

Minimising aggression in CD-1 and CD-1 background male mice with different enrichment types

AMY VENESS, CHIS COYLE, SIAN MURPHY, JAMIE REDDEN,
TINA O'MAHONY and ELENI M AMANITI

Sainsbury Wellcome Centre

Correspondence: a.veness@ucl.ac.uk

Based on a poster displayed at IAT Congress 22

Introduction

Cage-mate aggression is a well-known problem that is more prevalent in certain strains of mice and is one of the most common reasons for single housing, increased stress levels, premature death and euthanasia. It is known in the Animal Technology field that there is a higher-prevalence of aggression in strains with the following lineage; C3H, CBA and CD-1 (Lidster, K *et al.*, 2019).¹

From a retrospective review of health conditions between April 2020-April 2021 approximately one third of male mice on a CD-1 background were single housed due to aggression and fight wounds (Figure 1). This was observed both in CD-1 background males generated in the facility and those ordered in from an external supplier.

Numerous methods can be implemented to ameliorate and reduce aggression, including; reducing cage density

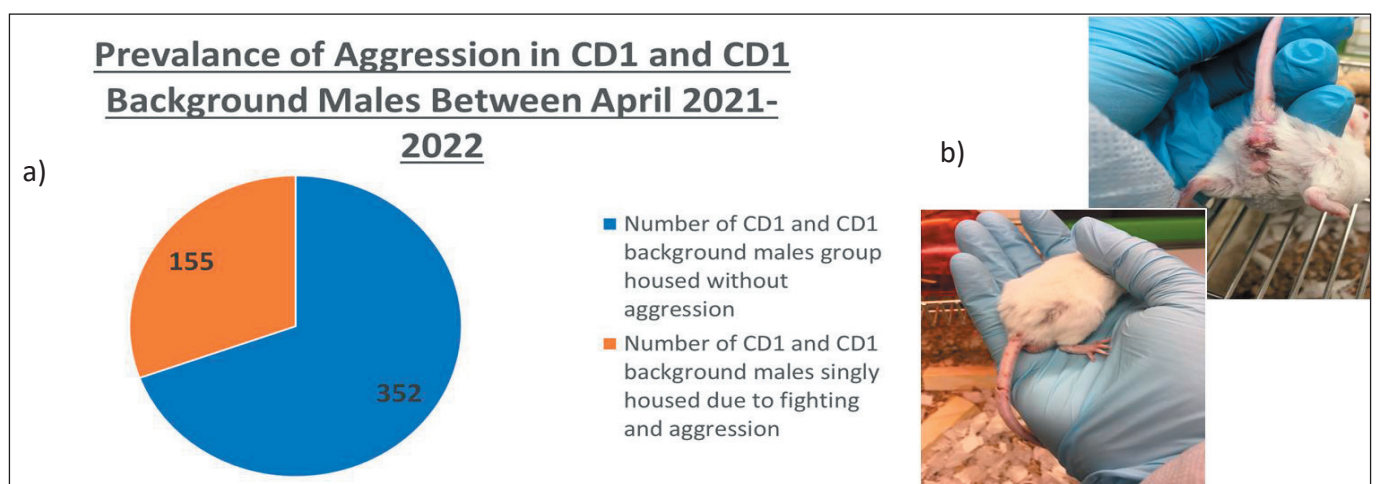


Figure 1. a) A pie chart of the amount of singly housed CD-1 and CD-1 background males b) Examples of fight wounds.

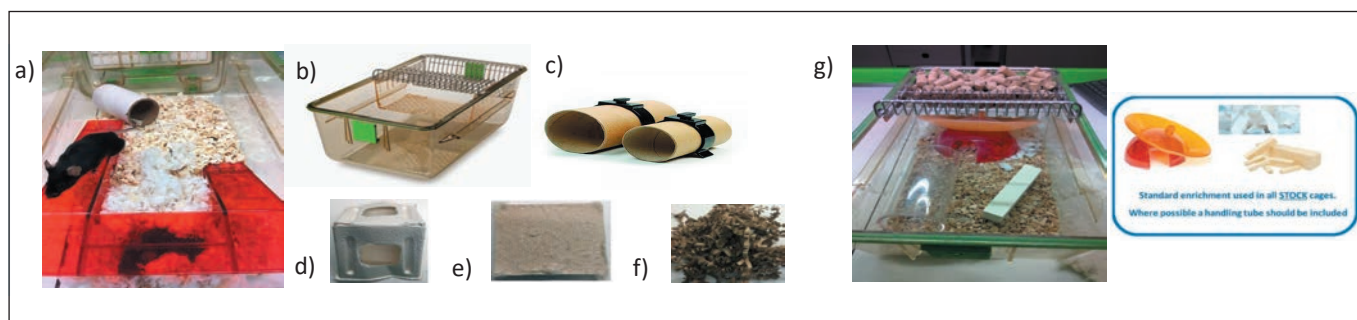


Figure 2. Examples of enrichment: a) Mezzanine. b) Mouse balcony. c) Tunnel clips. d) Cardboard house. e) Nestlet. f) Sizzle nesting. g) Standard enrichment used at the Sainsbury Wellcome Centre.

(number of mice per cage), altering cage cleaning routines and changing environmental enrichment. It is considered throughout the Animal Technology industry that single housing should be the last resort. Several combinations of environmental enrichment can be trialled, including tunnel clips, mezzanines, mouse loft/balconies and different nesting materials such as sizzle nesting and nestlets (Figure 2).

Method

- A data review between April 2020-2021 and April 2021-2022 was conducted using the animal facility software PyRAT. The criteria used were: 1) CD-1 (supplied by Charles River) and CD-1 background males, 2) Having the condition of ‘fight wounds’ and/or ‘aggressive’, 3) Single housed males. The total number of CD-1 and CD-1 background males included in this study was 507 (April 2020-2021) and 458 (April 2021-2022).
- Between February 2021 and April 2021 numerous environmental enrichments (Figure 2) were trialled/added to cages of CD-1 and CD-1 background males.

In April 2021, 10g of sizzle nest and 1 nestlet were added as part of standard enrichment to all cages housing CD-1 and CD-1 background males.

- Fisher’s exact test was used to determine if there was a significant difference between rates of aggression during April 2020-2021 and April 2021-22.

Results

- Tunnel clips, mezzanines and mouse loft/balconies had minimal effect on reducing observed aggression rates, therefore the use of these enrichment types was discontinued.
- Adding sizzle nest and a nestlet to cages with CD-1 and CD-1 background males considerably reduced the aggression rates and the amount of single housed males (Figure 3).
- The decrease of 27.51% in the aggression rates, observed in CD-1 and CD-1 background males between the two years, was statistically significant ($P < 0.0001$) (Figure 4).

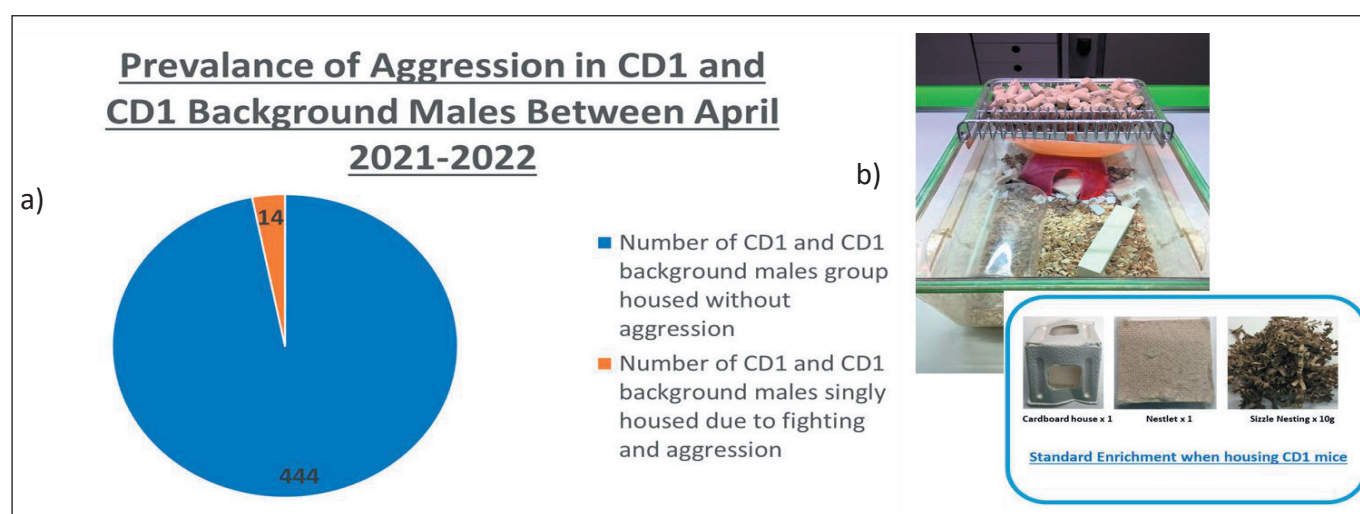


Figure 3. a) A pie chart of the amount of singly housed CD-1 and CD-1 background males. b) Example of a cage with sizzle nest and a nestlet and our current enrichment for CD-1 males.

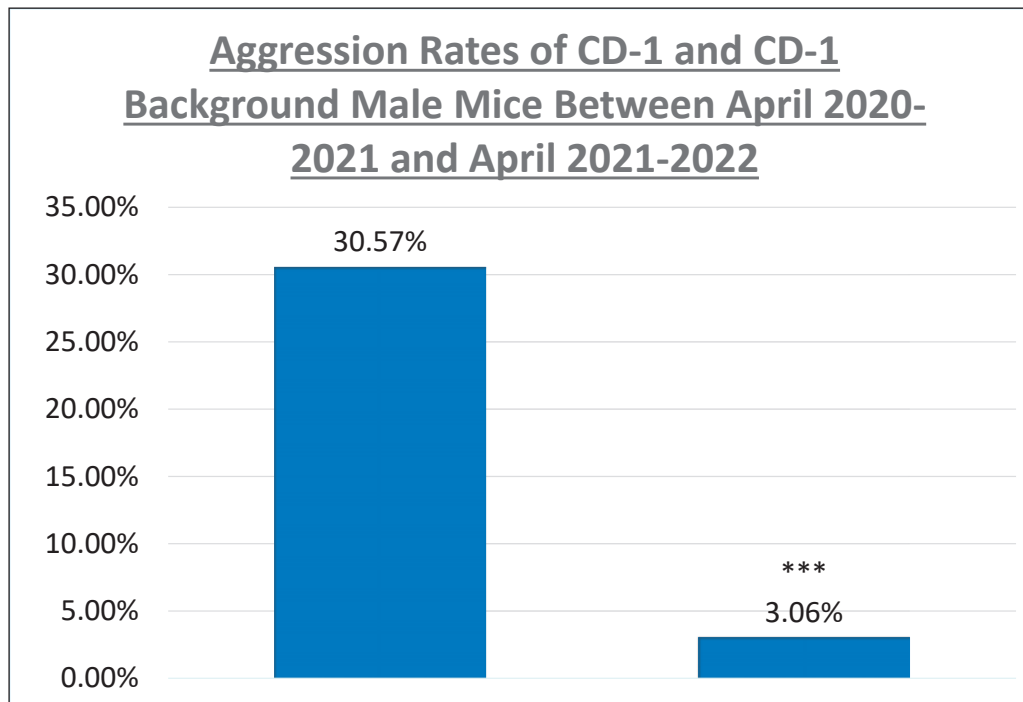


Figure 4. Bar chart showing the rates of aggression, Fisher exact test (***) $P < 0.0001$.

Discussion

Previous literature has suggested that certain enrichment such as wheel-igloos may increase aggression in CD-1 males due to them becoming territorial over it, however when used in conjunction with nesting material this aggression was reduced (Howerton, C.L *et al.*, 2008).² This may explain why there were higher levels of aggression seen between April 2020-2021, but the aggression was reduced after adding sizzle nest and nestlets.

These results support current literature that shows manipulatable enrichment reduces aggression seen in CD-1 and CD-1 background mice (Weber E.M., 2017: Jirkof, P, *et al.*, 2020).^{3,4}

This study accomplished its aim to reduce the aggression rates in group housed CD-1 and CD-1 background males and reduce the number of males single housed. In turn, Animal Welfare has increased as well as reducing the requirement for resources in the facility. Due to the results of this study, adding sizzle nest and nestlets to cages and strains showing aggression has become standard practice.

Acknowledgements

Yoh Isogai and NRF Team – Aaron Donaldson, Amy Lynch, Ben Foster, Georgie Spibey, Molly Pettengell, Olivia Adams, Rosie Keenan, Sandra Stojkovska.

References

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- ⁴ **Jirkof, P. *et al.***, (2020). Laboratory Animals. 54.2.