Male management: coping with aggression problems in male laboratory mice

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Summary

In a laboratory environment, aggressive interactions between male mice may exceed normal levels leading to negative effects both on the well-being of the animals and on the validity of experimental results. In this paper we review results from the literature and our own research with regard to coping with excessive aggressive behaviour in male laboratory mice. Based on this review practical recommendations concerning the housing and care of male laboratory mice are formulated. In short, it is recommended to avoid individual housing, to transfer odour cues from the nesting area during cage cleaning and to apply nesting material as environmental enrichment. Furthermore, group size should be optimized to three animals per cage. Further research, in particular into the effects of frequency, duration, type and severity of disturbances during an experiment on the degree of aggression, is recommended.

Keywords Male mice; aggression; social housing; husbandry; group size; environmental enrichment

Laboratory animals spend the vast majority of their life in their home cage. It is in the interest of both the animals and the researcher, that in this home cage an environment is created that provides for the animals' physiological and ethological needs. The animals will then be better able to cope with stressful procedures, and experimental results will be more reliable. Legally, we are also obliged to adapt the environment as well as possible to the physiological and behavioural needs of laboratory animals (EC Directive 1986). For these reasons, research into housing conditions for laboratory animals that also take into account experimental, economic, and ergonomic preconditions is highly prioritized (Rodent Refinement Working Party 1998, Kornerup-Hansen 1999).

The present standardized housing of laboratory animals often fulfils the needs of its inhabitants only marginally. As a result, the animals may behave abnormally and their well-being may be compromised to a great deal (Moberg & Mench 2000). One of the problems often encountered in laboratory animal facilities is excessive aggression in group-housed male mice. Since mice comprise about 40% of all animals used in research (Ministry of Health, Welfare and Sports 2001), a solution to this problem would have great impact on a large number of animals.

In this review we try to tackle the problem of excessive aggression in view of existing literature and our own experiments, and try to compose a set of recommendations for housing male mice in experimental settings balancing the behavioural and physiological needs of the animals with scientific, economic and ergonomic demands.

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Aggression: beneficiary or maladaptive?

In their natural habitat, male mice (*Mus musculus*) live in despotic social groups existing of one dominant male, females with their progeny, and subordinate males. The dominant male defends his territory containing resources such as females, food and a nest site. Familiar subordinate males are generally tolerated inside the territorial boundaries (Crowcroft 1966, Mackintosh 1970, 1973, Hurst *et al.* 1993). Intermale aggression within a socially stable group may be part of a broader behavioural strategy, in which all behaviour of an animal is aimed at a broader goal, namely to be fittest for a specific environment (Benus *et al.* 1990a,b, 1991, Sluyter *et al.* 1995). In the wild, mice show rhythmic population numbers, going up to a certain optimum level, followed by a rapid decline and an increase to the optimum again. It appears that when mice are living in small numbers, it is beneficial to possess a 'sturdy' character. Sturdy involves being aggressive and less flexible, showing routine-like behaviour, which enhances the chance of survival. When population size increases, however, it is beneficial to be more flexible. It appears that in the wild, mice of the more flexible type will migrate to unfamiliar grounds, and will be more successful in finding and maintaining a new territory (Busser *et al.* 1974).

Although potentially damaging, aggression can be regarded as having beneficiary effects in the short run. In such situations, physiological and behavioural mechanisms at work within and between animals prevent aggression from escalating to levels that are actually damaging. When male mice are group-housed in the laboratory, a certain level of intermale aggression can thus be regarded as normal or natural (Bisazza 1981, Brain & Parmigiani 1990). However, aggression levels may be so high that the welfare of the animals is jeopardized (Van Oortmerssen 1971, Bisazza 1981, Brain & Parmigiani 1990; Fig 1). Several factors are important in contributing to this specific welfare problem.

Fig 1 Male mouse subjected to severe aggression of a cage mate

Factors influencing aggressive behaviour

Genetic background

Laboratory mice differ from wild mice in their genetic composition. To achieve genetic standardization, intensive selective breeding has been used to create inbred strains of mice with minimal genetic variation. Furthermore, breeding schemes have been selected for several morphological, physiological or behavioural traits to create mouse models for human diseases. During the process of inbreeding, several mouse strains have become highly aggressive, either as a side effect of selective breeding, or because aggressive behaviour was the main selection criterion (Bisazza 1982, Mondragón *et al.* 1987, Guillot & Chapouthier 1998, Parmigiani *et al.* 1999). Odour cues, which are important for kin recognition and advertisement of social status and essential for the mediation of aggressive behaviour, appear to be similar between families within inbred strains of mice, which may hamper individual recognition (Nevison *et al.* 2000). Thus disturbed social behaviour, such as excessive aggression between group members, may have a genetic background.

Environmentally induced disturbed social behaviour

Laboratory mice that live in a barren confined space such as a laboratory cage may be unable to respond to each other in a proper social way. Subordinate mice are unable to flee from the dominant's sight, or migrate out of the territory. When the proper behavioural response is frustrated, the animal's attempt to cope can be deemed to fail, causing a state of suffering in the subordinate mouse. Furthermore, the dominant male may respond with more extreme aggression than naturally, in an attempt to achieve the desired effect (i.e. disappearance of the subordinate). In pigs kept at high stocking density, Ewbank and Bryant (1972) showed that the close proximity of the subordinate pig continued to elicit aggression from the dominant.

Frustration and lack of control and predictability

In a laboratory environment behavioural responses may be frustrated, i.e. they are triggered to be performed, but critical factors to complete it are missing. This may, for example, be the case when humans are approaching the cage, and escape out of sight is not possible. In these instances, an animal may direct its energy into aggression against nearby animals (Broom & Johnson 1993). In an experiment in which rats received electric foot shocks but were unable to escape, the rats' escape behaviour was redirected into aggression towards each other (Ulrich & Azrin 1962). Furthermore, unpredictable situations such as experimental procedures and routine husbandry procedures to which the animal is not able to anticipate may occur regularly in the laboratory. Aggression is a common response to such unpredictable situations (Broom & Johnson 1993). Cage cleaning, for example, often induces high levels of aggression in male mice (Gray & Hurst 1995).

Severe aggression in group-housed male mice may thus be an indicator of poor welfare of the aggressor, although this need not necessarily be the case in all situations. In any case, severe aggression can certainly be regarded as affecting the psychological and physiological well-being of the mice being the target of the aggression, either by frustrating an appropriate behavioural response or through pain and distress resulting from injuries (National Research Council 1992). Additionally, severe aggression may hamper the validity of experimental results. Different kinds of measures are taken to avoid severe aggression between male mice in the laboratory.

Use of females

From strains known to be aggressive, primarily female mice can be used for experimentation. This is common practice now and, mainly due to this gender preference, many male mice are euthanized before weaning (on average 65%, which can cumulate to 80% for highly aggressive strains such as FVB mice, Laboratory Animal Science Association 1998). This waste is highly undesirable since the killing of animals with no apparent purpose creates an ethical problem.

Use of docile strains

The use of males from a docile strain may, in some instances, be a good solution. However, mouse models should be suitable in the first place for solving the scientific problem being studied which could be in conflict with the choice of a docile strain. A wealth of literature has revealed that behavioural characteristics of an animal, such as high or low aggressiveness, do not act alone, but are part of a variety of characteristics that form the animal's coping style (see Koolhaas *et al.* 1997 for a review). Selecting a model on one characteristic may therefore bias the experimental outcome, render the experiment invalid, and the use of animals morally unjustifiable.

Separation of males or permanent individual housing

Males can be separated when aggression increases to unacceptable levels. Also, directly after weaning, male mice may be housed individually. Individual housing is recommended for several highly aggressive strains such as Swiss/CD-1 and FVB (Committee on Infectious Diseases of Mice and Rats 1991, Mouse Genome Database 2001). However, the lack of opportunity to interact with conspecifics greatly influences the behaviour and physiology of both mice and rats and has frequently been referred to as 'isolation stress' or 'isolation syndrome'. It should be noted at this point that 'individual housing' is not the same as 'isolation'. In the case of individual housing, the animals can still obtain visual, auditory and olfactory cues from conspecifics in adjacent cages. The term isolation should only be used in those cases where animals lack all sensory cues of conspecifics. Both terms, however, have been used in the past to describe effects of individual housing. Animals that suffer from 'isolation syndrome' are more difficult to handle and become more aggressive, sometimes show stereotyped behaviour patterns and may suffer from convulsions. Furthermore, several studies indicate that individual housing induces reduction of stress resistance and immunocompetence, higher tumour incidence, 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).

It is evident that individually-housed animals differ from socially-housed animals in behavioural, neuro-endocrinological and neuro-physiological parameters, and as such may not be appropriate as a research model. However, it has also been proposed that individual housing may not be as stressful for male mice as it may seem. Comparisons of socially-housed dominant male mice and individually-housed male mice revealed that several neuroendocrine parameters indicating sympathetic activity of the adrenal gland were similar between these males (Maengwyn-Davies *et al.* 1973, Haemisch & Gärtner 1996). The wild counterpart of most laboratory mice (*Mus musculus*) is territorial, and housing male mice individually may be regarded as providing them with their own territory (Brain 1975).

Evidence exists that both rats and mice prefer social to individual housing. In rats it has been shown that animals that were reared in group-housed conditions and were later individually housed would choose to feed and sleep in close proximity with others with maximal body contact rather than alone (Gärtner 1968a,b). In the same review, Gärtner concludes that individual housing of rats will have many undesirable effects and should be avoided if possible. Sherwin (1996) conducted a preference test in which laboratory mice were willing to spend increasing amounts of energy to obtain access to certain traits, one being companionship with the companion housed in a nearby cage.

In order to obtain further insight into what the animals would prefer, we investigated the social preferences of male BALB/c mice in relation to the level of aggression, dominance hierarchy, age, familiarity and kinship (Van Loo *et al.* 2001b, and unpublished data; Fig 2). These preference tests were conducted to further explore the question whether individual housing for male mice is acceptable or not. Results indicated that, in general, all mice preferred the vicinity of a cage mate during resting periods, especially when mice were familiar to each other (i.e. had been housed together previously). Social preference during active periods increased with age. During these periods, the mice clearly showed increasing appetitive behaviour: they regularly sniffed at the openings in and alongside the partition separating them from each other, and dug away the sawdust near it, as if attempting to establish social contact. One might assume that the inability to actually establish contact could influence the mice's choice of behaviour. The mice were, however, able to communicate by sight, hearing, smell and nose contact. The hierarchy between two male mice that are not able to be in bodily contact does not cease to exist when close olfactory and visual contacts are possible (Parmigiani *et al.* 1989, Hurst *et al.* 1993), and isolation effects on behaviour and physiology do not develop (Kudryavtseva 1991, 1994, 2000, Kudryavtseva *et al.* 2000). Furthermore, by preventing access to the cage mate, not all social interactions can take place, i.e. consummatory responses affecting the rewarding properties of social contact cannot interfere with the anticipatory behaviour which may indicate the need for social contact (Spruijt 2001). We therefore assume that the conducted tests provided valuable

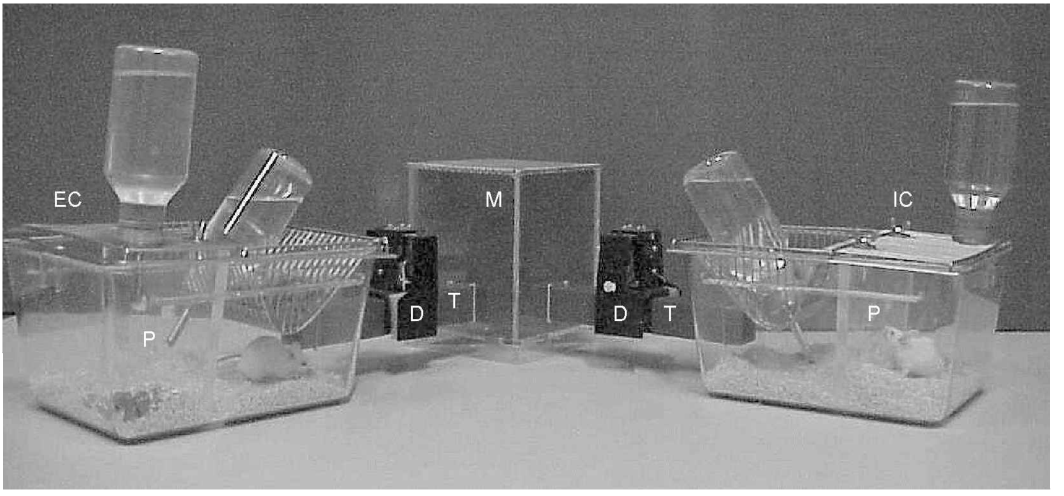

Fig 2 Preference test system. IC = inhabited cage with a mouse behind a partition, EC = empty cage, M = middle cage, T = tunnel, D = infrared detector, P = perspex partition with holes

information on the preferences of the mice themselves for social or individual housing.

The general conclusion drawn from these experiments was that male mice prefer social housing to individual housing, independent of their level of aggression and social status. Together with the existing evidence of negative side effects of individual housing on the well-being of mice these results support the recommendation that individual housing should be avoided whenever possible.

Individual housing may be required by the experimental set-up, e.g. in metabolic cages. However, is expected excessive aggression between male mice enough reason to make individual housing a necessity? It may be argued that excessive aggression in group-housed male mice provides a serious welfare problem for both dominant and subordinate mice, and that individual housing will be less costly from a welfare perspective. On the other hand, both individual housing and group housing in combination with excessive aggression have obvious negative consequences for the mice. These kinds of cost–benefit analyses can also be found in farm animal welfare research. In laying hens, the welfare benefit of preventing cannibalism is weighed against the welfare cost of beak trimming. To objectively make such cost–benefit analyses for animal welfare is an impossible task, due to the complexity and interdependence of aspects influencing animal well-being (Appleby 1997, cf. Broom 1997, Fraser 1997, Newberry & Estevez 1997). Since both alternatives may induce animal suffering, why should we opt for either of these? Fox (1983/84) points out that there is a limit to the extent that we can adapt an animal to a captive environment. It may therefore be better to seek for possibilities to adapt the environment to its inhabitants rather than the other way around.

Changes in housing and husbandry procedures

Controversies

Both scientific studies and anecdotal evidence from animal caretakers have been reported concerning the modification of housing and husbandry procedures to decrease aggression in group-housed male mice. Many of these studies and reports, however, contradict each other. Partial cage cleaning or transfer of dirty sawdust to a clean cage has been reported to increase aggression (Gray & Hurst 1995) but also to decrease aggression (McGregor *et al.* 1991). Others have investigated the availability of space (Poole & Morgan 1976, Vestal &

Schnell 1986, McGregor & Ayling 1990) and group size (Welch & Welch 1966, Greenberg 1972, Poole & Morgan 1973, Butler 1980, Barnard *et al.* 1994). In these studies too, both increases and decreases in aggression have been reported, and effects of group size and cage size seem to be interdependent. Most of the studies in this area, however, have focused on the effects of environmental enrichment on aggression, but again with varying success. Several authors have indicated that environmental enrichment leads to an increase in aggression when male laboratory mice are housed together, and conclude that the enrichment may actually be unfavourable to their well-being in this respect (McGregor & Ayling 1990, Haemisch & Gärtner 1994, Haemisch *et al.* 1994, Haemisch & Gärtner 1997). Others have found that cage enrichment or environmental complexity does not alter, or decreases the amount of aggression between male mice (Vestal & Schnell 1986, Chamove 1989a, Ward *et al.* 1991, Armstrong *et al.* 1998, Ambrose & Morton 2000).

The identification of factors explaining the apparent controversies in the studies mentioned above is complicated by the wide variety of parameters used to quantify aggression and by the differences in experimental design. Aggression levels are based on number of wounds, attack latency time, number or duration of fights or agonistic encounters. Furthermore, the degree of familiarity between the mice varies from newly acquainted to being familiar from birth, and test environments vary from novel to familiar.

In further experiments, we tested a selection of adaptations to the housing and husbandry of male laboratory mice for its modulating properties to aggression and social stress. The experiments have been described in original papers (Van Loo *et al.* 2000, 2001a,b, 2002, 2003, 2004). Adaptations were introduced in a variety of husbandry aspects that potentially influence intermale aggression. Evidence for this was primarily based on personal experience of animal caretakers and researchers, and on the scientific reports mentioned above. These husbandry aspects were routine husbandry procedures, group composition, cage size, and complexity of the cage. Apart from their alleged aggression modulating properties, the adaptations were chosen for three other main reasons. First, adaptations to laboratory animal facilities should preferably be economically and ergonomically feasible. The adaptations chosen can all be implemented in the existing housing design, with minimal costs with regard to material and personnel. Second, the success of changes in laboratory animal husbandry is directly related to the motivation of the animal care staff, where important factors are extra workload and obvious benefits to animal welfare (Benn 1995, Markowitz & Gavazzi 1995, Van de Weerd & Baumans 1995). With the choice of adaptations to be tested and recommended to modulate aggression, this has been kept in mind. Third, the aim was to provide a set of husbandry adaptations each capable of decreasing aggression and/or social stress, with a possible synergistic effect when combined. The variety of aspects in the mice's environment that was tested were not interdependent, which made a combination of any set of adaptations possible.

Cage cleaning

Cage cleaning, although essential for hygiene, disrupts odour cues and stimulates activity, leading to an increase in aggression (Gray & Hurst 1995, Rodent Refinement Working Party 1998). Aggression between male mice is mediated by social odour cues, and both source and distribution of odour cues appear to be important in this respect (Bishop & Chevins 1987, Hurst *et al.* 1993). Since mice keep their nests clean of urine and faeces (Blom *et al.* 1993), the nesting area will primarily contain pheromones originating from plantar and other body glands. In contrast to most urinary pheromones, these have an aggression-inhibiting effect (Mugford 1972, Jones & Nowell 1975, Stoddart 1980, Brown 1985). The first experiment (Van Loo *et al.* 2000) revealed that simple changes in routine cleaning procedures significantly affected the level of aggression. The transfer of used nesting material from the old cage to the new cage clearly reduced aggression, while the transfer of sawdust soiled with urine and/or faeces seemed to intensify aggression. The recommendation resulting from this experiment is that material derived from the nesting area should be transferred to the clean cage.

Group composition

Two aspects of group composition—kinship and group size (Van Loo *et al.* 2000, 2001a)—were tested for aggression modulating properties. Evidence exists that social interactions between male mice can be affected both by kinship (Kareem & Barnard 1982, Hayashi & Kimura 1983, Kareem 1983, Kareem & Barnard 1986) and by group size (Poole & Morgan 1973, Butler 1980, Barnard *et al.* 1994). In our experiments, however, kinship did not affect the level of aggression. A possible explanation is that familiarization between the non-littermates being housed together while they were still young, may have overruled effects of kin recognition (Kareem & Barnard 1982), or because kin recognition in inbred strains such as the BALB/c strain may be disturbed by inbreeding (Nevison *et al.* 2000). Group size on the other hand, affected aggression to a marked degree. Groups composed of three mice showed the least aggression as compared to groups of five or eight mice. In the latter, not only the dominant mouse, but also the subordinate mice showed more aggression than in groups of three mice. This seems to indicate that the dominance hierarchy is more stable in smaller groups. The housing of male mice in small groups is therefore recommended.

Cage size

For the effects of cage size on the level of aggression, the literature reports contradictory results (Welch & Welch 1966, Poole & Morgan 1976, Vestal & Schnell 1986, McGregor & Ayling 1990, Jones 1991). In the experiments described in Van Loo *et al.* (2001a), mice were housed in cages of two different sizes: one providing the minimal amount of space per mouse that is legally required, the other providing about 50% extra space. The smaller cages appeared to invoke less aggression than the larger cages. These results should be interpreted with care since the relationship between cage size and aggression may actually be curvilinear with an optimum (Polley *et al.* 1974). In different species evidence exists that crowding may induce a reduction in aggression (Welch & Welch 1966, Ewbank & Bryant 1972, Hughes & Wood-Gush 1977). Crowding effects reported in mice and rats are generally stress-related; these include increased corticosterone levels, impaired skin homeostasis and diminished immune response (Brown & Grundberg 1995, Csermely *et al.* 1995, Denda *et al.* 1998).

Environmental enrichment

Studies investigating the effect of cage complexity by the addition of environmental enrichment on the well-being of laboratory mice are numerous (cf. Van de Weerd & Baumans 1995). In the majority of cases, the increase of complexity or the addition of environmental enrichment enhances the well-being of the mice being studied. For male mice, however, several studies report an increase in aggression when cages are more complex (McGregor & Ayling 1990, Haemisch & Gärtner 1994), while others report no effect (Vestal & Schnell 1986) or a decrease in aggression (Chamove 1989a, Ward *et al.* 1991, Armstrong *et al.* 1998, Ambrose & Morton 2000). The effects of cage complexity on aggression were tested in an experiment (Van Loo *et al.* 2002) by adding two types of environmental enrichment to the cage (Fig 3). One type of enrichment (shelter) was rigid, and structured the cage into specific areas with properties meeting the mice's needs, i.e. a dark and secluded sleeping area, an escape route, wire mesh to eat from and to use as voiding area (Blom *et al.* 1993, Schlingmann *et al.* 1994, Van de Weerd & Baumans 1995, Baumans 2000). The other enrichment (nesting material) could be manipulated, and gave the mice the opportunity to build a nest for sleeping and thermoregulation (Van de Weerd *et al.* 1997, 1998). Results revealed that aggression and stress-related parameters increased when mice were housed with a shelter, while a significant decrease in aggression was apparent when mice were

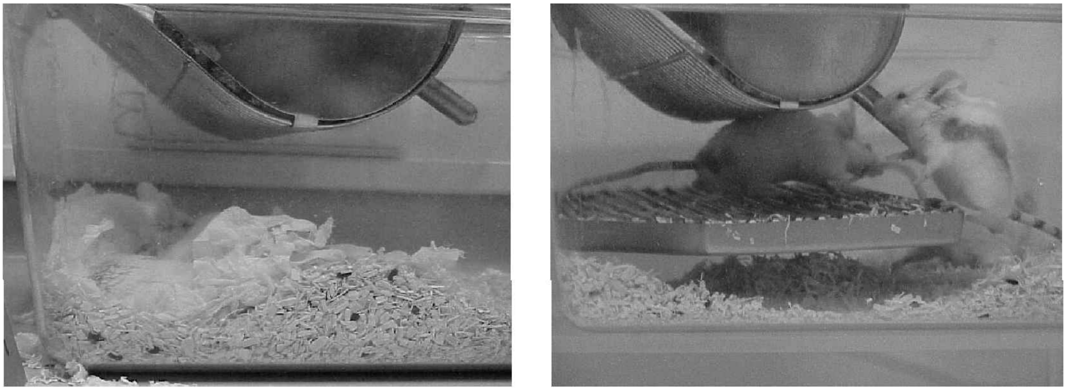

Fig 3 The two enrichment items tested: Kleenex tissues (left) and the Utrecht Shelter (right)

housed with nesting material. Both enrichments provided for the already mentioned important features in the mice's cage. Additionally, with the nesting material mice could actively structure their environment at their own will. It has been argued that active behaviour may have a reward value in itself, i.e. that an animal's needs may lie as much in the 'doing' as in the 'achieving' (Wemelsfelder 1997a). Items providing for this need, such as nesting material, would thus be more suitable enrichment items than rigid structures (Poole 1998). The recommendation resulting from this experiment is that male mice should be provided with nesting material to actively structure their cage.

Long-term consequences

When combining the results from the latter experiments, a reduction in aggression was expected to be most significant in groups of three mice, provided with nesting material that was transferred after cage cleaning. We have also tested this combination of husbandry adaptations with proven aggression-reducing properties (Van Loo *et al.* 2003, 2004). This experiment differed in two important aspects from the previous experiments, namely experimental design and choice of strain.

Experimental design

In the previous experiments, groups of mice were alternately subjected to different housing or husbandry procedures, with the exception of the experiment studying group size. The advantage of this experimental set-up was that groups of mice served as their own control and even slight changes in the usually quite variable levels of aggression could be detected. The disadvantage was, however, that no long-term effects of changes in housing and husbandry procedures on aggression and stress could be examined. Long-term effects may differ from short-term effects, since animals may habituate to the adapted situation or loose interest in introduced enrichment items after some time (Chamove 1989b, Bloomsmith *et al.* 1991, Van de Weerd & Baumans 1995). Therefore, husbandry procedures of experimental groups and control groups of mice were not changed during this experiment.

Choice of strain

For the previous experiments, BALB/c had been the strain of choice. Males of this strain are known to be moderately aggressive and animals may fight fiercely. The main goal of this study was to find ways of preventing excessive aggression arising in group-housed male mice. In previous experiments, however, levels of aggression hardly ever reached levels beyond what could be considered normal. It may thus be argued that the animal model used was not the best model to study environmental influences on excessive aggression since such levels of aggression never arose. However, mechanisms underlying the development of excessive aggression may not differ distinctly from mechanisms regulating normal levels of aggression. Excessive aggression merely may be a state in which the mechanisms to prevent normal levels of aggression to escalate, dysfunction. Environmental stimuli influencing normal levels of aggression may therefore also influence the development of excessive aggression. Furthermore, for aggression modulating properties of husbandry changes to be investigated, we needed to house male mice in groups for a relatively long period. When using a highly aggressive strain there would have been a distinct possibility that males would have to be separated early during the experiment due to excessive aggression. Still, it was considered of main importance to examine whether the aggression reducing adaptations to the husbandry procedures, as found in previous experiments, would also decrease aggression in strains known to be highly aggressive, to the extent that group housing is optional. Therefore, the combination of presumed aggression reducing husbandry adaptations were tested not only in the BALB/c strain, but also in a strain known to be highly aggressive (Swiss-derived CD-1; Parmigiani *et al.* 1999).

Results of this experiment indicated that long-term enrichment with nesting material and the repeated transfer of nesting material when cleaning the cages did not affect intermale aggression levels (Van Loo *et al.* 2003). Since aggression levels between groups were highly variable, and groups of mice could not serve as their own control, a possible effect on aggression may have been too low to detect. Another explanation may be that the novelty effect, rather than the differences in husbandry procedures *per se*, were responsible for the aggression decreasing effects found earlier. The perception of 'change' or 'novelty' may be regarded as important factors providing for variety in a captive animal's environment (Wemelsfelder 1997b, Poole 1998). Novel objects stimulate investigative or manipulative behaviour that decreases over time as the animal habituates to the presence of the object (Shepherdson *et al.* 1998), and frequent change of objects to avoid boredom has been recommended (Benn 1995, Markowitz & Gavazzi 1995). The alternate application of different housing and husbandry procedures earlier may have sustained the effect of novelty. From the results of this experiment, it is not possible to find out whether detection levels were too low, or whether aggression-inhibiting effects declined after the mice became familiarized with their enriched environment. Stress-related parameters such as corticosterone levels, thymus weight and food and water consumption, however, indicated reduced levels of stress in enriched-housed conditions (Van Loo *et al.* 2004). This implies that the husbandry changes as recommended in the previous experiments may also have beneficiary effects on the well-being of group-housed male mice in the long term.

Concluding considerations

An interesting phenomenon is that overall aggression levels were low in all experiments, both in BALB/c mice and in the Swiss derived CD-1 mice. In the experiment testing preference of male mice for social housing in which mice were housed in pairs, the highest levels of aggression were observed, and several pairs of mice needed to be separated or euthanized. In the experiment investigating group size, in several groups of five and eight mice, animals were badly wounded as well. In the final experiment, with a group size of three mice, only four out of 20 CD-1 groups were moderately wounded. In three of the latter, aggression returned to acceptable levels after several weeks. In the other groups of Swiss:CD-1 mice, all animals lived in harmonious groups. This is remarkable, since the general presumption is that adult Swiss males will kill each other when group housed (Bisazza 1981). The experiments in which aggression remained within acceptable levels differed in two aspects from the experiments in which aggression levels reached excessive levels and studies of others reporting high levels of aggression (cf. Parmigiani *et al.* 1999), namely group size and nature of disturbances.

Group size

In general, mice were housed in groups of three. As mentioned before, housing male mice in groups of three clearly invokes less aggression than in groups of five or eight mice, since the hierarchy in the latter groups appears to be less stable. The combined results of the social preference experiment and the experiment on group size imply that housing in groups of three would also invoke less aggression than pair housing. Evidence for this has also been provided by Bisazza (1981), who observed severe fighting in pairs of Swiss mice, while these mice had peacefully shared nests previously when they were housed in groups of three. When male mice are housed in pairs, the subordinate male is the only animal at which aggression can be directed. In groups of three mice, aggression can be directed at two subordinates. These subordinates may find social support with each other that may reduce the level of social stress they experience. Social support after encountering a (socially) stressful situation appears to be essential for successful coping in animals as well as in humans (Medalie 1985, Cowie 2000). Ruis *et al.* (1999) showed that male rats that had been subjected to social defeat without post-defeat social support developed depression-like behavioural and physiological symptoms, while rats that were group-housed after social defeat, were coping well. Keeney and Hogg (1999) used socially-defeated mice that lived individually while the unfamiliar mouse that defeated them lived opposite behind bars, as an animal model of depression. Mice that are socially housed immediately after surgery appear to recover quicker than mice that are housed individually (Baumans, personal communication).

Nature of disturbances

In most experiments described in this review, animals were subjected to routine handling such as cage cleaning, weighing and a visual daily check, and in some instances, a relatively short disturbance e.g. to obtain urine samples. These disturbances may be regarded as relatively low-stress situations. In the experiment on social preferences, however, pairs of mice were removed from their home cage and physically separated for a period of 48 h on three occasions. Evidence exists that intermale fighting in mice increases with progressive isolation, and that effects on social behaviour and aggression in both mice and rats already become apparent after a period of 24 h of isolation (Goldsmith *et al.* 1978, Cairns *et al.* 1985, Varlinskaya *et al.* 1999). Although the mice used in this experiment were not isolated completely, they were unable to perform normal social behaviour during the periods of separation. In general, more than 40% of the experiments conducted with mice in The Netherlands involve stressful procedures, such as inducing traumatic physical, chemical or psychological stimuli, inflammations or infections, subjection to radiation, etc. The degree of discomfort for these mice is categorized as moderate to extremely severe (Ministry of Health, Welfare and Sport 2000). Mild disturbances such as cage cleaning, already induce an increase in aggression between male mice (Gray & Hurst 1995, Van Loo *et al.* 2000, 2001a, 2002), and exposure to a repeated or even a single extreme stressor may lead to excessive aggression (Pant & Nath 1993). Once excessive aggression has arisen, it may not return to normal levels so easily. Therefore, refinement, in particular minimizing the number and duration of stressful procedures not only directly affects the well-being of the animals, but also indirectly, by reducing the chance of triggering excessive aggression.

Recommendations for the housing and care of male mice in laboratories

Based on the information presented in this review, the following recommendations can be put forward:

(1) Individual housing of male mice should be avoided whenever possible.
(2) When cleaning the mice's cages, material from the nesting area should be transferred to the clean cage. These materials seem to contain aggression-reducing odour cues. Transferring bedding or other materials contaminated with urine

and/or faeces should be avoided, since these may contain aggression-eliciting odour cues.

(3) Male mice can best be housed in groups of three animals. Larger group sizes may decrease the chance of a stable hierarchy developing, while pair housing may increase aggression, and lack of social comfort may induce depression-like symptoms in the subordinate.

(4) When applying environmental enrichment, nesting material would be the enrichment of choice. It provides the opportunity to indulge in species-specific behaviour. When first introduced, it decreases intermale aggression, and, in the long term, it may help the animals to better cope with stressful situations.

(5) Disturbances during an experiment should be limited as far as practically possible since they may create situations in which excessive aggression can arise. Further research is necessary to investigate if, and how frequency, duration, type and severity of disturbances influence the development of excessive aggression.

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