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Differential Sexual Activity of Isolated and Group-Housed Male Mice: Lack of Substantial Influence of Acute or Chronic Naloxone Administration

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DECATANZARO, D., A. DOUGLAS, J. GRIFFITHS AND C. MUIR. *Differential sexual activity of isolated and group-housed male mice: Lack of substantial influence of acute or chronic naloxone administration.* PHARMACOL BIOCHEM BEHAV. **55**(1) 169–174, 1996.—Influences of naloxone upon male sexual behavior were examined using two different baseline activity levels: individually and group-housed mice. In Experiment 1, single injections of 0, 12.5, or 50 μg per animal were administered before testing. Isolated mice showed more sexual activity than did grouped mice; naloxone failed to alter those differences. In Experiment 2, a similar result was obtained despite administration of 50 or 150 μg per animal of naloxone. In Experiment 3, 0 or 50 μg of naloxone was administered to isolated or grouped males daily on the 5 days before testing. Isolated mice showed performance superior to that of grouped mice, but there was no effect of the drug. In Experiment 4, doses of 0, 12.5, or 50 μg of naloxone were given to isolated or grouped males twice daily for 7 days prior to testing, producing little effect. These results suggest that the influences of prior social condition on male sexual activity are robust in the face of naloxone administration.

Male sexual behavior Mice Naloxone Isolation Grouping

IT is established that male mice that are chronically housed in groups are, on average, less likely to be sexually active in the presence of receptive females than are males that are housed individually (10–12). Presumably, unless such effects are due to conditioning processes that influence the onset of sexual activity, there is some physiological change occurring in group-housed mice that diminishes their sexual activity. It is established that chronic group housing can lead to increased pituitary-adrenal activation and changes in brain catecholamines (3–5,16,23,34), although the role of these systems in differential sexual behavior of isolated and group mice is unclear (18). Group housing can also lead to generally decreased pituitary-gonadal activity (5,26). We have previously examined whether controlled levels of androgens might influence effects of housing conditions, but found that castrated testosterone-treated group-housed males are substantially less active sexually than are castrated testosterone-treated isolated males when the same level of exogenous testosterone is given to both (10). Physiological mechanisms contributing to this effect, thus, are still not defined.

Laboratory studies have consistently reported inhibitory effects of opiate agonists and endogenous opioids on male copulatory behavior in rodents (19,22,27,28), and it is well known that opiates suppress human male sexual response (6,9). Endorphins are released during stressful stimulation (31). Because a role of stressful defeat in intermale aggression among grouped animals is related to sexual suppression among some grouped male mice (13), one hypothesis is that group housing might increase endogenous opiates, which in turn, might cause suppression of sexual activity.

Studies with rodent male sexual activity have suggested that treatment with the opiate antagonist, naloxone, can facilitate male sexual activity or alter its topography in laboratory rodents, although there are some discrepancies among results.

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One report (17) indicated that injections of naloxone induced substantial copulatory behavior in previously sexually inactive male rats. It has also been reported that there are reductions in the number of intromissions necessary to achieve ejaculation and reductions in ejaculation latency in sexually experienced male rats given acute injections of naloxone (29,30). On the other hand, other studies of male rats have found that naloxone increases the postejaculatory interval without affecting other aspects of sexual behavior (33), that it increases ejaculation latency (21), that it can diminish sexual response in a stressful context (32), and that it failed to affect copulatory activity in testosterone-treated castrated males (1). In male hamsters, it has been reported that naloxone decreased mount and intromission frequency while reducing the latency to ejaculation and increasing the length of the postejaculatory interval, while also failing to facilitate initiation of copulatory behavior in sexually inactive males (35).

Accordingly, we inquired whether naloxone might influence the copulatory activity in another rodent species, house mice (*Mus musculus*), and especially whether it might differentially influence socially isolated and group-housed male mice. To the best of our knowledge, there have not been any previous reports of studies of effects of naloxone on male sexual activity in this species. The topography of sexual behavior in mice differs substantially from that of the rat (10,25). Male mice tend to show intromissions that are variable in length but typically much longer than those of the rat. Mating is very variable in length but often protracted in this species, with multiple intromissions but fewer ejaculations than might be observed in rats in a typical experimental session.

EXPERIMENT 1

In the first experiment, we attempted to determine whether an acute dose of naloxone would influence male sexual activity in mice as has been demonstrated for other rodent species [e.g., (17,29,30)]. This was examined with two different baseline sexual activity levels, those of isolated and group-housed mice, to see if there would be differential responsiveness to such treatment.

Method

All mice were of C57BL/6J strain, obtained from Charles River Laboratories, La Prairie, Quebec, or bred in this laboratory from such stock and kept in like sex groups from weaning. They were maintained on a reversed 14 L:10 D cycle in standard cages measuring 28 × 16 × 11 cm, with continuous access to food and water.

Stimulus females were bilaterally ovariectomized and made receptive through procedures described in full elsewhere (11). Briefly, this procedure involved a repeated weekly regimen in which females acquired sexual experience with males 2 days after receiving 10 μg of 17β-estradiol benzoate and 6–8 h after receiving 500 μg of progesterone SC; after at least 3 weeks, the females were similarly primed with estrogen and progesterone and presented to experimental males.

Experimental males were housed in groups of four until commencement of the experimental conditions at about 70–85 days of age, with age counterbalanced across conditions. At that age, a random subset of the males was housed individually. Two weeks after commencement of differential housing, at 4–7 h after commencement of the dark phase of the colony light cycle, each male was transferred to an illuminated testing room maintained at the same temperature as the colony room. Each male received an injection of 0, 12.5, or 50 μg of naloxone hydrochloride (Sigma) IP in 0.05 cc of 0.9% saline. These dosages were approximately equivalent to 0, 0.5, and 2.0 mg/kg respectively; the mean body weight of males of this strain and age is measured at 25.2 ± 1.7 g in this laboratory and does not significantly differ between isolated and grouped males. Males within particular groups were assigned randomly to dosage conditions. Immediately after the injection, each male was placed alone in a 4-liter Pyrex beaker containing about 0.5 liters of bedding material. At 30 min after the injection, a sexually receptive female was placed in each beaker and testing for sexual behavior began. Each male–female pair was observed continuously for 2 h by a trained observer. Time of testing was counterbalanced across conditions. The number of mounts, intromissions, and ejaculations, measured as defined previously (25), and the latencies from session commencement to the first of each of these responses, were recorded for each animal.

Results

There were three deaths of group-housed males during the 2 weeks of differential housing, and two others were removed from the experiment before behavioral testing because of wounding caused by other males. Table 1 presents data for the number of mounts, intromissions, and ejaculations, the latencies of the first of each such response from session commencement, and the percentage of males showing each such response. Males not showing a particular response were assigned zero as the number of responses and 120 min (the session length) as the response latency to permit parametric statistical analyses. There was a clear effect of social isolation or grouping upon most measures, especially in the absence of naloxone treatment. Analyses of variance (2 × 3) were conducted on each of the measures of sexual activity. For all measures except number of intromissions, there was an effect of isolation/grouping below the 0.05 probability threshold: mounts, $F(1, 118) = 6.88$, $p = 0.009$; ejaculations, $F(1, 118) = 6.28$, $p = 0.013$; mount latency, $F(1, 118) = 8.04$, $p = 0.006$; intromission latency, $F(1, 118) = 15.33$, $p = 0.004$; and ejaculation latency, $F(1, 118) = 7.90$, $p = 0.006$. None of the measures showed significant main effects of naloxone dosage or the interaction, with associated probability values ranging from 0.18 to 0.97. For males showing at least one complete copulatory series, the difference between ejaculation latency and mount latency was also calculated (isolated males: 29 ± 11, 39 ± 10; grouped males: 38 ± 15, 46 ± 16, 36 ± 13, respectively, by dosage); this measure is not amenable to statistical analysis because of differential proportions of males from each condition, but no strong trend is apparent.

EXPERIMENT 2

The present experiment followed the same rationale as that of Experiment 1, except that a higher dosage range was examined.

Method

Procedures were identical to those of Experiment 1, except that dosages of 1, 50, and 150 μg of naloxone were examined in both isolated and group-housed mice.

Results

Table 2 presents all data for this experiment. There was a clear effect of social isolation or grouping upon most measures,

TABLE 1

MEAN (± SE) NUMBER OF MOUNTS, INTROMISSIONS, AND EJACULATIONS AND LATENCIES TO THE FIRST SUCH RESPONSES FROM SESSION COMMENCEMENT AFTER A SINGLE INJECTION OF VARIED DOSAGES OF NALOXONE IN EXPERMENT 1

		Dosage—μg (mg/kg)		
		0 (0)	12.5 (0.5)	50 (2.0)
Isolated	*n*	21	21	21
Mounts	Number	2.9 ± 0.5	3.6 ± 1.3	3.3 ± 0.8
	Latency (min)	28 ± 8	38 ± 10	23 ± 7
	% Responding	85.7	76.2	90.5
Intromissions	Number	21.4 ± 5.3	25.5 ± 7.6	27.8 ± 4.6
	Latency (min)	31 ± 8	42 ± 10	29 ± 7
	% Responding	85.7	76.2	90.5
Ejaculations	Number	0.5 ± 0.1	0.6 ± 0.2	0.6 ± 0.2
	Latency (min)	88 ± 10	84 ± 10	84 ± 10
	% Responding	42.9	47.6	47.6
Grouped	*n*	23	22	18
Mounts	Number	1.4 ± 0.4	2.3 ± 0.6	1.4 ± 0.4
	Latency (min)	66 ± 11	46 ± 11	45 ± 10
	% Responding	56.5	77.3	83.3
Intromissions	Number	13.5 ± 4.7	20.9 ± 6.0	26.5 ± 7.4
	Latency (min)	82 ± 10	60 ± 11	52 ± 9
	% Responding	43.5	72.7	83.3
Ejaculations	Number	0.2 ± 0.1	0.4 ± 0.1	0.3 ± 0.1
	Latency (min)	108 ± 6	102 ± 7	104 ± 7
	% Responding	21.8	31.8	27.8

TABLE 2

MEAN (± SE) NUMBER OF MOUNTS, INTROMISSIONS, AND EJACULATIONS AND LATENCIES TO THE FIRST SUCH RESPONSES FROM SESSION COMMENCEMENT AFTER A SINGLE INJECTION OF VARIED DOSAGES OF NALOXONE IN EXPERIMENT 2

		Dosage—μg (mg/kg)		
		0 (0)	50 (2.0)	150 (6.0)
Isolated	*n*	10	10	10
Mounts	Number	5.5 ± 1.8	6.3 ± 2.3	9.1 ± 2.9
	Latency (min)	28 ± 11	28 ± 12	36 ± 15
	% Responding	90	90	80
Intromissions	Number	25.4 ± 4.9	22.7 ± 4.2	33.8 ± 9.0
	Latency (min)	28 ± 11	28 ± 12	36 ± 15
	% Responding	90	90	80
Ejaculations	Number	0.7 ± 0.2	0.8 ± 0.1	0.4 ± 0.2
	Latency (min)	73 ± 14	64 ± 13	105 ± 9
	% Responding	70	80	30
Grouped	*n*	10	10	10
Mounts	Number	3.5 ± 1.4	1.0 ± 0.4	2.0 ± 1.0
	Latency (min)	62 ± 18	70 ± 14	89 ± 14
	% Responding	60	60	40
Intromissions	Number	30.1 ± 12.6	16.7 ± 8.5	17.5 ± 7.5
	Latency (min)	62 ± 18	77 ± 15	89 ± 14
	% Responding	60	50	40
Ejaculations	Number	0.6 ± 0.2	0.4 ± 0.2	0.1 ± 0.1
	Latency (min)	92 ± 13	100 ± 9	118 ± 2
	% Responding	50	40	10

especially in the absence of naloxone treatment. Analyses of variance were conducted on each of the measures of sexual activity as for Experiment 1. For each of the following measures, there was an effect of isolation/grouping below the 0.05 probability threshold: mounts, $F(1, 54) = 10.31$, $p = 0.003$; mount latency, $F(1, 54) = 13.79$, $p < 0.001$; intromission latency, $F(1, 54) = 15.11$, $p < 0.001$; and ejaculation latency, $F(1, 54) = 6.65$, $p = 0.012$. The comparable main effect for number of ejaculations approached this significance threshold, $F(1, 54) = 3.65$, $p = 0.058$. There were also significant main effects for naloxone dosage for number of ejaculations, $F(2, 54) = 3.24$, $p = 0.045$, and ejaculation latency, $F(2, 54) = 5.07$, $p = 0.009$, but these effects were in the opposite direction from those predicted, with drug-treated animals showing fewer ejaculations and a longer ejaculation latency. The interactions were not significant for any measure, with associated probability values ranging from 0.38 to 0.79.

EXPERIMENT 3

Single injections in Experiments 1 and 2 may have produced subtle, albeit largely nonsignificant, alterations in the topography of sexual activity, but did not alter the differential behavior of isolated and group-housed males. In general, major pituitary-gonadal influences of group housing have been observed after substantial periods of exposure to other males (5,26). The housing conditions that produced differential sexual activity in Experiments 1 and 2 and elsewhere (10,11) were in effect for weeks. Therefore, we inquired whether a chronic regimen of naloxone might influence differential sexual activity of grouped and isolated mice.

Method

Mice were obtained, maintained, and assigned to conditions of differential housing for 2 weeks as in Experiment 1. Commencing 6 days prior to scheduled testing with sexually receptive females, each isolated or grouped male was assigned to one of two dosage groups, 0 or 50 μg of naloxone administered as in Experiment 1. Each male received an injection at about 2 h after commencement of the dark phase of the light cycle on each of the 5 days preceding the day of testing. Males within groups were counterbalanced across dosage conditions, being given colored tail markings to identify condition. Behavioral testing and dependent measures were the same as in Experiment 1.

Results

Table 3 gives all data for this experiment. There was a very clear trend for isolates to show greater sexual activity than did group-housed males, regardless of whether or not the animals had received naloxone. Naloxone-treated males performed much as did saline-treated males. Analyses of variance indicated strong effects of housing condition for all measures: mounts, $F(1, 28) = 16.3$, $p < 0.001$; intromissions, $F(1, 28) = 17.4$, $p < 0.001$; ejaculations, $F(1, 28) = 20.7$, $p < 0.001$; mount latency, $F(1, 28) = 15.2$, $p < 0.001$; intromission latency, $F(1, 28) = 15.0$, $p < 0.001$; and ejaculation latency, $F(1, 28) = 18.0$, $p < 0.001$. None of the effects of dosage of the interactions of housing and dosage attained or approached significance.

TABLE 3

MEAN (± SE) NUMBER OF MOUNTS, INTROMISSIONS, AND EJACULATIONS AND LATENCIES TO THE FIRST SUCH RESPONSES FROM SESSION COMMENCEMENT AFTER DAILY INJECTIONS OF VARIED DOSAGES OF NALOXONE ON THE 5 DAYS BEFORE TESTING IN EXPERIMENT 3

		Dosage—μg (mg/kg)	
		0 (0)	50 (2.0)
Isolated	*n*	8	8
Mounts	Number	5.7 ± 1.3	5.9 ± 1.5
	Latency (min)	39 ± 18	22 ± 2
	% Responding	75	87.5
Intromissions	Number	33.1 ± 8.3	32.2 ± 8.5
	Latency (min)	41 ± 17	25 ± 14
	% Responding	75	87.5
Ejaculations	Number	0.6 ± 0.2	1.2 ± 0.2
	Latency (min)	79 ± 13	47 ± 12
	% Responding	62.5	87.5
Grouped	*n*	8	8
Mounts	Number	1.0 ± 0.6	1.2 ± 0.9
	Latency (min)	89 ± 17	97 ± 15
	% Responding	37.5	25
Intromissions	Number	6.6 ± 5.1	3.5 ± 2.9
	Latency (min)	89 ± 17	98 ± 15
	% Responding	37.5	25
Ejaculations	Number	0.1 ± 0.1	0.1 ± 0.1
	Latency (min)	110 ± 10	111 ± 9
	% Responding	12.5	12.5

EXPERIMENT 4

The present experiment followed the same rationale as that of Experiment 3, except that injections were made more frequent and two dosages were examined.

Method

Mice were obtained, maintained, and assigned to conditions of differential housing for 2 weeks as in Experiment 1. Commencing 8 days prior to scheduled testing with sexually receptive females, each isolated or grouped male was assigned to one of three dosage groups, 0, 12.5, or 50 μg of naloxone. Each male received two daily injections at about 2 and 9 h after commencement of the dark phase of the light cycle on each of the 7 days preceding the day of testing. Males within groups were randomly assigned to dosage conditions, being given colored tail markings to identify condition. Behavioral testing and dependent measures were as in Experiment 1.

Results

Table 4 presents all data for this experiment. Isolated mice showed greater sexual activity than did group-housed males, regardless of dosage of naloxone, which apparently was without effect. Analyses of variance showed effects of housing condition at values of < 0.05 probability for all measures except ejaculation latency: mounts, $F(1, 66) = 12.8$, ip < 0.001; intromissions, $F(1, 66) = 6.3$, $p = 0.014$; ejaculations, $F(1, 66) = 4.2$, $p = 0.044$; mount latency, $F(1, 66) = 7.3$, $p = 0.009$; and intromission latency, $F(1, 66) = 8.5$, $p = 0.005$. None of the effects of dosage or the interactions of housing and dosage attained or approached significance.

GENERAL DISCUSSION

These experiments replicate previous findings (10,11) that isolated male mice are, on average, more sexually active than are group-housed male mice. However, despite the examina-

TABLE 4

MEAN (± SE) NUMBER OF MOUNTS, INTROMISSIONS, AND EJACULATIONS AND LATENCIES TO THE FIRST SUCH RESPONSES FROM SESSION COMMENCEMENT AFTER TWICE DAILY INJECTIONS OF VARIED DOSAGES OF NALOXONE ON THE 7 DAYS BEFORE TESTING IN EXPERIMENT 4

		Dosage—μg (mg/kg)		
		0 (0)	12.5 (0.5)	50 (2.0)
Isolated	*n*	12	12	12
Mounts	Number	3.0 ± 1.2	7.3 ± 2.5	5.8 ± 1.6
	Latency (min)	31 ± 11	42 ± 12	13 ± 8
	% Responding	91.7	91.7	100
Intromissions	Number	44.8 ± 8.8	54.3 ± 11.8	53.2 ± 8.6
	Latency (min)	31 ± 11	42 ± 12	18 ± 8
	% Responding	91.7	91.7	100
Ejaculations	Number	0.5 ± 0.1	0.4 ± 0.2	0.9 ± 0.2
	Latency (min)	91 ± 10	94 ± 10	76 ± 11
	% Responding	50	41.7	66.7
Grouped	*n*	12	12	12
Mounts	Number	1.2 ± 0.3	0.9 ± 0.2	2.2 ± 0.9
	Latency (min)	53 ± 13	61 ± 14	54 ± 15
	% Responding	75	75	66.7
Intromissions	Number	37.9 ± 10.4	30.9 ± 10.0	24.5 ± 7.1
	Latency (min)	60 ± 14	62 ± 14	58 ± 14
	% Responding	66.7	75	66.7
Ejaculations	Number	0.6 ± 0.2	0.2 ± 0.1	0.2 ± 0.1
	Latency (min)	90 ± 10	107 ± 9	102 ± 9
	% Responding	50	16.7	25

tion of diverse dosages and regimens of naloxone administration, they fail to show any substantial influence of this drug upon male sexual activity in this species.

The failure of naloxone to elevate the sexual performance of group-housed male mice is contrary to a report (17) that sexually inactive male rats can be induced to copulate through administration of this drug. It is more comparable to a finding that sexually inactive hamsters remain so in the face of treatment with naloxone (35). Clearly, species and other parametric differences could account for these discrepancies. Nevertheless, many of the more recent reports have not indicated any substantial facilitatory effect of naloxone treatment upon male sexual activity in rodent species (1,8,21,32,33). Mice show a longer copulatory sequence, and repeated sequences are relatively uncommon compared to some other rodent species, so the prolonging effects of naloxone upon postejaculatory interval reported for rats and hamsters (33,35) could not be assessed here.

Group housing of mice is associated with intermale aggression [e.g., (4,13)], and some studies (14,15) indicate that defeat in aggressive encounters can diminish hypothalamic levels of luteinizing hormone (LH). There is also evidence that levels of LH are correlated with sexual arousal and activity in mice and rats (2,20). Administration of naloxone to adult male rats has been found to cause an increase in serum concentrations of LH within a half hour after injection (7,30). Accordingly, the naloxone-induced alterations in male sexual topography found by some other researchers remain plausible, despite one study (24) showing that serum LH levels did not correlate with naloxone-induced sexual behavior topography changes, and despite failure of the current data to show a substantial influence of this drug upon differential sexual activity of grouped and isolated male mice.

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