Applied Animal Behaviour Science 177 (2016) 70–76

Contents lists available at ScienceDirect

Applied Animal Behaviour Science

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

The effects of metabolic cage housing and sex on cognitive bias expression in rats

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ARTICLE INFO

Article history:
Received 17 December 2014
Received in revised form 6 January 2016
Accepted 17 January 2016
Available online 22 January 2016

Keywords:
Cognitive bias
Sex differences
Metabolic cage
Judgement bias paradigm

ABSTRACT

The use of metabolic cages is established housing protocol in small-animal, gastrointestinal research. Physiological data, as primarily observed through secretions of the hypothalamic–pituitary–adrenal axis has shown that rodents housed in this manner experience an increased stress response. Few studies have observed behavioural measures of stress, or the emotional impact, in response to metabolic cage housing in rodents. This study investigated the impact of moving rats from standard group housing to individual metabolic cage housing on the affective states of rats as measured through a judgement bias paradigm. It was assumed that a change from standard housing to metabolic cages would impact on the rats' affective state. It was therefore hypothesised that rats moved to metabolic cages would show fewer optimistic responses to an ambiguous stimulus compared to rats remaining in standard housing. Rats (*Rattus norvegicus*) ($n = 24$) were trained to learn the correct response needed to obtain a reward, given the type of stimulus present (rough versus smooth sandpaper). One stimulus was associated with a high-positive reward (chocolate), whilst the other was associated with a low-positive reward (cereal). Upon learning the discrimination, the rats were introduced to a stimulus intermediate between their learned stimuli (intermediate sandpaper) creating an ambiguous probe. Responses to the probe were regarded as optimistic if the rat responded to an ambiguous cue as if it were a positively rewarded stimulus, or pessimistic, if response to an ambiguous cue mimicked that of a negatively rewarded stimulus. Male rats moved to metabolic cages ($n = 6$) showed significantly fewer optimistic responses to the probes (0.16 ± 0.16), than control males that remained in open-top cages (4.5 ± 0.34) ($p = <0.001$). No differences in optimistic decisions were observed in females moved to metabolic cages ($n = 6$) compared to standard housing ($n = 6$) ($p = 0.0524$). This demonstrated, that upon being moved to metabolic cages, male rats responded with increased negative behavioural judgements. The data also demonstrated that gender can alter rodent judgement in cognitive experimentation. This has implications for all cognitive bias studies, and for the continued use of metabolic cages in small-animal research.

1. Introduction

Metabolic cages are an established housing practice in research studies that involve evaluation of drug pharmacology or gastrointestinal research in rodents (Hwang et al., 2013). The system allows for the accurate assessment of the output and input measures of the housed rodents. Metabolic cages designed for rodents are constructed to maintain the integrity of the data being measured. However, in doing so, the physical constraints of the cage and their subsequent limitations create a housing environment that does not provide for all aspects of good welfare (Greco et al., 1989; Manser et al., 1995; van Praag et al., 2000; Nagy et al., 2002; Eriksson et al., 2004; Whittaker et al., 2012; Ravenelle et al., 2014).

When evaluating metabolic cages as a complete unit, the majority of research conducted on rodent welfare in these cages has primarily focused on physiological responses. For example, it was first reported by Gomez-Sanchez and Gomez-Sanchez (1991) that metabolic cage housing led to increased corticosterone excretions in rats. This was confirmed by Kalliokoski et al. (2013) who reported that mice housed in this type of cage had ten times greater corticosterone output, measured in their faeces then control mice. Metabolic cages have been associated with increased weight loss and reduced immunoglobulin A (IgA) secretions in rats (Eriksson et al., 2004), both of which are indicative of a stress response (Guhad and Hau, 1996; Royo et al., 2004). Metabolic cages have also been associated with increases in catecholamine levels and endogenous monoamine-oxidase activity (Gil et al., 1999).

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http://dx.doi.org/10.1016/j.applanim.2016.01.018
0168-1591/

Physiological measures have had wide application in the assessment of rodent affective state in response to metabolic cage housing. However such techniques have associative flaws as they tend to be a measure of emotional arousal rather than emotional valence (Yeates and Main, 2008; Mendl et al., 2009). As reviewed by Yeates and Main (2008), emotional arousal is a measure of how strongly an emotional response is elicited by the animal, whilst emotional valence is a measure of whether the emotional response elicited by the animal is positive or negative in regards to the subjective experience of that animal. Therefore, without proper application of methods of evaluation that specifically measure emotional valence, there is no way to determine how an emotional response is perceived by an animal (Yeates and Main, 2008; Mendl et al., 2009). Behavioural observations, coupled with evaluation of physiological parameters provide some insight into the nature of the stress response in animals (Amir et al., 2005; Yeates and Main, 2008; Mendl et al., 2009; Salmeto et al., 2011). Despite this new understanding in the assessment of the stress response, few studies have been conducted into metabolic cage housing and the associated effects on rodent behaviour. This is largely due to the physical and social constraints that the cage imposes, making many behavioural tests inapplicable.

Research into human psychology has provided evidence that alterations in cognitive processing, so-called ‘cognitive biases’, are a reliable indicator of affective state and emotional valence (Wells and Matthews, 1996; Amir et al., 2005; Mendl et al., 2009). For example, subjects in a negative affective state (e.g. anxiety, depression) made negative judgements regarding ambiguous stimuli more often than subjects in a positive affective state (Amir et al., 2005).

This principle was adapted to animals in the rodent study by Harding et al. (2004). This work demonstrated that cognitive biasing as a result of affective state manipulation existed in multiple animal species. Therefore the measurement of cognitive biases presents a unique, under-studied protocol in the assessment of rodent affective state that is not limited by the physical and social constraints of the metabolic cage. As operationally defined by Douglas et al. (2012) and throughout the current study, optimism is defined as responding to an ambiguous cue as if it were a positively rewarded stimulus, whilst pessimism is defined as responding to an ambiguous cue as if it were a negatively rewarded stimulus.

Environmental enrichment has been demonstrated to induce optimism in animals such as rats, pigs and macaques (Bateson and Matheson, 2007; Matheson et al., 2008; Brydges et al., 2011; Bethell et al., 2012; Douglas et al., 2012). The removal of this enrichment has also been associated with pessimism in starlings and rats (Bateson and Matheson, 2007; Burman et al., 2008). Pessimistic judgement biases have also been associated with restraint in sheep (Doyle et al., 2010a,b; Bethell et al., 2012), pain in calves (Neave et al., 2013) and predatory threat in chickens (Salmeto et al., 2011), all three of which, are negatively valanced emotional states (Yeates and Main, 2008).

Previous studies have determined that environmental conditions can bias the cognitive processing of animals. (Bateson and Matheson, 2007; Matheson et al., 2008; Brydges et al., 2011; Bethell et al., 2012; Douglas et al., 2012; Wichman et al., 2012). Therefore, in the current study, the judgement-bias test for rodents as established by Brydges et al. (2011), was adapted with the primary aim of investigating the impact of re-housing in a metabolic cage on the affective state of rats using this judgement-bias paradigm. It was assumed that moving group-housed rats into metabolic cages would negatively impact their affective state. Therefore it was hypothesised that rats moved to a metabolic cage would exhibit a decreased number of optimistic responses to an ambiguous probe compared to rats that remained in standard open-top cages. Male and female rats were included in the study, and it was hypothesised that there would be no difference in cognitive bias expression between sexes, a theory that has never been tested, representing an under-studied area within the field of cognitive biases. Rats were housed in the metabolic cages for 7 nights (8 days). This period in the metabolic cage best represents the conditions in which these animals are housed when following typical laboratory work that involves the use of these cages (Mashtoub et al., 2013; Wang et al., 2013; Whittaker et al., 2015a).

The detection of cognitive biasing resulting from metabolic cage housing has profound implications for the use of metabolic cages in future studies involving rats. These biases would provide some of the first indications as to the valence of the emotional state of rats when moved to this housing system.

2. Materials and methods

Animal housing and experimental protocols were approved by the Animal Ethics Committee of the University of Adelaide and conducted in accordance with the provisions of the Australian Code for the Care and Use of Animals for Scientific Purposes (National Health and Medical Research Council, 2013).

2.1. Subjects and housing

Male ($n = 12$) and female ($n = 12$) Sprague Dawley rats were sourced from a barrier-maintained, specific-pathogen-free (SPF) facility (University of Adelaide, Laboratory Animal Services, Adelaide, Australia). This sample size ($n = 24$) was utilised based on previous literature using similar methodology (Brydges et al., 2011). At 3 weeks of age rats were housed in same sex groups of 3 in standard polycarbonate open-top rat cages (415 mm × 260 mm × 145 mm, Tecniplast, NSW, Australia) lined with paper based bedding (Animal Bedding, Fibrecycle Pty Ltd., Qld, Australia) and furnished with a chewing object (Nylabone Products, NJ, USA). Standard rat chow (Rat and Mouse Cubes, Specialty Feeds, WA, Australia) and potable reverse osmosis (RO) water were provided ad libitum. All animals were identified by marking the base of the tail with a non-toxic marker pen. During the testing phases, twelve rats (6 males, 6 females) were housed in metabolic cages (220 mm in diameter × 120 mm tall, Tecniplast, NSW, Australia), with a metal grid floor and no shelter. Room temperature remained at 21–23 °C. The photoperiod was set on a 12 h light/dark cycle.

2.2. Apparatus

The testing chamber was similar to that utilised by Brydges et al. (2011), and comprised two transparent perspex boxes (610 mm × 435 mm × 215 mm). The “start box” was connected by a PVC pipe 90 mm in diameter and 800 mm long, to the “goal box”. During the experiment this pipe was lined with either coarse (P80), or fine (P1200) sandpaper (Flexovit, NY, USA) according to the learned association of the rat (see below). The goal box contained one blue and one brown bowl (each 90 mm diameter, 20 mm deep), in either corner (Fig. 1), these bowls were present and their positions fixed throughout the testing. Milk chocolate baking chips (Cadbury, London, England) and cheerios (UncleToby’s, Victoria, Australia) were used for the high-positive and low-positive reward items respectively. Previous cognitive bias work see Harding et al. (2004) utilise a positive versus negative reward scheme, in that the negative reward is mildly aversive to the animal. However, this experiment follows from the work of Brydges et al. (2011) in using a high-positive versus low-positive reward item. The decision to use this reward scheme was two-fold. Firstly, it was discussed that attempted detection of a positive effect works most optimally when a positive or neutral reinforcer is utilised (Mendl et al., 2009). Secondly, as discussed by Brydges et al. (2011), repeated exposure to