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Naloxone Inhibits Mating and Conditioned Place Preference for an Estrous Female in Male Rats Soon After Castration

R. L. MILLER AND M. J. BAUM[1]

Department of Biology, Boston University

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MILLER, R. L. AND M. J. BAUM. *Naloxone inhibits mating and conditioned place preference for an estrous female in male rats soon after castration.* PHARMACOL BIOCHEM BEHAV **26**(4) 781–789, 1987.—Three experiments were conducted to assess the role of endogenous opioids in controlling mating behavior and sexual reward in the male rat. In Experiment 1 SC administration of naloxone (0.5, 1.0, 5.0, or 10.0 mg/kg) significantly reduced mounting and ejaculation in male rats tested 14, but not 7 days, after castration. In Experiment 2 naloxone (5.0 mg/kg) administered SC to gonadally intact males, which had ejaculated repeatedly with one female until they were sexually sated, significantly inhibited the resumption of mating after the reintroduction of a female partner. One interpretation of these results is that naloxone attenuated the reward experienced by castrated and sexually sated males in the presence of an estrous female, thereby disrupting males' coital performance. This hypothesis was tested in Experiment 3 using a conditioned place preference paradigm in which males copulated with an estrous female in an initially "non-preferred" (white) compartment, whereas on alternate days they remained alone in an initially "preferred" (black) compartment. After 10 such conditioning sessions, males were either castrated or sham-operated. They later were given free access to both compartments in the absence of an estrous female. Seven days after conditioning and surgery, sham-operated, naloxone-injected males and both groups of castrates spent significantly less time than sham-operated, saline-injected controls in the initially "non-preferred" compartment. Fourteen days after conditioning and surgery castrated, naloxone-treated males spent significantly less time in the "non-preferred" compartment than males in the other three groups. Endogenous opioids may play an important role in the interpretation by males of the incentive motivational stimuli which emanate from an estrous female.

Rat Sexual behavior Opioids Naloxone Reward

MUCH evidence suggests that endogenous opioid peptides participate in the regulation of several consummatory behaviors. Such a contribution of opioids is well-documented in studies involving the ingestion of sweet substances. For example, administration of the opiate receptor antagonist, naloxone, significantly reduced the consumption of chocolate milk by male rats and decreased rearing behavior in rats which had been trained to expect candy either 10 or 30 min after being placed into an observation chamber [17]. Naloxone also reduced the probability that males would remain at a site where they had previously been trained to expect candy. This latter result suggests that naloxone specifically reduced animals' interest in the expected tasty food, and that this effect was not due to a drug-induced reduction in either behavioral arousal or locomotor activity. In other studies [29,46] naloxone narrowed the range of saccharin concentrations preferred by both sated and food-deprived rats. Finally, administration of opiate receptor antagonists reduced lever pressing by male rats to obtain electrical stimulation of the pontine central grey, the periaqueductal grey, the zona compacta of the substantia nigra, and the nucleus accumbens [6,48].

Several lines of evidence also have implicated endogenous opioids in the control of masculine sexual behavior in the rat. Opiate receptor agonists, including morphine, methadone D-Ala-Ala-Met-enkephalin and β-endorphin reduced mounting and ejaculation in male rats and hamsters [20, 32, 38]; pretreatment with naloxone prevented these effects. In some studies administration of naloxone by itself reduced mount and intromission latencies and accelerated ejaculation sexually experienced, gonadally intact male rats [32,39] and facilitated mounting in persistent non-maters [20]. However, in other experiments [31, 44, 49] administration of opiate receptor antagonists failed to facilitate mounting and ejaculation but instead consistently lengthened the postejaculatory interval, i.e., the time after ejaculation prior to the resumption of mating.

Typically, male rats continue to exhibit coital behaviors in tests with estrous females for several weeks following castration [16], even though plasma testosterone levels decline within hours after sugery [22]. The cause of this temporary persistence of masculine sexual behavior is not known, but some evidence suggests that the stimulus qualities of the female parter are important mediating factors. For example,

[1]Requests for reprints should be addressed to Dr. M. J. Baum, Department of Biology, Boston University, 2 Cummington St., Boston, MA 02215.

TABLE 1

EFFECT OF NALOXONE ON PARAMETERS OF MASCULINE SEXUAL BEHAVIOR IN MALE RATS TESTED 7 DAYS AFTER CASTRATION OR SHAM OPERATION

Treatment	N	Mount Latency (min)	Intromission Latency (min)	Ejaculation Latency (min)	Postejaculatory Interval (min)	No. Intromissions Before Ejaculation
Sham-Operated/Saline	8	0.1 ± 0.0	0.2 ± 0.1	8.8 ± 1.3	6.0 ± 0.4	16.0 ± 1.2
Castrated/Saline	5	0.8 ± 0.5	2.8 ± 1.7	9.3 ± 1.4	10.4 ± 2.5	14.6 ± 1.6
Castrated/Naloxone (all doses)	9	2.2 ± 1.2	4.2 ± 1.6	13.3 ± 2.2	25.8 ± 7.2*	15.2 ± 1.9

Note: Data are based only on tests with ejaculation. The castrated/naloxone group is comprised of castrated males treated with naloxone at dosages of 0.5 (n=1), 1.0 (n=3), 5.0 (n=4), and 10.0 (n=1) mg/kg.

*$p<0.05$, Newman-Keuls comparison with the sham-operated/saline-injected group.

male rats tested shortly after castration with females which exhibited high levels of solicitational behavior continued to mount and ejaculate for a longer period of time than other males tested with females which displayed less intense solicitational behaviors [33]. In light of the evidence which points to an endorphinergic contribution to the ingestion of sweet substances, Lieblich *et al.* [28] proposed that endogenous opioids similarly may facilitate masculine sexual behavior when the stimulus qualities of the female partner are predominant determinants of males' sexual arousal. They raised this possibility after finding that naloxone (5.0 mg/kg, SC) significantly reduced mounting rates and ejaculation in male rats shortly after castration whereas no such effects was obtained in gonadally intact, sham-operated controls.

The first aim of the present research was to assess further the possibility that endogenous opioids mediate the persistence of sexual behaviors in male rats under circumstances when the females' incentive qualities are primary determinants of males' sexual activity. In Experiments 1 and 2 this situation was created using either recently castrated males or gonadally intact, sexually sated males. In each instance the ability of naloxone to inhibit mating was assessed. A second aim was to test the possibility that naloxone-induced reductions in males' sexual performance, which were observed in Experiments 1 and 2, could be attributed to an attenuation by naloxone of the females' incentive value to the male. This aim was accomplished in Experiment 3 by testing the ability of naloxone to inhibit the expression of a conditioned place preference response for an estrous female at times after castration when naloxone had previously been found to reduce males' coital performance.

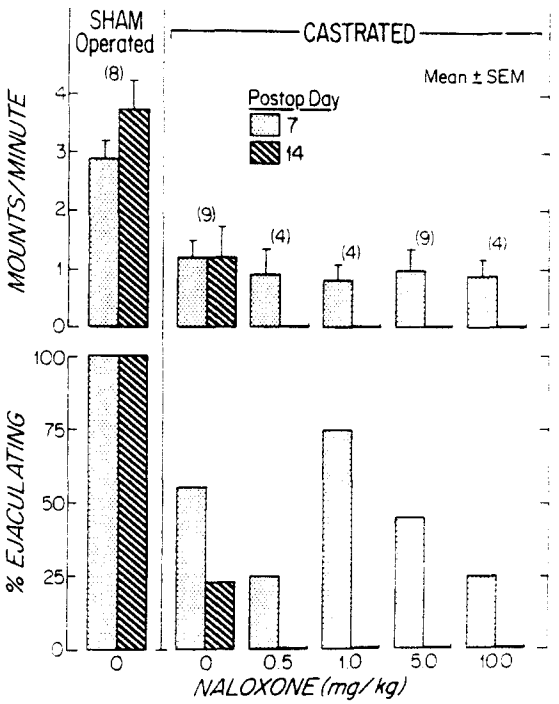

FIG. 1. Effects of different doses of naloxone or saline vehicle on mounting rates and the incidence of ejaculation in sexually experienced males which were castrated or sham-operated and tested 7 and 14 days later. The number of males in each group is given in parentheses.

GENERAL METHOD

Subjects

Adult male and female rats of the hooded Long Evans strain were purchased from Charles River Breeding Laboratory. They were housed alone (Experiment 1) or in pairs (Experiments 2, 3) in hanging wire mesh cages. Colony lights were off between 1200 and 2400 hr, and tap water and rat chow were available at all times. Males were 2–6 months old and weighed 300–600 g at the time of each experiment. Female rats weighing 200–300 g were ovariectomized and were made sexually receptive and proceptive by administering estradiol benzoate (10 μg/0.1 ml sesame oil, SC) and progesterone (500 μg/0.1 ml sesame oil SC) 48 and 4 hours prior to testing, respectively. Castrations and ovariectomies were performed via abdominal incisions using ether. Sham-castrations consisted of a ventral incision through the skin and muscle wall and manipulation of the testes without removal. All behavioral testing was carried out during the dark phase of the light/dark cycle under a dim 25 watt bulb. Tests of sexual behavior were carried out in ten gallon aquaria (25×47×29 cm) with sawdust bedding on the floor.

Drug Administration

Naloxone hydrochloride was dissolved in sterile 0.9% saline vehicle. Fresh solutions were always prepared one day prior to their administration. All injections were coded so that the experimenter did not know whether naloxone or

saline vehicle was actually being administered to animals. In all three experiments naloxone or saline vehicle were administered SC 5 min prior to the initiation of behavioral testing. In a previous study in which this interval was used [28] naloxone caused significant reductions in the coital performance of recently castrated males. Also, Cicero *et al.* [14] reported highly significant increments in plasma LH in male rats within 10 min after SC injection of naloxone. In the present studies behavioral testing invariably lasted at least 10 min after the injection of drug or saline vehicle (details given with the individual experiments).

Data Analysis

Data were subjected to analysis of variance (ANOVA), followed by Newman-Keuls' post hoc comparisons of individual group means. Chi-square tests also were performed where appropriate.

EXPERIMENT 1: EFFECTS OF A RANGE OF NALOXONE DOSES ON MALES' PERFORMANCE ONE AND TWO WEEKS AFTER CASTRATION

An experiment was conducted to extend the preliminary work of Lieblich *et al.* [28], who found that a single dose (5.0 mg/kg) of naloxone significantly reduced males' coital performance in the weeks after castration but not after sham operation. We examined the effects of a range of naloxone doses (0.5–1.0 mg/kg) on mounting rates and the incidence of ejaculation in male rats tested 7 and 14 days after castration.

Method

Males were allowed to ejaculate in at least one preliminary test, and then were distributed into six groups matched on the basis of mounting rates (see below). Males in five of these groups were castrated and later given naloxone in doses of 0.0, 0.5, 1.0, 5.0, or 10.0 mg/kg. The sixth group was sham-operated and received saline. Each male's mating performance was tested 7 and 14 days following surgery. On test days each male was injected SC with a particular dosage of naloxone or with saline and then was immediately placed into a test aquarium for five minutes. An estrous female was then introduced, and the male's mounts, intromissions, and ejaculation were scored using a Rustrak event recorder. Individual males were given 15 min to begin mounting. If a mount occurred, the male was given 15 additional min to intromit. If intromission occurred, the male was given another 30 min to ejaculate. A test was terminated when a male achieved the first intromission of a second ejaculatory series or failed to meet one of the above criteria. Several parameters of masculine sexual behavior were calculated: (a) mount and intromission latencies, (b) ejaculation latency (time elapsed between the first intromission and ejaculation), (c) postejaculatory interval (time between ejaculation and the first intromission of a second ejaculatory series), (d) number of intromissions prior to ejaculation, (e) percentage of rats in a group which ejaculate, (f) mount rate (the quotient of the total number of mounts, including mounts with penile intromission—but excluding the ejaculatory mount, displayed by the male and the time elapsed between the first mount and the ejaculation or the end of the test if no ejaculation occurred).

Results

A two-factor ANOVA performed on the mounting rates for the six groups of males (Fig. 1) yielded a significant Groups effect, $F(5,32)=11.32$, $p<0.001$. Post hoc tests indicated that on Day 7 each of the five groups of castrated males, regardless of whether they received saline or naloxone, mounted at significant lower rates than the sham-operated, saline-injected males ($p<0.01$). Mounting rates did not differ significantly among the five groups of castrates. On Day 14 mounting rates in each of the four groups of castrated males which received naloxone were significantly lower than in saline-treated, castrated males ($p<0.05$). The incidence of ejaculation (Fig. 1) also was significantly reduced by castration and naloxone treatments on Day 14, $\chi^2(5)=30.57$, $p<0.01$, but not on Day 7. Administration of naloxone 7 days after castration tended to lengthen the postejaculatory intervals in those males which ejaculated, although this effect did not reach statistical significance (Table 1). No other parameters of masculine coital performance were affected by naloxone. Too few males ejaculated 14 days after castration to allow a meaningful analysis of their mating parameters.

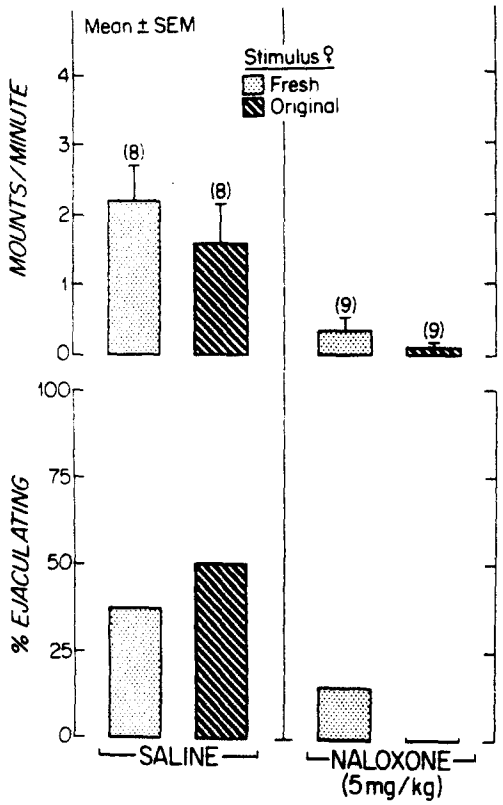

FIG. 2. Effects of naloxone or saline vehicle on mounting rates and the incidence of ejaculation in gonadally intact, sexually sated males after the reintroduction of either a fresh, unmated estrous female or the original female partner. The number of males in each group is given in parentheses.

Discussion

The present observation that administration of naloxone at doses ranging from 0.5–10.0 mg/kg inhibited mounting and ejaculation in tests given 14, but not 7 days after castration corroborates the findings of Lieblich *et al.* [28], who found that a single dosage (5.0 mg/kg) of naloxone significantly

TABLE 2

EFFECT OF NALOXONE ON MOUNT AND INTROMISSION LATENCIES OF SEXUALLY-SATED, GONADALLY INTACT MALE RATS AFTER THE REINTRODUCTION OF EITHER A FRESH, UNMATED ESTROUS FEMALE OR THE ORIGINAL FEMALE PARTNER

Treatment/Stimulus Female	N	Mount Latency (min) (maximum=30)	Intromission Latency (min) (maximum=30)
Saline			
Fresh Female	8	0.6 ± 0.2	7.6 ± 4.2
Original Female	8	8.8 ± 5.4	12.5 ± 5.9
Naloxone (5.0 mg/kg)			
Fresh Female	9	19.4 ± 4.8*	21.6 ± 4.3*
Original Female	9	20.3 ± 3.3*	26.7 ± 3.3*

*$p<0.01$. Significantly different from both saline-treated groups by ANOVA.

inhibited mating in male rats tested repeatedly for 4 weeks after castration. No such inhibitory effect of naloxone was observed by Lieblich *et al.* in gonadally intact males. Other investigators have also noted different effects of naloxone in rats, depending on whether sex steroids were circulating at the time this drug was administered. For example, the inhibitory effect of naloxone on feeding behavior was significantly greater in ovariectomized female rats given no steroid hormones than in ovariectomized females treated with estradiol [36]. Thus the ability of naloxone to inhibit feeding as well as masculine sexual behavior is greater in the absence of circulating sex steroids. Just the reverse is true of the effect of naloxone on the secretion of luteinizing hormone (LH). Administration of naloxone induced a rapid rise in plasma LH when given to gonadally intact or recently castrated male rats [14] whereas no such effect occurred in animals which had been gonadectomized for several weeks unless they were treated concurrently with sex steroids [7,40]. The fact that gonadectomy alters the behavioral and neuroendocrine responses to naloxone treatment in opposite ways suggests that these effects of sex hormone deprivation are not simply due to changes in the availability of the drug to the brain or even to steroid-induced changes in the concentration of opiate receptors [23]. Naloxone is a relatively non-specific opiate receptor antagonist, and these differential effects of sex steroids on the ability of naloxone to influence behavior versus LH secretion imply that different classes of opiate receptor and/or different opioid peptides may control these two functions.

EXPERIMENT 2: EFFECTS OF NALOXONE ON MATING PERFORMANCE IN GONADALLY INTACT, SEXUALLY SATED MALES

A second circumstance in which the persistence of coital performance in male rats is strongly influenced by the stimulus qualities of the female partner is after sexual exhaustion. In the male rat postejaculatory intervals become increasingly longer after successive ejaculations until a satiety criterion is reached, usually defined as a failure to reinitiate mating within 30 minutes of the last ejaculation [5]. It has been reported [13, 51, 53] that sexually sated male rats resumed mating more reliably when a fresh, unmated female was substituted for the original female partner. This phenomenon, referred to as the Coolidge effect, suggests that enhancing the stimulus qualities of the female can partially compensate for the male's diminished sexual arousal due to repeated ejaculation. This situation is analogous to that of castrated males which continue to mate for longer periods if tested with highly attractive, proceptive females [33]. In light of this similarity, an experiment was conducted to see whether naloxone could inhibit the resumption of mating by gonadally intact males after sexual satiation. This effect of naloxone was studied in sexually sated males after the introduction of either a fresh, unmated estrous female or the original partner.

Method

A new set of sexually experienced male rats ejaculated in two preliminary tests. Males then were distributed into four groups (n=8–9/group) matched on the basis of mounting rates during the second of these tests. Subsequently, each male was permitted to mate and ejaculate repeatedly with one estrous female until he achieved sexual satiety, i.e., failed to intromit within 30 minutes after an ejaculation [5]. Once a male attained this criterion, the female partner was removed, and the male was injected SC with either naloxone (5.0 mg/kg) or saline and returned to the test arena. Five minutes later either the original or a fresh (unmated for at least four days) estrous female was introduced into the aquarium. Mating was then scored, and the test was terminated either 30 minutes after the male ejaculated, 30 minutes after his first intromission if he failed to ejaculate within that time, or 30 minutes after the introduction of the female if he failed to mount or intromit within that period.

Results

Naloxone significantly reduced mount rates in sexually sated males, regardless of whether they were tested with a fresh or the original female partner (Fig. 2). A two-factor ANOVA performed on the mount rate data from the four independent groups yielded a significant Drug Treatment effect, $F(1,30)=20.52$, $p<0.01$; however, neither the Stimulus Female nor the Drug × Stimulus Female Interaction effects reached significance. The incidence of ejaculation was also significantly lower after naloxone, $\chi^2(1)=7.20$, $p<0.01$. Finally, naloxone significantly lengthened mount, $F(1,30)=12.35$, $p<0.01$, and intromission, $F(1,30)=10.20$, $p<0.01$, latencies of sexually sated males (Table 2), irrespective of whether males were tested with a fresh or the original female.

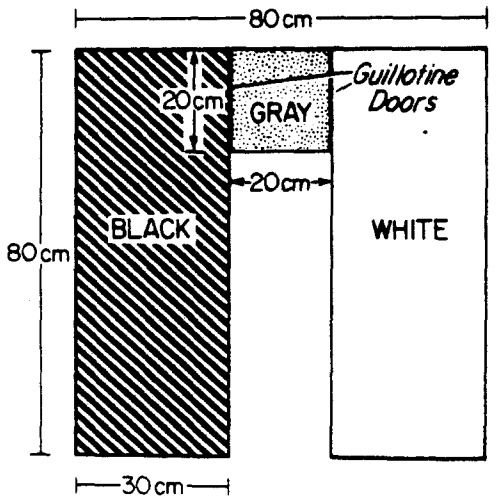

FIG. 3. Floor plan of the apparatus used to establish and assess male rats' conditioned place preference for an estrous female.

Discussion

Naloxone inhibited mating in gonadally intact, sexually sated males just as it inhibited mating in the castrated males studied in Experiment 1. Apparently naloxone's inhibitory effect on mating is not limited to circumstances in which circulating levels of testosterone are low. Sexual satiety is achieved when a male's postejaculatory interval is greatly exaggerated. Thus, the results of Experiment 2 compliment the results of previous studies [31, 44, 49] in which naloxone significantly extended the postejaculatory intervals of gonadally intact and castrated, testosterone-implanted males following an initial ejaculation. These data together with the results of Experiments 1 and 2 suggest that endogenous opioids may facilitate the male's ability to become sexually aroused in the presence of an estrous female at times when the tonic level of sexual arousal is low for any reason.

The data obtained in Experiment 2 provided no evidence of a significant Coolidge effect, even in males which received saline vehicle. Mounting rates and the incidence of ejaculation were equivalent in sexually sated males after the reintroduction of either the original female partner or a fresh estrous female into the test cage. The only hint of a Coolidge effect was the nonsignificant trend for mount and intromission latencies to be shorter in saline-injected males which were tested with fresh stimulus females (Table 2). It should be noted that others [24] also have reported difficulty in replicating the Coolidge effect. In that study, as in the present experiment, removal and subsequent reintroduction of any estrous female sufficed to reactivate mating in a substantial number of sexually sated males. Apparently any change in the stimulus condition associated with an estrous female, including her temporary absence, will suffice to increase the female's incentive value for a male. In the present study administration of naloxone to sexually sated males reduced their coital responsiveness to any female which was reintroduced into the testing cage.

EXPERIMENT 3: EFFECT OF NALOXONE ON THE EXPRESSION OF A CONDITIONED PLACE PREFERENCE RESPONSE FOR AN ESTROUS FEMALE IN CASTRATED AND GONADALLY INTACT MALES

As already discussed, the incentive qualities of the stimulus female are important determinants of masculine sexual arousal following either castration or sexual satiety. We hypothesize that naloxone attenuated the reward experienced by castrated (Experiment 1) and sexually sated (Experiment 2) males in the presence of an estrous female, and as a consequence their coital performance was inhibited. In order to test this hypothesis we studied the effect of naloxone on the expression of a conditioned place preference response for an estrous female in castrated versus gonadally intact males. In previous studies [27, 37, 43, 47] central injections of morphine or an enkephalin analogue, as well as intraperitoneal administration of morphine or heroin, to rats confined to an initially "non-preferred" (i.e., white) chamber of a test apparatus subsequently led them to spend more time in this chamber when later given free access to the entire apparatus in the absence of any drug treatment. In the present study a conditioned place preference was established for an initially "non-preferred' (white) compartment by allowing males to mate on several occasions with an estrous female in this site. After a series of conditioning sessions, males were either castrated or sham-operated. Subsequently they were given free access to the entire test apparatus in the absence of a female after injections of naloxone or saline 7 and 14 days following surgery.

Method

Twenty-five sexually experienced males used in Experiment 2 served as subjects. The test apparatus (Fig. 3) was designed to minimize a rat's ability to monitor visually the black or white compartment while standing in the other compartment. Thus a male had to enter a compartment in order to see whether it might contain a female partner. Two identical apparatuses were placed beside each other, and mirrors were positioned above each so that the rats' location could easily be monitored by an observer. Individual animals were tested in the same apparatus during all phases of the study.

The experiment was comprised of a series of preconditioning, conditioning, and postconditioning sessions. During preconditioning sessions (Days 1–4) each male was injected SC with 0.3 ml saline and placed for five minutes into an aquarium. He then was placed into the gray compartment of the place preference apparatus for 30 seconds with the guillotine doors lowered. The doors then were raised, and the male was allowed free access to all three interconnected compartments for 15 minutes. On the fourth preconditioning trial, the time spent inside each of the different compartments was recorded. All but two males preferred to spend more time in the black compartment.

The conditioning phase occurred over the next consecutive 10 days (Days 5–14). On the first and all subsequent odd-numbered days, each male was injected SC with 0.3 ml saline and placed into his initially "non-preferred" (usually white) compartment with the doors down. Five minutes later an estrous female was placed into the center of the compartment and the male's ejaculation latency and total test duration were recorded. The test was terminated 30 seconds after the male achieved an ejaculation. Males achieved an ejaculation in 98% of these tests. If a male failed to ejaculate, he was removed after spending 15 minutes with the female. On the second day of conditioning and all subsequent even-numbered days, each male was injected SC with 0.3 ml saline and was confined to his initially "preferred" (usually black) compartment with the doors lowered. Each male remained in

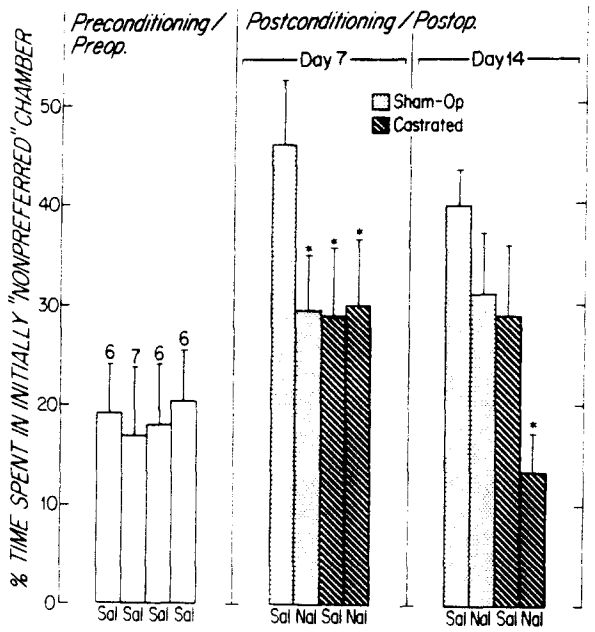

FIG. 4. Effects of naloxone (Nal) (5.0 mg/kg) or saline vehicle (Sal) on the expression by male rats of a conditioned place preference for an estrous female. Data are expressed means±SEM. The number of males in each group is given above the bars in the left panel. *$p<0.05$, Newman-Keuls comparisons with the sham-operated, saline-injected group (Day 7); Newman-Keuls comparisons with each of the three other groups (Day 14).

the initially "preferred" chamber alone for the same time which he had spent the previous day in the initially "non-preferred" compartment (i.e., 5 minutes plus the time spent with the estrous female). After the final conditioning session (Day 14), the males were divided into four groups matched on the basis of percentage of time spent in the initially "non-preferred" compartment during the last preconditioning trial (Day 4). Then half of the males were castrated and the other half were sham-castrated.

Postconditioning trials were conducted 7 and 14 days following conditioning and surgery. Half of the castrated and sham-operated males received naloxone (5.0 mg/kg SC) and the other half received saline prior to being placed in an aquarium for five minutes. Then each male was placed in the gray compartment of the place preference apparatus with the doors lowered. The doors were raised after 30 sec, and the time spent by the male in each of the three compartments of the apparatus was recorded for 15 minutes. No estrous female was present during these tests. Prior to each day of testing each apparatus was washed with soap and water followed by 70% ethanol. Any urine and feces were sponged from the floor of the apparatus between tests for individual males.

Results

Males acquired a place preference after mating repeatedly with an estrous female in the initially "non-preferred" compartment of the test apparatus (Fig. 4). A two-way ANOVA performed on the times rats spent in the initially non-preferred chamber before and after conditioning yielded a significant Repeated Measure effect, $F(2,42)=11.46$, $p<0.001$. Post hoc tests showed that the sham-operated, saline-injected control males spent significantly more time in the initially "non-preferred" compartment during each of the two postconditioning trials than prior to conditioning ($p<0.05$). Castration and naloxone treatments caused reductions in the expression of place preference responses, as indicated by a significant Groups × Repeated Measure Interaction effect, $F(6,42)=2.28$, $p=0.05$. Seven days after conditioning and surgery, sham-operated, naloxone-treated males and both groups of castrates spent significantly less time in the initially "non-preferred" compartment than the sham-operated, saline-treated control males ($p<0.05$). Fourteen days after conditioning and surgery the castrated, naloxone-treated males spent significantly less time in the initially "non-preferred" compartment than males in each of the other three groups ($p<0.05$).

Discussion

Male rats acquired a conditioned place preference for the initially "non-preferred" (white) compartment of the apparatus after being allowed to mate with an estrous female during a series of conditioning sessions. Naloxone treatment and castration both contributed to a reduction in the expression of this conditioned response. In tests given 7 days after the last conditioning trial sham-operated males given naloxone and both groups of castrated males spent significantly less time than control males in the initially "non-preferred" chamber in which mating had occurred during conditioning trials. This effect of castration correlates with the observation in Experiment 1 that males' mounting rates were significantly reduced in tests given 7 days after castration, regardless of whether naloxone or saline was given prior to the test. In tests given 14 days after the last conditioning trial (Experiment 3) naloxone-treated castrates spent significantly less time than males from the other three groups in the chamber in which mating had occurred during conditioning. This finding correlates with the observation in Experiment 1 that in tests given 14 days after castration mounting rates were significantly lower in males given naloxone as opposed to saline. These observations are consistent with, though not proof of the hypothesis that castration and administration of naloxone interact in male rats to reduce the reward derived from the incentive qualities of an estrous female, which in turn leads to a reduction in males' coital performance.

GENERAL DISCUSSION

As reviewed in the Introduction, much evidence suggests that endogenous opioids contribute to the control of ingestive behaviors under circumstances when taste is a predominant determinant of that behavior. Naloxone's suppressive effects on motivated behaviors are not limited, however, to the ingestion of sweet substances. Naloxone also inhibited the intake of rat chow and water in non-deprived rats [12]. In addition, naloxone inhibited the duration of eating [52] and drinking [11] and reduced the total consumption of nonsweetened substances without affecting the latency to initiate ingestive responses. Naloxone also began to extend rats' latency to eat in response to electrical stimulation of the lateral hypothalamus after several trials [26]. Wise and coworkers [26] proposed that naloxone first attenuates the incentive value of proximal food cues, such as taste and texture, which maintain eating behavior after it is initiated. They suggested that eventually, however, naloxone also at-

tenuates the incentive value of distal food cues, such as sight and smell, which are necessary for the onset of feeding.

Naloxone treatment and castration may interact to attenuate the incentive value of cues derived from an estrous female in a fashion that is analogous to the effect of naloxone on feeding. Thus in gonadally intact males naloxone has been found to delay the resumption of mating after an ejaculation [31, 44, 49]. Perhaps this effect results from a drug-induced reduction in the incentive value of proximal cues associated with repeated intromissions and ejaculation per se. As shown previously [28] and in Experiment 1, naloxone inhibits the initiation of mounting (and thus also inhibits ejaculation) 2–4 weeks after castration. As shown in Experiment 2, naloxone also inhibits the resumption of mounting in gonadally intact, sexually sated males. Perhaps in these circumstances naloxone reduces the incentive value of more distal cues emanating from estrous females, including olfactory signals and the observation of proceptive responses which normally facilitate masculine sexual arousal. The notion that castration and naloxone interact to reduce the incentive value of the estrous female is further supported by the results of Experiment 3, in which the expression of a conditioned place preference response for an estrous female was most strongly inhibited by administration of naloxone to males castrated 2 weeks earlier. In some ways this latter result resembles that of a previous study [17] in which naloxone caused rats to wait less time for candy which they had been trained to expect in a particular location. These diverse results suggest tha opioid peptides may be present in neural circuits which mediate incentive motivational cues essential for the expression of several kinds of consummatory behavior.

Naloxone has been reported to reduce rats' locomotor activity in a familiar test environment [50]. It seems unlikely, however, that such an effect can explain the observed inhibitory effects of naloxone on masculine sexual behavior. In Experiment 1 different doses of naloxone inhibited mounting and ejaculation only in tests given 14 days after castration. If naloxone inhibited males' sexual performance simply by reducing their locomotion, it should have also caused significant reductions in mount rates and ejaculation in tests given 7 as well as 14 days after castration. Likewise, it seems unlikely that the effects of naloxone on males' expression of a conditioned place preference response can be attributed to a drug-induced suppression of locomotion. First, inspection of Fig. 4 shows that in tests given both 7 and 14 days postoperatively there were groups of naloxone and saline-treated males which exhibited equivalent place preferences. As in the case of mating behavior, the strength of the naloxone effect on place preference depended on how many days after castration drug treatment and testing occurred. Second, there is no reason to expect that a drug-induced reduction in locomotion, had it occurred, would have caused rats selectively to spend greater amounts of the test time outside of the initially non-preferred (white) test chamber.

Several lines of evidence suggest that endogenous opioids and dopamine interact in the neural circuits that mediate reward. For example, both naloxone and the dopamine receptor blocker, pimozide, reduced the duration of eating in food-deprived rats [52], suppressed operant responding for electrical stimulation of the medial forebrain bundle [19,45], and blocked the expression of a conditioned place preference for heroin [8]. Moreover, opiate receptors have been localized on afferents in the dopamine-containing cells of the ventral tegmental area (vta) [42], and microinjections of opioid agonists, such as morphine and enkephalin, into the vta have produced reinforcing effects [9, 10, 41]. Other evidence suggests that enhanced transmission at central dopaminergic synapses facilitates the expression of masculine sexual behavior in the male rat. For example, systemic administration of dopamine receptor agonists, such as LY1633502 [18], RSD-127 [15], or apomorphine [34], facilitated mounting and ejaculation in gonadally intact rats and in castrated males given a low dosage of testosterone. Microinjections of apomorphine into the medial preoptic area and lateral ventricle of gonadally intact male rats increased mounting rates, accelerated the occurrence of ejaculation, and decreased the postejaculatory interval [25]. Finally, administration of dopamine receptor antagonists, such as haloperidol and spiperone, reduced mounting rates, decreased the number of intromissions and lengthened the postejaculatory interval [2,3]. One possibility which has not previously been considered is that dopamine receptor antagonists duplicate the inhibitory effect of naloxone on masculine coital activity by reducing the incentive motivational value for males of the proximal and distal cues emanating from estrous females. Clearly, more research is needed to determine whether a link exists among the endogenous opioid peptides, dopamine neurons, and motivational aspects of masculine sexual activity.

The incentive qualities of the female are especially important determinants of masculine sexual arousal in primate species [4,21]. Male primates typically restrict their sexual interest and activity to favorite females, with the female's sexual attractivity being determined by a variety of endocrine and social variables. It is interesting to note that in 2 primate species studied to date naloxone inhibited sexual activity in males which were gonadally intact. Thus in a group of talapoin monkeys, in which a dominant male copulated only with several dominant females, naltrexone significantly inhibited masculine sexual activity [35]. Likewise, both naloxone and naltrexone inhibited sexual behavior in gonadally intact, socially reared male rhesus monkeys [1]. In contrast to male primates, most gonadally intact male rats eagerly copulate with any estrous female, either in laboratory pair tests or when placed together in groups [30]. As discussed above, administration of naloxone to gonadally intact male rats exerts only a minimal inhibitory effect on mating (i.e., a lengthening of the postejaculatory interval) if, indeed, it affects coital performance at all. As shown in the present experiments, however, an inhibitory action of naloxone on mating is more clearly revealed in male rats after castration or sexual exhaustion. At such times sexual arousal in the male rat, as in the male primate, is especially dependent on the incentive qualities of a potential female partner. Endogenous opioids may play an important role in the interpretation by males of these incentive motivational stimuli.

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