Applied Animal Behaviour Science 183 (2016) 77–85

Contents lists available at ScienceDirect

Applied Animal Behaviour Science

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

He's getting under my skin! Comparing the sensitivity and specificity of dermal *vs* subcuticular lesions as a measure of aggression in mice

Brianna N. Gaskill[a,b,*], Aurora Stottler[c], Kathleen R. Pritchett-Corning[b,d], Lilian K. Wong[c,e], Jerome Geronimo[c], Joseph P. Garner[c,f]

[a] Purdue University Department of Comparative Pathobiology, West Lafayette, IN, United States
[b] Charles River, Wilmington, MA, United States
[c] Stanford University, Department of Comparative Medicine, Stanford, CA, United States
[d] Harvard University Faculty of Arts and Sciences, Office of Animal Resources, Cambridge, MA, United States
[e] Banfield, Portland, Oregon, United States
[f] Stanford University, (by Courtesy) Department of Psychiatry and Behavioural Sciences, Stanford, CA, United States

ARTICLE INFO

Article history:
Received 30 November 2015
Received in revised form 26 May 2016
Accepted 4 July 2016
Available online 19 July 2016

Keywords:
Mouse
Aggression
Wound
Skin
Method
Welfare

ABSTRACT

Aggression is the leading cause of death in young laboratory mice, representing a major welfare issue. Many of the experimental measures used in traditional aggression research, especially those focusing on territorial aggression (e.g., resident/intruder) are poorly suited to examining dominance or abnormal aggression in the home cage. Scoring of flank and tail wounds by observers is widely used in these experimental paradigms. Scoring external skin wounds is time consuming, subjective, and the constant handling of mice involved can affect experimental outcomes. Here we describe a variety of subcuticular signs of aggression that can be observed during necropsy, and develop a "Pelt Aggression Lesion Scale" (PALS) for standardized scoring of these signs. Inter-rater and test-retest reliabilities were assessed and were excellent (0.84, and 0.96, respectively). PALS showed significantly greater sensitivity (in terms of detecting unusually aggressive cages) and less error variance than external wound scores. PALS showed convergent validity with external wound scores (*i.e.* each could predict the other), and also discriminant validity (in that only PALS subscores for the posterior of the pelt, where aggressive biting is primarily directed, predicted external wound scores). Finally PALS shows specificity, in that PALS and its subscores did not generate false positive results when animals with ulcerative dermatitis were examined. Thus PALS is a reliable, sensitive, specific and validated measure of aggressive wounding in mice that avoids many of the confounds of traditional methods, is high-throughput, easy to perform, and particularly well suited to home-cage and welfare-related studies but limited in its use for behavioural management interventions.

1. Introduction

Aggression in laboratory mice is a significant problem that affects not only the welfare of animals, but the outcome of studies. Aggression is the second most common morbidity (after ulcerative dermatitis) in mouse facilities, and the most common morbidity in young mice (Marx et al., 2013). Aggression is also an important behavioural phenotype in mice, and varying aggression levels are recognized as strain background characteristics (*e.g.*, FVB, SJL) as well as associated with a variety of genetic manipulations (Nelson and Chiavegatto, 2000). While aggression is not a trait limited solely to male mice, males are generally more likely to have agonistic interactions that affect health and welfare. However, male mice are generally not indiscriminately aggressive, attacking anything nearby – rather, aggression is a strategic choice made by animals (Grant and Mackintosh, 1963; Crowcroft, 1966; Van Zegeren, 1979; Pocock et al., 2004; Howerton et al., 2008; Koolhaas et al., 2013), and given the right environmental and behavioural cues, mice establish dominance hierarchies which often allow them to live in bachelor groups (Crowcroft, 1966; Van Loo et al., 2000, 2001; Hurst, 2005). As granting agencies make clearer statements on the importance of including both sexes in studies (Clayton and Collins, 2014), and regulatory and oversight bodies continue to emphasize the importance of social housing (Institute of Laboratory Animal Resources (ILAR), 2010), aggression in mice is likely to continue to affect science.

* Corresponding author at: Animal Welfare Department of Comparative Pathobiology Purdue University 625 Harrison St G 170B West Lafayette, IN 47907, United States.
E-mail addresses: brikil@hotmail.com, bgaskill@purdue.edu (B.N. Gaskill).

http://dx.doi.org/10.1016/j.applanim.2016.07.001
0168-1591/

Measuring aggression in mice is challenging (Nelson and Chiavegatto, 2000; Howerton et al., 2008). Conventional measures, such as counting skin wounds through the fur, fall short in several important ways. Handling non-habituated mice is a stressor (Hurst and West, 2010) and the manipulations necessary to count visible wounds are likely to increase stress further. Mice may or may not acclimate to repeated handling, and acclimation changes behaviour: a classic case of the measurement changing the measured quantity. Daily handling is also impractical for longitudinal studies. Measuring aggression by observing animals interacting in the home cage is the gold standard, but this is also labour and equipment intensive. In addition, home cage observation is better suited to "*what kind of?*" questions ("*What kind of aggression is being exhibited by these mice?*") (e.g. Howerton et al., 2008) but is often overkill when applied to "*how much?*" questions. However, simpler experimental measures of aggression tend to be specific to particular kinds of aggression (*e.g.* the resident intruder test measures territorial and impulsive aggression) (Nelson and Chiavegatto, 2000; Koolhaas et al., 2013), rather than providing a general overall measure of aggression in the home cage more suited to examination of multifactorial welfare or management questions. Therefore, our goal was to develop and validate a simple, easily quantifiable measure of aggression in mice that would not depend on daily handling, be reliable both between and within observers, and allow us to determine how much aggression was occurring within groups of male mice.

Scoring wounds in some fashion are routinely used as a marker for severity of aggression (Litvin et al., 2007; Koolhaas et al., 2013). In our previous work, examining live mice for wounds proved to be difficult and time-consuming, and we have become increasingly concerned that, as shown by (Van Loo et al., 2003), the excessive handling might affect aggression. We suspected that the difficulty in examining the epidermis through the fur might over-emphasize animals that were severely wounded, and underplay those with minor wounds caused by less dramatic agonistic encounters. Examination of a live animal is difficult to perform consistently and systematically, with no two examiners reliably following the same pattern of exam, and individual wounds are easily overlooked. As a routine part of our studies, animals were sent for necropsy, during which we noted that the wounds found on the animals were much more visible in the subcutis, rather than through the fur.

The measure we devised, the Pelt Aggression Lesion Score (PALS) is based on these findings. We refined the method by standardizing the removal of pelts and the counting of injuries. We checked reliability by comparing both inter-and intra-rater reliability. Finally, we validated the measure by examining the sensitivity and specificity by correlating with externally visible fight wounds and by comparing PALS to other skin wounds, most notably ulcerative dermatitis. Thus the current study had two goals: (1) to validate the PALS measure; and (2) to test whether these subcuticular lesions (and the PALS score) were a record of chronic aggression in mice.

2. Materials and methods

All work was conducted at either Stanford University's or Charles River's AAALAC-accredited facility. Work conducted at each institution was approved by that institution's IACUC.

2.1. Behavioural pilot data

2.1.1. Animals and housing

In order to determine if escalated aggressive behaviour was correlated with wounding, we used archival behavioural data from an experiment using C57BL/6NCrl mice. Eight cages of 4 male mice were housed in standard micro-isolator shoebox cage (PC7115HT; Allentown Inc., Allentown, NJ USA) for 14 days. Mice were housed with Sani-chip hardwood bedding (vendor, location), had *ad libitum* food (Teklad 2018; Harlan, Indianapolis, IN USA) and water and were kept on a 12:12 (light:dark; lights on at 07:00). The cages were filmed continuously for 14 days using day/night cameras (720 × 480 resolution). Mice were examined for fight wounds by picking up the mice and brushing the fur backwards using a Q-tip. Wounds were counted and their character noted (scabbed or fresh) daily at 10:00 AM. The video was divided into 24 h periods between each examination for fight wounds. Only the 24-h period preceding the first sign of fighting was examined. A sign of fighting was defined as wounds for two or more mice in a single cage being observed. If a single mouse was injured, it might be due to cage conditions or a carry-over from aggression at the breeder or during shipping. By waiting until we saw at least two mice wounded, we could be certain that we would observe fighting in the video. For cages in which no wounds were observed, the first 24-h period the mice were housed together after being shipped to Stanford was examined. The video was watched at high speed until fighting was observed. The video was then rewound and each fight was re-watched in slow motion or on a frame-by-frame basis. The time each fight occurred was recorded and each mouse in the cage was scored using one/zero sampling for either giving or receiving an attack during the fight.

2.2. Reliability and validity

2.2.1. Animals and housing

The animals used in this study were drawn from ongoing aggression studies, or from animals euthanized for various morbidities by the veterinary services group at either Stanford University or Harvard University. In particular, we used data from a study conducted at Charles River where C57BL/6 male mice sourced from Charles River (CRL), Taconic, The Jackson Laboratory, and the National Cancer Institute were tested for prevalence of injurious intermale aggression. Although all mice tested in this manuscript were C57BL/6 strain mice, mice from different vendors have been separated long enough that they are now substrains of C57BL/6 mice and are known to have divergent behaviour and physiology. Substrain identification is not relevant to this methods paper but will be addressed in separate work. Lesions were assessed in the CRL study by brushing back the fur, similar to the behavioural pilot, but with the scorer's finger. All mice were individually marked on arrival by ear notch to follow individuals over the 2 week study and document the presence or absence of wounds on a daily basis (10:00 AM).

Animals were free of a list of common mouse pathogens on arrival; further details may be found at each vendor's health monitoring webpage. No further infectious agent testing was conducted. On arrival, at 7 weeks of age, mice were randomly assigned to treatments using the random integer generator found at random.org, weighed, and then ear notched. Mice were housed 5 per cage in disposable individually ventilated cages (Innovive, San Diego, CA; 37.3 × 23.4 × 14.0 cm). Ten cages per substrain were used. The cages were bedded with irradiated aspen shavings (NEPCO, Warrensburg, NY) and all cages were provided with 8–10 g of a long-fibre nesting material (EnviroDri, Shepherd Specialty Papers, Watertown, TN). Food (5L79, LabDiet, St. Louis, MO) and ultrafiltered hyperchlorinated water delivered via water bottle were provided *ad libitum*. The light cycle was 12 h:12 h light:dark, temperature was maintained at 21 °C ± 1 °C, and humidity was maintained between 30 and 70%.

2.2.2. Overall experimental design

We developed the PALS measure in four stages. First, using mice euthanized in the course of ongoing studies at CRL, we standard-