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Naloxone Facilitates Spatial Learning in a Water-Maze Task in Female, but Not Male, Adult Nonbreeding Meadow Voles

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GALEA, L. A. M., L. SAKSIDA, M. KAVALIERS AND K.-P. OSSENKOPP. *Naloxone facilitates spatial learning in a water-maze task in female, but not male, adult nonbreeding meadow voles.* PHARMACOL BIOCHEM BEHAV 47(2) 265–271, 1994. – The present study examined the effects of the opiate antagonist naloxone on spatial acquisition and retention in a water-maze task by adult, nonbreeding, male and female meadow voles (*Microtus pennsylvanicus*). Voles were required to learn the position of a hidden, submerged platform using distal visual cues. There were four trials per day for 6 days. Daily pretraining (15 min before first trial) systemic administrations of naloxone (1.0 mg/kg, IP) significantly facilitated spatial acquisition in female, but not in male, voles in a water-maze task on days 2, 3, and 4. There were two probe tasks given 1 day and 1 week after the last training trial. All groups acquired the spatial task by the end of the fifth day with no significant effects of naloxone on retention of the spatial task. There were also no significant sex differences in acquisition of the spatial task and task retention in control, nonbreeding adult voles. It is suggested that the lack of sex differences in basal spatial performance may be related to the low levels of testosterone in male nonbreeding voles. The obtained sex differences in the effects of naloxone on spatial acquisition are considered in relation to sex differences in stress, opiate responses, and gonadal steroid levels.

Naloxone Sex differences Meadow voles Morris water-maze Spatial acquisition Opiates Stress

ENDOGENOUS opioid peptides have been implicated in spatial-learning tasks, such that opiate antagonists facilitate spatial learning (6,10,12) and opiate agonists impair spatial learning (30). Because it is well established that endogenous opiate activity is augmented after exposure to various types of stressors (1), it seems reasonable that, to delineate the true nature of the effect of opiates on learning, one should use the least stressful paradigm to assess learning (14). There are at least two elements that may influence the findings of studies investigating the effects of opiates on learning: the amount of stress associated with the specific spatial learning paradigm and the sex and reproductive status of subjects.

Spatial learning paradigms have typically used various forms of the radial-arm maze (6,13) and versions of the Morris water-maze (10,30,32). Learning paradigms using radial-arm mazes usually involve chronic food deprivation (6,14) and are considered more stressful than water-maze paradigms as evidenced by elevated corticosterone levels in food-restricted rats (22). Further, opiate antagonists such as naloxone have been shown to suppress the consumption of food in rats (43) and thus could influence performance in tasks that use food as a reward. Such findings suggest that water-maze paradigms, which induce an acute, transient stress (3,33), may be better paradigms for examining the effects of opiate agonists and antagonists on spatial learning. Indeed, the results of past research suggest that the prototypic opiate antagonist naloxone exerts a stronger facilatory effect on radial-arm maze performance (6,14) than water-maze performance (10,30).

Relatively few studies have investigated the effects of opiates on spatial learning in the water-maze. Pretraining administration of an opiate antagonist, 5 min prior to trials in the water-maze, enhanced learning in male rats in the water-maze (10). McNamara and Skelton (30) found that morphine impaired spatial performance in male rats, as assessed by the Morris water-maze, when the water temperature was 22°C but found no significant effect of morphine on spatial perfor-

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mance when the water temperature was 10°C. Pretraining injections of naloxone (injected 15 min prior to testing) did not significantly alter performance in the water-maze compared to control animals in 22°C water.

There are also well-established sex differences in endogenous opioid activity and opiate-mediated behavioral responses, such as antinociception in rodents (2,20). Adult male rodents typically display greater exogenous opiate- and stress-induced analgesic responses than do adult females (2, 25,26,36). Gonadal hormones appear to be involved in the mediation of these sex differences in antinociception because the sex difference in antinociception is eliminated in gonadectomized adults (20,36). There are also substantial data indicating sex differences favoring adult male rodents on tasks of spatial ability and indicating that these differences are also hormonally mediated [see (44) for review]. This raises the possibility of an interaction between sex and hormonal status, opioid-mediated stress responses, and spatial performance. To date, none of the studies that have examined the effects of opiates on spatial learning in a water-maze have investigated the possibility of sex differences in the effects of opiate antagonists on spatial learning (6,10,14).

The meadow vole, *Microtus pennsylvanicus,* is a polygynous microtine rodent that displays pronounced sex differences, favoring reproductive males in symmetrical, spatial-maze tasks (12,15,23). Meadow voles are excellent swimmers (34) and make diurnal aquatic excursions in the wild (7,8). This suggests that the water-maze is an appropriate task for investigations of spatial learning in the meadow vole. Meadow voles are also photoperiod-determined breeders and are reproductively quiescent in the nonbreeding season (winter or short-day cycle) (5,19). Testosterone levels fluctuate across the seasons in voles, with higher levels of testosterone present during the breeding season relative to the nonbreeding season (4,38). Thus, by manipulating the photoperiod to simulate the nonbreeding season one could test spatial learning during a period of lower gonadal steroid levels and in particular "lower testosterone" levels in adult, male meadow voles. The use of testing during the nonbreeding season also serves to eliminate the possible developmental confounds of surgical manipulations involved in early gonadectomy. Testing during the nonbreeding season may potentially minimize sex differences in underlying basal opioid activity and spatial performance due to gonadal steroid effects. Accordingly, the present study examined the effects of the opiate antagonist, naloxone, on spatial performance in a Morris water-maze by adult, nonbreeding (nonreproductive), male and female meadow voles. Portions of this research were previously presented in abstract form (13).

METHOD

Subjects

Subjects were 21 female and 16 male naive, adult meadow voles. These meadow voles weighed approximately 30–60 g and were second- and third-generation offspring of wild-trapped animals. Voles were individually housed in polyethylene cages provided with hardwood bedding (Beta chips) and maintained in a colony room kept at 20 ± 1°C under a 10 L : 14 D cycle. Food (Purina Rat Chow) and tapwater were available ad lib. Voles had been held under the reproductively inhibitory short photoperiod for approximately 4 months, and were in a nonbreeding state. All males had testes that had regressed into the abdominal position.

Apparatus

Water-maze. The water-maze was a black, circular pool with a diameter of 90 cm and a height of 45 cm, filled with 21 ± 1°C water to a depth of 15.5 cm. The maze was divided geographically into four equal quadrants and release points that were designated at each quadrant as N, E, S, and W. A hidden circular platform (7 cm in diameter) was located in the center of the fourth quadrant, submerged 1 cm beneath the surface of the water. Fixed extramaze cues were present at various locations around the maze (i.e., refrigerator, television monitor, and videocassette recorder, experimenter, etc.). A television camera was mounted 2 m above the center of the maze. All trials were recorded on a videocassette recorder connected to the camera. A thin layer of hardwood chips (Beta chips) was sprinkled on top of the water to obstruct the view of the submerged platform. After each trial, fresh hardwood chips were sprinkled across the top of the water to reestablish an even layer of woodchips over the entire water surface.

Procedures

Subjects were divided into four groups: females injected with saline (n = 11); females injected with naloxone (n = 10); males injected with saline (n = 9); and males injected with naloxone (n = 7). Naloxone (Sigma Chemical Co., St Louis, MO) was dissolved in an isotonic saline solution and injected IP in a dose of 1.0 mg/kg. Physiological saline (0.9%) was administered in a volume of 10 ml/kg.

There were three phases of trials in this experiment: a baseline trial, acquisition trials, and probe trials. Trials in all phases were videotaped.

Baseline phase. The baseline trial consisted of a 3-min swim without the platform. Animals were injected with naloxone or saline and placed in a holding cage for 15 min before beginning the baseline trial. The 15-min interval was chosen from the results of previous studies that showed that naloxone had its maximum effects in meadow voles 15–30 min after injection (28,39). The baseline trial examined the effect of naloxone on swimming behavior.

The baseline phase was scored by dividing the pool into 20 equal areas and recording the number of times that each area was crossed by a subject during the 3-min trial. The total number of crossings of all areas was aggregated and used as a measure of total activity.

Acquisition phase. Training trials consisted of four trials per day over a period of 6 days. A total of 6 days was chosen on the basis of previous research in this laboratory that found that both male and female meadow voles reached a plateau in water-maze performance after 6 days (23). Animals were injected with either naloxone or saline and placed in a holding cage for 15 min prior to the first trial on each day. Each vole started a trial from each release point on each day, with the initial release point (N, S, E, W) varying from day to day. The maximum duration of each trial was 60 s, and voles that failed to locate the platform within 60 s were placed on the platform for 15 s. Those voles that found the platform prior to the 60-s time limit were allowed to remain on the platform for 15 s. Animals were kept in a holding cage during the intertrial interval (45 s). Groups of voles were either tested during a morning session or an afternoon session and these groups were counterbalanced. Speed data was collected during day 2. Distance was calculated for each of the four trials during day 2 using the digitizing program Java (Jandel Scientific). Speed during each of the four trials was calculated as an

indication of a possible effect on motor activity and indirectly motivation.

Probe phase. There were two probe trials. The first probe trial was conducted the day after the last training session and the second probe trial occurred 7 days later. There was no platform in place during the probe trials, and none of the animals received any drug treatments prior to being placed in the maze. Each vole was given a 60-s swim trial and time spent in each quadrant was recorded. During the probe phase, all animals were released from point A.

Data Analysis

Data from the three phases were analyzed separately. Baseline-phase results were analyzed using an analysis of variance (ANOVA) with sex and drug as the between-subject factors. The acquisition-phase data were analyzed using a mixed-design repeated-measures ANOVA, with sex and drug as between-subject factors and block and trial as within-subject factors. All posthoc tests were performed using Tukey's procedure. Speed data were analyzed with a mixed-design repeated-measures ANOVA with sex and drug as the between-subjects factors and trials on the second day as the within-subject factor. The probe-phase results were analyzed with a mixed-design repeated-measures ANOVA, with sex and acquisition-phase drug as between-subject factors and quadrant and probe trial as within-subject factors. All hypothesis testing used a significance level of 0.05.

RESULTS

Body Weights

There were no significant differences among the mean group body weights (female-saline, 44.6 ± 13.4; female-naloxone, 46.1 ± 13.9; male-saline, 40.8 ± 4.653; male-naloxone, 43.3 ± 8.5).

Baseline

An ANOVA of the total number of movements revealed a significant main effect of sex, favoring males in the number of total movements made, $F(1, 31) = 14.25$, $p = 0.001$, but no significant main effect of drug, $F(1, 31) = 2.98$, $p = 0.094$, nor a significant interaction effect, $F(1, 31) = 1.07$, $p = 0.308$. These data are presented in Fig. 1.

Acquisition

The repeated-measures ANOVA on latency to reach the platform obtained a significant four-way interaction, $F(15, 495) = 1.94$, $p = 0.018$. The following effects were also significant: main effect of training day, $F(5, 165) = 45.00$, $p < 0.001$; main effect of trial, $F(3, 99) = 16.76$, $p < 0.001$; day × trial interaction, $F(15, 495) = 3.24$, $p < 0.001$. None of the other main effects or interactions were significant.

Simple main effects on each day revealed that on day 1 there were significant main effects of drug, sex, and trial and a significant three-way interaction of sex × drug × trial. An analysis of day 2 found a significant main effect of trial, a sex × drug interaction, and a sex × drug × trial interaction. Posthoc comparisons revealed that naloxone-treated females had significantly ($p < 0.05$) shorter latencies in reaching the platform, relative to control females, while naloxone-treated males were not significantly different from control males. The simple main effects on day 3 found a significant sex × drug interaction with females injected with naloxone obtaining significantly shorter latencies than did control females and males injected with naloxone did not statistically differ from control males. There was a main effect of sex on day 4 with females of both groups outperforming males and almost a sex × drug interaction ($p < 0.14$). There were no significant main effects or interaction effects on days 5 or 6. Figure 2 illustrates mean group latencies for the four trials as a function of training days. The observation that all four groups exhibited high levels of task performance by days 5 and 6 and the lack of an effect of naloxone on these days could have been due to a floor effect.

Speed

An ANOVA on speed during each of the four trials during day 2 revealed a significant main effect of trial, $F(3, 99) = 15.55$, $p < 0.001$, and a significant interaction effect of sex × trial, $F(3, 99) = 3.00$, $p = 0.034$. There were no other

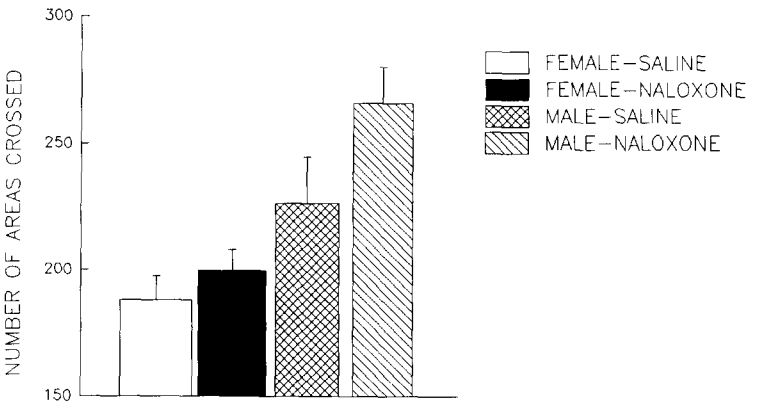

FIG. 1. Group mean area crossings in the 3-min baseline swim test. Male meadow voles made significantly more crossings than did female meadow voles. Control females injected with saline (10 ml/kg) ($n = 11$), naloxone-treated (1.0 mg/kg) females ($n = 10$), control males ($n = 9$), and naloxone-treated males ($n = 7$). All injections were given IP, and baseline test was run 15 min after injection. Error bars indicate SEM.

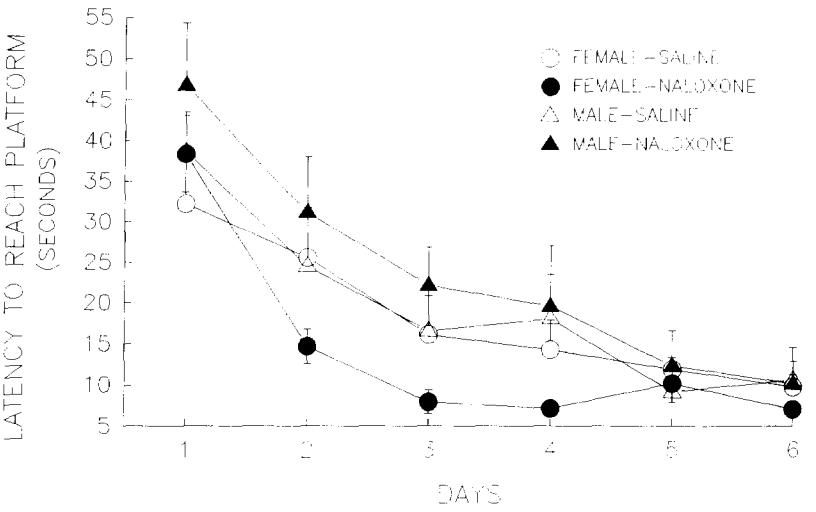

FIG. 2. Group mean latencies to reach platform in acquisition of the water-maze over the 6 training days (four trials per day) of male and female meadow voles IP injected with either naloxone (1.0 mg/kg; n = 7 males, n = 10 females) or saline vehicle (10 ml/kg; n = 9 males, n = 11 females). Trials were conducted 15 min after injection. Naloxone-treated females had significantly lower latencies to reach the platform on days 2, 3, and 4 than any of the other groups. Error bars indicate SEM.

significant main effects or interaction effects. Posthoc testing revealed that males were significantly faster in Trial 1 than were females; however, females were significantly faster in Trial 3 than were males. There were no significant drug effects on speed during any of the four trials.

Probe

There were no significant group differences in the probe trials. These data are presented in Fig. 3. The repeated-measures ANOVA found a significant main effect of quadrant, $F(3, 99) = 11.43$, $p < 0.001$, but no other significant effects. All groups spent significantly more time in the quadrant that had previously contained the platform ($p < 0.01$) than any other quadrant. These results provide additional evidence that animals had acquired the spatial task and retained this task for at least 1 week.

DISCUSSION

The major findings of the present study are the absence of sex differences in spatial learning in nonbreeding adult meadow voles but the presence of sex differences in the effects of naloxone on acquisition of the spatial task.

Pretraining injections of the opiate antagonist naloxone enhanced acquisition of the water-maze task only in female nonbreeding, adult meadow voles. There was no significant effect on performance in naloxone-treated males, although naloxone did appear to have a slightly detrimental effect on spatial task acquisition. This is the first demonstration of a sex difference in the effects of naloxone on spatial-task acquisition in nonbreeding adult rodents. This sex difference in the effects of naloxone on water-maze learning may be related to a number of factors, including: sex differences in swimming and swim stress, sex differences in naloxone-sensitive opioid systems or components, and/or sex differences in the levels of gonadal steroids.

A sex difference in the stress response to water-maze swimming may account for females displaying a facilatory effect of naloxone only on days 2–4 of testing. Results of studies of swim-stress-induced analgesia (SSIA) in rodents have indicated that the degree and nature of stress (opioid and nonopioid mediated) are affected by the duration of swim, the water temperature, and the strain of animal (29,45). There have been relatively few investigations of the impact of sex in SSIA, with the results of those studies yielding no sex differences in SSIA (33,45). However, SSIA tasks are qualitatively different from the swim stress induced by the water-maze task. SSIA tasks usually involve one 3-min swim (31,45) and, in contrast, water-maze tasks generally involve a number of 1-min swim trials over a number of days. The present study does, however, raise the possibility of sex differences in the stress associated with repeated swimming and handling procedures used in a water-maze task. In future studies, it may be important to determine when the learning paradigm is most stressful to the animal to determine if the effects of stress and opiate antagonists are related.

If there is significantly greater increase in the amount of stress associated with water-maze training in females relative to males, it is also possible that the naloxone-induced decrease in analgesia may serve to increase motivation rather than improve spatial learning. However, because there were no effects of naloxone on swim speed during day 2 (where there were significant effects of naloxone on latency) this suggests that naloxone did not have an effect on locomotor activity and indirectly suggests that motivation was not differentially affected for the two sexes. This adds to the findings from the baseline phase in which no significant drug effects were found on the total amount of movement in the maze itself. Thus, the facilitation of female spatial acquisition by naloxone does not appear to be simply due to an increase in swim speed or motivation as measured by swim speed.

There are sex differences in the relative density and levels

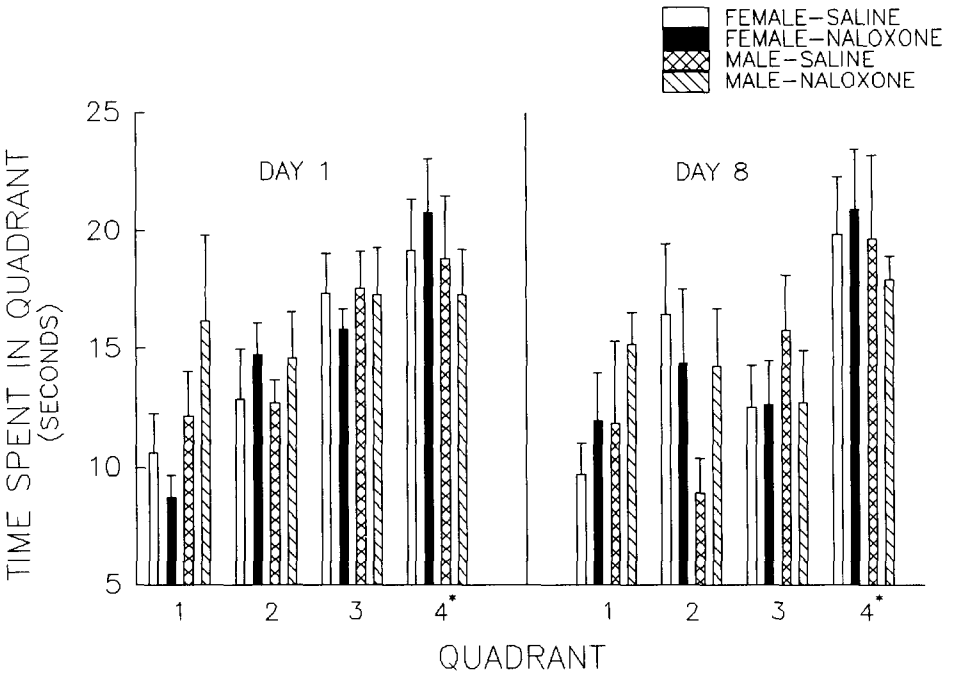

FIG. 3. Group mean time spent in each quadrant for the two 1-min probe tests by male and female voles that had previously received either naloxone (1.0 mg/kg; n = 7 males, n = 10 females) or saline vehicle (10 ml/kg; n = 9 males, n = 11 females) in the acquisition phase. All groups spent significantly more time swimming in the quadrant that had previously held the platform (marked as quadrant 4*) than any other quadrant. Error bars indicate SEM.

of opioid receptors in adult rats (18,42). Whether similar sex differences exist in nonbreeding meadow voles is not known. Similarly, whether there are sex differences in the relative distribution of various categories of opioid receptors is also not known. Because naloxone affects both μ-, δ-, and to a lesser extent κ-opiate receptors, it is possible that there is a sex difference in the relative levels and expression of these endogenous opioids and their receptors. There is some evidence suggesting that the μ-opioids have a facilitory effect on learning and that the δ- and κ-opioids have an inhibitory effect (41). Any sex difference in the relative levels and/or distributions of these receptors could result in differential effects of naloxone on spatial performance. As well, repeated administration of naloxone has been indicated to have an agonist effect on nociception in rodents (17), with the results of recent studies suggesting that this effect is greater in males than in females (in preparation). Whether or not this agonist action transcends all of the multiple opioid systems and extends to learning remains to be determined.

Naloxone had no significant effect on the spatial performance of nonbreeding males. This is partially consistent with results of previous studies (10,30). Decker et al. (10) found that naloxone improved spatial performance at doses of 3 mg/kg but had no facilitory effect at doses of 1 mg/kg. However, previous data suggests that higher doses of naloxone may have nonspecific effects (40), thus potentially confounding the interpretation of the prior finding. McNamara and Skelton (30) also found no significant effect of naloxone (2 mg/kg) on maze performance, although interestingly the antagonist did appear to have a detrimental effect on mean path length on days 3 and 4 (trials 12–16).

The sex differences in the effects of naloxone on spatial performance may also be influenced by and/or related to gonadal steroids. Testosterone levels have been suggested to affect the levels of opioid-mediated SSIA in male rodents (31,36,45). Results of a study with castrated male rats (31), which had lowered testosterone levels, suggested that there were decreased levels of SSIA and, more interestingly, decreased sensitivity to naloxone. Because in the present study we used adult males in a nonbreeding, presumably low-testosterone (4,38) state, it is possible that it may be the case that naloxone would facilitate spatial performance in breeding (high testosterone) males. However, reports on the effects of castration in males on SSIA have yielded equivocal results (31,36,45). Thus, it is unclear as to what effect naloxone would have on breeding adult males with higher levels of testosterone.

The effect of naloxone on female spatial performance may also be related to reproductive state and gonadal steroid levels. Estrogen and progesterone have been shown to affect opioid activity (9), opioid receptor density (21), and SSIA (31,36,45). In the present study, only nonbreeding females were tested and because female meadow voles are induced ovulators they presumably had relatively low levels of estrogen and progesterone. The effects of naloxone on spatial performance in the presumably low-estrogen females suggests effects that may be attributed to an organizational effect of estrogen and progesterone on SSIA (31).

In the present study, there was no sex difference in the acquisition of the spatial task in control animals. Sex differences favoring males in both water-maze tasks (12,23) and various symmetrical mazes tasks (15) have been reported in

breeding meadow voles held under long-day, reproductively stimulatory light cycles. Because breeding male voles have elevated testosterone levels (4,38), and because testosterone and its metabolites have been implicated in the mediation of spatial ability (16,27,44), it is likely that sexually dimorphic spatial ability varies across the seasons in meadow voles. Indeed, we have found that sexually dimorphic spatial ability varies across the breeding season in deer mice, with a sex difference in spatial performance apparent only during the breeding season and not during the nonbreeding season (11,24).

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