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Opiate Antagonists Enhance the Working Memory of Rats in the Radial Maze

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CANLI, T., R. G. COOK AND K. A. MICZEK. *Opiate antagonists enhance the working memory of rats in the radial maze.* PHARMACOL BIOCHEM BEHAV **36**(3) 521–525, 1990.—Two experiments tested the influence of the opiate antagonists naloxone and naltrexone on the spatial working memory of rats in a 12-arm radial maze. In Experiment 1, ten rats were serially forced to visit six randomly selected arms, then were removed from the maze for delays of either 30, 60, or 240 minutes, and then returned to the maze for a free-choice memory test with all 12 arms available. Five minutes into the delay, rats were injected intraperitoneally (IP) with either physiological saline or naloxone (1 mg/kg). When injected with naloxone the rats revisited forced-choice arms less often than when injected with saline during a subsequent free-choice test. In Experiment 2, twelve rats showed a similar facilitation of working memory when injected with the opiate antagonists naltrexone (0.3 mg/kg) and naloxone (1 mg/kg) in comparison to a saline control condition. These findings demonstrate the beneficial effects that opiate antagonists exert on working memory-based performance in the radial maze. They may also resolve conflicting reports about the influence of opiate antagonists on radial maze performance, by suggesting that the choice of measurement and testing conditions are crucial for detecting these effects in working memory procedures.

Spatial working memory Radial arm maze Opiate receptors Naloxone Naltrexone

THE opiate antagonist naloxone has been shown to enhance memory in both active and passive avoidance tasks (16,23). The specific cause of this enhancement is difficult to identify, however, because of the multiple effects of opiate antagonists in aversively motivated tasks. For example, research on stress-induced analgesia (13, 17, 20, 21) has shown that an organism will respond with the release of endogenous opiates to both unconditioned and conditioned aversive stimulation. Thus, the effects of opiate antagonists in memory tasks may also reflect contributions of processes related to aversive stimulation, in addition to their presumed capacity to modulate memory. It is therefore important to examine the memory-enhancing effects of opiate antagonists in appetitively motivated tasks also. Thus far, the evidence for the mnemonic role of opiate antagonists in appetitive tasks is mixed (2, 11, 25, 33). The experiments below report that both naloxone and naltrexone increased choice accuracy in a working memory procedure using the appetitively motivated radial maze task.

In the radial maze task an animal is placed on an elevated central platform in which a number (e.g., eight) of arms radiate out at equal angles. A small amount of food is available at the end of each arm. The animal is permitted a series of choices among the arms and is thus rewarded with food for the first visit to an arm. Since food is no longer available on previously visited arms, revisits to arms are considered to be "errors." It has been found that rats quickly discriminate and remember which arms they have chosen previously and which still contain food (26). Because it presumably requires the use of spatial information concerning the distribution of food reward, the radial maze has become widely used in studies of the cognitive and neural mechanisms of spatial orientation and memory.

Conflicting results have been reported about the influence of opiate antagonists on learning and memory in the radial maze. In one study (11) it was reported that naloxone-treated rats needed significantly less time to relearn a maze placed in a new spatial environment than a saline control group. In contrast, another study (2) failed to show that naloxone and naltrexone had any influence on memory-based choice accuracy in rats tested in eight-arm radial maze using a working memory procedure.

One possible reason for this inconsistency may be related to the robust nature of the working memory displayed by rats in the radial maze. It appears that stringent or challenging conditions are often necessary to demonstrate certain treatment effects in this task (5, 22, 28, 31). We decided to reexamine the effects of naloxone on the working memory using a more difficult and possibly more sensitive 12-arm radial maze.

EXPERIMENT 1

METHOD

Subjects

The subjects were 10 male Long-Evans rats (Charles River Laboratories, Wilmington, MA) between 6 and 8 weeks old. They

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were individually housed, and maintained at 90% of their free-feeding weight with free access to water in a room with a 12:12-hour LD cycle. Tests were conducted during the first four hours of the light phase.

The apparatus was a radial maze consisting of twelve arms radiating out from a center platform. The platform was 41 cm in diameter and the twelve arms were each 80 cm long and 10 cm wide. The surface of the maze was elevated 60 cm above the floor. Separate guillotine doors allowed the experimenter to control access to the arms. Along the side of each arm there were barriers (12.5 cm in height and 30 cm in length) preventing the rats from going to another arm without first returning to the center platform. Brown plastic food cups (5 cm in diameter) were located at the end of each arm. Two 45 mg sucrose pellets were used to bait the end of each arm at the beginning of a trial. The maze was located in an illuminated room with a variety of extra-maze cues, including a blackboard, cage rack, computer and desk, experimenter, and door.

Procedure

Training. Training began with one 20-minute familiarization session in which groups of three or four rats freely explored the maze and could eat from constantly rebaited food cups. Following this session, individual free-choice training started on a daily basis, with one training session being conducted per animal per day. In these free-choice trials, a rat was placed on the center platform with the entrances to all twelve arms open. Subjects were allowed to choose among the arms until all 12 arms had been visited, a total of 20 choices had been made, or 20 minutes had elapsed. A choice was defined as entering an arm and going out 30 cm (marked by the barrier) on to the arm. This training continued until the last animal reached the criterion of visiting all 12 arms within the first 14 choices on three consecutive days. On the average, subjects needed 10 training sessions until they reached criterion.

To prepare the animals for experimental trials, training was modified to include a forced choice procedure, a short delay of fifteen minutes, and a saline injection during the delay. Each trial was now divided into two parts, a forced-choice phase in which the animals were exposed to specific arms to be remembered, and a free-choice memory test phase in which the memory for these arms was measured. In the forced-choice phase of a trial, six arms were randomly selected and ordered by computer to be the "to-be-remembered" information. The subject was placed on the center platform with all the guillotine doors closed. In succession, each door for a selected arm was raised and the animal allowed to enter and eat the food from the arm. Upon returning to the center platform that door was lowered and the next arm was opened. After the animal had visited each of the selected arms, it was removed from the maze and placed in an individual holding cage, located in a nearby rack, for 15 minutes. The use of individual holding cages during the delay was to aid subjects with discriminating retention intervals from the end of a trial (indicated by a return to the home cage). Five minutes into each subject's delay, an intraperitoneal (IP) injection of saline was given. Immediately after the 15-minute delay, the subject was placed back in the maze for the free-choice test phase of the trial. In the free-choice phase access to all twelve arms was available, but only those arms which were not entered in the forced-choice phase were baited and considered to be correct. A total of 10 training trials of this type were conducted at a rate of one trial per subject per day. Experimental trials using naloxone started on the day following the tenth training trial.

Naloxone testing. In the experimental trials, two independent variables were manipulated: 1) naloxone (1 mg/kg) versus saline; and 2) the length of the delay between the forced-choice procedure and the free-choice testing, consisting of 30, 60 or 240 minutes. On experimental trials, animals were given six forced-choices and removed from the maze. Five minutes after the end of the forced-choice phase, the subjects received an intraperitoneal (IP) injection of either physiological saline or naloxone. During the delay, all subjects were kept in individual holding cages with free access to water. After either 30, 60 or 240 minutes, a free-choice test was conducted with the experimenter recording the sequence of arms chosen by the subject.

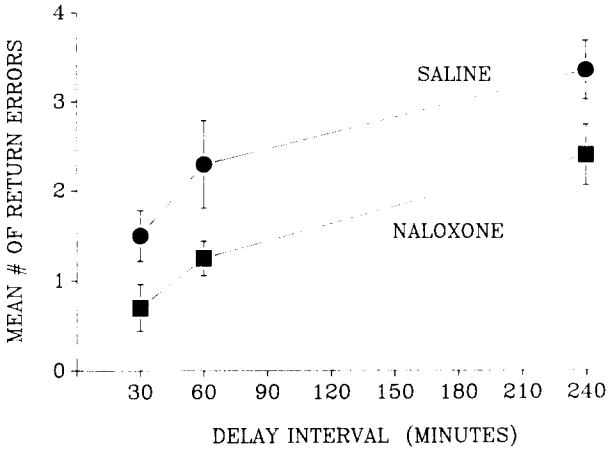

FIG. 1. Mean number of forced-choice returns as a function of delay length and drug treatment.

The experiment used a randomized block within-subject design. Each block consisted of six trials that tested all combinations of the two experimental variables. One trial was conducted daily and the testing order of the six conditions within a block was randomly determined for each rat. Three blocks of testing were conducted.

RESULTS AND DISCUSSION

There are two types of "errors" possible during the free-choice test. The first type are *forced-choice returns*, consisting of reentering one of the six forced-choice arms. The second type are *free-choice returns*, consisting of all reentries into arms previously visited during the free-choice phase. Only the number of forced-choice returns were analyzed, because they represent the best measure of the influence of delay and drug treatment on working memory. Free-choice returns were infrequent and also considered contaminated, since the source of the memories associated with these errors was not under the control of the experimenter, unlike for the forced-choice arms.

Figure 1 displays the effects of delay and drug treatment on number of revisits to forced-choice arms during the free-choice test. Working memory became poorer at longer delays as indicated by the increased number of return visits to forced-choice arms. More interestingly, after naloxone treatments, the rats avoided the forced-choice arms more often than when injected with saline treatment. A two-way ANOVA for repeated measures (Drug Treatment $\times$ Delay) was conducted on the number of forced-choice returns for each trial of the test phase. Both Drug Treatment, $F(1,9)=5.87$, $p<0.05$, and Delay, $F(2,18)=26.7$, $p<0.001$, had significant main effects on the number of forced-choice returns.

The results from Experiment 1 showed an enhancing effect of

naloxone on working memory-based performance in the radial maze. This finding is in agreement with the large body of previous work reporting the enhancement of learning and memory with naloxone in aversively motivated tasks (15, 16, 23, 24). It fails to confirm findings (2) that report no effect of naloxone on radial maze performance.

EXPERIMENT 2

Experiment 1 found that injections of naloxone improved memory for avoiding previously visited arms in the radial maze. We were curious if this reflected the action of naloxone specifically, or was more related to the family of opiate antagonists in general. For example, it has been shown that rats in a passive avoidance task show enhanced retention when injected with any of several opiate antagonists, including naloxone, naltrexone or diprenorphine (9). Such results suggest that the enhancement of learning and memory may be a general property of this class of drugs.

In the next experiment, the effects of another opiate antagonist, naltrexone, was examined. Naltrexone was chosen because it shares naloxone's property of binding to mu-receptors (3,7) but is different from naloxone in its potency and duration of action. If both naloxone and naltrexone treatments enhance memory in an appetitive task, it would suggest more strongly that memory-enhancement is a property of opiate antagonists, and may be related to their property of binding to mu-receptors.

METHOD

Subjects

The subjects were 10 male Long-Evans rats (Charles River Laboratories, Wilmington, MA) between 6 and 8 weeks old. They were individually housed, and maintained at 90% of their free-feeding weight with free access to water in a room with a 12:12-hour LD cycle. Tests were conducted during the first four hours of the light phase.

Apparatus

The apparatus was the same as in the previous experiment, but was located in a different room with similar dimensions and extra-maze cues as used in Experiment 1.

Procedure

Initial training. The initial familiarization and individual acquisition training were the same as in Experiment 1.

Delay and testing familiarization training. Preparation for memory testing differed slightly from Experiment 1. This experiment used a slightly different working memory test. The forced-choice phase and delay phase of each trial was the same as for Experiment 1. During the test-phase, however, two different types of memory tests were conducted, the previously used free-choice test and a new and additional 2-Alternative Forced-Choice (2-AFC) test which occurred in part with the free-choice testing. These memory tests were conducted in the following way. When a rat was returned to the maze after a delay, only two arms were available. One arm had been previously entered before the delay, the second arm had not been entered before on that trial. Because only the new arm was baited, the "correct" response was to enter the new arm. The "old" and "new" arms were chosen randomly from their respective set of possible arms, within the constraint that they be spatially separated by at least one arm. Following the return to the center platform after the 2-AFC choice, the free-choice test phase began and all the doors were raised simultaneously to allow access to the arms. In the data analysis, the 2-AFC choice was considered as the first choice of the free-choice test. The criterion for ending a trial was the same as for Experiment 1: 12 correct choices, 20 total choices, or 20 minutes in the maze.

TABLE 1

PERCENTAGE OF CORRECT RESPONSES IN THE 2-AFC TEST

	Test 1	Test 2	Test 3	Mean
		60-Minute Delay		
Naloxone	100	100	88	96
Naltrexone	91	92	63	82
Saline	83	92	100	92
		240-Minute Delay		
Naloxone	67	73	67	69
Naltrexone	67	50	75	64
Saline	75	67	92	78

Percentage of correct choices in the 2-AFC procedure of Experiment 2. There were no statistically significant main effects for tests, drug condition, or delay interval.

As in Experiment 1, a saline injection was given five minutes into each animal's delay in preparation for future experimental trials. Delay training with the combined 2-AFC and free-choice testing was conducted for seven days using a delay of 30 minutes. At the end of this training, each animal was making 12 correct choices within the first 14 total choices.

Experimental testing. The effects of two independent variables were investigated: 1) naloxone (1 mg/kg) or naltrexone (0.3 mg/kg) relative to saline and 2) the length of the delay, either 60 or 240 minutes. On experimental trials, animals were given six forced-choices and removed from the maze. Five minutes after the end of the forced-choice phase, the subjects received an intraperitoneal (IP) injection of either physiological saline, naloxone, or naltrexone. During the delay, all subjects were kept in individual holding cages with free access to water. After either 60 or 240 minutes, a 2-AFC/free-choice test was conducted, with the experimenter recording the sequence of arms chosen by the subject.

The experiment used a randomized block within-subject design. Each block consisted of six trials that tested all combinations of the two experimental variables. One trial was conducted each day, with the order of the conditions within a block randomly determined for each rat. Three blocks were conducted.

RESULTS

2-AFC Test

No clear or consistent effect of naloxone or naltrexone compared to saline was detected on the percentage of correct choices during the 2-AFC memory test procedure (see Table 1). A two-way ANOVA for repeated measures (Drug Treatment × Delay) revealed no significant effects for Drug Treatment or Delay.

Forced-Choice Returns

Figure 2 displays the effects of delay-length and drug treatment on memory for the forced choice arms. A two-way ANOVA for repeated measures (Drug × Delay) was conducted on the number of forced-choice returns for each trial in the test phase. Again the longer delay produced more forced-choice returns, as indicated by the significant main effect of Delay, $F(1,11)=23.4$, $p<0.001$.

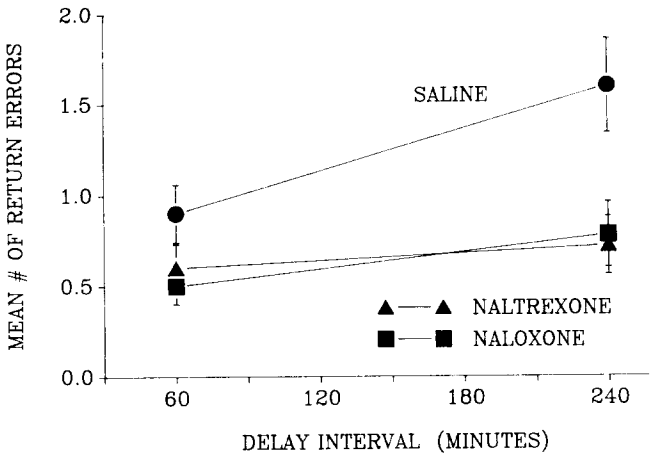

FIG. 2. Mean number of forced-choice returns as a function of drug treatment and delay interval.

Further, both opiate antagonists reduced the number of forced-choice returns during the free-choice phase as compared to saline, as indicated by the main effect of Drug Treatment, $F(2,22) = 10.64$, $p<0.001$. Two separate ANOVAs for each drug [(Drug vs. Saline) × Delay] confirmed this result. Rats made fewer forced-choice returns when injected with either naloxone, $F(1,11) = 11.68$, $p<0.01$, or naltrexone, $F(1,11) = 18.2$, $p<0.01$.

GENERAL DISCUSSION

Experiment 2 found that both naloxone and naltrexone improved retention in the radial maze, as indicated by fewer return visits during the memory test. Thus the results of Experiments 1 and 2 suggest that opiate antagonists enhance memory-based performance in the radial maze task. This finding is consistent with the growing body of research showing that opiate antagonists enhance processes related to learning and memory, both in aversively motivated memory tasks (23,24) and appetitively motivated tasks (11, 15, 33). The present study extends these findings by showing that opiate antagonists enhance choice accuracy in an appetitively motivated working memory task.

Furthermore, the present experiments indicate these drugs need not be present in the system during the presentation of to-be-remembered material, but that it is sufficient for them to be active immediately afterwards, perhaps during the "encoding" or consolidation period for the memories. This in combination with the fact that naloxone was unlikely to be present in the animal at the time of test in the 240-minute-delay condition (27), suggests that action of the drug was specifically confined to those neural and cognitive processes associated with the retention interval, and most likely working memory.

While the more specific question of whether opiate antagonists facilitate the encoding or the retrieval of information was not addressed in these experiments, evidence from aversive studies (1, 16, 23) suggests that naloxone and related antagonists act on the consolidation of memory. Future radial maze experiments could vary the time interval between the forced-choice phase and drug administration, for example, in order to examine whether performance, and presumably the degree of memory consolidation, would fail to benefit from the delayed application of the antagonists.

Experiment 2 further showed that the detection of these particular drug effects may critically depend on how they are measured. In this case within the same experiment, a 2-AFC memory test failed to reveal any influence of naloxone or naltrexone, while a free-choice test revealed robust drug treatment effects. This raises the possibility that previous failures (2) to detect the enhancing effect of opiate antagonists may be due to the methods involved in the measurement of these effects. It is also conceivable that the processes measured by the 2-AFC memory test are qualitatively different from those of the free-choice test, and the neural substrate underlying the cognitive processes of the 2-AFC test is not susceptible to the modulatory effects of opiate antagonists.

One area for immediate future research suggested by the present finding is the observation that both naloxone and naltrexone share a high affinity for mu-receptors. Identification of the receptors subtypes and sites involved with the enhancing effects of opiate antagonists will lead to a better understanding of the influences of opioids on learning and memory.

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