Physiology & Behavior 173 (2017) 124–131

Contents lists available at ScienceDirect

Physiology & Behavior

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

Female rats display fewer optimistic responses in a judgment bias test in the absence of a physiological stress response

T.H. Barker [a,*], L. Bobrovskaya [b], G.S. Howarth [a,c], A.L. Whittaker [a]

[a] School of Animal and Veterinary Sciences, The University of Adelaide, Roseworthy Campus, South Australia, Australia
[b] School of Pharmacy and Medical Sciences, University of South Australia, City East Campus, South Australia, Australia
[c] Gastroenterology Department, Children, Youth and Women's Health Services, Adelaide, South Australia, Australia

HIGHLIGHTS

- Metabolic cages induce pessimistic biases in housed rats.
- Significant differences observed between male and female cognitive expression.
- No correlation observed between cognitive biases and physiological controls.

ARTICLE INFO

Article history:
Received 17 December 2016
Received in revised form 19 January 2017
Accepted 5 February 2017
Available online 7 February 2017

Keywords:
Cognitive bias
Sex difference
Metabolic cage
Judgment bias paradigm

ABSTRACT

Metabolic cages are a type of housing used in biomedical research. Metabolic cage housing has been demonstrated to elicit behavioural and physiological changes in rodents housed within them. The nature of this effect has been characterized as anxiogenic. However, few studies have evaluated positive affect in response to metabolic cage housing and the interaction between this, sex and traditional physiological measures of stress. Cognitive biasing, as measured through a judgment bias paradigm has proven a reliable measure of animal affective state, particularly through its ability to measure positive affect. The current study investigated differences in cognitive biasing between male and female rats when transferred from open-top, grouped housing to a metabolic cage. Rats (*Rattus norvegicus*) ($n = 60$) were trained in a judgment bias paradigm previously validated for use in the rat model. Upon exposure to an intermediate, ambiguous probe rats responded with either an optimistic or pessimistic decision. The animals were also subjected to the sucrose preference test to identify the presence of anhedonia. Faecal corticosterone and changes in adrenal tyrosine hydroxylase were also measured to establish whether a stress-like state was experienced. There was a significant interaction between sex and metabolic cage housing on the number of optimistic decisions made $F(1, 56) = 7.461$, $p = 0.008$. Female rats that remained in control housing responded with a reduced number of days featuring an optimistic decision compared to males in control housing ($p = 0.036$). However, both males and females responded with significantly fewer optimistic decisions in the metabolic cage compared to control ($p < 0.001$). There was a significant negative correlation between treatment and sucrose consumption ($r_{pb} = -0.654$, $n = 195$, $p < 0.001$). There was also a significant sex effect for faecal corticosterone concentrations $F(1, 30) = 6.305$, $p = 0.018$) with female rats (4.050 ± 1.285), displaying greater corticosterone concentrations than males (2.291 ± 0.495). No differences between treatment were observed for either corticosterone or tyrosine hydroxylase levels. This data demonstrates that movement into a metabolic cage resulted in rats displaying significantly greater pessimistic cognitive biases as determined through the judgment bias test. Interestingly, male rats that remained in control housing demonstrated cognitive biases that were not equivalent to female rats. Furthermore, despite a behavioural change being evident, a physiological change in corticosterone or tyrosine hydroxylase levels was not observed.

1. Introduction

The classification of affective state in animals is an attempt to quantify the subjective feelings, emotions and experiences that individual animals engage when performing reward-motivated behaviours [1]. The detection of a positive affective state has been

* Corresponding author.
E-mail address: timothy.barker@adelaide.edu.au (T.H. Barker).

http://dx.doi.org/10.1016/j.physbeh.2017.02.006

most commonly achieved with cognitive bias tests, such as the judgment bias paradigm. This testing paradigm was first proposed by Harding et al. [2] and involved training animals to respond with two unique behaviours in response to two unique stimuli. These behaviours could be objectively classified as positive (rewarding or pleasurable) or negative (aversive or punishing). After the animal had learned these associations it was tested through introduction of a novel stimulus. If the animal responded to this ambiguity with the positive behaviour, the implication was that the animal behaved optimistically and vice versa. Optimism and optimistic-tendencies have been associated with animals being in a positive affective state, and the occurrence of optimistic judgments to an ambiguous probe identifies animals as such.

Negative affective states have been associated with the sensation of pain [3], restraint [4,5], threat [6], depression and anxiety [7–9]. Meanwhile environmental enrichment [10–13] and removal from a stressor [5,14] have been associated with the prevalence of positive affective states. The accurate assessment of these affective states has become increasingly important in animal welfare assessment [15].

Metabolic cages are commonly used in biomedical research [16]. Their utility in allowing the separation and collection of faeces and urine as well the controlled provision of food and water have made the cages popular choice for gastrointestinal and nutritional research in small rodents [17–20]. The employment of these cages however, has been associated with changes in rodent physiology, including increased corticosterone concentrations [21,22], reduced immunoglobulin A secretion [23] and increases in both catecholamine levels and monoamine-oxidase activity [24]. In addition, metabolic caged animals have responded with altered behavioural expression; when tested using a social interaction test, caged animals responded with a decreased movement time, fewer incidents of manipulating enrichment and rearing behaviours and were accompanied with a corresponding shift toward inactivity [16]. The use of these cages has therefore been strictly regulated by governing bodies through legislation [25]. Despite this legislative intervention, there has been little research into the effects of metabolic cage housing on animal affective state.

There has been one previous attempt to assess the impact of metabolic cage housing on positive affective state in rats [7]. This study indicated that female and male cognitive expression may not have been equivalent to one another, a finding scarcely reported within cognitive bias literature [14]. However, in the Barker et al. [7] study, no other correlative measures were used to assess if metabolic cage housing per se induced a stress response. This could suggest that the pessimistic biases expressed by the male metabolically housed animals may have been attributed to an external confounding factor. We aimed to determine if there was any difference in cognitive bias expression between male and female rats exposed to an identical stressor. We also wanted to compare the judgments made to an ambiguous probe with other, well-established controls indicative of a stress response. These included the concentrations of faecal corticosterone [22], adrenal tyrosine hydroxylase [26,27], and the sucrose preference test as an indicator of anhedonia [28]. Utilising a previously-validated judgment bias paradigm [12] it was hypothesised that animals moved to a metabolic cage from control housing would respond with a significant decrease in the number of optimistic biases expressed. It was also hypothesised that there would be no significant differences in the cognitive bias expression of the male rats compared to the females. We further hypothesised that animals moved to the metabolic cages would experience a significant increase in concentrations of faecal corticosterone and levels of tyrosine hydroxylase in the adrenal gland, and a significant decrease in the percentage of sucrose solution consumed in a sucrose preference test.

2. Methods

2.1. Ethics statement

All 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 [29].

2.2. Experimental design and apparatus

Treatment groups were randomly allocated; rats either remained in open-top group housing as controls ($n = 15$) or were separated and moved to singly-housed metabolic cages ($n = 15$). These allocations were counter-balanced between sexes. Rats were moved to the metabolic cages after demonstrating that they successfully completed the training phase (see below), and could be reliably tested in the judgment bias test. This training period took approximately 148 days, at which point the rats were approximately 21–23 weeks old. Whilst training in the judgment bias test, all animals were housed in open-top cages with two same-sex conspecifics.

The testing chamber and apparatus were identical to those utilised in the Barker et al. [7] study, adapted from the design of the original study by Brydges et al. [12]. They comprised a start box connected via a pipe to a goal box constructed from perspex. The pipe was lined with one of two-grades of sandpaper to act as the coarse (P80) and fine (P1200) training cues. The goal box contained a brown bowl in the right-hand corner filled with coriander scented sand (1% by weight of spice to sifted sand) and a blue bowl was placed in the left-hand corner filled with cinnamon scented sand (Fig. 1). 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.

Animals were randomly assigned an association to the reward items and rewarded locations as described in Barker et al. [7]. These associations were counter-balanced between sex and treatment and are summarised in Table 1.

2.3. Animals and housing

Male ($n = 30$) and female ($n = 30$) Hsd: Sprague Dawley rats were sourced at 3-weeks of age from a barrier-maintained Specific-Pathogen-Free facility (University of Adelaide, Laboratory Animal Services, Adelaide, Australia). This sample size was calculated to have a power of 80% based on the data obtained from the Barker et al. [7] study. All animals were housed in same sex groups of three, per their future testing parameters (e.g. metabolic cage or control) in standard polycarbonate open-top rat cages (415 mm × 260 mm × 145 mm, Tecniplast, NSW, Australia). Cages were lined with paper-based bedding (Animal Bedding, Fibercycle Pty Ltd., Qld, Australia) and furnished with cardboard boxes and PVC tubes as enrichment. All enrichment items were standardized between cages. Standard rat chow (Rat and Mouse Cubes, Specialty Feeds, WA, Australia) and water purified by reverse osmosis were provided ad libitum. All rats were identified by marking the base of the tail with a unique identifying number with a non-toxic marker pen. During the testing phases, 30 rats (15 male, 15 female) were moved into metabolic cages (220 mm in diameter × 120 mm tall, Tecniplast, NSW, Australia), with a metal grid floor and no shelter. Room temperature remained between 21 and 23 °C. The photoperiod was set on a reversed 12 h light/dark cycle.

2.4. Judgment bias test

Definitions of important procedures and milestones have been included in Table 2. Prior to training in the judgment bias test, rats were handled by the researchers for 5 days to habituate them to human