

Background of male mouse aggression

Social structure

One dominant male, multiple adult females with their offspring, subordinate males at the margin of territories using resources, whilst dominant male sleeps. Social hierarchy is reinforced with urinary and plantar gland odour cues.

Senses

Smell is a highly developed sense for mice, with a large proportion of olfactory receptors. Auditory cues are also significant, with upper range outside that of humans.

An important consideration as whilst we can hear some mouse communications those squeaks are at a low frequency and are used for long range communication. The high frequency short range ultrasonic calls we will not hear.

Territory

Size varies by resources e.g. farm Buildings <3m² to sparse natural habitats >100m². Challenge for territory would see the submissive males leave the area. This is not possible in a cage environment, resulting in repeated bouts of territorial challenge and/or social defeat resulting in fighting and to learned helplessness. (Ref [1 & 2](#)) (Fig. 1).



Fig 1.
Submissive mouse posture showing upright stance, 'freezing' and allowing other mouse access to vulnerable area.



Fig 2.
Signs of contented nest building and house sharing.

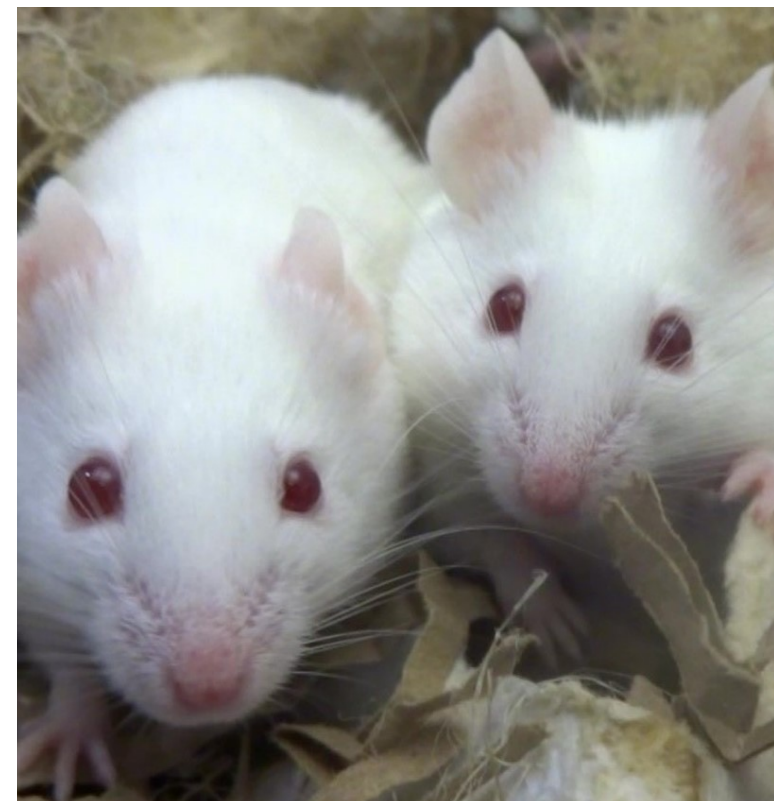


Fig 3.
White hairs and coat damage due to chronic fighting.

Footnotes

*Disinfectant agents, for example Quatricide, can have an intrinsic odor that can act as a deterrent to rodents. Caution should also be taken with harsh cleaners such as hydrogen peroxide which can damage acrylic and other plastics. Water is commonly used because it has no odor itself but may not be the most effective option for removing odors and microbes. Ethanol (70%) can be used but care must be taken to ensure that it has evaporated completely before placing an animal in the apparatus. Super oxidized or super hypochlorous water, has been demonstrated to be an effective odor removal agent with a weak intrinsic odour.

Mitigating male mouse aggression



Introduction

Mice are the most used species for AstraZeneca work, both internally and externally. In line with our 3Rs and Culture of Care commitments we are committed to refinement of all our animal work and have shared practices across the industry. There is no simple set of industry wide recommendations across laboratories.

This leaflet aims to help share background alongside examples and practical advice from our experience to help reduce the risk and impact of male mouse aggression; for use internally and with our external colleagues.

Aim is for a contented cage

Calm, no vocalizations, well-formed single nest, good clinical condition of individuals (body weight/condition score, coat condition) mice are happy to eat, drink and groom together/ near to cage mates (Fig 2.)

Signs & sequence of aggression

Signs of incompatibility (cage without single nest, multiple latrines, urine-soaked nesting material scattered in cage) will usually precede overt aggression signs (direct injury, broken nails and swollen digits, blood observed in cage and/or indirect body weight variances, poor coat condition or white hairs from chronic fighting – Fig 3.).

Sequence of escalation

1

Short infrequent bouts to remove male from territory. Low level of injury

2

More regular bouts, wounds across body often hidden by fur. Swollen digits and broken toenails.

3

Frequent targeted injuries to lower back, tail and genitalia to emasculate and render infertile.

Practical factors to reduce aggression

Before (at breeding facility)

- **Strain** – know the tendencies for the strain you are going to work with. Broadly outbred strains (eg CD1) are more prone to aggression than inbred strains. Transgenics often more so than their background strain, may see earlier age of onset too. Disease phenotypes can directly reduce tendency for aggression (i.e., due to impaired health or activity in obese strains) or indirectly impact likelihood e.g., reduced testicular size in BTBR homozygous mice. Be aware of over representation of cases e.g., nude mice; physical injury is easier to spot.
- **Age** – Compatible groups are more successful from a pre-sexual maturity age (Ref 3), compatible with scientific aims and statistical design animals should be ordered and grouped <6 weeks of age.
- **Transport** – length and mode of transport influence triggers for aggression. Mice need to recover in acclimation, which should be extended if transport stress is high at point of unpacking.

After (at point of study)

- **Caging density** – Literature varies with groups sizes from 3 to 11 males per cage (Ref 4–6)
- **Randomisation** – A known trigger due to change of social structure/harmonious groups. Consider statistician advice on whether other strategies are possible e.g., by cage rather than individual.
- **Cage enrichment** – Bedding, nesting and chewable items are our minimum standards. Some literature notes enrichment can increase aggressive behaviours; If noted, then more rotation of type or increase volume should be given to reduce undesired behaviours.
- **Cage change** – Commonly triggers territorial behaviours as olfactory cues need to be re-established. Efforts need to focus on reducing this stress and the volume of cues lost e.g.
 - Retain nesting material and recreate same layout at cage change.
 - Where nesting needs to be replaced rub the positive cues from previous nesting material to new cage
 - Change gloves with urine and plantar scenting between female/male mice handling and between cages. Ensure gloves cover cuffs of clothing.
- **Holding area** – as aggression is linked to increased disturbance (Ref 2) consider location of holding cages and movements to quieter areas to reduce noise/traffic. Also consider increased distance between genders where able.
- **Procedures** – acclimation (to facility, staff, practices), habituation (to routines, equipment, and procedures) and positive training programs are key parts of stress

reduction, and reduction of aggression. Equipment can retain olfactory cues from other mice so must be carefully cleaned* to reduce risk between cages of mice. Especially important where heat can increase scent effects e.g., warming chambers.

When to intervene

Intervening	Monitoring
• Persistent observed fighting • Obvious injuries • Lameness • Non healing bites • Dehydration • Body weight loss	• Mark mice to help identify aggressor • Keep cage records. These are useful for continual assessment. Indicate cages of concern. • Take photos (name and date these) of mice and bite marks/injuries – these should show improvements if fighting has stopped. • Body weight is a useful indicator. Weight loss or failure to gain weight when other mice in the cohort are doing so may give you further information. • Lack of nest formation and chaotic structure is another warning sign. • No obvious latrine.

Key learnings

- Increase efforts earlier for better outcomes (more observations to understand group dynamic, systematic review of signs seen for known triggers to ensure nothing missed)
- Do not assume harmonious group will remain so through sexual maturity, or routine change e.g., study procedures – focus efforts on known risk points.
- Consider dominance/aggression for clinical signs other than just fight injuries (body weight variance, urine soaked/scattered nesting, cage disarray)
- Early stages may not need single housing by default – try to correct triggers whilst monitoring welfare of individuals.
- If you alter group structure increase observations to ensure new dominance hierarchy established smoothly (this will involve short bouts of dominant/aggressive behaviour but should be low level and resolve within four days)