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# Behavioural Patterns and Corticosterone Levels Induced by Chronic Psychosocial Stress in the Four-Striped Mice (*Rhabdomys pumilio*)

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**Abstract:** This study presents, findings from investigations of the influence of chronic psychosocial stress on behavioural patterns and corticosterone levels in the four-striped mice, *Rhabdomys pumilio*. A modified resident-intruder paradigm was adopted in the study and involved the introduction of an intruder mouse (40-80 g) to a resident mouse (90-175 g) for 1 h for 5 consecutive days. Intruders interacted with different residents each day and behavioural responses were recorded. To determine if the resident-intruder paradigm induced an adrenocortical stress response, blood samples were collected after the last day of the paradigm via cardiac puncture from the control and intruder mice. The frequency of aggression (90±18 events day$^{-1}$) received from the residents and upright defense exhibited by intruders (43±9 events day$^{-1}$) differed significantly over repeated test days, however, there were no significant differences in the frequency of the resident climbing on the intruder's cage (104±21 events day$^{-1}$). Elevations of corticosterone levels were evident in the stressed mice. Our data indicate that behavioural and neuroendocrinological responses may have important implications in the four-striped mice and in the understanding of psychiatric disorders.

**Key words:** Resident-intruder-paradigm, behavioural interactions, corticosterone, four striped mice

## INTRODUCTION

Stress has been implicated as a major factor in the emergence of affective disorders such as depression and anxiety (Buwalda *et al*., 2005; Westenbroek *et al*., 2004). Epidemiological studies show that a large proportion of the global health burden is due to psychiatric disorders and it is most likely that this proportion will increase in many African countries engulfed by extreme social pressures, poverty and violence (Okasha, 2002).

Paradigms such as subordination stress, resident-intruder stress, foot shock, restraint stress, isolation, cold immobilization, cold swim and predator odour have all been employed within the laboratory to reflect Selye's concept of a physiological stress response pattern to a change in homeostasis (Buwalda *et al*., 2005). The modification of a resident-intruder paradigm, initially developed by Miczek in 1979 (Mitra *et al*., 2006), was therefore employed in this research.

The availability and relatedness of the stripe mice to non-human and human primates serves as a useful model for the study of social behaviour and stress. The four-striped mice (*Rhabdomys pumilio*) is the only species in its genus, *Rhabdomys*, a diurnal, murid rodent, probably the most common mammal within Southern Africa, as it is so widespread and can be found in habitats such as grasslands, deserts and forests (Schradin and Pillay, 2004). Our aim in this study was to evaluate the behavioural patterns and corticosterone levels in the four-striped mice (*Rhabdomys pumilio*) exposed to chronic psychosocial stress.

## MATERIALS AND METHODS

**Experimental animals:** The animals were treated and used according to the guidelines of the University of the Witwatersrand Animal Ethics and Screening Committee, which parallel those set down by the National Institute of Health (NIH) for use of animals in scientific experiments. Stripe mice from 7-82 weeks of age were obtained for this study obtained. The mice were allowed 7 days to adjust to the cages before any experimental procedures were conducted. All animals were singly housed in standard plastic cages (20.5×26.5×13 cm) in a temperature controlled room. Veterinary care was provided to the animals, including access to food and water, *ad libitum*.

**Resident-intruder paradigm:** Three residents and 3 intruders were used in the experiment. Residents were larger in weight (90-175 g) and older than the intruders

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(40-80 g). Two mice, one male with a similar weight to the intruders and a female with a similar weight as the residents were used as controls.

The social dominance paradigm used provides a model that is of greater relevance to human stress, compared to other laboratory stressors (Malatynska and Knapp, 2005). The stress period was 1 h each day. The resident was transferred to a larger cage (60×38×17 cm) and allowed 10 min to adjust to the new surroundings. The intruder was placed in a smaller cage (18.5×18.5×18.5 cm) and put in the centre of the larger cage of the resident, providing space for the resident to encircle and climb on top of the intruder's cage. All occurrences of 2-predefined categories of behaviour were recorded during the interaction; Aggression-resident bites, holds, or attacks the intruder and Upright defense-intruder exhibits a defensive upright posture in response to aggression from the resident (Bhatnagar and Vining, 2003; Mitra *et al*., 2006). The number of times the resident climbed on the intruder's cage was recorded. After the stress test, both the intruder and resident were returned to their home cages. The same procedures were repeated each morning between 08:00-10:00 for 5 consecutive days. Intruders were introduced to a different resident during each daily test session to prevent any form of familiarisation. The control mice were not exposed to the resident-intruder paradigm.

**Collection of blood samples:** To confirm that the resident-intruder test induced an adrenocortical stress response, blood samples were taken from the repeatedly stressed intruder mice (Mitra *et al*., 2006). Blood sampling was done on the day of completion of the resident-intruder paradigm. To minimize stress, mice were anaesthetised with 0.2 mL xyalazine and ketamine (4:1 ratio). Approximately, 2-3 min thereafter, 0.5-1.0 mL blood was taken by cardiac puncture. Mean duration for blood collection (including transport between rooms) was approximately 3-4 min. Blood samples were centrifuged and the serum fraction was transferred to a polypropylene tube. Samples were stored at -20°C prior to hormone quantification. Corticosterone was measured using a commercially prepared radio-immunoassay (Assay designs, USA). All samples were subjected to a single assay run. The sensitivity of the corticosterone assay was 30 pg $mL^{-1}$.

**Data analysis:** Statistical analyses were done with SPSS (Student version 9.0). All tests used were non-parametric and $p \leq 0.05$ was considered significant and tests were 2-tailed. Mann-Whitney tests and Kruskal Wallis tests were used for comparisons between groups.

## RESULTS

**Behavioural interactions:** The resident mouse immediately established contact and engaged in competition over territory with the intruder. A clear dominant (resident mouse) and subordinate (intruder mouse) relationship was soon established. All the intruders exhibited submissive behaviours toward the resident indicative of stress. The frequency of aggression on the intruder was 90±18 events $day^{-1}$. The Upright Defense (UD) was 43±9 events $day^{-1}$ and differed significantly over repeated test days. However, there were no significant differences in the frequency of the resident climbing over the intruder's cage, which was 104±21 events $day^{-1}$ (Fig. 1). There were significantly negative correlations between intruder mass, biting and cage climbing (Spearman's rho, $p<0.05$), suggesting that there was an increase in these behaviours with decreasing intruder body mass.

The sex of the intruder seem to have affected the aggression from the resident ($p = 0.19$), irrespective of the sex of the resident (Mann Whitney U test, $p<0.05$). Female intruders prompted a greater number of attacks from residents than the male intruder, however the sex of the intruder did not affect occurrence of upright defense ($p = 0.679$) by the intruder or the number of times the resident climbed on the intruder's cage ($p = 0.055$). It is

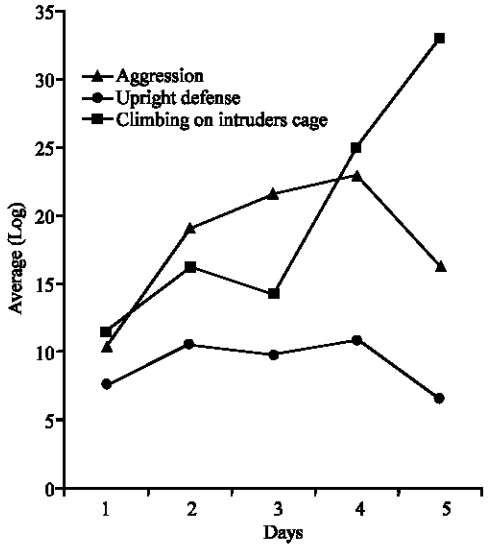


Fig. 1: Graphical representation of the average amount of behavioural interactions displayed during the 1 h resident-intruder paradigm by intruder mice (day 1-5). Aggression and upright defense increased progressively except on the 5th day, with the resident establishing dominance by climbing over the cage more

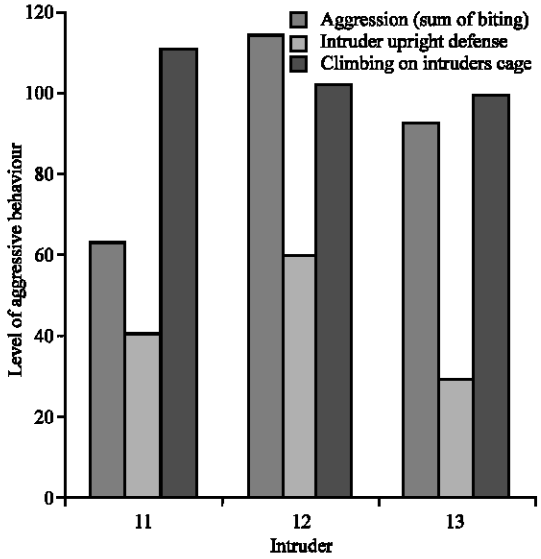


Fig. 2: Resident-Intruder behavioural interactions observed in the four-striped mice. The interaction was highest with the 2nd intruder possibly due to the male-female between contacts. I1, I2 and I3 = Intruder mice

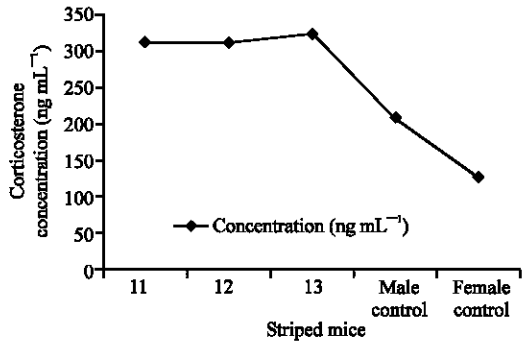


Fig. 3: Graphic representation of the plasma corticosterone concentrations in the four-striped mice. I1, I2 and I3 = Intruder mice

also, interesting to note that the intruders exhibited lesser amount of upright defense on the last day of chronic stress, even though the aggression on this particular day was notably high (Fig. 2).

**Corticosterone levels:** Chronic psychosocial stress using the resident-intruder paradigm induced an increase in the adrenocortical response in intruder mice. The corticosterone level in the intruder mice was 310-322.8 ng $mL^{-1}$ compared to 126.1-208.5 ng $mL^{-1}$ in control mice (Table 1 and Fig. 3). Higher corticosterone levels were observed in the female intruders compared to that produced by the male intruder (312-323 ng $mL^{-1}$ vs 310 ng $mL^{-1}$).

Table 1: Body weight, brain weight and plasma corticosterone levels in the four-striped mice

| Striped mice | Body weight (g) | Brain weight (g) | Corticosterone levels (ng $mL^{-1}$) |
|---|---|---|---|
| Female control | 78 | 0.9 | 126.1 |
| Male Control | 36 | 0.7 | 208.5 |
| Intruder 1 (Male) | 76 | 0.9 | 310.0 |
| Intruder 2 (Female) | 42 | 0.8 | 312.2 |
| Intruder 3 (Female) | 48 | 0.8 | 322.8 |

## DISCUSSION

Although, stress has traditionally been investigated by studying behaviour and mortality, quantitative measurements of the stress response make available data with greater objectivity and comparability (Ohe and Servheen, 2002). In this experiment, frequency of aggressive behaviour and the concentrations of corticosterone were recorded.

**Behavioural interactions:** The resident-intruder paradigm emphasizes aggression and sensitivity as the elements of dominant-submissive behaviour (Malatynska and Knapp, 2005). Compared to models such as foot shock, the resident-intruder paradigm produces behaviours that are more salient to affective disorders observed in humans (Thomas *et al*., 2007). An intruder of a smaller body weight is introduced to a 'resident' mouse of a greater body weight with a tendency to be more aggressive (Uno *et al*., 1989). Mice were not allowed to interact without a wire mesh cage separating them, which served as an ethologically relevant model and prevents extreme aggressive behaviour between the resident and intruder (Bhatnagar and Vining, 2003).

The time period and nature of the social stress test, as well as the species under investigation, appear to be important determinants of the types of changes that are examined and could be used to explain the behavioural patterns observed. Establishment of dominant and subordinate relationships between resident and intruder mice are dependant on body mass. Studies show that variations in body mass are correlated with dominance rank, fighting ability and gaining resources and that the stripe mice have a tendency to attack mice that are lighter than themselves (Schradin, 2004). Body weight within this study was an important component in the establishment of this relationship. There was a significant increase in the aggressive behaviour and cage climbing with decreasing intruder body mass.

Schradin (2004) reported that the striped mice attacks mice from other groups based on 3 factors. The sex of the other mouse: males are much more likely to attack strange males than strange females, whereas sex specific aggression was not observed in females; the body size of the opponent: striped mice have a tendency to attack an opponent that is lighter than themselves and the location

of the interaction: striped mice are much more likely to attack strangers, even those significantly heavier than themselves in front of the nest than at territory boundaries. Although, the stripe mice display much aggression toward opponents with a smaller weight, previous studies show that they also elicit a great amount of paternal care (Schradin and Pillay, 2003).

Frequency of aggression, upright defense and climbing on the intruder's cage may be influenced by a competition for space, stereotypic behaviours and/or the sex of the intruder. The frequency of aggression and upright defense generated by the stripe mice in this study was greater than that observed by Mitra *et al.* (2006) on Swiss-Webster male mice following a 2 h resident-intruder paradigm. Behavioural studies showed that the stripe mice (*Rhabdomys pumilio*) is a solitary, aggressive, territorial species (Schradin, 2004), which may explain the high frequencies of biting and attacks, as well as the high frequency of cage climbing exhibited daily by the resident, possibly resulting from competition for space (Perrin *et al.*, 2001).

The sex of the intruder seems to have had a significant effect on the aggressive interactions, irrespective of the sex of the resident. The different intruders provoked an aggressive-like behaviour from the residents especially, in the interactions with female intruders. It is interesting to note that in the natural environment, females protect food, nest-sites and offspring, males protect females from other males and food and nest sites, particularly at high population densities (Schradin, 2004).

It had long been proposed that although repeated encounters with resident animals often occur, aggressiveness and defeat are not necessary factors for the establishment and maintenance of social hierarchies and subtler behavioural relations between resident and intruder may occur (Christian, 1970). In this particular study, paternal care was evident and male residents were highly sociable toward younger mice. Captive male stripe mice have been shown to display a large amount of the same patterns of paternal behaviour as exhibited by female mice (huddling, grooming and retrieving pups). This behavioural interaction is believed to contribute to the development of the younger mice (Schradin and Pillay, 2003).

Affiliate interactions were observed between the resident and the intruder such as the resident sitting next to the intruder, grooming and licking the younger mouse. These behavioural patterns have been described as indicative of paternal care (Schradin and Pillay, 2003). Intruder mice displayed repetitive, invariant behavioural patterns such as back flipping in the cage during the stress paradigm. These stereotypies, previously reported in the four-striped mice are believed to allow the animal to cope with unfavourable environments (Schwaibold and Pillay, 2001). Whilst the coping hypothesis remains a popular explanation for stereotypies, experimental studies have not been successful in reproducing the stress reducing effect associated with the behaviour and lack of evidence renders this hypothesis inconclusive.

**Corticosterone levels:** The increase in the corticosterone levels in the intruder mice are a result of the facilitation of Hypothalamo-Pituitary-Adrenal (HPA) responses to the stress paradigm. The chronic stress paradigm is significant in that it shows that the HPA axis remains sensitive to a stress stimulus. It seems adaptive for an animal to produce a robust response to a stimulus whose threat to homeostasis is of an unknown magnitude (Bhatnagar and Vining, 2003). The facilitation of the HPA responses to chronic stress within our study is consistent with other experiments to a certain degree (Bhatnagar and Vining, 2003; Mitra *et al.*, 2006; Thomas *et al.*, 2007; Kim *et al.*, 2005), as there was increase in the corticosterone levels in the intruder mice.

Various factors may have contributed to this result. The species, nature and time period of the stress paradigm may affect the level of corticosterone. The species under investigation, time period and nature of the psychosocial stress paradigm is an important determinant of the changes observed in corticosterone levels. Studies, which show a robust adrenocortical response resulting from a resident-intruder paradigm include those by Thomas *et al.* (2007). Mitra *et al.* (2006) observed a corticosterone levels in the intruder Webster mice that were seven times greater compared to control mice. The duration of the stress paradigm may also account for the small elevation in corticosterone levels observed within our study. In a 1 h acute stress paradigm adult male Sprague Dawley rats induced plasma corticosterone levels greater than 6 fold (Thomas *et al.*, 2007). Another possible explanation for the slight increases in corticosterone levels within the chronic stress paradigm, when compared to other studies, may be the paternal care behaviour commonly observed in captive *Rhabdomys pumilio*.

An increase in plasma corticosterone, is an indication of a physiological response to stress (McEwen, 2000) and an elevated corticosterone level was induced in all the intruder mice within this study. It is important to consider that the stress response will be different among individuals as both genetics and the environment are important factors thus, explaining the different corticosterone concentrations. The significant difference in aggression received by intruders each day of the paradigm may also, account for the elevated corticosterone levels in female intruders compared to the male intruders.

## CONCLUSION

The frequency of aggression differed significantly over repeated test days however, there were no significant differences in the frequency of the resident climbing on the intruder's cage. This study has shown that the body mass of the intruder is an important determinant of the amount of aggression the resident mice received. In addition, intruder mice exhibited frequent stereotypic behavioural responses and female intruders received the greatest amount of aggression. Corticosterone levels were elevated in stressed mice compared to the control mice.

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