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Differential Sexual Activity of Isolated and Group-Housed Male Mice: Influence of Acute *d*-Amphetamine Sulfate Administration

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DECATANZARO, D. AND J. GRIFFITHS. *Differential sexual activity of isolated and group-housed male mice: Influence of acute* d-*amphetamine sulfate administration.* PHARMACOL BIOCHEM BEHAV **54**(3) 601-604, 1996.—It is established that group housing can impair sexual activity of male mice, and that central catecholamines are involved in male sexual response, but it is not known whether catecholamine mechanisms are involved in sexual impairment in grouped males. Injections of 0, 0.22, 0.67, 2.0, or 6.0 mg/kg of *d*-amphetamine sulfate were administered 1 h before testing to individually and group-housed male C57BL/6J mice. Isolated mice showed more mounts, intromissions, and ejaculations and shorter response latencies than did group-housed mice. The latencies to first mount, intromission, and ejaculation were nonmonotonically related to dosage, being shortest at the lowest dosage in isolated mice, but significantly elevated by the higher dosages in both isolated and grouped males. The number of ejaculations was significantly elevated by moderate dosages in both isolated and grouped mice, peaking at the 2.0 mg/kg dosage in isolated mice and at 0.67 mg/kg in grouped mice. Nevertheless, amphetamine treatment generally failed to eliminate differences between males from isolated and grouped backgrounds.

Amphetamine Male sexual behavior Isolation Group housing Mice

IT IS ESTABLISHED that male mice (*Mus musculus*) that are chronically housed in groups are, on average, less likely than isolated mice to be sexually active in the presence of receptive females (10,12,13). This may in part relate to intermale aggression among grouped males (14). Physiological substrates of this effect are not established. Chronic group housing can lead to generally decreased pituitary-gonadal activity in this species (6,7,22), but castrated group-housed males remain less active sexually than isolated males in the face of controlled levels of androgens (10). Social housing conditions can also modulate levels of pituitary-adrenal activity in mice (2,6,16,20), although the role of these systems in differential sexual behavior of isolated and grouped mice is not entirely clear (18).

Isolation or grouping of male mice can also influence brain biogenic amine turnover, in particular, the rates of synthesis and utilization of brain catecholamines (15,23,27-29). In grouped males in their home environment, catecholamine turnover is higher in comparison to isolates. However, in a novel arena or in response to novelty stress or human handling, higher reactivity and slower adaptation are found in isolated males, which suggests a more active arousal mechanism and greater catecholamine turnover. Such differences in endogenous catecholamine activity may be associated with differential dose-response in motor activity of isolated and grouped male mice to various pharmacological agents (30).

Much evidence indicates that catecholamine mechanisms may be involved in male sexual behavior in rodents (4). Male sexual behavior in rats can be augmented by administration of the catecholamine-activating drug, *d*-amphetamine sulfate (3,25). Apomorphine, a direct stimulator of brain dopamine receptors, can stimulate the copulatory behavior of male rats selected for their sluggishness; these effects are reversed by giving the dopamine receptor inhibitor, haloperidol (17,26). Sexual behavior of male rats is damaged by intraventicular administration of the catecholamine neurotoxin, 6-hydroxydopamine, or acute peripheral administration of α-methyl-*p*-tyrosine, a tyrosine hydroxylase inhibitor (9). Localized cen-

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tral administration of apomorphine can facilitate sexual reflexes in male rats, although this depends upon location and dosage (19,24). A role of general arousal mechanisms in male sexual behavior also receives support from evidence that application of a mild tail shock to sluggish male rats enhances sexual arousal in the presence of receptive females (1,8). Deficits in male sexual activity in rats induced by α-methyl-*p*-tyrosine or 6-hydroxydopamine can be overridden by pinching the males' tails during the test of sexual behavior (9), suggesting an interaction of nonspecific arousal mechanisms and central catecholamines in the control of male sexual behavior.

Accordingly, if catecholamine-mediated arousal mechanisms are involved in differential sexual activity of isolated and group-housed males, one would predict that treatment with catecholamine stimulants might elevate sexual activity, especially in group-housed males because of a lower baseline level. Here, we inquired whether amphetamine treatment might influence the copulatory activity of male mice, and especially whether it might differentially influence socially isolated and group-housed males.

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 after 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 (12). 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 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. Each male then received an intraperitoneal injection of 0, 0.22, 0.67, 2.0, or 6.0 mg/kg of *d*-amphetamine sulphate (Sigma); each dose was dissolved in approximately 0.05 cc of 0.9% saline with small variation according to body weight. Immediately after the injection, each male was placed alone in a 4-l Pyrex beaker containing about 0.5 l of bedding material. Testing for sexual activity began for each male 60 min after the injection when a receptive female was introduced into each beaker. 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 elsewhere (21), and the latencies from session commencement to the first of each of these responses, were recorded for each animal [see also (12)].

RESULTS

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 all measures, with greater average frequencies and shorter average latencies of responses in males that had been socially isolated. This effect was composed of two elements, both a reduction in the number of males showing sexual responses and a reduction in the number of responses shown by the average male that did respond. Most animals that mated showed only one copulatory series, with the only exceptions being among isolated males; at the 0.22 mg/kg dosage one showed two series, at the 0.67 mg/kg dosage one showed two series and another showed three series, and at the 2.0 mg/kg dosage two showed two series. Among isolated males, the mean latency to each response category was lowest at the 0.22 mg/kg dosage and highest at the 6.0 mg/kg dosage, while the number of ejaculations was above controls at moderate dosages but not at the highest dosage. In grouped males, the greatest difference from controls was evident at the 0.67 mg/kg dosage, where the greatest frequencies and shortest latencies were found in each response category. Analyses of variance (2 × 5) were conducted on each of the measures of sexual activity. For all measures, there was a significant effect of isolation/grouping: mounts, $F(1, 118) = 26.70$, $p < 0.0001$; intromissions, $F(1, 118) = 57.92$, $p < 0.0001$; ejaculations, $F(1, 118) = 25.98$, $p < 0.0001$; mount latency, $F(1, 118) = 108.70$, $p < 0.0001$; intromission latency, $F(1, 118) = 98.90$, $p < 0.0001$; and ejaculation latency, $F(1, 118) = 23.17$, $p < 0.0001$. The main effect of amphetamine dosage was significant for number of ejaculations, $F(4, 118) = 2.53$, $p = 0.0441$; mount latency, $F(4, 118) = 7.35$, $p = 0.0001$; intromission latency, $F(4, 118) = 6.26$, $p = 0.0003$; and ejaculation latency, $F(4, 118) = 3.48$, $p = 0.0103$. The interaction was significant for ejaculation latency, $F(4, 118) = 2.92$, $p = 0.0239$, and approached significance for mount latency, $F(4, 118) = 2.33$, $p = 0.0594$, and intromission latency, $F(4, 118) = 2.36$, $p = 0.0572$. Multiple comparisons (Duncan's test, $p < 0.05$) generally showed differences between isolated and grouped males for all measures. For isolated males, such multiple comparisons also showed a significant elevation above controls at the 2.0 mg/kg dosage in number of ejaculations, and significant decreases in ejaculation latency at the 0.22 and 2.0 mg/kg dosages. For grouped males, such multiple comparisons showed a significant increase above vehicle controls in number of ejaculations for the 0.67 mg/kg dosage, and significant increases in mount and intromission latency at the two highest doses. Curvilinear regressions, assessing linear, quadratic, and cubic effects of dosage, were conducted separately for isolated and grouped males for measures of number of ejaculations and mount and ejaculation latency. Significant quadratic trends emerged for isolates in mount latency, $r = 0.54$, $F(2, 57) = 11.91$, $p = 0.0002$, and ejaculation latency, $r = 0.35$, $F(2, 57) = 4.03$, $p = 0.0223$; for grouped animals in mount latency, $r = 0.36$, $F(2, 56) = 4.20$, $p = 0.0195$, but linear and cubic trends were generally not significant. For isolated males showing at least one complete copulatory series, the difference between ejaculation latency and mount latency decreased with amphetamine treatment (73 ± 15, 42 ± 8, 55 ± 14, 34 ± 5, and 46 ± 6, respectively, by dosage); this is not amenable to statistical analysis because of differential proportions of males from each condition and cannot be analyzed for grouped males because too few showed a complete copulatory series.

TABLE 1

MEAN (±SE) NUMBER OF MOUNTS, INTROMISSIONS, AND EJACULATIONS AND LATENCIES TO THE FIRST SUCH RESPONSES FROM SESSION COMMENCEMENT AFTER AN INJECTION OF *d*-AMPHETAMINE SULFATE

Dosage—mg/kg*		0	0.22	0.67	2.0	6.0
Isolated	*n*	12	12	12	12	12
Mounts	Number	11.8 ± 3.1	13.9 ± 3.5	8.7 ± 3.5	9.2 ± 2.1	6.6 ± 1.4
	Latency (min)	16 ± 10	3 ± 1	22 ± 10	25 ± 5	56 ± 8*
	% Responding	91.7	100	91.7	100	91.7
Intromissions	Number	23.2 ± 3.7	30.4 ± 5.3	28.0 ± 5.6	28.0 ± 2.9	18.7 ± 4.5
	Latency (min)	19 ± 9	9 ± 2	33 ± 13	31 ± 7	64 ± 9*
	% Responding	91.7	100	83.3	100	83.3
Ejaculations	Number	0.50 ± 0.15	0.83 ± 0.17	0.75 ± 0.28	1.00 ± 0.17*	0.33 ± 0.09
	Latency (min)	99 ± 9	64 ± 11*	91 ± 11	71 ± 10*	111 ± 5
	% Responding	50	75	50	83.3	33.3
Grouped	*n*	12	12	12	12	11
Mounts	Number	2.7 ± 0.7	2.7 ± 1.9	3.7 ± 1.1	3.5 ± 1.9	1.5 ± 1.1
	Latency (min)	74 ± 13	91 ± 14	57 ± 14	106 ± 6*	115 ± 3*
	% Responding	75	25	75	41.7	18.2
Intromissions	Number	8.4 ± 2.5	5.2 ± 3.2	12.3 ± 3.1	5.3 ± 2.7	4.8 ± 3.3
	Latency (min)	78 ± 13	96 ± 13	67 ± 13	110 ± 4*	116 ± 3*
	% Responding	66.7	25	75	33.3	18.2
Ejaculations	Number	0.08 ± 0.08	0.17 ± 0.11	0.50 ± 0.15*	0.08 ± 0.08	0.09 ± 0.09
	Latency (min)	113 ± 7	110 ± 8	100 ± 9	119 ± 1	120 ± 1
	% Responding	8.3	16.70	50	8.3	9.1

*Significant difference from respective (isolated or grouped) 0 mg/kg group.

DISCUSSION

These experiments replicate previous findings (10,12) that isolated male mice are, on average, more sexually active than are group-housed male mice. Amphetamine treatment had nonmonotonic dose-dependent influences on both isolated and grouped males, with facilitatory effects being more evident at lower dosages and inhibitory effects occurring at higher dosages. The quality of these dose-dependent influences depended upon the measure and whether males were isolated or grouped. In isolated males, response latencies were shortest at the lowest (0.22 mg/kg) dosage, significantly so for ejaculation latency, and longest at the highest dosage (6.0 mg/kg), significantly so for mount latency and intromission latency. The number of ejaculations for isolated males was elevated at moderate dosages and peaked at the 2.0 mg/kg dosage. In grouped males, the greatest frequencies and shortest latencies were found in each response category at the 0.67 mg/kg dosage, where the number of ejaculations was significantly elevated. The two highest dosages significantly increased mount latency and intromission latency in grouped males.

These data replicate previous studies [e.g., (3,17,25)] showing that catecholamine stimulants can facilitate certain aspects of male sexual response at moderate dosages in rodents. At the same time, they show some inhibition of sexual response at higher dosages, particularly reflected in longer response latencies. It should be noted that effects upon arousal of sexual behavior, as measured in the latency and number of mounts and intromissions, may be influenced by mechanisms that are somewhat independent of those affecting measures of ejaculation. The clearest facilitatory influences of amphetamine in the present study are upon measures of ejaculation. It should be noted that although the act of ejaculation is biologically critical for reproduction, a shorter ejaculation latency is not necessarily adaptive, in that longer mating durations are associated with greater fertility in mice (11).

The present data are consistent with a role of catecholamine-related mechanisms in male sexual behavior, but they do not provide definitive evidence that these mechanisms account for differences in performance of isolated and grouped males. Differential dose response of isolated and grouped males was predicted on the basis of established differences in reactivity to novel environments and associated endogenous catecholamine dynamics in isolated and grouped males of this species (5,23,27–29). Arousal-related factors were also suggested by the finding that periods of isolation as short as 1 day can have facilitatory influences upon the arousal of male sexual behavior in mice, as can cage-cleaning within the few hours before testing in group-housed male mice (13). However, the prediction that amphetamine treatment might have greater influences upon grouped males, because of a lower baseline, was not supported by the data. Grouped males in the present study given 0.67 mg/kg did resemble 0 mg/kg treated isolates in number of ejaculations, but it is noteworthy that in no other measure at any dosage did grouped males approach the performance of 0 mg/kg treated isolates. Furthermore, amphetamine treatment elevated performance of isolated males at least as much as that of grouped males, and at any particular dosage, isolated males exceeded the performance of grouped males.

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