Aggression in group-housed laboratory mice: why can't we solve the problem?

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Group housing is highly important for social animals. However, it can also give rise to aggression, one of the most serious welfare concerns in laboratory mouse husbandry. Severe fighting can lead to pain, injury and even death. In addition, working with animals that are severely socially stressed, wounded or singly-housed as a result of aggression may compromise scientific validity. Some general recommendations on how to minimize aggression exist, but the problem persists. Thus far, studies attempting to find solutions have mainly focused on social dominance and territorial behavior, but many other aspects of routine housing and husbandry that might influence aggressive behavior have been overlooked. The present way of housing laboratory mice is highly unnatural: mice are prevented from performing many species-typical behaviors and are routinely subjected to painful and aversive stimuli. Giving animals control over their environment is an important aspect of improving animal welfare and has been well-studied in the field of animal welfare science. How control over the environment influences aggression in laboratory mice, however, has not been closely examined. In this article, we challenge current ways of thinking and propose alternative perspectives that we hope will lead to an enhanced understanding of aggression in laboratory mice.

Mice are the most commonly used mammal in biomedical research, and are typically group-housed for good reasons. Group housing is important for the welfare of social animals such as mice and is mandated by law in many countries. Furthermore, external predictive validity (to humans) and internal construct validity[1] require that animal experiments be performed on a background of good general physical and mental health[2]. For instance, if a human patient has a stable environment and abundant social support, then an animal patient should receive the same. Besides its other welfare benefits, support from conspecifics markedly improves health outcomes, and therefore model quality, in mice[3–5]. However, social housing often gives rise to aggression, one of the most serious welfare concerns in laboratory mouse husbandry[6].

Severe fighting can lead to pain, injury and death. Daily visual inspections may fail to detect aggression problems until they have become quite severe, as mice can inflict extensive wounds rapidly. Both injured and very aggressive mice are generally separated and may be euthanized. These problems could be avoided by housing all mice singly[7,8], although this is clearly not optimal for welfare either. Ultimately, injuries, deaths and social isolation directly conflict with the 3Rs goals of reduction and refinement.

Aggression is not only an animal welfare concern, but may also compromise the scientific process. First, aggression can inflate the number of animals required to achieve sufficient statistical power. Deaths or separations as a result of aggression can undermine experimental design by altering the numbers of groups and of animals per cage. Aggression, pain and social isolation can also change several physiological parameters, particularly immune function[9,10]. Thus, working with animals that are severely socially stressed, wounded or singly-housed as a result of aggression creates additional variability that can reduce statistical power and may reduce external validity[11]. Second, fighting injuries and risks of aggression may complicate or render unfeasible certain research procedures. Third, some researchers may only use females in an attempt to avoid aggression[12]. This can lead to sex differences being overlooked, and runs counter to US federal policy that now requires that studies include both sexes where relevant[13].

Group housing without severe aggression is therefore the ideal from welfare and scientific standpoints. However, problematic aggression persists despite some general recommendations produced by the few studies attempting to find solutions to the problem[14,15]. We begin by examining the contexts in which aggression has been

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studied and then summarize what is currently known about the causes of aggression before turning to remaining areas of uncertainty. We end by discussing directions for future research. We hope to challenge current ways of thinking and propose alternative perspectives that will lead to an enhanced understanding of aggression in laboratory mice.

How aggression has been studied in mice

Our current understanding of mouse aggression is based on three distinct areas of the ethological literature. First, social interactions and relationships have been studied in free-ranging mice and mice housed in naturalistic settings. Second, aggression and social defeat have been studied using staged encounters between unfamiliar mice. Finally, dominance and aggression have been studied in social groups of caged mice, which represent typical laboratory conditions.

In the wild, the social organization of mice varies depending on local resource availability and distribution. Studies conducted in semi-natural enclosures have identified social organizations ranging from situations in which males individually defend established territories and attack male intruders (females may move freely between males' territories), to those in which multiple males occupy the same area and maintain dominant-subordinate relationships, and others in which a minority of males defend territories while males that do not have territories co-exist more or less peacefully in a 'no-man's land' between defended territories[16–18]. Thus, the nature and severity of aggression in wild and feral mice seems to depend on their social organization. Dominance requires the establishment and maintenance of social relationships, which are characterized by animals recognizing and consistently behaving differently and adaptively toward different individuals. In contrast, territorial aggression need not involve social relationships, with mice using olfactory and behavioral signals (for example, tail rattling) indiscriminately toward all intruders. Each context can involve mediated aggression (threats that are terminated by submissive behavior or fleeing, without physical damage) and escalated aggression (actual attacks or retaliation involving biting)[19], although the exact behaviors and signals may differ by context.

Mice are also used extensively as a model species to study aggression and social defeat. This literature has focused on territorial aggression, exemplified by the resident-intruder test, which measures the latency for a 'resident' mouse to attack an unfamiliar 'intruder' mouse introduced into the resident's home cage[20]. This reliably induces aggression, at least in males, specifically because it exploits ethological principles of territory ownership, defense and territorial aggression. Other protocols pair unfamiliar mice in a neutral testing chamber, often adding stimuli known to induce aggression (for example, shock-induced aggression)[21]. Because the chamber is neutral, it is assumed that ownership has not been established and territorial aggression is unlikely. Instead, these protocols are designed to induce stress, emotional conflict and frustration; aggression is often used as a readout of these states, rather than as the focus of the study.

Finally, some researchers have studied aggression in long-term group-housed animals, which is the context of this article (for example, see refs. 7,8,22,23). In the wild, the smallest mouse territories are seen when mice live commensally in human dwellings; these territories typically measure approximately 2 m^2 (ref. 24). Mice do not use this space evenly, however. Only some parts of a defensible territory will be used regularly, such as a sleeping area. A standard mouse cage housing 4–5 same-sex adults provides around 0.0525 m^2 of floor space. We do not know whether mice perceive this environment as sharing 2.6% of the minimum space they would inhabit in the wild, or individually occupying as little as 0.5% of that space. Their behavioral and genetic flexibility and adaptability is one of the primary reasons mice have become the most commonly used animal in research, and laboratory mice have been selected that reproduce and survive under these conditions. However, we do not know whether they can respond to such extreme space limitations without becoming fundamentally abnormal.

Because mice are not free to spatially disperse in the laboratory setting, it is difficult to establish what kind of social organization they maintain. Mice rarely meet unfamiliar mice in their home cage, suggesting that territorial aggression is not a major factor. One way to cope with living together in a cage might be to form a dominance hierarchy; a breakdown of which could be one cause of injurious escalated aggression[19]. However, it is unclear whether cases in which aggression does break out represent a failure of dominance relationships to mediate aggression (for example, lack of appropriate submissive behavior or recognition thereof, leading to escalated aggression), whether this is more closely related to territorial aggression (for example, one mouse establishing a territory and perceiving its cage mates as trespassers), or if it is a result of frustration or pain (for example, different animal ID methods, such as ear tags, trigger different risks of aggression[25]). Furthermore, we caution against the simplistic view of a fixed pecking order: real animals have complex social structures that are not linear, transitive or consistent across resources, context or time, which makes measuring dominance a non-trivial and sometimes irrelevant task, as the outcome of such measures might not accurately reflect the social dynamics in a group of undisturbed mice[26]. Finally, the social organization of lab mice could potentially be closer to that of males living in the no-man's land between other males' defended territories[18]: a type of social organization that has been universally overlooked in the mouse housing literature. These mice are in poor health and physical condition and live in a state of constant scramble competition, but do crowd into nest sites at densities similar to the lab environment. If this is how lab mice perceive their housing, then aggression might result from scramble competition for territory or other resources cued by salient changes in the environment (such as cage change), and if their physiology is affected as well, this raises additional welfare and quality-of-science concerns.

What we have learned about aggression in laboratory mice

Group composition modulates aggression in mice under normal husbandry conditions. Most studies of group composition manipulate stocking density, without considering the different influences of group size and cage size. Only one study has properly teased out these two effects to show that aggression is affected by group size and not by cage size[23]. Thus, aggression levels increase with group size, particularly in excess of three individuals in a standard

shoebox cage[23]. Establishing stable groups, such as keeping siblings or familiar mice together from weaning, generally decreases aggression[7,27]. Large differences in aggression also exist between strains, both in the resident-intruder test[28] and in the home cage[29], indicating that there is a possible genetic component.

In terms of the physical environment, temperature and bedding material may be important. Mice in the wild suppress territorial aggression in cold months[18], and mice show marked increases in aggression in the lab as temperatures increase from 20 to 25 °C[29]. Considering bedding material, mice show preferences for certain types of bedding[30], which may affect physical comfort; bedding may also have unexpected endocrine effects—corncob bedding contains estrogen disruptors that increase aggression in resident-intruder tests in *Peromyscus californicus*[31].

In terms of enrichment, transferring nesting material at cage cleaning decreases aggression, whereas transferring soiled bedding increases it[14]. However, the literature on structural enrichments, such as shelters, is mixed[32], with studies reporting both increases and decreases in aggression with structural enrichments. This topic is plagued with untested received wisdom, particularly the idea that shelters with multiple entry points do not cause aggression. In fact, providing a single multi-entrance shelter per cage can cause large increases in escalated aggression[19].

Given these findings, recommendations for minimizing aggression can be organized in a hierarchy of ease of implementation and strength of evidence. Minimum standard practice, supported clearly by empirical evidence, should be: ensuring that the nest site, but not soiled bedding, is transferred during cage change; maintaining cages at 20–22 °C and providing sufficient nesting material for mice to thermoregulate; avoiding mixing unfamiliar males, and keeping littermates together whenever possible.

We also strongly recommend some practices with a solid evidence base, but some logistical difficulty. First, group size should be limited to three animals in a standard cage. This may seem impractical, but aggression is so common that many four- or five-animal cages are split. This adds variability and disrupts experimental designs, as discussed earlier. Furthermore, the fecal output in a three-mouse cage is much less, and the *per diem* cost of a small group size is potentially offset by lengthening the cage change interval. Second, shelter enrichments should not be provided in circumstances in which these are known to increase aggression[19] (which is best assessed on a case-by-case basis in each facility). Note that providing multiple shelters per cage or providing shelters to smaller groups may have beneficial effects on aggression[33], although this has not been studied in males or in isolation from other factors.

Finally, evidence extrapolated from non-home cage data and/or other species suggests other housing and husbandry practices that may mitigate aggression: ensuring physical comfort, providing adequate pain control, using handling and identification methods that minimize stress and pain, and avoiding exposure to potential endocrine disruptors.

But mice still fight: possible explanations that have not been investigated

The environment of laboratory mice is extremely far removed from their ecological niche, and as such they are exposed to highly unnatural stimuli and prevented from performing many species-typical behaviors. In addition to the obvious spatial restriction, laboratory cages also lack the complexity of a natural environment. Wild mice spend a large amount of time searching for and hoarding food, but food is easily accessed and provided *ad libitum* in the laboratory. Mice burrow, but are typically kept on a minimal amount of unsuitable substrate that discourages burrowing. They are highly motivated to build nests, but are often kept with such small amounts of nesting material that formation of a fully enclosed nest is impossible, despite ambient temperatures being set below the mice's thermoneutral zone. These examples clearly illustrate that laboratory mice are routinely exposed to stressors and prevented from performing a wide range of their natural behaviors.

Captive environments elicit natural behaviors that are attempts by the animal to gain control over their situation, such as foraging if hungry, hiding if scared or nesting if cold. Animals that cannot perform highly motivated behaviors or control their environment can experience frustration, stress and boredom, which can lead to disturbed social behavior and increased aggression[34,35].

Furthermore, laboratory mice lack social control; they cannot choose their social group or escape from conspecifics. Mice may form dominance hierarchies as a way to cope with enforced proximity with conspecifics and avoid escalated aggression[36]. Formation of dominance hierarchies is predicated on submission, which may involve submissive postures, exiting line of sight of the mouse displaying dominant behavior or outright fleeing. The last two of these three methods to show submission are impossible in non-enriched laboratory cages. The remaining response to a threat might then be for the mouse to fight or to be attacked for failing to leave. An animal's control over its environment and how the lack thereof might contribute to aggression has not been examined.

Disturbance, pain and other aversive stimuli. Disturbing mice for experimental or management procedures may contribute to aggression. For example, mice are nocturnal and light sensitive, but are kept in brightly lit vivariums and are typically handled or otherwise used in experiments during the light phase, when they should be asleep[37]. A disturbed sleeping pattern can lead to stress, frustration and aggressive behavior[38]. Although cage cleaning is known to lead to flare-ups in aggression[22,39], the effects of other forms of disturbance have not been studied.

Aversive stimuli may also induce aggression. Mice sometimes turn and bite when held by forceps[40] or by the tail, and electric shocks can cause one mouse to attack another[41]. Fearful animals may become aggressive when they cannot escape, but fear may also suppress aggression, at least toward intruders[42]. Painful, frightening and aversive stimuli are routinely imposed on laboratory mice. Even standard procedures, such as ear marking, can be painful to mice[43], and mice lifted by the tail (a routine handling technique) avoid contact with humans and are more anxious when compared with mice lifted by using a tube[44]. Still, the effects of such common procedures on aggression under normal husbandry have not been investigated.

Effect of resource distribution. Environmental enrichment is potentially a powerful means of ameliorating many of the problems listed above. Additional cage furnishings, such as running

wheels, shelters, nesting materials and burrowing substrates could allow captive mice to engage in many highly motivated behaviors and to exercise some control over their environment and their exposure to aversive stimuli. However, as mentioned above, several studies have found that enrichment can increase within-cage aggression in male mice[19,45,46].

It is possible that insufficient enrichment is to blame. Only a small number of enrichment items fit inside a conventional mouse cage. Scarce, but important, resources are highly valued, and animals will compete over resources that they can monopolize. However, if resources are provided in abundance and/or spread out, one animal may not need to or be able to defend all of them. The extent to which food resources are clumped and defendable has already been shown to affect social organization in free-ranging mice[17] and many other species[47]. Given that most cages used in laboratory environments are otherwise barren, the enrichments provided in past experiments may have been very valuable and thus defended. Female mice fight less if enrichment items are dispersed[34], but the effects of providing multiple, dispersed enrichments on within-cage aggression have not been investigated in male laboratory mice.

Are lab mice deficient in social communication? Laboratory mice are usually weaned abruptly and at an unnaturally early age[48]. Early weaned mice may lack certain communication skills, and this could conceivably influence aggressive behavior[49]. In addition, some strains of laboratory mice are blind or severely visually impaired[50] and others have hearing deficiencies[51], both of which may affect certain components of communication, such as the perception of submissive displays.

However, even if early weaned mice are perfectly capable in social communication, does their social and physical environment allow them to communicate effectively? Mice use olfactory cues for communication and individual recognition[52]; however, the majority of mice used in biomedical research are inbred, and such genetically identical individuals may be difficult to distinguish by olfactory signals alone. Lack of individual recognition could potentially prevent or disrupt the formation and maintenance of dominance hierarchies, but has also been suggested to lower aggression between males of the same strain, as they are recognized as close kin[53]. The short-term effects of cage cleaning on aggression also seem to be modulated, at least in part, by removal or disruption of these odor cues. In addition to olfactory cues, the laboratory environment may also disrupt some visual cues. The visual range of mice is shifted toward short wavelengths, relative to that of humans, and includes the ultraviolet range[54]. Given that mouse urinary cues are visible in the ultraviolet, the lack of ultraviolet light sources in the laboratory may impede some forms of communication[55].

Discussion and conclusion

Laboratory mice live a fundamentally unnatural existence, with a housing environment unlike anything most would experience in the wild. For some social groups, these circumstances may be essentially incompatible with peaceful coexistence, but it is difficult to establish which components of captive life contribute to aggression. Instead of focusing on solving aggression as an isolated issue, the ultimate way forward might be to consider alternative ways to house, handle and experiment on mice that will take their natural behavior into account and give them opportunities to control social interactions with conspecifics.

A major part of the effort to make captivity more suitable for mice will be providing environmental enrichment. In a way, animals fighting over enrichment is a good sign: it shows that the resource provided was highly valued as a result of its rarity. The solution to fighting over a rare resource is to increase its abundance; providing more resources may decrease aggression[34]. Multiple resources may not only reduce competition, but also have other independent effects on aggression. For instance, although it is not possible to escape from the cage, shelters or visual barriers could allow mice to hide from a threatening conspecific.

Van Loo *et al.*[14] described abnormal levels of aggression. But how do we define what is abnormal? Everything we know about wild mice suggests that it is in fact the lack of aggression that is abnormal. This might explain why aggression is so unpredictable. We may have unwittingly created a perfect storm of unnatural cues that combine to suppress aggression when it would normally occur. Thus, changes in husbandry that appear benign to us may disrupt this fragile equilibrium with aggression, resulting in the resumption of normal mouse behavior.

Alternatively, the levels of aggression seen in the laboratory environment might be viewed as normal reactions to an unnatural situation in which the animal's ability to successfully cope is constantly challenged. In this scenario, we have managed to house mice in conditions adequate to suppress aggression, but again, a small perturbation may be the straw that breaks the camel's back. If so, there may be large-scale changes that further reduce the stress on the animals and give them enough resiliency to effectively control aggression. Although this is the scenario implicitly assumed in most existing work, it has been markedly ineffective at finding a reliable solution; to our frustration, we generally find ways of making aggression worse, not better.

Whichever scenario turns out to be correct, it may be impossible to completely remove aggression between mice while at the same time continuing with business as usual. It might be the case that fighting will persist if mice are housed and treated the way they are in laboratories, as they are not given the possibility to express a full repertoire of natural behaviors that would include those that would normally reduce fighting. If this is the case, truly prioritizing animal welfare and scientific validity may mean we must seriously reconsider the present way of housing laboratory mice.

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