Novel Non-invasive Physiological and Behavioural Indicators of Stress in Laboratory Rats (*Rattus norvegicus*)

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Introduction

Rattus norvegicus is a common experimental subject in biomedical research. Elevated stress levels in this species have been associated with the confinement and social challenges of "shoe-box" housing and human disturbance, which persist in many laboratories (Touma & Palme, 2005; Balcombe, 2006). Current Australian codes of practice state that "animals must be regularly assessed for signs of pain or distress" (NHMRC, 2004). Although some level of acute stress may be justified, few dispute that animals used in experimentation must be free from chronic stress, which constitutes poor animal welfare (Mostl & Palme, 2002; Balcombe, 2006).

Improved animal welfare depends upon accurate detection of stress; ideally, tests should not themselves act as stressors. This paper will review novel, non-invasive physiological and behavioural parameters of stress that have emerged in the past 12 months, and discuss their potential welfare benefits for *R. norvegicus.*

Discussion

Elevation of adrenocortical activity enhances an animal's physiological response to stressors, any challenging or threatening stimuli (Touma & Palme, 2005). Measurement of faecal corticosteroid metabolites (FCMs) is a non-invasive stress indicator in many animal species. However, prior to 2007, no studies using existing immunoassays had validated this method for *R. norvegicus* (Lepschy *et al*., 2007). Lepschy *et al*. (2007) aimed to determine corticosterone excretion routes in *R. norvegicus* and validate an immunoassay to detect FCMs. Eighteen rats were administered ^{3}H-corticosterone intravenously and orally and the faeces were collected for five days. A 5α-pregnane-3β,11β,21-triol-20-one immunoassay applied to faeces found 74.8±9.2% of administered corticosteroids appeared as FCMs. Adrenocortical activity was then stimulated (using synthetic ACTH) and suppressed (using dexamethasone) and significantly increased or decreased concentrations of FCM were measured 12 to 14 hours later (Lepschy *et al*., 2007).

This study demonstrates that subtle changes in adrenocortical activity are reflected in FCMs and can be detected in this immunoassay. Although males produced FCM concentrations six times greater than females, and untreated rats showed a pronounced diurnal rhythm in FCM concentration, this technique is an important indicator of stress in *R. norvegicus* (Lepschy *et al*., 2007). Faecal collection allows non-invasive, repeated sampling of individuals (Touma & Palme, 2005), avoiding labour-intensive handling and venipuncture, which cause increased corticosteroid concentrations and pain (Vahl *et al*., 2005; Lepschy *et al*., 2007). FCMs are also less affected by short-term fluctuations in corticosterone than blood samples, as single faecal samples incorporate hormones secreted over several hours (Touma & Palme, 2005). Although this measurement does not assess the aetiology of the chronic physical stress response, it facilitates behavioural monitoring, which may elucidate specific stressors (Mormède *et al*., 2007).

Sleep behaviour may be a valuable indicator of stress in group-housed *R. norvegicus*. Research has demonstrated that hypothalamo-pituitary-adrenal axis hyperactivity in chronic stress reduces total sleep frequency and duration and increases sleep interruptions (Abou-Ismail *et al*., 2007). Despite this, no studies prior to Abou-Ismail *et al.* (2007) have compared observations of sleep behaviour with physiological parameters of stress. In this study, 144 group-housed rats were maintained in identical conditions and were weighed weekly for five weeks. Observations of "sleep disruption" and "aggression" during sleep were recorded for individuals. After five weeks, all subjects were euthanased and blood samples, bodyweight, adrenal and thymus weights

recorded. The study found that increased sleep frequency and duration correlated positively with final bodyweight and weight gain, and negatively with adrenal weight.

This study indicates that stress experienced by laboratory rats can disrupt sleep patterns and these are associated with widely accepted physiological indicators of stress. A fault in this study, recognised by its designers, is the concept that increased "sleep frequency" is a definitive indicator of low stress levels. Increased sleep frequency may indicate that sleep was more interrupted, representing higher levels of stress (Abou-Ismail *et al*., 2007). Nevertheless, this study demonstrates that disrupted sleep has a physiological basis and observation of sleep behaviour facilitates understanding of social dynamics among group-housed rats.

Behavioural responses to ultrasonic vocalisations (USVs) emitted by rats may indicate stress in group-housed rats. Previous research has established two USVs produced by rats that correlate with the caller's emotional state: 50kHz is associated with rewarding behaviours and social contact, while 22kHz are emitted during aggressive, defensive behaviours (Burman *et al*., 2007). Burman *et al*. (2007) investigated the behavioural effects of these USVs on receiver rats. USVs recorded from randomly selected rats were played to individual subjects as they undertook an "emergence test", a test of anxiety examining the emergence of rats from shelter into an open arena. In rats exposed to 22kHz, the likelihood of emergence, latency to emerge, and percentage of total time spent emerged was reduced. This was significant compared with the control group exposed to background noise. One month later, the same rats were exposed to 50kHz but there was no significant difference in behaviour compared with a control group (Burman *et al*., 2007).

This experiment demonstrated alteration of behaviour in receiver rats according to the type of USV played; at 22kHz, emergence behaviour was inhibited, indicating anxiety (Burman *et al.*, 2007). Unlike adrenocortical activity, which is a non-specific stress response, "withdrawal" behaviour is a specific adaptation aimed at protecting the animal from a threat (Veissier & Boissy, 2007), the 22kHz USV. A fault in this study was the removal of individuals from the group prior to the emergence test. In addition to stress experienced by the individual being handled, Burman *et al.* (2008) have shown that remaining rats have increased FCM and vocalisation. Therefore, multiple stressors may have produced the anxiety seen in response to the 22kHz USV. Nevertheless, awareness of specific stress behaviours in rats allows informed changes to be made to group composition that will reduce stress in individuals.

Conclusion

Stress detection underpins improved welfare for *R. norvegicus* experimental subjects. Traditional physiological parameters of stress are invasive and difficult to measure, paradoxically causing further stress. Measurement of FCMs and USVs, together with observations of sleeping behaviour and receiver responses to USVs, facilitates an understanding of the animal's experience of its environment. Subsequently, husbandry procedures and animal welfare can be improved by minimising appropriate stressors that cause adverse physical and emotional states.

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