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Opiate Antagonists, Morphine and Spatial Memory in Rats

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BEATTY, W. W. *Opiate antagonists, morphine and spatial memory in rats.* PHARMACOL BIOCHEM BEHAV **19**(3) 397–401, 1983.—To assess the possible role of endogenous opioids on spatial memory, rats were administered morphine (1–15 mg/kg), naloxone (1–10 mg/kg), or naltrexone (0.1–10 mg/kg) at varying times after or prior to completing the first 4 choices in an 8 arm radial maze. None of these agents consistently affected retention, suggesting that endogenous opioid systems do not play a major role in modulating neural mechanisms that maintain accurate spatial memory.

Spatial memory Working memory Morphine Naloxone Naltrexone Opioids and memory

THE results of several recent studies suggest that endogenous opioids may modulate neural processes that are essential to memory consolidation. Time-dependent facilitation of retention has been observed following post training treatment with naloxone and several other opiate antagonists [5, 6, 7, 9, 11, 13, 15] while treatment with morphine and other opiate agonists often has the opposite effect under comparable testing conditions [9, 12, 13]. In the majority of experiments memory for active or passive avoidance conditioning has been studied, but limited data suggest that the influence of naloxone may be observed in situations that do not involve aversive stimulation [9,12].

In the present experiments I investigated the effects of morphine, naloxone, and naltrexone on spatial memory in rats. The long span of accurate spatial memory displayed by rats tested in the radial maze [3] offers a unique opportunity for assessing the effects of pharmacological treatments on a test of working memory since it is possible to administer treatments after the to-be-remembered event (TBRE). Such a strategy offers the possibility of dissociating drug effects on performance from more direct influences on memory processes which is very difficult to accomplish with more conventional short term memory paradigms. While spatial memory is very difficult to disrupt by pharmacological treatments, haloperidol causes a retention deficit which resembles natural forgetting [1]. Since haloperidol blocks memory facilitation caused by naloxone [11] in some tasks, it might be expected that spatial memory would also be influenced by drugs that alter brain opiate activity. In the present studies drugs were administered 0 or 2 hr after the TBRE since both haloperidol and ECS have time-dependent effects on spatial memory in this paradigm [1,16].

METHOD

Animals

Two groups of male albino rats, originally purchased from the Holtzman Co, Madison, WI were used. One group (N=16) had extensive behavioral and drug experience and were approximately 18 months old at the start of the present experiment. Previously they had served in experiments designed to evaluate the effects of cholinergic and various monoaminergic antagonists on spatial memory [1,8].

The rats in the other group (N=21) were experimentally naive at the outset of training (2.5 months of age). All animals were caged singly with free access to water in an air-conditioned animal room (22±3°C) that was illuminated from 0800 to 2200 hr by overhead fluorescent lights. They were maintained on a restricted feeding schedule of Purina lab chow pellets designed to maintain body weight at 80–85% of the free-feeding level adjusted for growth. Behavioral tests occurred during the daylight portion of the L:D cycle.

Apparatus

Behavioral testing was conducted in an elevated 8 arm maze made of wood painted white which was shaped like a rimless wagon wheel. Each arm (74×9 cm) extended from an octagonally shaped central hub (36 cm across). Black plastic sidewalls (3.5 cm high) extended the length of each arm. Small metal cups, mounted at the end of each arm, served as receptacles for reinforcers. Guillotine doors surrounded the hub and controlled access to each arm. The room housing the maze (3 m on a side) was cluttered with running wheels, a steam line with valves and hoses and other surplus equipment which provided a rich variety of extra-maze cues.

Behavioral Procedures

At the start of each session a single 190 mg Noyes pellet was placed into the food cup at the end of each arm. The rat was placed into the central hub and the guillotine doors were raised, permitting access to any of the arms. In the first 4 experiments the animal was allowed to choose 4 arms in any order that it wished. This constituted the TBRE. It was then returned to its home cage for the duration of the 5 hr interval. Completion of the first 4 choices almost never required more than 5 min. The retention interval began upon completion of the fourth choice. At the start of the retention test only the 4

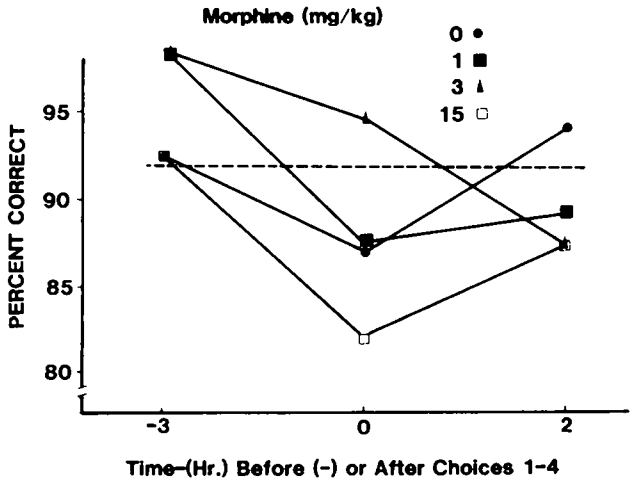

FIG. 1. Mean percent correct on choices 5–8 after varying doses of morphine sulfate during Experiment Morphine 1. Broken horizontal line indicates performance during the no treatment condition.

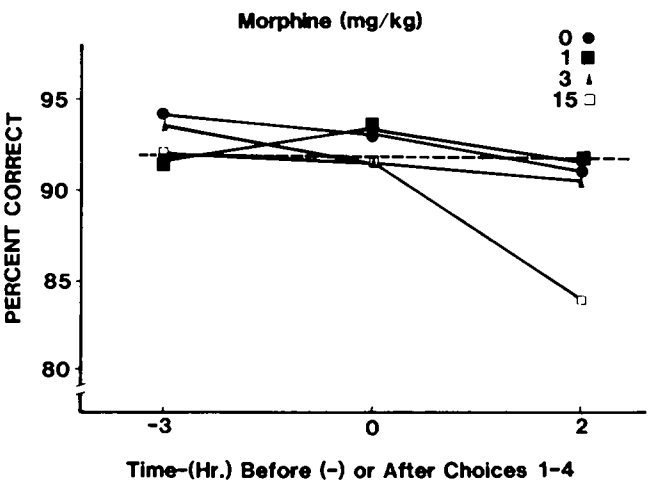

FIG. 2. Mean percent correct on choices 5–8 after varying doses of morphine sulfate during Experiment Morphine 2. Broken horizontal line indicates performance during the no treatment condition.

arms that the rat had not already visited contained food. Since food was never replenished during a daily test session, the rat was required to learn a win-shift food-searching strategy and avoid arms previously visited to achieve optimal performance. The retention test continued until the rat succeeded in finding all of the pellets or 10 min elapsed. Reentries into an arm previously visited on that test day were counted as errors. Since testing continued until the rat collected all of the pellets, the total number of errors was potentially unlimited. Retention errors (reentries into arms previously visited during the retention test) were also recorded, but this type of error rarely occurred under any condition.

By virtue of their extensive prior training the older rats were already quite proficient at performing in the maze at the 5 hr-long retention interval. Hence it was only necessary to retrain them for about 1 week to reestablish baseline performance.

The younger group was adapted to the maze and trained until they performed accurately when no delay was imposed during the run. Then a delay was imposed between choices 4 and 5, which was gradually extended to 5 hr. By the end of pretraining, which lasted about 2 months, these rats were maintaining performance of about 85% correct on choices 5–8 at the 5 hr long retention interval.

Drug Treatments

All drugs were dissolved in physiological saline and administered IP at a volume of 1 ml/kg. Doses are expressed as the weight of the salt. In general the rats received some dose of the active drug under study every third day, no treatment on the following day and saline on the remaining day. Drug doses and times of administration were partially counterbalanced among subjects to control for order effects. Specific details of the individual experiments are given below. The studies were conducted in the order they are described.

Naloxone 1

In this experiment the effect of 1 or 10 mg/kg naloxone HCl (Endo Labs) was studied using the older rats. Doses were administered 0 or 2 hr after the rat completed its first 4 choices or 3 hr before the procedures used in the first 4 choices. In contrast to subsequent studies, 2 days without treatment were interposed between drug tests. Saline was administered 0 or 2 hr after the first 4 choices as part of the counterbalanced sequence of naloxone treatments.

Morphine 1

This experiment began when the older rats were about 21 months of age. Morphine sulfate (Lilly) (1, 3, or 15 mg/kg) was given 0 or 2 hr after, or 3 hr before the first 4 choices. The latter condition was included as a control for possible proactive effects of the drug treatments. Between the end of Naloxone 1 and the start of Morphine 1 the rats served in a study of the effects of amphetamine on spatial memory [2]. During this experiment one rat died and its partial data are exluded. Prior to the experiment one other rat was excluded because of respiratory disease.

Naltrexone 1

In this and all subsequent experiments the younger rats were used. Naltrexone HCl (0.1, 1, or 10 mg/kg) was administered 0 or 2 hr after, or 3 hr before the first 4 choices.

Morphine 2

This experiment was a direct replication of Morphine 1. Prior to the start of the study 2 rats were eliminated because of chronic respiratory disease.

Morphine 3

Morphine sulfate (15 mg/kg) was given 0 or 2 hr after, or 3 hr before the first 4 choices. After the completion of Morphine 2, I noted that nearly 50% of the rats were consistently entering 4 adjacent arms on the first 4 choices, a pattern that was not observed in the older rats. Since by patterning its responses the rat could potentially simplify its memory assignment, in effect converting a spatial task into a nonspatial problem, the testing procedure was modified slightly for Morphine 3 and all subsequent studies. The rats were forced

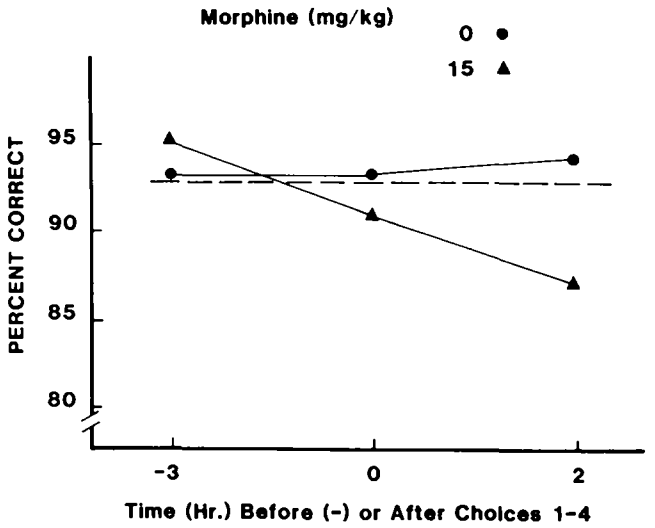

FIG. 3. Mean percent correct on choices 5–8 after morphine sulfate treatment during Experiment Morphine 3. Broken horizontal line indicates performance during the no treatment condition.

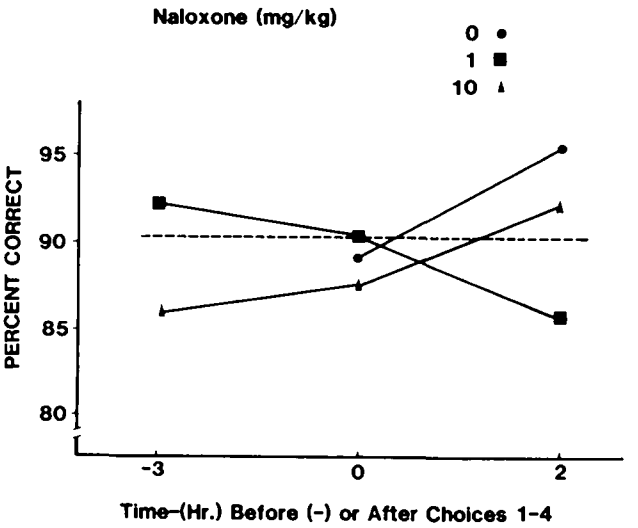

FIG. 4. Mean percent correct on choices 5–8 after varying doses of naloxone HCL during Experiment Naloxone 1. Broken horizontal line indicates performance during the no treatment condition.

to enter a random sequence of arms during the first 4 choices. The sequence varied for each rat over days and for different rats on a particular day. One rat died of respiratory collapse after the receiving the highest morphine dose and its partial data were excluded.

Morphine 4

The length of the retention interval extended to 7 hr and 15 mg/kg morphine sulfate was administered 2 hr after the first 4 choices.

Naloxone 2

With a retention interval of 5 hr no improvement in retention was observed after treatment with opiate antagonists. Since this failure might have arisen because of a ceiling effect on choice accuracy, I lengthened the retention interval to 12 hr and reexamined the effect of administering 10 mg/kg naloxone HCl 0 or 2 hr after the first 4 choices.

Naltrexone 2

With a 12 hr-long retention interval 10 mg/kg naltrexone HCl was administered 0 or 2 hr after the first 4 choices.

In general, each rat received each drug at each dose at each temporal condition. In Morphine 3 and Morphine 4 morphine was tested twice at each temporal interval.

Data Analysis

Initially the data were inspected for evidence of systematic changes in performance on the no treatment and saline tests. No such changes were observed so the average performance for each of these control conditions was determined for each rat. Repeated measures analyses of variance were computed for each experiment on the percent correct on Choices 5–8 as well as for errors per session. Since these measures yielded comparable effects, only the data for percent correct are reported.

RESULTS

Morphine

Figure 1 describes the effects of various doses of morphine on the accuracy of spatial memory at the 5 hr long delay (Morphine 1). Although differences among treatment conditions were modest, there was a significant treatment effect, $F(12,156)=2.24$, $p<0.02$. Subsequent analyses demonstrated that there was no reliable difference between the no treatment and saline conditions so these data were pooled to provide a single control condition. Additional analyses indicated that administering 1 or 3 mg/kg morphine 3 hr before the first 4 choices enhanced performance, $Fs(1,13)>10.12$, $p<0.01$, while giving 15 mg/kg morphine immediately after the first 4 choices decreased accuracy, $F(1,13)=13.11$, $p<0.01$. No other comparisons reached significance.

My attempt to replicate these intriguing results (Morphine 2) failed as seen in Fig. 2. Overall there was no reliable treatment effect ($F<1$). Further, there was no suggestion of facilitation after treatment with low doses of morphine before the TBRE or of impairment when the highest dose was given immediately after the TBRE. Instead, there was a tendency, albeit insignificant, for the highest morphine dose to impair performance when given 2 hr after the TBRE.

To examine this trend, the effects of 15 mg/kg morphine were studied in Morphine 3 (Fig. 3). Overall the treatment effect was not reliable, $F(6,102)=1.67$, but again performance was slightly depressed when the drug was given 2 hr after the TBRE (3 hr before the retention test).

The slight effect of the highest morphine dose could be indicative of a weak influence on memory or simply a performance change, perhaps because the rats were still mildly sedated at the time of the retention test. The results of Morphine 4 support the latter view. When the retention interval was extended to 7 hr performance under no treatment condition averaged 95.1% correct versus 93.1% correct when saline was given 2 hr after the TBRE and 91.0% when the 15 mg/kg morphine dose was given 2 hr after the TBRE. These differences were statistically insignificant.

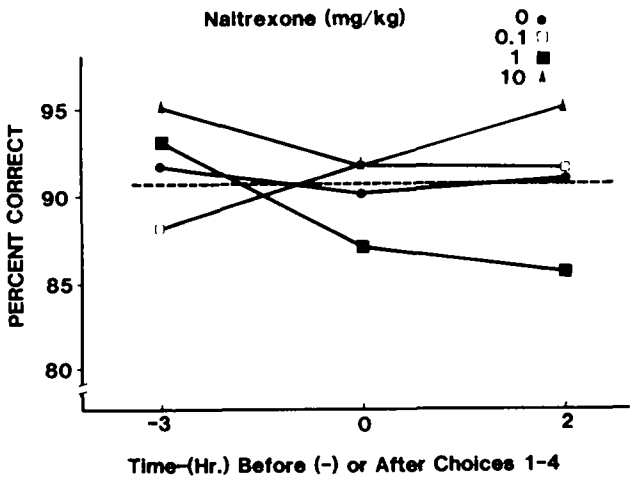

FIG. 5. Mean percent correct on choices 5–8 after varying doses of naltrexone HCl during Experiment Naltrexone 1. Broken horizontal line indicates performance during the no treatment condition.

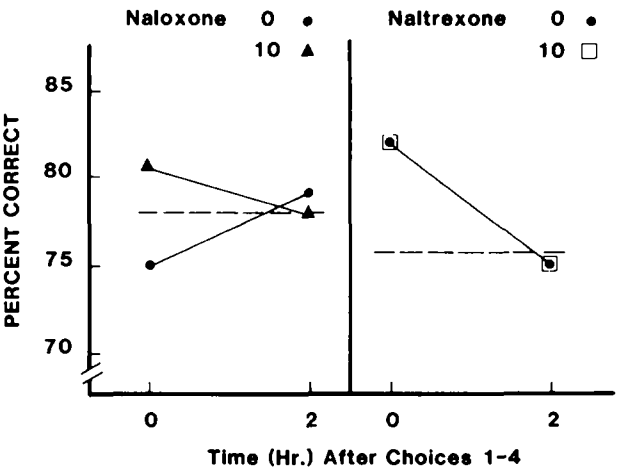

FIG. 6. Mean percent correct on choices 5–8 after naloxone HCl or naltrexone HCl treatment in Experiments Naloxone 2 and Naltrexone 2. Drug doses are in mg/kg. The retention interval was 12 hr. Broken horizontal line indicates performance during the no treatment condition.

Opiate Antagonists

With a 5 hr retention interval (Naloxone 1, Naltrexone 1) neither naloxone (Fig. 4) nor naltrexone (Fig. 5) reliably affected performance ($Fs < 1.55$).

It might be argued that the failure to observe the expected facilitation of retention after opiate antagonist treatment arose because of a ceiling effect on retention accuracy. To test this idea I extended the retention interval to 12 hr and retested both drugs (Naloxone 2, Naltrexone 2). As seen in Fig. 6, retention accuracy declined at the longer retention interval, but neither opiate antagonist facilitated performance ($Fs < 1$).

DISCUSSION

The present findings suggest that endogenous opioids do not play a major role in modulating processes essential to maintaining accurate spatial memory. A subtle influence of morphine upon the performance of older rats cannot be ruled out, especially since opiate receptor concentrations in some brain areas decline with age and the effects of naloxone on retention of avoidance behavior vary with age [14]. But overall the data suggest that morphine has little effect on performance in the radial maze. Whatever slight effect it may have is unlikely to reflect a direct action on neural mechanisms that maintain spatial memory.

Despite employing a wide range of doses and times of administration I could find no evidence that opiate antagonists improved retention. It is not likely that this failure arose because of a ceiling effect imposed by the highly accurate performance under control conditions at the 5 hr delay. Extending the retention interval to 12 hr reduced retention accuracy, but it did not unmask a drug effect. Moreover, even when performance is highly accurate, the task is sensitive enough to detect small improvements in retention as the results of Morphine 1 demonstrate.

Perhaps the present results indicate that the endogenous opioids are primarily involved in modulating the consolidation of memory into long term storage and are not involved in maintaining short term or working memory. However, spatial memory in rats has a number of properties such as longevity and resistance to retroactive interference [3,4] that set it apart from other forms of working memory. Until the effects of manipulating endogenous opioids on other working memory tasks are studied, no conclusion can be drawn.

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