Available online at www.sciencedirect.com
SCIENCE DIRECT®
Behavioural Processes 70 (2005) 168–176
BEHAVIOURAL PROCESSES
www.elsevier.com/locate/behavproc

# Urinary corticosterone measures: Effects of strain and social rank in BKW and CD-1 mice

Ann E. Fitchett [a], Sarah A. Collins [a], Howard Mason [b], Christopher J. Barnard [a], Helen J. Cassaday [c,*]

[a] *School of Biology, University of Nottingham, University Park, Nottingham, NG7 2RD, UK*
[b] *Health & Safety Laboratory, Harpur Hill, Buxton, Derbyshire, SK17 9JN, UK*
[c] *School of Psychology, University of Nottingham, University Park, Nottingham, NG7 2RD, UK*

Received 14 February 2005; received in revised form 9 June 2005; accepted 29 June 2005

**Abstract**

We used urinary assays as a non-invasive method to examine corticosterone levels in two outbred strains of male laboratory mice (BKW and CD-1). Measures were taken before and after 2 weeks of pair housing, to examine the effects of social stress. We found that CD-1 mice had significantly higher corticosterone levels compared to BKW mice both before and after pairing. Behavioural measures provided evidence that, when paired, both strains of mice polarised into dominants and subordinates, with a higher overall incidence of aggressive acts in the BKW mice.

Some pairings had to be separated to prevent injuries so the pairing procedure introduced a selection for non-aggressive socially tolerant mice. Social status was nevertheless found to be associated with pre-existing differences in urinary corticosterone in the CD-1 strain: mice that later became dominant had overall lower levels of urinary corticosterone compared to subordinates. In conclusion, urinary corticosterone levels indicated clear differences in physiology, likely to be related to the adrenal stress response, dependent on both strain and social status. Thus, this non-invasive measure could help to predict the welfare outcomes of social housing and how these may depend on dominance status, rather than overall levels of aggression, in different strains of mice.


*Keywords:* BKW; CD-1; Corticosterone; Mouse; Social rank; Strain difference

## 1. Introduction

Whilst group housing is generally considered beneficial for a range of laboratory animals, social stress can also raise husbandry issues. In general, we need to be able to quantify the likely reproductive costs and benefits of social living before we can evaluate its impact on

* Corresponding author. Tel.: +44 115 951 5124; fax: +44 115 951 5324.
*E-mail address:* helen.cassaday@nottingham.ac.uk (H.J. Cassaday).

0376-6357/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.beproc.2005.06.006

the welfare of group-housed individuals (Barnard and Hurst, 1996; Hurst et al., 1997). In the present study, we report evidence relating housing conditions to a putative physiological welfare indicator (urinary corticosterone) by using this to complement behavioural measures in drawing conclusions about welfare.

### *1.1. The costs and benefits of social living*

It has been proposed that the stress caused by an animal's social environment could be as severe as any form of experimental stressor. For example, in rodents, compared to electric foot-shocks, restraint stress and food and water deprivation, the greatest plasma corticosterone response was observed in response to social defeat (Koolhaas et al., 1997). Typically, the male mice used in scientific research are individually housed because of problems with aggression levels when group housed. However, single housing may also be problematic and, in the extreme, results in 'the isolation syndrome' (Valzelli, 1973), which includes heavier adrenal glands, higher plasma corticosterone and hypertension and behavioural consequences (on later exposure to other individuals), e.g., compulsive aggressive behaviour and deviated or decreased sexual activity. Isolation-induced aggression is most commonly reported in outbred albino strains (Goldberg et al., 1973) of the kind used here.

Even in the absence of the opportunity to reproduce, there is evidence that mice show preferences for social living. For example, controlled studies have shown that male mice opt to sleep with a familiar cage mate and that the need for social contact increases with age (Van Loo et al., 2004). Similarly, dominant mice have been found to prefer soiled bedding from familiar subordinate males and this preference is greater than that shown for bedding from females (Rawleigh et al., 1993). This shows a preference for cues associated with conspecifics in the absence of any direct reproductive benefit, though clearly dependent on likely advantage in terms of social rank.

### *1.2. What mediates the detrimental effects of single housing?*

One possibility is that single housing constitutes a barren housing environment that blocks natural behavioural responses that depend on environmental features, thus inducing abnormal behaviour such as stereotypies (Würbel, 2001). Secondly, the mismatch between postnatal environment (social with littermates) and adult environment (when animals are separated) is likely to disrupt habitat-dependent adaptation. In other words, the littermate rearing environment does not prepare the animal for adult life in standard laboratory conditions (Würbel, 2001). All this suggests that, notwithstanding any stress that may be caused by the immediate social environment, the welfare of laboratory rodents should be enhanced by the use of group housing.

### *1.3. How are the benefits of group housing influenced by social rank?*

Particularly under laboratory conditions, where the opportunity to escape is limited, social stress is typically assumed to have a greater effect on subordinate compared to dominant animals, presumably due to the constant threat of defeat by the dominant (Creel et al., 1996). Adopting a subordinate social role, and even just the acute experience of social defeat by a conspecific, have both been shown to cause a number of physiological and behavioural changes in mice (Martinez et al., 1998). These include impaired sexual behaviour (D'Amato and Pavone, 1992), a depressed immune response (De Groot et al., 1999) and increased tendency to modulate the secretion of immunodepressive steroid hormones (Barnard et al., 1996), decreased territorial marking and ultrasonic courtship vocalisations (Lumley et al., 1999), an increase in core body temperature (Keeney et al., 2001) and changes in body weight (Bartolomucci et al., 2004). This raises a dilemma for animal husbandry: at what point and for which mice, do the costs of social living outweigh the benefits?

### *1.4. Corticosterone levels as a measure of stress*

Corticosterone is released from the adrenal cortex after activation of the hypothamalamic-pituitary-adrenal axis in response to, among other things, stress (De Kloet, 2000). Elevated corticosterone levels have been seen in subordinates in a number of species: mice (Avitsur et al., 2001; Keeney et al., 2001; Louch and Higginbotham, 1967), rats (Blanchard et al., 1993), trout (Sloman et al., 2002) and lizards (Summers et al., 2003). However, there are some inconsistencies in the