ .10). Incubation with Ca^{2+} caused an increase in [^{3}H]glutamate binding in all samples analyzed (range 59–93%; comparable to that shown in Fig. 5 of Ref. (2)). The only significant difference between groups was in dorsal entorhinal cortex where Group Sal showed a larger Ca^{2+}-induced increase in binding than that of the leupeptin group ($F(1/17) = 6.32$, $p < .025$), but given the number of pairwise comparisons possible, we view this effect as a possible Type I statistical error.

TABLE 2
[^{3}H]Glutamate Bound in Various Brain Regions (pmol/mg Protein)

		Hippocampus		Entorhinal	
	N	Dorsal	Ventral	Dorsal	Ventral
[^{3}H]Glutamate binding (B°)					
Saline	8	2.36 ± 0.19	2.66 ± 0.47	3.09 ± 0.33	3.01 ± 0.56
Leupeptin	11	2.25 ± 0.14	3.09 ± 0.47	4.14 ± 0.62	3.46 ± 0.38
Ca^{2+}-stimulated [^{3}H]glutamate binding (B^{Ca2+})					
Saline	8	4.46 ± 0.36	5.01 ± 0.86	5.48 ± 0.57	5.24 ± 0.88
Leupeptin	11	4.10 ± 0.21	5.62 ± 0.72	6.61 ± 1.03	5.84 ± 0.55
% Ca^{2+}-induced increase					
Saline	8	89 ± 6	93 ± 13	77 ± 7	78 ± 10
Leupeptin	11	86 ± 1	91 ± 11	59 ± 4*	73 ± 8

* $p < .025$ compared to saline.

Experiment 2

Behavioral Training

The behavioral performance of the trained group was comparable to that of the saline-treated group of Experiment 1. Over trials 1–15, escape latency declined from 120 to 9.7 s. The group showed a significant spatial bias toward the training quadrant during the retention test (42.2% of time spent there), and, as in Experiment 1, this test caused a disruption of performance on the first trial of reinstatement (mean latency = 53.0 s). Animals in the Random group were removed from the pool at various different locations over trials, as intended in the experimental design. They showed no spatial bias during the transfer test ($p > .10$). Thus, these two groups had equivalent experience of swimming in the pool, differing only in whether any spatial learning had occurred.

Effect of Behavioral Training on [^{3}H]Glutamate Binding

The [^{3}H]glutamate binding data are summarized in Table 3. Tissue from hippocampal and entorhinal regions were analyzed, as in Experiment 1. The values obtained from both baseline (B°) and Ca^{2+}-stimulated (B^{Ca2+}) binding for all three groups were comparable to those obtained in Experiment 1 ($p > .10$), but were expressed as a percentage of the B° binding for the Homecage group, for each area of brain, for the purposes of clarity. No differences among groups were observed. Ca^{2+} again caused between 64 and 108% increased binding across the four brain regions samples. Cross-correlations of several behavioral performance measures with both B° and B^{Ca2+} measures of binding were nonsignificant in all cases.

TABLE 3

	N	Hippocampus		ECX	
		Dorsal	Ventral	Dorsal	Ventral
[^{3}H]Glutamate binding (B°) expressed as % of Home Cage Group					
Homecage	8	100.0 ± 5.3	100.0 ± 14.4	100.0 ± 9.6	100.0 ± 18.4
Random	8	102.9 ± 8.8	98.2 ± 18.7	100.5 ± 9.5	129.9 ± 26.9
Trained	11	89.3 ± 5.8	82.8 ± 13.4	96.8 ± 10.3	138.3 ± 25.7
Ca^{2+}-stimulated [^{3}H]glutamate binding (B^{Ca2+}) as % of Home Cage B°					
Homecage	8	203.6 ± 11.1	180.0 ± 29.7	160.1 ± 11.9	191.7 ± 41.0
Random	8	213.3 ± 10.9	178.9 ± 28.7	159.0 ± 10.5	246.4 ± 48.5
Trained	11	183.6 ± 10.5	169.8 ± 29.9	161.4 ± 14.6	241.1 ± 58.4

DISCUSSION

Leupeptin induced a partial impairment of spatial learning, as characterized by a deficit specific to the first trial of each day during acquisition, no deficit in retention of well-learned spatial information, but less disruption of performance after the retention test. The biochemical results indicate that neither training in itself nor leupeptin treatment modified the Cl-dependent [^{3}H]glutamate binding to hippocampal or entorhinal cortex membranes.

As expected, leupeptin induced a much more subtle effect upon performance than that caused by lesions of the hippocampus (18,29). However, the effects of the drug were also less dramatic than those found previously using radial maze learning (28), and weaker than those induced by intraventricular infusion of the *N*-methyl-D-aspartate receptor antagonist D,L-AP5 (19). Nevertheless, the trial 1 specificity points to a possible functional locus of impairment—specifically, leupeptin may impede the transfer of information between working or intermediate memory (WM, ITM) (20,24) and long-term memory (LTM). In order to escape rapidly on trial 1 of each day, an animal must retrieve information as to the location of the platform from LTM. On trials 2 and 3, WM/ITM already will be "primed" with the relevant information, assuming, as has been claimed (11), that WM can hold spatial information for at least 4 h without "resetting." If leupeptin affected the interaction between these memory systems, without necessarily affecting the operation of either on its own, a deficit specific to trial 1 would emerge.

There are indications, in the present data, that the deficit is due to a failure of storage into LTM, rather than of retrieval from it. A partial deficit restricted to storage would explain why the trial 1 deficit declined across days of training as the rats reached asymptote (the latency difference on Day 2 between saline and leupeptin rats was 30.9 s on Day 5, the difference was only 12.5 s). It would also account for the good performance

of the leupeptin-treated rats at the start of the retention test. A storage deficit can also, albeit speculatively, explain the apparent *reversal* of disruption seen on trial 1 of reinstatement after the retention test, a test which is more complicated than was originally (15) envisaged. One of us (L.N.) has pointed out that it is also an extinction trial, and that animals which visit the correct location at the beginning of the 60-s test, find the platform absent, and then search elsewhere immediately should be classified as rapid learners rather than as rats with a weak spatial bias. By this criterion, the saline rats trend toward searching elsewhere during the course of the retention test indicates that they were better than the leupeptin group in learning that the platform was now absent. Thus, both they and the Trained group of Experiment 2 searched elsewhere in the pool on trial 1 of reinstatement, and thus showed longer escape latencies. If leupeptin-treated rats were less capable of storing information about the absence of the platform, they would have continued to retrieve unaltered information from LTM. This account explains their better performance at the start of reinstatement, but we recognize that it is ad hoc and would require further experimentation before it could be accepted.

From a neurobiological perspective, this account is a modification of that proposed by Lynch and Baudry (9), more in keeping with recent proposals concerning "memory consolidation" (27). If leupeptin impairs changes in synaptic efficacy, as the calpain theory must predict, these changes in efficacy should perhaps be construed as a means of altering the threshold at which neural activity in one brain region can trigger activity in another, rather than as elements of memory itself (8,12). Such an account would, of course, be more persuasive if definitive changes in synaptic efficacy had been found following spatial training. Unfortunately, such evidence was not obtained. This failure cannot be put down to an insensitivity of the glutamate-binding assay, because it showed the usual stimulation of binding by Ca^{2+}. The lack of any differences in binding to hippocampal membranes contrasts with the results reported by Mamounas et al. (13) in which nictictating membrane conditioning in the rabbit did cause measurable increases in Cl-dependent binding. Rabbits which had received contiguous CS and US presentations were compared (blind) with others receiving these stimuli in a random sequence. Effects upon binding were specific to training. What might be the reason for this discrepancy? It could be due to effects upon binding requiring extensive training in order to become detectable by biochemical assay. Mamounas et al. used 117 trials whereas, in the present study, only 15 spaced training trials and 4 reinstatement trials were given. Although these were sufficient to produce a spatial bias toward the training quadrant, the bias was smaller than has been obtained in experiments using more extensive, massed training trials (16,18). A second possibility is that even extensive training on the water task would be insufficient to observe measurable

changes in [^{3}H]glutamate receptor binding, either because the calpain theory is incorrect (3,5), or because learning induced changes in binding are likely to be subtle and difficult to measure. McNaughton (12) and Morris and Baker (17) have noted that the ease with which learning-induced changes in plasticity can be measured in a brain region should be inversely related to its storage capacity. In the case of the hippocampus and spatial processing, unit-recording studies (7,22) have shown that place cells are relatively quiescent in the freely moving animal outside the circumscribed spatial field in which they protypically respond. Thus, only a small proportion of cells within the entire hippocampus may be involved in processing the spatial associations that subserve learning about the relative location of cues within an environment. Extensive training may do no more than strengthen these spatial associations, leaving the vast majority of cells and their synaptic connections unaffected. The Sharpe et al (25) report of only modest changes in the field-potential slope function (an extracellular index of synaptic current flow) following extensive exposure to a complex environment supports this argument. Moreover, Lynch et al. (10) found it necessary to use numerous stimulation sites, and dissected minislices of hippocampus, to measure changes in binding after high-frequency activation. In nictitating membrane conditioning, on the other hand, large numbers of pyramidal cells are recruited during conditioning (2), which may be why changes in binding are seen with that task even though it is unaffected, in its simplest forms, by hippocampal lesions. A third possibility would be that the Cl-dependent binding of [^{3}H]glutamate may not, in fact, be as good a measure of postsynaptic changes as had been thought previously, subject to problems associated with transport into membrane vesicles (23). A different biochemical assay measuring binding restricted to postsynaptic densities rather than, as here, crude membrane fractions would be worth exploring further.

REFERENCES

1. Baudry, M., Bundman, M., Smith, E., & Lynch, G. (1981). Micromolar levels of calcium stimulate protolytic activity and glutamate receptor binding in rat synaptic membranes. *Science,* **212,** 937–938.
2. Berger, T. W., & Thompson, R. F. (1978). Neuronal plasticity in the limbic system during classical conditioning of the rabbit nictictating membrane response. I. The hippocampus. *Brain Research,* **145,** 323–346.
3. Bliss, T. V. P., & Lynch, M. A. (1986). Long-term potentiation of synaptic transmission in the hippocampus: Properties and mechanisms. In P. W. Landfield & S. Q. Deadwyler (Eds.), *Synaptic potentiation in the brain: A critical analysis.* New York: Liss.
4. Bradford, M. (1976). A rapid and sensitive method for the quantification of microgram quantities of proteins utilizing the principle of protein-dye binding. *Analytical Biochemistry,* **72,** 248–254.
5. Foster, A. C., & Fagg, G. E. (1984). Acidic amino acid binding sites in mammalian

neuronal membranes: Their characteristics and relationship to synaptic receptors. *Brain Research Reviews* **7**, 103–164.

6. Jarrard, L. E. (1983). Selective hippocampal lesions and behaviour: Effects of kainic acid lesion on performance of place and cue tasks. *Behavioural Neuroscience*, **97**, 1119–1127.
7. Kubie, J. L., & Ranck, J. B., Jr. (1983). Sensory-behavioral correlates in individual hippocampus lesions in three-situations: Space and context. In W. Siefert (Ed.), *The neurobiology of the hippocampus*. London: Academic Press.
8. Levy, N. B., & Steward, O. (1979). Synapses as associative memory elements in the hippocampal formation. *Brain Research*, **175**, 233–245.
9. Lynch, G., & Baudry, M. (1984). The biochemistry of memory: A new and specific hypothesis. *Science*, **224**, 1057–1063.
10. Lynch, G., Halpain, S., & Baudry, M. (1982). Effects of high-frequency synaptic stimulation on glutamate receptor bindings studies with a modified *in vitro* hippocampal slice preparation. *Brain Research*, **244**, 101–111.
11. Maki, W. S., Brokofsky, S., & Berg, B. (1979). Spatial memory in rats: Resistance to retroactive interference. *Animal Learning and Behaviour*, **7**, 25–30.
12. McNaughton, B. L. (1983). Activity dependent modulation of hippocampal synapatic efficacy: Some implications for memory processes. In W. Siefert (Ed.), *The neurobiology of the hippocampus*. London: Academic Press.
13. Mamounas, L., Thompson, R., Lynch, G., & Baudry, M. (1984). Classical conditioning of the rabbit eyelid response increases glutamate receptor binding in hippocampal synaptic membranes. *Proceedings of The National Academy of Science (USA)*, **81**, 2848–2852.
14. Mishkin, M. (1982). A memory system in the monkey. *Philosophical Transactions of the Royal Society of London, B*, **298**, 85–95.
15. Morris, R. G. M. (1981). Spatial localisation does not depend on the presence of local cues. *Learning and Motivation*, **12**, 239–260.
16. Morris, R. G. M. (1984). Developments of a water-maze procedure for studying spatial learning in the rat. *J. Neuroscience Methods*, **11**, 47–60.
17. Morris, R. G. M., and Baker, M. (1984). Does long-term potentiation/synaptic enhancement have anything to do with learning or memory? In N. Butters & L. R. Squire (Eds.), *The neuropsychology of memory*. New York: Guilford.
18. Morris, R. G. M., Garrud, P., Rawlins, J. N. P., & O'Keefe, J. (1982). Place-navigation impaired in rats with hippocampal lesions. *Nature (London)*, **297**, 681–683.
19. Morris, R. G. M., Anderson, E., Lynch, G. S., & Baudry, M. (1986). Selective impairment of learning and blockade of long-term potentiation ny an *N*-methyl-D-aspartate receptor antagonist, AP5. *Nature (London)*, **319**, 774–776.
20. Olton, D. S., Becker, J. T., & Handelmann, G. E. (1979). Hippocampus, space and memory. *The Behavioural and Brain Sciences*, **2**, 313–315.
21. Olton, D. S., Walker, J. A., & Gage, F. H. (1978). Hippocampal connections and spatial discrimination. *Brain Research*, **139**, 295–308.
22. O'Keefe, J. (1979). A review of the hippocampal place cells. *Progress in Neurobiology*, **13**, 419–439.
23. Pin, J.-P., Bockaert, J., and Recasens, M. (1984). The Ca^{2+}/Cl-dependent L-[^{3}H]-glutamate binding: A new receptor or a particular transport process? *FEBS Letters*, **175**, 31–36.
24. Rawlins, J. N. P. (1985). Associations across time: The hippocampus as a temporary memory store. *Behavioural and Brain Sciences*, **8**, 479–528.
25. Sharpe, P. E., McNaughton, B. L., & Barnes, C. A. (1983). Spontaneous synaptic enhancement in hippocampi of rats exposed to a spatially complex environment. *Society for Neuroscience Abstracts* **9**, S.191–197.

26. Siman, R., Baudry, M., & Lynch, G. (1985). Glutamate receptor regulation by proteolysis of the cytoskeletal protein, fodrin. *Nature (London)*, **313**, 225–228.
27. Squire, L. R., Cohen, N., & Nadel, L. (1984). The medial temporal region and memory consolidation: A new hypothesis. In H. Weingartner & E. Parder (Eds.), *Memory consolidation*. Hillsdale, NJ: Erlbaum.
28. Staubli, U., Baudry, M., & Lynch, G. (1984). Leupeptin, a thiol-proteinase inhibitor, causes a selective impairment of spatial maze performance in rats. *Behavioural and Neural Biology*, **40**, 58–69.
29. Sutherland, R. J., Whishaw, I. Q., & Kolb, B. (1983). A behavioural analysis of spatial localisation following electrolytic, kainate-, or colchicine-induced damage to the hippocampal formation in the rat. *Behavioural Brain Research*, **7**, 133–153.
30. Urquhart, J., Fara, J. W., & Willis, K. L. (1984). Rate-controlled delivery systems in drug and hormone research. *Annual Review of Pharmacology and Toxicology* **24**, 199–236.