# ISOLATION-INDUCED FACILITATION OF MALE SEXUAL BEHAVIOR IN MICE

by

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## Abstract

The present study examined the effects of housing and social conditions on the sexual performance of male house mice (Mus musculus). Specifically, mice housed postpubertally in social isolation were compared to others housed in all-male groups. Following periods of differential housing, males were tested in the presence of ovariectomized females made receptive with exogenous estrogen and progesterone. In five series of experiments, effects of isolation/grouping and related parameters were delineated, and physiological and social mechanisms underlying these effects probed.

In the first series of experiments, the basic effects of isolation and grouping were examined. In Experiment 1A, animals housed 1, 3, or 12 per cage were given repeated weekly tests with females. Performance in isolates was consistently superior and reached an asymptote twice that of grouped animals. In Experiment 1B, reversal of housing conditions reversed performance. Experiment 2 varied intervals of isolation between subjects, finding facilitation at several intervals. Experiment 3 compared animals under different population densities. Density did not alter the effects of isolation and grouping. In all experiments, additional tests with target males indicated that aggressive and sexual performance were moderately correlated and responded similarly to parametric manipulations.

In the second series, the strain and species generality of isolation/grouping differences in sexual activity was studied. Experiment 4 examined male Swiss-Webster, C57, and DBA mice housed individually or grouped for 2 weeks. Within each strain, social isolates showed more mounts,

intromissions, and ejaculations and shorter latencies to first mount and intromission. Experiment 5 involved a similar comparison of isolated and grouped male rats, hamsters, and gerbils. Isolation produced no major effect in hamsters but reduced performance in rats and gerbils. Results suggest that facilitation of sexual activity by isolation is characteristic of the mouse species. Decrements accompanying postpubertal isolation in the rat resemble effects of prepubertal isolation in this species. These species differences may parallel differences in physiology and social behavior.

In the third series, the minimum period of isolation required to produce isolation/grouping differences was established. Experiment 6 compared sexual performance of male mice isolated or grouped for periods of 1 day or 2 weeks. Isolation facilitated performance equally at both intervals; this differs from effects of isolation on other behavioral and physiological variables. Experiment 7 examined animals isolated for intervals ranging from 1 hour to 1 week. Isolates showed greater performance at all intervals exceeding 12 hours. Simple cleaning of grouped animal's cages increased their performance at 1- and 4-hour intervals. In Experiment 8, grouped males were observed continuously for 24 hours preceding testing. Intermale mounting was rare and neither it nor aggression correlated with subsequent sexual performance.

In the fourth and fifth series, some possible physiological mediators of isolation/grouping effects were studied. In Experiment 9A, adrenalectomized and non-adrenalectomized mice were compared. Following adrenalectomy, grouped mice showed elevated sexual activity while isolates declined. In Experiment 9B, corticosterone treatment failed to reverse effects of adrenalectomy. In Experiment 10, ACTH treatment restores mating

activity to preadrenalectomy levels in adrenalectomized isolates but had little effect on intact isolates. Results suggest an adrenal involvement in isolation effects but do not specify its nature. In Experiment 11, castrated males given replacement testosterone were compared to intact males. Isolation/grouping differences were present in intact but not testosterone treated mice, suggesting gonadal hormone involvement in the phenomenon.

Social interactions among grouped male mice appear to suppress their subsequent sexual activity with females. Intermale aggression, particularly, may stress group members, producing physiological changes conducive to low sexual activity. Furthermore, the presentation of stimulus females may be relatively more novel for isolates and consequently produce higher levels of general arousal in these mice.

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# INTRODUCTION

There is growing evidence that the biology and behavior of male house mice (Mus musculus) are strongly affected by manipulations of social variables and housing conditions. One major focus of investigation has involved a comparison of adult male mice housed either in social isolation or in all-male groups. This investigation has produced a considerable amount of data indicating the existence of major physiological and behavioral differences between isolated and grouped mice.

The experimentation described here involved an examination of male sexual behavior within the isolation/grouping paradigm. While there have been numerous reports of effects of isolation and grouping on subsequent intermale interactions and on associated physiological variables, there are no comparable reports involving male-female interactions. In the present study male mice that were housed differentially during postpubertal development were measured for sexual activity. Measurement occurred in the presence of females made sexually receptive through ovariectomy and treatment with estrogen and progesterone. The effects of the basic isolation/grouping and related parameters were

probed. Subsequently, physiological and social mediators of these effects were examined through techniques involving glandular extraction, treatment with exogenous hormones, and systematic observation of intermale interactions.

Effects of Isolation on Behavior and Physiology

The most frequently studied effect of social isolation upon male mice is isolation-induced aggression (Scott, 1966; Valzelli, 1969). Mice isolated for several days in adulthood frequently show intense levels of aggression when subsequently placed with target mice. Isolation-induced aggression is found almost exclusively in males of the species and is usually directed only toward other males. The effects of isolation on intermale aggression are known to be progressive; they increase with time in isolation (Brain & Nowell, 1970; Valzelli, 1969). Some inbred genetic strains of mice show higher levels of aggression than do other strains (Karczmar & Scudder, 1969; LeDouarec & Broussy, 1969). Isolation-induced aggression may occur in a relatively mild and attenuated form in some other rodent species (Blanchard & Blanchard, 1977; Conner, 1972; Edwards & Rowe, 1975; Thiessen & Yahr, 1977). In mice, however, it is generally quite intense; mice may bite and attack one another quite vigorously, inflicting large lacerations and occasionally causing death, when placed together following long periods of isolation.

General activity levels may also be affected in male mice by social isolation. Essman (1968) and Brain, Haley, and Nowell (1971) have found that isolates show more locomotor activity than do grouped animals upon exposure to a novel environment. However, isolated mice are usually less active than grouped mice while in their home cages

without disturbance (Welch & Welch, 1969a; 1969b).

There is considerable evidence that the pituitary-adrenocortical system is affected by social isolation, although the reported results and interpretations are often at variance. Isolated male mice generally show lower adrenal weights than do grouped males when neither have been subjected to stressors (e.g., Benton, Goldsmith, Gamal-El-Din, Brain, & Hucklebridge, 1978; Brain & Nowell, 1971; Bronson, 1967; Christian, 1959). Experiments reporting higher adrenal weights for isolates (e.g., Sigg, 1969) tend to be those in which the animals are subjected to stressful experiences such as fighting before the gland weights are obtained. Assays of circulating corticosterone levels have indicated a lack of difference between unstressed isolated and grouped mice (Benton et al., 1978; Goldsmith, Brain, & Benton, 1976). However, isolates may show higher levels of circulating corticosterone when stressed by fighting or other means (Goldsmith et al., 1976; Sigg, 1969). Results from a study by Brain and Nowell (1971) are somewhat inconsistent with this picture. Brain and Nowell found that both basal and stress corticosterone levels were lower in isolated mice, however, their group size (16 mice) was somewhat larger than in most studies. Adrenocorticotrophic hormone, which is more difficult to assay because it is relatively unstable and its levels may change rapidly, appears to be involved in control of isolation-induced aggression (Brain & Poole, 1974; Leshner, Walker, Johnson, Kelling, Kreisler, & Svare, 1973) and may also differ between isolated and grouped mice.

Adrenal medullary function is also affected. There are a number of reports (Anton, 1969; Anton, Schwartz, and Kramer, 1968; Benton et al., 1978; Welch & Welch, 1968) that there are no significant differences in adrenal catecholamine levels between isolated and grouped mice. However,

one study (Welch, 1965) has reported that grouped mice have higher medullary levels of epinephrine. It also appears that the turnover of epinephrine is greater in grouped animals (Welch & Welch, 1968; 1971). The percentage of total adrenal catecholamine constituted by norepinephrine has been reported both to be higher in isolates (Welch, 1965) and not to differ between isolated and grouped male mice (Anton, 1969; Anton et al., 1968).

Many studies of mice report an increase in gonadal activity as a consequence of isolation. Studies contrasting isolated and group-housed males have indicated that the former have heavier sex accessories than the latter (Benton et al., 1978; Brain, 1971; Brain & Nowell, 1971; Christian, 1955; Vandenbergh, 1960). Sizes of testes, preputials, prostates, and seminal vesicals may all be increased by social isolation. Differences between isolated and grouped animals in gonadal activity may relate to differences in pituitary-adrenocortical activity because levels of testosterone are known to decrease as a consequence of pituitary-adrenocortical activation (Bullock & New, 1971; Desjardins & Ewing, 1971).

Neurotransmitter systems also appear to be affected by social isolation and group housing. Marked effects have been observed in the levels and turnover rates of many of the putative neutrotransmitters and their precursors and metabolites in the central nervous system. Isolated mice have been reported to show lower levels of the brain catecholamines, norepinephrine and dopamine, while serotonin levels are generally not affected (Garattini, Giacalone, & Valzelli, 1969; Welch & Welch, 1969a; 1969b). Decreased levels of neural N-acetyl-L-aspartic

acid have also been observed in isolates (Marcucci, Mussini, Valzelli, & Garattini, 1968). Turnover or utilization rates of norepinephrine, dopamine, and serotonin may all be higher in isolated than in grouped mice when these animals are exposed to stressful or novel stimuli (Garattini et al., 1969; Welch & Welch, 1969a; 1969b). Increased neurotransmitter turnover may relate to higher levels of aggressive behavior and general activity observed in isolates (Essman, 1968; Welch & Welch, 1971). Differences in neurotransmitter levels and utilization between isolated and grouped animals may also relate to differences in pituitary-adrenocortical activity; for example, increased ACTH levels may produce increased turnover of catecholamines and serotonin (Dunn & Gispen, 1977).

In summary so far, there is evidence that isolates are less active in their home cage, but more active and prone to be aggressive when exposed to novel or stressful environments. Also, isolates show lower baseline pituitary-adrenal and catecholaminergic activity, but higher activity of these systems when stressed. Baseline gonadal activity is increased in isolates.

## Relationship of Isolation to Stress, Dominance, Defeat, and Territoriality

The physiological differences between isolated and group-housed mice can be interpreted as indicating greater "stress" in the grouped animals. "Stress" is generally defined physiologically as involving pituitary-adrenocortical and adrenal medullary functioning, with increased activation of these systems indicating increased stress (Levine, 1976; Selye, 1956). A variety of physiological and psychological stimuli, which usually are aversive and tax the organism's ability to cope, will activate these physiological systems; such stimuli are commonly called stressors.

Adrenal activation adapts the organism to demanding situations through a number of biochemical and visceral changes that increase the organism's energy levels. Prolonged adrenal activation is usually associated with reductions in other physiological activity, such as immunological and reproductive activity. Recent evidence indicates that changes in neurochemical systems occur concomitant with stress-related pituitary-adrenal change. Brief stressors may produce immediate increases in central catecholaminergic activity, followed by a decrease in catecholaminergic activity below baseline and an increase in cholinergic activity (Anisman, 1978). During more prolonged stress there may be an increase in synthesis of catecholamines, a slowdown in utilization of catecholamines, or both, while very severe stress may produce very low levels of brain catecholamines (Weil-Maherbe, 1972; Yuwiler, 1971).

The indications, discussed above, of increased pituitary-adrenocortical, adrenal medullary, and central catecholaminergic activity, and decreased gonadal activity in grouped male mice are thus indicative of greater stress levels in these animals (see Brain, 1975 for further discussion of this point). This stress presumably arises from intermale aggression and competition for common resources among grouped animals. Paradoxically, however, isolates may show greater immediate stress reactions to novel stimuli. This increased physiological reactivity is accompanied by increased behavioral activation.

Male mice in long established groups usually demonstrate considerable variance in intermale interactions and behavioral response to a number of experimental tasks. Such inter-individual differences have frequently been interpreted as indicating the presence of dominance

hierarchies. "Dominance" level has been rated through observation of relative success of group members in obtaining reinforcers, such as food, when they must compete for a single source (e.g., Messeri, Eleftheriou, & Oliverio, 1975). The occurrence of submissive postures has also been used; these are characterized by upright stances with eyes open, ears erect, and occasional vocalization (e.g., Benton et al., 1978). Animals that are more aggressive are also generally considered more dominant, and the occurrence of scars on the flanks and hindquarters, inflicted by other aggressive males, is considered an especially reliable sign of subordination (Benton et al., 1978; DeFries & McClearn, 1970; 1972; McKinney & Desjardins, 1973a; 1973b). There is a substantial amount of evidence indicating that isolated male mice are similar in terms of both physiology and behavior to more dominant and territorial grouped males. Like isolates, dominant grouped males show glandular and hormonal indications of relatively low adrenal medullary and pituitary-adrenocortical activity, and relatively high gonadal activity (Benton, et al., 1978; Brain, 1975; McKinney & Desjardins, 1973a; 1973b). Among grouped mice, then, dominants may be less stressed than subordinates. The major defining feature of dominance, aggressiveness, is also an attribute of isolates. Brain (1975) has recently suggested that both isolates and dominants are territorial animals and that their aggressiveness reflects this.

These dominance studies are complemented by studies of biochemical and behavioral effects of defeat in fighting bouts. Subjecting mice to defeat increases adrenocortical activity (Brain & Nowell, 1970; Bronson & Eleftheriou, 1965) as does grouping. Defeat also alters whole brain, hypothalamic, amygdaloid, and frontal cortical levels of norepine-

phrine and serotonin (Eleftheriou & Church, 1968; Welch & Welch, 1971). Changes in hypothalamic levels of luteinizing hormone also follow defeat (Eleftheriou & Church, 1967; 1968), while prolonged aggressive interactions may suppress weights of sex accessory glands and decrease production of testicular androgens in subordinate but not dominant mice (McKinney & Desjardins, 1973a; 1973b). Subjecting mice to defeat may also elevate adrenal medullary tyrosine hydroxylase and phenyl-ethanolamine-N-methyl transferase activity (Maengwyn-Davies, Johnson, Thoa, Weise, & Kopin, 1973) and adrenal and plasma levels of epinephrine and norephinephrine (Hucklebridge, Nowell, & Dilks, 1973; Welch & Welch, 1971). Pituitary-thyroidal activity may also be influenced by defeat in mice (Eleftheriou, Church, Norman, Pattinson, & Zolovick, 1968). Since intermale fighting is common among grouped male mice, these studies of defeat and dominance may help explain the condition of grouped animals. As increasing numbers of male mice are housed per cage, the number of defeated or subordinate mice may increase (Brain, 1975), thereby increasing the number of stressed animals. Isolated animals may be relatively unstressed because they remain unexposed to defeat and other social stressors.

Differential stress and other isolation/grouping effects may also be explained by the relationship of these housing conditions to the natural social structure of the species. House mice generally live in small demes, with one dominant male defending a territory (Crowcroft & Rowe, 1963; MacIntosh, 1970; Reimer & Petras, 1967; Rowe & Redfern, 1969). Individual housing in the laboratory may better approximate natural environmental and social conditions than does the confinement of several males together in a limited space. Each individually housed mouse may

constitute a territorial and dominant animal. However, Reimer & Petras (1967) report that some subordinate males may be found living in proximity with more dominant males in natural demes of house mice; thus it is not yet clear that laboratory grouped-mouse social structures are unnatural. Nonetheless, subordinate grouped animals in the laboratory may be particularly stressed because they cannot escape the aggression of dominant males as they might under more natural conditions.

Isolation and Male Sexual Behavior

The present study extends the isolation/grouping literature through an examination of the effects of this parameter on male sexual behavior. As discussed above, there have been numerous examinations of effects of postpubertal social isolation and grouping on subsequent intermale interactions (see reviews by Brain, 1975; Scott, 1966; Valzelli, 1969). In contrast, there are very few reports of effects of isolation on male-female interactions.

King (1956) reported that isolation extending from weaning-age well into adulthood did not affect male sexual behavior in mice; his study differs, however, from the isolation studies discussed above, where isolation begins in adulthood. A number of studies in other species have concerned effects of postweaning, prepubertal isolation. Such isolation has been reported to impair adult sexual performance in rats (Folman & Drori, 1965; Gerall, Ward, & Gerall, 1967; Gruendel & Arnold, 1974) and guinea pigs (Valenstein, Riss, & Young, 1955), although Beach (1958) reported an opposite effect in rats. The differences in species and the developmental stage at which isolation is introduced, however, make these studies incomparable to those concerned with postpubertal isolation in mice.

The present study is concerned with the effects of postpubertal social isolation and grouping on male sexual behavior directed toward estrous females. Although effects of this parameter on sexual behavior have not previously been reported, there are related reports that indirectly suggest that such effects might occur. As discussed above, both locomotor and aggressive activity are higher in isolates when animals are exposed to novel situations; this trend might also occur with sexual activity. Also discussed above, testicular function, which exerts some control over male sexual behavior (Gorzalka & Mogenson, 1977), may be suppressed by prolonged grouping. Numerous studies (see Christian, 1971) indicate that high population density and grouping decrease natality; this may in part be due to induced low levels of sexual activity. In female mice, grouping may suppress estrus and lengthen diestrus (Whitten, 1959), effects which would reduce sexual receptivity and which might be paralleled in males. When females are placed with grouped males, dominants may sire the majority of offspring (DeFries & McClearn, 1970; 1972); these effects may relate to differential sexual activity, and if isolates resemble dominants, may suggest high levels of sexual activity in isolates. Finally, male mice subjected to defeat may show poor sexual activity (Kahn, 1961); since grouped males are exposed to aggression they may show sexual activity deficits. All of these findings suggest that isolates might show higher levels of sexual activity than do grouped mice.

Male sexual behavior in mice has been described by McGill (1965) and more recently by Mosig and Dewsbury (1976). This behavior is comprised of three basic elements: mounts, intromissions, and ejaculations. When a male mouse is presented with an estrous female he usually begins investigatory behavior, consisting of nudging and sniffing the female's body,

particularly her genital area. This usually culminates in mounting behavior. A mount is scored when the male straddles the female's back with his forelimbs. The male may terminate a mount within a few seconds of its inception, or he may begin to palpate the female's sides with his forepaws, simultaneously executing a series of rapid, probing pelvic thrusts. An intromission is scored when the male gains vaginal penetration. The male's success in achieving mounts and intromissions is highly dependent on female receptivity. A fully receptive female raises her hindquarters and tail and maintains a rigid posture until the male dismounts. When an intromission is terminated both animals engage in cleaning of their own genitalia. After from one to more than 100 intromissions the male may ejaculate. During the ejaculatory intromission the tempo of pelvic thrusting may increase until the male displays a convulsive quivering of the hindquarters, clutching the female with his forelimbs. Often the male will fall to one side, carrying the female with him. The ejaculatory intromission may terminate with the female struggling to release herself and vocalizing. Occasionally the male may attack and bite the female immediately following an ejaculation. The initial ejaculation usually terminates all sexual behavior for at least 15 minutes. Although many males may be capable of a second ejaculation within one hour, recovery from a second ejaculation usually requires considerably longer than one hour. The time required to recover mounting behavior after an ejaculation, like many other aspects of sexual behavior in mice, is dependent on genetic strain (Levine, Barsel, & Diakow, 1966; McGill, 1965).

An examination of the effects of social isolation and all-male grouping on sexual behavior might be of scientific value for several reasons. Since a considerable amount of evidence specifies the physiological

and behavioral effects of the isolation/grouping parameter, such an examination might improve understanding of the relationship between sexual behavior and several other physiological and behavioral variables. While the effects of isolation on many other variables are well delineated, the relationship of sexual behavior to this profile of effects is not known.

The study of isolation and grouping effects on sexual behavior might particularly elucidate an hypothesized stress-sex antagonism. There have been allusions to such an antagonism in both the human clinical and the behavioral-ecological literatures, but there have been a few relevant experimental examinations. There are a number of human clinical observations that physical and mental fatigue, as well as any other type of stressful experience, diminishes sexual desire and may induce temporary impotence (Selye, 1961). It has also been suggested that population regulatory mechanisms may reduce reproductive activity in times of high density, and that these mechanisms may operate specifically by reducing the reproductive activity of stressed members of the population (Christian, 1971; Gray, 1971). Little is known regarding the mechanisms that might underlie these phenomena. Because group-housing produces stress-characteristic hormonal and neurochemical conditions, isolation/grouping comparisons might provide a model for the study of a stress-sex antagonism.

The comparison of animals differing in endogenous hormonal and neurochemical levels may have advantages, for the study of stress effects, over other methods such as those attempting to mimic stress states through exogenous hormones and drugs. The study of animals exposed to different levels of social stress, as are isolated and grouped male mice, may elucidate complex natural interactions among physiological and behavioral response to stress. Isolated and grouped mice also provide two differing

baseline physiological preparations for examination of behavioral effects of a variety of hormonal, pharmacological, and other physiological manipulations.

The comparison of sexual performance of isolated and grouped mice might also provide information regarding an hypothesized relationship between sex and aggression. Bindra (1959) has suggested that sex and aggression covary insofar as they both respond to changes in the level of arousal. In seasonally polyestrous or monestrous mammals, fighting among males increases dramatically during the breeding season (Bermant & Davidson, 1974). Taylor (1976), Lagerspetz & Hautojärvi (1967), and Kahn (1961) all reported interactions between sexual and aggressive behavior in the laboratory. In males of many mammalian species, both sex and aggression are dependent on prenatal and circulating levels of androgens (Quadagno, Briscoe & Quadagno, 1977). There is a considerable amount of evidence regarding physiological, social, and environmental control of aggressive behavior in mice, and most of it is derived from studies within the isolation/grouping paradigm (see reviews by Brain, 1975; Scott, 1966; Valzelli, 1969). Parallel manipulations of isolation/grouping effects on sexual behavior and comparisons with results in aggression studies might provide information regarding common or differential control of sex and aggression.

An examination of isolation/grouping differences in sexual behavior might also elucidate the sociobiology of the mouse species. An examination of the response of sexual activity to the presence or absence of conspecific males may clarify the necessary conditions for reproductive success in this species. It might also provide information about the relationship of social structure, social status, and population density to reproductive behavior.

## SECTION I: EFFECTS OF BASIC ISOLATION/GROUPING PARAMETERS ON SEXUAL AND AGGRESSIVE BEHAVIOR

Within the isolation/grouping paradigm, considerable attention has been paid to the effects of prior housing upon behavior in intermale encounters. Specifically, it has been demonstrated that mice isolated for several days in adulthood show intense levels of aggression when subsequently placed with target mice (e.g., Scott, 1966; Valzelli, 1969). Isolation-induced aggression is found almost exclusively in male mice and is usually directed only toward other male mice (Scott, 1966). Several physiological systems are known to be altered by isolation and associated with isolation-induced aggression. These include, among others, pituitary-gonadal, pituitary-adrenocortical, and brain biogenic amine systems (see review by Brain, 1975).

While behavioral investigations have delineated the effects of isolation versus grouping upon intermale interactions, little attention has been given to the effects of this parameter upon male-female interactions. A few studies of male rats (e.g., Gerall et al., 1967; Gruendel & Arnold, 1974) have indicated that preadolescent social isolation can reduce subsequent sexual behavior, although effects of periods of isolation

in adulthood were not reported. King (1956) reported that isolation at weaning did not affect sexual performance of male mice in adulthood, but did not investigate the effects of postpubertal isolation and grouping. The present series of studies investigated male sexual behavior in mice that were either isolated or housed in groups in adulthood. Sexual behavior was compared as a function of number of animals per cage, length of time in particular housing conditions, and population density. Since aggressive behavior has been extensively examined within this paradigm and is relatively well understood, the degree of correlation between sexual and aggressive responses within this context was also examined.

## Experiment 1A

The first experiment was designed to examine development of sexual behavior in sexually-naive males housed with different numbers of conspecific males. Because sexual performance might also vary with experience and maturation, behavior was measured weekly until it reached an asymptote.

### Method

Subjects - Thirty-six male CD-1 mice, obtained from Canadian Breeding Farms, St. Constant, Que., served as experimental subjects. After receipt from the breeder and before commencement of the experimental housing conditions, all animals were housed for 3 days in groups of 6 in cages measuring 28 x 16 x 11 cm. Also, obtained from the same breeder, were two groups of 9 CD-1 stimulus females each and one group of 6 CD-1 stimulus males, each group being housed in a cage measuring 26 x 47 x 14 cm. All animals were maintained under a reversed 12-hr dark/12-hr light cycle at 21 $\pm$ 1$^{o}$C. Animals were tested 5-8 hr after commencement of their dark phase in an

illuminated room.

Preparation of stimulus females - Stimulus females were prepared so as to induce maximum receptivity according to a procedure adapted from studies by Gorzalka and Whalen (1974, 1976). These females were bilaterally ovariectomized at approximately 60 days of age. Two weeks following surgery an injection schedule was begun in which each female received 10 µg of estradiol benzoate in .05 cc of peanut oil sc followed 48 hr later by 500 µg of progesterone in .05 cc oil sc. This injection schedule was repeated weekly. For the first five weeks, stimulus females were presented in groups of 6 for one hour to a group of 6 group-housed males 6 hrs following progesterone treatment. On the sixth and subsequent weeks, females were presented to the experimental animals 6 hrs following progesterone, each group of females being used for 3 successive hourly tests with experimental males.

Procedure - At 50 days of age, experimental males were separated into 3 group conditions. These consisted of one group of 12, 4 groups of 3, and 12 animals housed individually. These were housed in standard polypropylene cages, manufactured by Carworth Lab Cages and measuring approximately 28 cm x 16 cm x 11 cm with straight-wire tops each containing a built-in feeder and water dispenser which together displaced 952 $cm^3$. Each cage contained one liter of commercial bedding material (San-i-cel) and one 24 x 24 cm paper towel torn into several pieces. This bedding was changed as required to maintain all cages at an approximately equivalent level of cleanliness.

At 7-day intervals beginning one week following the introduction of the differential housing conditions, animals were presented with receptive females for 1-hr sessions. During each session, 6 males were observed

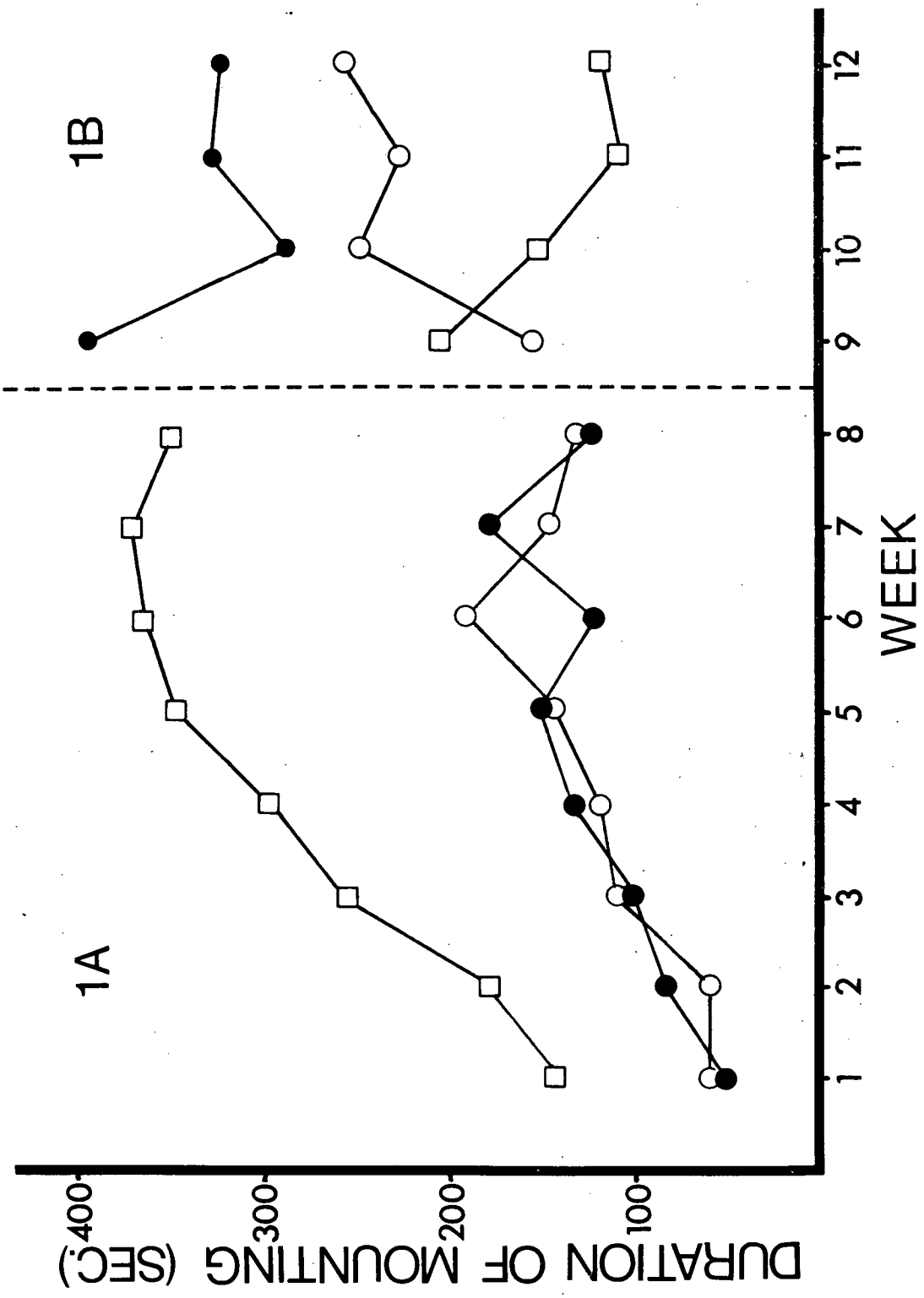
simultaneously in adjacent Plexiglas enclosures measuring 23 cm x 12 cm x 22 cm (height). Each enclosure contained 0.5 liter bedding material. Internal walls of the enclosures were covered so that an animal could not see events in adjacent enclosures. Testing times were counterbalanced so that each housing condition was represented equally in each set of animals observed simultaneously. Stimulus females were rotated among the enclosures every 10 min of each hour session so that animals representing each condition received each female for an equal period of time. During sessions, the frequency and duration of mounts without intromission (mounts) and mounts with intromission and pelvic thrusting (intromissions), and the occurrence of any tail-rattles, bites, and non-biting attacks were recorded via an Esterline-Angus event recorder.

Three days following each session with receptive females, experimental males were each presented with a single group-housed target male in an experimental enclosure of the same dimensions as those used in tests with females. Sessions with males were limited to 15 min to prevent severe damage to target males. A composite aggression score, adapted from Leshner et al. (1973) was calculated, weighting tail rattles by 1 and bites and attacks each by 2. The number and duration of any mounts oriented toward target males were also measured. In the event of a death of an experimental group-housed animal, that animal would be removed and replaced with a surrogate that would not be included in testing.

## Results and Discussion

Figure 1 shows the mean total duration of mounting per hour session for all conditions. This measure is calculated by summing, for each animal, the duration of all mounts with and without intromission,

Figure 1: The mean total duration of mounting, with or without intromission, in weekly repeated measures of animals in Experiment 1. The squares represent mice individually housed in part 1A, then grouped in 3's in part 1B. The open circles represent mice housed in 3's for part 1A, then isolated in part 1B. The closed circles represent mice grouped in 12 for part 1A, then isolated in part 1B.


DURATION OF MOUNTING (SEC.)
400
300
200
100
1A
1B
1
2
3
4
5
6
7
8
9
10
11
12
WEEK

and provides a summary statistic for male murine sexual behavior in that it contains information about both mounts and intromissions and duration of these responses. After one week in the housing conditions about twice as much activity was evident in isolated animals as in animals in groups of 3 or 12. This pattern continued in successive weekly tests, with activity increasing in parallel until it reached an apparent asymptote at weeks 7 and 8. A two-factor analysis of variance indicated a significant effect of number of animals per cage ($F = 7.37$, $df = 2/231$, $p = .002$). Subsequent Newman-Keuls comparisons ($p < .05$) indicated two homogeneous subsets: the groups of 3 and 12 versus the isolates. The within-subjects factor, weeks, was also significant ($F = 6.85$, $df = 7/231$, $p < .001$) while the interaction factor was not significant.

Table I gives the results for all remaining measures in Experiment 1. Trends in the numbers of mounts and intromissions were identical to those in the duration measure. There was a significant effect of number of animals per cage with both mounts ($F = 9.56$, $df = 2/231$, $p < .001$) and intromissions ($F = 6.98$, $df = 2/231$, $p = .004$), while both mounts ($F = 5.53$, $df = 7/231$, $p < .001$) and intromissions ($F = 7.36$, $df = 7/231$, $p < .001$) increased over weeks. In both measures subsequent comparisons indicated that the isolates differed from grouped animals while there were no differences between the groups of 3 and 12. A few animals showed some aggressive responses toward receptive females. Despite a low level of such responses in all groups, there was a significant effect of number of animals per cage ($F = 3.56$, $df = 2/231$, $p = .039$), with comparisons indicating that the difference lay between isolates and grouped animals.

In the 15-min tests with stimulus males, more aggression was evident in isolates than in either set of grouped animals. Composite

TABLE I

Mean Scores in Weekly Repeated Measures of Sexual and Aggressive Responses to Stimulus Females and Males in Experiment 1.

| Measure | n per cage | EXPERIMENT 1A Week 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | n per cage | EXPERIMENT 1B Week 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Mounts | 1 | 15.83 | 22.92 | 28.00 | 32.33 | 34.00 | 36.08 | 42.33 | 40.00 | 3 | 28.42[b] | 10.40[b] | 16.30[c] | 17.20[c] |
| | 3 | 6.42 | 5.84 | 9.50 | 8.58 | 14.50 | 12.75 | 14.58 | 10.92 | 1 | 24.36[b] | 20.82[b] | 37.09[b] | 33.64[b] |
| | 12 | 3.42 | 6.33 | 14.16 | 16.67 | 9.45 | 7.09 | 21.18 | 8.82 | 1 | 35.18[b] | 46.27[b] | 47.73[b] | 45.64[b] |
| Intromissions | 1 | 8.00 | 13.60 | 22.00 | 25.42 | 33.92 | 32.92 | 33.67 | 25.84 | 3 | 15.92[b] | 14.10[b] | 8.90[c] | 10.90[c] |
| | 3 | 3.42 | 4.75 | 8.42 | 10.84 | 13.75 | 17.60 | 13.33 | 11.75 | 1 | 13.55[b] | 21.55[b] | 15.55[b] | 18.64[b] |
| | 12 | 3.02 | 6.42 | 5.08 | 9.33 | 12.64 | 8.27 | 13.27 | 7.00 | 1 | 43.96[b] | 22.09[b] | 21.36[b] | 24.55[b] |
| Stimulus Females | | | | | | | | | | | | | | |
| Aggression Score | 1 | 9.08 | 3.33 | 1.00 | 0.17 | 1.00 | 2.17 | 1.33 | 0.33 | 3 | 0.00[b] | 0.00[b] | 0.00[c] | 0.00[c] |
| | 3 | 0.33 | 0.67 | 0.00 | 0.00 | 0.17 | 1.33 | 0.50 | 0.00 | 1 | 4.45[b] | 0.36[b] | 0.73[b] | 0.18[b] |
| | 12 | 0.83 | 0.00 | 0.50 | 0.00 | 0.00 | 2.00 | 0.00 | 1.45 | 1 | 0.33[b] | 0.18[b] | 0.00[b] | 0.00[b] |
| Aggression Score | 1 | 21.75 | 35.16 | 39.08 | 29.00 | 40.33 | 52.33 | 41.84 | a | 3 | 11.83[b] | 10.40[b] | 12.40[c] | 6.90[c] |
| | 3 | 0.00 | 1.00 | 1.30 | 12.75 | 8.25 | 6.50 | 8.33 | a | 1 | 29.64[b] | 42.45[b] | 49.09[b] | 44.27[b] |
| | 12 | 0.00 | 0.67 | 0.00 | 1.64 | 0.18 | 0.00 | 7.64 | a | 1 | 42.91[b] | 63.18[b] | 62.91[b] | 60.73[b] |
| Stimulus Males | | | | | | | | | | | | | | |
| Duration of Mounting (Sec.) | 1 | 0.58 | 0.75 | 0.25 | 2.17 | 0.25 | 0.25 | 1.83 | a | 3 | 0.00[b] | 0.00[b] | 0.00[c] | 0.40[c] |
| | 3 | 0.00 | 0.25 | 0.08 | 0.17 | 0.00 | 0.00 | 0.00 | a | 1 | 1.55[b] | 1.64[b] | 1.09[b] | 0.91[b] |
| | 12 | 0.00 | 0.00 | 0.00 | 0.00 | 0.08 | 0.00 | 0.64 | a | 1 | 5.09[b] | 0.73[b] | 0.55[b] | 0.73[b] |

[a]Data not collected due to recent introduction of reversal conditions.

[b]n = 11

[c]n = 10

aggression scores revealed significant differences in the number of animals per cage factor ($F$ = 7.92, $df$ = 2/198, $p$ = .002), but not in the weeks and interaction factors. Comparisons indicated again that group differences lay between the isolates on the one hand and the groups of 3 and 12 on the other. Very few animals mounted stimulus males; this measure showed no significant differences. A Pearson product-moment correlation, calculated between the total duration score with stimulus females for each animal and the composite aggression score with stimulus males for that animal, indicated a moderate positive relationship ($r$ = .33, $n$ = 36, $p$ = .023). When the scores for the individual grouping conditions were correlated separately ($n$ = 12) no significant correlations were obtained.

These results indicate that isolation in male mice not only increases aggressiveness, but also produces a marked increase in the quantity of sexual behavior. This effect is maintained despite increases in sexual behavior in all groups with successive repeated measures of behavior.

## Experiment 1B

If isolation is the primary factor responsible for higher levels of sexual behavior observed in the individually-housed animals of Experiment 1A, then it should be possible to increase behavior in the grouped animals by isolating them and to decrease behavior in isolates by grouping them. Part B of Experiment 1 attempted such a reversal.

### Method

Subsequent to the eighth week's measure of behavior in the presence of receptive females, animals that were isolated in Experiment 1A were rehoused in groups of 3, while animals formerly in groups of 3 and 12

were rehoused individually. Behavior was measured according to the procedures of Experiment 1A, with weekly 1-hour tests with receptive females and 15-min tests with group-housed target males 3 days after each sexual test. Behavior was again tested weekly until performance in all conditions appeared to have stabilized. All other procedures were identical to those of Experiment 1A.

## Results and Discussion

Weeks 9-12 in Figure 1 and Table I give results for all measures following reversal of conditions. One week following reversal there were two to three times as many mounts, intromissions, and seconds spent mounting than found the previous week in the isolated animals that were formerly grouped in 12. This increase was sustained during the three subsequent weekly tests. Isolated animals that were formerly grouped in 3 did not show a similar immediate increase, but by the third and fourth week following reversal (weeks 11 and 12) these animals performed at levels substantially higher than any they had previously shown. The formerly isolated animals showed a large decrease in performance one week following reversal, with further decreases occurring during subsequent weeks. Two animals in this condition died, apparently as a result of wounding incurred by other animals. Individual analyses of variance performed on measures from weeks 11 and 12 indicated differences between conditions of the number of animals per cage (duration of mounting: $F = 9.84$, $df = 2/29$, $p = .001$; mounts: $F = 5.23$, $df = 2/29$, $p = .011$; intromissions: $F = 3.85$, $df = 2/29$, $p = .033$). Newman-Keuls comparisons ($p < .05$) in each case indicated two homogeneous groups: isolated vs. grouped animals. There were no significant differences in the composite aggression scores toward females.

In the aggression tests with target males, a reversal in performance was evident at the first test 10 days after reversal of conditions and was maintained in subsequent weeks. An analysis of variance on data from weeks 11 and 12 indicated differences between conditions ($F = 3.06$, $df = 2/29$, $p = .044$), while comparisons indicated that both groups of isolates showed more aggression than did grouped animals. Only a few animals showed sexual behavior toward males; there were no significant differences in this measure. A correlation calculated by pairing the total duration score (with females) from weeks 11 and 12 with the aggression score for that animal on the subsequent test with males showed a significant relationship ($r = .40$, $n = 32$, $p = .002$).

This reversal in performance pursuant to reversal of isolation/grouping would appear to indicate that the effect of differential housing upon sexual performance is not permanent and is more dependent upon current housing conditions than upon prior experience. Furthermore, it suggests that the performance of sexually experienced mice is affected by grouping or isolation as much as is the performance of naive animals. It is noteworthy that a similar reversal occurred in aggressive behavior.

## Experiment 2

It has been reported that isolation-induced aggression (Valzelli, 1969) and biochemical changes (see Brain, 1975) are a function of the length of time that animals have been housed individually. In general, it has been found that some changes occur within a few days of the introduction of isolation and that prolonged isolation produces a more marked effect. While Experiment 1 examined sexual behavior within subjects at different intervals following isolation, differences in performance in successive measures may have been due to experience and age. Experiment 2

examined whether differences in sexual performance were a function of the length of time in isolation, this variable being manipulated between subjects with age at time of testing held constant across conditions. Also, since ejaculations were not systematically recorded in Experiment 1 and might vary between conditions in a manner dissimilar to those of mounts and intromissions, these responses were measured in both this and subsequent experiments. Murine ejaculatory responses have been described by McGill (1965).

## Method

CD-1 stimulus females were prepared, housed, and made receptive according to the procedure described for Experiment 1A. Experimental males consisted of 144 CD-1 males obtained at 50 days of age and housed in groups of 6 until commencement of the experimental housing conditions. All animals were without previous sexual experience and were tested at 77-82 days of age, each age being equally represented in all conditions. Subjects were divided into 12 conditions, each of which contained 12 subjects. Conditions consisting of animals housed with 1, 3, or 12 animals per cage were formed at the beginning of each of four intervals prior to testing: 3, 7, 14, and 28 days. Cages and bedding were provided and maintained according to the procedures of Experiment 1A. A 1-hr test session in the presence of stimulus females was given to each animal. These sessions were conducted as in Experiment 1, with the occurrence and duration of mounts, intromissions, and ejaculations recorded on an Esterline-Angus event recorder. Aggressive attacks, bites, and tail-rattles oriented toward stimulus females were also recorded. A 15-min test session in the presence of a group-housed target male was given to each experimen-

tal animal 3 days following its session with stimulus females. During these tests, bites, non-biting attacks, tail rattles, mounts, and duration of mounting were recorded.

## Results and Discussion

Figure 2 presents the mean total duration of mounting, with or without intromission, for all conditions. At the 3-day interval, isolated animals showed marginally more activity than animals in groups of 3, which in turn showed marginally more activity than animals in groups of 12. At the 7, 14, and 28 day intervals, more activity was evident in the isolates than at the 3 day interval. Animals grouped in 3 and 12 showed similar levels of performance at all intervals, except at 7 days when groups of 3 performed at a higher level, and always exhibited less activity than isolates. A two-factor analysis of variance indicated a significant main effect of the number of animals per cage ($F = 10.26$, $df = 2/131$, $p < .001$), while other effects were not significant. Subsequent Newman-Keuls comparisons ($p < .05$) indicated that this significant effect was due to differences between the isolates and the animals in groups of 3 and 12.

Table II gives the results for all remaining measures in Experiment 2. The patterns in measures of mounts and intromissions were comparable to the pattern in the duration measure. There was a significant effect of number of animals per cage in mounts ($F = 15.90$, $df = 2/131$, $p < .001$) and intromissions ($F = 4.67$, $df = 2/131$, $p = .011$) while other effects were not significant. Comparisons indicated that the isolates exceeded both sets of grouped animals for mounts, while the isolates exceeded the animals grouped in 12 for intromissions. Only a small number

Figure 2: The mean total duration of mounting, with or without intromission, in between-group tests at different intervals following isolation or grouping in Experiment 2. The squares represent animals housed individually, the open circles groups of 3, and the closed circles groups of 12.

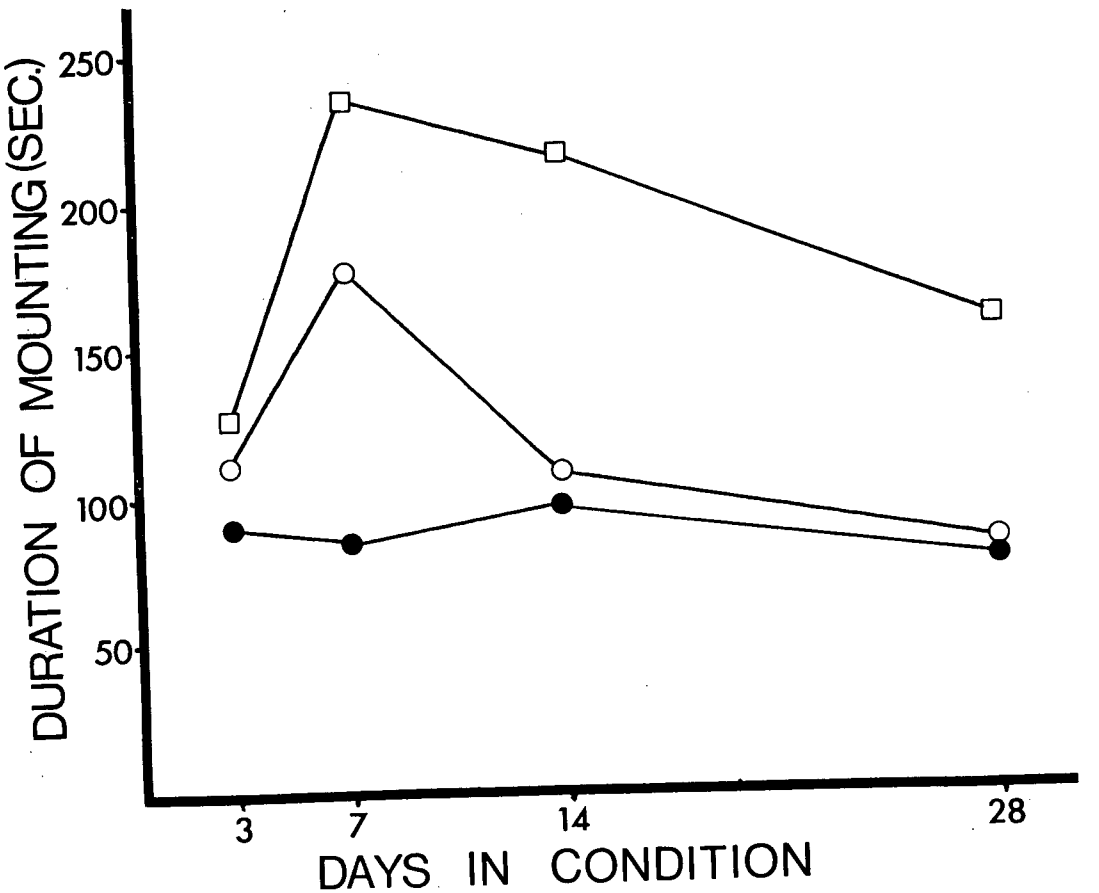

DURATION OF MOUNTING (SEC.)
250
200
150
100
50
3
7
14
28
DAYS IN CONDITION

TABLE II

Mean Scores on Sexual and Aggressive Measures with Stimulus Females and Males at Intervals from Isolation/ Grouping in Experiment 2

| | Measure | n per cage | Days in Condition 3 | 7 | 14 | 28 |
|---|---|---|---|---|---|---|
| Stimulus Females | Mounts | 1 | 18.33 | 27.75 | 25.90[a] | 25.58 |
| | | 3 | 16.50 | 16.16 | 9.67 | 9.50 |
| | | 12 | 13.92 | 8.00 | 8.67 | 7.16 |
| | Intromissions | 1 | 12.58 | 15.67 | 15.81[a] | 9.75 |
| | | 3 | 8.83 | 15.58 | 11.25 | 6.75 |
| | | 12 | 6.50 | 8.67 | 6.00 | 6.83 |
| | Ejaculations | 1 | 0.08 | 0.33 | 0.54[a] | 0.17 |
| | | 3 | 0.17 | 0.50 | 0.08 | 0.08 |
| | | 12 | 0.17 | 0.33 | 0.17 | 0.08 |
| | Aggression Score | 1 | 0.67 | 0.07 | 11.36[a] | 3.50 |
| | | 3 | 0.00 | 6.00 | 0.33 | 1.16 |
| | | 12 | 0.00 | 7.83 | 5.00 | 4.33 |
| | | | 6 | 10 | 17 | 31 |
| Stimulus Males | Aggression Score | 1 | 33.67 | 44.92 | 31.50[a] | 36.08 |
| | | 3 | 15.08 | 20.83 | 2.58 | 15.25 |
| | | 12 | 6.42 | 8.58 | 3.67 | 0.75 |
| | Duration of Mounting (Sec.) | 1 | 0.17 | 4.00 | 1.36[a] | 1.00 |
| | | 3 | 0.08 | 0.00 | 0.50 | 0.00 |
| | | 12 | 0.00 | 0.08 | 0.00 | 0.00 |

[a] n = 11

of ejaculations were observed. This appeared to be not so much due to the length of sessions as to the fact that some animals showed very little sexual behavior while others would cease responding before achieving an ejaculation. There were no significant differences in this measure. There were also no significant differences in the composite aggression score toward females.

In the 15-min tests with stimulus males, more aggression was evident in isolates than in either set of grouped animals. Statistical analysis indicated a significant effect of number of animals per cage ($F$ = 14.25, $df$ = 2/131, $p$ <.001) but no significant effect of interval nor an interaction effect. There was also a significant effect of number of animals per cage in the duration of mounting with stimulus males ($F$ = 8.19, $df$ = 2/131, $p$ <.001), although only a few animals showed such responses. In both these latter measures comparisons indicated that differences were due to higher performance in isolates but that no difference occurred between the two sets of grouped animals. A correlation calculated between each animal's duration of mounting with females score and its aggression score with males indicated a moderate relationship ($r$ = .25, $n$ = 143, $p$ = .001). When isolates and animals in groups of 3 were each considered separately, no significant relationship was obtained. However, when animals in groups of 12 were considered a positive correlation was obtained ($r$ = .28, $n$ = 48, $p$ = .027).

These results suggest that differences between individually- and group-housed naive male mice are evident at a wide range of intervals between isolation and testing. While there is some indication that differences may not be fully developed before one week of isolation, this question requires more extensive investigation. Similarly, a study of intervals exceeding 4 weeks may reveal results which differ from those obtained here.

Nevertheless, these results indicate that isolation-induced facilitation of male sexual behavior is a robust rather than a transient phenomenon and that it occurs under a fairly wide variety of experimental conditions. One qualification is that results may have been influenced by the arbitrary pre-treatment housing of all animals in groups of 6. It seems conceivable that if all animals had been isolated or housed in different group sizes prior to treatment, different results may have been obtained.

## Experiment 3

In both of the previous experiments treatment involved placing 1, 3, or 12 animals in cages of identical dimensions for all conditions. This practice has been common in studies of the behavioral and physiological effects of isolation. These studies confound two variables however; the number of animals per cage and the amount of space per animal. Since either factor might be responsible for isolation-induced facilitation of male sexual behavior, the present experiment endeavored to vary both parameters systematically.

### Method

Stimulus females employed in Experiment 2 were maintained on their weekly injection schedule and re-used in this experiment. Experimental males were 108 CD-1 males obtained at 55 days of age and housed in groups of 6 until commencement of the experimental housing conditions. Two weeks prior to testing with receptive females at 75-80 days of age, males were divided into 9 conditions of 12 subjects each. These conditions consisted of 3 group sizes (1, 3, or 12 animals per cage) at each of 3 different population densities (331 $cm^3$, 1325 $cm^3$, or 3976 $cm^3$ per animal). These volumes per animal were equivalent, respectively, to the

volumes per animal in Experiments 1 and 2 in the 1, 3, and 12 animals per cage conditions. These volumes included space filled with bedding but not space displaced by the feeder and water dispenser. Special cages were constructed from polypropylene and Plexiglas where cages of the required dimensions were not available. All cages were 11 cm high.

In the 331 $cm^3$ condition, the 12-animal group was placed in a pre-manufactured cage with floor dimensions 28 x 16 cm, with a feeder displacing 952 $cm^3$ and equipped as described in Experiment 1A. The 3-animal groups were housed in an identical cage that was subdivided into 4 compartments of equal volume by polypropylene dividers. Each compartment allowed access to the feeder and was equipped with a separate water dispenser. The isolated animals in this condition were also housed in an identical cage; this was subdivided into 12 compartments and equipped with a specially-constructed wire-grid top with 1 $cm^2$ holes in the grid. Each compartment allowed access to food through the grid and was equipped with its own water dispenser. All 331 $cm^3$ animals were given .083 liter bedding material and one 4 x 12 cm piece of paper towel per animal.

In the 1325 $cm^3$ condition, the 3-animal groups were each housed as described for Experiment 1A (also identical to the housing of the 331 $cm^3$ 12-animal group). The 12-animal group was housed in 4 such cages which had side walls removed and were adjoined, while the isolated animals were housed in 4 such cages each subdivided into 3 compartments. All 1325 $cm^3$ per animal cages were provided with .333 liter bedding material and one 12 x 12 cm piece of paper towel per animal in the cage.

In the 3976 $cm^3$ condition, the isolated animals were each housed as described for Experiment 1A. The 3-animal groups were housed in cages

with floor dimensions 47 x 26 cm, each equipped with a large straight-wire top with a built-in feeder that displaced 1514 $cm^3$. The 12-animal group was housed in a specially-constructed Plexiglas cage with floor dimensions 104 x 47 cm and roofed with 4 large straight-wire tops which in total displaced 6056 $cm^3$. All 3976 $cm^3$ per animal cages were provided with one liter bedding material and one 24 x 24 cm paper towel per animal. Bedding was changed as required to maintain cages in all conditions at an approximately equivalent level of cleanliness.

Testing procedures with receptive females were the same as in Experiments 1 and 2, with conditions counterbalanced across times of testing. Number and duration of mounts, intromissions, and ejaculations, and bites, attacks, and tail rattles were measured in a single 1-hr test with receptive females two weeks following introduction of the housing conditions. Also, as in the previous experiments, a 15-min session with a stimulus male was given to each animal 3 days following its test with females; during this session bites, attacks, tail rattles, and number and duration of mounts were recorded.

## Results and Discussion

Figure 3 gives the mean total duration of mounting, with or without intromission, for each condition. Performance in isolates was higher than that of either set of grouped animals at all volumes of space per animal. There was a significant main effect of the number of animals per cage ($F = 5.99$, $df = 2/99$, $p = .004$) but no significant effect of space per animal and no interaction. Newman-Keuls comparisons ($p < .05$) indicated that the isolates differed from the animals grouped in 3 and 12, but that there was no difference between the two sets of grouped animals.

Figure 3: The mean total duration of mounting, with or without intromission, of animals housed with different volumes of space per animal in each cage in Experiment 3. The squares indicate animals housed individually, the open circles groups of 3, and the closed circles groups of 12.

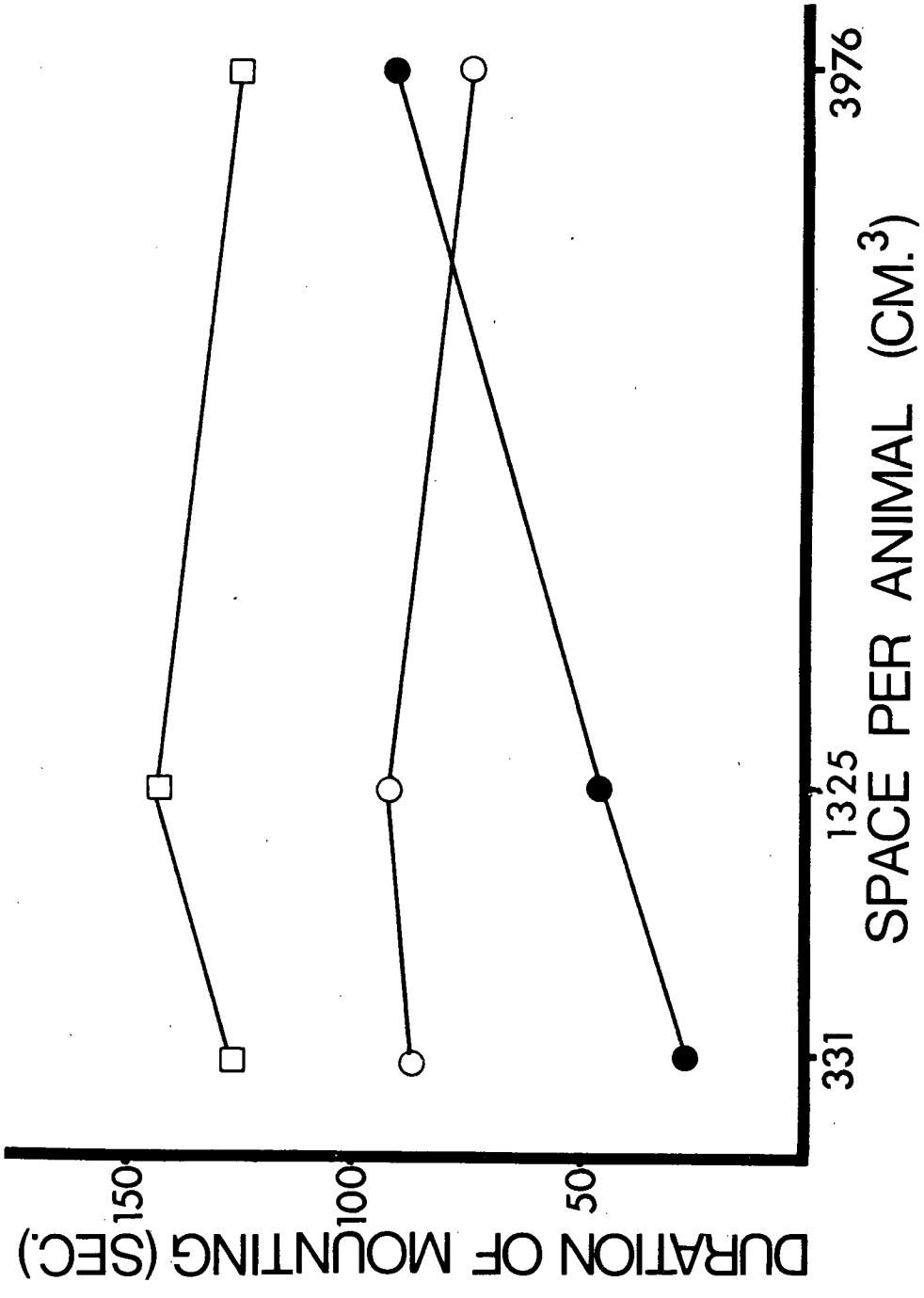

DURATION OF MOUNTING (SEC.)
150
100
50
331
1325
3976
SPACE PER ANIMAL (CM.3)

Table III shows means for all remaining measures in Experiment 3. The trends in the mounts measure were identical to those in the duration measure, with a significant effect of number of animals per cage ($F$ = 9.16, $df$ = 2/99, $p$ <.001) and comparisons indicating a difference between isolates and grouped animals. The intromissions measure also showed a significant effect of number of animals per cage ($F$ = 3.65, $df$ = 2/99, $p$ = .029). Comparisons showed a difference between isolates and the animals grouped in 12, but no differences between animals grouped in 3 and either of the other grouping conditions. Only a few animals showed ejaculations; there were no significant differences in this measure. There were also no significant effects in the composite aggression score with females.

The composite aggression score with males showed a significant interaction ($F$ = 7.48, $df$ = 4/99, $p$ = .021). Comparisons showed that the three individually-housed groups and the 3976 $cm^3$ group of 12 formed one homogeneous subset, while all remaining groups formed a second subset. There were very few mounts directed toward males, although this measure showed a significant effect of number of animals per cage ($F$ = 5.67, $df$ = 2/99, $p$ = .005). A correlation between the duration of mounting with females measure and the aggression score with males, calculated by pairing each animal's scores on these measures, indicated a positive relationship ($r$ = .32, $n$ = 108, $p$ = .001). No correlations were evident when isolates and groups of 3 were each considered separately, although a significant correlation did occur in the groups of 12 ($r$ = .62, $n$ = 36, $p$ = .001).

It appears, then, that the primary factor responsible for differential sexual performance is the number of animals per cage rather than

Table III

Mean Scores on Measures of Sexual and Aggressive Responses to Stimulus Females and Males Under Different Densities in Experiment 3

| Measure | n per cage | Space per animal ($cm^3$) 331 | 1325 | 3976 |
|---|---|---|---|---|
| Stimulus Females | | | | |
| Mounts | 1 | 27.67 | 21.25 | 24.95 |
| | 3 | 16.53 | 14.08 | 9.67 |
| | 12 | 6.17 | 6.00 | 10.33 |
| Intromissions | 1 | 7.08 | 9.25 | 8.42 |
| | 3 | 6.00 | 6.00 | 5.00 |
| | 12 | 1.25 | 3.58 | 5.33 |
| Ejaculations | 1 | 0.17 | 0.08 | 0.17 |
| | 3 | 0.08 | 0.17 | 0.17 |
| | 12 | 0.00 | 0.17 | 0.08 |
| Aggression Score | 1 | 4.75 | 10.75 | 8.50 |
| | 3 | 0.00 | 3.42 | 1.42 |
| | 12 | 1.83 | 0.00 | 6.42 |
| Stimulus Males | | | | |
| Aggression Score | 1 | 15.50 | 23.75 | 14.17 |
| | 3 | 1.50 | 2.83 | 0.00 |
| | 12 | 0.00 | 0.00 | 22.58 |
| Duration of Mounting (sec) | 1 | 0.83 | 0.00 | 1.33 |
| | 3 | 0.00 | 0.00 | 0.08 |
| | 12 | 0.00 | 0.00 | 0.00 |

the amount of space allotted per animal. This must be qualified, however, in that volumes per animal outside the range studied might produce different results. Volumes sufficiently small as to constitute severe restraint stress might impair sexual performance despite individual housing, while extremely large volumes might reduce contact between grouped animals to an extent equivalent to individual housing. Clearly the results of Experiments 1 and 2 cannot be attributed to differential volume or density. It is interesting that an interaction occurred in the measure of aggression with males but not in measures of sexual behavior. This interaction, which appeared in part due to high levels of aggression in the low density group of 12, may reflect some differential control of sex and aggression.

## General Discussion

These experiments demonstrate a substantial facilitation of male sexual behavior in mice when they are housed individually rather than in groups. This facilitation is positively correlated with isolation-induced aggression and responds similarly to most of the parametric manipulations investigated. Isolation-induced facilitation of male sexual behavior occurs in both naive and experienced animals, and can be reversed by reversing conditions. A strong effect is produced within one week of isolation, while the effect is present in naive animals after periods of isolation as prolonged as four weeks. Within limits, isolation-induced facilitation of male sexual behavior is independent of population density and amount of space allotted per animal. Several variables may play a role in producing this phenomenon.

One possibility is that the poorer performance of grouped animals is due to stress-related variables. There is evidence that grouped male mice are more stressed than individually-housed mice, insofar as grouped

mice show high baseline levels of pituitary-adrenocortical activity (Brain, 1975; Leshner et al., 1973). This higher pituitary-adrenocortical activity is thought to be due to competition and intermale fighting, and is particularly evident in regularly defeated animals (Brain, 1975; Bronson & Eleftheriou, 1965). There have been suggestions, both in the human clinical (e.g., Selye, 1961) and behavioral-ecological (e.g., Christian, 1971) literatures that an antagonism exists between stress and sexual performance in mammals. While it has been demonstrated that social stressors may inhibit other aspects of reproductive functioning (see Christian, 1971), there has been little examination of sexual performance per se within this context. It may be that increased pituitary-adrenocortical activity acts through some unknown physiological mechanism to depress sexual functioning, and that this mechanism accounts for the present phenomenon. If this position holds, this phenomenon might provide an experimental paradigm for investigation of such a mechanism.

A second, related possibility involves differential pituitary-gonadal functioning in isolated and grouped mice. An increase in gonadal activity, as a consequence of isolation, has been indicated in many studies of male mice. Studies contrasting isolated and group-housed males have indicated that isolates have heavier sex accessories, such as testes, seminal vesicles, prostate glands, and preputial glands (e.g., Brain & Nowell, 1971; Christian, 1955). Also, levels of testosterone are known to decrease as a function of pituitary-adrenocortical activation (Bullock & New, 1971; Desjardins & Ewing, 1971), which has been demonstrated, as discussed above, in grouped animals. Thus, it may be that higher levels of gonadal activity in isolates lead to higher levels of sexual performance. The mechanism by which such an effect could occur is not as straightforward

as might be assumed. There is little evidence, for example, to suggest that changes in mammalian testosterone levels, above a certain threshold level, are correlated with changes in sexual performance (Gorzalka & Mogenson, 1977). Nor has any means been established by which decreased glandular weight might effect neural organization of sexual behavior. Nonetheless, the possibility remains that decreased pituitary-gonadal functioning might produce social stress- and housing-dependent deficits in sexual performance.

A third possibility is that a higher level of general activity accounts for both enhanced sexual performance and increased aggression in isolates exposed to conspecifics. In this light, Essman (1968) and Brain, Haley, and Nowell (1971) have found that isolates show more locomotor activity than do grouped animals upon exposure to a novel environment. General activity and behavioral activation are thought to be partially regulated by levels and utilization rates of biogenic amines (Bennett & Rosenzweig, 1971). Although isolates may show lower baseline levels and utilization of norepinephrine and dopamine, and lower utilization of serotonin, they may exhibit very high levels of utilization of these amines in stressful and novel situations (Garattini et al., 1969; Welch & Welch, 1969a; 1969b). It may be that increased turnover of biogenic amines mediates enhancement of sexual performance in isolates, either by increasing non-specific activity levels or by directly facilitating sexual performance through some unknown mechanism. Some evidence suggesting dopaminergic and serotonergic involvement in male sexual behavior has been presented by Gessa and Tagliamonte (1975).

A fourth possibility is that the tactile and olfactory stimuli which presumably elicit sexual behavior in mice are more novel to animals

that are individually housed and thus may be more powerful in controlling their behavior. Since animals housed in groups in small enclosures constantly receive stimuli associated with conspecifics, similar stimuli might be less salient when animals are exposed to receptive females in a novel environment. Receptive females may be insufficiently distinct from other males to elicit sexual behavior from experimental males. Similarly, a sufficient level of intermale mounting may occur in grouped animals to make these animals sexually satiated when they are exposed to females. Intermale mounting is commonly observed in mammalian species and was occasionally observed in the present study when experimental animals were exposed to target males. Such mounting might be unlikely to lead to sexual satiety, however, since mounted males might not be sufficiently receptive to allow responses analogous to intromission. In the present study, the few cases of intermale mounting were usually repulsed by the mounted male and frequently evoked an attack. A fairly high level of intermale mounting would presumably be required to produce the deficits in performance observed in group-housed animals.

Finally, social learning variables may in some manner inhibit the occurrence of sexual action patterns in group-housed animals. For example, since sexual behavior oriented towards other males in a group may evoke attacks against the sexually aroused animal, the probability of mounting behavior may be decreased in the aroused animal. The effects of punishment of sexual overtures by evoked attacks may generalize to other situations, in that the probability of sexual responses on the part of group-housed males toward receptive females could be reduced.

One consideration for future research is that the topography, as well as the quantity, of murine sexual behavior may be affected by isolation. For example, it may be that more mounts and intromissions are required for each ejaculation on the part of isolates. The proportions of ejaculations to mounts and intromissions observed in Experiments 2 and 3 appeared comparable for all conditions. More ejaculations occurred in the isolates than in the groups of 12, and usually more in isolates than in the groups of 3. However, the sample of ejaculatory responses was too small for statistical analysis to reveal significant differences. Future examination might test animals under conditions where a sufficient number of ejaculatory responses occur to provide an analysis of this question.

The moderate positive correlations between sexual performance and aggression suggest that some variable affected by the manipulations of this study influences these two response classes commonly. Since the correlation was usually absent when conditions were considered separately, sex and aggression may only covary insofar as they respond similarly to the parameters studied. These correlations may not, however, entirely reflect the relationship between sex and aggression. The order of testing, which in the present study involved aggression tests three days after sex tests, may have influenced the obtained relationship between these response classes. Lagerspetz and Hautojärvi (1967) demonstrated that prior aggressive experiences can affect sexual performance in mice, and vice versa. Previous aggressive experience with a male was found to decrease sexual behavior in male mice, while previous sexual experience was found to decrease aggression toward other males. This factor, then, could have reduced aggressive behavior in animals which were sexually active in the

previous test with females, thus lowering the correlation values obtained. This factor might also have affected the results of Experiment 1, where several tests of sexual and aggressive behavior were given to the same animals. However, it might be more likely to reduce differences in sexual performance between conditions than to increase such differences, in that greater aggressive experience would accordingly reduce sexual performance in isolates. Also, Lagerspetz and Hautojärvi (1967) found their results were limited to inexperienced animals and attributed effects to a lack of differentiation of responses to males and females. In Experiment 1 of the present study responses to males and females were highly differentiated, particularly after the experimental animals had had the experience of several sessions.

In another relevant experiment, Kahn (1961) has found that isolated mice trained to be aggressive by a dangling technique were subsequently more active sexually than other isolated males made submissive by successive defeats by a trained fighter. Although only a few subjects were examined, this study suggests a further means by which aggression may interact with sexual behavior. Also, these results could indicate a mechanism accounting for facilitation of sexual performance in isolates. Since grouped male mice normally fight frequently and establish dominance hierarchies (DeFries & McClearn, 1970; Messeri et al., 1975), it may be that individuals at different strata of a hierarchy show different levels of sexual performance. Regularly defeated, more subordinate males in a group may show poor performance, while victorious, dominant males may show performance comparable to that of isolates. Such an interpretation might be comparable with some of the biochemical interpretations presented above, insofar as defeated and subordinate mice show increased adrenocortical

activity (Bronson & Eleftheriou, 1965), changes in neurotransmitter levels (Welch & Welch, 1971; Eleftheriou, 1971) and decreased testosterone levels (McKinney & Desjardins, 1973a; 1973b), effects which are absent in dominant, victorious mice. It is also consistent with the interpretation that social learning is involved, in that behavior oriented toward dominant males might be punished and the subsequent suppression of behavior generalize to situations involving receptive females. In this regard, DeFries and McClearn (1970, 1972) have found that dominant male mice in a group sire the majority of offspring when females are introduced into the group.

There have been a number of previous suggestions that sexual and aggressive behavior may be motivationally linked. Both aggressive and sexual behavior are known to be dependent upon prenatal and circulating levels of androgens in many species including mice (Quadagno et al., 1977). Accordingly, the relationship between sex and aggression in the present study might be dependent upon divergence in androgen levels subsequent to separation into isolated and grouped colonies. Studies by Leshner et al. (1973) and Brain and Poole (1974) might contradict such a position, however, since they indicate that differences between isolated and grouped animals in aggression result more from levels of ACTH than from levels of androgens. Bindra (1959) has suggested that covariation of sex and aggression is due to changes in the level of arousal, in that both types of behavior are normally performed in a state of high arousal and thus are more likely to occur when arousal is already high. Such a position might be concordant with an interpretation that isolation leads to increased levels of arousal in novel situations, which consequently increases aggressive, sexual, and general activity levels in these situations. Such arousal might be mediated by increased utilization of central and peripheral biogenic amines as discussed above, with its orientation being determined by salient stimuli in

the novel environment.

A number of naturalistic studies provide a further perspective that may help to explain a motivational link between sex and aggression. Turner and Iverson (1973) found that aggressive acts in males sampled from natural populations of voles (Microtus pennsylvanicus) increased in frequency as males became reproductively active and decreased as the breeding season ended. In other seasonally polyestrous or monestrous mammals, fighting among males increases dramatically during the breeding season (Bermant & Davidson, 1974). Taylor (1976) has found that, in rats, exposure to estrous females has a direct influence upon intermale interactions, in that it increases the probability that a male will approach other aggressive males. These results are significant since they indicate that increased intermale fighting at breeding season is not simply an indirect result of the attraction of several males to an area occupied by a female. This motivational link may have survival value in that a male that is aggressive during the mating season may be more likely to pass on his genes than would a non-aggressive male.

## SECTION II: STRAIN AND SPECIES GENERALITY

In the previous experiments, social isolation of male mice facilitated their sexual performance. The experiments of the present section examined the strain and species generality of isolation/grouping differences in male sexual performance. Because of cross-species variation in social structure, various social arrangements may differentially affect the behavior of members of different species. Furthermore, there are indications that physiological variables may be differentially affected by social isolation in different species (Bennett & Rosenzweig, 1971; Sahakian, Robbins, Morgan, & Iverson, 1975; Stolk, Conner, & Barchas, 1974), effects which may in turn produce differences in sexual performance. Previous investigation of isolation-induced facilitation of male sexual behavior (Experiments 1, 2, & 3) was limited to CD-1 strain mice. It is thus important to examine whether the phenomenon is characteristic of this strain, of mice in general, or of a number of species. Furthermore, differential sexual responsiveness to isolation or grouping can be compared to differential physiological responsiveness and social differences among species. Such a comparison might provide indirect information regarding the relationship of sexual behavior to social and physiological variables.

## Experiment 4

There is considerable evidence that inbred strains of house mice may respond differentially to social isolation. Differences may occur in aggressiveness (Karczmar & Scudder, 1969; LeDouarec & Broussy, 1969), pituitary-adrenocortical and pituitary-gonadal response (see Brain, 1975), and neurochemistry (Karczmar & Scudder, 1969). Furthermore, the distribution and relative frequencies of sexual action patterns may vary among strains. Several researchers (Levine et al., 1966; McGill, 1962; 1965; Mosig & Dewsbury, 1976) have provided descriptions of strain differences in sexual performance. On this basis it becomes necessary to establish whether or not a phenomenon found in one strain is characteristic of only that strain. The present experiment examined the effects of isolation and grouping upon the sexual performance of three commonly studied strains of mice.

### Method

Experimental animals consisted of 24 male C57Bl/6NCr1BR and 24 male DBA/2NCr1BR mice obtained from Canadian Breeding Farms, and 24 male Swiss-Webster mice obtained from Biobreeding Laboratories, Ottawa. All animals were sexually naive. After receipt from the breeders at 55 days of age, animals were housed in groups of 6 of homogeneous strain until commencement of the experimental housing conditions. Each group was caged and maintained as in Experiment 1A. Animals were tested 5-8 hr after the commencement of their dark phase in an illuminated room. Stimulus animals consisted of 40 group-housed CD-1 females obtained from Canadian Breeding Farms. Females from a fourth strain were employed to avoid confounding of male strain differences with strain differences in

female receptivity (see Gorzalka & Whalen, 1976). Stimulus females were prepared according to the procedure outlined for Experiment 1A. Females were used for no more than two successive hourly tests.

At 60-65 days of age, 12 of the animals from each strain were rehoused in clean cages in groups of 6 while the remaining 12 animals were housed individually in clean cages. Groups of 6 were constituted by the same animals as they had been since receipt from the breeder. Cages were those manufactured by Carworth Lab Cages from polypropylene described for Experiment 1A. Cages were cleaned as required to maintain all conditions at an approximately equivalent level of cleanliness, but under no circumstances were they disturbed during the 3 days immediately preceding testing. After 2 weeks in these conditions, a period previously established as sufficient to produce effects in CD-1 mice (Experiment 2), animals were tested for sexual performance. This test consisted of a 1-hr session during which each animal was presented with receptive females in the testing enclosures described for the previous experiments. Six animals were tested simultaneously in adjacent enclosures, with one animal from each treatment combination present at each session. After 5 min of adaptation to the chamber, a single receptive female was presented to each animal. At 10-min intervals females were rotated so that each treatment combination received each female for an equivalent amount of time. During sessions, the frequency, duration, and latency of mounts without intromission (mounts) and mounts with intromission and pelvic thrusting (intromissions), and the number and latency of ejaculations were measured. All latencies were measured from the commencement of the session. Responses were recorded via an Esterline-Angus event recorder by a trained observer who was unaware of the purpose of the experiment. Mounts, intromissions, and ejaculations

were defined as described by McGill (1965).

## Results and Discussion

Figure 4 shows the results for measures of duration of mounting, with or without intromission, and number of mounts, intromissions, and ejaculations. Table IV reports remaining measures of copulatory performance. The measure of copulatory efficiency is adapted from Parrott (1975) and is calculated using all mounts and intromissions preceding the first ejaculation or the end of the session as follows:

$$\text{if } M + I > 0,\ CE = \frac{I}{M+I}$$

$$\text{if } M + I = 0,\ CE = 0$$

where M = number of mounts, I = number of intromissions, CE = copulatory efficiency. Individually-housed mice of all strains showed substantially more mounts, intromissions, and time spent mounting than did same-strain group-housed mice. There were only a few ejaculations in any condition, but these were also more common in isolated than group-housed mice. Latencies to first mount and intromission were on the average shorter in isolated mice, while the percentage mounting, intromitting, and ejaculating was in all cases higher in isolated animals within each strain. DBA mice showed substantially less sexual activity than either C57 or Swiss-Webster mice; this may in part be due to their smaller relative size compared to CD-1 females.

Parallel two-factor (housing condition and strain) analyses of variance were conducted on all measures except percentages of responses. The isolation/grouping factor was significant in total duration ($F = 7.54$, $df = 1/66$, $p = .008$), number of mounts ($F = 10.94$, $df = 1/66$, $p = .002$), number of intromissions ($F = 4.62$, $df = 1/66$, $p = .033$), mount latency

Figure 4: Mean performance of isolated and grouped Swiss-Webster (Swiss), C57B1/6NCr1BR (C57), and DBA/2NCr1BR (DBA) male mice on measures of sexual behavior in the presence of receptive females in Experiment 4. Lines above the bars indicate standard errors.

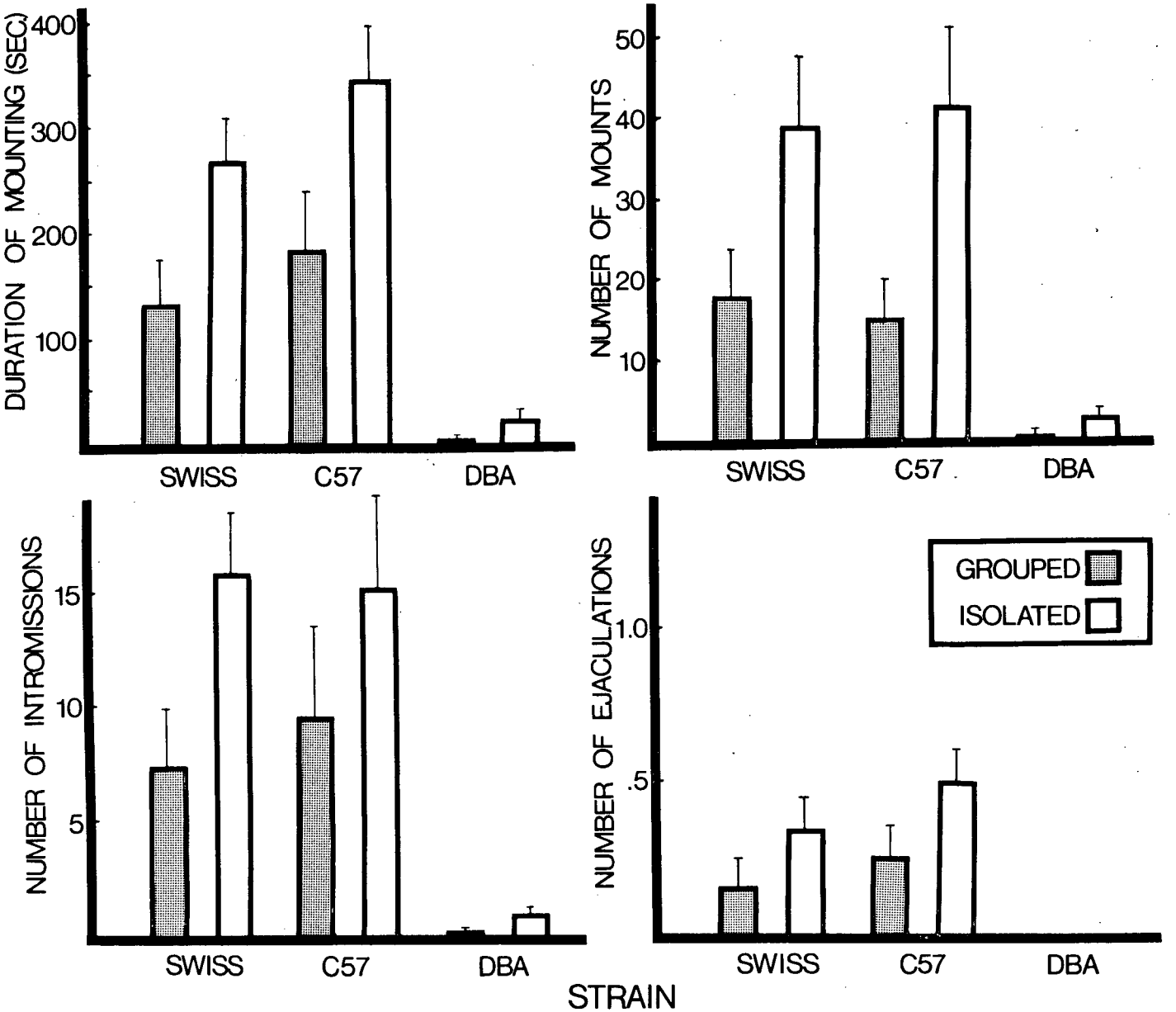

DURATION OF MOUNTING (SEC)
400
300
200
100
SWISS
C57
DBA
NUMBER OF MOUNTS
50
40
30
20
10
SWISS
C57
DBA
NUMBER OF INTROMISSIONS
15
10
5
SWISS
C57
DBA
NUMBER OF EJACULATIONS
1.0
.5
SWISS
C57
DBA
GROUPED
ISOLATED
STRAIN

Table IV

Means and Standard Errors of Response Latencies (in sec) and Copulatory Efficiency, and Percent Responding in Experiment 4

| Measure | n per cage | Strain | | |
|---|---|---|---|---|
| | | Swiss | C57 | DBA |
| Mount Latency | 1 | 592±140 | 344±100 | 2105±471 |
| | 6 | 1088±327 | 1163±356 | 3315±285 |
| Intromission Latency | 1 | 985±247 | 1265±354 | 2986±285 |
| | 6 | 1664±399 | 1881±375 | 3225±275 |
| Ejaculation Latency | 1 | 3234±224 | 3304±163 | 3600±0 |
| | 6 | 3226±258 | 3314±151 | 3600±0 |
| Copulatory Efficiency | 1 | .318±.036 | .256±.047 | .119±.048 |
| | 6 | .258±.053 | .214±.062 | .021±.021 |
| % Mounting | 1 | 100 | 100 | 50 |
| | 6 | 92 | 92 | 8 |
| % Intromitting | 1 | 100 | 92 | 42 |
| | 6 | 83 | 67 | 8 |
| % Ejaculating | 1 | 25 | 50 | 0 |
| | 6 | 17 | 25 | 0 |

($F$ = 11.25, $df$ = 1/66, $p$ = .001), and intromission latency ($F$ = 4.16, $df$ = 1/66, $p$ = .043). There was not a significant effect of this factor in other measures. There was a significant effect of strain in all measures except ejaculation latency: duration ($F$ = 16.10, $df$ = 2/66, $p$ <.001), mounts ($F$ = 13.30, $df$ = 2/66, $p$ <.001), intromissions ($F$ = 10.87, $df$ = 2/66, $p$ <.001), ejaculations ($F$ = 6.03, $df$ = 2/66, $p$ = .004), mount latency ($F$ = 25.87, $df$ = 2/66, $p$ <.001), intromission latency ($F$ = 18.44, $df$ = 2/66, $p$ <.001), and copulatory efficiency ($F$ = 12.03, $df$ = 2/66, $p$ <.001). In all cases Newman-Keuls tests ($p$ <.05) indicated that the DBA mice showed lower performance than both the C57 and Swiss-Webster mice, but that these latter two strains did not differ. There were no significant strain by isolation/grouping interactions.

These results suggest that facilitation of sexual behavior by isolation is characteristic of the species. Isolation affects several components of sexual behavior. An effect is evident in all measures, although a few may not reach statistical significance under these conditions. There is considerable variability among group animals; some show no sexual response whatsoever, others show performance comparable to that of isolates. In the Swiss-Webster and C57 mice, the major effect of isolation appeared to be an increase in the average amount of sexual behavior, with most grouped animals showing some response. In the DBA strain, the major effect of isolation was to increase the number of animals showing any response to females.

## Experiment 5

House mice normally live in small demes composed of a dominant male, several females, and a few subordinate males (Reimer & Petras, 1967). Male mice may consequently adapt readily to individual housing, but be

relatively stressed under group-housing conditions, an interpretation that is supported by hormonal comparisons of isolated and grouped mice (see review by Brain, 1975). It has been hypothesized that stress may antagonize sexual behavior (Christian, 1971; Gray, 1971; Selye, 1961); the findings of Experiment 1 may support this hypothesis.

Other rodent species differ from mice in social organization. The rat (Rattus norvegicus), for example, is a fairly social species with individuals living in large colonies that share nesting sites and feeding grounds (Barnett, 1975). The golden hamster (Mesocricetus auratus) is believed to be solitary in its natural environment (Eibl-Eibesfeldt, 1953; Johnson, 1975). The Mongolian gerbil (Meriones unguiculatus) is reported to show small personal distances and aggregate peacefully in seminatural environments (Eisenberg, 1967; Thiessen & Yahr, 1977). These species may also respond differently to isolation and housing conditions with respect to levels of aggressiveness (cf., Blanchard & Blanchard, 1971; Conner, 1972, 1972; Edwards & Rowe, 1975; Scott, 1966; Thiessen & Yahr, 1977) and physiology (see Bennett & Rosenzweig, 1971). Moreover, the topography of male sexual behavior differs among these species (cf., Gorzalka & Mogenson, 1977; Kuehn & Zucker, 1968; Larsson, 1956, McGill, 1965). Because of these species differences, there is reason to suspect that male sexual behavior might respond differentially in different species to the isolation/grouping manipulation. Therefore, in order to test the generality of previous findings, male rats, gerbils, and hamsters were examined under conditions known to produce isolation/grouping differences in mice.

## Method

Subjects - Twenty-four sexually naive male Long-Evans rats, obtained from Canadian Breeding Farms, were housed in groups of 6 in standard triple wire-mesh cages until commencement of the experimental housing conditions at about 120 days of age. Twenty-four sexually naive male golden hamsters, obtained from Charles River Laboratories, Newfield, N.J., were housed in groups of 6 in polycarbonate cages measuring 46 x 25 x 15 (height) cm equipped with large straight-wire tops until commencement of experimental housing conditions at about 100 days of age. These cages each contained 2 liters of bedding material and one paper towel. Twenty-four sexually naive male gerbils, obtained from High Oak Ranch, Goodwood, Ont., were housed in the manner described for hamsters until about 120 days of age. These ages were chosen because almost all males of these species should be sexually mature by these ages. All animals were housed under a reversed 12-hr dark/12-hr light cycle and kept in rooms maintained at 21 $\pm$ 1°C. Animals were tested 5-8 hr after commencement of their dark phase in an illuminated room.

Stimulus females - Adult females of each species were obtained and group-housed. These were bilaterally ovariectomized at least 3 weeks prior to testing. On the first and second days before testing females were given 5 $\mu$g estradiol benzoate in .05 cc peanut oil sc. On the day of testing females were given 500 $\mu$g progesterone in .05 cc oil sc 5 hr prior to testing for rats and hamsters and 9 hr prior to testing for gerbils. Females were used for no more than two successive hourly tests.

Procedure - Two weeks prior to testing with receptive conspecific females, males were placed into two conditions, each of which contained 12 animals of each species: animals grouped in 6 and animals housed individually. All groups of 6 were simply placed in clean cages at this point with each group being constituted by the same individuals as it had been since animals were received from the breeders. Individually-housed rats were each housed in standard laboratory single wire-mesh cages. Individually-housed gerbils and hamsters were each housed in cages identical to those described for mice in Experiment 1A. Animals were tested for 1 hr in cylindrical Pyrex testing jars measuring 45 cm in height with a diameter of 29 cm. Each species was tested at separate times. Six animals, three isolates and three grouped animals, were observed simultaneously. After 5 min adaptation to the jar, each animal was presented with a receptive conspecific female. Females were transferred to adjacent jars every 10 min so that grouped and isolated conditions received each female for identical lengths of time. During these sessions the number and latencies of mounts, intromissions, and ejaculations were recorded via an Esterline-Angus event recorder. The sexual responses of rats were defined as described by Larsson (1956), those of hamsters as described by Gorzalka and Mogenson (1977), and those of gerbils as described by Kuehn and Zucker (1968).

## Results and Discussion

Figure 5 gives the results for measures of mounts, intromissions, and ejaculations. Remaining measures are given in Table V. A duration of mounting measure is not reported since most mounts and intromissions are very brief and of about the same duration within each of these species.

Figure 5: Mean performance of isolated and grouped male rats, hamsters, and gerbils on measures of sexual behavior in the presence of receptive conspecific females in Experiment 5. Lines above the bars indicate standard errors.

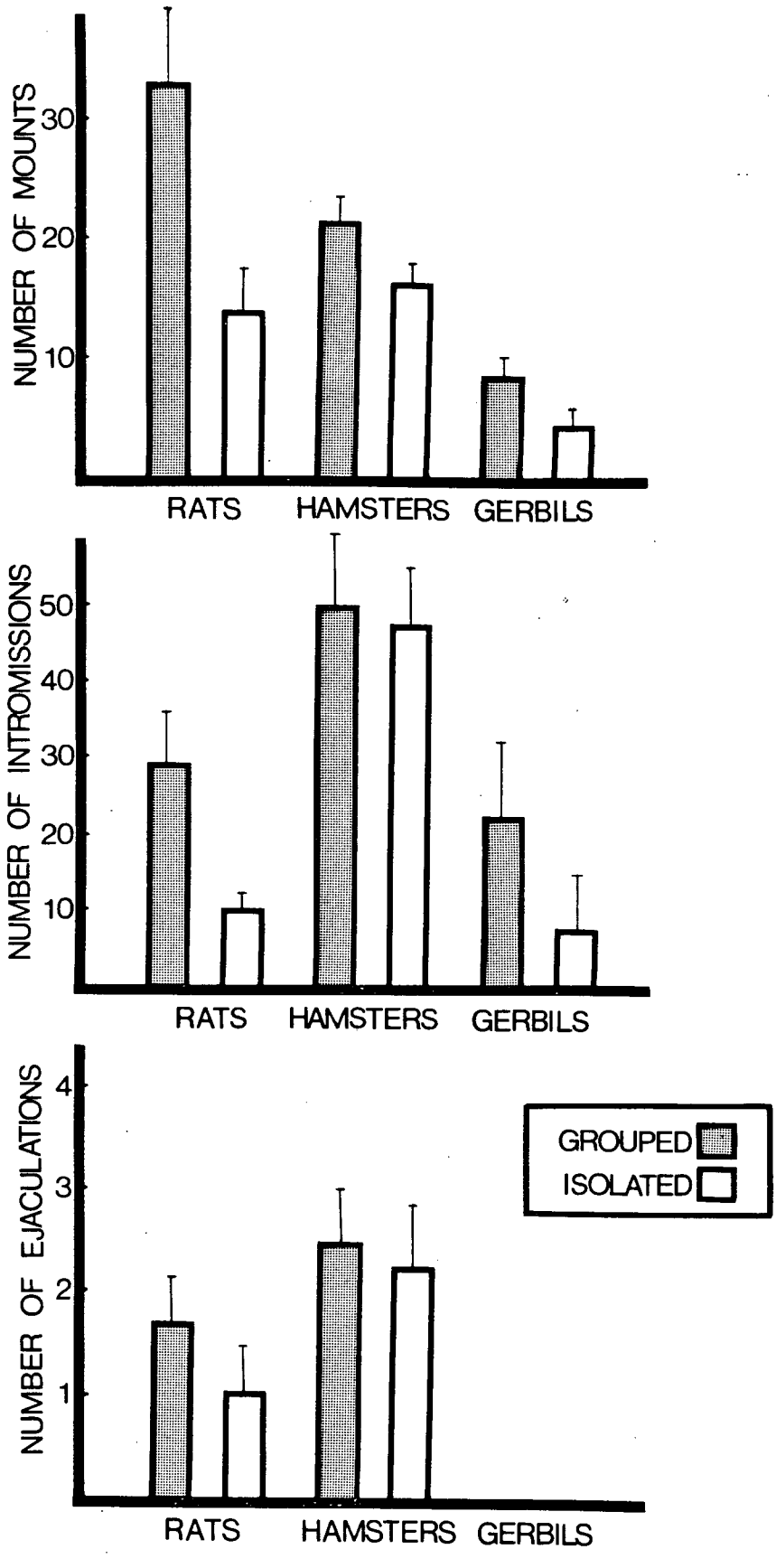

NUMBER OF MOUNTS
30
20
10
RATS
HAMSTERS
GERBILS
NUMBER OF INTROMISSIONS
50
40
30
20
10
RATS
HAMSTERS
GERBILS
NUMBER OF EJACULATIONS
4
3
2
1
RATS
HAMSTERS
GERBILS
GROUPED
ISOLATED

Table V

Means and Standard Errors of Response Latencies (in sec) and Copulatory Efficiency, and Percent Responding in Experiment 5

| Measure | n per cage | Species | | |
|---|---|---|---|---|
| | | Rats | Hamsters | Gerbils |
| Mount Latency | 1 | 1494±354 | 207±68 | 1635±410 |
| | 6 | 838±370 | 99±23 | 1276±388 |
| Intromission Latency | 1 | 1801±378 | 418±130 | 3018±391 |
| | 6 | 1079±394 | 192±48 | 2198±440 |
| Ejaculation Latency | 1 | 2867±293 | 1808±406 | 3600±0 |
| | 6 | 2216±351 | 1230±373 | 3600±0 |
| Copulatory Efficiency | 1 | .338±.070 | .660±.057 | .094±.070 |
| | 6 | .529±.076 | .620±.053 | .321±.112 |
| % Mounting | 1 | 83 | 100 | 82 |
| | 6 | 92 | 100 | 83 |
| % Intromitting | 1 | 75 | 100 | 18 |
| | 6 | 92 | 100 | 50 |
| % Ejaculating | 1 | 50 | 75 | 0 |
| | 6 | 66 | 92 | 0 |

More mounts, intromissions, and ejaculations were evident in grouped rats and more mounts and intromissions evident in grouped gerbils than in socially isolated conspecifics. There was little difference in performance between isolated and grouped hamsters, although there was a slight trend in the same direction as found in rats and gerbils. Gerbils showed relatively few mounts and intromissions and no ejaculations. One isolated gerbil died prior to testing.

An analysis of variance on the measure of mounts indicated a significant difference between grouped and isolated animals ($F = 7.54$, $df = 1/65$, $p = .007$). Newman-Keuls tests ($p < .05$) on individual species indicated a significant difference between the grouped and isolated conditions in rats and gerbils but not hamsters. Analyses of variance indicated that the difference between grouped and isolated animals approached significance, but did not reach the accepted level, for intromissions ($F = 3.75$, $df = 1/65$, $p = .054$) and copulatory efficiency ($F = 3.61$, $df = 1/65$, $p = .059$), while a similar analysis of intromission latency was significant ($F = 4.18$, $df = 1/65$, $p = .042$). The isolation/grouping factor was not significant in other measures. In all analyses there were also significant differences among species: mounts ($F = 10.37$, $df = 2/65$, $p < .001$), intromissions ($F = 12.06$, $df = 2/65$, $p < .001$), ejaculations ($F = 14.96$, $df = 2/65$, $p < .001$), mount latency ($F = 9.68$, $df = 2/65$, $p < .001$), intromission latency ($F = 23.66$, $df = 2/65$, $p < .001$), ejaculation latency ($F = 24.41$, $df = 2/65$, $p < .001$), copulatory efficiency ($F = 15.68$, $df = 2/65$, $p < .001$). None of the interaction factors was significant.

These results indicate that, contrary to findings with mice, postpubertal social isolation may decrease sexual performance in rats and gerbils

and have little effect in hamsters. The low level of performance observed in gerbils may relate to the fact that adults of this species frequently form monogamous pairs (Theissen & Yahr, 1977). This may limit interpretations of findings with this species.

## General Discussion

The present results indicate that the effects of isolation and group-housing upon male sexual behavior vary considerably across different rodent species. The results of Experiment 4 suggest that findings in the previous experiments of facilitation of male sexual patterns in CD-1 mice by isolation can be generalized to other mouse strains. While the different strains investigated here showed markedly different levels of sexual behavior, within each of the three strains isolated animals showed performance that exceeded that of group animals. By contrast, in Experiment 5, isolated male rats, gerbils, and hamsters showed poorer performance than group-housed conspecifics. This latter effect was most marked in rats and gerbils and only slightly evident in hamsters.

In most cases the measures of duration of mounting, mounts, intromissions, and some of the latency measures reached significance, while measures of ejaculation did not. It thus remains possible that specific components of the sexual response pattern, rather than the quantity of all responses, are sensitive to the presence or absence of conspecific males. However, in all cases where ejaculations occurred this measure reflected a strong trend in the same direction as was found with other measures. The lack of significance in the ejaculation measure was usually due to a low number of such responses in all conditions, which reduced statistical power.

The finding in Experiment 5 of decreased sexual performance in isolated male rats and gerbils is consistent with many (but not all, e.g., Beach, 1958) previous investigations of copulatory behavior following isolation. The literature abounds with reports of deficits in copulatory behavior following isolation. For example, isolation has been reported to impair sexual performance in rats (Folman & Drori, 1965; Gerall et al., 1967; Gruendel & Arnold, 1974) and guinea pigs (Valenstein et al., 1955). However, studies of this type have employed postweaning, prepubertal isolation. These deficits have been typically interpreted as evidence that contact between conspecifics prior to puberty is essential for normal adult sexual performance. In the present study, animals were isolated during adulthood after having been group-housed during development. The results of Experiment 5 open the possibility that isolation per se, rather than isolation during a critical period, produces deficits in adult sexual performance. Although these data do not rule out the potential importance of prepubertal social interactions, earlier studies may nevertheless have confounded the concept of critical periods with the intrinsic effects of isolation.

The species differences observed here are consistent with findings of other differences between these species in behavioral responses to social isolation. Aggressiveness is also increased in male mice by isolation (Scott, 1966), while isolation-induced aggression is either absent (Conner, 1972) or very mild and attenuated in rats (Blanchard & Blanchard, 1977) and less pronounced in hamsters (Edwards & Rowe, 1975) and gerbils (Edwards & Rowe, 1975; Thiessen & Yahr, 1977) than in mice. Moreover, these effects may relate to the natural social ecology of the

different species. Although laboratory rats and mice have been bred under unnatural laboratory conditions through several generations, it is likely that many of their natural social predispositions survive (cf., Boice, 1977; Boreman & Price, 1972). Since, as discussed above, rats are naturally found in larger groupings than are mice, it is consistent that they adapt more readily to group housing. It may be that the suppression of sexual performance in mice in the presence of conspecific males relates to the population dynamics of natural house mice.

The species differences, at least those between mice and rats, are also consistent with differences between these species in their physiological response to social isolation. Isolated mice exhibit less pituitary-adrenocortical and more pituitary-gonadal activity than do grouped mice (Benton et al., 1978; Brain, 1975; Brain & Nowell, 1971; Christian, 1955; 1959; McKinney & Desjardins, 1973a; 1973b). Isolated mice may also show lower baseline levels and utilization rates of brain catecholamines, and lower utilization of serotonin than grouped mice, but greater utilization of these amines in stressful and novel situations (Garattini et al., 1969; Welch & Welch, 1969a; 1969b). There would appear to have been fewer investigations of the effects of isolation on physiological mechanisms in rats; those that exist indicate effects which are qualitatively different or relatively weaker (cf. Bennett & Rosenzweig, 1971; Sahakian et al., 1975; Stolk et al, 1974). For example, isolation in rats may produce an increase in baseline turnover of catecholamines and minimal changes in serotonin metabolism relative to grouped subjects (Stolk et al., 1974). These differences in physiological response to isolation may be relevant in light of evidence that pituitary-adrenal hormones (Bertolini, Gessa, & Ferrari, 1975), pituitary-gonadal hormones

(Gorzalka & Mogenson, 1977), and central catecholamines and serotonin (Gessa & Tagliamonte, 1975) may all influence male sexual behavior.

The present results suggest, then, that the modulation of male sexual behavior by isolation may be dependent on the social ecology of the species and the profile of physiological changes accompanying individual housing. One possibility is that physiological determinants of sexual behavior are constant across species but that social mechanisms affecting them are not. The pituitary-adrenal and neurochemical effects of group-housing in mice are consistent with the interpretation that grouping may produce some level of stress in this species (Brain, 1975). In more social species, such as the rat, isolation of previously grouped animals may constitute a stressor and thereby produce sexual performance deficits. Future research correlating physiological responses and social mechanisms might therefore help to explain behavioral effects of social isolation.

## SECTION III: BRIEF PERIODS OF ISOLATION OR GROUPING

As outlined in the General Discussion of Section I, several physiological and behavioral mechanisms may mediate differences in sexual performance between isolated and grouped mice. Isolated mice exhibit less pituitary-adrenocortical and more pituitary-gonadal activity than do grouped mice (Benton et al., 1978; Brain, 1971; 1975; Christian, 1955, 1959; McKinney & Desjardins, 1973a; 1973b). Isolated and grouped mice also show different utilization rates and levels of the central catecholamines, norepinephrine and dopamine, and serotonin (Garattini et al., 1969; Welch & Welch, 1969a; 1969b). Both catecholaminergic and serotonergic activity can influence male sexual behavior (Gessa & Tagliamonte, 1975). One behavioral possibility is that grouped mice might be relatively satiated when presented with females if high levels of intermale mounting occur in the group. Alternatively, intermale fighting among grouped animals may punish approach behavior oriented toward other group members, with consequent suppression of responding generalizing to situations with females.

Since these possibilities could require different time minima to influence mating behavior, an investigation of the temporal parameters of this phenomenon could provide information about causation. The gross changes in weights of adrenals, testes, seminal vesicles, prostates, and

preputials that accompany social isolation generally require several weeks of individual housing to develop (Benton et al., 1978; Brain, 1971; Brain & Nowell, 1971; Christian, 1955; 1959; McKinney & Desjardins, 1973a; 1973b). Neurotransmitter changes, however, can occur within one or two days of isolation (Garattini et al., 1969; Giacalone, Tanzella, Valzelli, & Garattini, 1968). Intermale mounting would have to occur shortly before testing to produce satiation, while learned suppression of approach behavior might require several trials and thus longer periods of grouping or isolation.

Experiments 1, 2, and 3 indicated that isolation-induced facilitation of sexual activity in male-female interactions paralleled isolation-induced aggression in intermale interactions in several parametric manipulations. Isolation-induced aggression is a progressive phenomenon that usually requires periods of isolation greater than one week to develop (Brain, 1975; Scott, 1966). An examination of effects of different periods of isolation on sexual behavior might thus also provide further information regarding a possible common control of sex and aggression in mice.

In Experiment 2 it was found that isolation facilitated sexual behavior at isolation-test intervals of 1-4 weeks, while results at a shorter interval (3 days) were unclear. The present series of experiments investigated briefer periods of isolation than those previously examined to establish the minimum period necessary to produce effects on mating. Once this period was established, behavior of grouped animals during this minimum period was examined and correlated with subsequent sexual performance to elucidate possible mechanisms underlying isolation/grouping differences.

## Experiment 6

Experiment 2 indicated that the maximum effect of isolation upon male sexual behavior is evident after about two weeks of social isolation. The present experiment examined animals after one day of isolation, a period much shorter than those previously examined, and compared these to animals isolated for 2 weeks.

### Method

Seventy-two male CD-1 mice, obtained from Canadian Breeding Farms, at 55 days of age, were housed until commencement of the experiment in groups of 6. Stimulus animals consisted of CD-1 females, obtained from the same breeder and made receptive according to procedures outlined for Experiment 1A. All animals were housed, maintained, and tested under the same conditions as in Experiment 1A.

At 60 days of age, half of the experimental males were divided into three conditions. These consisted of one group of 12, four groups of three and 12 isolated animals. The remaining experimental males were divided into three identical conditions at 73 days of age. All cages were those manufactured from polypropylene by Carworth Lab Cages equipped as described for Experiment 1A. At 74 days of age, after they had been housed in these conditions for 2 weeks or 1 day, animals were presented with receptive females. Test sessions were conducted in the Plexiglas enclosures described for Experiment 1A. Each experimental male was adapted to the enclosure for 5 min, presented with a receptive female, and observed for 1 hr. During these sessions the number, latency, and duration of mounts without intromission (mounts), mounts with intromission and pelvic thrusting (intromissions), and ejaculations were recorded via an Esterline-Angus event recorder. All latencies were taken from the

commencement of the session.

## Results and Discussion

Table VI gives the results for all measures. At both intervals (1 day and 2 weeks) individually-housed animals showed more mounts and intromissions and a longer duration of mounting than animals housed in groups of 3 or 12. Animals in groups of three showed somewhat more mating than animals in groups of 12. Mount and intromission latencies (latencies to the first mount and intromission) were shorter in isolates than in grouped animals. Individual analyses of variance on each measure indicated a significant effect of number of animals per cage for duration of mounting ($F = 5.91$, $df = 2/66$, $p = .004$), number of mounts ($F = 7.66$, $df = 2/66$, $p = .001$), number of intromissions ($F = 3.81$, $df = 2/66$, $p = .027$), mount latency ($F = 5.62$, $df = 2/66$, $p = .006$), and intromission latency ($F = 9.33$, $df = 2/66$, $p < .001$). Subsequent Newman-Keuls comparisons ($p < .05$) indicated that the isolates exceeded both animals grouped in three and animals grouped in 12 in mounts, and that isolates exceeded animals grouped in 12 in duration of mounting and intromissions. Comparisons in mount latency indicated that isolates showed shorter latencies than grouped animals. In intromission latency isolates showed shorter latencies than animals grouped in 3 which in turn showed shorter latencies than animals grouped in 12. There were no significant effects of interval length nor were any of the interaction factors significant. There were also no significant effects in measures of number of ejaculations or ejaculation latency.

The present results extend previous findings in that they indicate that isolation produces as strong an effect at 1 day as is found at

Table VI

Means and Standard Errors of Measures of
Male Mice Isolated or Grouped in 3 or 12 for
24 Hours or 2 Weeks in Experiment 6

| Measure | n per cage | Isolation Test Interval 24 Hours | 2 Weeks |
|---|---|---|---|
| Duration of Mounting (sec) | 1 | 145.08 ± 30.48 | 127.83 ± 27.83 |
| | 3 | 77.25 ± 35.82 | 90.25 ± 37.13 |
| | 12 | 38.42 ± 29.13 | 28.25 ± 13.67 |
| Mounts | 1 | 25.33 ± 6.35 | 24.42 ± 6.08 |
| | 3 | 9.25 ± 4.46 | 14.17 ± 5.97 |
| | 12 | 5.50 ± 3.17 | 6.08 ± 2.57 |
| Intromissions | 1 | 10.33 ± 2.64 | 8.50 ± 2.58 |
| | 3 | 6.00 ± 3.63 | 6.00 ± 3.09 |
| | 12 | 2.92 ± 2.33 | 1.25 ± 0.79 |
| Ejaculations | 1 | 0.25 ± 0.13 | 0.17 ± 0.11 |
| | 3 | 0.08 ± 0.08 | 0.17 ± 0.11 |
| | 12 | 0.08 ± 0.08 | 0.00 ± 0.00 |
| Mount Latency (sec) | 1 | 1085 ± 371 | 1215 ± 302 |
| | 3 | 2469 ± 472 | 2124 ± 435 |
| | 12 | 2290 ± 399 | 2303 ± 367 |
| Intromission Latency (sec) | 1 | 1761 ± 327 | 1815 ± 361 |
| | 3 | 2535 ± 444 | 2519 ± 380 |
| | 12 | 3285 ± 275 | 3251 ± 222 |
| Ejaculation Latency (sec) | 1 | 3314 ± 151 | 3398 ± 136 |
| | 3 | 3568 ± 110 | 3226 ± 258 |
| | 12 | 3575 ± 83 | 3600 ± 0 |

the longer intervals previously investigated. These results are contrary to expectation because many of the major physiological changes accompanying social isolation, particularly those involving gonads and peripheral reproductive tissues, require several days of social isolation to develop (Benton et al., 1978; Brain, 1971; Brain & Nowell, 1971; Christian, 1955). Moreover, other behavioral changes with isolation, such as increases in aggressiveness, require more prolonged periods of isolation (Brain, 1975).

## Experiment 7

The time course for development of facilitation of sexual behavior by isolation remains to be determined. The present experiment compared the effects of periods of isolation ranging from 1 hour to 1 week. Also, because it is possible that pre-treatment grouping of animals and the provision of a clean cage might affect behavior, a comparison set of grouped animals was provided with clean cages at each of the times before testing that animals were isolated.

### Method

Subjects were 216 male CD-1 mice received at 55 days of age and housed in groups of 6 until commencement of the experimental conditions. Preparation of females and other pre-experimental conditions were the same as in Experiment 1A. All animals were tested at 72-75 days of age. At intervals prior to testing of 1 hr, 4 hr, 12 hr, 1 day, 3 days, and 7 days, animals were housed individually in clean cages. At each of these intervals other animals were placed in clean cages but remained in the same groups of 6 as they had been since receipt from the breeder. There was an equal number of animals in each treatment combination. One hour test

sessions in the presence of receptive females were conducted as described for Experiment 1A. Behavior was recorded via an event recorder by a trained observer who was unfamiliar with previous findings and the purpose of the experiment.

## Results and Discussion

Figure 6 gives results for the total duration of mounting, with or without intromission. Table VII presents results for all remaining measures. Most of the measures reflect the same trend. Individually-housed animals showed marginally more sexual activity at intervals of 12 hr or longer than at shorter intervals. Group-housed animals showed more sexual activity at 1 and 4 hr intervals, where they exceeded the isolates, than they did at longer intervals, where they showed poorer performance than the isolates. Separate analyses of variance were conducted on each measure. There was a significant interaction in duration of mounting ($F = 3.13$, $df = 5/204$, $p = .010$), number of mounts ($F = 2.43$, $df = 4/204$, $p = .036$), number of intromissions ($F = 3.12$, $df = 5/204$, $p = .010$), and intromission latency ($F = 3.13$, $df = 5/204$, $p = .010$). Subsequent examination of simple main effects revealed a significant effect of interval among grouped animals for each of these measures (duration: $F = 3.73$, $df = 5/102$, $p = .004$; mounts: $F = 2.74$, $df = 5/102$, $p = .023$; intromissions: $F = 4.02$, $df = 5/102$, $p = .002$; intromission latency: $F = 3.58$, $df = 5/102$, $p = .005$). Newman-Keuls comparisons ($p < .05$) indicated that 4 hr differed from 12 hr, 1 day, 3 days, and 7 days in the duration and intromission measures. 1 hr differed from 3 days in the mounts measured, while 1 hr differed from 7 days and 4 hr differed from 3 and 7 days in the intromission latency measure. Corresponding analyses of isolated animals indicated that the trends over intervals did not reach

Figure 6: The mean total duration of mounting, with or without intromission, in mice isolated or grouped for different intervals in Experiment 7. The open squares represent isolated animals that were formerly in groups of 6. The closed circles represent groups of 6 whose cages were cleaned at the specified intervals before testing. Vertical lines indicate standard errors.

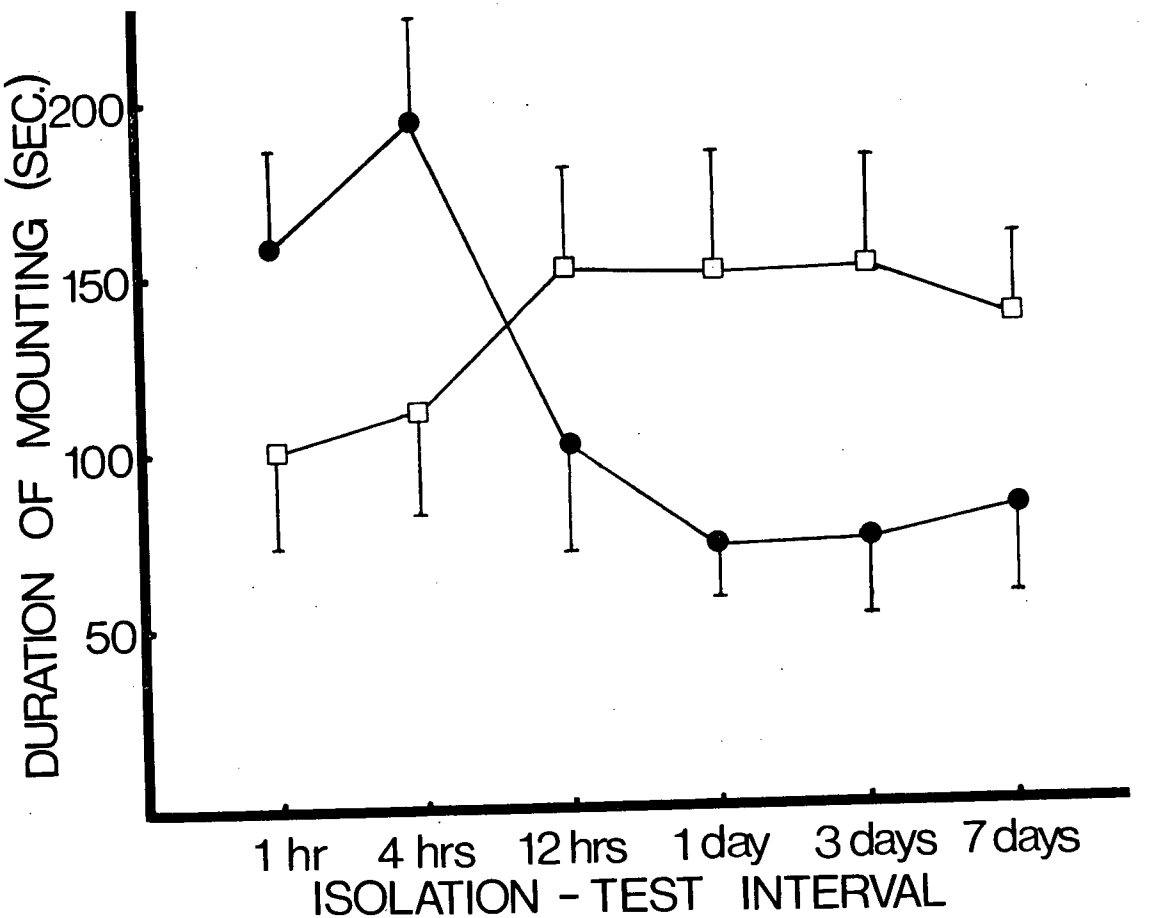

DURATION OF MOUNTING (SEC.)
200
150
100
50
1 hr
4 hrs
12 hrs
1 day
3 days
7 days
ISOLATION - TEST INTERVAL

Table VII

Means and Standard Errors of Measures of Male Mice Isolated or Grouped for Different Intervals in Experiment 7

| Measure | | Isolation-Test Interval | | | | | |
|---|---|---|---|---|---|---|---|
| | | 1 hr | 4 hrs | 12 hrs | 1 day | 3 days | 7 days |
| Mounts | 1 | 16.28±4.23 | 14.89±3.84 | 19.72±5.07 | 23.28±4.95 | 19.67±3.44 | 26.61±5.65 |
| | 6 | 22.67±4.36 | 14.83±3.57 | 14.00±3.78 | 10.33±2.49 | 7.06±1.86 | 10.11±3.04 |
| Intro-missions | 1 | 6.94±2.17 | 7.94±2.32 | 10.72±2.16 | 10.28±2.81 | 10.67±2.78 | 8.67±1.64 |
| | 6 | 11.72±2.24 | 15.56±3.05 | 6.94±2.25 | 4.72±1.12 | 5.06±1.90 | 5.28±2.27 |
| Ejacula-tions | 1 | 0.17±0.09 | 0.11±0.08 | 0.06±0.06 | 0.00±0.00 | 0.00±0.00 | 0.06±0.06 |
| | 6 | 0.17±0.09 | 0.28±0.11 | 0.11±0.08 | 0.00±0.00 | 0.11±0.08 | 0.17±0.12 |
| Mount Latency (sec) | 1 | 1518±350 | 990±296 | 1053±284 | 1134±269 | 977±295 | 810±149 |
| | 6 | 1034±304 | 928±190 | 1361±296 | 1418±321 | 1636±326 | 1924±352 |
| Intro-mission Latency (sec) | 1 | 1882±333 | 2093±330 | 1519±324 | 1415±288 | 1534±320 | 1646±249 |
| | 6 | 1364±306 | 1089±227 | 1895±316 | 1737±313 | 2312±307 | 2593±312 |
| Ejacula-tion Latency (sec) | 1 | 3374±161 | 3385±152 | 3590±10 | 3600±0 | 3600±0 | 3585±12 |
| | 6 | 3302±166 | 3240±163 | 3436±152 | 3600±0 | 3489±83 | 3481±105 |

significance. Examination of the simple main effect of isolation/grouping at each interval indicated differences at 4 hr in intromission latency ($F$ = 6.30, $df$ = 1/34, $p$ = .016), at 24 hr in duration ($F$ = 4.28, $df$ = 1/34, $p$ = .044) and mounts ($F$ = 5.47, $df$ = 1/34, $p$ = .024), at 3 days in mounts ($F$ = 10.41, $df$ = 1/34, $p$ = .003), and at 7 days in mounts ($F$ = 6.65, $df$ = 1/34, $p$ = .014) and intromission latency ($F$ = 5.62, $df$ = 1/34, $p$ = .022).

This experiment suggests that isolation-grouping differences require at least 12-24 hr to develop. It would thus appear that some behavioral and/or physiological transition occurs in male mice during the 24 hr interval after they are isolated from conspecifics. Furthermore, this experiment suggests a second phenomenon, that sexual behavior of grouped animals is facilitated for a few hours following provision of clean cages. The behavior of grouped animals whose cage has recently been cleaned resembles that of animals isolated for at least 24 hr.

## Experiment 8

If behavioral events in grouped animals reduce their performance relative to isolates, these events should be observable during the 24 hr period preceding testing. Numerous possibilities exist. If grouped animals participate in intermale mounting, one might expect some degree of satiation which would inhibit subsequent performance with females. Conversely, intermale mounting could be found in animals that are more active sexually, since mounts without intromission may increase the probability of a male approaching a female (Bermant & Davidson, 1974), and inhibition in remaining animals be caused by other factors. A number of previous studies (DeFries and McClearn, 1970; Kahn, 1961; Lagerspetz & Hautojärvi,

1971) suggest that intermale aggression and dominance may be correlated with sexual performance. In the present experiment, the aggressive and sexual behavior of group-housed males was continuously recorded during the 24 hr prior to testing with females. Animals grouped in 12 were examined because these animals have displayed the poorest performance of those examined in previous experiments.

## Method

Subjects consisted of male CD-1 mice obtained at 60 days of age. These were housed in groups of 6 until 72 days of age. At this time a single group of 12 was formed and housed in a cage similar to those described for Experiment 1A. This group was transferred to a partially illuminated testing room and remained there for 24 hr. Mice within the group were labelled with colored markings on their tails and observed continuously by the author and three other experimenters taking shifts. Experimenters were pre-trained to use the same measuring system. This involved recording the number and duration of mounts, and the number of non-biting attacks, bites, and tail-rattles. Both the animal exhibiting and the animal receiving mounts or aggressive responses were recorded. Immediately following this 24 hr period, all animals were tested with receptive females according to the procedures of Experiment 1A.

## Results and Discussion

Table VIII gives the results of sexual tests with females. Results are similar to those obtained for the group of 12 in Experiment 6. Table IX gives the results for measures of intermale mounting and aggression during the 24 hr prior to testing. Aggressive responses were summarized through the composite aggression score employed in Experiments 1,

Table VIII

Sexual Performance and Stepwise Regression Between Intermale Aggression and Mounting and Subsequent Sexual Measures in Experiment 8

| Measure | Mean ± S.E. | Regression (Includes only significant measures) | | Variables not in equation: Exhibition of Aggression | Receipt of Aggression | Exhibition of Mounting | Receipt of Mounting |
|---|---|---|---|---|---|---|---|
| Duration of Mounting (sec) | 40.42 ±15.78 | $R^2$ = .351<br>F = 5.40<br>p = .041 | Partial R | .177 | .075 | signif. | .227 |
| | | | p | .608 | .810 | | .507 |
| Mounts | 3.50 ± 1.11 | $R^2$ = .000 | Partial R | .178 | .039 | .514 | .157 |
| | | | p | .584 | .871 | .085 | .629 |
| Intromissions | 2.25 ± 0.95 | $R^2$ = .000 | Partial R | .191 | .043 | .370 | .242 |
| | | | p | .559 | .863 | .236 | .453 |
| Ejaculations | 0.08 ± 0.08 | $R^2$ = .987<br>F = 756.00<br>p = .000 | Partial R | .109 | .307 | signif. | .100 |
| | | | p | .744 | .361 | | .762 |
| Mount Latency (sec) | 2282 ±310 | $R^2$ = .000 | Partial R | .249 | .031 | .482 | .231 |
| | | | p | .440 | .887 | .110 | .476 |
| Intromission Latency (sec) | 2864 ±291 | $R^2$ = .462<br>F = 8.59<br>p = .015 | Partial R | .189 | .202 | signif. | .248 |
| | | | p | .582 | .558 | | .468 |
| Ejaculation Latency (sec) | 3490 ±11 | $R^2$ = .987<br>F = 756.10<br>p = .000 | Partial R | .109 | .307 | signif. | .100 |
| | | | p | .744 | .360 | | .762 |

Table IX

Means and Standard Errors of Measures of Intermale Aggression and Mounting in Experiment 8

| | | Exhibition of Aggression[b] | Receipt of Aggression[b] | Exhibition of Mounting (sec) | Receipt of Mounting (sec) |
|---|---|---|---|---|---|
| 1-4 h | Mean | 11.67 | 11.33 | 0.50 | 0.50 |
| | S.E. | 10.13 | 2.83 | 0.50 | 0.29 |
| | n[a] | 7 | 12 | 1 | 3 |
| 5-8 h | Mean | 5.83 | 5.83 | 1.25 | 1.25 |
| | S.E. | 5.12 | 2.69 | 0.95 | 1.25 |
| | n[a] | 3 | 8 | 2 | 1 |
| 9-12 h | Mean | 2.17 | 2.17 | 1.25 | 1.25 |
| | S.E. | 1.03 | 1.22 | 1.25 | 1.25 |
| | n[a] | 5 | 4 | 1 | 1 |
| 13-16 h | Mean | 3.00 | 3.00 | 0.00 | 0.00 |
| | S.E. | 1.90 | 0.97 | 0.00 | 0.00 |
| | n[a] | 3 | 8 | 0 | 0 |
| 17-20 h | Mean | 1.00 | 1.00 | 0.00 | 0.00 |
| | S.E. | 0.83 | 0.39 | 0.00 | 0.00 |
| | n[a] | 2 | 5 | 0 | 0 |
| 21-24 h | Mean | 8.08 | 7.67 | 0.25 | 0.25 |
| | S.E. | 6.56 | 2.71 | 0.25 | 0.25 |
| | n[a] | 6 | 9 | 1 | 1 |
| Total | Mean | 31.75 | 31.00 | 3.25 | 3.25 |
| | S.E. | 24.72 | 5.04 | 2.91 | 2.90 |
| | n[a] | 11 | 12 | 2 | 3 |

[a]number of animals (of 12 total) showing response
[b]Exhibition of Aggression may exceed Receipt of Aggression because tail rattles occurred.

2, and 3. Sexual responses were summarized by taking a total duration of mounting score. A considerable amount of aggression occurred during the initial 4-hr period in which the animals were observed. There were occasional aggressive responses during the remaining period of observation. Only two animals mounted other males and these responses were infrequent in these animals. Exhibition of aggression, receipt of aggression, exhibition of mounts, and receipt of mounts were treated as predictor variables in a stepwise multiple regression analysis performed on each dependent measure (see Kerlinger & Pedhazur, 1973). The results of these analyses are presented in Table VIII. Regression analyses indicated that the exhibition of intermale mounting showed a significant relationship to four measures, notably ejaculation number and latency. This occurred because the one high-scoring animal that ejaculated was also the only animal that showed a substantial amount of mounting of males. Other variables did not reach significance, although exhibition of aggression and receipt of mounting each showed small relationships to most measures.

These results suggest that intermale mounting is uncommon and insufficient to account for isolation-grouping differences. There is, however, some indication that ejaculation frequency in grouped animals relates to intermale mounting. This may be because intermale mounts increase the likelihood of ejaculation or because some sexually active males may mount other males. In neither case could this relationship account for the poor performance of other males that showed no intermale mounting. While intermale aggression levels were relatively high, none of the relationships between them and behavior with females reached statistical significance. These results leave open the possibility that recent

intermale aggression reduces the sexual performance of grouped animals as a whole, but suggest that any effects it has on intra-group variance in sexual performance are small.

## General Discussion

The present findings extend results of the previous experiments by demonstrating that only a brief period of isolation is necessary to facilitate mounting and intromitting. Males from both recently formed (Experiment 6) and long-established (Experiment 7) social groups show fewer mounts and intromissions than do isolates. The effects of these manipulations on ejaculations remain unclear due to the low incidence of these responses. Results of Experiment 8 indicate that effects of grouping on ejaculation frequency may be complicated by effects of intermale mounting on ejaculations.

It was previously suggested (Section I) that sexual and aggressive patterns respond in parallel to parametric manipulations of environmental and social variables. Since major effects of isolation on aggressiveness generally require several days of individual housing to develop (Brain, 1975; Scott, 1966), this study suggests some differential control of sex and aggression.

These results may narrow the range of hypotheses that could account for isolation/grouping differences in mating. The findings of Experiment 8 appear to rule out more visible aspects of intermale social interaction as determinants of the poor performance of grouped animals. Clearly, intermale mounting occurs much too infrequently to produce relative sexual satiation in group-housed males. Differential participation in intermale aggression does not appear to account for intra-group

variance in subsequent sexual performance. It remains possible, however, that exposure to or observation of attacks from other males reduces the subsequent sexual performance of all group-housed males, and is thus involved in the isolation/grouping differences. Indeed, evidence presented by Kahn (1961) suggests that exposure to attack does reduce subsequent sexual performance with females.

A lack of a strong relationship between differential participation in intermale aggression and subsequent intra-group variation in sexual performance raises some interesting questions. It has been argued (Benton et al., 1978; Brain, 1975) that isolates are similar to dominant, territorial grouped males in several behavioral and physiological respects. On this basis one would predict that more aggressive or dominant grouped males would be more active sexually than other grouped males. Indeed, DeFries and McClearn (1970) have found that dominant males sire the vast majority of offspring when females are introduced into a group. Kahn (1961) found that more aggressive males mated more; although Lagerspetz and Hautojärvi (1967) found a transient effect in the opposite direction. The absence of a significant correlation between aggression and subsequent intra-group variance in mating here would contradict these studies. However, it is possible that this lack of significance reflects the small sample size or the recomposition of the group 24 hours prior to testing. Additional work relating dominance and aggressiveness to mating is needed.

The present results suggest that the large differences in endocrine weight that accompany prolonged isolation and grouping (Benton et al., 1978; Brain, 1971; Brain & Nowell, 1971; Christian, 1955, 1959; McKinney & Desjardins, 1973a; 1973b) are probably not responsible for differential sexual behavior, since the time factors involved in the two effects differ

substantially. However, these results do not rule out involvement of hormones produced by these glands and suggest a need for examination of gonadal and adrenal activity at short isolation/grouping intervals. Investigations of endocrine activity following isolation or grouping have tended to employ one week as the minimum interval. For example, Goldsmith et al. (1976) measured plasma corticosterone at intervals of one week and longer following introduction of experimental housing conditions. Although they failed to find differences between isolated and grouped mice, differences might have occurred at shorter intervals. Since environmental manipulations can significantly alter steroid levels in a matter of minutes (e.g., Macrides, Bartke, & Dalterio, 1975), it would be worthwhile to investigate effects of short-interval housing on adrenal and gonadal steroids. Intermale aggression in grouped animals is known to produce fairly rapid pituitary-adrenocortical activation among more subordinate group members (Brain & Nowell, 1970; Bronson & Eleftheriou, 1965), while defeat in aggressive encounters may also produce changes in hypothalamic levels of luteinizing hormone (Eleftheriou & Church, 1967, 1968). It is possible that the occurrence of these hormonal events, as a result of aggressive encounters, produces the relatively low levels of sexual behavior in grouped males through some unknown mechanism.

If biochemical events are responsible for isolation/grouping differences it must be demonstrated that these undergo transitions within 24 hours of isolation. At least in the case of central neurotransmitters, such evidence exists, since brain catecholamines and serotonin undergo rapid changes following isolation (Garattini et al., 1969; Giacalone et al., 1968). Isolated mice may show greater utilization of the neurotransmitters nore-

pinephrine, dopamine, and serotonin in novel situations than do grouped mice (Garattini et al., 1969; Welch & Welch, 1969a; 1969b). This evidence is complemented by pharmacological evidence that catecholamine agonists increase and antagonists decrease sexual behavior, while serotonin agonists decrease and antagonists increase such behavior (Gessa & Tagliamonte, 1975). It is conceivable that biogenic amine utilization rates also account for the high levels of sexual performance observed in group-housed animals 4 hours after cage-cleaning. It is a common observation that male mice will fight shortly after cage cleaning, even when group interactions have formerly been stable. This phenomenon is quantified in Experiment 8, albeit with a reconstituted group. Aggressive interactions are known to be accompanied by high levels of turnover of biogenic amines (Garattini et al., 1969; Welch & Welch, 1969a; 1969b). Increased turnover of biogenic amines, particularly the catecholamines, norepinephrine and dopamine, might carry over into subsequent tests with receptive females. Both isolation-induced and cage-cleaning-induced facilitation of male sexual behavior might thus be accounted for by behavioral activation due to increased catecholamine utilization or perhaps some serotonergic mechanism.

These data may also suggest an involvement of pheromonal variables. There is evidence (see Bronson, 1971) that male mice secrete pheromones in their feces and urine. Isolating mice might remove them from the immediate vicinity of pheromones secreted by conspecific males. Such pheromones could conceivably act to reduce the sexual performance of some males, an effect which may relate to the population dynamics of the species. Such pheromones could also be involved in the transient increase in performance of grouped animals observed in Experiment 7 following cage-cleaning. Cage cleaning temporarily removes much of the odor of conspecific males, but as

feces and urine accumulate these odors return. One problem with this interpretation is the relatively low level of performance of isolates after 1 and 4 hr isolation in Experiment 7. This might be explained, however, by other variables involved in early adaptation to isolation. Pheromonal suppression of sexual behavior in grouped male mice might parallel effects found elsewhere (Whitten, 1959) with female mice, where grouping may suppress estrus and lengthen diestrus.

## SECTION IV: PITUITARY-ADRENAL MEDIATION

The experiments of the present section test the hypothesis that pituitary-adrenal factors are involved in differences in sexual activity between isolated and grouped mice. This research follows from evidence that isolation in mice frequently lowers pituitary-adrenocortical activity (Brain, 1975; Burge & Edwards, 1971; Leshner et al., 1973), adrenalectomy or ACTH decreases while corticosterone restores isolation-induced aggression in adrenalectomized mice (Burge & Edwards, 1971; Gorzalka & Caira, 1979; Leshner et al., 1973), and aggression and sexual activity are increased in parallel by individual housing in male mice (Section I).

The present section is concerned, in addition to isolation/grouping differences, with the general role of pituitary-adrenocortical activity in the expression of sexual responding. A good deal of evidence suggests that male sexual behavior is probably not dependent on adrenal hormones. Adrenalectomy fails to modify copulatory behavior in gonadally intact rats (Bloch & Davidson, 1968) and castrated rats receiving testosterone propionate (Gorzalka, Rezek, & Whalen, 1975). Neither adrenalectomy nor treatment with desoxycorticosterone acetate appreciably influences mating activity in hamsters (Warren & Aronson, 1956). Furthermore, adrena-

lectomy fails to reduce the post-castrational copulatory behavior that is evident in male cats (Cooper & Aronson, 1958) and dogs (Schwartz & Beach, 1954).

Since adrenalectomy leads to a sustained increase in pituitary ACTH levels (Gemzell, van Dyke, Tobias, & Evans, 1951), one might reasonably predict that ACTH administration would also fail to alter male sexual behavior. The few studies of this issue have yielded contradictory results. Intravenously administered ACTH inhibits copulatory activity in the male rabbit (Korányi, Endröczi, & Tárnok, 1966) while ACTH administered intraventricularly facilitates sexual activity in the same species (Bertolini et al., 1975). ACTH injections facilitate sexual activity in male rats (Bertolini et al., 1975). However, $ACTH^{4-10}$, a peptide largely devoid of adrenocorticotropic activity but possessing the effectiveness of ACTH on avoidance behavior (deWied, 1969), increases the latency of male rats to exhibit copulatory responses (Bohus, Hendrickx, van Kolfschoten, & Krediet, 1975). Thus, while adrenalectomy-induced increases in ACTH would appear to have no effect on sexual behavior, exogenous ACTH seems to increase or decrease mating, depending on methodology. Because experimental paradigms and species have varied widely in the literature, it would seem appropriate to examine the effects of adrenalectomy and exogenous ACTH within a single study.

## Experiment 9A

Adrenalectomy both increases ACTH levels and removes the primary source of corticosteroids. If pituitary-adrenocortical mechanisms are involved in the differential performance of grouped and isolated mice, it

is likely that there will be an interaction between adrenalectomy and sexual behavior under different housing conditions. The present experiment compared the effects of adrenalectomy and sham adrenalectomy on the mating responses of grouped and isolated male mice.

## Method

CD-1 male mice were obtained from Canadian Breeding Farms at 55 days of age. Pre-experimental housing and general testing conditions were like those of Experiment 1A. Stimulus animals consisted of CD-1 females, obtained from the same breeder and made receptive according to the procedures of Experiment 1A.

At about 60 days of age, surgery was performed on experimental males administered 2.5 mg. Nembutal (Sodium Pentobarbital, Abbott) anesthesia. Half of the animals were bilaterally adrenalectomized while remaining animals were sham-adrenalectomized. All animals were returned to groups of 6. Adrenalectomized animals were given access to both water and 0.9% saline solution. One week after surgery, both adrenalectomized and sham-adrenalectomized animals were either isolated or housed in groups of 6. All cages were like those described for Experiment 1A.

After 2 weeks in these conditions, animals were given their first test of sexual behavior in the presence of receptive females. Test sessions were conducted for 1 hr as in the previous experiments. During sessions the number, latency, and duration of mounts without intromission (mounts), mounts with intromission and pelvic thrusting (intromissions), and ejaculations were recorded via an Esterline Angus event recorder. Each animal was returned to its isolation or group housing condition and retested one and two weeks following this first test. Of nonadrenalectomized animals,

11 isolated and 9 grouped animals survived to finish all tests, while 9 isolated and 9 grouped adrenalectomized animals survived. When grouped animals died they were replaced by surrogates that were treated in the same manner as other animals in the group but not included in testing sessions.

## Results and Discussion

Figure 7 gives the results for a measure of duration of mounting. Table X gives results for the remaining measures. Isolated sham-adrenalectomized animals showed substantially more sexual activity than grouped sham-adrenalectomized animals. Grouped adrenalectomized animals showed more activity than isolated adrenalectomized and grouped sham-adrenalectomized animals. Individual analyses of variance were conducted on each measure, with two between-subjects factors (adrenalectomized/sham, isolated/grouped) and one within-subjects factors (weekly test). There was a significant adrenalectomy-sham by isolation-grouping interaction in duration of mounting ($F = 7.66$, $df = 1/33$, $p = .009$), mounts ($F = 8.71$, $df = 1/33$, $p = .006$), ejaculations ($F = 5.26$, $df = 1/33$, $p = .028$), mount latency ($F = 6.32$, $df = 1/33$, $p = .016$), intromission latency ($F = 8.13$, $df = 1/33$, $p = .007$), and ejaculation latency ($F = 5.11$, $df = 1/33$, $p = .031$). Examination of simple main effects revealed significant sham isolation-grouping differences in duration ($F = 5.11$, $df = 1/18$, $p = .036$), mounts ($F = 4.59$, $df = 1/18$, $p = .046$), mount latency ($F = 5.36$, $df = 1/18$, $p = .033$), and intromission latency ($F = 4.59$, $df = 1/18$, $p = .046$). There were adrenalectomized isolation-grouping differences only in mounts ($F = 4.58$, $df = 1/15$, $p = .049$). Comparisons of sham isolate and adrenalectomized isolate conditions revealed effects in duration ($F = 5.68$, $df = 1/17$, $p = .029$), mounts

Figure 7: The mean total duration of mounting, with or without intromission, in adrenalectomized (Adx) or sham-adrenalectomized (Sham), isolated (Isol) or grouped (Gp) mice in Experiment 9A. Vertical lines indicate standard errors.

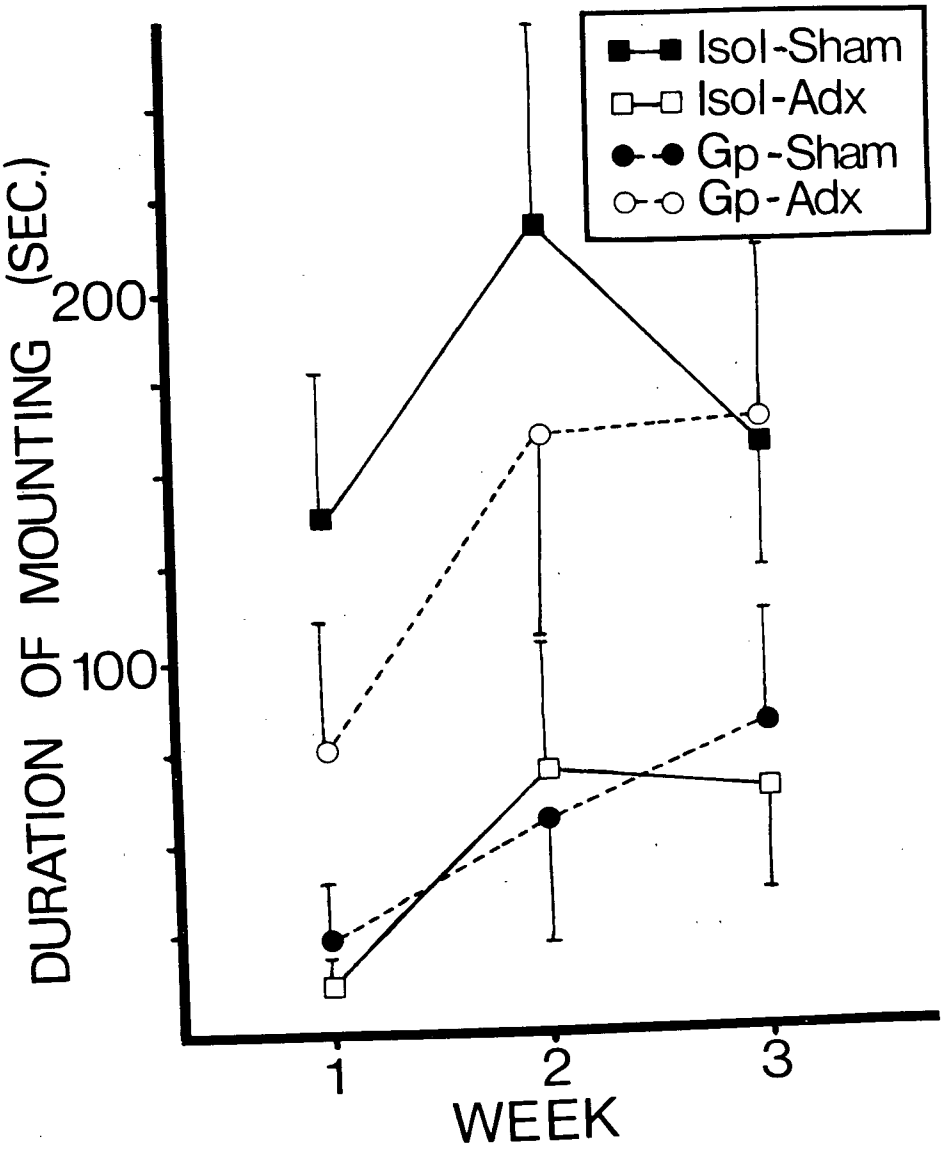

Isol-Sham
Isol-Adx
Gp-Sham
Gp-Adx
DURATION OF MOUNTING (SEC.)
200
100
1
2
3
WEEK

Table X

Means and Standard Errors of Measures of Sexual Performance in Adrenalectomized or Sham-Adrenalectomized, Isolated or Grouped Male Mice in Experiment 9A

| Measure | Week | Sham | | Adrenalectomized | |
|---|---|---|---|---|---|
| | | isolated | grouped | isolated | grouped |
| Mounts | 1 | 19.09±4.60 | 5.56±4.60 | 0.88±0.52 | 10.00±3.92 |
| | 2 | 29.18±8.64 | 9.00±4.61 | 4.63±2.24 | 25.67±8.89 |
| | 3 | 21.09±4.52 | 12.89±5.63 | 8.75±4.07 | 13.44±5.03 |
| Intromissions | 1 | 5.00±1.67 | 1.82±1.13 | 0.50±0.38 | 3.11±1.63 |
| | 2 | 8.89±3.77 | 5.18±3.01 | 4.00±3.34 | 6.22±2.72 |
| | 3 | 6.33±1.67 | 4.37±1.71 | 2.50±1.71 | 6.44±2.64 |
| Ejaculations | 1 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 |
| | 2 | 0.18±0.12 | 0.00±0.00 | 0.00±0.00 | 0.11±0.11 |
| | 3 | 0.36±0.20 | 0.11±0.11 | 0.12±0.12 | 0.44±0.18 |
| Mount Latency (sec) | 1 | 1221±431 | 2803±431 | 2962±375 | 1414±491 |
| | 2 | 696±567 | 2047±293 | 1900±340 | 1280±580 |
| | 3 | 1127±547 | 1523±355 | 1013±389 | 1005±497 |
| Intromission Latency (sec) | 1 | 2202±370 | 3123±379 | 3186±321 | 2759±401 |
| | 2 | 1654±501 | 2944±363 | 3085±396 | 1790±493 |
| | 3 | 1137±344 | 2190±564 | 2468±480 | 1226±465 |
| Ejaculation Latency (sec) | 1 | 3600±0 | 3600±0 | 3600±0 | 3600±0 |
| | 2 | 3349±233 | 3600±0 | 3600±0 | 3494±106 |
| | 3 | 2931±349 | 3550±50 | 3370±230 | 2987±310 |

($F$ = 11.18, $df$ = 1/17, $p$ = .004), mount latency ($F$ = 5.42, $df$ = 1/17, $p$ = .033), and intromission latency ($F$ = 6.26, $df$ = 1/17, $p$ = .023). All other effects did not reach significance.

It is apparent from these results that adrenalectomy affects a mechanism that contributes to the development of isolation-grouping differences in sexual performance. Since grouped animals actually showed a facilitation of activity following adrenalectomy, it is unlikely that the results of this experiment can be attributed entirely to nonspecific debilitation. The mating deficits observed in isolates subsequent to adrenalectomy could be attributed to elevated ACTH levels, reduced adrenal steroid levels, general debilitation, or some combination of these factors. However, none of these possibilities would account for the effects of adrenalectomy on grouped mice. This would suggest the absence of a simple monotonic relationship between pituitary-adrenocortical activity and sexual behavior observed under different housing conditions.

## Experiment 9B

It has been shown that corticosterone, a major adrenal steroid, reverses the deficits in isolation-induced aggression following adrenalectomy (Leshner *et al.*, 1973). If the aggression and facilitation of sexual behavior that follow isolation are under similar endocrine control, exogenous corticosterone should have similar effects on mating in adrenalectomized mice. The present experiment examined whether corticosterone treatment would restore normal isolation-grouping effects in the adrenalectomized animals examined in Experiment 9A.

## Method

Subjects from Experiment 9A were maintained under the housing conditions described for that experiment. Following the last test with receptive females in Experiment 9A, daily injections of 200 µg corticosterone sc in .05 cc peanut oil were given to all adrenalectomized animals. This dose was derived from other experiments (Leshner et al., 1973; Moyer & Leshner, 1976) demonstrating restoration of nonsexual behavior, such as aggression, in adrenalectomized mice with corticosterone. Sham-adrenalectomized animals were given .05 cc oil daily for comparison purposes with Experiment 9A. After 1, 2, and 3 weeks of this injection regimen animals were tested according to procedures of Experiment 9A. Eight animals in each condition survived to complete all tests.

## Results and Discussion

Table XI gives results for all measures. The patterns evident in Experiment 9A were essentially unchanged by treatment with either corticosterone or oil. Analyses of variance on individual measures revealed a significant adrenalectomy-sham by isolation-grouping interaction in mounts ($F$ = 5.11, $df$ = 1/28, $p$ = .032) and mount latency ($F$ = 7.86, $df$ = 1/28, $p$ = .009), while this interaction approached significance for duration of mounting ($F$ = 3.48, $df$ = 1/28, $p$ = .072) and intromission latency ($F$ = 2.99, $df$ = 1/28, $p$ = .095). Analyses of simple main effects revealed significant differences between sham isolates and adrenalectomized isolates in mounts ($F$ = 11.90, $df$ = 1/14, $p$ = .004) and mount latency ($F$ = 6.58, $df$ = 1/14, $p$ = .022), differences approaching significance between sham isolates and sham grouped animals in mounts ($F$ = 4.15, $df$ = 1/14, $p$ = .061) and mount latency ($F$ = 4.39, $df$ = 1/14, $p$ = .055), and differences approaching significance between adrenalectomized isolates and adrenalectomized

Table XI

Means and Standard Errors of Measures of Sexual Performance of Adrenalectomized Corticosterone-Treated and Sham-Adrenalectomized Oil-Treated Mice in Experiment 9B

| Measure | Week | Sham-Oil | | Adrenalectomized-Corticosterone | |
|---|---|---|---|---|---|
| | | isolated | grouped | isolated | grouped |
| Duration of Mounting (sec) | 1 | 119.38±31.19 | 74.13±29.77 | 31.63±23.69 | 115.75±41.61 |
| | 2 | 133.25±29.84 | 76.25±40.31 | 36.37±26.98 | 91.88±30.83 |
| | 3 | 101.13±36.29 | 48.25±19.12 | 36.00±29.26 | 48.00±18.02 |
| Mounts | 1 | 28.38±7.32 | 10.50±5.26 | 4.88±2.90 | 13.13±5.74 |
| | 2 | 22.50±5.10 | 10.25±6.45 | 5.25±2.66 | 6.50±4.06 |
| | 3 | 18.50±5.26 | 5.63±3.60 | 2.13±1.49 | 9.38±5.25 |
| Intromissions | 1 | 5.25±2.18 | 2.88±1.56 | 1.50±1.22 | 5.75±2.97 |
| | 2 | 6.00±1.44 | 4.75±2.87 | 1.25±1.00 | 5.13±2.95 |
| | 3 | 4.00±1.63 | 1.38±0.73 | 1.75±1.61 | 1.75±0.77 |
| Ejaculations | 1 | 0.00±0.00 | 0.25±0.16 | 0.00±0.00 | 0.50±0.19 |
| | 2 | 0.00±0.00 | 0.13±0.12 | 0.00±0.00 | 0.63±0.26 |
| | 3 | 0.25±0.16 | 0.38±0.18 | 0.25±0.16 | 0.38±0.18 |
| Mount Latency (sec) | 1 | 706±161 | 1373±515 | 2426±507 | 651±428 |
| | 2 | 686±183 | 2154±481 | 1538±515 | 1293±575 |
| | 3 | 1297±575 | 2416±584 | 2530±541 | 926±487 |
| Intromission Latency (sec) | 1 | 2274±365 | 2220±542 | 2846±471 | 1199±506 |
| | 2 | 1981±423 | 2381±478 | 3040±385 | 1547±516 |
| | 3 | 2000±505 | 2454±565 | 2731±526 | 2362±534 |
| Ejaculation Latency (sec) | 1 | 3600±0 | 3093±332 | 3600±0 | 2358±506 |
| | 2 | 3600±0 | 3339±261 | 3600±0 | 2293±549 |
| | 3 | 3321±219 | 2801±391 | 3060±378 | 2578±511 |

grouped animals in mount latency ($F$ = 3.64, $df$ = 1/14, $p$ = .077). Interaction factors were not significant for other measures but the isolation-grouping main effect was significant for ejaculations ($F$ = 7.00, $df$ = 1/28, $p$ = .013) and ejaculation latency ($F$ = 6.94, $df$ = 1/28, $p$ = .014). All other effects were not significant. Many of the statistical differences between these analyses and those of Experiment 9A may be attributable to loss of power due to reduced sample size.

The failure of corticosterone to reverse sexual activity in adrenalectomized mice at a dosage sufficient to restore isolation-induced aggression opens the possibility of a differential hormonal control of these behaviors. Since corticosterone normally reduces ACTH levels, it seems less probable that the deficits seen in adrenalectomized isolates can be attributed to elevated levels of ACTH. However, the possibility that corticosterone, under the present regimen, failed to restore endogenous ACTH to pre-adrenalectomy levels cannot be excluded. Rather, the deficits may reflect an absence of other adrenocortical hormones. Recently it was demonstrated that desoxycorticosterone was effective while corticosterone was ineffective in facilitating sexual receptivity in ovariectomized, estrogen-treated rats (Gorzalka & Whalen, 1977). Although the adrenal cortex produces considerably greater quantities of corticosterone than desoxycorticosterone, there is no reason to assume that the major steroid is necessarily the most effective for every behavioral response. Alternatively, the possibility that a different corticosterone regimen would have reversed the sexual performance of adrenalectomized mice cannot be excluded.

## Experiment 10

The results of Experiment 9A suggest, among other arguments, that either removal of adrenal steroids or elevation of ACTH levels could mediate

the effects of adrenalectomy upon sexual performance. Results from Experiment 9B suggest that corticosterone treatment, which presumably normalizes ACTH levels, does not influence sexual responding in adrenalectomized animals. This leads to another possibility, namely, that appropriate levels of both ACTH and specific adrenal corticoids are necessary for the facilitation of sexual activity in isolates. In other words, pituitary ACTH may play a regulatory role but the behavioral expression of this mechanism would require a permissive action of specific adrenal corticosteroids. This hypothesis can be tested by comparing the behavior of adrenalectomized and sham-adrenalectomized mice following exogenous ACTH. If effects of ACTH are dependent on adrenal steroids, they would only be evident in sham animals. If ACTH is capable of acting alone to regulate mating then effects might be observed in both adrenalectomized and sham mice. The present experiment examined the effects of ACTH treatment in both adrenalectomized and sham-adrenalectomized isolated male mice.

## Method

CD-1 males were obtained at 55 days of age and housed in groups of 6. At 60-65 days of age, about half of the animals were bilaterally adrenalectomized and the remainder sham-adrenalectomized. Subsequent to surgery, all animals were housed individually in cages like those described for Experiment 1A. All adrenalectomized animals were given access to both water and 0.9% saline solution. Beginning one week following surgery, approximately half of the adrenalectomized and sham animals were given daily injections of .5 IU ACTH (Calbiochem.) dissolved in .2 cc physiological saline of pH 7.2. Other work in our laboratory has shown this to be the

minimally effective ACTH dose for influencing sexual behavior in the mouse. Remaining animals received the saline vehicle alone. All injections were given ip. One, two, and three weeks after commencement of injections, all animals were tested for one hour in the presence of receptive females. Females were prepared and sessions conducted according to the procedures of Experiment 1A. Eleven sham saline, 12 sham ACTH, 9 adrenalectomized saline, and 6 adrenalectomized ACTH animals survived to complete all tests of sexual behavior.

## Results and Discussion

Figure 8 presents results for duration of mounting, with and without intromission. Table XII presents results for all remaining measures. Adrenalectomized animals given ACTH showed the highest scores on most measures. These strongly exceeded scores of adrenalectomized saline animals. Sham saline animals showed more behavior than adrenalectomized saline animals and marginally more behavior than sham ACTH animals. An analysis of variance was conducted on each measure. In the duration measure there were significant main effects of hormone treatment ($F$ = 4.93, $df$ = 1/34, $p$ = .033) and weekly test ($F$ = 6.70, $df$ = 1/34, $p$ = .002), and significant weekly test by hormone treatment ($F$ = 4.54, $df$ = 2/68, $p$ = .014) and adrenalectomy-sham by hormone treatment ($F$ = 5.07, $df$ = 1/34, $p$ = .031) interactions. Analyses of simple main effects for the latter interaction indicated a significant effect of hormone treatment in adrenalectomized animals ($F$ = 12.50, $df$ = 1/13, $p$ = .004) and a difference between ACTH-treated sham and ACTH-treated adrenalectomized animals which approached significance ($F$ = 4.26, $df$ = 1/16, $p$ = .056). Analyses of simple main effects in the weeks by hormone interaction revealed effects at week 2 ($F$ = 10.06, $df$ = 1/34, $p$ = .003). In the mounts measure the main effects of hormone treatment

Figure 8: The mean total duration of mounting, with or without intromission, in adrenalectomized (Adx) or sham-adrenalectomized (Sham) isolated mice given daily saline (Sal) or ACTH in Experiment 10. Vertical lines indicate standard errors.

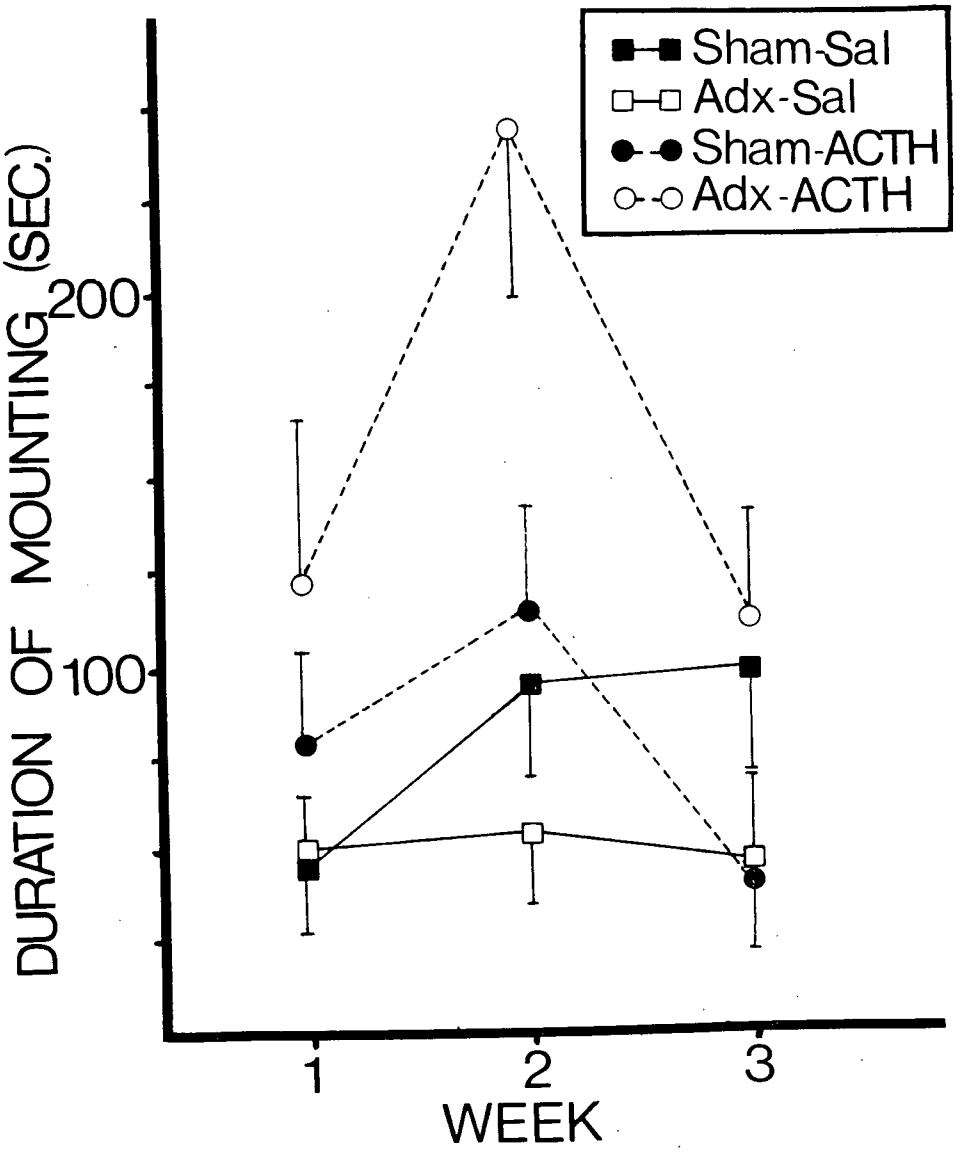

Sham-Sal
Adx-Sal
Sham-ACTH
Adx-ACTH
DURATION OF MOUNTING (SEC.)
200
100
1
2
3
WEEK

Table XII

Means and Standard Errors of Sexual Performance of Adrenalectomized and Sham-Adrenalectomized Mice Treated with ACTH or Saline in Experiment 10

| Measure | Week | Sham | | Adrenalectomized | |
|---|---|---|---|---|---|
| | | Saline | ACTH | Saline | ACTH |
| Mounts | 1 | 13.81±4.38 | 12.08±2.41 | 10.67±3.23 | 23.00±9.10 |
| | 2 | 9.73±4.18 | 14.83±3.19 | 9.67±3.00 | 25.83±5.70 |
| | 3 | 9.27±3.77 | 10.33±4.28 | 5.67±2.03 | 13.00±3.78 |
| Intromissions | 1 | 1.64±0.77 | 4.91±1.45 | 2.78±1.50 | 8.67±5.79 |
| | 2 | 4.55±1.69 | 5.83±1.97 | 2.11±0.98 | 10.00±2.29 |
| | 3 | 5.36±2.58 | 2.17±1.34 | 1.67±0.71 | 3.83±1.01 |
| Ejaculations | 1 | 0.00±0.00 | 0.17±0.11 | 0.00±0.00 | 0.17±0.17 |
| | 2 | 0.09±0.91 | 0.08±0.08 | 0.22±0.15 | 0.33±0.21 |
| | 3 | 0.27±0.19 | 0.00±0.00 | 0.33±0.24 | 0.50±0.22 |
| Mount Latency (sec) | 1 | 1781±439 | 1139±350 | 1582±326 | 735±335 |
| | 2 | 1809±455 | 1011±259 | 1022±322 | 505±162 |
| | 3 | 1515±393 | 2060±398 | 1747±442 | 769±222 |
| Intromission Latency (sec) | 1 | 3140±236 | 2157±386 | 2754±376 | 2169±554 |
| | 2 | 2389±376 | 2116±347 | 2363±453 | 1190±511 |
| | 3 | 2248±443 | 2848±339 | 2620±414 | 1536±478 |
| Ejaculation Latency (sec) | 1 | 3600±0 | 3413±134 | 3600±0 | 3423±177 |
| | 2 | 3404±195 | 3525±74 | 3104±331 | 3327±175 |
| | 3 | 3220±264 | 3600±0 | 3388±183 | 2854±412 |

($F$ = 4.14, $df$ = 1/34, $p$ = .050) and weeks ($F$ = 3.93, $df$ = 2/68, $p$ = .024) were significant. In the intromissions measure there was a significant interaction between test weeks and hormone treatment ($F$ = 3.19, $df$ = 2/68, p = .047). Analysis of simple main effects revealed effects of ACTH at weeks 1 ($F$ = 4.18, $df$ = 1/34, $p$ = .049) and 2 ($F$ = 6.04, $df$ = 1/34, $p$ = .019). None of the remaining effects reached significance.

ACTH appears to have had a strong effect upon performance of adrenalectomized isolates, but relatively little effect upon sham-adrenalectomized isolates. These results are contrary to expectation since one would assume an effect either on sham and adrenalectomized mice or on sham mice only. Clearly one hypothesis can be eliminated, namely, that behavioral effectiveness of ACTH is dependent on some minimal level of adrenal corticoids. As with the results of Experiment 9, the data from the present experiment indicate that the relationship between ACTH and sexual behavior is not a monotonic one.

## General Discussion

This series of experiments again confirms the finding that isolation facilitates the display of mounts and intromissions in male mice. Adrenalectomy inhibited mating in isolates but produced the opposite effect in group-housed animals (Experiment 9A). This finding suggests a rather complex relationship between pituitary-adrenocortical activity and male sexual behavior in this species. By contrast, adrenalectomy neither facilitated nor inhibited mating activity in gonadally intact hamsters (Warren & Aronson, 1956) and rats (Bloch & Davidson, 1968). This finding in the mouse is, however, consistent with most reports on the effect of adrenalectomy on isolation-induced aggression (e.g. Brain, Nowell & Wouters, 1971),

although Burge and Edwards (1971) find no effect of adrenalectomy on aggression. In Experiment 9B, it was shown that corticosterone failed to restore mating to preadrenalectomy levels in isolates. This contrasts with isolation-induced aggression where deficiencies produced by adrenalectomy can be restored by corticosterone treatment (Leshner et al., 1973). Experiment 10 demonstrated that exogenous ACTH elevated sexual responding in adrenalectomized isolates and had less of an effect on sham adrenalectomized isolates. These results also suggest a differential control of sexual and aggressive behavior since administration of ACTH reduces rather than elevates isolation-induced aggression (Brain et al., 1971).

Experiment 9A indicated that adrenalectomy may, under some circumstances, significantly reduce sexual behavior in singly-housed mice. In Experiment 10, the same trend was evident in the second and third weeks of testing. The absence of this trend in the first week may relate to the fact that testing occurred at a shorter interval following adrenalectomy and the inception of isolation. Furthermore, daily intraperitoneal injections in Experiment 10 may have constituted a stressor, reducing performance of sham isolates through pituitary-adrenal or other biochemical effects. Comparable effects of injection stress on intermale aggression have recently been reported (Brain & Bowden, 1978). In this paradigm, high levels of ACTH may act to protect against injection stress. This could explain the difference in performance between ACTH- and saline-injected adrenalectomized mice in Experiment 10 and the difference in performance between isolated, adrenalectomized mice in Experiments 9A and 10. According to this model, one need not assume a direct relationship between sexual activity and ACTH titer.

Experiments 9A and 10 indicate that adrenalectomy has significant

effects on at least some components of sexual responding in mice. There has been at least one report that adrenalectomy does not reduce the percentage of isolated mice that ejaculate (Thompson, McGill, MacIntosh, & Manning, 1976). There is also no significant difference in this measure in the present study. Another group of investigators find no significant effect of adrenalectomy on intromission frequency, mount frequency, and the percentage of mice intromitting (Wallis & Luttge, 1975). However, these investigators sampled considerably less behavior, employing two 20-minute tests as compared to three 1 hour tests in the present study. Furthermore, differences in Experiment 9A were found in four measures: duration of mounting, mount frequency, mount latency, and intromission latency. Three of these measures were not reported in earlier studies (Thompson et al., 1976; Wallis & Luttge, 1975).

It is conceivable that adrenalectomy produces nonspecific deficits which interfere with sexual performance. For example, the thermoregulatory dysfunction that accompanies adrenalectomy (Tanche, 1976) could have contributed to a decline in the performance of isolates. In grouped animals, the symptoms may have been ameliorated by the animals' tendency to huddle together in their home cage. This explanation could partially account for the differential effects of adrenalectomy on isolated and grouped mice in Experiment 9A. However, the results of Experiment 9B tend to argue against this interpretation. Corticosterone treatment did not facilitate recovery in isolates. Moreover, ACTH alone permitted high levels of mating in adrenalectomized isolates in Experiment 10. There is little evidence that exogenous ACTH, in the absence of the adrenal cortex, has a health-promoting property.

Despite the alternative explanation of injection stress, both the effects of adrenalectomy on grouped mice in Experiment 9A and ACTH treatment in Experiment 10 are consistent with the idea that ACTH potentiates sexual responding. The results of adrenalectomy on isolates in Experiment 1 suggests an inhibitory action of ACTH. These paradoxical findings can be reconciled if one assumes a U-shaped function with ACTH titer as abscissa and sexual responding as ordinate. Isolates, with asymptotic copulatory activity and relatively low pituitary ACTH activity, could be plotted on a peak of this graph. Group-housed mice, with depressed sexual activity and elevated ACTH levels, would appear at a descending point on the curve. Assuming that adrenalectomy potentiates ACTH levels equally in isolates and group-housed mice, isolates could now be distributed on the trough while grouped animals could appear on an ascending point on the curve. Administration of ACTH to adrenalectomized mice would place these animals on the ascending peak of the graph. According to this model, one would expect somewhat lower levels of sexual activity in adrenally-intact mice receiving ACTH since total ACTH titers would be lower than in adrenalectomized mice. Figure 8 and Table XII show a marked trend that is consistent with this prediction. A U-shaped function might also explain seemingly contradictory results in other species. For example, an intraventricular injection of ACTH facilitates sexual activity in the male rabbit (Bertolini et al., 1975) while an intravenous injection is inhibitory (Korányi et al., 1966). This might simply reflect quantitative differences in ACTH levels at appropriate neural sites.

Since the modulating effect of ACTH on murine sexual behavior is not necessarily mediated by adrenocortical activation, the pituitary hormone may be acting directly on the brain. In recent years there has been much

speculation as to the mechanism of action of ACTH on the brain. Evidence indicates that ACTH has profound effects on enzymes associated with the metabolism of cyclic AMP, RNA, and protein as well as on the neurotransmitters dopamine, norepinephrine, serotonin, and GABA (Dunn & Gispen, 1977). For example, catecholamine turnover can be significantly modified with either exogenous ACTH or treatments which change endogenous levels of ACTH (Dunn & Gispen, 1977). Other data indicate that neurotransmission processes are influenced also by isolation. Chronic isolation in mice has been shown both to reduce turnover of catecholamines and serotonin (Valzelli, 1974; Welch & Welch, 1969a; 1969b) and to produce relatively greater increases in turnover when animals are exposed to novel situations (Welch & Welch, 1968). Since dopaminergic and serotonergic systems appear to be involved in male sexual behavior (Gessa & Tagliamonte, 1975), they may provide a common mechanism for the effects of isolation and ACTH.

## SECTION V: PITUITARY-GONADAL MEDIATION

### Experiment 11

It has been established in previous studies that isolated mice show higher pituitary-gonadal activity than do group housed mice (Benton et al., 1978; Brain, 1971; Brain & Nowell, 1971; Christian, 1955, Vandenbergh, 1960). This is reflected especially in higher weights of testes, preputials, prostates, and seminal vesicles. Increases in pituitary-gonadal activity in isolates may occur consequent to decreases in pituitary-adrenal activity in these animals (Benton et al., 1978; Bullock & New, 1971; Desjardins & Ewing, 1971).

Because pituitary-gonadal activity is strongly involved in the control of male sexual behavior (Gorzalka & Mogenson, 1977; Quadagno et al., 1977), it is conceivable that differences in activity of this system mediate isolation/grouping differences in sexual performance. The present experiment was designed to test whether levels of the major male gonadal steroid, testosterone, might influence isolation/grouping effects. The design involved comparison of isolated and grouped mice that were intact and given oil placebo injections with others castrated and given either of two doses of testosterone. If isolated and grouped mice differ when intact but do not when their testosterone level is controlled, gonadal activity

may be involved in isolation/grouping effects.

## Method

Experimental animals consisted of 120 male CD-1 mice, received from Canadian Breeding Farms at 50-55 days of age. Animals were housed, maintained, and tested under the conditions described for Experiment 1A. Stimulus females were prepared as described for Experiment 1A.

At 55-60 days of age, 72 of the mice were orchidectomized under 2.5 mg. Nembutal (Sodium Pentobarbital, Abbott) anaesthesia. These were returned to groups of 6. One week following this surgery, both intact and operated mice were housed 1, 6, or 12 per cage. Cages were identical to those described for Experiment 1A. Commencing on the same day as differential housing and daily throughout the remainder of the experiment, subjects were given sc injections. Half of the operated animals received 25 µg testosterone propionate in .05 cc oil, while the other half received 500 µg testosterone propionate in .05 cc oil. All intact animals received the oil vehicle only. The low dose of testosterone was chosen as a dose just below the threshold level required to maintain full sexual behavior, while the high dose was well above this threshold (Champlin, Blight, & McGill, 1963). Two weeks after the introduction of differential housing and commencement of injections, males were tested for 1 hr in the presence of receptive females according to the procedures of the previous experiments. This testing was repeated one and two weeks following the first test. An animal's injections were omitted on each day that it was tested to avoid possible effects of recent injection stress on performance.

## Results

Figure 9 gives the results for the measure of duration of mounting. Table XIII gives all remaining measures. Intact, oil-treated iso-

Figure 9: The mean total duration of mounting, with or without intromission, in intact oil-treated and castrated testosterone-treated mice in Experiment 11. The open squares represent isolated mice, the open circles mice grouped in 6, and the closed circles mice grouped in 12. Vertical lines indicate standard errors.

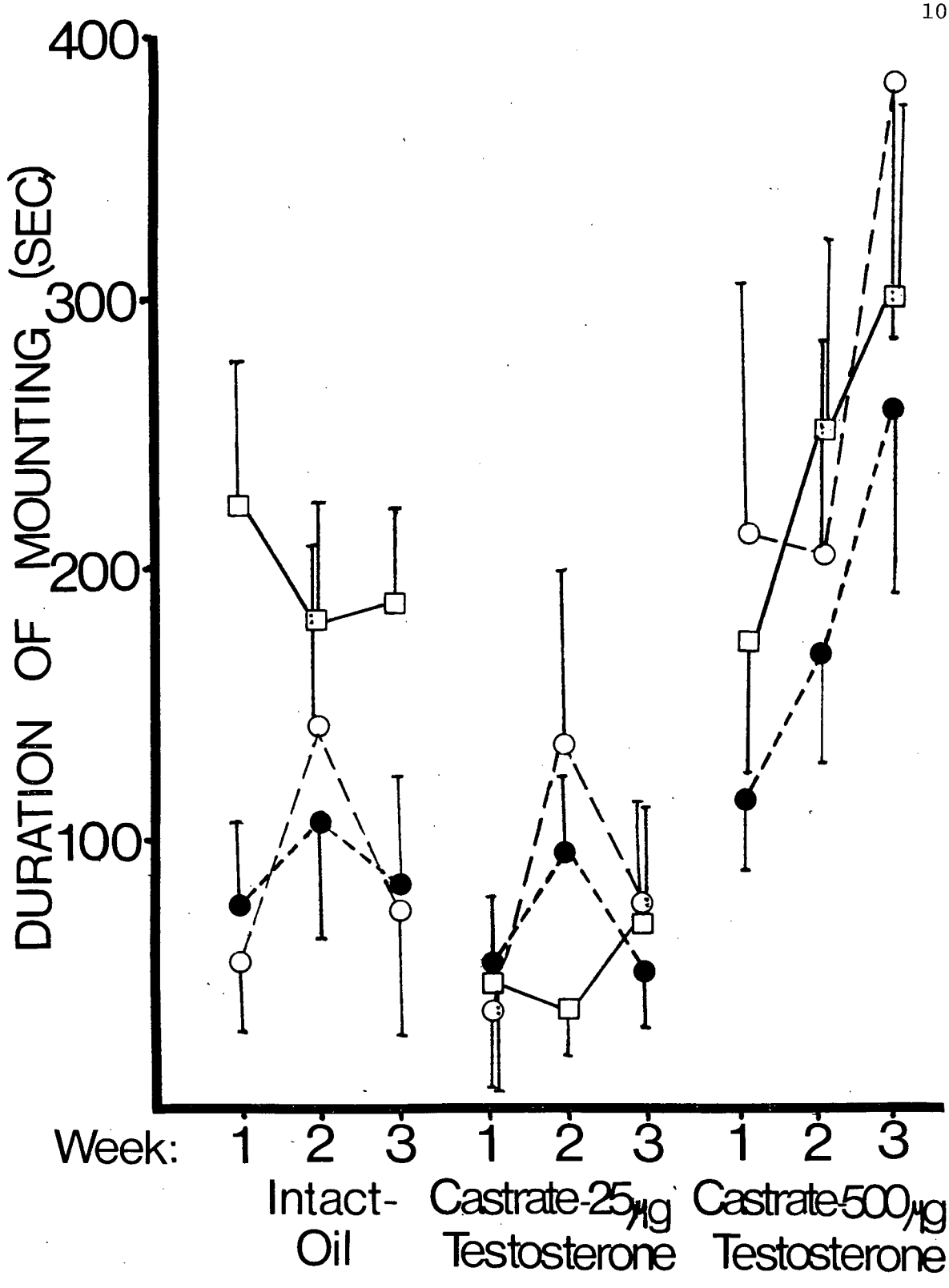

400
300
200
100
DURATION OF MOUNTING (SEC)
Week: 1 2 3 1 2 3 1 2 3
Intact-Oil
Castrate-25 µg Testosterone
Castrate-500 µg Testosterone

Table XIII

Means and Standard Errors of Sexual Performance of Intact or Castrated Male Mice Treated With Oil or Testosterone Propionate in Experiment 11

| | | Intact-Oil | | | Castrate-25mg Testosterone | | | Castrate-500mg Testosterone | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | n per cage: | 1 | 6 | 12 | 1 | 6 | 12 | 1 | 6 | 12 |
| Measure | Week | | | | | | | | | |
| Mounts | 1 | 25.36±5.05 | 7.42±3.65 | 9.67±4.55 | 5.27±4.33 | 2.67±1.99 | 4.75±2.24 | 14.17± 5.27 | 16.08± 6.42 | 11.70±3.53 |
| | 2 | 16.24±3.61 | 13.67±7.05 | 8.00±3.83 | 9.82±3.56 | 9.00±3.60 | 7.58±2.49 | 38.67±10.42 | 38.42±14.90 | 11.70±2.62 |
| | 3 | 24.80±4.30 | 6.50±3.25 | 8.00±3.34 | 14.27±8.58 | 15.17±3.11 | 9.33±4.12 | 46.58±10.32 | 35.50± 9.06 | 19.70±4.67 |
| Intromissions | 1 | 8.96±2.09 | 2.08±1.14 | 4.17±1.85 | 1.73±1.73 | 1.25±1.01 | 3.08±1.26 | 8.50± 2.37 | 10.75±4.67 | 7.50±1.72 |
| | 2 | 9.36±2.54 | 5.67±4.22 | 7.00±2.98 | 1.27±1.01 | 4.58±2.69 | 6.17±1.97 | 12.00± 3.35 | 13.33±5.14 | 13.90±3.12 |
| | 3 | 9.16±1.72 | 2.33±0.92 | 6.00±2.64 | 3.73±2.39 | 2.33±1.14 | 2.67±0.98 | 13.17± 3.35 | 18.50±5.06 | 20.70±5.28 |
| Ejaculations | 1 | 0.08±0.06 | 0.08±0.08 | 0.17±0.11 | 0.00±0.00 | 0.08±0.08 | 0.00±0.00 | 0.00± 0.00 | 0.17±0.11 | 0.20±0.13 |
| | 2 | 0.32±0.10 | 0.08±0.08 | 0.25±0.13 | 0.00±0.00 | 0.17±0.11 | 0.17±0.11 | 0.00± 0.00 | 0.00±0.00 | 0.10±0.10 |
| | 3 | 0.16±0.07 | 0.33±0.14 | 0.17±0.11 | 0.09±0.09 | 0.08±0.08 | 0.08±0.11 | 0.00± 0.00 | 0.25±0.18 | 0.40±0.22 |
| Mount Latency (sec) | 1 | 1149±198 | 2690±315 | 2139±442 | 2773±431 | 3098±339 | 2324±394 | 1932±385 | 1901±448 | 1371±268 |
| | 2 | 1547±265 | 2308±407 | 2383±451 | 1909±396 | 2373±379 | 1818±309 | 1711±400 | 1469±400 | 1228±378 |
| | 3 | 1232±242 | 2387±423 | 1771±473 | 2726±522 | 1376±325 | 1663±361 | 1031±361 | 996±362 | 452±200 |
| Intromission Latency (sec) | 1 | 2045±261 | 3049±268 | 2501±428 | 3311±289 | 3142±310 | 2658±358 | 2375±344 | 2470±417 | 1897±336 |
| | 2 | 2159±283 | 3025±304 | 2405±441 | 3406±130 | 2766±374 | 2484±336 | 2043±399 | 2439±429 | 1762±396 |
| | 3 | 1779±278 | 2960±258 | 2472±436 | 2949±275 | 2899±343 | 2680±368 | 1825±402 | 1742±427 | 550±201 |
| Ejaculation Latency (sec) | 1 | 3517± 66 | 3518± 82 | 3565± 26 | 3600± 0 | 3509± 91 | 3600± 0 | 3600± 0 | 3399±165 | 3520± 59 |
| | 2 | 3251±142 | 3496±104 | 3465± 89 | 3600± 0 | 3524± 74 | 3500±100 | 3600± 0 | 3600± 0 | 3409±200 |
| | 3 | 3425± 83 | 3177±229 | 3448±152 | 3555± 45 | 3548± 52 | 3292±210 | 3600± 0 | 3335±187 | 2898±413 |
| n per condition: | | 25 | 12 | 12 | 11 | 12 | 12 | 12 | 12 | 10 |

lates showed about twice as much sexual activity as did intact, oil-treated males grouped in 6 or 12. There were no evident differences between gonadectomized isolated and grouped males treated with either dose of testosterone. Animals from all housing conditions showed low levels of activity in the 25 µg testosterone propionate condition. Animals from all housing conditions showed high levels of activity in the 500 µg testosterone propionate condition; this performance was comparable to that of the isolated, intact, oil treated mice.

A 3 (no. of animals per cage) x 3 (surgery and dose) x 3 (weekly test) analysis of variance was conducted for each measure, treating the last factor as within subjects. Despite an apparent interaction between the number of animals per cage and surgery dose treatments in many of the measures, this factor reached significance only for the measure of mount latency ($F = 2.66$, $df = 4/108$, $p = .037$). In the other measures the values for this interaction were as follows: duration of mounting ($F = 1.02$, $df = 4/109$, $p = .400$), mounts ($F = 1.18$, $df = 4/109$, $p = .325$), intromissions ($F = 1.09$, $df = 4/109$, $p = .367$), ejaculations ($F = .75$, $df = 4/109$, $p = .563$), intromission latency ($F = 1.89$, $df = 4/109$, $p = .117$), and ejaculation latency ($F = 1.43$, $df = 4/109$, $p = .228$). The surgery and dose factor was significant for duration of mounting ($F = 8.69$, $df = 2/109$, $p < .001$), mounts ($F = 9.01$, $df = 2/109$, $p < .001$), intromissions ($F = 12.12$, $df = 2/109$, $p < .001$), mount latency ($F = 6.43$, $df = 2/109$, $p = .002$), and intromission latency ($F = 7.61$, $df = 2/109$, $p < .001$). In all cases Newman Keuls tests ($p < .05$) indicated a significant difference between the high and low dose of testosterone conditions. There was a significant main effect of number of animals per cage in mounts ($F = 4.19$, $df = 2/109$, $p = .018$);

Newman Keuls tests ($p < .05$) identified a difference here between isolated males and those grouped in 12. There was also a significant effect of weekly repeated test for most measures: duration of mounting ($F = 6.70$, $df = 2/218$, $p = .002$), mounts ($F = 10.38$, $df = 2/218$, $p < .001$), intromissions ($F = 9.13$, $df = 2/218$, $p < .001$), mount latency ($F = 12.74$, $df = 2/218$, $p < .001$), intromission latency ($F = 8.18$, $df = 2/218$, $p < .001$), and ejaculation latency ($F = 5.58$, $df = 2/218$, $p = .004$).

The simple main effects of number of animals per cage were also examined in separate analyses of variance for each surgery and dose level. In the oil-treated intact males there was a significant effect of number of animals per cage in mounts ($F = 4.32$, $df = 2/46$, $p = .019$) and mount latency ($F = 4.67$, $df = 2/46$, $p = .014$); Newman Keuls tests ($p < .05$) indicated that these differences were between the isolated animals on the one hand and the animals grouped in 6 and 12 on the other. These same effects approached significance in the duration of mounting ($F = 2.38$, $df = 2/46$, $p = .104$) and intromission latency ($F = 3.03$, $df = 2/46$, $p = .058$) measures.

## Discussion

There is an apparent trend in these data indicating that controlling the level of testosterone eliminates isolation/grouping differences. This is suggested particularly by the high level of performance of group-housed castrated males given the high dose of testosterone. It is furthermore suggested by the presence of major isolation/grouping differences only in intact, oil treated animals. However, this trend receives strong statistical support only in the mount latency measure, where a significant interaction obtained between the number of animals per cage and the surgery/dose factors. The existence of the apparent trend is not strongly supported in

the statistical analyses of all of the other measures. The absence of a significant interaction in these measures does not necessarily demonstrate a lack of involvement of testosterone in the isolation/grouping effects usually found with these measures. Rather, there may have been insufficient statistical power to make the apparent trend reach significance in all measures. There is a large amount of variance in the scores within each treatment combination, which weakens statistical power despite fairly large differences among the means. Thus, further research is required to establish firmly whether testosterone level differences relate to isolation/grouping differences, but these data suggest such a relationship.

The finding that exogenous testosterone maintains high levels of sexual activity in castrated males is consistent with the findings of numerous other studies (see Gorzalka & Mogenson, 1977). However, the indications that testosterone might increase the performance of animals that are sexually sluggish, in this case the grouped males, are novel. In other studies, testosterone treatment has not appreciably raised the performance of intact males. In one experiment, Grunt and Young (1953) divided male guinea pigs into "high", "medium", and "low" sexual behavior groups on the basis of initial tests. Animals from all of the groups were then castrated, eliminating the group differences and greatly reducing sexual behavior. However, when animals were all administered equal amounts of testosterone, the group differences returned. Studies of male sexual behavior in rats (Larsson, 1966; Whalen, Beach, & Kuehn, 1961) have similarly found that preoperative differences between "copulators" and "non-copulators" are maintained after castration and administration of exogenous testosterone. These other studies suggest that differences in plasma testosterone levels do not contribute to inter-individual variance in sexual

activity in these species.

Batty (1978a; 1978b) has recently examined endogenous plasma testosterone levels in house mice displaying different amounts of sexual behavior. She actually obtained a negative correlation between measures of sexual activity and plasma testosterone in a comparison of different genetic strains of the species. Within each of the strains there was a wide variation in plasma testosterone among individuals, and these levels were not related to any measures of sexual behavior. However, when she examined acute changes in plasma testosterone in a sexual context, there was a positive relationship to behavior. Plasma testosterone levels were higher in copulators than non-copulators when blood samples were removed immediately after a test. Blood samples removed at particular stages of sexual behavior suggested that testosterone levels were greatest at the initiation of mounting responses and declined during copulation. These findings are consistent with other findings in the literature (e.g. Macrides, Bartke, & Dalterio, 1975; Purvis & Haynes, 1974) that exposure to sexual stimuli produces an acute increase in testosterone. This acute increase is correlated with the onset of sexual activity, but does not prove a causal relationship between testosterone increases and commencement of sexual behavior. It is also important to note that changes in testosterone are the product of a sequence of neuroendocrine events, and that any of the preceding links may be involved in the control of sexual behavior. Thus a correlation of sexual activity with testosterone implies a correlation with pituitary gonadotropins, such as luteinizing hormone, and hypothalamic releasing factors, such as luteinizing hormone releasing hormone.

The evidence from other studies, then, suggests that sustained differences in testosterone titer do not produce differences in sexual performance. However, a major reduction in testosterone level and other pituitary-gonadal activity in adulthood, for example through castration or treatment with antiandrogens, does reduce or eliminate male sexual activity (Gorzalka & Mogenson, 1977). It might be concluded, then, that variation in testosterone above a certain minimal threshold level does not affect male sexual performance.

Nevertheless, testosterone levels may be involved in isolation/grouping differences in sexual activity. A lack of correlation between testosterone levels and natural variation in sexual activity among animals housed in uniform environments would not necessarily exclude an involvement of testosterone in environmentally-induced sexual deficits. Variability among animals housed in uniform environments (as in Batty, 1978a; 1978b; Grunt & Young, 1953; Larsson, 1966; Whalen et al., 1961) may relate to such factors as genetic differences and be independent of pituitary-gonadal activity. Environmental variability, however, might induce variability in gonadal activity and consequently in sexual activity. Stressful environments may suppress gonadal activity (Rose, 1969; Rose, Bourne, Poe, Mougey, Collins, & Mason, 1969) as may group housing in mice (Benton et al., 1978; Brain, 1971; Brain & Nowell, 1971; Christian, 1955; Vandenbergh, 1960). If gonadal activity is suppressed to below the threshold level necessary to maintain normal sexual activity, grouping may reduce sexual performance via effects on the pituitary-gonadal system.

If treatment with exogenous testosterone entirely eliminates deficits in sexual activity induced by group-housing, this does not necessarily implicate testosterone level differences as the exclusive mediators

of isolation/grouping sexual activity differences. It remains possible that high doses of exogenous testosterone alter the activity of other hormonal and neurochemical systems that more directly mediate these effects, For example, Kitay (1968) has found that exogenous testosterone may alter pituitary-adrenal activity. This consideration notwithstanding, such results would suggest that testosterone level is at least one of several variables influencing isolation/grouping effects.

# OVERALL DISCUSSION AND CONCLUSIONS

## The Nature of Isolation Effects on Sexual Behavior

The present research has demonstrated that postpubertally isolated male mice show higher levels of sexual activity than do conspecific males housed in groups. This facilitation of sexual activity by isolation occurs when animals are sexually naive or experienced and is not substantially diminished by repeated weekly measurement of animals. It occurs equally after brief (24 hr) and long (4 week) periods of social isolation. Performance of grouped and isolated mice reverses when their housing conditions are reversed. The phenomenon relates specifically to isolation versus group-housing rather than to differences in volume of space per animal. There is some indication that performance of males housed in larger groups is poorer than that of males in smaller groups (Experiments 2, 3, & 6), but this effect did not always occur (see Experiment 1A) and in no case reached statistical significance.

The effect was demonstrated in all inbred genetic strains of mice that were measured, including CD-1 (Experiments 1, 2, 3, 6, 7, 8, 9, 10, & 11) and C57/Bl, DBA2, and Swiss-Webster (Experiment 4) mice. By contrast, the effect was absent in male rats, hamsters, and gerbils. Indeed, in rats and gerbils an opposite effect occurred; males of these species showed more

sexual activity when group-housed. This species difference may relate to differences in social dynamics and physiological response to social isolation (see Section II, General Discussion). Decrements in sexual activity in rats induced by postpubertal social isolation may parallel decrements in this species induced by prepubertal isolation (cf. Folman & Drori, 1965; Gerall et al., 1967; Gruendel & Arnold, 1974).

Facilitation of sexual activity by social isolation in mice was usually reflected in several measures, including the duration of mounting, number of mounts, number of intromissions, latency to mount, and latency to intromit. The results for number of ejaculations and latency to ejaculate are not clear. In several cases where intact isolated and grouped mice were compared for number of ejaculations, there was a trend toward more such responses in the isolates (Experiment 2 at 2 and 4 weeks, Experiments 4, 6, & 9A). However, in none of these cases did such a trend reach statistical significance. In some of the other experiments (3, 7, & 11) there was no apparent trend, while in Experiment 9B there was a trend toward more ejaculations in the grouped males. Comparisons on measures of ejaculation generally did not reach statistical significance because of the infrequency of such responses and the large number of animals in all treatment conditions that failed to ejaculate. This occurred despite several repeated measures of performance taken in some of the experiments (9 & 11) and large numbers of grouped and isolated animals compared in others (2, 3, 4, 6, & 7). In other experimentation in our laboratory, it has been found that even when 3-hour tests of behavior are employed, the number of ejaculations remains small. Thus, ejaculation measures may not have shown significant trends in the same direction as other measures because of a lack of statistical power.

The situation with ejaculations may be complicated further by other factors. For example, Experiment 8 indicates that intermale mounting may correlate with subsequent ejaculation frequency with stimulus females. It is conceivable that intermale mounting may reduce the latency to ejaculate for some grouped animals but not affect other measures. The threshold to ejaculate could be lowered in these few animals, with mounting of males acting in the same manner as mounting of females as a precursor of an ejaculation in the copulatory sequence. Furthermore, Gorzalka et al., (1975) have noted that treatments affecting ejaculations do not necessarily influence mounts and intromissions, suggesting differential physiological control of these response classes. It remains possible that ejaculations do not differ in the comparisons of the present study because the variables mediating different response classes are differentially affected by isolation and grouping.

In most of the experiments, socially isolated mice showed an average of two or three times as many mounts and intromissions as did group-housed mice. Nevertheless, there was a large amount of variance in performance within each condition. A few isolates showed little or no sexual activity, while some grouped males showed very high levels of such activity. Despite the reliability and magnitude of the isolation/grouping differences, this variance reduced statistical power. Thus it is in the experiments where factorial designs pooling large numbers of subjects (e.g. Experiments 2, 3, 4, & 6) and experiments employing repeated measures (e.g. Experiments 1 & 9) that statistical significance was greatest. This variance also substantially reduced the ability of factorial designs to demonstrate significant interactions. For example, the means in many measures of Experiments 3 and 11 would appear to indicate the presence of interactions, yet these did

not reach statistical significance. Unfortunately, this variance imposes limitations on the types of experimental questions that can be asked about this phenomenon, in that statistical power for some designs may not be adequate without the use of very large numbers of subjects.

The measuring system for the sexual behavior of mice employed here differs somewhat from that employed elsewhere. In some other studies (e.g. McGill, 1965; Mosig & Dewsbury, 1976) multiple criteria for commencement and termination of behavioral observation have been employed. For example, an animal may be excluded from study if it has not mounted within 20 minutes of commencement of observation. Also, measurement of animals is often terminated in these other studies differently dependent on the particular animal's performance; for example, when an animal ejaculates he may no longer be tested, while non-ejaculating animals may be measured for a fixed period of time. In the present study, all animals were tested, regardless of their performance, in fixed 1-hr sessions. The present procedure has the advantages of treating all animals in an equivalent manner and allowing the generalization of findings to the entire population from which animals are sampled. Moreover, the present results might not be obtainable using a procedure that eliminates low scoring animals from testing. Indeed, such a procedure would probably eliminate more grouped than isolated animals from testing and thus tend to equate the two conditions. Part of the purpose of the present study was to identify causes of variability in sexual performance; the elimination of poorly performing animals would defeat this purpose.

## The Mediation of Isolation Effects

There are some indications in the present data of possible mediators of isolation/grouping differences in sexual performance. These indi-

cations, taken with data from experimentation performed in other laboratories, present a fairly consistent picture but clearly suggest a need for future research. The mediation of this phenomenon appears to occur on two levels. First, differences in behavioral experience between grouped and isolated mice may produce differences in physiology and reactivity to novel stimuli. Second, physiological differences and differences in reactivity may produce different levels of sexual activity.

Grouped male mice are exposed to a variety of stimuli that are absent from the environments of socially isolated mice. Grouped mice must compete for space and other resources; this competition is often evident in high levels of intermale aggression. Grouped mice will also occasionally engage in intermale mounting and thus may have different sexual experience than isolates. Furthermore, grouped mice are constantly exposed to a variety of tactile, visual, auditory, and olfactory stimuli related to the presence of male conspecifics. Isolated mice, by contrast, lead rather sedate lives.

Experiment 8 indicates that one form of social interaction among male mice, intermale mounting, is probably not involved in isolation/grouping differences in mount and intromission frequency and latency. Intermale mounting did not occur with sufficient frequency to account for the relative deficits in sexual activity in grouped males that can develop within 24 hours of differential housing. However, as mentioned above, intermale mounting may affect ejaculation latency and frequency.

The studies of Section III also suggest that odors of males may act as pheromones, affecting the behavior of other males. It is conceivable that such odors act to suppress male sexual activity in grouped males. Accordingly, isolates may show higher performance levels because they are

not exposed to such odors. Similarly, the cage-cleaning manipulation of Experiment 7 may have increased the sexual activity of grouped males because it removed these odors. One finding that is inconsistent with this notion is that isolates did not show facilitated performance after intervals as short as those necessary for the cage-cleaning phenomenon in grouped males.

Perhaps the strongest argument can be made for an involvement of intermale aggression. In Experiment 8, aggression in grouped males occurred with a sufficiently high frequency to account for relative sexual deficits developing in these animals within 24 hours of differential housing. Further support comes from the study by Kahn (1961), wherein mice subjected to defeat in aggressive encounters showed poor sexual performance. This reinforces the notion that intermale aggression mediates isolation/grouping differences in sexual performance because many grouped males must be regularly subjected to defeat. Data from several other studies suggest how intermale fighting among grouped males could produce physiological conditions conducive to poor sexual performance. Intermale aggressive encounters can produce fairly rapid pituitary-adrenocortical activation (Brain & Nowell, 1970; Bronson & Eleftheriou, 1965), changes in hypothalamic levels of luteinizing hormone (Eleftheriou & Church, 1967; 1968), and changes in utilization rates of norepinephrine, dopamine, and serotonin (Garattini et al., 1969; Welch & Welch, 1969a; 1969b). These physiological changes occur sufficiently soon after aggression, and may be reversed sufficiently rapidly after social isolation, to account for the development of isolation/grouping differences within 24 hours of differential housing. The possible involvement of these physiological factors in the control of sexual performance will be discussed below.

Experiments 9A and 11 indicate that both adrenal and testicular hormones may be involved in isolation/grouping differences in sexual activity. Unfortunately, these experiments do not specify the nature of such involvement. Several possibilities remain open, particularly in view of the fact that various hormonal and neurochemical systems interact in complex manners. Also, effects of the manipulations of these experiments, glandular extractions and administration of exogenous hormones, may be diffuse and indirect. Accordingly, effects of the techniques may not always implicate the affected hormonal system in the natural phenomena under investigation. For example, it is difficult to rule out entirely the possibility that observed effects of adrenalectomy (see Experiment 9) are due to some non-specific effect of this manipulation.

However, the results of Experiment 9A, indicating that adrenalectomy reverses isolation/grouping differences, do suggest an involvement of the pituitary-adrenal system. Mediation of isolation/grouping sexual activity differences by this system is also plausible because prolonged isolation alters adrenal weight (Benton et al., 1978; Brain & Nowell, 1971; Bronson, 1967), intermale aggression alters pituitary-adrenal activity (Brain & Nowell, 1970; Bronson & Eleftheriou, 1965), and this system is involved in isolation-induced aggression (Leshner et al., 1973). Experiment 9B suggests that differences in sustained levels of corticosterone, the principal adrenal steroid in this species, are not responsible for the effects of adrenalectomy. This does not eliminate the possibility that acute changes in corticosterone levels during sexual activity might be involved, since these would remain absent in adrenalectomized animals given chronic corticosterone. Other adrenal steroids might also be involved.

Experiment 10 suggests that ACTH does not mediate isolation/grouping differences in sexual activity, although such an interpretation of this experiment must be qualified. ACTH preparations tend to vary in biological effectiveness (Brain & Poole, 1974), and the particular preparation employed in Experiment 10 may not have been adequate to test the hypothesis under study. Moreover, there is some question as to whether peripherally-introduced ACTH reaches all of the target tissues of this hormone (see Dunn & Gispen, 1977); thus the ACTH administered in Experiment 10 may not have mimicked all of the effects of endogenous ACTH.

There is not much other evidence in the literature of direct effects of pituitary-adrenal manipulations on sexual activity (see Section IV, General Discussion). For example, other studies (Bloch & Davidson, 1968; Gorzalka et al., 1975) report no effect of adrenalectomy on male sexual behavior. Such effects may only be present when animals are housed in certain limited environmental conditions and show particular levels of pituitary-adrenal activation. It may thus be necessary to measure animals housed in a variety of environments, as in Experiment 9, to observe effects of adrenal manipulations on sexual behavior.

One pituitary-adrenal effect that is relatively well documented is that ACTH cannulated into the ventricular system of the brain may stimulate male sexual activity (Bertolini et al., 1975). It is conceivable that this effect could help to explain isolation/grouping differences in sexual activity. Although there are indications that isolates show lower baseline pituitary-adrenal activity than do grouped males, as evidenced especially by their lower adrenal weights (Benton et al., 1978; Brain & Nowell, 1971), they may show a greater pituitary-adrenal response to novel and stressful stimulation (see Brain, 1975; Goldsmith et al., 1976).

Presentation of stimulus females would probably constitute a novel situation and may accordingly stimulate greater release of endogenous ACTH in isolates, producing higher level of sexual activity in these animals.

One other strong possibility is that the effects of adrenalectomy on differential sexual performance are indirect. Levels of pituitary-adrenal activity affect both the pituitary-gonadal (Bullock & New, 1971; Desjardins & Ewing, 1971) and central catecholaminergic (Dunn & Gispen, 1977) systems; these systems are known to have fairly direct influences on male sexual activity (Gessa & Tagliamonte, 1975; Gorzalka & Mogenson, 1977).

The results of Experiment 11 suggest, but do not demonstrate, an involvement of testosterone levels in isolation/grouping sexual activity differences. Other support for the notion that testosterone may mediate these differences comes from experiments indicating that testicular activity is greater in isolated than in grouped mice (Benton et al., 1978; Brain, 1971; Christian, 1955; Vandenbergh, 1960). However, the mechanism of such possible mediation is unclear. There is little evidence that testosterone levels, above a certain threshold level, are correlated with differences in sexual activity (cf. Batty, 1978a; 1978b; Gorzalka & Mogenson, 1977; Grunt & Young, 1953; Larsson, 1966; Whalen et al., 1961). Levels of testosterone would probably need to be exceedingly low in some group members to account for the sexual activity deficits accompanying group-housing.

Further physiological factors that were not directly explored here and might mediate isolation/grouping differences involve central neurochemicals. The activity of the catecholamines, norepinephrine and dopamine, and the indoleamine, serotonin, is modified by social isolation (Garattini et al., 1969; Welch & Welch, 1969a; 1969b). The catecholamines, particularly, show higher utilization rates in isolates than grouped animals

in novel and demanding contexts. Increased utilization of dopamine may increase sexual behavior in males (Gessa & Tagliamonte, 1975; Malmnäs, 1976). Furthermore, the time frame for changes in neurochemical utilization (Garattini et al., 1969; Giacalone et al., 1968) may be adequate to account for the time frame of isolation effects on male sexual performance (Experiments 6 & 7). In addition, isolation/grouping neurochemical utilization differences may result from intermale aggression (Garattini et al., 1969; Welch & Welch, 1969a; 1969b) and may interrelate with pituitary-adrenocortical events, particularly ACTH levels (Dunn & Gispen, 1977).

In any event, it seems likely that isolation/grouping differences in sexual activity result from an interaction of several factors rather than any one particular factor. A number of interrelating physiological and behavioral variables are affected by social isolation and grouping. Many of these variables may independently modulate sexual activity, while the total isolation/grouping effect may result from their additive or synergistic effects.

General Implications

Isolation-induced facilitation of male sexual behavior in mice is an intrinsically interesting phenomenon for several reasons. Isolated and group-housed male mice provide two different preparations for the study of the effects of a variety of manipulations of behavior and physiology. These different preparations may facilitate examination of interactions between sustained environmental conditions and effects of other manipulations. This is evident, for example, in Experiment 9A, where the effect of adrenalectomy on sexual activity was dependent on the animals' environment. The phenomenon is also important in that it indicates the need for careful con-

trol of environmental and social variables in future study of murine sexual behavior. It furthermore provides several indications about the social and physiological determinants of sexual activity and the way in which sexual motivation interrelates with other motivational variables. The isolation/grouping manipulation is a convenient and easily effected manipulation that may thus facilitate the study of sexual behavior.

There are at least two larger contexts that may help to explain the apparent relationships among environmental events, physiology, and subsequent sexual activity levels. These involve the concept of "stress" and the related concept of "arousal". The study of isolation/grouping differences in sexual activity may in turn elucidate these larger issues.

There have been a number of suggestions that stress antagonizes sexual performance. Stress is generally defined as involving activation of the pituitary-adrenal system in response to environmental demands (Levine, 1975; Selye 1956). Selye (1961) briefly discussed some human clinical indications that impotence and sexual disinterest in males might relate to stress. Christian (1971) reviewed a large amount of mammalian literature indicating that reproductive functioning in general declines under stress; it might be inferred that one component of this declining reproductive functioning involves sexual behavior. Gray (1971) explicitly hypothesized an antagonism between stress and mammalian sexual behavior. There have, however, been few previous experimental demonstrations of a stress-sex antagonism (but see also Anderson, 1938a; 1938b; Hediger, 1965; Ward, 1972); the present study may constitute such a demonstration. Since grouped males show physiological patterns characteristic of stress (see Brain, 1975), including the pituitary-adrenal, pituitary-gonadal, and

neurochemical conditions discussed above, their sexual performance deficits might be construed as being stress-induced.

The concept of arousal may also help to explain these data. Isolated mice presented with receptive females may be viewed as being simply more aroused in this situation because of its relative novelty. A grouped mouse is constantly in the presence of active conspecifics; to an isolated mouse a presented female may be a relatively salient novel feature of the environment. Increased arousal might be mediated by both the pituitary-adrenal and neurochemical activation discussed above. Indeed, there is evidence that simple arousal increases male copulation; several reports (e.g. Barfield & Sachs, 1968; Caggiula & Eibergen, 1969; Sachs & Barfield, 1974) indicate that mild electric shocks delivered to the skin will evoke sexual activity in male rats. Increased general arousal in isolates in novel situations might help to explain not only the present phenomenon but also isolation-induced aggression (see Scott, 1966; Valzelli, 1969) and isolation-induced locomotor activity (see Brain et al., 1971; Essman, 1968).

The present results may also shed light on possible sex-aggression interactions. Many of the manipulations of the present study parallel those that have been carried out in investigating isolation-induced aggression. As evident in Section I and numerous other studies (see Brain, 1975; Scott, 1966; Valzelli, 1969), isolation increases aggression in subsequent intermale interactions as it does sexual behavior in subsequent male-female interactions. As indicated in Experiments 1 and 3, aggression and sexual activity respond similarly to isolation, both reverse when housing conditions are reversed, and are perhaps only moderately affected by area of space per animal. However, isolation-induced facilitation of male sexual behavior may

require briefer periods of isolation than does isolation-induced aggression (see Section III). Pituitary-adrenal activity is involved in both phenomena, but peripheral ACTH suppresses isolation-induced aggression but not isolation-induced sexual behavior (cf. Brain & Poole, 1974; Leshner et al., 1973; Section IV). The results of Experiment 11 suggest a possible role of testosterone levels in isolation induced sexual behavior; adequate testosterone levels are necessary for aggression in mice but may not mediate isolation/grouping differences in this behavior (see Brain, 1975; Brain & Poole, 1976; Leshner et al., 1973).

Another issue raised by findings here is that of the relationship of social dominance to intragroup variance in sexual performance. In the present study it was noted that there was frequently a large amount of inter-individual variability in the performance of grouped males. This variability is similar to that found among grouped males in several other behavioral and physiological variables, including aggressiveness, activity levels, adrenal functioning, and gonadal functioning (see Benton et al., 1978; Brain, 1975; Messeri et al., 1975). Brain (1975) has concluded that isolated males resemble more dominant grouped males according to several behavioral and physiological indices. This suggests that the more dominant males in a group might show higher levels of sexual activity than more subordinate males.

Indeed, DeFries and McClearn (1970; 1972) found that dominant males sired the vast majority of offspring when females were housed with several males. Dominance level in these experiments was indicated by the presence or absence of scarring on the hindquarters and flanks; animals with more scars were assumed to be subject to more attacks and classified as being more subordinate. These results may suggest that dominant males

are more sexually active, but are subject to other interpretations. It remains possible that they indicate an active prevention of mating of subordinates by dominants rather than intrinsic differences in activity among group members. The semen of dominants may also be more fertile than that of subordinates. Furthermore, differences in social position were confounded with differences in genetic strain in the studies of DeFries and McClearn. The question of the relationship of dominance level to sexual activity in this species thus remains open.

If differences in sexual performance among mice at different levels of social hierarchies do obtain, they might be of considerable importance in elucidating the natural sociobiology of this species. Knowledge of which social members are successful in passing their genes into future generations might improve understanding of the evolutionary selective pressures affecting the species. This may be particularly true if intrinsic changes in subordinate mice produce suppression of their sexual activity. Christian (1971) and Gray (1971) have suggested that the suppression of reproduction among socially stressed and subordinate animals might require group selection processes like those outlined by Wynne-Edwards (1962). This issue is controversial (cf. Wiens, 1971; Wilson, 1975; Wynne-Edwards, 1971); phenomena like those of the present study might be of value in addressing such controversy.

It would seem important that future work relate the findings of the present study to animals living in more natural conditions. Animals here were housed in contrived, albeit well-controlled, environments. These environments may not resemble the living conditions of natural populations of the species (cf. Crowcroft & Rowe, 1963; MacIntosh, 1970; Reimer &

Petras, 1967; Rowe & Redfern, 1969), although it could be argued that the strains employed here are adapted specifically to the laboratory environment. Studies in laboratory environments allow systematic and rigorous analysis of the effects of various parameters, but should be complemented by studies in more naturalistic environments. Future investigation might thus focus on mice in more naturally dispersed populations and natural social aggregations.

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