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Acquisition of a Morris Water Maze Task Is Impaired During Early But Not Late Withdrawal From Morphine

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DOUGHERTY, K. D., T. J. WALSH, S. BAILEY, S. SCHLUSSMAN AND K. GRASING. *Acquisition of a Morris water maze task is impaired during early but not late withdrawal from morphine.* PHARMACOL BIOCHEM BEHAV **55**(2) 227–235, 1996.—Behavioral changes in male Sprague–Dawley rats during early and late withdrawal from morphine were investigated. Morphine-treated subjects (M) were implanted (SC) with osmotic pumps containing 2.0 ml of a 159 mg/ml morphine sulfate solution while control subjects (C) received sham implants. Implants were removed after 7 days. M subjects exhibited a significant decrease in body weight during withdrawal that recovered by 21 days after pump removal. Beginning 1 or 21 days following pump removal, subjects were tested for 8 days in a Morris water maze (MWM) task. M subjects trained in the MWM during early withdrawal exhibited significantly longer escape latencies than C subjects. However, during sequential probe trials, the same subjects exhibited a significant preference for the target quadrant of the maze and executed accurate searches for the escape platform. Though these subjects failed to locate the platform as efficiently as controls during training trials, they learned the location of the escape platform. M rats trained during late withdrawal exhibited no deficits in any measure of MWM performance relative to C subjects. The data suggest that a variety of processes involved in the acquisition and performance of the MWM task are differentially affected during early withdrawal from morphine. **Copyright © 1996 Elsevier Science Inc.**

Morphine withdrawal Morris water maze Opiates Declarative memory Procedural memory Spatial memory

A wide variety of hormones, growth factors, and drugs can affect neurotransmitter systems and influence memory. The available evidence supports the proposal that central opioid systems modulate learning and memory processes because acute injection of opiate agonists impairs memory while opiate antagonists enhance memory (3,6–8,35). Acute posttraining administration of opiate antagonists, like naloxone, naltrexone, and diprenorphine, enhances retention of recently acquired shock-motivated avoidance responses (7,8), while posttraining administration of morphine impairs such retention (10). Similarly, acute administration of naloxone and naltrexone prior to daily training enhance (3,6,8,33), while morphine impairs Morris water maze (MWM) acquisition and performance (18). Such morphine-induced impairments are reversed by naloxone (18). Although these data indicate that acute modulation of opiate activity influences memory, they present interpretive difficulties. Decrements in task performance may be attributed to shifts in the reward value of either aversive or appetitive stimuli rather than interference with memory (35). Further, any task requiring locomotion is problematic given the ataxia and sedation produced by acute morphine administration (13). In contrast, with a chronic dosing regimen tolerance to opiates develops and their acute behavioral effects diminish (13,32).

With the depressant and analgesic effects of morphine virtually eliminated in opiate tolerant subjects, it is possible to observe behavioral deficits that may be specifically related to opiate modulation of memory. For example, Spain and Newsom (34) established morphine tolerance in rats prior to behavioral testing. While continuing to receive daily doses of morphine, the rats exhibited lasting impairments in the

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acquisition of a radial arm maze task (RAM), widely considered to be a highly sensitive measure of working memory (23). They were also profoundly impaired in acquiring the Y maze choice escape task, which is considered to provide an assessment of rule-based learning (11,34). Subjects that had been trained in the RAM prior to the establishment of morphine tolerance failed to display RAM impairments, indicating intact working memory even while receiving daily doses of morphine. When chronic morphine treatment was discontinued, all subjects that had previously received morphine displayed an increase in the number of errors per trial in the RAM task during the first week of morphine withdrawal. In subsequent weeks, RAM performance of these subjects gradually improved and approximated that of controls at the end of testing (34). In summary, while generating data largely in agreement with that of acute administration studies, these more recent studies suggest that the opioid hypofunction that occurs during withdrawal (1,29,31) is also associated with behavioral impairments. Spain and Newsom's findings are in agreement with other animal studies and with research conducted in clinical settings. Systemic as well as ICV injection of naloxone can alter learning in a dose-dependent manner with high doses impairing performance in both spatial (18) and aversively motivated tasks (7). Clinical studies also show that high naloxone doses impair certain types of memory (4,36). For example, human subjects who received either acute or chronic doses of naloxone ranging between 2 and 4 mg/kg exhibited significant impairment on free recall tasks (4), while lower doses (0.3, 0.4) had no effects on free recall (4,36). Therefore, results from both clinical and basic research suggest that naloxone, and perhaps opiate antagonists in general, modulate memory processes in a complex manner that may be characterized by an inverted U-shaped function, rather than a simple linear relationship (7). The failure to clearly characterize this relationship may be due to the fact that most studies employ acute pre or posttraining naloxone doses within a limited range between 0.1 and 3.0 mg/kg (3,6).

The findings of Spain and Newsom (34) as well as those of Cohen (4) and Tariot (36), further suggest that the opioid system may modulate substrates that mediate specific types of memory. For instance, the results of Spain and Newsom suggest that opiate activity does not interfere with working memory as assessed on the RAM, but rather with the acquisition of rules or procedures necessary to perform both the RAM and Y maze tasks (34). In contrast, normal human subjects given 2 mg/kg of the opiate antagonist naloxone exhibited impairments on a number of cognitive measures, such as free recall of word lists and recognition of previously heard words, both of which might be considered assessments of working memory (4). However, these subjects also exhibited other signs of behavioral toxicity, reporting altered mental states, confusion, irritability, loss of appetite, and depression in the evening and days following naloxone administration (4). Consequently, it is difficult to conclude that these impairments reflect selective deficits in working memory rather than nonspecific behavioral toxicity. Though the results of both animal and human investigations suggest that opiates modulate memory, the nature of the memory system or systems affected remains to be determined.

Given the strength of the existing evidence that opiates bidirectionally modulate memory, it is also of particular interest to test this hypothesis under more clinically relevant conditions. Behavioral testing is most often conducted following acute administration of opiate agonists or antagonists. Similarly, behavioral effects associated with withdrawal are most often studied during naloxone-precipitated opioid hypofunction. Alternatively, a drug cessation approach may be useful because it is more likely to create parameters of opioid hypofunction that more closely resemble those experienced in human opiate users. While it has been shown that both naloxone-induced withdrawal and drug cessation lead to rebound decreases in striatal dopamine release and concommittant increases in acetylcholine release in a variety of brain regions (1,29,31), the intensity and time course of naloxone-induced withdrawal is biochemically distinct from the withdrawal syndrome that develops upon abrupt halt of opiate administration (2). It follows that observations conducted under drug cessation conditions may be more applicable to human subjects undergoing opiate withdrawal.

In the present investigations, the behavioral effects of abrupt withdrawal of chronic morphine administration in male rats were explored using the MWM task, which is sensitive to disruptions in spatial memory. Control subjects rapidly acquire this task, therefore allowing possibly transient spatial memory deficits limited to early morphine withdrawal to be observed. This task is also useful in the differential assessment of qualitatively distinct components of memory, such as learning the spatial location of the escape platform (i.e., declarative memory) and the acquisition of efficient strategies in searching for the escape platform (i.e., procedural memory) (24).

EXPERIMENT 1

The present experiment assessed acquisition of the MWM task in subjects, which were experiencing the first 7 days of morphine withdrawal.

Method

Subjects. Male Sprague–Dawley rats (Harlan–Sprague–Dawley, Inc., Indianapolis, IN) approximately 60–65 days of age and weighing 250–275 g at the start of testing served as subjects. They were singly housed in suspended wire mesh stainless steel cages in a climate controlled colony room with a 12 L:12 D cycle, with lights on at 0700 h. Food (Purina Rat Chow, #1086) and water were available ad lib. Body weights were monitored daily throughout the study.

Drug Administration. Subjects were anesthetized with a ketaset/rompun solution (Schein, Inc.; Sigma Chemical Co.) injected intramuscularly. In addition, a 1.0 mg/kg dose of naloxone was administered prior to implanting pumps to prevent additive respiratory depressant effects of morphine and anesthetics. To avoid dissolution of morphine, drug solution was heated prior to filling pumps and then maintained at 37°C until implantation. In subjects assigned to morphine treatment (M), an approximately 4 cm incision was made along the midline at the back of the neck and an osmotic minipump (Alza model 2ML1, Palo Alto, CA) filled with 2.0 ml of 159 mg/ml morphine sulfate (National Institute on Drug Abuse, Rockville, MD), equivalent to 60 mg/ml free base was subcutaneously implanted. The pumps were allowed to deliver 1.89 mg morphine sulfate per hour over a period of seven days. Control subjects (C) received an identical implantation procedure except that two sterile microcentrifuge tubes were used in place of osmotic minipumps. After 7 days, pumps and sham implants were removed under an additional period of ketamine/rompun anesthesia without naloxone treatment. Wounds were closed with stainless steel wound clips. Subjects were allowed to regain consciousness before being returned to their home cages.

Apparatus. The MWM consisted of a metal circular pool 120 cm in diameter and 56 cm deep. The pool, which was painted entirely white, was filled with tap water made opaque by the addition of nontoxic white paint (Pearl, Tempera). Four

equally spaced start points around the outer edge of the pool were designated as start points and divided the pool into four equally spaced imaginary quadrants. A removable platform was placed in a given quadrant of the pool. The platform consisted of a steel base column having a height of 35 cm, topped by a grooved, circular Plexiglas disk 15.24 cm in diameter. The area occupied by the escape platform surface was defined as the target. The target quadrant was defined as that quadrant of the pool in which the escape platform consistently occurred throughout training. Water level of the pool was maintained so that the disk was two cm below the surface and water temperature was maintained at 19–21°C. The pool was located in a small observation room adjacent to the colony room that contained many prominent extra maze cues, such as a chair, shelving, and wall hangings, which remained in the same location throughout testing.

MWM Training. Twenty four hours following pump removal rats were habituated to the MWM. For each day of training, subjects were placed in nalgene holding bins, with four subjects to each bin, and placed in the adjacent behavioral testing room. For the habituation trial, a subject was placed into the center of the pool and allowed to swim for 60 s. The rat was then removed from the water, the target placed in the center of the pool and the rat placed onto the target. After sitting on the target for 30 s, the subject was placed into a stainless steel cage, exactly the same type as used for home cages, with a wire mesh lid, for a 15-s intertrial interval (ITI). The rat was then returned to the group holding bin until all subjects had experienced an habituation trial, then returned to its home cage.

For the next 8 consecutive days, each rat was trained in the MWM for four trials/day. The hidden platform (i.e., target) was located in the center of a given quadrant of the pool (i.e., target quadrant) throughout training and subjects were required to locate the target to escape from the water. A trial began by placing the subject into the pool with its snout facing the wall of the pool. The subject was allowed to search for the target for up to 60 s. If the rat failed to find the target within 60 s, it was placed there by the experimenter for a period of 30 s. After 15 s in the ITI bin, a new trial was initiated. This was repeated until four trials had been completed. In this manner, all subjects experienced each start point in the same pseudorandom order on each day of training. Latency to reach the target was recorded for each trial. Each subject's ability to retain and recall information about target location learned during the previous day's training was expressed as percent of the previous day's fourth trial latency (i.e, [latency on the first trial of a given training day] divided by [latency on the fourth trial of the previous day]. In this way, each subject obtained seven forgetting scores. Low scores indicate little or no forgetting of information learned the previous day. However, high scores indicate that forgetting has taken place, because such scores occur only when latency on the first trial of a given day approaches or exceeds that of the fourth trial of the previous day.

On days 1, 4, and 8 of MWM training, probe trials were conducted in addition to the four regular training trials. As a result, MWM training days 1, 4, and 8 were referred to as probe days. Probe trials occurred between the second and third training trials for a given day and served to allow assessment of each subject's acquisition of the target location. During a probe trial, the target was removed from the pool and the rat, placed into the pool at a randomly selected start point, was allowed to swim for 30 s. The rat was then removed from the pool and placed into the ITI bin for 15 s. During this time the target was replaced to its usual position. The next training trial was then initiated. Each subject's performance during both probe and training trials was recorded on days 1, 4, and 8 using a video camera interfaced with a personal computer and a digital tracking device (Chromotrack, San Diego Instruments). Analysis of the digitized training trials yielded: 1) average pathlength (PL) across all four training trials and 2) average swim speed (SS) across all four training trials. Analysis of the digitized probe trials, during which the target was not available for escape, yielded average distance from the target (ADT) and percent time in the target quadrant (%T) (probe). Further analysis of the digitized probe trials was conducted to more thoroughly characterize target search behavior and swimming patterns across training. For this analysis, the maze was conceptualized as divided into three concentric annuli: 1) an outer annulus occupying the space between the pool wall and the edge of the target (15 cm wide); 2) a middle annulus (24 cm wide), which contained the target; and 3) an inner annulus, occupying the center-most portion of the pool (40 cm wide). The amount of time spent and the number of entries into each annulus were recorded for each of the probe trials, conducted on days 1, 4, and 8. Finally, the number of times a subject crossed over the target location during each probe trial was recorded.

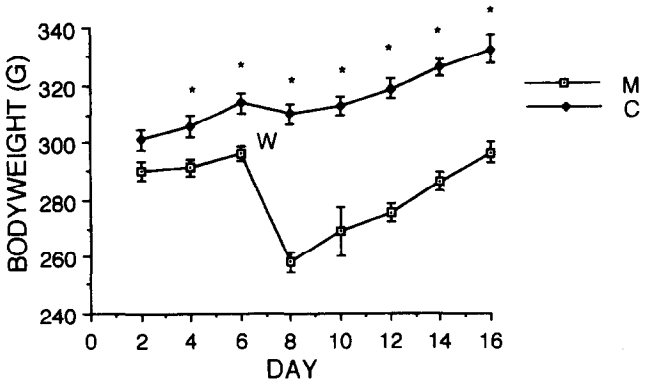

FIG. 1. Morphine (M)-treated rats displayed significant decreases in body weight, which persisted throughout early withdrawal testing. *Significantly different from morphine (M), $p < 0.05$, Fisher's PLSD tests. W = Withdrawal of osmotic pumps, day 7; C = controls.

Results

Body Weight. An independent groups *t*-test demonstrated that body weight did not differ between the M or C groups prior to pump implantation, $t(21) = 0.32, p > 0.05$. A 2 (M vs. C) × 16 (day) repeated measures ANOVA on postsurgery body weights demonstrated that there was a main effect for drug treatment, with M-treated rats maintaining significantly lower body weights than C-treated rats, $F(1, 21) = 37.59, p < 0.01$. There was also a significant day effect, indicating that subjects gained weight over time, $F(15, 315) = 18.30, p < 0.01$. However, a significant drug × day interaction was also observed, $F(15, 315) = 18.72, p < 0.01$, indicating that significant body weight differences between the M- and C-treated subjects emerged during testing. Fisher PLSD post hoc comparisons showed that M rats weighed significantly less than C rats beginning on day 4 of drug administration and throughout the remainder of testing (Fig. 1).

MWM Training. Latency: During early withdrawal, M- and C-treated subjects displayed comparable latencies to reach the escape target on the first trial of MWM training (mean M

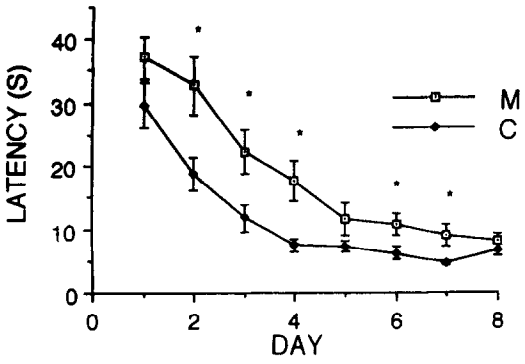

FIG. 2. Morphine (n = 12)-treated rats exhibited significantly longer latencies to reach the escape target than control subjects (n = 10) across MWM training days. All subjects displayed similar latencies on the last day of testing. *Significantly different from controls (C). $p < 0.05$, Fisher's PLSD tests.

latency = 36.37 ± 7.06 s; mean C latency = 41.08 ± 5.61), $t(21) = 0.53$, $p > 0.05$. Latency data were analyzed in one, two, and four trial blocks using two-factor repeated-measures ANOVAs. Because the outcomes of each of these ANOVAs were comparable, only the results of the latency by four trial block analysis are presented here. A significant main effect for drug treatment was observed. M-treated rats exhibited significantly longer average latencies (s) to reach the target than control subjects, $F(1, 12) = 8.17$, $p < 0.01$. However, all subjects displayed decreases in latency to reach the target over training, $F(7, 84) = 29.26$, $p < 0.01$. There was an interaction between drug treatment and MWM training day, $F(7, 84) = 2.69$, $p < 0.01$, indicating that C- and M-treated subjects learned to reach the target at different rates. Posthoc Fisher PLSD tests showed that on training days 2, 3, 4, 6, and 7, M subject latencies were significantly greater than those of controls. The latencies of the groups did not differ on the last day of MWM training (Fig. 2).

Forgetting Scores: A 2 (M vs. C) × 7 (training day) repeated-measures ANOVA indicated that M- and C-treated subjects displayed similar differences in latency between the last trial of a given training day and the first trial of the following day, $F(1, 21) = 0.67$, $p > 0.05$. Scores also did not change across the period of MWM training, $F(6, 126) = 0.741$, $p > 0.05$, indicating that performance was stable from the end of training on one day to the start of training on the next day. No interaction between drug treatment and training day was observed, $F(6, 126) = 1.028$, $p > 0.05$.

Percent Time in Target Quadrant (%T): During 30-s probe trials conducted on days 1, 4, and 8, early withdrawal M-and C-treated rats displayed comparable preferences for the target quadrant of the pool. No main effects for drug treatment were found in the percent time subjects searched the target quadrant of the pool during the 30 s probe, $F(1, 19) = 0.03$, $p > 0.05$. Similarly, all subjects displayed significant increases in %T across the probe days 1, 4, and 8, $F(2, 38) = 17.88$, $p < 0.01$. There was no significant interaction between drug treatment and probe day, $F(2, 38) = 0.99$, $p > 0.05$ (Fig. 3a).

Average Distance From Target (ADT): A 2 (M vs. C) × 3 (probe day) repeated-measures ANOVA demonstrated that during early withdrawal M- and C-treated subjects swam at similar distances from the target during the intermittent probe trials, $F(1, 19) = 0.12$, $p > 0.05$. All subjects displayed significant decreases in ADT across the three probe trials, $F(2, 38) = 64.52$, $p < 0.01$. There was no significant interaction between drug treatment and probe day, $F(2, 38) = 1.95$, $p > 0.05$ (Fig. 3b).

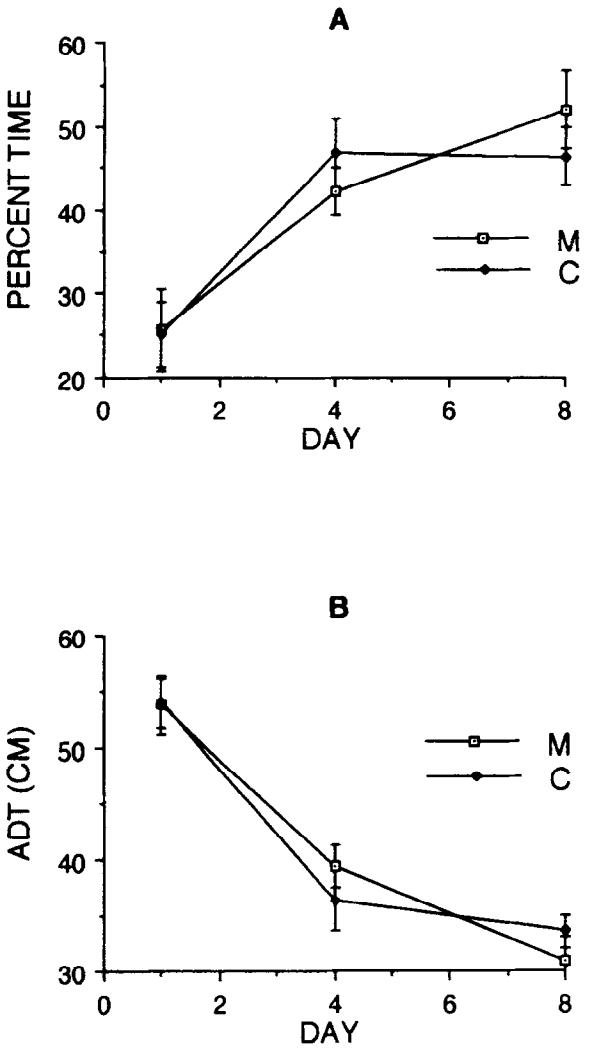

FIG. 3. (A) During 30-s probe trials conducted on training days 1, 4, and 8, C- and M-treated rats spent a comparable percent of their time (%T) in the area of the pool that previously contained the escape platform. (B) Early withdrawal M-treated rats also swam a similar average distance from the target (ADT) area as controls (C) during probe trials.

Pathlength: During early withdrawal, M-treated rats displayed significantly longer average pathlengths to reach the target during training trials on probe days 1, 4, and 8 than those displayed by control subjects, $F(2, 42) = 4.80$, $p < 0.05$. Post hoc comparison revealed that on day 4 M-treated rats displayed significantly longer average pathlengths than C-treated subjects (Fig. 4a). However, a significant main effect for probe day was also obtained, indicating that both M- and C-treated rats displayed significant decreases in average pathlength across probe days, $F(2, 42) = 61.41$, $p < 0.01$. There was no significant interaction between drug treatment and probe day, $F(2, 42) = 1.56$, $p > 0.05$.

Swim Speed: During early withdrawal, a 2 (M vs. C) × 3 (probe day) repeated measures ANOVA demonstrated that

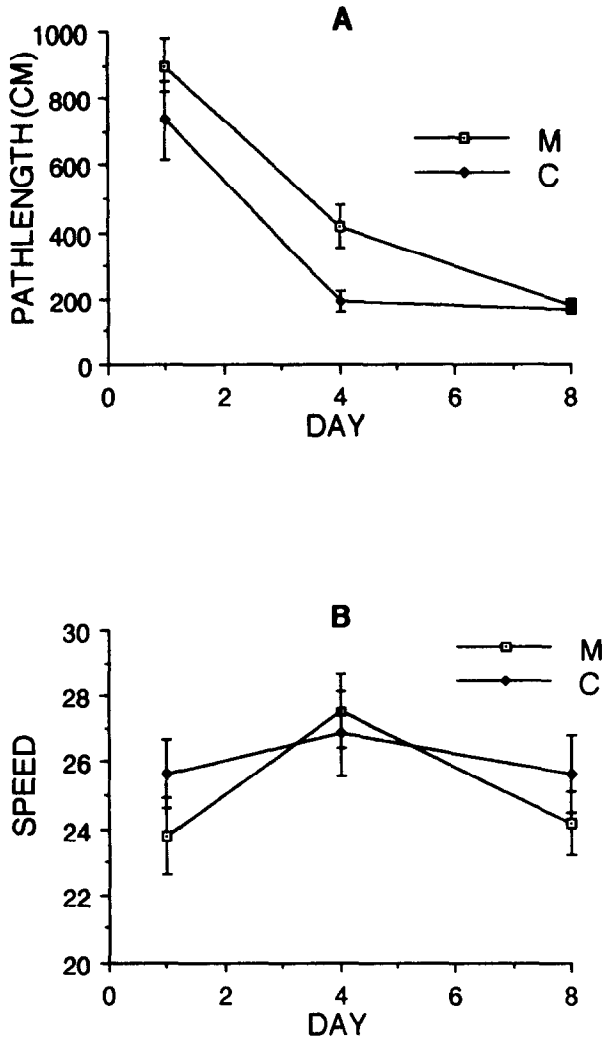

FIG. 4. (A) M-treated rats displayed significantly longer average pathlengths to reach the escape platform during training trials on days 1, 4, and 8 than those displayed by C subjects. (B) M and C subjects swam at comparable speeds during training trials on days 1, 4, and 8.

M- and C-treated rats swam at comparable average speeds during training trials conducted on probe days 1, 4, and 8, $F(1, 21) = 0.65, p > 0.05$. While a main effect for probe day was found, $F(2, 42) = 4.02, p < 0.02$, no interaction between drug treatment and probe day was observed, $F(2, 42) = 0.95$, $p > 0.05$. Fisher PLSD post hoc analysis showed that swim speeds for all subjects were faster on the fourth day of training than other days on which swims were tracked (Fig. 4b).

Outer Annulus: A 2 (M vs. C) × 3 (probe day) repeated-measures ANOVA revealed no effect of drug treatment, $F(1, 20) = 0.631, p > 0.05$, and no interaction, $F(2, 40) = 0.493$, $p > 0.05$, between treatment and probe day on the amount of time spent in the outer annulus during probe trials. All subjects spent less time in the outer annulus across the three probe trials, $F(2, 40) = 101.965, p < 0.05$. Interestingly, a main effect of drug treatment on the number of outer annulus entries was observed, $F(1, 20) = 4.51, p < 0.05$, revealing that M-treated rats executed significantly fewer entries into the outer annulus than did controls. However, all subjects regardless of drug treatment, tended to execute progressively fewer outer annulus entries as training progressed, $F(2, 40) = 3.54$, $p < 0.05$. A significant interaction between drug treatment and probe day was observed, $F(2, 40) = 3.62, p < 0.05$ (Fig. 5a and b).

Middle Annulus: Drug treatment was not found to have an effect on the amount of time spent in the middle annulus during probe trials, $F(1, 20) = 0.418, p > 0.05$. All subjects spent increasing amounts of time in the middle annulus, which contained the target location, across probe trials, $F(2, 40) = 96.850, p < 0.05$. A significant interaction between drug treatment and probe day was observed, $F(2, 40) = 3.07, p < 0.05$, with M-treated subjects spending less time than controls in the middle annulus on probe day 1, but behaving similarly to controls throughout the rest of training (Figs. 5c and d). Based on a 2 (M vs. C) × 3 (probe day) repeated-measures ANOVA, no main effect of drug treatment on number of middle annulus entries was observed, $F(1, 20) = 3.52, p > 0.05$, nor was a significant interaction between drug treatment and probe day demonstrated, $F(2, 40) = 0.36, p > 0.05$. All subjects executed increasing numbers of middle annulus entries across training, $F(2, 40) = 19.39, p < 0.05$.

Inner Annulus: No main effect of drug treatment on time spent in the inner annulus was observed, $F(1, 20) = 1.03, p > 0.05$, nor was an interaction between drug treatment and probe day demonstrated, $F(2, 40) = 2.75, p > 0.05$. All subjects spent more time in the inner annulus during the probe trials, $F(2, 40) = 17.89, p < 0.05$. Similarly, number of inner annulus entries was not effected by drug treatment, $F(1, 20) = 1.625$, $p > 0.05$, and there was no interaction between drug treatment and probe day, $F(2, 40) = 0.966, p > 0.05$. All subjects executed increasing numbers of inner annulus entries across the probe trials, $F(2, 40) = 10.37, p < 0.05$.

EXPERIMENT 2

It has been shown that changes in body weight following from withdrawal of chronic morphine administration return to levels resembling those of control subjects by 21 days after drug withdrawal. Changes in spatial memory processing that may have accompanied early withdrawal toxicity would also be expected to return to control levels at this time point. In Experiment 2, the MWM task was used to assess whether spatial memory processing of subjects that have experienced over 21 days of morphine withdrawal resembles that of sham-treated subjects.

Method

Subjects. Male Sprague–Dawley rats of the same age and weight upon arrival as those used in Experiment 1 served as subjects. They were housed and fed in exactly the same manner as those subjects in Experiment 1. Body weights were monitored daily.

Drug Administration. Subjects underwent surgical procedures and a morphine dosing regimen identical to those used for Experiment 1.

MWM Apparatus and Training. The apparatus, behavioral testing, and data analysis were as described in Experiment 1, except that rats began MWM testing 21 days following morphine withdrawal.

Results

Body Weight. Using a 2 (M vs. C) × 27 (day) repeated-measures ANOVA, no main effects for drug treatment on

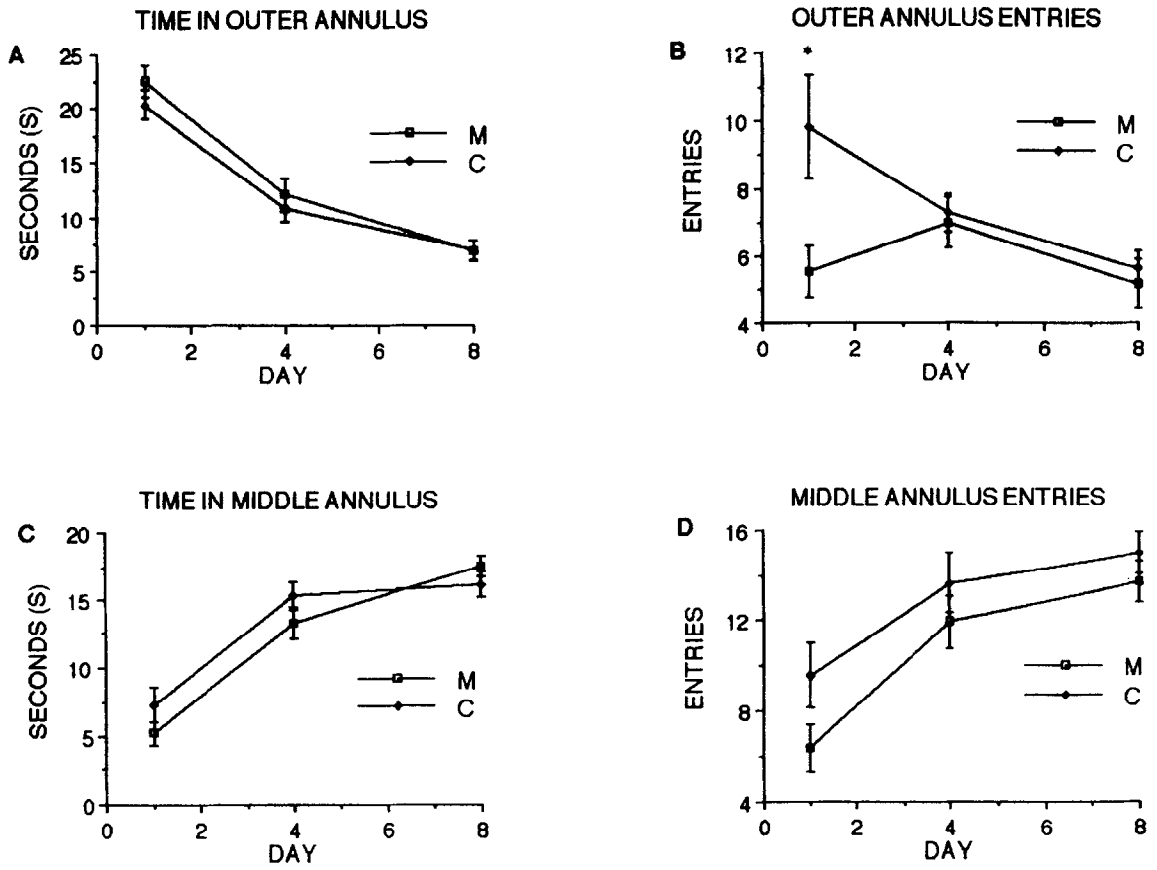

FIG. 5. (A) Both M- and C-treated subjects spent comparable, progressively decreasing amounts of time in the outer annulus across training. (B) M-treated rats executed significantly fewer entries into the outer annulus during the first probe trial than did C subjects. *Significantly different from C subjects, $p < 0.05$, Fisher's PLSD test. (C) M-treated subjects spent less time in the middle annulus than did C-teated subjects during the first probe trial. Time in the middle annulus was comparable throughout the remainder of testing. (D). M- and C-treated subjects executed comarable, progressively increasing numbers of middle annulus entries across MWM training.

body weight were observed, $F(1, 20) = 2.88$, $p > 0.05$. This difference between the results of Experiment 1 can be accounted for by considering the fact that in Experiment 2 body weights were monitored over a longer period than in Experiment 1 and that during much of this time, the body weights of M-treated rats did not differ from those of controls. As in Experiment 1, a significant day effect was observed, indicating that all subjects gained weight during the investigation period, $F(26, 480) = 116.66$, $p < 0.01$. However, a significant drug $\times$ day interaction was demonstrated, $F(26, 520) = 6.32$, $p < 0.01$. Based on Fisher PLSD post hoc analysis, significant decreases in body weight in the M-treated group were evident beginning on testing day 8 and continuing through testing day 16. Thereafter, body weights of M-treated subjects were similar to those of controls (Fig. 6).

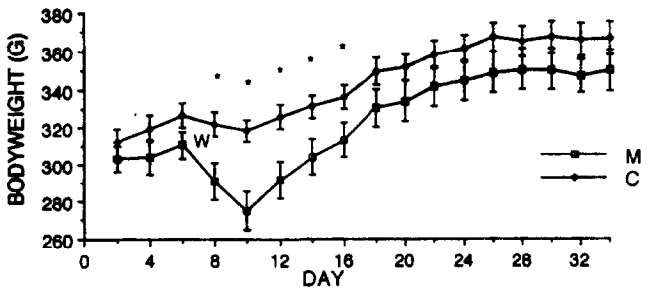

FIG. 6. Morphine-treated rats displayed a transient decrease in body weight through the first 16 days of withdrawal. Body weights of morphine-treated subjects were similar to those of controls during MWM training. W = morphine withdrawal, day 7. *Significantly different from controls, $p < 0.05$, Fisher PLSD.

MWM Training. Latency: M-treated rats experiencing MWM training during late withdrawal exhibited latencies to reach the escape platform similar to those of controls throughout training, $F(1, 22) = 0.72$, $p > 0.05$. Training had a significant effect on latency, $F(7, 154) = 44.88$, $p < 0.01$. There was no significant interaction between drug treatment and training, $F(7, 154) = 1.12$, $p > 0.05$.

Forgetting Scores: Based on a 2 (M vs. C) $\times$ 7 (training day) repeated-measures ANOVA, neither drug treatment, $F(1, 21) = 0.008$, $p > 0.05$, nor training day, $F(6, 126) = 1.333$, $p > 0.05$, was found to have an effect on retention scores. No interaction between drug treatment and training day was demonstrated, $F(6, 126) = 1.660$, $p > 0.05$.

Percent Time in Target Quadrant (%T): No main effects for drug treatment were found in the percent time subjects

searched the target quadrant of the pool during the 30 s probe, $F(1, 18) = 0.25$, $p > 0.05$. Similarly, all subjects displayed significant increases in %T across probe trials, $F(2, 36) = 16.45$, $p < 0.01$. There was no significant interaction between drug treatment and training, $F(2, 36) = 0.73$, $p > 0.05$.

Average Distance from Target (ADT): During late withdrawal, M- and C-treated subjects swam at similar average distances from the target during the intermittent probe trials, $F(1, 18) = 0.76$, $p > 0.05$. All subjects displayed significant decreases in ADT across the three probe trials, $F(2, 36) = 58{,}59$, $p < 0.01$. There was no significant interaction between drug treatment and training, $F(2, 36) = 1.69$, $p > 0.05$.

Pathlength: During late withdrawal no significant main effects for drug treatment or drug × MWM day interaction based on pathlength measures were found. All subjects displayed similar decreases in average pathlength to the target with training, $F(2, 34) = 63.08$, $p < 0.01$. There was no significant interaction between drug treatment and training, $F(2, 34) = 0.74$, $p > 0.05$.

Swim Speed: Swim speed measures during late withdrawal were submitted to a 2 (M vs. C) × 3 (probe trial) repeated-measures ANOVA. No main effect for drug treatment was observed, indicating that all subjects tested during late withdrawal swam at comparable speeds during probe trials, $F(1, 18) = 0.41$, $p > 0.05$. Interestingly, both groups exhibited significant increases in swim speed across training during late withdrawal, $F(2, 36) = 8.41$, $p < 0.01$. There was no significant interaction between drug treatment and training, $F(2, 36) = 0.07$, $p > 0.05$.

Subjects Excluded from Further Behavioral Analysis: Following initial analysis, 11 subject's digitized probe trial data were lost due to a computing error. Consequently, these subjects could not be included in the analysis of annulus search patterns.

Outer, Middle and Inner Annuli Searches: Using a series of 2 (M vs. C) × 3 (probe day) repeated measures ANOVAs, it was shown that neither drug treatment, nor an interaction between drug treatment and probe day had a significant effect upon either amount of time spent or number of entries into any of the annuli (all Fs < 1.0, all p-values > 0.05). While time spent in the outer annulus was found to decrease across probe days, $F(2, 16) = 1.587$, $p < 0.05$, number of entries into the outer annulus did not change across testing, $F(2, 16) = 0.682$, $p > 0.05$. Both time spent and number of entries into the middle annulus increased across probe days, $F(2, 16) = 10.6$, $p < 0.05$; $F(2, 16) = 25.458$, $p < 0.05$. Similarly, both time spent and number of entries into the inner annulus increased across probe days, $F(2, 16) = 9.948$, $p < 0.05$; $F(2, 16) = 22.1$, $p < 0.05$.

Target Crossings: A 2 (M vs. C) × 3 (probe day) repeated-measures ANOVA demonstrated that drug treatment had no effect on the number of target crossings executed during each of the probe trials, $F(1, 8) = 0.001$, $p > 0.05$. No interaction between drug treatment and probe day was observed, $F(2, 16) = 0.65$, $p > 0.05$. All subjects executed increasing numbers of target crossings across probe days, $F(2, 16) = 9.72$, $p < 0.05$.

DISCUSSION

Acquisition of the MWM task was impaired during early, but not late, withdrawal from morphine. Control subjects in both experiments and morphine-treated subjects trained during late withdrawal rapidly acquired the MWM task, with latencies to reach the hidden platform averaging between 3–8 s by day 6 of training. These subjects also demonstrated steady increases in %T, as well as decreases in ADT and average pathlength, indicating that controls accurately swam to and searched for the target on probe trial days. M-treated rats trained during early withdrawal demonstrated probe trial %T and ADT performance similar to that of controls. Both groups of rats also displayed significant increases in the number of times they crossed over the target location during probe trials. This clearly suggests that these M-treated subjects did learn the location of the platform, because they searched in the target area the same amount of time and as accurately as control subjects. The ability to retain information about the target location also remained apparently intact among M-treated rats during early withdrawal. If M-treated subjects took consistently longer to locate the target on the first training trial relative to their previous day's performance, one would predict that M-treated rats would earn significantly higher forgetting scores. However, such scores were comparable between M- and C-treated subjects. This indicates that the longer latencies observed among M-treated subjects can not be attributed to poor performance limited to only the first training trial of a given day. Analysis of search patterns indicated that, while early withdrawal M-treated rats spent amounts of time in the outer annulus comparable to those of controls during probe trials, M-treated subjects at the start of training made significantly fewer entries into (and, therefore, fewer exits from) the outer annulus. The M-treated subjects executed significantly less flexible searches than controls. During the first probe trial, control subjects consistently weaved into and out of both the outer and middle annuli, because they exhibited high numbers of entries into these regions of the MWM. In contrast, M-treated rats, while spending amounts of time in the outer annulus that were comparable to control levels, executed fewer entries into this annulus. M subjects also tended to enter the middle annulus less often than did controls during the first probe trial. This suggests that M-treated subjects possibly searched consistently first in one annulus, then in another, with significantly less travelling between the outer and middle annuli. This search pattern evidence, in addition to both the longer average latencies and pathlengths to reach the target displayed by early withdrawal M rats suggest, not that these rats failed to display spatial bias for the target location, but rather that they executed searches for the target area that were significantly different from those of controls. However, upon locating the target area, their behavior was similar to that of controls.

It has been argued that the MWM task assesses both declarative and procedural memory (24). Declarative memory might be defined as the subject's ability to recall specific stimulus events or facts, while procedural memory might be thought of as the ability to learn motor strategies or response patterns needed to efficiently perform a task. A clear dissociation of these processes using the MWM has previously been demonstrated. Subjects treated with the cholinergic neurotoxin AF64A displayed significant decreases in latency to reach the target but failed to display a significant preference for the target location during a probe trial conducted on the final day of training (24). Based on these findings, one might infer that AF64A-treated subjects learned to efficiently search for the platform, indicating intact procedural memory. However, they failed to learn the spatial location of the platform, indicating impaired declarative memory. In the present investigation, MWM behavioral measures such as %T, ADT, and number of target location crossings may be considered assessments of declarative memory, because they directly measure a given subject's knowledge about the relationship between the target

area and the likelihood of escape. On the other hand, annulus search patterns, latency, and pathlength may be considered assessments of procedural memory, because they directly reflect the efficiency and accuracy with which a subject locates the target area.

The present data demonstrate that deficits in MWM acquisition observed during early withdrawal do not involve the subject's ability to learn the escape target location. It seems that these deficits may instead be attributed to changes in the subject's ability to efficiently search for the escape platform, demonstrated by longer pathlengths and escape latencies than those exhibited by control subjects as well as initial search patterns that significantly differ from those of controls.

Based on the results of MWM testing, it is plausible to propose that during early withdrawal from morphine, transient deficits may occur in procedural but not declarative memory. Such deficits were not evident in M-treated rats trained after day 21 of morphine withdrawal. It has been argued that these qualitatively distinct types of memory processing are mediated by different brain systems (14,21,22,30,41). Many reports suggest that the hippocampus and caudate nucleus may be two such memory substrates (17,25,26). For instance, lesions of the hippocampus and fimbria-fornix, as well as pharmacological manipulations of the septohippocampal cholinergic system, typically impair performance of declarative memory tasks like the RAM while damage to the caudate nucleus does not (25). On the other hand, both Packard and McGaugh (27), and McDonald and White (15,16) argue that procedural memory is dependent on the caudate nucleus because damage to this nucleus reliably leads to impairment of tasks that are believed to depend on intact procedural memory processing, such as both tactile and spatial discrimination tasks (5,28,40). For instance, rats sustaining caudate-putamen lesions have been found to display impaired selection of navigation strategies, with a tendency to rely on motor response (taxon) strategies rather than the distal cues/place strategy used by controls (37). In the MWM task, rats with caudate DA depletions, display slower latencies to locate the platform, though they exhibit preferences for the target quadrant that resemble those of controls (9). More recently, it was shown that naive rats with caudate lesions were impaired on the procedural memory component of a tactile discrimination T-maze task, but not impaired on the variable goal-arm component of the task (declarative memory) (5).

The available data suggest that the caudate is involved in the acquisition of information that is invariant throughout training. In contrast, hippocampal lesions usually fail to impair performance of such tasks (19,13). Together, these observations provide evidence for the existence of separate neural systems that support different types of memory. The results of the present study suggest that these systems are differentially affected during the early stages of morphine withdrawal. A transient compromise of caudate nucleus function associated with opiate withdrawal may account for the behavioral changes observed in the present study. Further experiments should help to define the functional and neurobiological sequelae of opiate withdrawal as well as to elucidate brain mechanisms and substrates involved in different aspects of memory.

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