ARTICLE IN PRESS

Applied Animal Behaviour Science xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Applied Animal Behaviour Science

journal homepage: www.elsevier.com/locate/applanim

Breaking up is hard to do: Does splitting cages of mice reduce aggression?

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ARTICLE INFO

Keywords:
Mouse
Aggression
Wound
Welfare
3Rs
Husbandry

ABSTRACT

Injurious aggression in group housed male laboratory mice is a common welfare issue that can also negatively affect study outcomes. Often, one mouse in the cage appears unwounded, and the current standard practice is to remove this presumed aggressor. This procedure is not based on empirical evidence and may impede welfare by singly housing animals. We experimentally tested the hypothesis that the apparently uninjured mouse is indeed the aggressor, and that aggression is reduced in his absence. We separated cages of four or five male mice, reported for fight wounds to our university's veterinary service, into cages of two or three mice containing either only wounded mice ("wounded" treatment) or both wounded and unwounded mice ("mixed" treatment). We recorded aggressive behavior for 30 min immediately pre- and post-separation, and scored wound severity at separation and over two weeks after. We predicted that if unwounded mice are aggressors: mice in the wounded treatment would show less escalated aggression (involving biting) than mice in the mixed treatment, and would be wounded less and/or heal faster during the two weeks following separation. Wound scores decreased significantly after separation in both treatments (wounded: $p < 0.0001$; mixed: $p = 0.011$), but mice in the wounded treatment healed faster than those in the mixed treatment ($p = 0.006$). There was no significant effect of treatment on duration of escalated aggression in the 30 min following separation ($p = 0.240$), nor did treatment predict which cages would be re-separated due to continued aggression ($p = 0.104$). Our results support the hypothesis that the unwounded mouse is the aggressor, as mice in cages with an unwounded mouse healed more slowly than those without. Both types of groups healed significantly over time, suggesting that separation into groups of two or three is a possible management alternative to social isolation of the presumed aggressor. By identifying spontaneous cases of severe aggression in an existing colony, we obtained a heterogeneous and representative sample of clinical cases, bolstering the generalizability of our conclusions.

1. Introduction

Wounding due to aggression is the second most common clinical condition in mice, after ulcerative dermatitis (Marx et al., 2013). Effective treatment is available for the latter, but not the former (Adams et al., 2016; Weber et al., 2017). Wounding is a welfare problem in terms of health, pain, and distress, and can also affect experimental outcomes. Severe wounding can lead to unplanned euthanasia, requiring the use of additional animals. Fighting can additionally suppress the immune system thus introducing variability, increasing required sample sizes (Barnard et al., 1996; Weber et al., 2017), and creating the possibility of both false positive and false negative results (Garner, 2014; Garner et al., 2017).

There is surprisingly little literature on home cage aggression in mice (Weber et al., 2017). Warm environments, cage cleaning, transfer of soiled bedding and large cage populations are known to exacerbate aggressive behavior (Gray and Hurst, 1995; Greenberg, 1972; Van Loo et al., 2003, 2001; Weber et al., 2017). Procedures like ear punching or

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https://doi.org/10.1016/j.applanim.2018.06.003
Received 29 March 2018; Received in revised form 23 May 2018; Accepted 5 June 2018
0168-1591/

Please cite this article as: Blankenberger, W.B., Applied Animal Behaviour Science (2018), https://doi.org/10.1016/j.applanim.2018.06.003

snipping that cause mice pain may also elicit aggressive behavior (Gaskill et al., 2017; Weber et al., 2017). Effects of environmental enrichment on aggressive behavior are highly varied: for example, there is mixed evidence regarding the effects of providing shelters on home cage aggression (Howerton et al., 2008; Olsson and Dahlborn, 2002; Weber et al., 2017). Transferring nesting material during cage change and keeping siblings or familiar mice together from weaning generally decreases aggression (Van Loo et al., 2001, 2003). Male mice are more aggressive than females, and clear strain differences exist (Greenberg, 1972; Guillot and Chapouthier, 1996; Van Loo et al., 2003). Our limited understanding of home cage aggression in male mice is keeping us from resolving the problem, and its apparent intractability has led us to suggest that aggression may be the normal behavior for captive male mice, with the lack of aggression being an abnormal, albeit desirable alternative (Weber et al., 2017).

Wounding due to aggression can lead to a separate welfare issue when animals are separated and singly housed (Van Loo et al., 2003). Mice, including sexually mature males, are social animals that prefer to be socially housed (Van Loo et al., 2004). Singly housed mice show abnormal physiology and immunology compared to group housed mice (Kerr et al., 1997; Pham et al., 2010; Pyter et al., 2014; Van Loo et al., 2007, 2003). Thus singly housing mice to avoid aggression is simply swapping one welfare problem for another and one set of scientific confounds for another.

The work of Emond et al. (2003) suggests that removing the mouse engaging in the most aggressive interactions at the time of group formation can reduce the incidence of wounding, death and euthanasia. While promising, this result relies on behavioral observations prior to wounding. Veterinarians typically do not have such data when faced with clinical reports of mice with fight wounds. Current standard practice is to remove the presumed aggressor from the cage (Lockworth et al., 2015); often all mice in a cage but one are wounded, and the unwounded animal is presumed to be the aggressor (e.g. Emond et al., 2003; Marx et al., 2013). However, there is no published evidence that aggressors can actually be identified based on the absence of wounds, nor that there is necessarily one primary aggressor per cage. Without supporting data, it is unclear what the implications of being the sole unwounded mouse within a cage are. Intuitively, it makes sense for aggressors who frequently engage in fights to be most heavily wounded as a consequence of other animals fighting back: this seems to be the case among socially housed pigs (e.g. Turner et al., 2006). Unwounded mice might then be those who are able to avoid being attacked because they are particularly socially competent, or perhaps not worth competing with due to their social position. Alternatively, the unwounded mouse may indeed be the aggressor who wins every fight while avoiding injury detectable by humans – while this “Bruce Lee” scenario may seem implausible, it is the received wisdom in mouse husbandry (e.g. Emond et al., 2003; Marx et al., 2013).

The goal of this study was to evaluate best practices in separating cages of wounded mice. We aimed to test the hypothesis that the unwounded mouse is the aggressor, and that aggression is reduced in his absence. We separated cages of fighting mice into smaller groups in new cages and predicted that if the unwounded mouse is the aggressor, then escalated aggression (involving biting) would be more frequent in new cages containing unwounded mice than in new cages containing only wounded mice, and that mice housed with unwounded mice would be re-wounded more and/or heal more slowly during the two weeks following separation. Understanding the likely outcomes of cage separation would allow researchers and veterinarians to more successfully manage mouse aggression when it arises.

The approach to clinical veterinary research adopted here closely parallels that seen in human clinical research: we enrolled and attempted to treat a heterogeneous sample of animal patients spontaneously presenting with a disorder, which by definition is representative of and generalizable to the population targeted for treatment (Garner, 2014; Garner et al., 2017). The ethical cost of the study is extremely low, as it entails no additional animal use or disease induction beyond ongoing levels in our university’s facilities. We have previously used this approach to develop multiple effective treatments for ulcerative dermatitis, another serious welfare problem in mice (Adams et al., 2016; George et al., 2015).

2. Materials and methods

2.1. Animals and housing

Mice used in this study were drawn from “fighting mice” clinical cases at Stanford University – reported by caretaker staff and confirmed as fighting by registered veterinary technicians – from June 2016 through November 2016. All mice in the study were on approved Stanford University IACUC protocols; if investigators no longer wished to use these mice, they were transferred to our own approved Stanford IACUC protocol. Twenty two cases containing a total of 100 mice were housed in two AAALAC-accredited animal facilities in recyclable, individually ventilated Innocage® cages (29.5 W x 17.7 L x 12.7 H cm; Innovive, San Diego, CA) prefilled with 3.2 mm corncob bedding and were kept on a 12:12 light:dark cycle (lights on at 07:00). Environmental enrichment varied depending upon the animals’ scientific use and investigator preference. Cages were provided with either Innorichment™ nesting material (Innovive, San Diego, CA), Innorichment™ and one or two paper tubes (Custom Paper Tubes, Cleveland, OH), or a square of Nestlet™ nesting material (Ancare, Bellmore, NY) and a paper tube. Water and food (18% protein 2018 Teklad Global Diet®, Envigo, Indianapolis, IN) were provided ad libitum. After separation, wounded mice were administered trimethoprim/sulfadiazine antibiotics (TMS/Uniprim® TD6596 antibiotic diet, Envigo, Indianapolis, IN) and analgesic carprofen (5–10 mg/kg subcutaneous Rimadyl, Parsippany, NJ) as needed, determined by veterinary staff. All experimental subjects were adult males in seemingly robust health (excluding fight wounds). None displayed non-specific signs of illness, nor had obvious signs of surgery. There were seven cages of white mice, nine cages of black mice, and six cages containing a mixture of either black, white, or agouti mice. Further information concerning strain, age, relatedness, or clinical status was unavailable from the original investigators.

2.2. Experimental design

To be included in the study, cages had to contain four or five mice including at least one with visible wounds. We used this criterion so that all new cages would contain at least two animals after separation. Once a case was reported, the cage was moved to an observation bench where it was video-recorded for 30 min using a camcorder (Panasonic HC-V770 HD, Newark, NJ). Next, we individually weighed and examined animals by holding them by their tail on a horizontal surface and probing their fur using a cotton swab to identify and score wounds. We then separated animals into two new cages of two or three mice each. Enrichment and bedding were not transferred from the original cage to prevent pheromone transfer into new cages. We recorded video for an additional 30 min after this separation. Barring early separation or euthanasia, we examined each animal for fight wounds four more times, approximately every three to four days for two weeks. We followed standard institutional management practices of further separating and singly housing mice if we observed life-threatening aggression (relentless attacks) at any time following separation, or euthanized mice if they had severe wounds. In five mice who had to be re-separated and singly housed, we opportunistically continued to assess wound severity over 2 weeks following isolation to establish benchmark healing rates in mice who had no opportunity to fight and suffer fresh wounds.

At separation, we assigned mice to new cages according to a semi-randomized scheme (Table 1). We designated new cages containing