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Microinjection of Dynorphin Into the Hippocampus Impairs Spatial Learning in Rats

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McDANIEL, K. L., W. R. MUNDY AND H. A. TILSON. *Microinjection of dynorphin into the hippocampus impairs spatial learning in rats*. PHARMACOL BIOCHEM BEHAV **35**(2) 429–435, 1990.—The effect of hippocampal dynorphin administration on learning and memory was examined in spatial and nonspatial tasks. Bilateral infusion of dynorphin A(1–8) (DYN; 10 or 20 μg in one μl) into the dorsal hippocampus resulted in a dose-related impairment of spatial working memory in a radial maze win-stay task. Subsequent experiments found that acquisition of a reference memory task in the water maze was impaired by DYN injections (20 μg/μl) in the dorsal hippocampus, but not in the ventral hippocampus, and that this impairment could be blocked by naloxone. In a nonspatial task, posttraining DYN injections in the dorsal hippocampus had no effect on retention of step-through passive avoidance. These results suggest that dynorphin specifically interferes with spatial learning and memory, and that this effect is mediated by opioid receptors in the dorsal hippocampus.

Dynorphin Spatial memory Radial maze Water maze Rat

PREVIOUS studies have shown opioid peptide systems to be involved in learning and memory (8). Much of the work involving the opioid peptides in learning and memory has been performed using opiate receptor antagonists. For example, naloxone has been shown to improve memory retention in both positively and negatively reinforced procedures including the radial-arm maze and a passive avoidance task (3, 7, 9, 30). Other studies have reported that morphine and opioid peptides including Leu- and Met-enkephalin and β-endorphin can impair memory following either systemic or intraventricular administration and that this effect can be blocked by opiate antagonists (15, 18, 19, 29).

The hippocampus has been shown to play an important role in spatial learning and memory processes in the rat. Spatial memory has been described in terms of working memory and reference memory processes. Working memory is used to store information that is trial-specific and retained only for correct responding on the next trial. Reference memory, or long-term memory, stores information that remains constant and is useful for all subsequent trials (13). Previous studies have found animals with hippocampal lesions to be impaired in both working and reference memory tasks (1,31). Further studies have been shown that lesions of the CA3 area of the hippocampus are sufficient to disrupt spatial memory (11). Lesions in the dorsal hippocampus produced a greater impairment in a place-memory task than did lesions in the ventral hippocampus (36). In a recent study, Collier *et al.* (4) found an impairment in spatial memory after electrical stimulation of the dentate gyrus granule cells in the dorsal hippocampus. They hypothesized that the observed impairment may be due to the release of opioid peptides at the mossy fiber-CA3 synapse. They also found that naloxone not only blocked the impairment produced by electrical stimulation but also reduced in slight improvement in performance.

Dynorphin is an endogenous opioid peptide which has been found to act on both mu and kappa receptors in the rat hippocampus (28,32). Specifically, dynorphin is localized in the mossy fiber system between the dentate gyrus and the CA3 region (27,39). The function of dynorphin in the central nervous system has been found to include modulation of locomotor activity (37), reward (17) and memory (16). In order to further assess the effect of this opioid peptide on memory function, dynorphin A(1–8) was injected into the CA3 region of the hippocampus. The effects on spatial working and reference memory were measured using the radial-arm maze and the Morris water maze task, respectively. The effects of intrahippocampal dynorphin in a nonspatial learning and memory task were also determined.

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EXPERIMENT 1

The first experiment examined the effects of dynorphin on spatial working memory using a win-stay task in the radial arm-maze.

METHOD

Animals

Nineteen male rats of the F-344 strain obtained from Charles River Breeders (Raleigh, NC) were used. They were housed in individual cages in an environmentally controlled animal room. The animals were on a 12-hour light/dark cycle with lights on at 0700. The animals weighed approximately 300 grams at the time testing began.

Surgery

All animals were implanted bilaterally with 22-gauge stainless steel cannulas (Plastic One Co., Roanoke, VA) into the dorsal hippocampus. The animals were anesthetized with Nembutal (50 mg/kg). The cannulas were stereotaxically positioned at 3.3 mm posterior and 2.7 mm lateral to bregma. The cannula was cut to obtain a depth of 3.8 mm from top of skull. The position of the cannula tip was aimed at the CA3 region using coordinates obtained from the atlas of Paxinos and Watson (33). The cannula was permanently anchored to the skull using miniature screws and dental acrylic, and sealed with a dummy injection cannula (28-gauge stainless steel; Plastics One Co.).

Procedure

The animals were tested in an elevated eight-arm radial maze made of black Plexiglas. The maze consisted of a central area from which eight equally spaced arms (58 cm long, 10 cm wide, and 5 cm high) radiated. At the end of each arm was a food cup (1 cm high). The food reward used was a single 94 mg Noyes pellet. The maze was located in an enclosed room within the laboratory. Several extramaze cues were located throughout the testing room. A fan was placed above the maze to circulate air to help control for olfactory cues. The animals were observed from a closed circuit monitor connected to a video camera located above the maze.

Training in a 2-trial win-stay was similar to that described by Collier *et al.* (4). Animals were handled and food deprived to 85% of their body weight for 4–10 days before habituation in the maze began. Habituation consisted of acclimating the rat to the maze for five minutes with food either scattered throughout the maze or only in the food cups. Training began after eight days of habituation. A training session consisted of two trials. On the first trial, a single arm was baited and an animal was placed in the center of the maze and allowed to explore until the pellet was found or five minutes has elapsed. The arm that the rat faced upon being placed in the maze was randomly selected as was the baited arm. After trial 1 the animal was returned to its home cage for one minute, at which time the maze was cleaned with a solution of 05% acetic acid to control for olfactory cues and the same arm rebaited. The rat was then returned to the center of the maze in the same location it was placed in trial 1 and allowed to find the pellet during the second trial. The number of arm entries required to find the pellet was recorded for trials 1 and 2. The animals were trained for 23 days of one session per day before surgery was performed. After a recovery period of seven days they were retrained for ten days of two sessions per day. Fourteen animals which had learned to return directly to the baited arm on trial 2 were then tested for seven days of one session per day with a twenty-minute delay between trials. After exhibiting good performance under the twenty-minute delay condition drug treatment began. Drug injections were administered immediately after trial 1, and trial 2 was started twenty minutes from time of injection. Injections consisted of slowly infusing 1 μl of either normal saline or dynorphin A(1–8) (DYN; Peninsula Laboratories, Belmont, CA) dissolved in saline through a 26-gauge injector that extended 0.5 mm beyond the cannula tip, using a syringe pump (Sage Instruments, Cambridge, MA). The animals were tested once daily on five consecutive days. The order of the treatment was saline, saline, DYN (10 μg), saline, and DYN (20 μg).

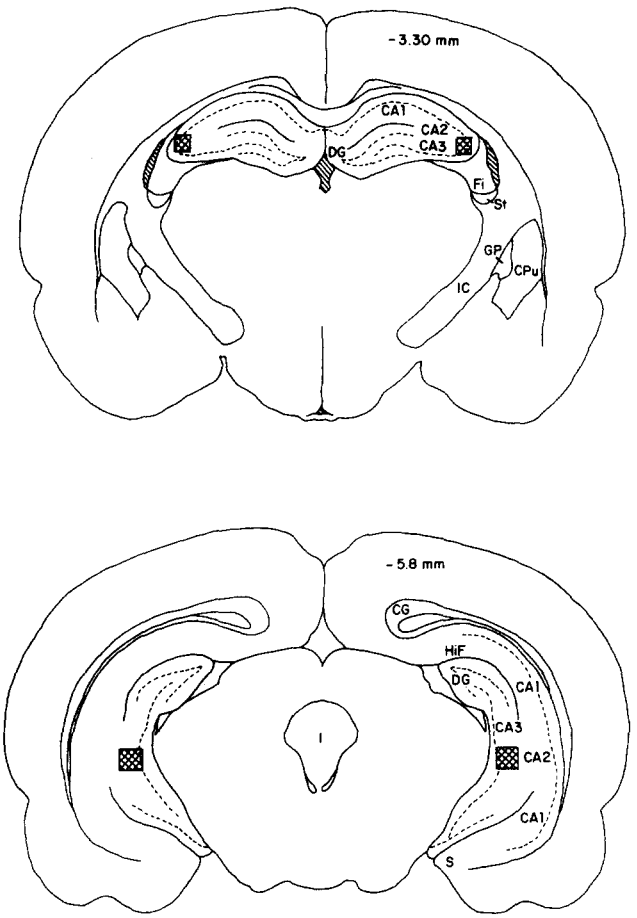

FIG. 1. Schematic drawings of coronal sections through dorsal (top) and ventral (bottom) hippocampus, adapted from Paxinos and Watson (33). Cross-hatched areas show injection sites for dynorphin. CA1, CA2, CA3, field of Ammon's horn; CG, cingulum; CPu, caudate putamen; DG, dentate gyrus; Fi, fimbria; GP, globus pallidus; HiF, hippocampal fissure; IC, internal capsule; S, subiculum; ST, stria terminalis.

Histology

Upon completion of the experiments, cannula placement was confirmed by injecting 1 μl of trypan blue stain into each cannula followed by decapitation and rapid brain removal. The brain was then sliced in a coronal plane at the point where the cannulas had been located. The presence of dye was noted as well as the position of the cannula tracks. All cannula tips were found to be located in the CA3 region of the dorsal hippocampus (Fig. 1).

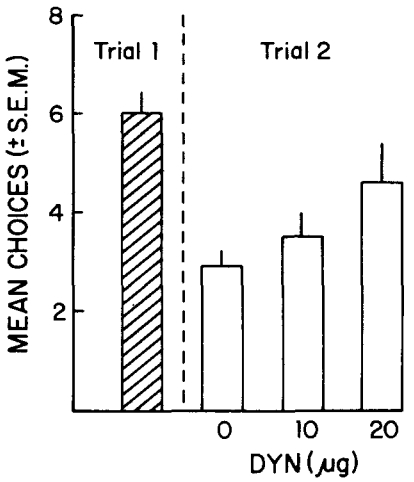

FIG. 2. Effect of dynorphin on a working memory task in the radial maze. Dynorphin was infused bilaterally into the dorsal hippocampus immediately after finding the baited arm in trial 1. After a 20-min interval, rats were returned to the maze for trial 2. The number of arm choices to find the baited arm was recorded.

RESULTS

Prior to surgery the mean number of arms entered (±S.E.M.) during trial 2 was 3.4±0.8. By the end of the retraining period after surgery the mean number of arms entered during trial 2 was 3.0±0.6. Thus, the presence of a cannula and damage accompanying implantation did not affect spatial memory performance. The results of drug testing in the win-stay task are shown in Fig. 2. Animals visited approximately 6 arms before finding the food pellet in trial 1. Under control conditions rats required approximately 3 arm choices to relocate the baited arm during trial 2. Increasing doses of DYN resulted in an increased number of arm choices during trial 2. Because a scattergram of individual data points indicated that the data were nonparametric in nature, the Jonckheere test was used to test for a dose-response relationship. The results ($Z=2.06$, $p<0.02$) indicated a significant trend, with increasing doses of DYN resulting in increased arm choices during trial 2. These results suggested that the highest dose used significantly impaired working memory. For all the following experiments, a dose of 20 μg was used.

EXPERIMENT 2

This experiment examined the effects of DYN on a spatial, reference memory task in the Morris water maze. Because opioid peptides can have differential effects in the dorsal and ventral hippocampus (24) our studies included injections in both sites.

METHOD

Animals

Sixty naive male Fischer-344 rats were used. The animals were maintained as described in Experiment 1.

Surgery

The surgery was performed as described in Experiment 1 for placement of the cannula bilaterally into the dorsal hippocampus. For injections into the ventral hippocampus the cannulas were bilaterally positioned at 5.8 mm posterior and 4.5 mm lateral to bregma. The cannulas were cut to obtain a depth of 6 mm from top of skull. The position of the cannula tip was aimed at the CA3 region of the ventral hippocampus. Cannula placement was confirmed by the procedure described in Experiment 1 (Fig. 1).

Procedure

The testing apparatus was a galvanized steel tank (57 cm high, 139 cm diameter), located in a test room containing several extramaze cues. The tank was filled with water (28°C) to a depth of 40 cm. Powdered milk was added to make the water opaque. The tank was divided into four quadrants by four equally spaced points around the edge of the pool (N,S,E,W). Animals were trained to swim to a transparent platform (10 cm diameter) submerged 2 cm below the surface.

Rats received one daily session of four trials per day. All four starting points (N,S,E,W) were used each day, with the order of starting differing over the test days. The animal was placed into the tank facing the wall at the starting point and allowed to swim until the platform was found or sixty seconds had elapsed. If a rat did not escape onto the platform within that time it was placed on the platform where it remained for 15 sec. For each rat the quadrant where the platform was located remained fixed throughout testing. There were four groups of animals. Immediately following trial 4, saline or dynorphin dissolved in saline (20 μg/1 μl) was infused bilaterally through a 26-gauge injector that extended 0.5 mm beyond the cannula tip. Naloxone (Endo Laboratories, Inc.) at a dose of 3 mg/kg or saline was injected subcutaneously 15 minutes prior to trial 1. Group I received saline only, group 2 received naloxone and saline, group 3 received saline and DYN, and group 4 received naloxone and DYN. The animals were tested for five consecutive days. They were given injections only on the first four days with no injections on day 5. On day six the platform was removed for a 60-sec free-swim test to determine the extent of spatial learning. The time spent swimming in each of the four quadrants during the free swim was recorded for each rat.

RESULTS

Dorsal Injections

The effects of DYN on mean latency to escape onto the platform are shown in Fig. 3A. Escape latencies for all groups decreased with training. A three-way repeated measures analysis of variance (ANOVA) (treatment × pretreatment × session) indicated a significant effect of session, $F(4,96)=3529$, $p<0.001$, and a significant treatment by pretreatment interaction, $F(1,24)=8.27$, $p<0.01$. Post hoc analysis using Fischer's LSD test showed that DYN treatment significantly impaired spatial learning compared to saline-treated controls ($p<0.005$). This effect was antagonized by pretreatment with naloxone. Naloxone pretreatment alone had no significant effects on spatial learning, although escape latencies were higher than controls during sessions 3 and 4.

The time spent swimming in the training quadrant during the free-swim trial, in which the platform is absent, is shown in Fig. 3B. One-way ANOVA indicated a significant effect of treatment on this measure of spatial learning, $F(3,12)=7.24$, $p<0.005$. Control animals spent a majority of their time (>50%) swimming in the quadrant formerly containing the platform. In contrast, animals receiving DYN during acquisition spent significantly less time in the training quadrant compared to controls ($p<0.005$). This effect was completely antagonized by naloxone. Animals receiving naloxone alone during acquisition showed a small, but significant, decrease in time spent in the training quadrant compared to controls ($p<0.05$).

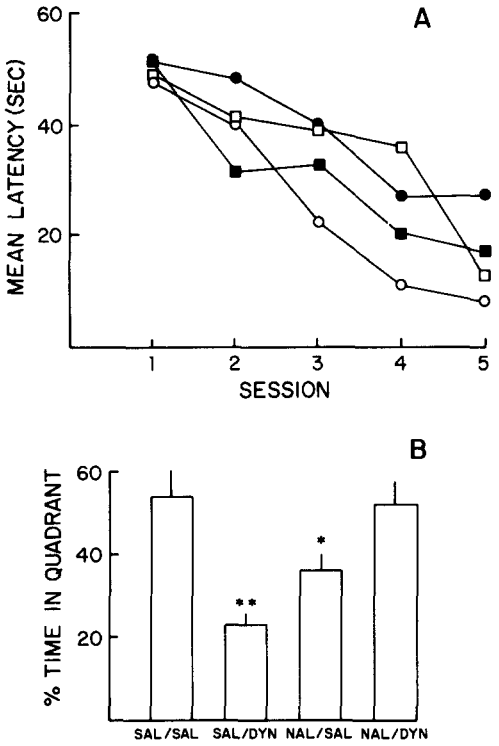

FIG. 3. Effect of dynorphin injections in the dorsal hippocampus on spatial navigation. (A) Mean latencies to escape onto a hidden platform averaged over 4 trials per session. Rats were pretreated with saline or naloxone (3 mg/kg, SC) 15 min prior to testing and received bilateral infusions of saline or dynorphin (20 μg/1 μl) immediately after the last trial. There was no drug treatment on session 5. ○: SAL/SAL (n=8); ●: SAL/DYN (n=8); □: NAL/SAL (n=4); ■: NAL/DYN (n=8). (B) Percent time (±S.E.M.) spent swimming in the training quadrant during the 60-sec free-swim trial. Significantly different from SAL/SAL control (*$p<0.05$; **$p<0.005$).

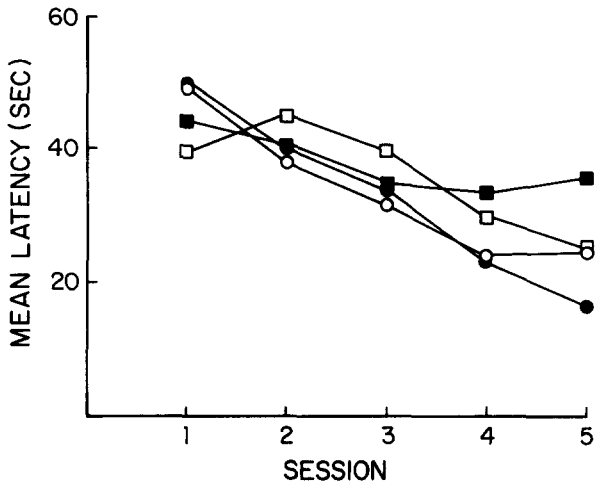

FIG. 4. Effect of dynorphin injections in the ventral hippocampus on spatial navigation. Data are mean latencies to escape onto a hidden platform averaged over 4 trials per session. Rats (n=8/group) were pretreated with saline or naloxone (3 mg/kg, SC) 15 min prior to testing and received bilateral infusions of saline or dynorphin (20 μg/μl) immediately after the last trial. There was no drug treatment on session 5. ○: SAL/SAL; ●: SAL/DYN; □: NAL/SAL; ■: NAL/DYN.

Ventral Injection

The effects of DYN injection into the ventral hippocampus in the water maze task are shown in Fig. 4. All groups showed a decrease in escape latencies with training [significant effect of session, $F(4,112)=17.8$, $p<0.0001$]. However, treatment with DYN had no effect on spatial learning (effect of treatment, pretreatment, and interactions not significant). A free-swim test was not performed since no differences were observed during acquisition.

Experiment 3

This experiment examined the effect of DYN on the retention of a nonspatial task using a passive avoidance procedure.

METHOD

Animals

Sixteen naive male F-344 rats were used. The animals were maintained as was described for Experiment 1.

Surgery

The surgery was identical to that done in Experiment 1. Thus, cannula tips were aimed at the CA3 region of the dorsal hippocampus (Fig. 1).

Procedure

The apparatus consisted of a trough-shaped stainless steel alleyway. The alleyway was divided into two compartments by a black guillotine door that could be raised or lowered by the observer. The small compartment (16 cm long) was illuminated while the larger compartment (33 cm long) was darkened.

Training consisted of placing the animal into the lighted compartment facing away from the door. After five seconds had elapsed the panel was raised and the latency for the animals to cross into the darkened compartment was recorded. Once the animals had crossed over, the door was lowered and a shock of 0.4 mA delivered for 3 seconds. Immediately following the shock the animal was removed and given bilateral infusions of either saline (n=8) or dynorphin (20 μg/1 μl, n=8). Forty-eight hours later the animals were given a retention test. The rat was placed into the lighted compartment facing away from the dividing door. After 10 sec had elapsed the door was raised and the latency to enter the dark compartment recorded to a maximum of 300 sec.

RESULTS

The results of testing DYN in a nonspatial task are shown in Fig. 5. On both the training and testing day there was no significant difference in step-through latencies between the saline- and DYN-treated animals (Mann-Whitney U-test, $p>0.20$).

DISCUSSION

Recent studies by Collier and co-workers (3,4) have shown that low-intensity stimulation of granule cells in the dorsal hippocampus results in memory disruption in a radial maze working memory task. In this procedure rats were trained to find food hidden in one arm of the maze on the first trial, and then return directly to the baited arm on the second trial. Stimulation of granule cells immediately after trial 1 with a 20-min interval before trial 2,

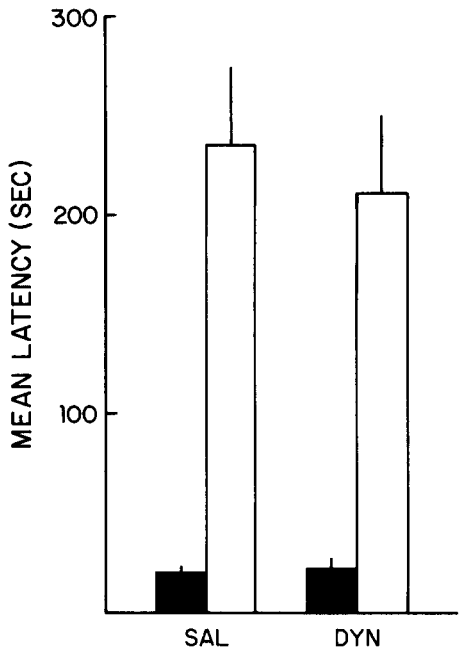

FIG. 5. Effects of dynorphin injections in the dorsal hippocampus on the retention of a step-through passive avoidance task. Saline or dynorphin (20 μg/1 μl) was injected bilaterally immediately after the training trial (n = 8/group). Data are mean latencies to enter the dark compartment during the training (filled bars) and test trials (open bars). Vertical lines indicate S.E.M.

resulted in a retrograde amnesia for the information learned in trial 1, which could be prevented by the administration of naloxone (4). Because the granule cells and their mossy fibers contain high levels of opioid peptides (6, 27, 39) and the effects of stimulation were naloxone-reversible, Collier *et al.* (4) suggested that opioid release at the mossy fiber-CA3 synapse mediated the amnesia effect. In order to directly test this hypothesis, we examined the effect of dynorphin injections into the CA3 region of the dorsal hippocampus on working memory using the procedure described above. Dynorphin is the opioid peptide which is found in the highest level in the granule cells and mossy fibers (27). In the present study we have shown that bilateral injections of dynorphin immediately after trial 1 result in a dose-related memory impairment when animals were tested 20 min later in trial 2. These results, therefore, support the hypothesis that opioid peptides are responsible for the retrograde amnesia observed after granule cell stimulation in previous studies (3,4). However, in the present study drug treatments were administered at a relatively short interval before testing on trial 2, and proactive drug effects on task performance cannot be ruled out.

Examination of specific choice behavior 20 min after drug treatment showed that while animals had poor memory for the baited arm, they did not make repeated arm entries during trial 2. The finding suggests that dynorphin injections impaired memory for information acquired during trial 1, but did not interfere with performance or memory for the arms they had just visited during trial 2.

The results described above suggest that dynorphin injection into the hippocampus interferes with spatial working memory. In order to determine whether endogenous hippocampal opioid peptides can influence spatial reference memory, we examined the effect of hippocampal dynorphin injections on the acquisitions of a Morris water maze task. Because the drug treatment was given immediately after animals were removed from the maze, with a relatively long (24 hr) interval before testing was resumed on the next day, it was unlikely that dynorphin would have any proactive effects on task performance. We found that postsession dynorphin injections into the dorsal hippocampus impaired acquisition of this spatial reference memory task, and that this effect could be blocked by naloxone.

Two aspects of the results from the Morris water maze task suggest that dynorphin was acting specifically on memory processes. First, animals receiving dynorphin injection during sessions 1 through 4 continued to perform poorly on session 5 in the absence of drug treatment, suggesting that poor performance was not related to nonspecific factors including handling and the manipulations associated with drug injection. Second, animals treated with dynorphin also performed poorly during the free-swim test on day 6, showing little bias for the quadrant of the maze that had previously contained the platform. Because the percentage of time spent in the training quadrant is not a latency measure, the free swim provides an assessment of spatial learning which is not confounded by nonspecific effects on performance or locomotor ability.

Systemic naloxone administration alone appeared to have a small but significant effect on spatial learning. This effect was less evident during the ventral injection study. It is possible that this dose of naloxone administered systemically is acting at sites other than the CA3-mossy fiber synapse to interfere with learning in this task. Further studies will be necessary to examine the influence of systemically and centrally administered naloxone on spatial learning in the water maze.

In contrast to dorsal administration, injection of dynorphin into the CA3 region of the ventral hippocampus had no effect on spatial learning in the water maze. The impetus for examining both dorsal and ventral sites in the hippocampus arose from the studies of Lee *et al.* (24) which demonstrated that regional differences in opioid-induced hippocampal excitation. Specifically, Lee *et al.* (24) showed that the injection of a mu agonist into the ventral, but not dorsal, hippocampus produced convulsions in rats. The present results, showing a a behavioral effect of dynorphin after injection into the dorsal, but not ventral, hippocampus suggest a functional dichotomy between the different regions of the hippocampus. Lesion studies also suggest functional differences between the dorsal and ventral hippocampus. Lesions of the dorsal hippocampus produced deficits in a place-learning task (36) and DRL performance (23) while ventral hippocampal lesions had little effect. However, there are alternative explanations for the lack of effect of dynorphin in the ventral hippocampus. In the present study, the effects of dynorphin in the ventral hippocampus were only examined in one spatial task. In addition, a single dose and site of administration was used. The possibility remains that the ventral hippocampus is involved in spatial information processing, but is relatively less sensitive to pharmacological manipulation with dynorphin.

Our results showing that hippocampal dynorphin administration impairs spatial learning in the Morris water maze are interesting in light of a recent study by Jiang *et al.* (22) on age-related changes in hippocampal dynorphin content. In initial experiments, Jiang *et al.* (22) observed that dynorphin was significantly elevated in the hippocampus of aged rats compared to young controls. Subsequent behavioral experiments revealed an age-related deficit in acquisition of a spatial task in the water maze. When the aged animals were subdivided into an impaired group (which performed outside the range of the young controls) and an unimpaired group (which acquired the task as well as young controls), it was found that hippocampal dynorphin levels were significantly elevated in the aged/impaired group, but not in the aged/unimpaired group compared to young controls. These re-

sults, in combination with those of the present study, indicate that spatial learning is impaired by high levels of hippocampal dynorphin, and suggest that spatial learning deficits in aged animals may be related to a neuronal dysfunction which leads to elevated levels of dynorphin in the hippocampus. One possibility involves the basal forebrain cholinergic system, which exhibits age-related neurodegeneration (10,26), and has been shown to influence the level of opioid peptides in the hippocampus (12).

The hippocampus is believed to be important for spatial learning and memory in rats. Our results show an impairment of memory in a spatial task, but no impairment in a nonspatial task. Posttraining injection of dynorphin into the dorsal hippocampus had no effect on the retention of a step-through passive avoidance task. These results are in accord with previous studies examining the effects of dynorphin in avoidance tasks in rats. Izquierdo *et al.* (19) found that posttraining intraperitoneal administration of dynorphin did not affect retention of active or passive avoidance tasks. Similarly, Tilson *et al.* (37) reported that posttraining intracerebroventricular injection of dynorphin had no effect on retention of a passive avoidance task. These results suggest that the effects of dynorphin may be specific to tasks with a large spatial component. However, intraperitoneal administration of dynorphin in mice significantly impaired retention of a passive avoidance task, without altering retention of a Y-maze discrimination task or habituation of exploratory activity (16). Thus, the effect of dynorphin appears to be dependent on the species of animal used, task, and route of administration.

The data reported here suggest that dynorphin acts in the hippocampus to alter spatial memory specifically. This effect is reversible by naloxone, indicating a site of action at the opiate receptors. The hippocampus contains mu, delta, and kappa opioid binding sites which have discreet distributions. The mu-sites are present at the highest concentration, while delta-sites are less dense and there are very few kappa-sites (35,39). While it has become apparent that dynorphin may be the endogenous ligand for the kappa receptor (2,14), it is not selective and also binds to mu-sites (21,38). Recent studies suggest that dynorphin acts at mu and kappa receptors in the hippocampus to increase the excitability of hippocampal pyramidal cells in CA1 (32,34) via the disinhibition of inhibitory interneurons. The effects of dynorphin may be similar in CA3 (25). Thus, the changes in memory observed in the present study may be related to an increase in pyramidal cell excitability in the CA3 region of the dorsal hippocampus. The role of dynorphin in the modulation of neuronal excitability and information processing in other regions of the hippocampus remain to be determined.

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