Hippocampal 3H-CPP binding and spatial learning deficits in aged rats

MARY ANN PELLEYMOUNTER, GEORGE BEATTY, and MICHELA GALLAGHER
University of North Carolina at Chapel Hill, Chapel Hill, North Carolina

Recent evidence has shown that either intraventricular or intrahippocampal injection of the NMDA antagonist D-2-amino-5-phosphonovalerate (AP-5) produces a spatial learning deficit. This deficit is similar to that observed in aged animals. The spatial learning capacity of aged rats could, then, be related to changes in the status of hippocampal NMDA receptors. This study assessed hippocampal NMDA receptor binding in young and aged rats using the potent NMDA antagonist 3H [(±)-2-carboxypiperazin-4-yl]propyl-1-phosphonic acid (CPP). Initial characterization experiments indicated that 3H-CPP bound selectively to a class of receptors in hippocampal tissue with high affinity (39.5 nM). The 3H-CPP receptor binding capacity was significantly reduced in experimentally naive aged rats (24-25 months) as compared with young animals (5 months). A comparable reduction in 3H-CPP binding was found in a comparison of aged and young rats that had been trained on a spatial task in the Morris water maze. Furthermore, the reduction in 3H-CPP binding was correlated with the severity of the learning deficit present within the aged group. It was concluded that a reduction in hippocampal NMDA sites, as measured by 3H-CPP binding, may contribute to the emergence of cognitive deficits in aged animals.

Glutamate receptors that are activated by N-methyl-D-aspartate (NMDA) are highly concentrated in the hippocampal formation (Monaghan & Cotman, 1985; Monaghan, Holets, Toy, & Cotman, 1983; Monaghan, Yao, Olverman, Watkins, & Cotman, 1984), a neural system long considered to be crucial for certain memory processes (Morris, Garrud, Rawlins, & O'Keefe, 1982; O'Keefe & Nadel, 1978). Recent evidence has also demonstrated the involvement of a hippocampal NMDA mechanism in synaptic plasticity (Cotman & Monaghan, 1988; Errington, Lynch, & Bliss, 1987; Harris, Ganong, & Cotman, 1984; Morris, Anderson, Lynch, & Baudry, 1986), thus linking this type of receptor to long-term changes in hippocampal function. Activation of NMDA receptors mediates the induction of long-term potentiation (LTP) following high-frequency stimulation, as evidenced by the blockade of hippocampal LTP (but not normal synaptic transmission) by the selective NMDA antagonist aminophosphonovaleric acid (AP5) (Cotman et al., 1988; Errington et al., 1987; Harris et al., 1984; Morris et al., 1986). LTP is widely considered to be a form of activity-dependent strengthening of synaptic transmission that may provide a mechanism for information storage in the mammalian brain (Bliss & Lomo, 1973; Tyler, 1986).

A case for the involvement of hippocampal NMDA-dependent LTP in information storage is further supported by studies that have demonstrated a selective learning impairment produced by AP5 at doses that also interfere with LTP. An initial study reported that intraventricular AP5 administration, at doses sufficient to block hippocampal LTP in rats, impaired learning of a spatial task in the Morris water maze; this task is highly sensitive to a variety of manipulations that disrupt normal hippocampal function. The deficit produced by AP5 was selective because it spared learning of another task, visual discrimination, which is also relatively insensitive to hippocampal dysfunction (Morris et al., 1986). More recently, a spatial learning deficit was observed in animals that received microinjections of AP5 directly into the hippocampus (Morris, Halliwell, & Bowery, 1989). Spatial learning deficits have also been reported following administration of noncompetitive NMDA antagonists (Shapiro & Caramanos, in press; Robinson, Crooks, Shinkman, & Gallagher, 1989).

Spatial learning deficits that are similar to those produced by NMDA antagonists are often found in aged rats. For example, many studies have reported an age-related deficit in spatial learning in the Morris water maze (Gage, Dunnett, & Bjorklund, 1984; Pelleymounter, Smith, & Gallagher, 1987; Rapp, Rosenberg, & Gallagher, 1987). Furthermore, aged rats exhibit deficits in a variety of other spatial learning tasks, including the Barnes circular hole board and the radial-arm maze (Barnes, 1979; Barnes & McNaughton, 1980; Barnes & McNaughton, 1985; De Toledo-Morell, Morell, & Fleming, 1984; Wallace, Krauter, & Campbell, 1980). The dynamics of LTP are also affected by age: LTP appears to reach its maxi-

This work was supported by National Research Service Award AG-05407 to M.A.P. and a National Institute of Mental Health Research Scientist Development Award (KO2-MH00406) and NIMH Grant MH39180 to M.G. The authors are grateful to Michael McKinney for his insightful comments and suggestions regarding the manuscript. Correspondence should be addressed to Mary Ann Pelleymounter, Neuroscience Program, Oberlin College, Oberlin, OH 44074.

mum more slowly (and decay more rapidly) in old rats than in young ones (Barnes et al., 1980; Barnes et al., 1985).

In light of these similarities, this study examined the possibility that spatial learning deficits exhibited by aged rats are associated with altered NMDA binding in the hippocampal formation. The present study, then, compares binding of the highly potent NMDA antagonist 3H-3-[(+)-2-carboxypiperazin-4-yl]propyl-1-phosphonic acid (CPP; Lehman et al., 1987; Murphy, Schneider, Boehm, Lehman, & Williams, 1987) in experimentally naive young and aged rats. In addition, groups of young and aged rats were trained on a spatial task in the Morris water maze, and hippocampal 3H-CPP binding was assessed for these subjects in order to compare the binding characteristics of the aged animals with their performance in the behavioral task.

Method

Subjects

Fourteen young (5 months) and 22 aged (24–25 months) male Long-Evans rats obtained from Charles River Laboratories were subjects in the study. The animals in the aged groups were obtained as pathogen-free retired breeders at 8–9 months of age. The young pathogen-free animals were resident in the Department of Psychology vivarium at least 1 month prior to the experiment. The vivarium is climate controlled at 25°C. All animals were housed in single cages on a 12:12-h light:dark cycle (lights on at 7:00 a.m.). Food and water were provided ad lib.

Radioligand Binding Assay

3H-CPP (specific activity = 30.2 Ci/mM) was obtained from New England Nuclear. D and L-AP5 were obtained from Cambridge Biochemical Corporation. L-glutamate, kainic acid, and quisqualic acid were obtained from Sigma Chemical Company. Unlabeled CPP was obtained from Research Biochemicals, Natick, MA.

Following decapitation of the animals, hippocampi were rapidly dissected on an ice-cold block and frozen on dry ice. Hippocampi were stored at -70°C for approximately 4–7 days prior to receptor-binding analysis. Tissue preparation and receptor-binding procedures were conducted by using a modification of the methods described by Olverman, Jones, and Watkins (1984) and Wong and Threlkeld (1987). Tissue was homogenized in 10 vol of a buffered 0.32 M sucrose solution (50 mM Tris acetate; pH 7.4), using a Polytron (10 sec at setting 5). The homogenate was centrifuged for 10 min at 3,000 rpm (1,000×g); all centrifugation steps were carried out at 4°C. The resulting supernatant was then centrifuged for 20 min at 12,000 rpm (18,000×g) to obtain the "P2" fraction. The P2 pellet was resuspended in 40x d-H₂O and incubated for 30 min at 37°C to facilitate the removal of endogenous glutamate. Following incubation, this suspension was centrifuged for 10 min at 19,500 rpm (48,900×g). The resulting pellet was washed three times (40x d-H₂O; 10 min at 48,900×g). The final pellet was resuspended in 25x Tris-acetate buffer (50 mM Tris base titrated to a pH of 7.6 with acetic acid). The protein concentration of this final "working" suspension was approximately 0.58 mg/ml, as determined by the method of Lowry, Rosebraugh, Farr, and Randal (1951).

Aliquots of the suspension (100 µl) were placed into 7-ml polyethylene scintillation vials that contained 700–800 µl of the 50 mM Tris-acetate buffer. L-glutamate (10 µM final concentration) was then added to half of the vials, and all vials were preincubated for 3 min at 25°C. Various concentrations of 3H-CPP (3.1–100 nM) were then added to the vials so that the total incubation volume was

1 ml. Nonspecific binding was defined as that radioactivity remaining in the presence of 10 µM L-glutamate. Nonspecific binding was linear and represented 60%–70% of the "total" bound radioactivity at a saturating concentration (100 nM). Specific binding was shown to be linearly dependent on protein in the range of 18–90 µg ($r = 0.98$).

In the drug inhibition studies, eight concentrations of drug were added (0.1 nM–1.0 mM) in the presence of 40 nM 3H-CPP. As in the saturation studies, 10 µM L-glutamate was used to define nonspecific binding, and the average final protein concentration in the inhibition studies was 58 µg. Vials were then incubated for 25 min at 25°C. Incubation was stopped by centrifugation (10 min at 37,000×g), as described by Murphy et al. (1987). The cold (4°C) supernatant was immediately removed from the pellet by vacuum aspiration over ice. After it was rinsed with ice-cold buffer, the pellet was dissolved in Scintiverse E (Fisher). Approximately 8 h later, radioactivity was counted by liquid scintillation spectrometry. No age difference was found in hippocampal protein content, as determined by the methods of Lowry et al. (1951). Therefore, 3H-CPP binding is expressed as pmol/mg protein.

Behavior

The water maze apparatus used in the behavioral training was a circular steel tank (diam = 1.83 m, depth = 0.58 m) with a removable white escape platform (diam = 10.2 cm, height = 34.5 cm) centered in the SE quadrant of the tank. The tank was filled to a depth of 35.5 cm with water (27°C) opacified by the addition of 0.9 kg powdered milk so that the escape platform was camouflaged and submerged 1 cm below the water surface. The maze was surrounded by white muslin curtains intersecting at each quadrant for ease of access. Dark extramaze patterns were affixed to the curtains at all times to provide an arrangement of spatial cues. During training, the subjects were videotaped with a Panasonic WV-241 P closed circuit TV camera with a 4-mm wide-angle lens suspended above the center of the maze. Training trials were observed by the experimenter on an RCA 12-in. monitor screen.

All subjects were handled (approximately 2 min/day) for 7 days prior to training. On Day 1 of training, the animals were habituated to the water maze by being placed, for 90 sec, in the water tank, with the escape platform removed. The animals were then returned to their home cages.

Starting on Day 2, each animal completed three trials per day for a maximum of 12 consecutive days of testing. During a trial, each animal was placed in the maze facing the wall at one of four equally spaced entry points (entry points corresponded with the North, South, East, and West poles of the maze). These entry points varied from trial to trial in such a way that an animal was placed in the maze at each entry point once across every four trials. During a training trial, the animal was allowed to swim for a total of 120 sec or until it reached the escape platform, where it was allowed to remain for 20 sec. Escape latencies were measured as the time elapsed from the animal's release into the water until its escape onto the platform. If an animal failed to reach the platform within 120 sec, the experimenter placed it onto the platform for 20 sec. Following the 20-sec interval on the platform, the animal was placed into a dry holding cage for 60 sec, at which point the second trial began. After the third trial, the animal was returned to its home cage. During training, the escape platform remained in the same location in the maze across trials.

Every sixth trial consisted of a probe trial during which the escape platform was removed and the animal was allowed to swim for 30 sec. These trials were used to assess the development of a spatial bias for the location of the escape platform. Two measures were used in evaluating spatial bias: quadrant time and annulus crossings. Both measures were obtained from videotapes of the probe trials. Quadrant time refers to the number of seconds an animal spent in the quadrant of the maze that had formerly contained the escape platform. Similarly, annulus crossings are the number of

times the animal traversed the exact former location of the escape platform. A criterion performance was achieved when an animal spent at least 10 sec in the training quadrant and crossed the training annulus at least twice during a probe trial. This criterion represents significant spatial learning; animals trained with a varying location for the escape platform do not exhibit a comparable spatial bias on probe trials (unpublished observations). After each rat achieved criterion performance in this study, behavioral testing of that rat was terminated.

Approximately 10 days after the completion of behavioral testing, the subjects were sacrificed. Naive animals were sacrificed immediately after removal from their home cages. Necropsy data were obtained by grossly inspected the pituitary gland of each aged animal for evidence of tumors. Blood samples from a subset of the aged subjects ($n = 5$) were screened for a panel of murine viral antibodies.

RESULTS

All aged animals included in the data analysis were healthy, as determined by routine assessment during the experiment and necropsies performed at sacrifice. Tests for murine viral antibodies performed on blood samples from a subset of the aged subjects were negative, confirming the absence of pathogens in this colony as indicated by routine screening.

Under the conditions used in this assay, 3H-CPP bound to washed hippocampal tissue from young, naive rats in a saturable and specific manner, with an average $B_{\max}$ of

2.47 (± 0.36) pmol/mg protein and an average apparent K_d of 39.5 nM (± 7.5). A saturation curve representing four preliminary experiments where 3H-CPP was incubated with hippocampal tissue from young rats is shown in Figure 1. Drug inhibition studies indicated that 3H-CPP binding was specific to NMDA-type glutamate receptors, in that the IC_{50} values for unlabeled CPP, L-glutamate, and D-AP5 were 73.0, 75.8, and 171.8 nM, respectively. IC_{50} values for non-NMDA glutamatergic agonists, such as quisqualate and kainic acid, were much higher, ranging from 12.4–15.2 μ M, respectively. 3H-CPP appeared to bind to NMDA-type receptors in a stereospecific manner, since the L-isomer of AP5 competed for 3H-CPP sites with an IC_{50} of 50.1 μ M, in contrast to the D-isomer, which exhibited an IC_{50} of 171.8 nM. IC_{50} values were derived from Hill plots for each of the drugs tested. Competition curves for each of the drugs tested were monophasic, suggesting that these compounds were competing for a single class of noncooperative sites labeled by 3H-CPP. None of the Hill coefficients generated from these characterization studies were significantly different from 1.0. These characterization data are summarized in Table 1. 3H-CPP was also dependent upon pH, in that binding was maximal at pH of 7.6, and was decreased by more than half in buffers as acidic as pH 6.0 or as basic as pH 9.0. 3H-CPP binding was also decreased by half

Figure 1. Specific binding of 3H-CPP to hippocampal tissue pooled from young animals in four separate experiments. Nonspecific binding was defined in the presence of 10 μ M L-glutamate and represented 30%–40% of total binding at 3H-CPP concentrations near the K_d and 60%–70% of total binding at saturating concentrations of 3H-CPP. The inset illustrates a Scatchard plot of 3H-CPP binding using a concentration range of 3.1–300 nM 3H-CPP. The average $B_{\max}$ in this plot was 2.35 pmol/mg protein and the K_d was 27.0 nM.

Table 1
Competition Experiments with 3H-CPP (40 nM)

	IC ₅₀ ($\pm$ SEM)	Hill Coefficient
L-glutamate	75.8 $\pm$ 15.4 nM	0.88
CPP	73.0 $\pm$ 6.92 nM	0.93
D-AP5	171.8 $\pm$ 69.7 nM	1.16
L-AP5	50.1 $\pm$ 12.2 μ M	0.82
Quisqualate	12.4 $\pm$ 3.5 μ M	0.97
Kainic acid	15.2 $\pm$ 4.85 μ M	0.83
Flunitrazepam	889.0 $\pm$ 205.4 nM	0.93

Note—All values represent the average of 4 separate experiments. Eight concentrations (3.1 nM–1.0 mM) of glutamate or nonglutamatergic ligands were incubated with hippocampal tissue (ave. = 58 μ g) and 40 nM 3H-CPP. Nonspecific binding was defined by L-glutamate (10 μ M). Inhibition constants were determined using Hill plots. Hill coefficients were not statistically different from 1.0.

in the presence of 2.0 mM Ca⁺⁺ and by two-thirds with the addition of 120 mM Na⁺ to the incubation buffer (data not shown).

The behavioral assessment in this experiment was included in order to evaluate the relationship between impaired learning capacity and the status of NMDA binding in aged rats. With the exception of 1 aged rat, all animals reached criterion by the sixth probe trial. For the purpose of data analysis, this animal was assigned a score of 7. As shown in Figure 2, many aged animals required more training to reach criterion than did the young subjects. The average number of training trial blocks required to reach criterion for the young and aged groups was $2.1 \pm .3$ and $4.0 \pm .5$, respectively. Statistical comparison of these data revealed a significant age difference ($t = 2.67$, $p < .02$), indicating that the aged group learned

at a slower rate than did the young group. A proportion of the aged rats, however, learned at a rate that was similar to that of the young rats, whereas others were distinctly impaired, falling outside the entire range of performance for the young group.

Analysis of 3H-CPP binding for these trained groups revealed B_{max} values that were lower for the aged animals than for their younger counterparts ($t = 2.99$, $p < .01$; see Table 2). K_d values did not differ significantly between the two age groups. Similarly, 3H-CPP binding experiments in a separate group of naive animals indicated that maximal binding was significantly decreased in the aged hippocampus ($t = 2.41$, $p < .05$), with no significant age difference in affinity for 3H-CPP (this is also illustrated in Table 2). Furthermore, B_{max} values for the trained aged animals correlated with the number of trials required to attain criterion ($r = -0.58$, $p < .01$). This modest, but significant, correlation indicates that larger decreases in 3H-CPP binding were associated with more pronounced learning deficits among the aged subjects. As Figure 3 illustrates, 3H-CPP binding for aged animals depended on how rapidly they acquired the task. Although 3H-CPP binding was similar in young and aged animals that required no more than 15 training trials to reach criterion, it was significantly reduced in the aged animals that required more extended training ($p < .01$).

DISCUSSION

Our initial characterization studies indicated that 3H-CPP binds to a single class of sites in a manner that is consistent with that of an NMDA receptor. In general,

Figure 2. Percent of animals in the young or old age group that attained spatial learning criterion at each of the six probe trials. Only 1 animal required more than six probe trials to attain criterion. Eight young and 14 aged animals are represented in the graph. Aged animals required significantly more training trials than did young animals to reach spatial learning criterion ($p < .01$).

Table 2
3H-CPP Binding in Young and Aged Rats
(pmol/mg protein $\pm$ SEM)

	Naive		Trained	
	Bmax	Kd (nM)	Bmax	Kd (nM)
Young	2.47 ($\pm$.36) N=6	39.5 ($\pm$ 7.5)	2.39 ($\pm$.26) N=8	45.8 ($\pm$ 3.0)
Aged	1.37* ($\pm$.29) N=8	24.2 ($\pm$ 6.7)	1.33** ($\pm$.17) N=14	34.2 ($\pm$ 5.1)

*Differs significantly from young, $p < .05$. **Differs significantly from young, $p < .01$.

these characterization data are in agreement with earlier reports (Murphy et al., 1987; Wong & Threlkeld, 1987). The K_d values for 3H-CPP binding in our assay, however, were lower (almost 10-fold) than those reported by Murphy et al. (1987), although they were similar to those reported by Wong et al. (1987). This seems reasonable, since the majority of our assay conditions were similar to those of Wong and Threlkeld (1987). A recent study reported that unlabeled CPP was capable of displacing 3H-flunitrazepam binding in a filtration assay at lower concentrations than it displaced 3H-CPP in a centrifugation assay (White, Bender, & Swinyard, 1988). We found that unlabeled flunitrazepam competed for sites labeled by 3H-CPP with an average IC_{50} of 889 nM (a 10-fold lower affinity than unlabeled CPP), using a centrifugation assay. This indicates that, under our assay conditions, it is unlikely that 3H-CPP binding represents labeling of benzodiazepine sites.

The two major findings of this study are that (1) maximal 3H-CPP binding in the hippocampal formation was markedly reduced in aged rats and (2) the magnitude of this reduction was related to the severity of the spatial learning deficit in the aged animals. Hippocampal 3H-CPP binding was reduced approximately 45% in aged animals whether they were naive or trained in the Morris water maze; in fact, at each age, 3H-CPP binding capacity was very similar for trained and naive rats. This indicates that training itself did not affect 3H-CPP binding. 3H-CPP binding, however, did correlate significantly with the number of trials to criterion for the aged subjects and was significantly lower in "impaired" aged rats than in both their "unimpaired" aged cohorts and young rats. Taken together, these data suggest that the reduction in hippocampal 3H-CPP binding may contribute to the spatial learning deficit observed in aged animals.

The decrease in hippocampal 3H-CPP binding in aged rats that were impaired in the water-maze task agrees with other work demonstrating that the function of NMDA receptors is critical in spatial learning tasks (Morris et al., 1986; Shapiro et al., in press; Robinson et al., 1989). It has further been suggested that hippocampal NMDA receptors may be important in spatial learning because they are necessary for the induction of long-term potentiation (LTP). This suggestion is based on the observa-

tion that a chronic regimen of AP5 administration blocks the induction of LTP at perforant/dentate synapses in vivo and impairs spatial learning in the water maze (Morris et al., 1986). Thus, altered NMDA binding may contribute to a decline in the plastic properties and information storage capacity of hippocampal circuitry with age.

The present results do not indicate whether the loss of 3H-CPP binding is particularly pronounced in any sub-region(s) of the hippocampal formation. There is normally a high concentration of NMDA binding sites in the dentate gyrus (Monaghan et al., 1985), sites that are presumably localized on neurons that are targets of perforant path innervation. This innervation becomes sparser in the aged rats' brain (Barnes, 1979), and a marked loss of perforant path innervation is observed in Alzheimer's Disease (Hyman, Van Hoesen, Damasio, & Barnes, 1984; Hyman, Van Hoesen, & Damasio, 1987). Thus, decreased 3H-CPP binding might occur in conjunction with perforant path denervation. There is also the possibility that a decrease in 3H-CPP binding in our homogenate assay reflects neuron loss in the hippocampus proper. Decreased numbers of neurons in the CA1 and CA3 regions of the hippocampus were previously observed in aged Long-Evans rats (Meaney, Aitken, van Berkel, Bhatnagar, & Sapolsky, 1988). In a more recent study, it was found that aged Long-Evans rats with spatial learning impairments had substantially greater cell loss in these regions

Figure 3. Average B_{max} values of 3H-CPP binding in young and aged animals that were trained in the water maze. All animals in the young group reached criterion in three probe trials. The aged "unimpaired" animals also reached criterion by the third probe trial, whereas the aged "impaired" group required an excess of three probe trials to reach criterion. Analysis of these data indicated a significant difference between groups [$F(2,19) = 12.2$, $p < .001$]. Post hoc comparisons revealed that B_{max} values for the aged impaired group differed significantly from B_{max} values for the young and aged unimpaired groups ($ps < .01$). The young and aged unimpaired groups, however, did not differ from one another.

than did either young rats or aged animals with a relatively intact spatial learning capacity (Issa, Gauthier, Rowe, & Meaney, 1989; M. Meaney, personal communication). The topography of NMDA binding in the aged brain could be resolved in further studies using in vitro autoradiography techniques. The present data suggest that defining the behavioral capacities of aged animals used in such an analysis would be highly desirable.

A growing number of studies indicate that age-related changes in neurobiological parameters within the hippocampal formation are associated with learning deficits on spatial tasks. These include the rate of decay for LTP (Barnes, 1979; Barnes et al., 1985), the rate of kindling (DeToledo-Morell et al., 1984), the complement of perforated synapses in the dentate gyrus (Geinesman, DeToledo-Morell, & Morell, 1986a, 1986b), neurochemical content (Jiang, Owyang, Hong, & Gallagher, 1989), and neural activity as reflected in altered hippocampal 2-deoxyglucose metabolism (Gage, Kelly, & Bjorklund, 1984). For each of these measures of hippocampal integrity and/or function, the severity of change in the aged brain bears a predictable relationship with the degree of spatial learning impairment. The present results provide added detail to an emerging role for hippocampal dysfunction underlying the mild/moderate cognitive impairments that often accompany normal aging.

REFERENCES

- BARNES, C. A. (1979). Memory deficits associated with senescence: A neurophysiological and behavioral study in the rat. *Journal of Comparative & Physiological Psychology*, **93**, 74-104.
- BARNES, C. A., & McNAUGHTON, B. (1990). Spatial memory and hippocampal synaptic plasticity. In D. Stein (Ed.), *The psychobiology of aging: Problems and perspectives* (pp. 253-272). New York: Elsevier/North-Holland.
- BARNES, C. A., & McNAUGHTON, B. (1985). An age comparison of the rates of acquisition and forgetting of spatial information in relation to long-term enhancement of hippocampal synapses. *Behavioral Neuroscience*, **99**, 1040-1048.
- BLISS, T., & LOMO, T. (1973). Long lasting potentiation of synaptic transmission in the dentate area of the anesthetized rabbit following stimulation. *Journal of Physiology*, **232**, 331-356.
- COTMAN, C., & MONAGHAN, D. (1988). Excitatory amino acid neurotransmission: NMDA receptors and Hebb-type synaptic plasticity. *Annual Review of Neuroscience*, **11**, 61-80.
- DE TOLEDO-MORELL, L., MORELL, F., & FLEMING, S. (1984). Age-dependent deficits in spatial memory are related to impaired hippocampal kindling. *Behavioral Neuroscience*, **98**, 902-907.
- ERRINGTON, M., LYNCH, M., & BLISS, T. (1987). Long-term potentiation in the dentate gyrus: Induction and increased glutamate release are blocked by D-amino-phosphonovalerate. *Neuroscience*, **20**, 279-284.
- GAGE, F., DUNNETT, S., & BJORKLUND, A. (1984). Spatial learning and motor deficits in aged rats. *Neurobiology of Aging*, **5**, 43-48.
- GAGE, F., KELLY, P., & BJORKLUND, A. (1984). Regional changes in brain glucose metabolism reflect cognitive impairments in aged rats. *Journal of Neuroscience*, **4**, 2856-2865.
- GEINESMAN, Y., DE TOLEDO-MORELL, L., & MORELL, F. (1986a). Aged rats need a complement of perforated axospinous synapses per hippocampal neuron to maintain a good spatial memory. *Brain Research*, **398**, 266-275.
- GEINESMAN, Y., DE TOLEDO-MORELL, L., & MORELL, F. (1986b). Loss of perforated synapses in the dentate gyrus: Morphological substrate of memory deficits in aged rats. *Proceedings of the National Academy of Science, USA*, **83**, 3027-3031.
- HARRIS, E., GANONG, A., & COTMAN, C. (1984). Long-term potentiation in the hippocampus involves activation of N-methyl-D-aspartate receptors. *Brain Research*, **323**, 132-137.
- HYMAN, B., VAN HOESSEN, G., & DAMASIO, A. (1987). Alzheimer's disease: Glutamate depletion in the hippocampal perforant pathway zone. *Annals of Neurology*, **22**, 37-40.
- HYMAN, B., VAN HOESSEN, G., DAMASIO, A., & BARNES, C. (1984). Alzheimer's disease: Cell-specific pathology isolates the hippocampal formation. *Science*, **225**, 1168-1170.
- ISSA, A., GAUTHIER, S., ROWE, W., & MEANEY, M. (1989). Hypothalamic-pituitary-adrenal dysfunction is associated with cognitive impairment in aged rats. *Society for Neuroscience Abstracts*, **15**, 1080.
- JIANG, H.-K., OWYANG, V., HONG, J.-S., & GALLAGHER, M. (1989). Elevated dynorphin in the hippocampal formation of aged rats: Relation to cognitive impairment on a spatial learning task. *Proceedings of the National Academy of Science, USA*, **86**, 2948-2951.
- LEHMAN, J., SCHNEIDER, J., MCPHERSON, S., MURPHY, D., BERNARD, P., TSAI, C., BENNETT, D., PASTOR, G., STEEL, D., BOEHM, C., CHENEY, D., LIEBMAN, J., WILLIAMS, M., & WOOD, P. (1987). CPP, a selective N-methyl-D-aspartate (NMDA)-type receptor antagonist: Characterization in vitro and in vivo. *Journal of Pharmacology & Experimental Therapeutics*, **240**, 737-746.
- LOWRY, O., ROSEBRAUGH, N., FARR, A., & RANDAL, R. (1951). Protein measurement with the Folin reagent. *Journal of Biological Chemistry*, **193**, 265-275.
- MEANEY, M., AITKEN, D., VAN BERKEL, C., BHATNAGAR, S., & SAPOLSKY, R. (1988). Effect of neonatal handling on age-related impairments associated with the hippocampus. *Science*, **239**, 766-768.
- MONAGHAN, D., & COTMAN, C. (1985). Distribution of N-methyl-D-aspartate sensitive L-[³H]-glutamate binding sites in rat brain. *Journal of Neuroscience*, **5**, 2909-2919.
- MONAGHAN, D., HOETS, V., TOY, D., & COTMAN, C. (1983). Anatomical distributions of four pharmacologically distinct ³H-L-glutamate binding sites. *Nature*, **306**, 176-179.
- MONAGHAN, D., YAO, D., OLVERMAN, H., WATKINS, J., & COTMAN, C. (1984). Autoradiography of D-³H-2-amino-5-phosphonopentanoate binding sites in rat brain. *Neuroscience Letters*, **52**, 253-258.
- MORRIS, R., ANDERSON, E., LYNCH, G., & BAUDRY, M. (1986). Selective impairment of learning and blockade of long-term potentiation by an N-methyl-D-aspartate receptor antagonist, AP5. *Nature*, **319**, 774-776.
- MORRIS, R., GARRUD, P., RAWLINS, J., & O'KEEFE, J. (1982). Place navigation impaired in rats with hippocampal lesions. *Nature*, **297**, 681-683.
- MORRIS, R., HALLIWELL, R., & BOWERY, N. (1989). Synaptic plasticity and learning: II. Do different kinds of plasticity underlie different kinds of learning? *Neuropsychologia*, **27**, 41-59.
- MURPHY, D., SCHNEIDER, J., BOEHM, C., LEHMAN, J., & WILLIAMS, M. (1987). Binding of ³H-3-(2-carboxypiperazin-4-yl)propyl-1-phosphonic acid to rat brain membranes: A selective, high affinity ligand for N-methyl-D-aspartate receptors. *Journal of Pharmacology & Experimental Therapeutics*, **240**, 778-784.
- O'KEEFE, J., & NADEL, L. (1978). *The hippocampus as a cognitive map*. Oxford, England: Clarendon Press.
- OLVERMAN, H., JONES, A., & WATKINS, J. (1984). L-glutamate has higher affinity than other amino acids for ³H-D-AP5 binding sites in rat brain membranes. *Nature*, **307**, 460-462.
- PELLEYMOUNTER, M. A., SMITH, M., & GALLAGHER, M. (1987). Spatial learning impairments in aged rats trained with a salient configuration of stimuli. *Psychobiology*, **15**, 248-254.
- RAPP, P., ROSENBERG, R., & GALLAGHER, M. (1987). An evaluation of spatial information processing in aged rats. *Behavioral Neuroscience*, **101**, 3-12.

- ROBINSON, G., CROOKS, G., SHINKMAN, P., & GALLAGHER, M. (1989). Behavioral effects of MK801 mimic deficits associated with hippocampal damage. *Psychobiology*, **17**, 156-164.
- SHAPIRO, M., & CARAMANOS, Z. (in press). NMDA antagonist MK801 impairs acquisition but not performance of spatial working and reference memory. *Psychobiology*.
- TYLER, T. (1986). Memory: Electrophysiological analogs. In J. Martinez & R. Kesner (Eds.), *Learning and memory: A biological view* (pp. 237-260). Orlando: Academic Press.
- WALLACE, J., KRAUTER, E., & CAMPBELL, B. (1980). Animal models of declining memory in the aged: Short term and spatial memory in the aged rat. *Journal of Gerontology*, **35**, 355-363.
- WHITE, H., BENDER, A., & SWINYARD, E. (1988). Effect of the selective N-methyl-D-aspartate receptor agonist 3-(2-carboxypiperazin-4-yl)propyl-1-phosphonic acid on 3[H]-flunitrazepam binding. *European Journal of Pharmacology*, **147**, 149-151.
- WONG, D., & THRELKELD, P. (1987). ³H-CPP binding to NMDA receptors in hippocampal membranes. *Society for Neurosciences Abstracts*, **13**, 382.

(Manuscript received November 15, 1989;
revision accepted for publication March 15, 1990.)