Chapter 2

Do male mice prefer or avoid each other's company?

Influence of hierarchy, kinship and familiarity

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Influence of hierarchy, kinship and familiarity

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Abstract

In the laboratory, individual housing of male mice that 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 cage mate 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 cage or an empty cage have shown that the mice preferred the proximity of another male to individual housing. No differences in this respect were found between dominant and subordinate males, or between littermates and non-littermates. 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 intermale aggression.

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Introduction

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, mice 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 *et al.* 1994). Group housing of males is actually 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 1994a). The effects of individual housing on behaviour 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 behaviour patterns, suffer from convulsions, and are nervous and difficult to handle. Physiologically, they may show reduced immunocompetence, higher tumour incidence, gastric ulceration, hypersensitivity to toxic agents, and increased pathology such as 'scaly tail' (Chance & Mackintosh 1962, Ader & Friedman 1964, Hatch *et al.* 1965, Barrett & Stockman 1966, Gärtner 1968a, b, Baer 1971, Brain 1975, Haseman *et al.* 1994). Many of these effects are known stress-responses (Manser 1992).

For social species such as the mouse and rat, social contact may be a behavioural need. Preference testing has provided more insight into the behavioural 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 *et al.* 1997a, 1998b) and soiling site (Sherwin 1996c). Gärtner (1968a, b) 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.

Methods – general

Animals and husbandry

Sixty-six male mice of the BALB/cAnNCrlBR strain were used. This strain generally is moderately aggressive towards cage mates with wounding to the tail and back of subordinates being common (Van Loo *et al.* 2001b). Extreme fighting causing severe injury or death, however, is rare; thus the chance that experiments had to be terminated prematurely was minimised. All mice previously had been observed in behavioural 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 cm^2 or 825 cm^2, respectively, Tecniplast, Milan, Italy) 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 L:D, white light on at 07.00 h, approximately 200 lux at 1 m above the floor, and red light on at 19.00 h, 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}).

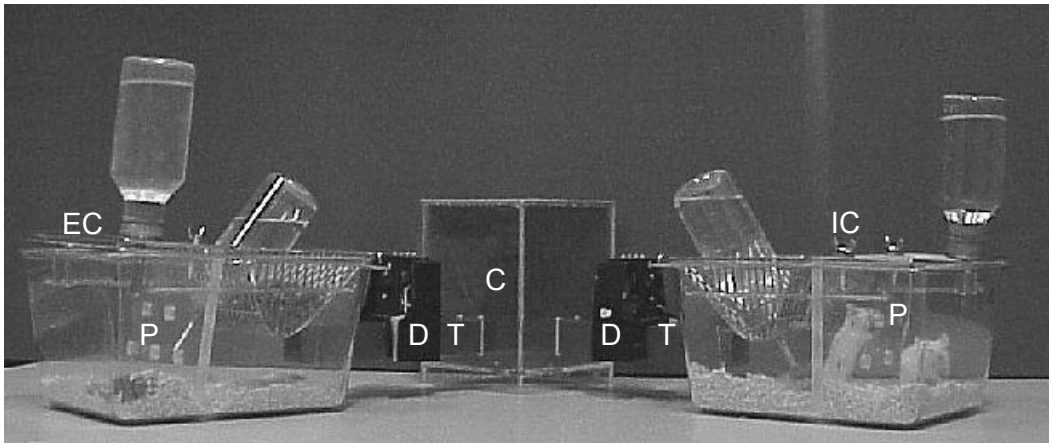

Figure 1 Preference test system. IC = inhabited cage with a mouse behind a partition; EC = empty cage; C = central cage; T = tunnel; D = infrared detector; P = perspex partition with holes.

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 (15x15x18 cm) by non-transparent tubes (PVC, inner dimensions: 2.6x2.6x25 cm). The test cages were Makrolon Type II cages, divided in two 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 minimise any external influences on choice behaviour, 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 dwelling times per cage (software: Gate-Watch, Metris System Engineering, Wassenaar, The Netherlands). Mice were introduced into the test system between 15.00h and 15.30h and their activity monitored for a period of 48 h. 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 h) were analysed 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 non-littermates 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 analysed 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 analyse the behavioural data.

Methods - experiment 1

Animals

Thirty-six males were housed in groups of three from weaning until twelve weeks of age when the subordinate animal in each group was removed for another behavioural study. The remaining twelve 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 non-littermates. 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 behaviour 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 analysed, and animals were categorised as dominant or subordinate depending on the number of initiated and won aggressive encounters (Table I). One pair of littermates and one pair of non-littermates 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 cage mate behind a partition.

Table I Number of initiated aggressive encounters in four 10-min periods (Exp. 1) or during 30 min after cage cleaning (Exp. 2) for mice classified as dominant or subordinate.

	Experiment 1		Experiment 2	
Group	Dominant	Subordinate	Dominant	Subordinate
1	18	2	42	2
2	15	0	37	2
3	11	0	22	5
4	9	4	20	12
5	9	0	18	1
6	7	4	15	7
7	6	0	15	0
8	6	0	12	8
9	5	0	11	5
10	2	0	10	7
11	0[a]	0[a]	8	3
12	0[a]	0[a]	6	2

[a]omitted from analyses

Methods - experiments 2 & 3

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 two subordinate males were removed for another behavioural 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.

Assessment of dominance

Two weeks before preference testing, each group was recorded on videotape for 30 min. after cage cleaning. Aggressive behaviour between male mice is known to rise after cage cleaning (Van Loo *et al.* 2000). Video recordings were analysed and animals were

categorised as dominant or subordinate depending on the number of initiated and won aggressive encounters (Table I). For groups in which the existing hierarchy could not be determined accurately, a second 30-min. video recording after cage cleaning was analysed 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 cage mate and an empty cage (Experiment 2). The remaining 18 subordinate mice from groups of 5 mice (6x3 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.

Behaviour

In Experiments 2 and 3, the behaviour of two dominant mice and one subordinate mouse was scored during the final 24 h of preference testing. Behaviour was recorded with a time lapse video recorder (Panasonic AG-6024), recording 24 h on a 3 h videotape. Tapes were analysed by scan-sampling every 5 sec (= 45 sec. real time) with the aid of the Observer (version 3.0 for Windows, Noldus Information Technology bv, Wageningen, The Netherlands). Next to the position of the mouse (empty, inhabited or central cage) the following behaviours were scored: eating and drinking (eat), digging (dig), grooming (gro), social interaction (sin), sleeping (sle), climbing (cli), rearing (rea), and locomotion (loc). If the mouse was not in view or his behaviour difficult to determine, this also was noted (inv).

Table II Food and water consumption of mice in Experiments 1, 2 and 3 in both test cages.

		Food consumption (g ± SEM)		Water consumption (ml ± SEM)	
Experiment	Mouse type	Inhabited cage	Empty cage	Inhabited cage	Empty cage
1	Subordinate littermates	3.9 ± 0.9	3.9 ± 1.0	5.1 ± 0.7	5.3 ± 0.6
1	Subordinate non-littermates	4.4 ± 0.4	3.7 ± 0.4	5.1 ± 0.5	5.1 ± 0.7
2	Familiar dominant	5.2 ± 0.5	5.1 ± 0.6	5.9 ± 0.7	5.5 ± 0.4
3	Unfamiliar subordinate	5.4 ± 0.7	6.7 ± 0.5	4.2 ± 0.4[a]	7.7 ± 0.8[a]

[a] $P < 0.05$

Results

Experiment 1: Choice of subordinate males for their dominant cage mate

Littermates and non-littermates did not differ significantly in their preference. Data of these groups could thus be combined to analyse overall preference. For the 24-hour analysis, the subordinate mice showed a clear preference for their dominant cage mate ($P < 0.01$). Figure 2 illustrates this preference, both for littermates and for non-littermates. Data analysis of the night period was consistent with the overall analysis: A clear preference was shown for the inhabited cage ($P < 0.01$). Data analysis of the day period, however, revealed this preference only marginally because of a large spread in the data as one mouse (non-littermate) chose to sleep in the empty cage ($P < 0.1$). No differences were found in food and water consumption between the two test cages (Table II). From the two non-aggressive couples, a subordinate mouse was chosen at random and tested in the preference test but omitted from further analyses. One mouse preferred his cage mate, and the other chose to sleep in the empty cage.

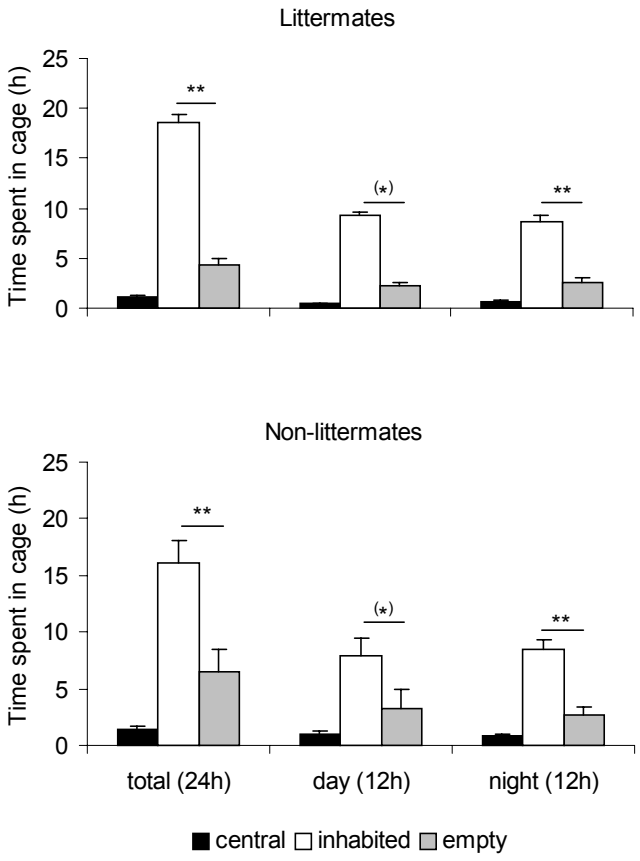

Figure 2 Experiment 1: Mean dwelling time in hours of subordinate male mice in the two test cages for (a) littermates and (b) non-littermates for the final day of the preference test (24h), a light period of 12h (day) and a dark period of 12h (night).
$^{**}P < 0.01$, $^{(*)}P < 0.1$

Experiment 2: Choice of dominant males for their subordinate cage mate

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 cage mate ($P < 0.01$), and data analysis of the night period was consistent with the overall analysis: A clear preference was shown for the inhabited cage ($P < 0.01$). Again, data analysis of the day period, revealed this preference only marginally due to one mouse's choosing differently ($P < 0.1$). No differences were found in food and water consumption between the two test cages (Table II). Behavioural analysis of two dominant mice revealed that, in concordance with preference data, mice spent more time in the inhabited cage. 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).

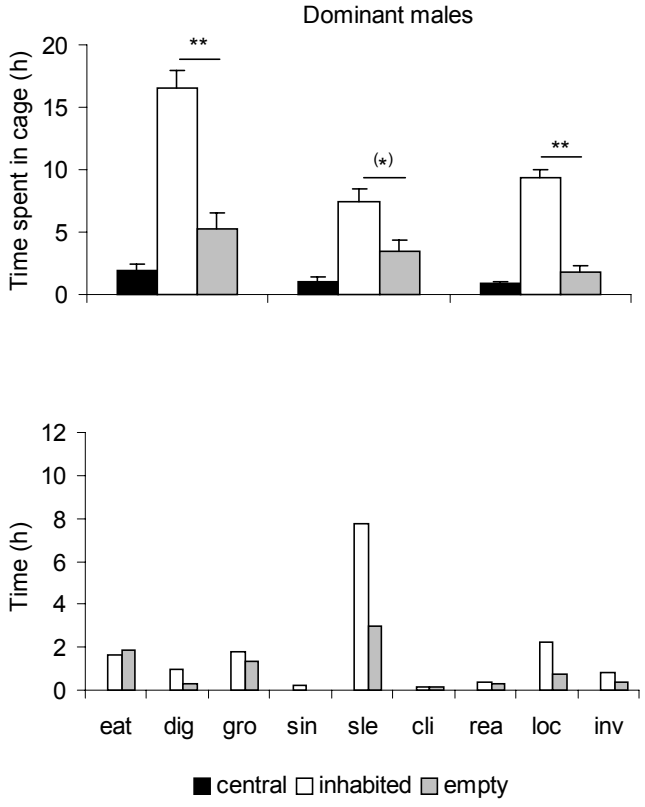

Figure 3 Experiment 2: (a) Mean dwelling time in hours of dominant male mice in the two test cages for the final day of the preference test (24h), a light period of 12h (day) and a dark period of 12h (night). (b) Mean time budget of two dominant mice separated for behaviour in the empty and inhabited cage. $^{**}P < 0.01$, $^{(*)}P < 0.1$

Experiment 3: Choice of subordinate males for an unfamiliar cage mate

Preference of subordinate males for an unfamiliar cage mate was less obvious than in the previous two experiments (Figure 4a). Although 6 of the 9 mice tested showed a strong preference to be near the other male, 3 mice divided their time equally across both cages, with a slight preference for the empty cage. Consequently, overall preference tended to be towards the inhabited cage ($P = 0.05$) but was significant during the light period ($P < 0.05$). During the dark (active phase), no significant preference for either cage was present. Water consumption was significantly higher in the empty cage ($P < 0.05$). Food consumption was equal for both cages (Table II). Behavioural 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).

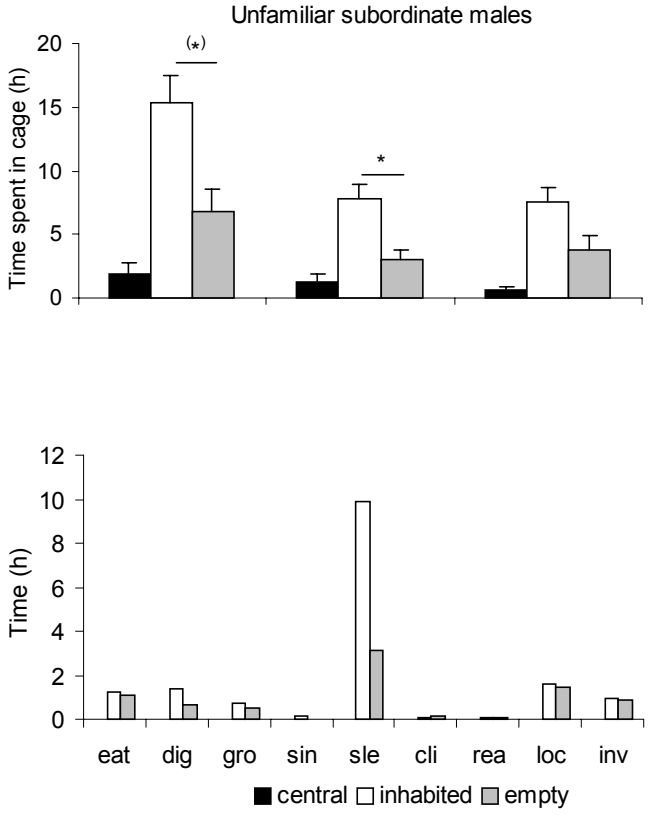

Figure 4 Experiment 3: (a) Mean dwelling time in hours of unfamiliar subordinate mice in the two test cages for the final day of the preference test (24h), a light period of 12h (day) and a dark period of 12h (night). (b) Mean time budget of one of the unfamiliar subordinate mice separated for behaviour in the empty and inhabited cage.
$^{*}P < 0.05$, $^{(*)}P < 0.1$

Discussion

In all three experiments the male mice showed a clear preference for the inhabited cage. In Experiment 1, only two of the twelve subordinate mice made their nests in the empty cage, one of whom came from an almost non-aggressive 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 cage mate (Figure 2). Of twelve dominant mice in Experiment 2, only one made his nest in the empty cage (moderately aggressive) and one mouse seemed to have switched cages during testing (low aggressive). All other mice made their nests in the cage inhabited by their subordinate cage mate (Figure 3a). In Experiment 3, one of nine subordinate mice chose to be alone, whereas two 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, b) reported that formerly group-housed rats rather than eat and sleep alone, actively seek company of other rats.

This experimental set up 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 (Parmigiani *et al.* 1989, Hurst *et al.* 1993). In fact, Kudryavtseva (1991) used a similar set-up, known as the sensory contact model, to investigate aggressive and submissive behaviour in male mice. In spite of this, both the subordinate and the dominant mice independent of levels of aggression that were scored before preference testing chose to be in the vicinity of another mouse for the majority of time. This is partly 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 & 3a). In a similar experiment with female rabbits, Held *et al.* (1995) gave low ranking does 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 cage mate 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 behaviour from their wild counterparts. Poole (1992) suggested that several solitary species such as polecats and orang-utans opt to socialise 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 littermates 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 & 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 behaviour of the videotaped subordinate mouse were found in sleeping and sleeping-related behaviours (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 *et al.* (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, b), a behaviour 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.

Conclusions and recommendations

The results described in this article favour 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 experiment 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 favour 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.