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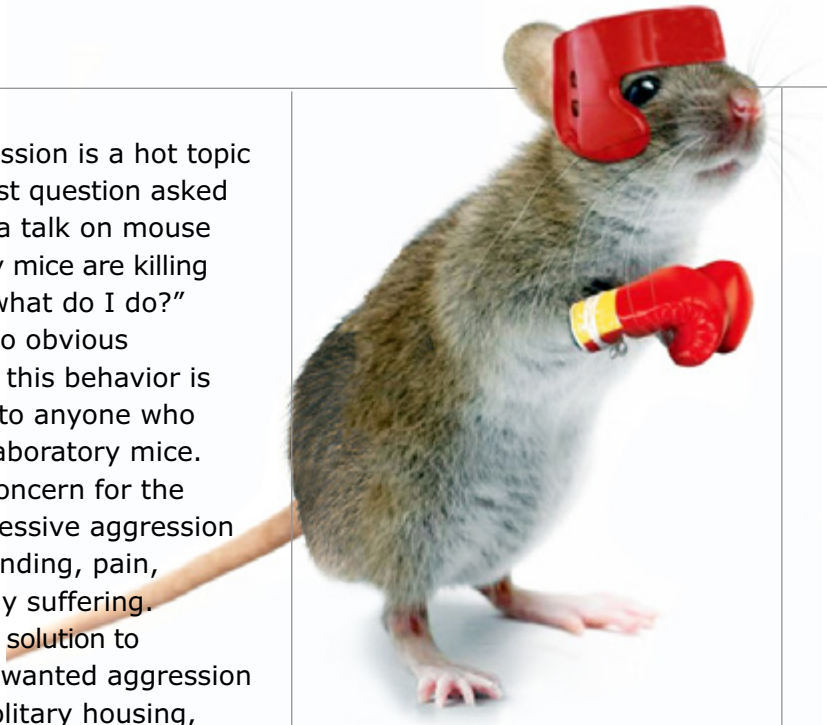
Aggression in Laboratory Mice: Potential Influences and How to Manage It



Mouse aggression is a hot topic and is the first question asked when I give a talk on mouse behavior. "My mice are killing each other, what do I do?" There are two obvious reasons why this behavior is so troubling to anyone who works with laboratory mice. The first is concern for the animals. Excessive aggression leads to wounding, pain, and inevitably suffering. The common solution to alleviating unwanted aggression is through solitary housing, which is likely to cause other sources of stress for mice[1], which are social animals. Second, is concern for the science. Aggression, pain, and social isolation can alter physiological parameters, such as circadian rhythm, glucocorticoid levels, and immune function[2-6], creating variability and issues of scientific validity. The best solution for aggression is prevention instead of reaction. However, before aggression can be prevented, we need to know what the purpose of the behavior is, and what causes and influences it.

Aggression is a natural behavior related to territories and the resources they contain[7-8]. Resources could include food, a water source, or access to breeding females. The spatial and temporal distributions of these resources strongly influence the development of territoriality even within the same species. If resources are in short supply or spread out, this will increase the amount of space needed to support an individual or group[7]. For instance, territory size can be as large as 80,000 m^2 for mice in wheat fields where resources are patchy or scarce[9] and as small as a few square meters in mice living commensally with people where high quality resources are frequently found[10]. Because of the stability of resources found in close proximity to humans, commensal territories remain relatively stable and are more vigorously defended compared to wild territories where it is more difficult to patrol and defend vast areas[10]. Other aspects of the environment, such as temperature, influence the rigidity of territory boundaries[11]. Dominant mice owning a territory are likely to tolerate an outsider when temperatures are low and both mice benefit thermally from huddling[11]. Similar results have been found in laboratory mice; less aggression occurs in cooler temperatures[12]. Unfortunately, laboratory temperatures already induce cold stress[13-14], which can effect various physiological systems altering scientific results[15-18]. Thus cooling laboratories further is not a solution to reducing or alleviating aggression.

The natural history of the wild house mouse also provides insight into motivations and needs of the domesticated laboratory mouse. Vulnerability to predation has shaped the behavior and life history strategies of mice[19]. They are nocturnal, showing peaks of activity at dawn and dusk[20-22], and avoid brightly lit and open spaces[19,23]. Mice live in complex and variable social systems.

They generally live in loose kin groups called 'demes' which include a male, 1-2 breeding females (which are usually related), subadults, and pups[10-11]. One male owns a territory but other adults, including females, will defend it fiercely[11]. Groups of bachelors can be found when dispersion is restricted or there are no available territories to claim[11]. These animals tend to be subordinate to animals holding territories and relatively more active during the day when territory holders are asleep[11].

As a social animal, mice exhibit many types of behaviors that maintain social structure. Affiliative behaviors, such as grooming others, function to strengthen social bonds. Mediated aggression is an agonistic interaction which solidifies dominance hierarchies but avoids fighting. An example of this is a dominant mouse mounting a subordinate, eliciting a submissive behavior from the subordinate. This type of aggression can account for up to 15% of daily activity[24]. If a subordinate responds to a threat with aggressive behavior instead of submission, the mice engage in escalated aggression. This particular type of aggression can result in bite wounds, castration, or death if a mouse does not display an appropriate subordinate signal to end the fight[24-27]. In the wild, aggressive interactions between territory holders (dominant mice) and intruders (subordinates) consist of a frontal attack by the owner and the intruder fleeing. Chasing ceases once line of sight is broken or the intruder has left the boundaries of the territory[11]. Since exiting a territory or breaking line of sight is difficult to achieve within the confines of a standard laboratory cage, chasing duration may be exacerbated in the laboratory. Interactions between two territory holders are more likely to result in injurious fighting[11], but this is unlikely to happen in the laboratory, where territories are limited to cages. Inter-male dominance hierarchies are found, again when territories are restricted[11]. Van Loo et al.[28] found that group size influenced agonistic interactions and wounding more than housing density. In that particular study, conducted with one inbred strain, groups of 5 or 8 mice had more wounds than groups of 3[28]. Dominance hierarchies in large groups of mice are less stable[29], thus resulting in more status fluctuations and likely escalated aggression. Kinship and familiarity have also been found to reduce aggressive social interactions[30-31]. Aggression toward strangers has been documented as early as 32-36 days of age[32]. Therefore, it may be beneficial to keep siblings together throughout life or combine unrelated groups before this age.

A physical structure or cover is necessary for territory formation[11,33]. Aspects of physical structures in the environment are utilized by wild mice to ambush intruders through holes, choke-points, and elevated platforms[11]. Research on the provision of retreat spaces in laboratories has produced mixed results. Cardboard tubes have been found to reduce wire-gnawing stereotypies[34] but the authors could not directly attribute the reduction to the fact that the mice utilized the tube as a retreat space since the tube was also used as nest building substrate. Nest boxes and hard plastic shelters are very popular, easily cleaned, and make the cage seem "enriched". These structures generally increase male aggressive interactions[24,35] but have also been found to increase longevity[35]. Providing a shelter increased aggression, indicating that mice perceived this item as a coveted resource that should be defended[24]. Howerton et al,[24] found that shelters increased the incidence of escalated, injurious aggression and destabilized the dominance hierarchy within the cage. This study and only a few others[36-37] have utilized home cage behavioral monitoring instead of separating, disturbing, or placing mice in an unfamiliar

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setting. When aggression is measured after disturbing or stressing the mice or altering the environment, this may potentially be measuring different types of aggression or motivations, making comparisons to normal husbandry difficult[24].

Nesting material is preferred by mice over nest boxes[38] and mice are willing to work to gain access to it[39-40]. Different materials can be manipulated and combined to create a flexible structure that decreases cold stress and provides both structural and occupational enrichment for mice. Nesting material and its transfer at cage change has been found to reduce aggressive interactions[31,41]. It is important to note that one study did report increased aggression when mice were provided with compressed cotton nesting material[42]. However, the mice in that study did not spend much time in contact with the material and usage was not described. The mechanism behind the mitigation of aggression by nests has not been specifically tested. Van Loo et al[31] postulate that specific pheromones deposited within the nest may be the reason for this behavioral response compared to behaviors seen after the transfer of soiled bedding. This is not surprising since scent and pheromones play such an instrumental role in rodent communication[43-44]. Urinary pheromones are used to determine the "maleness"of rivals, mark territory boundaries, and can elicit aggression[43-44]. Importantly, nest sites are kept clean of urine and feces and perhaps free of these aggression eliciting pheromones [31,45].

Another potential influence on mouse aggression is weaning and the early life experiences leading up to it. In the wild, weaning is a gradual process. Pups begin to eat solid food between 10-17 days of age and nursing is significantly reduced after 21 days[10,46]. Maternal interactions, however, can continue up to 4 weeks after pups are born. Even after pups are weaned, they are likely to remain in the natal nest until sexual maturity, continuing to interact with adult females and the dominant male. Curley et al.[46] found that pups weaned at 28 days were more likely to engage in longer bouts of social interaction than pups weaned at 21 days of age. They also found that pups were increasingly mounted by their dam as they neared weaning[46]. This is likely the adult demonstrating their dominance over the pup, while exposing the pup to social cues. Whether pups need this exposure prior to separation to properly react to certain social cues has not been formally tested. If it is necessary, it's possible that mice without those experiences do not understand how to stop aggression before it escalates to injurious fighting. One study did find that pups weaned early (14 days of age) had more wounds than pups weaned at 21 days[47]. This particular study, unlike normal husbandry, isolated mice for 4 weeks before regrouping them with their wean group. Therefore, these isolation-induced results may not be directly applicable to aggression seen in continuously housed mice.

Aggression is a naturally occurring behavior of mice that can provide benefit to those that display it in the wild[11]. Many factors appear to influence these negative social interactions between mice, making mitigation in the laboratory more complicated. Based on the current literature and the natural history and motivations of mice, a few management strategies may help keep escalated aggression in the laboratory to a minimum[41]. First combine small stable groups at 3-4 weeks of age, so they are familiar with one another prior to puberty. Avoid providing mice, males in particular, with items or enrichments that can be monopolized and guarded by dominant mice, such as rigid shelters. Last, transferring nesting material at cage change may maintain some olfactory cues related to identification without transferring aggression-eliciting pheromones.

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