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Do Male Mice Prefer or Avoid Each Other’s Company? Influence of Hierarchy, Kinship, and Familiarity

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In the laboratory, individual housing of male mice who otherwise show aggression is common practice. Because mice are a social species, the question arises whether this procedure is right from the animals’ point of view. This study tested the preference of subordinate animals for their dominant cagemate, and vice versa, and the preference of subordinate animals for an unknown subordinate partner. Experiments that allowed male mice with different histories to choose either an inhabited or an empty cage have shown that the mice preferred the proximity of another male over individual housing. No differences in this respect were found between dominant and subordinate males, or between littermates and nonlittermates. The preference was most obvious when mice who were previously housed together were tested. The study concludes that separation and single housing for mice are not attractive solutions for overcoming aggression in group-housed male mice and that alternative approaches, such as improving the housing conditions, should be explored as a way of tempering intramale aggression.

In almost all laboratories, male mice are housed together after weaning in groups of 6 to 10. When used for an experiment, usually at 6 to 8 weeks of age, groups often will be regrouped with unfamiliar males. Group housing of male mice is not natural, as in the wild males form despotic territories, and no male from another deme will be tolerated inside those boundaries (Crowcroft, 1966; Mackintosh, 1970, 1973). When forced to live together in a confined space, however, male mice will form dominance relationships (Poole & Morgan, 1973, 1976).
In many cases, depending on strain and age, the hierarchy will be stable, and the animals will live together with relatively low social stress. In other cases, fighting may occur frequently (Bisazza, 1981). To a certain degree, fighting can be regarded as normal, but some groups show such high levels of aggression that housing animals individually is necessary to prevent further injury and stress (Haseman, Bourbina, & Eustis, 1994). Group housing of males actually is advised against for several strains known to be highly aggressive (Mouse Genome Database, 2001). Individual housing, on the other hand, frequently has been reported to be stressful for mice (Claassen, 1994). The effects of individual housing on behavior and physiology in rats and mice, referred to as “isolation stress” or “isolation syndrome,” had become apparent as early as the 1960s. Individually housed mice and rats become more aggressive, may show stereotyped behavior patterns, suffer from convulsions, and are nervous and difficult to handle. Physiologically, they may show reduced immunocompetence, higher tumor incidence, gastric ulcerations, hypersensitivity to toxic agents, and increased pathology such as “scaly tail” (Ader & Friedman, 1964; Baer, 1971; Barrett & Stockman, 1966; Brain, 1975; Chance & Mackintosh, 1962; Gärtner, 1968a, 1968b; Haseman et al., 1994; Hatch et al., 1965). Many of these effects are known stress responses (Manser, 1992).

For social species such as the mouse and rat, social contact may be a behavioral need. Preference testing has provided more insight into the behavioral needs of animals (Blom et al., 1992; Fraser, 1996). Mice of both sexes, for example, show a strong preference for nesting material (Van de Weerd, Van Loo, Van Zutphen, Krogh, & Baumann, 1997, 1998) and soiling site (Sherwin, 1996). Gärtner (1968a, 1968b) reported that rats choose to eat and sleep in close proximity with others, with maximal body contact, rather than alone.

To test whether male mice also prefer dwelling near other males to staying alone, we conducted a series of preference tests in which male mice could choose between an empty cage or a cage inhabited by another male but separated by a partition. The history and relationships of the males differed between experiments.

**MATERIALS AND METHODS**

**General**

Sixty-six male mice of the BALB/cAnNCrlBR strain were used. This strain generally is moderately aggressive toward cagemates with wounding to the tail and back of subordinates being common (Van Loo, Mol, Koolhaas, Van Zutphen, & Baumann, 2001). Extreme fighting causing severe injury or death, however, is rare; thus, the chance that experiments had to be terminated prematurely was minimized. All mice previously had been observed in behavioral studies; hence, groups of males and age were predefined at the time of testing. All groups were housed in wire-topped Makrolon Type II or III cages (375 cm² or 825 cm², re-
spectively, Tecniplast, Milan), provided with sawdust (Lignocel 3/4, Rettenmaier & Söhne, Ellwangen-Holzmühle, Germany) and Kleenex tissues (Kimberly-Clark Corporation®, Europe). Tap water and food pellets (RMH-B, Hope Farms, Woerden, The Netherlands) were provided ad libitum. The animal rooms had a controlled photoperiod (12:12 light to dark, white light on at 07.00 hr, approximately 200 lux at 1 m above the floor, and red light on at 19.00 hr, approximately 5 lux at 1 m above the floor), temperature (23–24 °C), relative humidity (60 ± 5%), and ventilation (18–20 air changes × h⁻¹).

Preference Testing

The preference test system (Figure 1) used in this study has been validated and described in detail by Blom et al. (1992). In short, a housing system was used consisting of two test cages connected to a clear Perspex central cage (15 cm × 15 cm × 18 cm) by nontransparent tubes (PVC, inner dimensions: 2.6 cm × 2.6 cm × 25 cm). The test cages were Makrolon Type II cages, divided by a wire mesh (Experiment 1) or a Perspex wall with holes (Experiments 2 and 3). Each test cage was provided with 50 g of sawdust, and each half of the cages was provided with food pellets and tap water in a bottle. The central cage had no food, water, or bedding. A total of six of these housing systems were used to allow simultaneous testing of six pairs of mice. To minimize any external influences on choice behavior, each system rotated slowly during testing. Photoelectric devices in the passage tubes automatically detected the movements of the mice between the test cages. The signals were sent to a computer that calculated dwell-

![Image](figure1.jpg)

**FIGURE 1** Preference test system. IC = inhabited cage with a mouse behind a partition; EC = empty cage; M = middle cage; T = tunnel; D = infrared detector; P = Perspex partition with holes.
ing times per cage (Gate-Watch software, Metris System Engineering, Wassenaar, The Netherlands).

Mice were introduced into the test system between 15.00 and 15.30 hr and their activity monitored over 48 hr. Food and water in each test cage were weighed before and after the experiment.

Statistical Analysis

Data on dwelling time on the final test day (24 hr) were analyzed by distinguishing three time frames: total dwelling time per cage, dwelling time during the light period (12 hr), and dwelling time during the dark period (12 hr). As data were not always distributed normally, dwelling times were compared using a Wilcoxon matched-pairs signed rank test. Differences between littermates and nonlittermates were tested using a Mann–Whitney U test. Levels of aggression and dwelling time were correlated by means of a Pearson’s test. Data on food and water intake were analyzed by means of a paired t test. All statistical tests were carried out using SPSS for Microsoft Windows, Release 9.0. Because only three animals were observed, descriptive statistics were used to analyze the behavioral data.

EXPERIMENT 1:
SOCIAL PREFERENCE OF SUBORDINATE MALES FOR THEIR DOMINANT CAGEMATE

Animals

Thirty-six males were housed in groups of three from weaning until 12 weeks of age when the subordinate animal in each group was removed for another behavioral study. The remaining 12 couples were left undisturbed for 3 weeks to enable dominance hierarchies to be re-established. Six couples consisted of littermates, and six couples consisted of nonlittermates. The animals were individually marked on the tail with a black waterproof marker. The mark was renewed weekly. At the time of preference testing, the mice weighed 26.1 ± 0.3 g.

Assessment of Dominance

One week before testing, all couples were separated for a period of 30 min and then placed together in a novel environment. The behavior of the animals was recorded on videotape for a period of 10 min. This procedure was repeated daily for 4 days. All 10-min video recordings were analyzed, and animals were categorized as dominant or subordinate depending on the number of initiated and won aggressive encounters (Table 1). One pair of littermates and one pair of
nonlittermates showed no aggressive interactions at all. Therefore, they were omitted from further statistical analyses. Subsequently, the subordinate animals were submitted to a preference test with the choice being between an empty cage or a cage with their dominant cagemate behind a partition.

EXPERIMENTS 2 AND 3: SOCIAL PREFERENCE OF DOMINANT MALES FOR THEIR SUBORDINATE CAGEMATE AND SOCIAL PREFERENCE OF SUBORDINATE MALES FOR AN UNFAMILIAR SUBORDINATE MALE

Animals

Seventy-eight male mice, 6 weeks of age, were housed in six groups of 5 and six groups of 8 animals. At 20 weeks, the dominant male and 2 subordinate males were removed for another behavioral study. The remaining mice were left for 15 weeks (now comprising groups of 2 and 5 animals, respectively) to enable dominance hierarchies to be re-established. At the time of preference testing, the mice weighed 28.3 ± 0.2 g.

### TABLE 1
Number of Initiated Aggressive Encounters in Four 10-Min Periods (Experiment 1) or During 30 Min After Cage Cleaning (Experiment 2) for Mice Classified as Dominant or Subordinate

<table>
<thead>
<tr>
<th>Group</th>
<th>Experiment 1</th>
<th></th>
<th>Experiment 2</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dominant</td>
<td>Subordinate</td>
<td>Dominant</td>
<td>Subordinate</td>
</tr>
<tr>
<td>1</td>
<td>18</td>
<td>2</td>
<td>42</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>15</td>
<td>0</td>
<td>37</td>
<td>2</td>
</tr>
<tr>
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<td>4</td>
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<td>20</td>
<td>12</td>
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<tr>
<td>5</td>
<td>9</td>
<td>0</td>
<td>18</td>
<td>1</td>
</tr>
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<td>6</td>
<td>7</td>
<td>4</td>
<td>15</td>
<td>7</td>
</tr>
<tr>
<td>7</td>
<td>6</td>
<td>0</td>
<td>15</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>6</td>
<td>0</td>
<td>12</td>
<td>8</td>
</tr>
<tr>
<td>9</td>
<td>5</td>
<td>0</td>
<td>11</td>
<td>5</td>
</tr>
<tr>
<td>10</td>
<td>2</td>
<td>0</td>
<td>10</td>
<td>7</td>
</tr>
<tr>
<td>11</td>
<td>0(^a)</td>
<td>0(^a)</td>
<td>8</td>
<td>3</td>
</tr>
<tr>
<td>12</td>
<td>0(^a)</td>
<td>0(^a)</td>
<td>6</td>
<td>2</td>
</tr>
</tbody>
</table>

\(^a\)Omitted from analyses.
Assessment of Dominance

Two weeks before preference testing, each group was recorded on videotape for 30 min after cage cleaning. Aggressive behavior between male mice is known to rise after cage cleaning (Van Loo, Kruitwagen, Van Zutphen, Koolhaas, & Baumans, 2000). Video recordings were analyzed, and animals were categorized as dominant or subordinate depending on the number of initiated and won aggressive encounters (Table 1). For groups in which the existing hierarchy could not be determined accurately, a second 30-min video recording after cage cleaning was analyzed 1 week later. Subsequently in the preference test, 12 dominant mice (1 in each group) were given the choice between the most frequently attacked subordinate cagemate and an empty cage (Experiment 2). The remaining 18 subordinate mice from groups of 5 mice (6 × 3 mice) were used for Experiment 3. In this preference test, 9 of these subordinate males were given a choice between an unfamiliar subordinate male (from another cage) and an empty cage.

Behavior

In Experiments 2 and 3, the behavior of two dominant mice and one subordinate mouse was scored during the final 24 hr of preference testing. Behavior was recorded with a time-lapse video recorder (Panasonic AG–6024), recording 24 hr on a 3-hr videotape. Tapes were analyzed by scan-sampling every 5 sec (45 sec real time). Next to the position of the mouse (empty, inhabited, or middle cage), the following behaviors were scored: eating and drinking, digging, grooming, social interaction, sleeping, climbing, rearing, and locomotion. If the mouse was not in view or his behavior difficult to determine, this also was noted.

RESULTS

Experiment 1: Choice of Subordinate Males for Their Dominant Cagemate

Littermates and nonlittermates did not differ significantly in their preference. Data of these groups thus could be combined to analyze overall preference. For the 24-hr analysis, the subordinate mice showed a clear preference for their dominant cagemate ($p < .01$). Figure 2 illustrates this preference, both for littermates and for nonlittermates. Data analysis of the night period was consistent with the overall analysis: A clear preference was shown for the inhabited cage ($p < .01$). Data analysis of the day period, however, revealed this preference only marginally because of a large spread in the data as one mouse (nonlittermate) chose to sleep in the empty cage ($p < .1$). No differences were found in food and water consumption between the two test cages (Table 2).
FIGURE 2  Experiment 1: Mean dwelling time in hours of subordinate male mice in each of the test cages for (a) littermates and (b) nonlittermates for the final day of the preference test (24 hr), a light period of 12 hr (day) and a dark period of 12 hr (night).

**p < .01. *p < .05. (*)p < .10.

TABLE 2
Food and Water Consumption of Mice in Experiments 1, 2, and 3 in Each of the Test Cages

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Mouse Type</th>
<th>Food Consumption (g ± SEM)</th>
<th>Water Consumption (ml ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Inhabited Cage</td>
<td>Empty Cage</td>
</tr>
<tr>
<td>1</td>
<td>Subordinate littermates</td>
<td>3.9 ± 0.9</td>
<td>3.9 ± 1.0</td>
</tr>
<tr>
<td>1</td>
<td>Subordinate nonlittermates</td>
<td>4.4 ± 0.4</td>
<td>3.7 ± 0.4</td>
</tr>
<tr>
<td>2</td>
<td>Familiar dominant</td>
<td>5.2 ± 0.5</td>
<td>5.1 ± 0.6</td>
</tr>
<tr>
<td>3</td>
<td>Unfamiliar subordinate</td>
<td>5.4 ± 0.7</td>
<td>6.7 ± 0.5</td>
</tr>
</tbody>
</table>

*<p < .05.
From the two nonaggressive couples, a subordinate mouse was chosen at random and tested in the preference test but omitted from further analyses. One mouse preferred his cagemate, and the other chose to sleep in the empty cage.

Experiment 2: Choice of Dominant Males for Their Subordinate Cagemate

Preference of dominant males showed many similarities with preference of subordinate males in the previous experiment (Figure 3a). Mice clearly preferred to be near their subordinate cagemate ($p < .01$), and data analysis of the night period was consistent with the overall analysis: A clear preference was shown for the inhabited cage ($p < .01$). Again, data analysis of the day period revealed this preference only marginally because of one mouse’s choosing differently ($p < .10$). No differences were found in food and water consumption between the two test cages (Table 2). Behavioral analysis of two dominant mice revealed that, in concordance with preference data, mice spent more time in the inhabited cage.

![Figure 3](image)

**FIGURE 3** Experiment 2: (a) Mean dwelling time in hours of dominant male mice in each of the test cages for the final day of the preference test (24 hr), a light period of 12 hr (day), and a dark period of 12 hr (night). (b) Mean time budget of two dominant mice separated for behavior in the empty and inhabited cage.

**$**p < .01. *p < .05. (*$p < .10**.
Differences were most obvious for sleeping, locomotion, digging, and grooming (Figure 3b). The amount of aggression before preference testing was not significantly correlated with dwelling time in the inhabited cage ($r = 0.053$, $ns$).

Experiment 3: Choice of Subordinate Males for an Unfamiliar Cagemate

Preference of subordinate males for an unfamiliar cagemate was less obvious than in the previous two experiments (Figure 4a). Although six of the nine mice tested showed a strong preference to be near the other male, three mice divided their time equally over both cages, with a slight preference for the empty cage. Consequently, overall preference tended to be toward the inhabited cage ($p = .05$) but was significant during the light period ($p < .05$). During the dark (active phase), no significant preference for either cage was present. Water consumption was significantly higher in the empty cage ($p < .05$). Food consumption was

![Graph showing time spent in each cage](image)

**FIGURE 4  Experiment 3: (a) Mean dwelling time in hours of unknown subordinate mice in each of the test cages for the final day of the preference test (24 hr), a light period of 12 hr (day), and a dark period of 12 hr (night). (b) Time budget of one of the unknown subordinate mice separated for behavior in the empty and inhabited cage.**

**$**p < .01. *p < .05. (*)p < .10.**
equal for both cages (Table 2). Behavioral analysis of one subordinate mouse confirmed that the mouse spent most of his time in the inhabited cage. Differences were most obvious for sleeping, digging, and grooming (Figure 4b).

**DISCUSSION**

In all three experiments, the male mice showed a clear preference for the inhabited cage. In Experiment 1, only 2 of the 12 subordinate mice made their nests in the empty cage, 1 of whom came from an almost nonaggressive pair (omitted from analyses), the other from a moderately to highly aggressive pair. All other mice made their nests in the cage near their dominant cagemate (Figure 2). Of 12 dominant mice in Experiment 2, only 1 made his nest in the empty cage (moderately aggressive), and 1 mouse seemed to have switched cages during testing (low aggressive). All other mice made their nests in the cage inhabited by their subordinate cagemate (Figure 3a). In Experiment 3, 1 of 9 subordinate mice chose to be alone, whereas 2 mice did not show a strong preference for either of the cages. Six mice clearly showed a preference for the unfamiliar subordinate mouse (Figure 4a). These results accord with results found in rats in that Gärtner (1968a, 1968b) reported that formerly group-housed rats, rather than eat and sleep alone, actively seek the company of other rats.

This experimental setup did not allow physical contact between the test mouse and the mouse behind the partition while preference was measured. The mice may have been aware of this, which may have influenced the choice of the test mice. The hierarchy between two male mice unable to be in bodily contact, however, does not cease to exist when close olfactory and visual contact is possible (Hurst, Fang, & Barnard, 1993; Parmigiani, Mainardi, Brain, Haug, & Brunoni, 1989).

In fact, Kudryavtseva (1991) used a similar setup, known as the sensory contact model, to investigate aggressive and submissive behavior in male mice. In spite of this, both the subordinate and the dominant mice, independent of levels of aggression scored before preference testing, chose to be in the vicinity of another mouse for the majority of time. This partly is in concordance with Kudryavtseva (1994), who found that mice who repeatedly had won an encounter with their partners (comparable with the dominants in this test) spent a lot of time approaching the partition separating them from their partners. Losers (subordinates) did this to a lesser degree, but whether losers would have avoided the partition by moving to another cage was not tested.

As preference is measured by dwelling time, the cage in which the animals make their nests and sleep, by definition, is the most preferred cage. Experiments 1 and 2, however, clearly showed that, during the active night period, the mice seek company for the majority of time (Figures 2 and 3a). In a similar experiment with female rabbits, Held, Turner, and Wootton (1995) gave low-ranking rabbits a
choice between a barren solitary pen or group pen and they showed a strong preference for the group pen. For dominant mice, this preference during the active period also may indicate a true preference for company. Another explanation may be that the dominant mouse prefers to stay in close proximity to his subordinate cagemate to control the other male and defend his own territory (Poole & Morgan, 1973). The hypothesis that dominant males prefer to be alone because they do not tolerate other males in their territory in the wild (Brain, 1975) is not supported by the results of this study. Animals in confined spaces may exhibit different social behavior from their wild counterparts. Poole (1992) suggested that several solitary species such as polecats and orangutans opt to socialize in captivity and sleep in close proximity in the nesting area. The same may be true for male mice.

It is important to note that the preference for company of brothers and weanlings from different litters (Experiment 1, Figure 2) is equally strong, whereas the preference of unfamiliar subordinate mice for each other (Experiment 3) is clearly less striking than when familiar mice were tested (Experiments 1 and 2). This indicates that familiarity, not kinship, is a main factor for company preference. Indeed, Bisazza (1981) found that unfamiliar mice were much less tolerant of each other and chose different nest boxes to sleep. In this study, however, the preference for company of familiar mice was most obvious during the dark period (Figures 2 and 3a), but for unfamiliar mice the preference for company was most obvious during the light period (Figure 4a). This might indicate that the unfamiliar mice prefer to sleep together while spending a considerable amount of time alone when active. Indeed, the largest differences in behavior of the videotaped subordinate mouse were found in sleeping and sleeping-related behaviors (digging and grooming; Figure 4b). These results do not agree with those of Kudryavtseva (1994), who found that mice separated by a partition spent more time near the partition when the familiar mouse behind a partition was replaced by an unfamiliar one.

The unfamiliar subordinate mice in this study had a preferred cage for water but not for food consumption. All other mice had no preferred cage for food and water consumption. This is in accord with the results of Blom, Van Tintelen, Van Vorstenbosch, Baumann, and Beynen (1996), who, in preference tests for bedding material, found that mice showed a clear preference for one of the test cages, whereas food and water intake was similar for four test cages. Many social mammals, including rodents, prefer to eat and drink together (Gärtner, 1968a, 1968b), a behavior known as social facilitation. On the other hand, dominant mice have been reported to defend resources and restrict the movements of subordinates (Poole & Morgan, 1973). These results support neither of these two possible scenarios.

The results described in this article favor the idea that male mice prefer each other’s company to individual housing, at least when precautions are taken so that the mice are unable to injure one another. Male mice of the BALB/c strain are moderately aggressive when housed in groups. When extrapolating results to other, more aggressive mouse strains, we should keep in mind that the mice used in this experi-
ment had been successfully group-housed for a relatively long time before testing and that no extreme injuries were observed. This may have biased the results in favor of social contact. Nevertheless, we may argue that other approaches, such as improvement of the housing conditions, should be explored to decrease the incidence of injury in group-housed male mice without depriving them of social contact. Research on this subject currently is being conducted in our laboratory.

REFERENCES


