STEREOTYPY, PERSONALITY AND ENVIRONMENTAL ENRICHMENT

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DECLARATION

I hereby declare that this thesis is my own unaided work and that recognition has been given to the references used. It is being submitted for the Degree of Doctor of Philosophy in the Faculty of Science at the University of the Witwatersrand

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ABSTRACT

Animals in captivity are often raised in suboptimal environments, which lead to abnormal behaviours, such as stereotypic behaviour. Environmental enrichment can reduce or eliminate these behaviours to some extent. However, enrichments are not always successful in their intended purpose, which may be attributed to differences among individuals (i.e. personality). The overarching aim of my study was to investigate how environmental enrichment affected the expression of stereotypic behaviour in my study model, the African striped mouse, *Rhabdomys dilectus*, and to ascertain whether personality modulated the responses to enrichment. I conducted four experiments to test these aims. Firstly, I tested whether personality was associated with the development and expression of stereotypic behaviours. Results indicated that stereotypic striped mice were bold and showed a proactive coping style, while non-stereotypic striped mice were less bold and showed a reactive coping style. Furthermore, having a proactive coping style did not predict the onset of stereotypic behaviours. Nevertheless, individual differences in personality were observed even within stereotypic and non-stereotypic striped mice so that each group was not homogeneous for personality. Secondly, I tested whether personality was associated with the responses of stereotypic striped mice to enrichment. While stereotypic behaviours were reduced in enriched cages, individuals were not consistent in their behaviour, indicating flexible behavioural responses to the different cage complexities. Interestingly, these responses occurred irrespective of personality differences. There were no treatment-related differences in the behavioural responses of non-stereotypic striped mice. Thirdly, I examined whether the age at which striped mice were introduced to the environmental enrichment influenced their behavioural responses. Age did not affect the behavioural responses of stereotypic or non-stereotypic mice to the cages of different complexity. Surprisingly, while stereotypic behaviours were reduced in the enriched treatments, not all stereotypic mice responded to enrichment in the same manner, implying flexible behavioural responses. Moreover, these behavioural responses also occurred regardless of the individual's personality type. There were no age-related differences in the behavioural responses of non-stereotypic striped mice. Finally, I investigated the purpose of wheel running, either as an enrichment or as a re-directed stereotypic behaviour, in stereotypic striped mice, because there is much debate about its use as an enrichment. Due to individual differences in responses to the running wheel, wheel running appeared to be both an enrichment and a re-directed behaviour. In conclusion, my study provides the first empirical data for the theory that stereotypic animals have different personalities to non-stereotypic animals. Nonetheless, this dichotomy between stereotypic and non-stereotypic striped mice at the group level masked individual responses within groups, with individuals flexibly altering their behaviour, depending on the environment to which they were exposed, which in turn affected the efficacy of environmental enrichment. My study suggests that the welfare and well-being of animals requires an assessment of individual trajectories in the development of stereotypic behaviours.

DEDICATION

I dedicate this thesis to my late grandparents Mr N. K. Joshi and Mrs K. N. Joshi and to the little stars that made this project feasible- the striped mice.

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CHAPTER ONE

General Introduction

Rationale for the study

Animals in captivity are often raised in suboptimal environments, which lead to the development of abnormal behaviours, such as stereotypic behaviour. The addition of enrichments (i.e. behavioural or physical) to captive environments can, to some extent, reduce or eliminate these behaviours. However, the question, which then arises, is why not all animals respond to environmental enrichment in the same way? The aim of my study was therefore to investigate how environmental enrichment affects the expression of stereotypic behaviour in the striped mouse, and to ascertain whether the influence of enrichment is modulated by the individual differences in response (i.e. personality) in my study model, the African striped mouse *Rhabdomys dilectus*. Studies in the past have independently looked at the interaction between stereotypic behaviours and environmental enrichment as well as the interaction between personality and environmental enrichment. However, to date, no studies have explored how personality influences the stereotypy-enrichment relationship at both group (stereotypic vs. non-stereotypic animals) and individual level (between and within groups).

Stereotypic behaviour

Stereotypic behaviours, described as any abnormal, repetitive and invariant behaviours are caused by frustration, repeated attempts to cope and/or central nervous system (CNS) dysfunctions (Latham and Mason, 2008). They are assumed to be an indirect response to deprived (physical and behavioural) environmental conditions created by captivity (Mason, 1991a; Wiedenmayer, 1997) and are mediated by changes in forebrain function, particularly changes in the neural pathways between the cortex and basal ganglia, key in inhibiting inappropriate behaviours and maintenance of behavioural flexibility (Lewis et al., 2006; Graybiel, 2008). Stress induced by the aversive conditions also alters the functioning of dopamine, a neurotransmitter implicated in the development of stereotypic behaviours (McBride and Hemming, 2009). Thus, an altered functioning of neuronal structures utilizing dopamine lead to the development and maintenance of stereotypic behaviours (McBride and Hemmings, 2009). Stereotypic behaviours are apparently completely restricted to captive animals but also occur in human patients with psychiatric dysfunctions, with lesions in the brain, and with the administration of stimulant drugs (Graybiel, 2008).

Stereotypic behaviours are by far the most common form of abnormal repetitive behaviour (ARB), the other being impulsive or compulsive behaviours. Impulsive behaviours involve repetition of an inappropriate goal with varying goal-directed behaviour, for example barbering in mice (Garner et al., 2004). Stereotypic behaviours include: (1) locomotor stereotypic behaviour and (2) oral stereotypic behaviour. Locomotor stereotypies are thought to arise from frustrated locomotor behaviour, whereby normal behaviours are thwarted due to limited space (Carlstead, 1998), whereas oral stereotypies might develop from restriction to feeding or foraging behaviours (Terlouw et al., 1991). Some examples of locomotor stereotypic behaviour are route tracing in blue tits, *Parus caeruleus* and marsh tits, *Parus palustris* (Garner et al., 2003), repetitive pacing in circus tigers, *Panthera tigris* (Krawczel et al., 2005), rhythmic head-swinging in elephants, *Elephas maximus* (Rees, 2004), bar biting in laboratory mice, *Mus musculus* (Nevison et al., 1999) and bank voles, *Clethrionomys glareolus* (Garner et al., 2003). Examples of oral stereotypic behaviour are tongue playing in dairy cattle (Redbo, 1998) and weaving and cribbing in stabled horses (McAfee et al., 2002; Ninomiya, 2007). Whereas oral stereotypic behaviour is influenced more by dietary and feeding related restrictions than environmental variables, locomotor stereotypic behaviours are usually affected by environmental variables, such as access to conspecifics and the number of hours kept indoors (Bashaw et al., 2001). For example, when free-ranging red deer, *Cervus elaphus,* stags are restricted to smaller pens, a higher percentage of these males displayed pacing and vertical/ horizontal head movements (Bashaw et al., 2001). It is evident that stereotypic behaviours are a result of an abnormal animal-environment interaction (Carlstead, 1998).

Development of stereotypic behaviour

Stereotypic behaviour is known to have an underlying genetic basis, as shown by studies of bank voles, *Clethrionomys glareolus* (Schoeneker and Heller, 2000), striped mice *Rhabdomys* spp, (Schwaibold and Pillay, 2001; Jones et al., 2008) and mink *Mustela vison* (Jeppesen et al., 2004; Svendsen et al., 2007). For example, Schwaibold and Pillay (2001) found that striped mouse young from stereotypic mothers were more likely to display stereotypic behaviour than young of non-stereotypic mothers, both when raised by their own mother or a non-stereotypic foster mother. However, the importance of non-genetic factors (e.g. maternal deprivation) cannot be excluded. Rhesus macaques, *Macaca mulatta,* raised in the absence of their mothers showed high levels of stereotypy, indicating that the particular behaviour was not learnt (at least not from the mother; Latham and Mason, 2008). Social influence and learning may also be important in the development of stereotypy. Cooper and Nicol (1993) showed that visual contact with a stereotypic bank vole, *Clethrionomys glareolus*, demonstrator accelerates the development of stereotypy in a neighbouring bank vole, and this behaviour persists even when the demonstrator is absent. The same was observed in horses, whereby individuals housed facing stereotypers tended to display higher levels of weaving than those that had faced away from the stereotypers (Ninomiya et al., 2007).

Stereotypic behaviours are often suggested to occur because of aversive, unsuitable and stressful environments (Mason, 1991b) and are believed to result from restraint of movements and frustration from not being able to perform species-specific behaviours (Hogan, 2007; Würbel, 2006). Moreover, stereotypic behaviours often arise from species-typical behaviours, which may be incorporated into normal behavioural patterns (Mason and Mendl, 1993). For example, stereotypy in carnivores is highly prevalent in individuals housed in smaller enclosures with limited opportunities for movement (Clubb and Mason, 2003). However, stereotypic behaviours do not develop in all individuals that are housed in barren conditions (Mason, 1991b), and are affected by the age and context (i.e. life history and rearing environment) of the animal (Mason, 1993).

The development of stereotypic behaviours can also be related to the structure of the physical environment (Würbel et al., 1998), which implies that the underlying cause of stereotypic behaviours is the inability to cope with adverse environmental conditions. For example, stereotypic digging in the corners of standard laboratory cages in the Mongolian gerbil, *Meriones unguiculatus*, is primarily the result of being prevented from burrowing in the cages, which would eventually enable them to retreat to a safe area (Wiedenmayer, 1997). Similarly, the development of locomotor stereotypies observed in bank voles, *Clethrionomys glareolus*, may occur because of unsuccessful attempts to climb out of the cage (Wiedenmayer, 1997). The captive environment therefore may create circumstances that are very different to the natural habitat and so normal behaviours (e.g. exploration, play and social behaviours) are gradually replaced by abnormal behaviours, such as stereotypic or apathetic behaviours (Mason, 1991a; Rushen, 1993; Wiedenmayer, 1997; Hogan and Tribe, 2007).

Stereotypic behaviour and perseveration

Positive relationships between stereotypy frequency and perseveration (i.e. recurrence of a behaviour in the absence of the original eliciting stimulus) have been noted in captive bank voles, blue tits, marsh tits (Garner et al., 2003) and orange-wing Amazon parrots, *Amazona amazonica* (Vickery and Mason, 2005). This relationship has been proposed to occur because the captive environments that cause stereotypy modify features of behavioural organisation by affecting the functioning of the dorsal striatum in the forebrain (Vickery and Mason, 2005). The dorsal striatum is part of the basal ganglia, which is involved in the selection and ordering of behavioural patterns, inhibition of inappropriate behaviours as well as the maintenance of behavioural flexibility (Garner et al., 2003; Vickery and Mason, 2005; Garner, 2006, Graybiel, 2008). The altered functioning of these neural structures and thus the inability to inhibit inappropriate behaviours, contribute to the development and long-term maintenance of stereotypic behaviours (Garner et al., 2003; McBride and Hemmings, 2009; McBride and Parker, 2015). For example, older voles, *Clethrionomys glareolus*, show stronger perseveration of stereotypic behaviours than younger voles after environmental enrichment (Cooper et al., 1996), and established stereotypic behaviours in voles are easily increased following an arousing or stressful stimulus (Ödberg, 1987; Cooper and Nicol, 1991). Stereotypic behaviours become perseverative (or bad habits) if they become centrally controlled (i.e. with repetition, behaviour shifts into a form of automatic processing; Mason and Latham, 2004) and over time, the behavioural efforts to cope with the aversive situation slowly become more rigid and increase in frequency and duration (Würbel and Stauffacher, 1997).

Stereotypic behaviour and coping

A variety of responses (termed “coping strategies”), combining physiological and behavioural mechanisms, are shown by captive or farm animals in response to challenges or changes posed by the environment they inhabit (Levine, 1985; Groothuis and Carere, 2005). The coping hypothesis states that an organism develops stereotypy in order to cope with the hostile conditions in which it is housed (Rushen, 1993). In addition, coping is an individual’s response to a stressor (Schouten and Wiepkema, 1991), whereby detrimental physiological outcomes of the stressor can be minimised. Whilst some studies show that stereotypic behaviours are associated with a decrease in physiological measures of stress and can be regarded as a successful coping behaviour, other experimental studies have been unsuccessful in showing the stress-reducing effects of stereotypy. For example, Wechsler (1995) showed that increases in stereotypic behaviour of individual rats sensitised to amphetamine were associated with a decrease in plasma corticosterone levels, while Terlouw et al. (1991) found no relationship between the level of post-feeding stereotypic behaviour and plasma cortisol levels, and also no increase in cortisol concentrations in sows prevented from performing stereotypic behaviours. Therefore, the coping hypothesis remains questionable and it is essential that stereotypic behaviours should only be considered as a warning of suffering but not necessarily a sole indicator thereof (Mason and Latham, 2004).

Adversaries of the coping hypothesis dispute the concept of coping and state that not all forms of stereotypy are responses to stress (Mason, 1991a; Cooper and Nicol, 1993). For instance, once established, stereotypic behaviours become less dependent on the initiating stimulus (Würbel et al., 1996; Wiedenmayer, 1997). It has been suggested that some stereotypic behaviours might reflect poor welfare of captive animals (Mason and Latham, 2004) and that they do not result in coping. For example, chain-manipulation by sows showed no activation of the pituitary-adrenal system, which is usually initiated in response to an aversive situation (Mason, 1991a). In bank voles, stereotypic rather than non-stereotypic individuals were apprehensive and tended to flee on exposure to loud noise (Ödberg, 1987), which might mean that the stereotypic voles are not coping.

Personality

To understand how individual animals cope with particular circumstances, it is essential to comprehend the differences between individuals and how they respond to stressful conditions. Behavioural and physiological differences are common between individuals of the same species and vary within and between age classes and between sexes. For example, female rats show a greater inclination to novelty than males and therefore display higher levels of exploration and reduced levels of anxiety or fearfulness (Aguilar et al., 2003; Øverli et al., 2006). Individuals may differ in the way they perceive stressors and how these eventually affect them, which ultimately reflect the differences in personalities (Dall, 2004).

Personality is defined as inter-individual variation but intra-individual consistency across situations or contexts (Schuett and Dall, 2009; Gosling, 2001) of several behaviours, such as boldness, neophobia, coping styles and behaviour (Dall, 2004; Sih et al., 2004). These individual differences are attributed to temperament or behavioural styles and can lead to differences in exploration, intraspecific aggression and other social behaviours, all of which may influence fitness (Dall, 2004; Sih et al., 2004; Hadley et al., 2006; Svartberg et al., 2005). Furthermore, many studies show that personality is a heritable trait (Benus et al., 1991; Dingemanse et al., 2002; Drent et al., 2003; Van Oers et al., 2004) or may result from epigenetic changes mediated by variation in pre- or post-natal environmental conditions (Carere et al., 2005; Macrì and Würbel, 2006; Macrì and Würbel, 2007).

Personality and coping

Since personality describes consistent behavioural differences across contexts or situations, often such a description can be interchangeably used with terms such as temperament (Gosling, 1998), behavioural syndrome (Sih et al., 2004) and coping styles (Benus et al., 1991; Verbeek et al., 1996; Koolhaas et al., 1999). While, these terms are very similar, the definition of behavioural syndromes and coping styles places emphasis on the existence of "suites of correlated behaviours" (i.e. correlations between different personality traits across contexts or situations; Sih et al., 2004). Personality characteristics are highly correlated within individuals. For example, individual great tits, *Parus major,* vary in their response to stressors and novelties along a behavioural continuum from shy (more fearful and docile) to bold (less fearful, aggressive and risk-taking in exploring novel environments and/or predators), which in turn also co-varies with other behavioural traits, such as aggression, exploration, risk-taking, fearfulness and reactivity (Carere and Van Öers, 2004; Dingemanse and Réale, 2005). Great tits that quickly explored novel environments also immediately explored novel objects (Dingemanse et al., 2002), which shows a consistency of a personality trait.

There are two types of coping styles (i.e. an alternative response in reaction to a stressor), namely proactive (active) and reactive (passive) styles (Koolhaas et al., 1999; Koolhaas et al., 2010). Behaviourally, proactive animals are characterized by developing routines, being more aggressive and bold, and, on encountering defeat with a particular challenge, tend to show active avoidance (Janczak et al., 2003) and show behavioural responses, which are independent of environmental stimuli (Benus et al., 1988). In contrast, reactive individuals are dependent on environmental cues, are less aggressive and freeze or display apathetic behaviours when faced with a challenge (Wechsler, 1995; Janczak et al., 2003). For example, in a T-maze task, proactive coping piglets were less successful in reversal learning than reactive coping piglets. Furthermore, proactive copers had more difficulties in preventing their previously reinforced response, implying that proactive animals depend on previous experience and develop routines.

The underlying mechanism in the ability of individuals to adopt proactive or reactive coping styles could be attributed to behavioural flexibility (Coppens et al., 2010). Both neuroendocrine and neurobiological factors can explain the mechanisms underpinning behaviour flexibility (Koolhaas et al., 2010; Coppens et al., 2010). While the proactive coping style is controlled through the activation of the sympathetic adrenomedullary system, the reactive coping style is controlled by the activation of the pituitary-adrenocortical system (Wechsler, 1995; Koolhaas et al., 1999). Neurobiologically, individual variation arises from changes in the prefrontal cortex, responsible for behavioural flexibility and behavioural inhibition. In particular, individual variation in the serotonergic input to the medial prefrontal cortex may explain the individual variation in coping styles, since serotonin is involved in behavioural flexibility (Koolhaas et al., 2007; Koolhaas, et al., 2010; Coppens et al., 2010).

Recently, Ijichi et al. (2013) proposed that personality might play a part in the development of stereotypic behaviours. They suggest that because stereotypic animals show an active response to stress by attempting to exert control over the external stressor (e.g. sub-optimal housing), they have a proactive (flight-fight response) coping style, while non-stereotypic animals would show a reactive (conservation-withdrawal response) coping style, and be unable to exert control over the stressor. Furthermore, Ijichi et al. (2013) suggest that due to the many behavioural and physiological similarities between proactive and stereotypic individuals, these phenomena may be linked. For example, both stereotypic and proactive individuals have the propensity to develop routines (Benus et al., 1988; Koolhaas et al., 1999; Bolhuis et al., 2004). Mechanistically, dopamine, which has been implicated in the development of stereotypic behaviour, is also high in proactive individuals. Another neurotransmitter, serotonin that promotes behavioural flexibility is low in both proactive and stereotypic individuals (De Boer and Koolhaas, 2003).

Environmental Enrichment

The improvement of the lives of captive animals (termed environmental enrichment) has received much attention. Environmental enrichment can be defined as using objects (wheels, toys, tunnels) and cage designs to enhance the quality of life of captive animals, thus providing an alternative to the monotonous environment created by captive conditions (Pietropaolo et al., 2004; Friske and Gammie, 2005; Simpson and Kelly, 2011). In practice, there are many different ways of enriching the physical and social environments of captive animals. These include introducing biologically relevant features such as tunnels, designing more suitable exhibits in zoos, increasing the number and diversity of behavioural opportunities, by allowing the animals to perform more natural behaviours such as foraging or exploration, providing shelters so that animals can escape from perceived threats, stimulating animals cognitively through training (novel-object recognition), and housing social animals in groups rather than individually (Newberry, 1995; Shepherdson, 1998; Mellen and MacPhee, 2001; Young, 2003).

Environmental enrichment is said to ameliorate some of the problems created by captivity, such as stereotypic and other abnormal behaviours, by changing the animal's environment in a way that promotes the performance of behaviours that are within the normal range of the animal's species-specific repertoire, as well as improving health and reproductive success (Newberry, 1995; Van de Weerd et al., 1997; Young, 2003). Enriched environments are also associated with structural and biochemical changes in the brain of captive animals, by increasing the number and density of neurons and synapses as well as by increasing dendritic arborisation (Van Praag et al., 2000; Würbel, 2001). The functional consequences of these changes include enhanced memory, learning, and, perhaps the most crucial, the ability to cope with environmental challenges (Newberry, 1995; Young, 2003). Despite the positive outcomes of environmental enrichments, there are also drawbacks associated with such implementations and a question that arises is why environmental enrichment not always effective in its intended goal (Swaisgood and Shepherdson, 2006). Some animals may find novelty frightening and more stressful since it might not allow for normal behaviours to take place (Jordan, 2005) or these complex environments may still present an unnatural degree of monotony. For example, stereotypy was still prevalent in ICR mice (outbred albino strain of laboratory mice) *Mus musculus*, after being enriched (Balcombe, 2006). Furthermore, Wistar rats housed under enriched conditions actually showed higher levels of resting plasma corticosterone levels, larger adrenal glands and an increase in corticosterone release in response to a buspirone challenge in comparison to the controls, implying that some animals can actually find novelty frightening (Moncek et al., 2004). Environmental enrichment may also make abnormal behaviours even more complicated by bringing about aggression or territoriality. For example, shelters and complex cages may benefit some mice, but these items may induce territoriality and aggression and hence become deleterious to others (Marashi et al., 2003; Garner, 2005; Mason et al., 2007). Furthermore, environmental enrichments might sometimes enhance stereotypic behaviour. For example, increasing cage size in Arctic blue foxes, *Alopex lagopus*, and presenting minks, *Mustela vison*, with 'playballs' increased stereotypic behaviour (Korhonen et al., 2001).

Another factor that might potentially affect the efficacy of environmental enrichment is habituation. For example, stump-tailed macaque, *Macaca arctoides* and Barbary macaque, *Macaca sylvanus* provided with a device filled with food lost interest in it after a couple of hours despite food still available in the device (Vick et al., 2000). Furthermore, conditions experienced during early life can also have important effects on subsequent life stages (Lindstrom, 1999; Lummaa and Clutton-Brock, 2002). For example, it has been shown in humans, that children who have been raised under impoverished conditions or have been raised by single rather than both parents form birth show better cognitive and behavioural outcomes (Jaffee et al., 2003). Similarly, in other mammals, the quality of rearing environment, for example changes in social environment during early life (Würbel and Stauffacher, 1997; Jones et al., 2010) can result in behavioural changes in adults such as poor social interaction (Pryce et al., 2005), stereotypic behaviours (Würbel, 2006) as well as individual differences in coping responses to stress (Branchi et al., 2011; Chapman et al., 2010; Caldji, 2000). Those reared in more enriched conditions exhibit reduced anxiety related behaviours (Fares et al., 2013) and an increase in locomotory and exploratory activities, object exploration and learning ability (Marashi et al., 2003).

Increasing species-specific behaviours can best be achieved by determining which behaviours naturally occur in the wild (e.g. exploration, activity levels, foraging behaviours) and thereafter providing an enrichment that particularly reinforces/provides opportunities for expression of these behaviours (Tarou and Bashaw, 2007). Before introducing environmental enrichment to reduce stereotypic behaviour, it is important to consider the natural behaviour of the animal (Mason et al., 2007). For example, stereotypic cage digging in Mongolian gerbils, *Meriones unguiculatus*, may not mean that these animals need a digging substrate, since in nature, this behaviour may have occurred while trying to construct a tunnel-like burrow to retreat into a safer area, and therefore it might be more suitable to provide these animals with tunnel-like dens, rather than other environmental enrichment devices (Wiedenmayer, 1997).

Environmental enrichments are usually tailored for a particular target species. Hansen and Berthelson (2000) raised the back of the cage of rabbits, *Oryctolagus cuniculus*, since these rabbits prefer to perch in high areas to survey their surroundings. When allowed access to artificial burrows, the development of stereotypic digging in Mongolian gerbils, *Meriones unguiculatus*, was completely eliminated (Wiedenmayer, 1997). Furthermore, provision of hay and twigs significantly reduced stereotypy in bank voles, *Clethrionomys glareolus*, as the presence of cover may have decreased the incentive to escape or have given them the opportunity to hide (Ödberg, 1987; Cooper et al., 1996).

Several studies indicated that increasing cage complexity and the addition of environmental enrichments reduced stereotypic behaviours and promoted more episodes of natural behaviours (e.g. bank voles, *Clethrionomys glareolus*, Ödberg, 1987; deer mice, *Peromyscus Maniculatus*, Powell et al., 1999, 2000; dairy cattle, Redbo, 1990; lion-tailed macaques, *Macaca Silenus*, Mallapur et al., 2005). Environmental enrichments, in terms of food provision, were helpful for stabled horses, since horses in their 'natural' environments spend a large proportion of time foraging (Winskill et al., 1996). However, additional space or environmental complexity is not always successful in reducing stereotypic behaviour. For example, stereotyping chimpanzees, *Pan troglodytes,* and chickens continued to exhibit stereotypic behaviours even after being transferred from barren housing to a complex environment, while horses, which crib bite and wind suck in the stable, still showed this behaviour at pasture (reviewed in Cooper et al., 1996). Some studies have shown that environmental enrichment can also protect against (provide neuroprotection) stereotypic behaviour even after the enrichment is removed (Ödberg, 1986; Powell et al., 1999, 2000; Jones et al., 2011). For example, when bank voles, *Clethrionomys glareolus*, were transferred from enriched to barren conditions after 60 days, they did not develop higher rates of stereotypy. The same was found in deer mice, *Peromyscus maniculatus*, whereby both early (at weaning) and late (after day 60 of age) exposure to environmental complexity resulted in lower rates of stereotypic behaviour (Powell et al., 1999, 2000).

Enriched animals have been observed to explore new environments quickly and to approach novel items faster than control animals in tests of anxiety and exploratory behaviour (open field and elevated plus maze, Friske and Gammie 2005). In addition, enriched house mice show an increase in locomotory and exploratory activities, object exploration and learning ability (Marashi et al., 2003). When provided with novel objects for environmental enrichment, orange-winged Amazon parrots, were less fearful (i.e. showed shorter latencies to approach), and approached and interacted with the novel objects many times, and performed many other behaviours (Meehan and Mench, 2002). This indicates that environmental enrichment can be a useful strategy to reduce anxiety-related behaviours and sensitivity to environmental stressors, such as novelty and human handling (Fox and Millam, 2006).

Study species

The African striped mouse *Rhabdomys dilectus*

The African striped mouse, genus *Rhabdomys*, is a diurnal murid rodent, with an adult weight of 40-80 g in nature. It is widespread in many biomes in southern Africa, such as grassland, desert, semi-deserts and forests (Skinner and Chimimba, 2005). Across its range, the striped mouse displays a diurnal, bimodal activity pattern, with most activity concentrated around the mornings and evenings (Schradin, 2005). Striped mice are opportunistic, omnivorous rodents and their behavioural plasticity (Schradin et al., 2012; Schradin and Pillay, 2006) is a likely explanation for their wide distribution in southern Africa.

In the grassland regions of southern Africa, the striped mouse is a seasonal breeder and is reproductively active from the austral spring (September to November) to the austral autumn (February to April). It has a gestation period of 22-23 days and litter sizes vary from five pups in nature (Brooks, 1982) to approximately seven pups in captivity (Pillay, 2000). Young start eating solid food at 10 days, start exploring outside the nest from 12 days and are weaned at approximately 16 days of age. Striped mice reach sexual maturity at approximately five to six weeks (range 34-90 days, Brooks, 1982). The striped mouse species, *Rhabdomys dilectus*, used in this study originated from the Highveld grasslands of central South Africa, where it is solitary living (Schradin and Pillay, 2005). Females rear their litters alone without help from the father (which is common in a sister species Rhabdomys pumilio), and both sexes overlap their territories with that of the opposite, but not the same, sex (Schradin and Pillay, 2005).

Striped mice are suitable study subjects because they breed readily in captivity, have short generation times and are easy to house and handle. They are also a suitable model for investigating stereotypic behaviour because, whilst few wild caught adult striped mice develop stereotypy in captivity, approximately 50% of captive born individuals become stereotypic as a consequence of housing in standard laboratory cages (Schwaibold and Pillay, 2001), without a drug challenge, and without a specific eliciting stimulus (Schwaibold and Pillay, 2001; Van Lierop, 2005). Stereotypic behaviours appear early in development, sometimes as early as weaning, and persist throughout the lifespan (Würbel and Stauffacher, 1997).

Objectives

My study is concerned with assessing the relationship between stereotypic behaviours, personality and environmental enrichment. Apart from a theoretical study (Ijichi et al., 2013) suggesting the link between personality and the development of stereotypic behaviours, I am not aware of any studies that have empirically tested this relationship.

- The first objective of my thesis was therefore to test the proposal made by Ijichi et al. (2013). From this study, it became apparent that the stereotypic striped mice were bolder than non-stereotypic mice and displayed a proactive coping style.
- The second objective was to establish whether personality modulated how stereotypic striped mice interact with environmental enrichment to evaluate the proposition that the success of environmental enrichment in reducing stereotypical behaviours is related to individual differences (i.e. personality). The findings showed that both stereotypic and non-stereotypic striped mice showed flexible behavioural responses to environments of different cage complexity irrespective of the personality type.
- The third objective was to ascertain whether the age at which the striped mice were exposed to varying levels of environmental complexity as well as the personality of striped mice influences how they interact with the cage complexities, as the ages at which striped mice were exposed to treatments of different cage complexities might influence their behavioural responses. The findings suggested that age does not affect behavioural responses of striped mice to the different treatments and individual differences (i.e. personality) did not associate with the behavioural responses to cages of different complexities in stereotypic mice.
- The fourth objective was to investigate the use of running wheels and whether it is a form of environmental enrichment or a stereotypic/re-directed behaviour. This aim followed from the second objective of the study, which showed that wheel running increased the overall activity in stereotypic striped mice. Results revealed that individuals differed in the use of the running wheel and it could not be explicitly concluded whether wheel running was a re-directed behaviour or enrichment.

Layout of thesis

This thesis comprises an introductory chapter (Chapter 1), four experimental chapters (Chapters 2-5), and a discussion and conclusion chapter (Chapter 6). Each of the experimental chapters is written in a manuscript format for publication, with Chapter 2 (Association between personality and stereotypic behaviours in striped mice, *Rhabdomys dilectus*) submitted to the journal *Applied Animal Behaviour Science*. Each chapter has its own reference list, with consequent repetition of references, and some introduction and discussion material. Tables and figures are numbered sequentially within each chapter and not for the thesis as a whole. However, the pages for the entire thesis are numbered in sequence and line numbers are provided continuously within chapters.

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CHAPTER TWO

Association between personality and stereotypic behaviours in striped mice, *Rhabdomys dilectus*

Abstract

Stereotypic behaviours, which are abnormal, repetitive and invariant behaviours caused by frustration and/or central nervous system dysfunction, develop as a result of sub-optimal captive conditions that provide inadequate motor and sensory stimulation. However, not all individuals housed under such conditions develop stereotypic behaviours. One hypothesis to explain such variation is personality differences (i.e. individual differences). This hypothesis was tested in the African striped mouse, *Rhabdomys dilectus* and it was predicted that stereotypic individuals would show a bolder personality and a proactive coping strategy than non-stereotypic animals. Two experiments were conducted. In the first experiment, adult stereotypic and non-stereotypic striped mice were tested for their personality using three tests (i.e., light-dark, startle-response and novel-object tests). Subsequently, the behaviours of individuals were recorded every second day for 30 days in standard laboratory housing. Stereotypic striped mice were proactive and showed a bolder personality type by spending longer time in the light compartment after a startle response, and showed greater manipulation of cage objects and were more active than non-stereotypic individuals in standard housing. In the second experiment, the personality of juvenile striped mice was tested, and their stereotypic status was ascertained later at adulthood. Again, the startle response test predicted the development of stereotypic behaviour, with stereotypic mice that spent more time in the light compartment (i.e. bolder) showing a greater likelihood of displaying stereotypic behaviours later. Although the data provides support for the association between personality and stereotypic behaviour, these group-level effects (stereotypic vs. non-stereotypic mice) were not evident at the individual level, particularly for stereotypic mice. Therefore, having a proactive coping style does not predict the onset of stereotypic behaviour for all individual striped mice, highlighting individual trajectories for the development of stereotypic behaviours.

Keywords: Coping styles, Ontogeny, Personality, Sub-optimal housing, Stereotypic behaviour, Striped mice

Introduction

Stereotypic behaviours are abnormal, repetitive and invariant behaviours that are caused by frustration and/or central nervous system (CNS) dysfunctions (Mason, 2006). They typically develop under impoverished captive housing, which provides inadequate motor and sensory stimulation, thereby exposing animals to uncontrollable stress and frustration, by preventing animals performing behaviours in their normal repertoire (Mason, 1991 a, b). For example, bar biting in laboratory mice, *Mus musculus* and bank voles, *Clethrionomys glareolus* (Nevison et al., 1999; Garner and Mason, 2002) arise from repeated attempts to escape, and pacing in zoo-housed carnivores is apparently linked to the motivation to roam, quantified by the species' home range size as well as the daily distance travelled (Clubb and Vickery, 2006).

Intriguingly, not all individuals housed under such impoverished conditions develop stereotypic behaviours, which may imply that there are other explanations for the development of stereotypic behaviours. Apart from environmentally induced effects, stereotypic behaviours have a genetic basis (Schoenecker and Heller, 2000; Schwaibold and Pillay, 2001; Jones et al., 2008), making some individuals genetically predisposed to stereotypic behaviour. Another explanation is the individual variation in behavioural responses, which in itself might be genetically determined. Such variation is encapsulated in the concept of personality, which describes variation in the behavioural and physiological responses of individuals of the same sex to a particular challenge or environmental perturbation (Dall, 2004, Sih et al., 2004; Réale et al., 2007). Personality considers the intra-individual consistency and inter-individual variation (Schuett and Dall, 2009) of several behaviours, notably aggression, activity levels, boldness and exploratory tendencies often retained over time in different situations or contexts (Koolhaas et al., 1999; Gosling, 2001; Sih et al., 2004; Groothuis and Carere, 2005; Réale et al., 2007). Like stereotypic behaviours (Schwaibold and Pillay, 2001; Jones et al., 2008; Hemmann et al., 2014), certain aspects of personality are also heritable (Dingemanse et al., 2002, Drent et al., 2003, Van Oers et al., 2004), or may result from epigenetic changes mediated by variation in pre- or post-natal environmental conditions (Carere et al., 2005; Macrì and Würbel, 2006; Macrì and Würbel, 2007). Since stereotypic behaviours are a consequence of the stressful and aversive environmental conditions and are a way of coping with such environments, Ijichi et al. (2013) hypothesized that personality might be associated with the development of stereotypic behaviours. In particular, they suggest that because stereotypic animals show an active response to stress by attempting to exert control over the external stressor (e.g. sub-optimal housing), they have a proactive (flight-fight response) coping style, as defined by Benus et al. (1991). In contrast, non-stereotypic animals would show a reactive (conservation-withdrawal response) coping style, and are unable to exert control over the stressor (Koolhaas et al., 1999). The proximate mechanisms underlying the ability of individuals to respond to environmental challenges can be explained to arise from changes in the prefrontal cortex, responsible for behavioural flexibility and behavioural inhibition. In particular, individual variation in the serotonergic input to the medial prefrontal cortex may explain the individual variation in coping styles, since serotonin is involved in behavioural flexibility (Koolhaas et al., 2007; Koolhaas, et al., 2010; Coppens et al., 2010).

The aim of this study was to test the hypothesis proposed by Ijichi et al. (2013), which has not been empirically tested to date. The personality type of the stereotypic individuals of my study model, the African striped mouse, *Rhabdomys dilectus,* was investigated. Striped mice readily display stereotypic behaviours, with approximately half of captive striped mice born in our colony at University of the Witwatersrand exhibiting stereotypic behaviours because of housing in standard laboratory cages (Schwaibold and Pillay, 2001). There is a strong indication that stereotypic behaviour in striped mice has a genetic basis (Schwaibold and Pillay, 2001) and stereotypic mothers are five times more likely to produce stereotypic than non-stereotypic offspring (Jones et al., 2008).

Striped mice have both stereotypic and non-stereotypic forms, making it an ideal model for investigating the role of personality in the development of stereotypic behaviours. Two sets of experiments were conducted. In the first experiment, stereotypic and non-stereotypic individuals were exposed to three personality tests, after which their behaviour was recorded in standard laboratory housing for 30 days. It was predicted that stereotypic striped mice would show a proactive coping style which is characterized by being bolder (i.e. greater propensity of an individual to take risks, be quick to approach novel objects, explore in novel environments and show more activity; Wilson et al., 1993), while non-stereotypic striped mice would be less bold and show a reactive coping style. In the second experiment, juveniles at 30 days of age were subjected to two personality tests and the emergence of stereotypic behaviour was monitored 50 days later when they were adult. It was predicted that a bolder, proactive personality type would lead to the development of stereotypic behaviour (Ijichi et al., 2013).

Materials and Methods

Striped mice, *Rhabdomys dilectus*, used in this study were captive born F1 and F2 individuals from a lab colony established in 2010, originating from a grassland population in Pretoria (25º 40’ S; 28º 30’ E), South Africa. They were housed in the Milner Park Animal Unit, University of the Witwatersrand under partially controlled environmental conditions: 14L: 10D light: dark cycle (lights on at 05h00); 22ºC-24ºC and 30-60% rH. Subjects were housed singly in clear Lab-o-tec™ cages (L × H × W: 300 mm × 200 mm × 150 mm). Wood shavings (± 3 cm) were provided as bedding with a handful of *Eragrostis* sp. grass (± 20 g) and ± 5 g of shredded tissue paper for nesting material. PVC nest-boxes (L × H × W: 100 mm × 100 mm × 150 mm, open at both ends) were also provided in each cage. Epol® mouse cubes and water were available *ad libitum*. Approximately 5 g of fresh fruit (apples, pears) or vegetables (lettuce, carrots, broccoli) and ± 5 g of mixed seed were supplied daily per individual.

Experiment 1

Preliminary observations

Video-recordings were made of 38 adult (>100 days of age) males and females housed singly, in order to identify stereotypic and non-stereotypic individuals and the form of stereotypy displayed by all individuals. The behaviours of individuals were video-recorded for 15 minutes a day per individual every other day for five days. Video-recordings were made between 09h00-12h00, as striped mice are most active during these times (Pillay, 2000); no human observers were present in the room during this time. From these video-recordings, 26 stereotypic mice (14 males, 12 females) and 12 non-stereotypic mice (6 males, 6 females) were identified for our study. All stereotypic mice displayed locomotor stereotypic behaviours (i.e. circuit runners and somersaulters); only striped mice with locomotory stereotypy were used, to account for variation in the underlying aetiology of different forms of stereotypy (Mason, 1991a; Würbel, 2006). Stereotypic individuals were those that exhibited 10 or more bouts of stereotypy per observation session, each with three or more repetitions (after Jones et al., 2008). Individuals that did not exhibit any stereotypic behaviour were classified as non-stereotypic and were used as a comparison with the stereotypic individuals. Since stereotypic behaviour is an ‘all or nothing’ occurrence in striped mice (i.e. an individual either displayed or did not display stereotypies). Only the absence or presence of stereotypic behaviours was recorded (see Jones et al., 2008); non-stereotypic mice never displayed stereotypic behaviours. Thereafter, the 26 stereotypic and 12 non-stereotypic striped mice underwent three conventional personality tests (see Miller et al., 2006), as described below. These tests have been routinely performed on striped mice in captivity (Rymer et al., 2008; Jones et al., 2011) and in nature (Yuen et al., 2015).

Light-dark test

A glass tank divided into two equal-sized compartments (L × H × W: 300 mm × 225 mm × 300 mm), using a Perspex partition: one-half of the tank was painted black (dark compartment); with black walls and a black lid whilst the other side was transparent with clear walls and a clear lid (light compartment) was used. The partition had a small opening at the base for the test individual to move from the light compartment to the dark compartment. The tank was cleaned with disinfectant soap and air-dried between tests to reduce carry-over odour effects of test subjects.

At the start of each test, the test subject was placed in the dark compartment, facing away from the opening leading to the light compartment. Its behaviour was video-recorded for 5 minutes immediately thereafter. Using Observer software (version 5.0; Noldus Information Technology), the following variables were later scored from the video-recordings: latency (in seconds) to move from the dark to the light compartment (latency to emerge from dark compartment); latency (in seconds) to return to the dark compartment after first entry into the light compartment (latency to emerge from light compartment); total time spent in the light and dark compartments. Behaviours were only recorded in the light compartment of the test tank because the subject was not visible in the dark compartment.

Startle response test

The startle response test followed immediately after the light-dark test. On the test subject's subsequent entry into the light compartment (after the end of the 5 minute light-dark test), the mouse was startled by clapping hands next to the tank, upon which it immediately retreated into the dark area. The same parameters in the light-dark test were scored in this test for a further 5 minutes.

Novel object test

This test was conducted 24 hours after the light-dark and startle tests. For this test, a glass tank (L x H x W: 600 mm × 300mm × 250 mm) with opaque sides was used. A novel round plastic object (± 60 mm diameter) was placed in the opposite corner of the tank furthest away from the subject. The behaviour of the individual was video-recorded for 10 minutes, and the following behaviours were scored: latency (in seconds) to approach the novel object, behaviours displayed on approaching the novel object, including object manipulation (biting and sniffing of novel object) and time spent within 5 cm of the novel object. The tank was cleaned with disinfectant soap between tests. Between personality tests, individuals were always returned to their standard laboratory housing.

Behaviour in the home cage

Striped mice were housed individually in their standard laboratory housing, furnished as described above for a further 30 days and their behaviours recorded every second day for 15 days. Every two weeks, the cages and the PVC nest boxes were cleaned and replaced. Using Observer software, the duration of three behaviours (inactivity, activity and stereotypic behaviour if applicable; see Table 1) and the frequency of six behaviours (inactivity, activity, manipulation, feeding, grooming and stereotypic behaviour if applicable) were scored between 09h00 to 12h00 using continuous sampling. The data were summed for all days for data analyses.

Table 1. Ethogram of striped mice behaviours scored in the standard laboratory housing

Behaviour	Definition
Inactive	Individual motionless and resting or out of sight
Active	Non-stereotypic movement or running on the cage floor usually digging in the wood shavings
Object manipulation	Manipulating enrichments (e.g. biting or nudging of cardboard tubes and wheels)
Feeding/Drinking	Manipulating or chewing of mouse cubes, seeds or vegetables/fruits and drinking from a water bottle
Stereotypic behaviour	A repetitive and invariant behaviour > 3 times in succession
Grooming	Squatting on hind legs, grooming head, body, tail, and/or genitals

Experiment 2

In this experiment, I investigated whether personality predicted the onset of stereotypy at a later age. Since stereotypy is observed as early as 40 days of age in striped mice (Jones, 2012), juveniles that were weaned and housed alone at 30 days were screened for this study. A haphazard sample of 40 (20 of each sex), each from a different litter was selected for study. They were observed for 6 hours over 2 days to ensure that none showed any stereotypic behaviour. Their responses in the light-dark and startle response tests were then tested twice on two sequential days, using the protocol described earlier. These two tests were chosen based on the outcome of experiment 1 (see Results). At 80 days of age (48 days after personality tests), the incidence of stereotypic behaviour was recorded daily for 10 days from 09h00 to 12h00. It was also recorded whether or not an individual displayed stereotypic behaviour and, if so, the type of stereotypic behaviour. These observations were conducted at 80 days of age since captive striped mice reach adulthood then and 90% of striped mice that develop stereotypy do so at this age (Jones, unpublished data).

Of the 40 individuals monitored, 19 (10 male, 9 female) showed locomotor stereotypic behaviour (circuit running, somersaulting) and 14 (9 male, 5 female) did not show any stereotypy. The remaining 7 individuals displayed non-locomotor stereotypy and were excluded from the analysis.

Data Analyses

Data were checked for normality and homogeneity of variance using the Shapiro-Wilk and Levene's tests. Analyses were done using Statistica (version 7). All statistical tests were two-tailed, with the model level significance set at $\alpha = 0.05$. Fishers posthoc tests were used to identify specific trends when predictors were significant. The data set was analysed at the group level (i.e. stereotypic vs non-stereotypic striped mice) and the individual level (intra-group) to assess variation in behavioural responses.

Experiment 1

A general linear model (GLM) for multiple dependents was used to analyse the behavioural responses of stereotypic and non-stereotypic mice (stereotypy status) and sex for each personality test separately. The latency to approach the novel object, time spent with novel object (novel-object test), latency to emerge from the dark compartment, time spent in the dark compartment (light-dark test), latency to emerge from the dark compartment and time spent in the light compartment (startle test) were included as dependant variables.

To assess whether personality scores were consistent among personality tests, a Pearson's product-moment correlation coefficient was used to evaluate correlations between the different variables of the personality test scores (as above) separately for stereotypic and non-stereotypic mice.

To investigate behavioural differences between the stereotypic and non-stereotypic striped mice of both sexes in the standard laboratory housing, a GLM for multiple dependents was run using six behaviours (Table 1).

Since personality describes individual variation in behaviour, the relationship between personality and the behaviours of individual stereotypic and non-stereotypic striped mice in the standard laboratory housing was assessed, using a polynomial multivariate regression (for multiple dependents); data for the sexes were pooled based on the GLM analyses. The frequency of the six behaviours and the duration of only activity, inactivity and stereotypic behaviours were included as dependent variables and the personality scores (as above) were used as the continuous predictors; other behaviours occurred infrequently and were too short to be scored. Separate tests were conducted for non-stereotypic individuals since they do not exhibit stereotypic behaviours. For all dependent variables, the homogeneity of slopes of the continuous predictors and their interaction were examined first to determine whether a single test with multiple dependent or separate regressions were required. For both tests, the coefficient of determination, F- and P-values and parameter estimates for linear and polynomial decomposition are reported. The beta coefficients, calculated by standardising all variables to a mean of 0 and a standard deviation of 1 were also examined. The polynomial variables are reported as linear and quadratic functions, indicated with a "^{2}".

Experiment 2

I averaged the personality scores per test over the two days of testing. I then used a GLM for multiple dependents to analyse the personality test scores of individuals that later developed stereotypy and those that did not. Stereotypy status and sex were categorical predictors.

Results

Experiment 1

Personality

There was a significant stereotypy status effect on the behaviours in the startle response test (Table 2), with stereotypic striped mice spending significantly more time in the light compartment of the light-dark tank after a startle response (Figure 1). There were no significant differences between stereotypic and non-stereotypic mice in the latency to approach the novel object, time spent with the novel object, latency to emerge from the dark

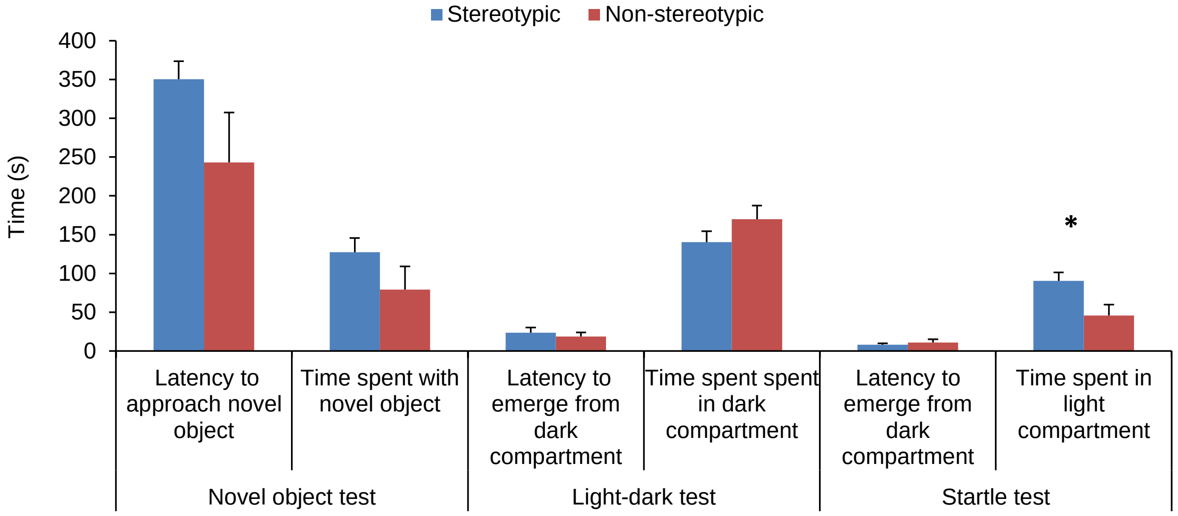

Figure 1. Time (s) of behaviours (mean + SE) in the novel object, light-dark and startle personality tests for stereotypic and non-stereotypic mice. Bars with * above them are significantly different (Fishers post hoc tests).

compartment and time spent in the dark compartment (light-dark test) and latency to emerge from the dark compartment (startle test; Table 2).

Table 2. Statistical output for the analysis of the stereotypic status (stereotypic vs non-stereotypic striped mice) and sex in three personality tests. Significant predictors are shown in bold.

Personality test	Variable	GLM	P
Novel object test	Stereotypy status	$F_{2, 33} = 2.47$	0.100
	Sex	$F_{2, 33} = 0.02$	0.982
	Stereotypy status*sex	$F_{2, 33} = 2.93$	0.067
Light-dark test	Stereotypy status	$F_{2, 33} = 0.70$	0.505
	Sex	$F_{2, 33} = 0.33$	0.720
	Stereotypy status*sex	$F_{2, 33} = 0.61$	0.548
Startle test	**Stereotypy status**	$\mathbf{F_{2, 33} = 3.59}$	**0.039**
	Sex	$F_{2, 33} = 0.44$	0.647
	Stereotypy status*sex	$F_{2, 33} = 1.29$	0.289

Pearson’s product-moment correlation coefficient analyses showed negative correlations between time spent in the dark compartment (Light-dark test) and (i) latency to approach novel object (Novel-object test) and (ii) latency to emerge from the dark compartment (Light-dark test) in stereotypic striped mice (Table 3). This indicated that

Table 3. Statistical output for the Pearson’s product-moment correlation coefficient analyses for three personality tests in stereotypic (above the diagonal) and non-stereotypic (below the diagonal) striped mice. Values in bold are significant at p<0.05.

		Stereotypic					
		Latency to approach novel object	Time spent with novel object	Latency to emerge from dark compartment	Time spent in dark compartment	Latency to emerge from dark compartment	Time spent in light compartment
Non-stereotypic	Latency to approach novel object		0.20	0.26	**-0.48**	0.19	0.18
	Time spent with novel object	0.54		-0.27	-0.23	0.36	**0.64**
	Latency to emerge from dark compartment	0.21	0.18		**-0.52**	-0.05	-0.27
	Time spent in dark compartment	-0.13	-0.34	0.19		-0.23	-0.38
	Latency to emerge from dark compartment	0.42	-0.10	-0.38	0.03		0.38
	Time spent in light compartment	0.28	0.49	-0.05	-0.49	0.05	

stereotypic mice that spent more time the dark compartment also showed a shorter latency to approach the novel object and a shorter latency to emerge from the dark compartment. There was one positive correlation between the time spent in the light compartment (Startle test) and time spent with the novel object (Novel-object test). This indicated that stereotypic mice that spent more time in the light compartment also spent more time with the novel object. There were no correlations between personality test scores in non-stereotypic mice.

Behaviour in the home cage

There was a significant stereotypy status effect on the frequency of five behaviours ($F_{2, 33} = 6.66$, $p<0.001$). Non-stereotypic striped mice were frequently more inactive than stereotypic mice, while stereotypic striped mice showed higher counts of object manipulation. There were no differences in the frequencies of activity, grooming and feeding between stereotypic and non-stereotypic striped mice (Figure 2). Sex ($F_{2, 33} = 1.24$, $p=0.313$) and stereotypy status * sex ($F_{2, 33} = 0.36$, $p=0.869$) were not significant predictors of the behaviours.

Stereotypy status influenced the duration of behaviours ($F_{2, 33} = 25.66$, $p<0.001$), with non-stereotypic striped mice displaying higher levels of inactivity than stereotypic striped

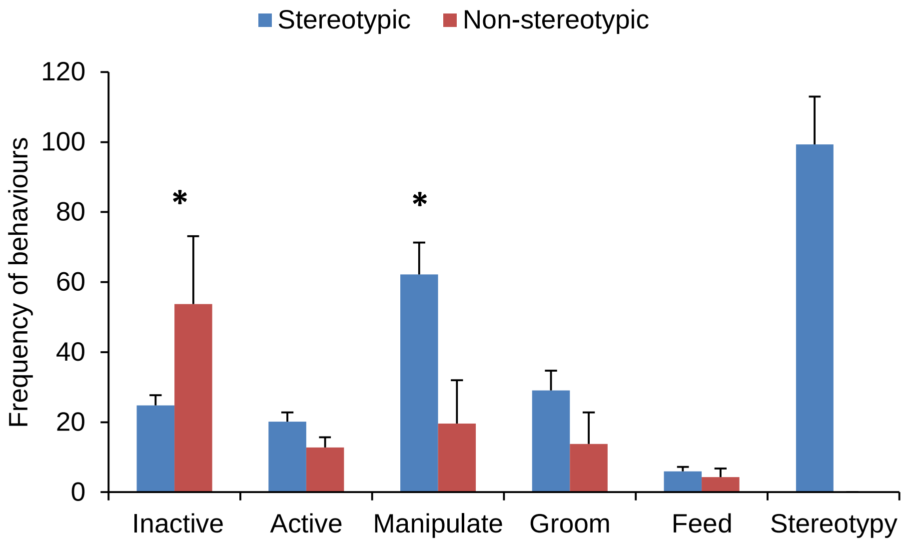

Figure 2. Mean (± SE) frequency of behaviours displayed by stereotypic and non-stereotypic striped mice in standard housing. Bars with * are significantly different (Fishers post hoc tests). Stereotypy was observed in stereotypic striped mice only and shown here for comparison.

mice. However, there were no significant differences in activity between stereotypic and non-stereotypic striped mice (Figure 3). Sex ($F_{2, 33} = 1.27$, p=0.294) and stereotypy status*sex ($F_{2, 33} = 1.102$, p=0.344) were not significant predictors of the behaviours.

Though stereotypic behaviours were not statistically analysed because non-stereotypic striped mice do not display stereotypic behaviours, stereotypic behaviour was a predominant constituent of the ‘general activity’ of stereotypic individuals (Figure 2 and Figure 3).

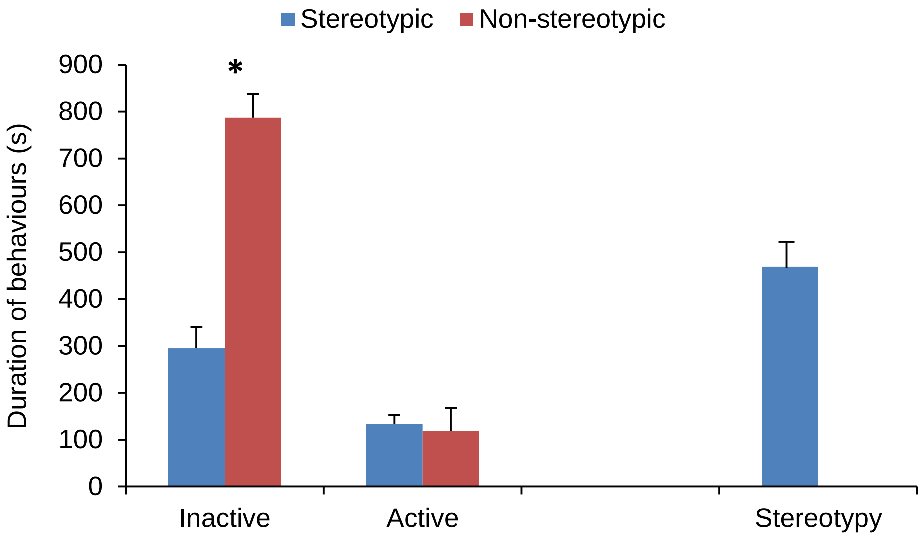

Figure 3. Mean (± SE) duration (s) of behaviours displayed by stereotypic striped and non-stereotypic striped mice in standard housing. Bars with * are significantly different (Fishers

post hoc tests). Stereotypy was observed in stereotypic striped mice only and shown here for comparison.

Personality vs stereotypic behaviours

Polynomial multivariate regression analyses were conducted on the frequency of stereotypic behaviours (stereotypic mice), activity, inactivity, object manipulation, feeding and grooming and the duration of stereotypic behaviours (for stereotypic mice), activity and inactivity versus the personality scores (as before) for each individual in the treatment. Since there were many zeroes in the data set for the non-stereotypic mice and the model did not run, behaviours (latency to approach the novel object and time spent with the novel object) from the novel-object personality test were excluded. This was not an issue for the stereotypic mice and thus I report results for all tests for these individuals.

An examination of the beta coefficients allows for an assessment of the relative contribution of each independent variable on the dependent variable. For the stereotypic mice, there was a significant association between the frequency of object manipulation and the time spent in the light compartment; a greater time spent in the light compartment in the personality test was correlated with a linear increase in the frequency of object manipulation but a non-random decrease in the frequency of object manipulation (**Supplementary material**: S1). There were 36 positive and 34 negative non-significant associations between the personality scores and frequency of behaviours. There were no significant associations between personality test scores and the duration of behaviours in the home cage. However, there were 18 positive and 18 negative non-significant associations between the personality tests scores and the duration of behaviours in the home cage.

For the non-stereotypic striped mice, an examination of the beta coefficients indicated that there were 2 significant positive associations between the personality test scores and the frequency of behaviours and 4 significant associations between personality tests scores and the duration of behaviours. There were 2 positive associations between latency to emerge from the dark compartment (light-dark test) and frequency of the polynomial of feeding and time spent in the light compartment^2 (startle test) and frequency of object manipulation (**Supplementary material**: S2). Furthermore, an examination of the beta coefficients revealed that there were significant associations between latency to emerge from the dark compartment (light-dark test) and duration of activity and duration of inactivity. With an increase in the latency to emerge from the dark compartment, there was a linear increase in activity and a linear decrease in inactivity but a non-random decrease in activity and a non-random increase in inactivity. There were six negative and six positive non-significant associations between the personality test scores and the behaviours.

Experiment 2

Stereotypy status ($F_{2, 28}$ = 2.01, p=0.153), sex ($F_{2, 28}$ = 0.61, p=0.555), stereotypy status*sex ($F_{2, 28}$ = 0.32, p=0.727) were not significant predictors of the latency to emerge from the dark and time spent in the dark compartment (Figure 4). In contrast, stereotypy status ($F_{2, 28}$ = 13.67, p<0.001) was a significant predictor of behaviours in the startle test, with stereotypic striped mice spending more time in the light compartment, post-startle (Figure 4). This significant difference between the groups was not a result of two distinct groupings. The range of scores for stereotypic striped mice (29 to 233 s) overlapped with those of the non-stereotypic striped mice (11 to 91 s). There was no significant difference in the latency to emerge into the light compartment. Sex ($F_{2, 28}$ = 2.11, p=0.139) and stereotypy*sex ($F_{2, 28}$ = 0.24, p=0.786) were not significant predictors.

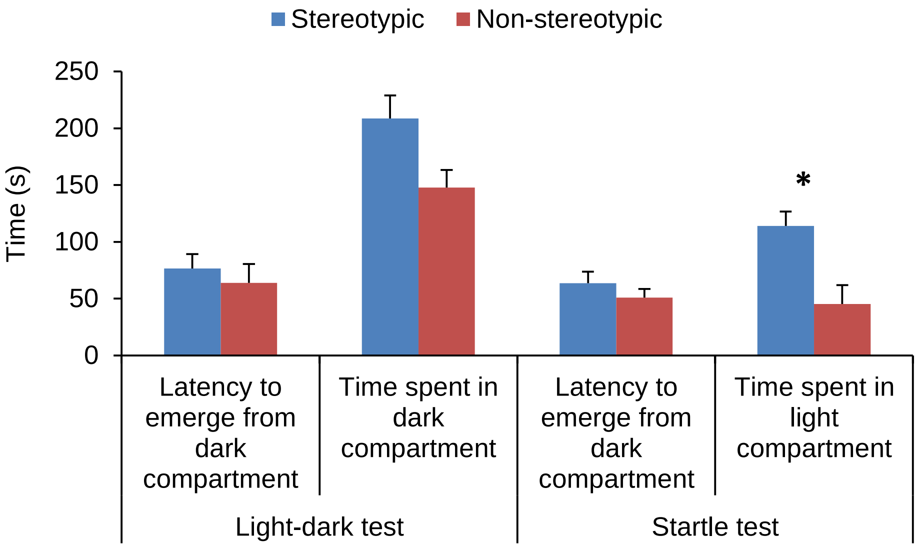

Figure 4. Time (s) of behaviours (mean ± SE) in the light-dark and startle personality tests for stereotypic and non-stereotypic mice respectively. Bars with * are significantly different (Fishers post hoc tests).

Discussion

The personality of stereotypic striped mice was studied to assess whether they show a proactive coping response (i.e. an active response) to stress, as proposed by Ijichi et al.

(2013). Two experiments were conducted in which standard personality tests were used to ascertain whether stereotypic striped mice display a bolder personality, typified by more exploratory behaviour, activity and reduced anxiety when faced with a novel-object or environment (Wilson et al., 1993). In contrast, non-stereotypic striped mice were expected to show a less bold personality and either retreat or become vigilant when confronted with novelty and also show a reactive coping style (i.e. conservation-withdrawal response), resulting in greater anxiety, fear and inactivity (Meagher et al., 2013; Meagher and Mason, 2012).

In experiment 1, stereotypic striped mice had a quicker recovery time following a startle and spent a longer time in the light compartment after a startle response compared to the non-stereotypic striped mice. In the home cage, stereotypic mice displayed more object manipulation while non-stereotypic striped mice showed a greater frequency and duration of inactivity. Although stereotypic behaviours could not be compared between the groups, stereotypic behaviours made up a large part of the general activity of stereotypic striped mice. Taken together, the data suggest that stereotypic striped mice are bolder, at least in the startle test, and have a proactive coping style compared to reactive coping style of non-stereotypic striped mice.

In experiment 2, the personality was measured at an early age (about 30 days old) and individuals were monitored to assess which became stereotypic later. The startle response test showed that bolder individuals (i.e. those spending more time in the light compartment) were likely to become stereotypic later. None of the other behavioural measures in the startle and light-dark tests predicted later onset of stereotypy. Similarly, Jones (2012) showed that behavioural responses in the light-dark box correspond to the development of stereotypic behaviours in striped mice.

The findings from both experiments indicate that only the startle response test could separate stereotypic and non-stereotypic striped mice, suggesting that this is a reliable test for assessing personality of striped mice. The responses following a startle are probably one of the most commonly measured traits in personality studies (Conrad et al., 2011), which measures boldness/fearfulness/anxiety by assessing how quickly an individual recovers from negative stimuli or a mild stressor (Miller et al., 2006). Individuals, which venture into open spaces of the light-dark box, are considered less anxious than those that spend time in “protected” spaces (Dellu et al., 1993). Our results are comparable to that of Van Oers et al. (2004) who showed that in great tits, *Parus major,* individuals that were selected from the ‘fast’ exploration line (i.e. proactive/bold) returned quickly to a feeding table with mealworms after being startled compared to the individuals selected from the 'slow' exploration line (i.e. reactive/less bold).

The most likely explanation for why the Novel object or the Light-dark tests did not influence the behaviour of stereotypic mice could be that stereotypic striped mice were probably including the novel object and cage divisions in the light-dark tests as part of their stereotypic behavioural routine and not actual interactions with the novel object per se. This questions the reliability of personality tests in stereotypic individuals. However, the difference in the Startle test could be attributed to the fact that by startling the stereotypic striped mice, the stereotypic behavioural routine was disrupted which eventually snapped them out of their stereotypic routine, enabling them to 'explore' the environment and spending more time in the light compartment after the startle. Similar results were found by Miller et al. (2006), who showed that the latency to peck the novel object correlated with pacing behaviour in the Japanese quail *Coturnix coturnix japonica*. This may suggest that interaction with the novel object was actually incorporated in the stereotypic behavioural routine. Miller et al. (2006) found that only three out of six behaviours that measured fearfulness were valid and thus fearfulness was not consistent through different contexts. Other reasons could be that different personality tests may measure different behavioural traits (Carter et al., 2013) or the same test could vary across species (Weiss and Adams, 2013).

There were two correlations between personality tests for the time spent in the dark compartment (Light-dark test) and (i) latency to approach novel object (Novel-object test) and (ii) latency to emerge from the dark compartment (Light-dark test) as well as between the time spent in the light compartment (Startle test) and time spent with the novel object (Novel-object test) for stereotypic striped mice and none for non-stereotypic striped mice. In addition, the novel object and light-dark tests could not separate stereotypic and non-stereotypic animals. It appears that behavioural responses were not consistent across contexts, a defining attribute of personality (Gosling, 2001). Indeed, Carter et al. (2013) suggested that the use of multiple personality tests might be measuring different behavioural traits altogether, making interpretation difficult. Nonetheless, the absence of a consistent response is surprising given that it has been shown in *R. pumilio* (a sister species of *R. dilectus*) from a semi-arid environment (Yuen et al., 2015), highlighting possible phylogenetic and habitat differences in responses (Rymer and Pillay, 2012), and suggesting that not all standard personality tests predict the onset of stereotypic behaviour in the genus.

Behavioural differences in personality tests are due to extrinsic (environment) and intrinsic factors (e.g. age), which may influence how animals vary their degree of boldness (Chapman et al., 2010; Bell and Stamps, 2004). The responses of stereotypic and non-stereotypic striped mice in personality tests were similar for adults (experiment 1) and juveniles (experiment 2). Personality traits are not always consistent over time and, in some species, personality traits may be plastic within or between life stages (Guenther et al., 2014). For example, juvenile dumpling squid, *Euprymna tasmanica*, showed consistent levels of boldness before and after sexual maturity i.e. adulthood. However, at sexual maturity, boldness varied depending on the context the squid were exposed (Sinn et al., 2008).

Since personality describes individual variation in behaviour, which is consistent across contexts (Réale and Dingemanse, 2012), the behaviour of individuals were compared to complement the group level (stereotypic status) effects in order to assess whether behaviour in the home (standard) cage was associated with personality test scores. I expected stereotypic individuals to show a proactive coping style, as shown by increased activity, quicker time to approach novel objects and increased exploration of novel environments, while non-stereotypic mice would show greater inactivity. As expected, at a group level, stereotypic mice showed greater activity and increased object manipulation while non-stereotypic mice exhibited greater inactivity. Nevertheless, at an individual level, both stereotypic and non-stereotypic mice showed flexible behavioural responses.

Multivariate regressions indicated two significant associations between personality scores and behaviours for stereotypic striped mice, compared to 6 significant associations for non-stereotypic mice. These results indicate that the behaviours in the personality tests are uncoupled from behaviours in the home cage for both stereotypic and non-stereotypic striped mice. In other words, despite being more or less bold in the personality tests (based on scores of the startle test), both stereotypic and non-stereotypic striped mice did not maintain this pattern in the Standard treatments. Thus, striped mice which were bold in the personality tests were not necessarily bold in the Standard treatment but rather varied their behaviours in the home cage. Furthermore, bolder stereotypic mice (i.e. those spending more time in the light compartment) showed a linear increase in object manipulation, and a non-random decrease in object manipulation, implying inconsistency in behaviours. Although flexible behavioural responses have not been shown in bold rodents, bold rainbow trout, *Oncorhynchus mykiss,* were generally plastic in their levels of neophobia and activity depending on the challenge (Frost et al., 2007). Similarly, bolder non-stereotypic mice (i.e. those spending more time in the light compartment) showed positive associations with object manipulation. There was an unexpected negative relationship between the latency to emerge from the dark compartment of startle test and activity and inactivity. In addition, less bold non-stereotypic striped mice (emerging slowly from the dark compartment) showed a linear increase in activity but a non-random decrease in activity, a linear decrease in inactivity and a non-random decrease in inactivity. It is possible that less bold non-stereotypic animals assess the situation before displaying behaviour and are less predictable in their behaviours. Similarly, when exposed to a shock by an electrified probe, non-aggressive wild house mice, *Mus musculus domesticus*, which showed a longer attack latency (LAL) and regarded as less bold reacted both proactively and reactively. In a familiar and less aversive environment i.e. home cage sawdust, these mice showed a reactive coping style by active defensive burying, while in a stressful and unfamiliar (fresh sawdust) environment, they showed a proactive coping style by showing immobility (Sluyter et al., 1996). In sum, it appears again that individual stereotypic and non-stereotypic striped mice do not show consistent behaviours across contexts.

Based on behavioural and physiological similarities between proactive and stereotypic individuals, Ijichi et al. (2013) proposed that a proactive coping style might predispose the onset of stereotypic behaviour, depending on environmental conditions. This study shows that coping styles do not predict behavioural responses under varying conditions and tend to vary in different contexts. This contradicts the view that stereotypic animals are not dependent on environmental stimuli to exhibit behaviours and thus resilient to changes (Fentress, 1976). While stereotypic striped mice showed a proactive and a bolder personality, our data indicate that this group level effect is not consistent at the individual level because of inconsistency across contexts, indicating flexibility across contexts. Non-stereotypic striped mice showed the requisite behaviours for a less bold personality and reactive coping style, and interestingly, their behaviour was consistent (less flexible) across contexts at the group level, while there was some degree of flexible behavioural responses at the individual level. In terms of an individual’s propensity to take risks in novel environments, these findings contradict those of other studies that bold or proactive individuals are comparatively ‘inflexible’ in their behavioural responses compared to shy/less bold or reactive individuals which display greater flexibility (Benus et al., 1987; Benus et al., 1991; Koolhaas et al., 1999; Sih et al., 2004, Bolhuis et al., 2005; Ruiz-Gomez et al., 2011), suggesting that the coping hypothesis may not hold in all cases. While the discrepancies in findings could be due to species differences, these findings are not surprising, as behavioural flexibility is well known in *Rhabdomys* spp. which show flexible social organisation (i.e. social flexibility; Schradin et al., 2010; Schradin et al., 2012), flexible mating strategies (Schradin, 2008) and flexible development of exploration (Rymer and Pillay, 2012). Although stereotypic animals are predicted to be proactive, not all proactive individuals are expected to show stereotypy when the eliciting triggers are absent (Ijichi et al., 2013) or have not reached a particular threshold (Koolhaas et al., 2010). The eliciting triggers could probably be the impoverished environments and genetic preposition for displaying stereotypies and having different personalities.

The findings in experiment 2 indicate that the onset of stereotypy could be statistically predicted based on the latency to recover from a startle. However, stereotypic and non-stereotypic striped mice did not form distinct groups in the startle response and there was overlap between individuals in a group, such that some individuals, which spent comparatively more time in the light compartment, did not develop stereotypic behaviours. In support, Jones (2012) proposed that the behavioural trajectory for the development of stereotypic behaviour and the frequency of later stereotypic behaviour performance in stereotypic individuals is not predicted by the measures of anxiety/ fearfulness assessed in juveniles before the onset of stereotypic behaviours.

Conclusions

This study provided the first experimental test for the model developed by Ijichi et al. (2013) that personality might be associated in the development of stereotypic behaviours and stereotypic behaviours indicate a proactive coping response to stress. This data provide general support for the idea of proactivity, including a bold personality associated with stereotypic behaviour in striped mice. These effects were demonstrated at a group level and in one personality test. Inconsistencies at the individual level are surprising, suggesting that personality and stereotypy should be evaluated at the individual level or that the personality tests were not able to detect personality differences. These are avenues for future study, which should also consider whether complexity of the housing environment modulates the personality-stereotypy relationship.

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Supplementary material

Table S1. Multivariate regression analysis of the frequency of stereotypic behaviour, activity, inactivity, object manipulation, feeding and grooming and the duration of stereotypic behaviour, activity and inactivity with latency to approach the novel object, time spent with the novel object (Novel-object test), latency to emerge from the dark for both light-dark and startle tests, time spent in the dark compartment (Light-dark test) and time spent in the light compartment (Startle test) as the predictor variables in stereotypic mice. Linear and polynomial decomposition are reported. Values in bold are significant.

FREQUENCY							
Personality test	**Behaviour**	**Parameter Estimates**	**P**	**β**	**R^2**	**F**	***df***
Latency to approach novel object	Stereotypy	0.053	0.902	0.09	0.64	0.22	6,8
	Active	0.069	0.486	0.59	0.52		
	Inactive	0.093	0.421	0.72	0.47		
	Manipulate	0.122	0.592	0.31	0.77		
	Feed	0.060	0.301	1.06	0.31		
	Groom	0.194	0.394	0.80	0.43		
Time spent with novel object	Stereotypy	-0.516	0.541	-0.69	0.64	0.32	6,8
	Active	-0.038	0.840	-0.26	0.52		
	Inactive	-0.073	0.743	-0.45	0.47		
	Manipulate	-0.473	0.294	-0.96	0.77		
	Feed	-0.009	0.936	-0.12	0.31		
	Groom	-0.336	0.448	-1.09	0.43		
Latency to emerge from dark compartment	Stereotypy	-0.512	0.686	-0.25	0.64	0.28	6,8
	Active	-0.048	0.866	-0.12	0.52		
	Inactive	-0.069	0.837	-0.16	0.47		
	Manipulate	-0.403	0.546	-0.30	0.77		
	Feed	-0.124	0.463	-0.64	0.31		
	Groom	-0.702	0.297	-0.84	0.43		
Time spent in dark compartment	Stereotypy	-0.496	0.678	-0.51	0.64	0.39	6,8
	Active	-0.254	0.356	-1.32	0.52		
	Inactive	-0.369	0.257	-1.73	0.47		
	Manipulate	-0.451	0.476	-0.70	0.77		
	Feed	-0.173	0.286	-1.84	0.31		
	Groom	-0.427	0.497	-1.06	0.43		
Latency to emerge from dark compartment	Stereotypy	3.510	0.601	0.46	0.64	0.14	6,8
	Active	-0.804	0.597	-0.53	0.52		
	Inactive	-0.950	0.595	-0.57	0.47		
	Manipulate	1.427	0.685	0.28	0.77		
	Feed	-0.163	0.854	-0.22	0.31		
	Groom	-1.721	0.623	-0.54	0.43		
Time spent in light compartment	Stereotypy	1.293	0.335	1.06	0.64	1.03	6,8
	Active	0.519	0.100	2.16	0.52		
	Inactive	0.667	0.075	2.51	0.47		
	Manipulate	**1.484**	**0.048**	**1.84**	**0.77**		
	Feed	0.251	0.168	2.15	0.31		

	Groom	0.813	0.250	1.61	0.43		
Latency to approach novel object²	Stereotypy	0.000	0.918	0.07	0.64	0.21	6,8
	Active	0.000	0.635	-0.36	0.52		
	Inactive	0.000	0.468	-0.58	0.47		
	Manipulate	0.000	0.915	-0.05	0.77		
	Feed	0.000	0.453	-0.68	0.31		
	Groom	0.000	0.586	-0.45	0.43		
Time spent with novel object²	Stereotypy	0.003	0.275	1.17	0.64	0.82	6,8
	Active	0.000	0.707	0.45	0.52		
	Inactive	0.000	0.718	0.46	0.47		
	Manipulate	0.003	0.086	1.51	0.77		
	Feed	0.000	0.950	0.09	0.31		
	Groom	0.001	0.352	1.25	0.43		
Latency to emerge from dark compartment²	Stereotypy	0.005	0.488	0.42	0.64	0.41	6,8
	Active	0.001	0.676	0.29	0.52		
	Inactive	0.001	0.771	0.21	0.47		
	Manipulate	0.003	0.392	0.42	0.77		
	Feed	0.001	0.455	0.63	0.31		
	Groom	0.004	0.329	0.76	0.43		
Time spent in dark compartment²	Stereotypy	0.001	0.725	0.45	0.64	0.40	6,8
	Active	0.001	0.336	1.43	0.52		
	Inactive	0.001	0.248	1.83	0.47		
	Manipulate	0.002	0.417	0.83	0.77		
	Feed	0.001	0.255	2.05	0.31		
	Groom	0.001	0.543	0.98	0.43		
Latency to emerge from dark compartment²	Stereotypy	-0.076	0.727	-0.29	0.64	0.15	6,8
	Active	0.038	0.445	0.74	0.52		
	Inactive	0.042	0.473	0.73	0.47		
	Manipulate	-0.038	0.739	-0.22	0.77		
	Feed	0.006	0.836	0.24	0.31		
	Groom	0.043	0.704	0.40	0.43		
Time spent in light compartment²	Stereotypy	-0.006	0.325	-0.87	0.64	1.19	6,8
	Active	-0.002	0.113	-1.67	0.52		
	Inactive	-0.003	0.098	-1.86	0.47		
	Manipulate	**-0.008**	**0.031**	**-1.64**	**0.77**		
	Feed	-0.001	0.152	-1.80	0.31		
	Groom	-0.003	0.307	-1.15	0.43		
DURATION							
Latency to approach novel object	Stereotypy	0.162	0.932	0.08	0.34	2.03	3,11
	Active	1.260	0.094	1.52	0.50		
	Inactive	-1.508	0.380	-0.77	0.50		
Time spent with novel object	Stereotypy	2.524	0.498	1.04	0.34	0.76	3,11
	Active	-0.771	0.580	-0.74	0.50		
	Inactive	-1.530	0.643	-0.61	0.50		
	Stereotypy	-1.117	0.841	-0.17	0.34	0.83	3,11

Latency to emerge from dark compartment	Active	-2.221	0.297	-0.78	0.50		
	Inactive	3.044	0.542	0.45	0.50		
Time spent in dark compartment	Stereotypy	3.555	0.503	1.12	0.34	0.35	3,11
	Active	-2.067	0.304	-1.51	0.50		
	Inactive	-1.495	0.750	-0.46	0.50		
Latency to emerge from dark compartment	Stereotypy	12.736	0.666	0.51	0.34	0.13	3,11
	Active	-6.932	0.533	-0.65	0.50		
	Inactive	-5.570	0.832	-0.22	0.50		
Time spent in light compartment	Stereotypy	1.636	0.779	0.41	0.34	0.54	3,11
	Active	1.585	0.471	0.93	0.50		
	Inactive	-3.350	0.521	-0.82	0.50		
Latency to approach novel object[2]	Stereotypy	0.001	0.826	0.19	0.34	0.94	3,11
	Active	-0.001	0.310	-0.79	0.50		
	Inactive	0.001	0.809	0.19	0.50		
Time spent with novel object[2]	Stereotypy	-0.006	0.610	-0.73	0.34	0.62	3,11
	Active	0.003	0.460	0.92	0.50		
	Inactive	0.002	0.836	0.26	0.50		
Latency to emerge from dark compartment[2]	Stereotypy	0.034	0.303	0.86	0.34	0.92	3,11
	Active	0.006	0.620	0.35	0.50		
	Inactive	-0.038	0.201	-0.94	0.50		
Time spent in dark compartment[2]	Stereotypy	-0.007	0.695	-0.68	0.34	0.53	3,11
	Active	0.009	0.198	1.99	0.50		
	Inactive	-0.002	0.911	-0.17	0.50		
Latency to emerge from dark compartment[2]	Stereotypy	-0.483	0.616	-0.57	0.34	0.18	3,11
	Active	0.268	0.460	0.73	0.50		
	Inactive	0.212	0.804	0.24	0.50		
Time spent in light compartment[2]	Stereotypy	-0.006	0.827	-0.26	0.34	0.57	3,11
	Active	-0.009	0.387	-0.90	0.50		
	Inactive	0.015	0.528	0.65	0.50		

Table S2. Multivariate regression analysis of the frequency of activity, inactivity, object manipulation, feeding and grooming and the duration of activity and inactivity with latency to approach the novel object, time spent with the novel object (Novel-object test), latency to emerge from the dark for both light-dark and startle tests, time spent in the dark compartment (Light-dark test) and time spent in the light compartment (Startle test) as the predictor variables in non-stereotypic mice. Linear and polynomial decomposition are reported. Values in bold are significant.

FREQUENCY							
Personality test	**Behaviour**	**Parameter Estimates**	**P**	**β**	**R^2**	**F**	***df***
Latency to emerge from dark compartment	Active	0.788	0.644	1.38	0.43	1.73	3,1
	Inactive	1.442	0.761	0.38	0.90		
	Manipulate	1.781	0.221	0.74	0.98		
	Feed	1.056	0.042	2.22	0.97		
	Groom	0.504	0.754	0.29	0.95		
Time spent in dark compartment	Active	0.650	0.758	3.89	0.43	0.60	3,1
	Inactive	3.315	0.584	3.01	0.90		
	Manipulate	2.231	0.221	3.17	0.98		
	Feed	0.825	0.123	5.91	0.97		
	Groom	2.445	0.276	4.73	0.95		
Latency to emerge from dark compartment	Active	1.855	0.807	2.50	0.43	1.01	3,1
	Inactive	10.440	0.631	2.13	0.90		
	Manipulate	9.183	0.177	2.93	0.98		
	Feed	3.712	0.077	5.99	0.97		
	Groom	9.281	0.257	4.04	0.95		
Time spent in light compartment	Active	-0.263	0.702	-1.25	0.43	0.73	3,1
	Inactive	-1.834	0.373	-1.32	0.90		
	Manipulate	-0.673	0.247	-0.76	0.98		
	Feed	-0.021	0.879	-0.12	0.97		
	Groom	-1.054	0.175	-1.62	0.95		
Latency to emerge from dark compartment^2	Active	-0.012	0.644	-1.10	0.43	0.88	3,1
	Inactive	-0.021	0.774	-0.29	0.90		
	Manipulate	-0.019	0.364	-0.41	0.98		
	Feed	-0.013	0.073	-1.40	0.97		
	Groom	0.001	0.978	0.02	0.95		
Time spent in dark compartment^2	Active	-0.002	0.736	-3.95	0.43	0.57	3,1
	Inactive	-0.010	0.558	-2.99	0.90		
	Manipulate	-0.006	0.218	-2.95	0.98		
	Feed	-0.002	0.130	-5.32	0.97		
	Groom	-0.007	0.263	-4.52	0.95		
Latency to emerge from light compartment^2	Active	-0.037	0.787	-2.62	0.43	1.03	3,1
	Inactive	-0.191	0.625	-2.05	0.90		
	Manipulate	-0.166	0.175	-2.79	0.98		
	Feed	-0.068	0.072	-5.80	0.97		
	Groom	-0.164	0.263	-3.76	0.95		
	Active	0.001	0.663	1.31	0.43	3.02	3,1

Time spent in light compartment^2	Inactive	0.015	0.180	2.02	0.90		
	Manipulate	0.007	0.049	1.56	0.98		
	Feed	0.001	0.362	0.70	0.97		
	Groom	0.008	0.070	2.31	0.95		
DURATION							
Latency to emerge from dark compartment	**Active**	**41.529**	**0.024**	**4.25**	**0.92**	6.13	2,2
	Inactive	**-40.256**	**0.026**	**-4.08**	**0.92**		
Time spent in dark compartment	Active	20.044	0.199	7.00	0.92	0.96	2,2
	Inactive	-19.098	0.215	-6.61	0.92		
Latency to emerge from dark compartment	Active	98.21	0.113	7.73	0.92	2.09	2,2
	Inactive	-89.993	0.134	-7.01	0.92		
Time spent in light compartment	Active	4.346	0.353	1.20	0.92	0.87	2,2
	Inactive	-4.95	0.299	-1.36	0.92		
Latency to emerge from dark compartment^2	**Active**	**-0.552**	**0.035**	**-2.91**	**0.92**	4.47	2,2
	Inactive	**0.541**	**0.037**	**2.82**	**0.92**		
Time spent in dark compartment^2	Active	-0.051	0.224	-6.04	0.92	0.80	2,2
	Inactive	0.049	0.236	5.77	0.92		
Latency to emerge from dark compartment^2	Active	-1.868	0.1	-7.73	0.92	2.33	2,2
	Inactive	1.714	0.119	7.03	0.92		
Time spent in light compartment^2	Active	-0.025	0.287	-1.30	0.92	0.95	2,2
	Inactive	0.028	0.248	1.42	0.92		

CHAPTER THREE

Does personality influence responses to environmental enrichment in stereotypic African striped mice, *Rhabdomys dilectus*?

Abstract

Environmental enrichment is used to enhance the well-being of captive animals and to prevent or reduce stereotypic and other abnormal behaviours. However, environmental enrichment does not always succeed in its intended purpose. The present study aims to investigate whether personality (i.e. consistent individual variation in behaviour) influences how stereotypic mice interact with environmental enrichments, since this could explain why enrichments vary in their efficacy. Stereotypic and non-stereotypic individuals of both sexes were tested for their personality using three standard tests (i.e. novel-object, light-dark and startle-response tests) as sub-adults at 43 days of age. Thereafter, mice were introduced individually to cages of different complexity i.e. Standard (standard laboratory housing provided with nest box with bedding), Standard-enriched (same as Standard treatment provided with enrichment - one/two cardboard tubes), and Enriched (large tank with nest box with bedding and enrichment - running wheel, one/two cardboard tubes, Habitrail ^{TM} PVC tunnels and balls) treatments in a random sequence, so animals would have experienced different treatments at different ages. At a group level, stereotypic striped mice were bolder than non-stereotypic mice, showing greater activity in all treatments and a significant reduction in stereotypic behaviours in the Enriched treatment. At an individual level, while stereotypic individuals showed a decrease in stereotypic behaviours in the Enriched treatment, not all stereotypic mice responded to the Enriched treatment in the same way. Stereotypic striped mice showed flexible behavioural responses to cages of varying complexity, with individuals that spent more time in the light compartment (i.e. bolder) showing a linear increase in stereotypic behaviours from the Standard to Standard-enriched treatments and decrease in stereotypic behaviours in the Enriched treatment. While non-stereotypic individuals were relatively fixed in their responses in all the treatments, there was some indication of flexible behavioural responses with individuals showing different patterns of activity, inactivity and object manipulation in the treatments. These findings provide novel evidence in rodents that behavioural responses of stereotypic striped mice to environmental enrichment were influenced by their personality.

Key words: Environmental enrichment, Stereotypic behaviour, Personality, Striped mice

Introduction

Captive animals are raised in impoverished environments, which provide inadequate motor and sensory stimulation and restrict behaviours in the normal behavioural repertoire (Mason, 1991a). These environments can initiate behavioural thwarting and motivational conflicts, which may elicit redirected behaviours and displacement activities from which maladaptive and stereotyped behaviours normally arise (Mason, 1991a; Würbel, 2006). Stereotypic behaviours are traditionally described as any abnormal, repetitive and invariant behaviours that are caused by frustration, repeated attempts to cope and/or central nervous system (CNS) dysfunctions (Latham and Mason, 2008). Stereotypic behaviours are indicative of impaired welfare because they are rife in individuals housed under deprived (physical and social) conditions, which expose them to unavoidable stress or fear (Mason, 1991b; Wiedenmayer, 1997; Mason et al., 2007).

Environmental enrichment is used to ameliorate some of the problems created by captivity, such as stereotypic and other abnormal behaviours, by changing the animal's environment in a way that promotes behavioural diversity and expression of 'normal' or species-typical behaviours, such as foraging and exploration (Young, 2003; Swaisgood and Shepherdson, 2006; Abou-Ismail, 2011), as well as improving health and reproductive success (Newberry, 1995; Van de Weerd et al., 1997; Young, 2003). Moreover, enrichment can also increase sensory and motor functioning by stimulating animals cognitively through training (e.g. novel-object recognition) (Pietropaolo et al., 2004; Nithianantharajah and Hannan, 2006; Simpson and Kelly, 2011).

Animals kept in enriched environments have been observed to explore novel environments quickly and to approach novel items faster than control animals in tests of anxiety and exploratory behaviour (Friske and Gammie, 2005). For example, when provided with novel objects for environmental enrichment, orange-winged Amazon parrots, *Amazona amazonica*, were less fearful (i.e. showed shorter latencies to approach the novel object), and approached and interacted with the novel objects many times, and performed many other behaviours, such as preening and feeding (Meehan and Mench, 2002). Therefore, environmental enrichment can be useful to reduce anxiety-related behaviours to environmental stressors, such as novelty and human handling (Fox and Millam, 2007), decrease performance of stereotypic behaviours (Swaisgood and Shepherdson, 2006) and reduce the release of corticosterone in response to stress (Belz et al., 2003). Moreover, enriched environments are also associated with structural and biochemical changes in the brain of captive animals, by increasing the number and density of neurons and synapses as well as by increasing dendritic arborisation (van Praag et al., 2000; Würbel, 2001). The functional consequences of these changes include enhanced memory, learning, and, perhaps crucially, the ability to cope with environmental challenges (Newberry, 1995; Young, 2003).

Despite the plethora of studies that demonstrate the advantages of environmental enrichment, it is intriguing that environmental enrichment does not always succeed for its intended purpose. It is possible that in these cases, a particular environmental enrichment is not tailored to the unique behavioural needs of a particular target species (Mellen and MacPhee, 2001). However, this does not explain intra-specific variation in behavioural responses, which could reflect the differences in personalities (Dall, 2004, Sih et al., 2004; Réale et al., 2007). Recently, Ijichi and colleagues (2013) hypothesized that personality may have a role in the development of stereotypic behaviours, since stereotypic behaviours are a result of environmental stressors and are thought to be a coping strategy (i.e. both physiological and behavioural mechanisms used by individuals to minimise the effects of stressful events or environmental conditions).

The coping style theory predicts that bolder animals should display rigid and invariant behaviours while less bold animals display flexible behaviours (Koolhaas et al., 1999). In their review, Coppens et al. (2010) suggested that behavioural flexibility is an integral component of coping styles and determines how an individual responds and adjusts its behaviour to environmental stimuli. This explains the low flexibility and tendency to develop routines in proactive individuals and ability of reactive individuals to readjust their behaviours by responding to environmental cues and thus show greater behavioural flexibility.

The aim of my study was to investigate whether stereotypic and non-stereotypic African striped mice, *Rhabdomys dilectus*, respond differently to cages of varying complexities and whether personality of individuals is associated with this response. Striped mice readily display stereotypical behaviours in captivity with approximately half of captive born individuals exhibiting stereotypic behaviours when housed in standard laboratory cages (Schwaibold and Pillay, 2001). There is a strong indication that stereotypic behaviour in striped mice has a genetic basis (Schwaibold and Pillay, 2001) and stereotypic mothers are five times more likely to produce stereotypic than non-stereotypic offspring (Jones et al., 2008).

In an earlier study (Chapter 2), I demonstrated that stereotypic striped mice displayed a proactive coping style and a generally bolder personality than non-stereotypic behaviour. These differences at the group level (stereotypic vs non-stereotypic) were not present at the individual level, which I specifically investigated to test the assumptions of personality theory (i.e. individual variation in behaviour; Gosling, 2001). At an individual level, both stereotypic and non-stereotypic striped mice were flexible in their behavioural responses, varying their behaviours irrespective of their personality type. In the current study, individuals were subjected to three personality tests, after which they were exposed in a random order to three treatments differing in the level of enrichment (i.e. Standard, Standard-enriched and Enriched housing conditions). At a group level, I predicted that stereotypic mice would show higher levels of stereotypic behaviours in the standard treatment and lower levels in the enriched treatments. Stereotypic striped mice would also show an increase in activity and object manipulation in the enriched treatments since they are bolder (see Chapter 2), while non-stereotypic mice would show decreased activity. At an individual level, since both stereotypic and non-stereotypic striped mice show flexible behaviour in different contexts regardless of personality differences (Chapter 2), I predicted that individual stereotypic and non-stereotypic striped mice would change their behaviour depending on the housing conditions.

Materials and Methods

Striped mice used in this study were captive born F1 and F2 individuals, originating from a population in Pretoria (25º 40ʿʿ S; 28º 30ʿʿ E), South Africa. They were housed in the Milner Park Animal Unit, University of the Witwatersrand under partially controlled environmental conditions: 14L: 10D light: dark cycle (lights on at 05h00); 22ºC - 24ºC and 30 - 60% rH. Subjects were housed singly in clear Lab-o-tec™ cages (L × H × W: 300 mm × 200 mm × 150 mm; Standard housing). Wood shavings (± 3 cm) were provided as bedding and a handful of *Eragrostis* grass (± 20 g) and ± 5 g of shredded tissue paper were provided as nesting material. PVC nest-boxes (L × H × W: 100 mm × 100 mm × 150 mm) were also provided in each cage. Epol® mouse cubes and water were available *ad libitum*. Approximately 10 g of fresh fruit (apples, pears) or vegetables (lettuce, carrots, broccoli) and ± 5 g of mixed seed were provided daily per individual.

Stereotypic behaviours have a genetic basis in striped mice (Schwaibold and Pillay, 2001; Jones et al., 2008), so to increase the chance of producing stereotypic and non-stereotypic individuals, seven stereotypic and seven non-stereotypic pairs (i.e. both male and female were either stereotypic or not; as described below) were established under standard laboratory conditions. The male was separated from the female prior to parturition. The pups were separated from the mother at 22 days of age and housed singly in Lab-o-tec™ cages. These offspring were used in experiments (Figure 1).

Observations for stereotypy and personality tests

Starting from 22 days, observations were made of young, twice a day for a total of half an hour, in order to establish the absence/presence of stereotypic behaviour. Stereotypic individuals were those that exhibited at least 10 or more bouts of stereotypy per observation session, each with three or more repetitions (after Jones et al., 2008). This method is routinely used in our lab. Only individuals that exhibited locomotor stereotypic behaviours were used in this study. Individuals that did not exhibit any stereotypic behaviour were classified as non-stereotypic and were used as a comparison for the stereotypic individuals. Stereotypic behaviour is an ‘all or nothing’ occurrence in striped mice (i.e. an individual either displayed or did not display stereotypies). Only the absence or presence of stereotypic behaviours was recorded (see Jones et al., 2008); non-stereotypic mice never displayed stereotypic behaviours. At 43 days of age (Figure 1), both stereotypic (male, n=8: female, n=7) and non-stereotypic (male, n= 3: female, n=4) striped mice siblings from six different litters underwent three conventional personality tests (see Miller et al., 2006), namely Light-dark, Startle and Novel-object tests to establish their personality types (described in Chapter 2).

Environmental enrichment and stereotypy

Following the personality tests, both stereotypic and non-stereotypic striped mice were subjected to three treatments in a haphazard manner, which are described below. The starting ages (45 days) and the duration (45 days) of the treatments were fixed (Figure 1). This exposure began when individuals were sub-adult showing stereotypic behaviours and continued into adulthood (> 60 days).

Treatment 1. (Standard housing/ baseline) - test subjects were housed individually in their original Lab-o-tec cages™ (See above).

Treatment 2. (Standard-enriched housing) - test subjects, were housed individually in their original Lab-o-tec™ cages as above but with the addition of one to two small cardboard tubes (± 50 mm diameter) for enrichment.

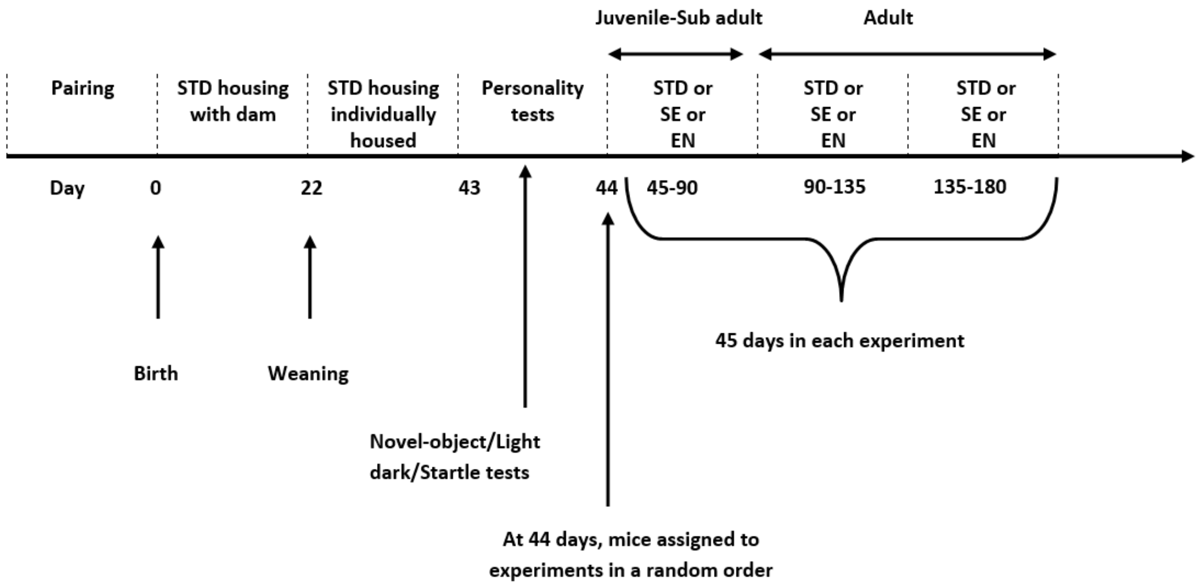

Figure 1. Timeline showing the ages (days) at which striped mice were weaned, exposed to personality tests and assigned in a random order to Standard (STD), Standard-Enriched (SE) and Enriched (EN) treatments.

Treatment 3. (Enriched housing) - test subjects were housed individually in a larger tank (L × H × W: 600 mm × 410 mm × 300 mm), provided with a nest box with the same dimensions as in Standard housing), a deep layer of wood shavings as bedding (± 40 g) and *Eragrostis* grass (± 20 g). In addition to cardboard tubes, several enrichment devices, namely a running wheel (± 15 cm diameter), Habitrail™ PVC tunnels and balls were provided.

The behaviour of all animals was video-recorded in all treatments between 09h00 to 12h00 every second day (i.e. 22 days of recording). Before starting video-recording of behaviours in each treatment, all individuals were allowed to acclimatize to the new treatment for 24 hours. Using Observer software (version 5.0; Noldus Information Technology), the frequency of six behaviours (Table 1) were scored using continuous sampling. In addition, I also recorded the frequency of the wheel running behaviour displayed by test subjects in the Enriched treatment (i.e. Treatment 3). The cages/tanks, PVC tunnels and all the contents were cleaned and the cardboard tubes replaced every two weeks.

Table 1. Ethogram of striped mice behaviours scored in three treatments.

Behaviour	Definition
Inactive	Individual motionless and resting or out of sight
Active	Non-stereotypic movement or running on the cage floor usually digging in the wood shavings + wheel running in Treatment 3
Object manipulation	Manipulating enrichments (e.g. biting or nudging of cardboard tubes and wheels)
Feeding/Drinking	Manipulating or chewing of mouse cubes, seeds or vegetables/fruits and drinking from a water bottle
Stereotypic behaviour	A repetitive and invariant behaviour > 3 times in succession
Grooming	Squatting on hind legs, grooming head, body, tail, and/or genitals

Data Analyses

Data were checked for normality and homogeneity of variance using the Shapiro-Wilk and Levene's tests. All the statistical tests were two-tailed with statistical significance accepted at $p \leq 0.05$ and were analysed using R (Ver. 2.13.0; R Development Core Team, 2011) or Statistica (version 7 Statsoft, USA) software. Utilising the pwr.chisq.test function in the *pwr* package (Blomberg, 2014), a power analyses was used to assess effect size. The data set was analysed at the group level by comparing stereotypic vs non-stereotypic striped mice, and at the individual level (intra-group) variation in behavioural responses.

Previously (Chapter 2), I showed that the startle response test was a reliable personality test in measuring the stress responses in striped mice, and that some personality tests were correlated. There were negative correlations between time spent in the dark compartment and (i) latency to approach novel object (Novel object test), and (ii) latency to emerge from the dark compartment (Light-dark test). In addition, there was a positive correlation between time spent in the light compartment (Startle test) and time spent with the novel object (Novel object test). Based on these findings, I used only the startle response (time spent in the light compartment after a startle) and novel object (latency to approach the novel object) tests in the subsequent analyses in the current study.

To compare the frequency data between stereotypic and non-stereotypic striped mice, feeding and drinking were pooled as 'Feed' to simplify the analyses and because they rarely occurred. Behaviours were combined into a single variable by using the *cbind* function in the *stats* package R, which was then used as a response variable. The *cbind* function takes into account the number of values (in this case, the number of observations being active, inactive, groom, feed/drink, object manipulation and stereotypy that make up each ratio for the response variable, and is a suitable technique of dealing with a non-normal error structure and a non-constant variance (Crawley, 2007). A generalised linear mixed-effects model (GLMZ), using *lmer* function in the *lme4* package (Bates and Maechler, 2009), was used to analyse the influence of stereotypy status (stereotypy or non-stereotypy), treatment and sex (fixed factors) on behaviour. To account for repeated measures of the same individual and the use of individuals from the same litter, mouse and litter identity, were used as random factors (random intercepts only) in the model. The two personality scores were used as continuous predictors in the model. For all generalized linear analyses, Wald χ^2 analysis of deviance type III testing was used to determine significance of the categorical predictors. For each model, I present estimates of the model coefficient (β), their standard errors and *p*-values. These estimates were calculated using the pvals function from the language R library (Baayen, 2009). A GLMZ comparing both stereotypic and non-stereotypic mice together was used to establish whether stereotypic status predicted behaviours. Since there was a stereotypy status and treatment effect on behaviours (See results), two more GLMZ on stereotypic mice (males and females) and non-stereotypic mice (males and females) were conducted separately to assess where and in which treatments the differences occurred. A separate GLMZ was ran for the stereotypic males and females to establish differences between the sexes.

Since the duration of the other behaviours occurred infrequently and too short to be scored, only three behaviours (active, inactive and stereotypic behaviour (for stereotypic mice)) were considered for the analysis of duration data. Using the *cbind* function, behaviours were combined and used in the model as a response variable. The same models as for the frequency data analyses were used for the duration data analyses. The frequency and duration data are presented as total proportions, which I suggest, are a better representation as to how striped mice apportioned their time among different behaviours. Since non-stereotypic striped mice do not display stereotypic behaviours, I analysed stereotypic behaviours separately for stereotypic mice using the same model described above.

To assess the relationship between the two personality scores (time spent in the light compartment and latency to approach the novel object (continuous predictors) and the behaviours (dependent factor), I ran two separate multiple regression tests for both frequency and duration data for stereotypic striped mice and non-stereotypic mice. Since only stereotypic mice display stereotypic behaviours, a linear regression was conducted to assess the relationship between personality test scores and stereotypic behaviours for stereotypic striped mice, using Statistica (version 7 Statsoft, USA).

At the individual level, the relationship between personality and the behaviours of individual stereotypic and non-stereotypic striped mice in the Standard, Standard-enriched and Enriched treatments were analysed using a polynomial multivariate regression for multiple dependents. The frequency and the duration of behaviours were included as dependent variables. The startle response and novel object personality test scores were used as the continuous predictors while the treatments (Standard, Standard-enriched and Enriched) were used as categorical predictors. Separate tests were conducted for both stereotypic and non-stereotypic individuals. For all dependent variables, the homogeneity of slopes of the continuous predictors and their interaction were examined first to determine whether a single test (multivariate regression) or separate regressions were required. For both tests, I report the coefficient of determination, F- and P- values and parameter estimates for linear and polynomial decomposition. I also examined the beta coefficient, obtained when all variables are standardized to a mean of 0 and a standard deviation of 1. The polynomial variables are reported as linear and quadratic functions indicated with a “^{2}”.

Results

Group-level comparisons

Stereotypic behaviour

I analysed stereotypical behaviours separately because of its occurrence in stereotypical striped mice only. Treatment was a significant predictor of the frequency of stereotypic behaviour. Stereotypic behaviours were highest in the Standard-enriched treatment, followed by the Standard treatment and lowest in the Enriched treatment (Figure 2a). Sex was a significant predictor of the behaviours (Table 2). Male striped mice displayed higher levels of stereotypic behaviour than female striped mice (Table 2). Sex*treatment was a significant predictor of stereotypic behaviour. Females displayed higher levels of stereotypic behaviours in the Standard treatment while males displayed higher levels of stereotypic behaviours in the Standard-enriched treatment. There were no significant differences in the levels of stereotypic behaviours in the Enriched treatment for stereotypic males or females (Figure 2a).

The continuous predictor, time spent in the light compartment, was a significant predictor of stereotypic behaviour (Table 2). Linear regression analyses showed that personality test scores were not correlated with the frequency of stereotypic behaviours in the

Table 2. Beta estimates, standard errors and *p*-values generated from a generalized linear mixed model (GLMZ) for contrasts for the frequency of stereotypic behaviours in the Standard, Standard-enriched and Enriched treatments for stereotypic striped mice. Values in bold are significant.

Variables	β Estimates	Standard Error	Wald χ^2	*df*	P
Standard	**-0.22**	**0.07**	**9.94**	**2.0**	**0.002**
Standard-enriched	-0.11	0.06	9.94	2.0	0.101
Enriched	0.11	0.06	9.94	2.0	0.101
Sex	**-0.23**	**0.07**	**11.22**	**1.0**	**0.001**
Sex*Standard	0.05	0.09	4.49	2.0	0.560
Sex*Standard-enriched	**0.19**	**0.09**	**4.49**	**2.0**	**0.036**
Sex*Enriched	**-0.19**	**0.09**	**4.49**	**2.0**	**0.036**
Time spent in light compartment	**0.00**	**0.00**	**11.36**	**1.0**	**0.001**
Latency to approach novel object	0.00	0.00	3.68	1.0	0.056

three treatments: time spent in light compartment (Standard: $R^2 = 0.02$, $F_{1, 15} = 0.33$, p= 0.572; Standard-enriched: $R^2 = 0.02$, $F_{1, 15} = 0.25$, p= 0.625; Enriched: $R^2 = 0.02$, $F_{1, 15} = 0.25$, p= 0.627).

Unlike frequency, treatment, sex and sex*treatment were not significant predictors of the duration of stereotypic behaviour (Table 3 and Figure 2b). The time spent in the light compartment (continuous predictor) was a significant predictor of stereotypic behaviour (Table 3). Linear regression analysis showed that time spent in the light compartment was not correlated with stereotypic behaviours in the three treatments (Standard: $R^2 = 0.01$, $F_{1, 15} = 0.0958$, p= 0.761; Standard-enriched: $R^2 = 0.03$, $F_{1, 15} = 0.43$, p= 0.523; Enriched: $R^2 = 0.04$, $F_{1, 15} = 0.67$, p= 0.425).

Group-level comparisons: all behaviours except stereotypic behaviours

Stereotypy status was a significant predictor of frequency of behaviours (Table 4). I found a moderate power of 0.56, indicating that the small number of test animals affected the analysis. Compared to the non-stereotypic striped mice, stereotypic striped mice showed greater levels of activity, grooming, feeding and object manipulation and lower levels of inactivity (Figure 3).

The GLMZ also revealed that treatment had a significant influence on behaviour (Table 4). Striped mice showed an increase in activity, grooming and feeding behaviours in the Standard treatment compared to the Standard-enriched and Enriched treatments, regardless of stereotypic status (Figure 3); there were no differences in these behaviours

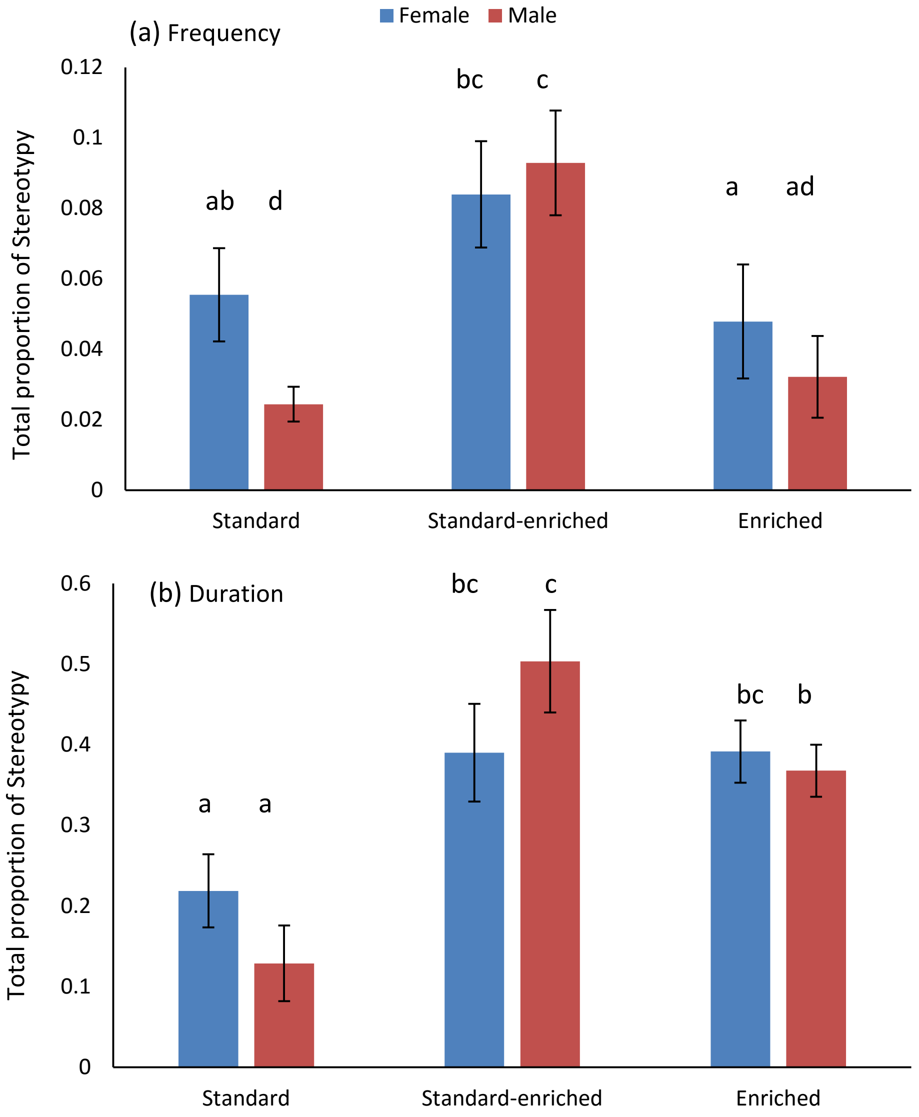

Figure 2. Total proportion of the (a) frequency and (b) duration of stereotypic behaviours for stereotypic males and females in three treatments (Standard, Standard-enriched, Enriched). Bars denote proportions generated through a generalized linear mixed effects model for each of the behaviours observed. Whiskers denote 95% confidence limits. Letters above bars marked denote significant differences in treatments.

between the Standard-enriched and Enriched treatments. The levels of object manipulation differed significantly in the three treatments and were higher in the Standard treatment and lower in the Standard-enriched treatment. There were no differences in the levels of inactivity in the Standard and Standard-enriched treatment but the level of inactivity was significantly reduced in the Enriched than the other treatments (Figure 3).

Stereotypy status*treatment was a significant predictor of behaviour (Table 4). Levels of activity were significantly higher in stereotypic striped mice than non-stereotypic mice in

Table 3. Beta estimates, standard errors and *p*-values generated from a generalized linear mixed model (GLMZ) for contrasts for the duration of stereotypic behaviours in the Standard, Standard-enriched and Enriched treatments for stereotypic striped mice. Values in bold are significant.

Variables	β Estimates	Standard Error	Wald χ2	*df*	P
Standard	-43.73	26.55	3.94	2.0	0.100
Standard-enriched	41.24	23.29	3.94	2.0	0.077
Enriched	-41.24	23.29	3.94	2.0	0.077
Sex	-11.43	26.81	0.18	1.0	0.670
Sex*Standard	-31.96	35.89	0.85	2.0	0.373
Sex*Standard-enriched	21.81	32.05	0.85	2.0	0.497
Sex*Enriched	-21.80	32.05	0.85	2.0	0.497
Time spent in the light compartment	**-0.57**	**0.15**	**14.31**	**1.0**	**<0.001**
Latency to approach novel object	-0.22	0.08	6.73	1.0	0.061

Table 4. Beta estimates, standard errors and *p*-values generated from a generalized linear mixed model (GLMZ) for contrasts for frequency of behaviours in the Standard, Standard-enriched and Enriched treatments for stereotypic and non-stereotypic striped mice. Values in bold are significant.

Variables	β Estimates	Standard Error	Wald χ2	*df*	P
Stereotypy status	**0.74**	**0.16**	**20.41**	**1.0**	**<0.001**
Standard	**-0.59**	**0.16**	**17.94**	**2.0**	**<0.001**
Standard-enriched	**-0.57**	**0.16**	**17.94**	**2.0**	**0.001**
Enriched	**0.57**	**0.16**	**17.94**	**2.0**	**0.001**
Sex	**-0.40**	**0.20**	**3.89**	**1.0**	**0.049**
Stereotypy status*sex	0.15	0.23	0.40	1.0	0.528
Sex*Standard	0.23	0.24	1.09	2.0	0.340
Sex*Standard-enriched	0.21	0.25	1.09	2.0	0.409
Sex*Enriched	-0.21	0.25	1.09	2.0	0.409
Stereotypy status*Standard	-0.37	0.21	6.31	2.0	0.081
Stereotypy status *Standard-enriched	**-0.49**	**0.21**	**6.31**	**2.0**	**0.018**
Stereotypy status *Enriched	**0.49**	**0.21**	**6.31**	**2.0**	**0.018**
Time spent in light compartment	0.00	0.00	2.57	1.0	0.109
Latency to approach novel object	0.00	0.00	0.01	1.0	0.915

in the Standard and Standard-enriched treatments. There was no difference in activity in the Enriched treatment in stereotypic or non-stereotypic mice. Feeding was greater in stereotypic mice in all three treatments (Figure 3). Grooming was greater in stereotypic mice in all three treatments than for non-stereotypic mice in the three treatments (Figure 3). Inactivity was significantly lower in stereotypic mice in all three treatments than for non-stereotypic mice.

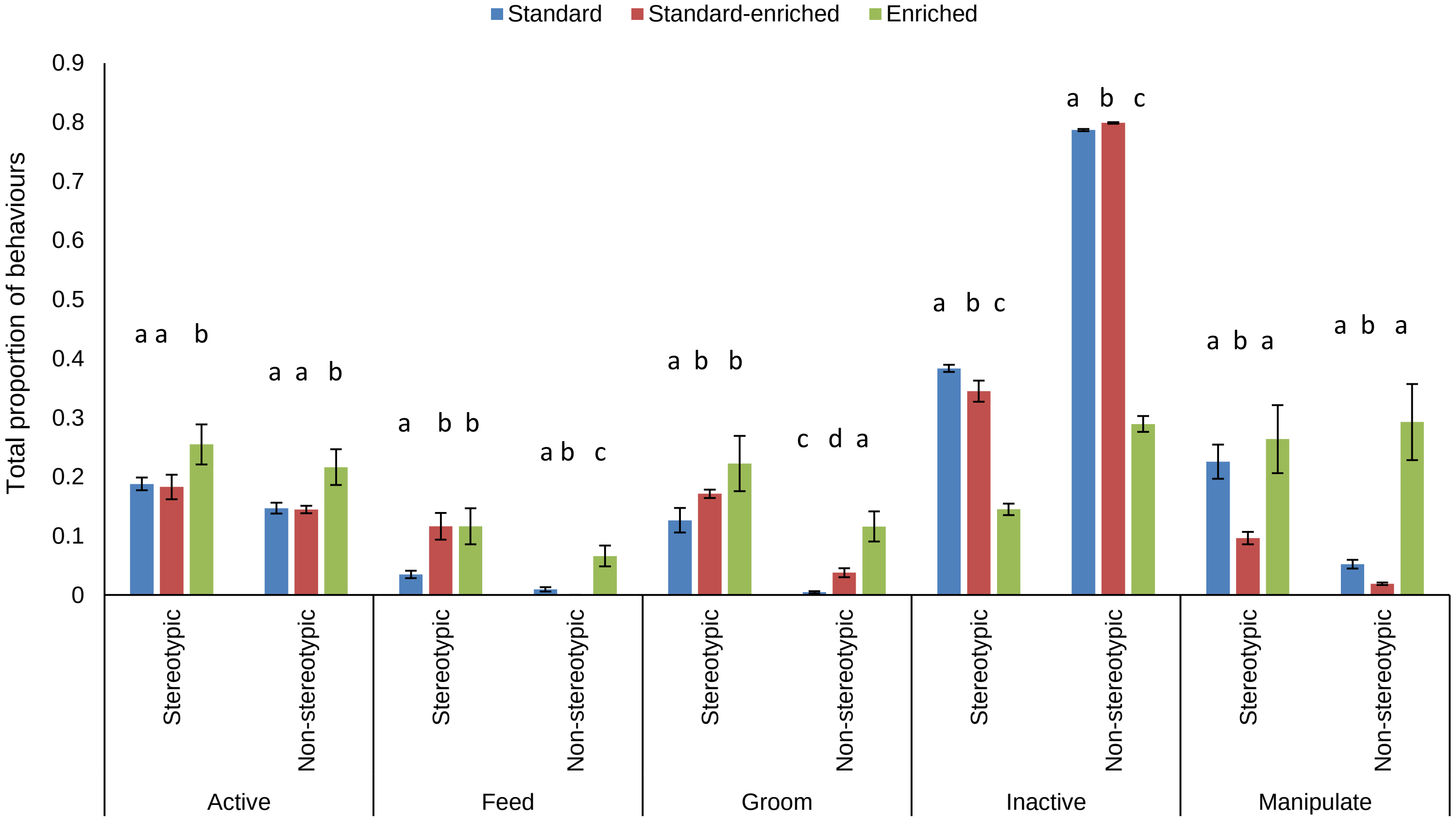

Figure 3. Total proportion of the frequency of behaviours in stereotypic and non-stereotypic striped mice in three treatments (Standard, Standard-enriched, Enriched). Bars denote proportions generated through a generalized linear mixed effects model for each of the behaviours observed. Whiskers denote 95% confidence limits. Letters above bars marked denote significant differences in treatments.

Object manipulation was greater in stereotypic mice in the Standard and Standard-enriched treatments than non-stereotypic mice, while there was no difference in the Enriched treatment in stereotypic or non-stereotypic mice (Figure 3).

Sex was a significant predictor of the behaviours (Table 4; Figure 4). Females displayed higher levels of inactivity in the Standard-enriched treatment, while males displayed greater inactivity in the Standard and Enriched treatments, regardless of stereotypic status (Figure 4). Females showed greater object manipulation in the Standard treatment than males but there was no difference in object manipulation in the Standard-enriched and Enriched treatments. There were no sex differences in levels of activity and feeding in all treatments. Grooming was high in females in the Standard treatment. However, there were no differences in the Standard-enriched and Enriched treatments. Sex*stereotypic status, sex*treatment, stereotypic status*treatment, latency to approach novel object (novel-object test) and time spent in the light compartment (startle test) did not significantly influence behaviour (Table 4).

A multiple regression analysis revealed that the two personality test scores were not correlated with the frequency of behaviours in all three treatments in stereotypic and non-stereotypic mice (Table 5).

For the duration of behaviour, only two behaviours (activity and inactivity) were considered. Treatment significantly influenced behaviour (Table 6). Activity was greater in the Enriched than the Standard and Standard-enriched treatments (Figure 5). There were no differences in the levels of inactivity in the Standard and Standard-enriched treatments. Sex, stereotypy status, sex*stereotypy status, sex*treatment, stereotypy status*treatment, latency to approach novel object and time spent in light compartment were not significant predictors of behaviour (Table 6).

A multiple regression analysis revealed that the time spent in the light compartment was significantly correlated with behaviours in the Standard treatment in stereotypic striped mice (Table 7). None of the personality scores were associated with the duration of behaviours in non-stereotypic mice in all three treatments.

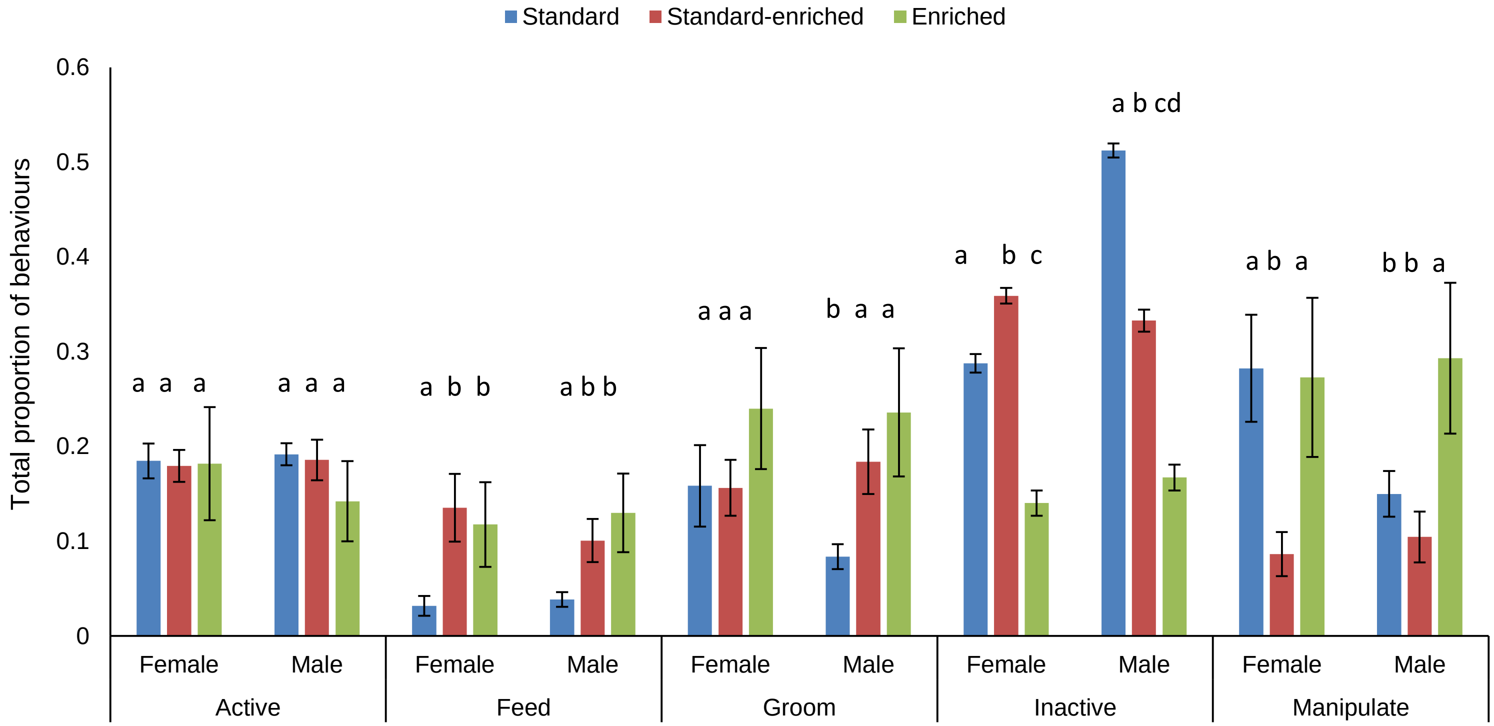

Figure 4. Total proportion of the frequency of behaviours combined for stereotypic and non-stereotypic females and males across three treatments (Standard, Standard-enriched, Enriched). Bars denote proportions generated through a generalized linear mixed effects model for each of the behaviours observed. Whiskers denote 95% confidence limits. Letters above bars marked denote significant differences in treatments.

Table 5. Multiple regression results for the frequency of behaviours and for the time spent in the light compartment (startle test) and latency to approach the novel object (novel object test) as the predictor variables for stereotypic and non-stereotypic striped mice in the Standard, Standard-enriched and Enriched treatments.

Stereotypy status	Treatment	Personality test	P	R²	F	*df*
Stereotypic	Standard	Time spent in light compartment	0.217	0.10	1.66	1,15
		Latency to approach novel object	0.964	0.00	0.00	1,15
	Standard-enriched	Time spent in light compartment	0.771	0.01	0.09	1,15
		Latency to approach novel object	0.610	0.02	0.27	1,15
	Enriched	Time spent in light compartment	0.990	0.00	0.00	1,15
		Latency to approach novel object	0.583	0.02	0.31	1,15
Non-stereotypic	Standard	Time spent in light compartment	0.244	0.19	1.62	1,5
		Latency to approach novel object	0.900	0.00	0.02	1,5
	Standard-enriched	Time spent in light compartment	0.788	0.02	0.08	1,5
		Latency to approach novel object	0.840	0.01	0.05	1,5
	Enriched	Time spent in light compartment	0.787	0.02	0.08	1,5
		Latency to approach novel object	0.416	0.14	0.78	1,5

Table 6. Beta estimates, standard errors and *p*-values generated from a generalized linear mixed model (GLMZ) for contrasts for duration of behaviours in the Standard, Standard-enriched and Enriched treatments for stereotypic and non-stereotypic striped mice. Values in bold are significant.

Variables	β Estimates	Standard Error	Wald χ2	*df*	P
Stereotypy status	-60.80	39.82	2.33	1.0	0.127
Standard	6.59	43.25	20.65	2.0	0.879
Standard-enriched	**145.46**	**40.84**	**20.65**	**2.0**	**<0.001**
Enriched	**-145.46**	**40.84**	**20.65**	**2.0**	**<0.001**
Sex	20.16	52.08	0.15	1.0	0.699
Stereotypy status*sex	-31.54	61.44	0.26	1.0	0.608
Sex*Standard	-7.85	67.50	0.02	2.0	0.908
Sex*Standard-enriched	7.81	64.28	0.02	2.0	0.903
Sex*Enriched	-7.81	64.28	0.02	2.0	0.903
Stereotypy status*Standard	12.36	56.05	0.32	2.0	0.826
Stereotypy status *Standard-enriched	-27.95	50.16	0.32	2.0	0.577
Stereotypy status *Enriched	27.95	50.16	0.32	2.0	0.577
Time spent in the light compartment	0.13	0.10	1.72	1.0	0.190
Latency to approach novel object	-0.04	0.06	0.58	1.0	0.445

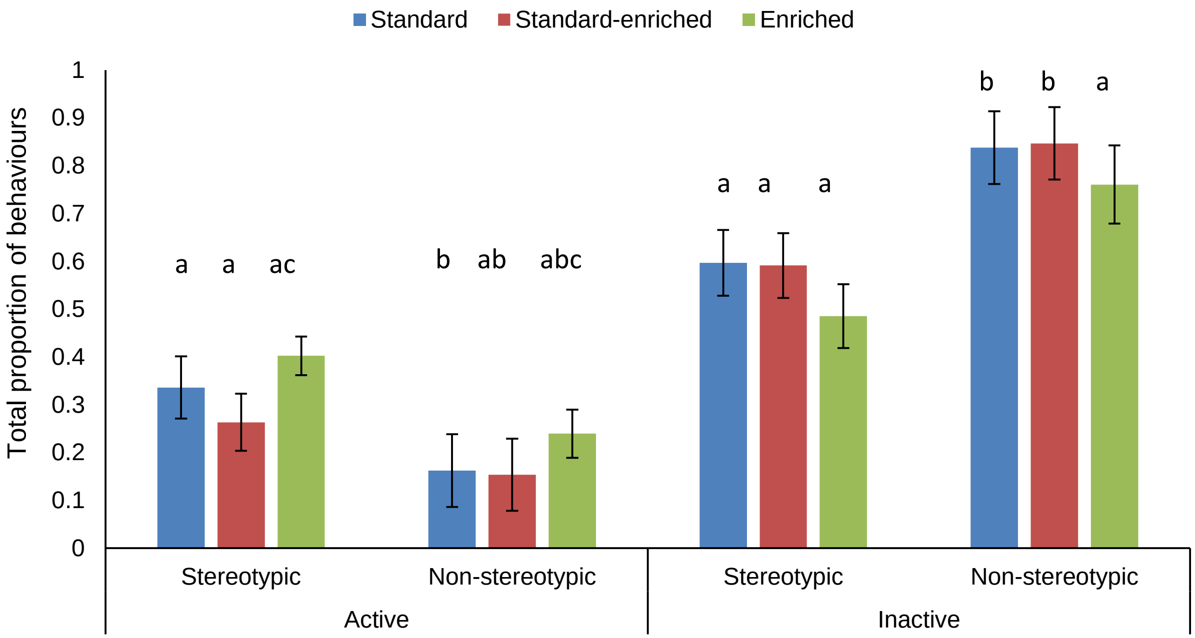

Figure 5. Total proportion of the duration (s) of behaviours in stereotypic and non-stereotypic striped mice across three treatments (Standard, Standard-enriched, Enriched). Bars denote proportions generated through a generalized linear mixed effects model for each of the behaviours observed. Whiskers denote 95% confidence limits. Letters above bars marked denote significant differences in treatments.

Table 7. Multiple regression results for the duration of behaviours and the time spent in the light compartment (startle test) and latency to approach the novel object (novel object test) as the predictor variables for stereotypic and non-stereotypic striped mice in the Standard, Standard-enriched and Enriched treatments. Value in bold is significant.

Stereotypy status	Treatment	Personality test	P	R^2	F	*df*
Stereotypic	Standard	**Time spent in light compartment**	**0.007**	**0.39**	**9.68**	**1,15**
		Latency to approach novel object	0.132	0.15	2.54	1,15
	Standard-enriched	Time spent in light compartment	0.588	0.02	0.31	1,15
		Latency to approach novel object	0.393	0.05	0.77	1,15
	Enriched	Time spent in light compartment	0.886	0.00	0.02	1,15
		Latency to approach novel object	0.168	0.12	2.09	1,15
Non-stereotypic	Standard	Time spent in light compartment	0.610	0.05	0.29	1,5
		Latency to approach novel object	0.577	0.06	0.35	1,5
	Standard-enriched	Time spent in light compartment	0.980	0.00	0.00	1,5
		Latency to approach novel object	0.672	0.03	0.19	1,5
	Enriched	Time spent in light compartment	0.830	0.01	0.05	1,5
		Latency to approach novel object	0.384	0.13	0.88	1,5

Individual-level assessment

Stereotypic striped mice

Although there were no significant associations between the personality test scores and the frequency of behaviours in stereotypic mice (**Supplementary material**: S1), an examination of the beta coefficients allows for an assessment of the relative contribution of each independent variable on the dependent variable. There were 13 positive and 11 negative non-significant associations (Table 8). An examination of beta coefficients revealed that there was a significant association between duration of stereotypic behaviour and time spent in the light compartment: a greater time spent in the light compartment in the personality test was correlated with a linear increase in the duration of stereotypic behaviour but a non-random decrease in the duration of stereotypic behaviour. In support, Figure 6a shows that although there was a general increase in the duration of stereotypic behaviour in 8 of 10 stereotypic striped mice from Standard to Standard-enriched treatment, there was nevertheless a decrease in stereotypic behaviour from Standard-enriched to Enriched treatment in 10 out of 15 individuals. However, 5 individuals showed an increase in stereotypic behaviours from Standard-enriched to Enriched treatment. Furthermore, 6 individuals showed an increase in stereotypic behaviours from Standard to

Standard-enriched treatment (Table 8). There were five positive and six negative non-significant associations between the personality scores and duration of behaviours.

An examination of the individual responses in the treatments showed that although not significant, the changes in the frequency of stereotypic behaviours mirrored that of the duration pattern, increasing from Standard to Standard-enriched and decreasing in the Enriched treatment (Figure 6b). There was a decrease in duration of activity in 9 of 15 individuals from Standard to Standard-enriched treatment, which then increased from Standard-enriched to Enriched in 10 individuals (Figure 6c). Frequency of activity was low in the Standard and Standard-enriched treatments in most individuals, and increased in the Enriched treatment (Figure 6d). Duration of inactivity increased in most individuals from the Standard to the Standard-enriched treatments, while it decreased in almost half of the individuals in the Enriched treatment (Figure 6e). The frequency of inactivity for all individuals was similar across treatments (Figure 6f). Frequency of object manipulation increased from the Standard-enriched treatment to the Enriched treatment in most (13 of 15) individuals, while in 2 it decreased in the Enriched treatment (Figure 6g). The frequency of feeding was low in the Standard treatment in most of the individuals and increased in the Enriched treatment (Figure 6h). Figure 6i shows that not all individuals showed the same levels of grooming patterns: it varied in most individuals in the Standard and Standard-enriched treatments but increased from Standard-enriched to Enriched in most individuals.

Non-stereotypic striped mice

There was a significant negative correlation between frequency of inactivity and the latency to approach the novel object (**Supplementary material**: S2). Though the linear component was not significant, the polynomial component of inactivity was significant and thus non-random, so that the longer the latency to approach the novel object in the personality test, the lower was the frequency of inactivity, which is unexpected. There were 11 positive and 8 negative non-significant associations between the personality tests scores and the frequency of behaviours. There were no significant associations between the personality test scores and duration of behaviours, but there were four positive and four negative non-significant associations. The duration of activity varied in the Standard and Standard-enriched treatments, but increased in most individuals in the Enriched treatment (Figure 7a).

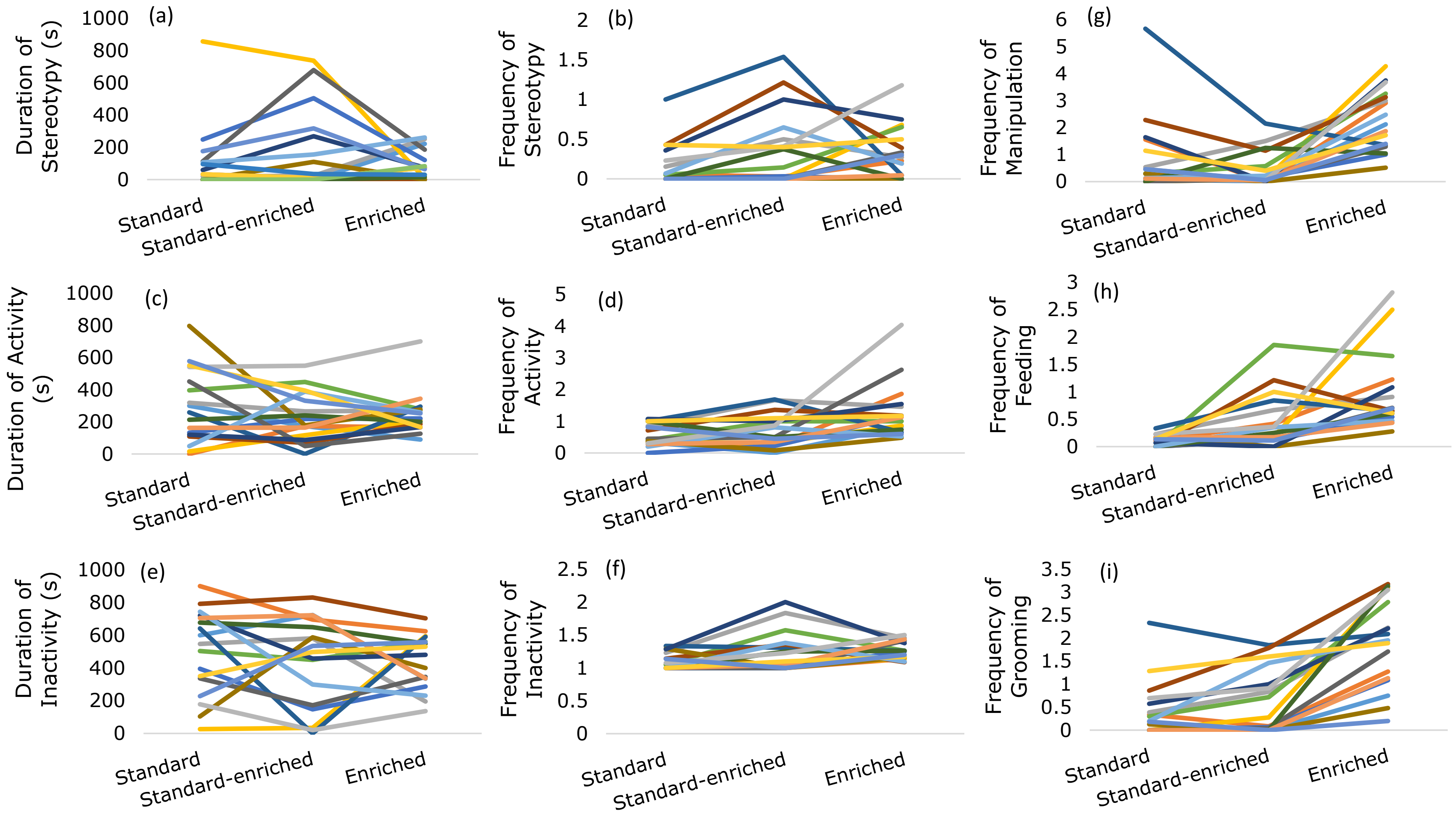

Figure 6. Changes in behaviour of individual stereotypic striped mice in the Standard, Standard-enriched and Enriched treatments. The data for each individual for each treatment is connected by a different coloured line. (a)- (f) represent the duration and frequency data and (g)-(i) show the frequency data of behaviours.

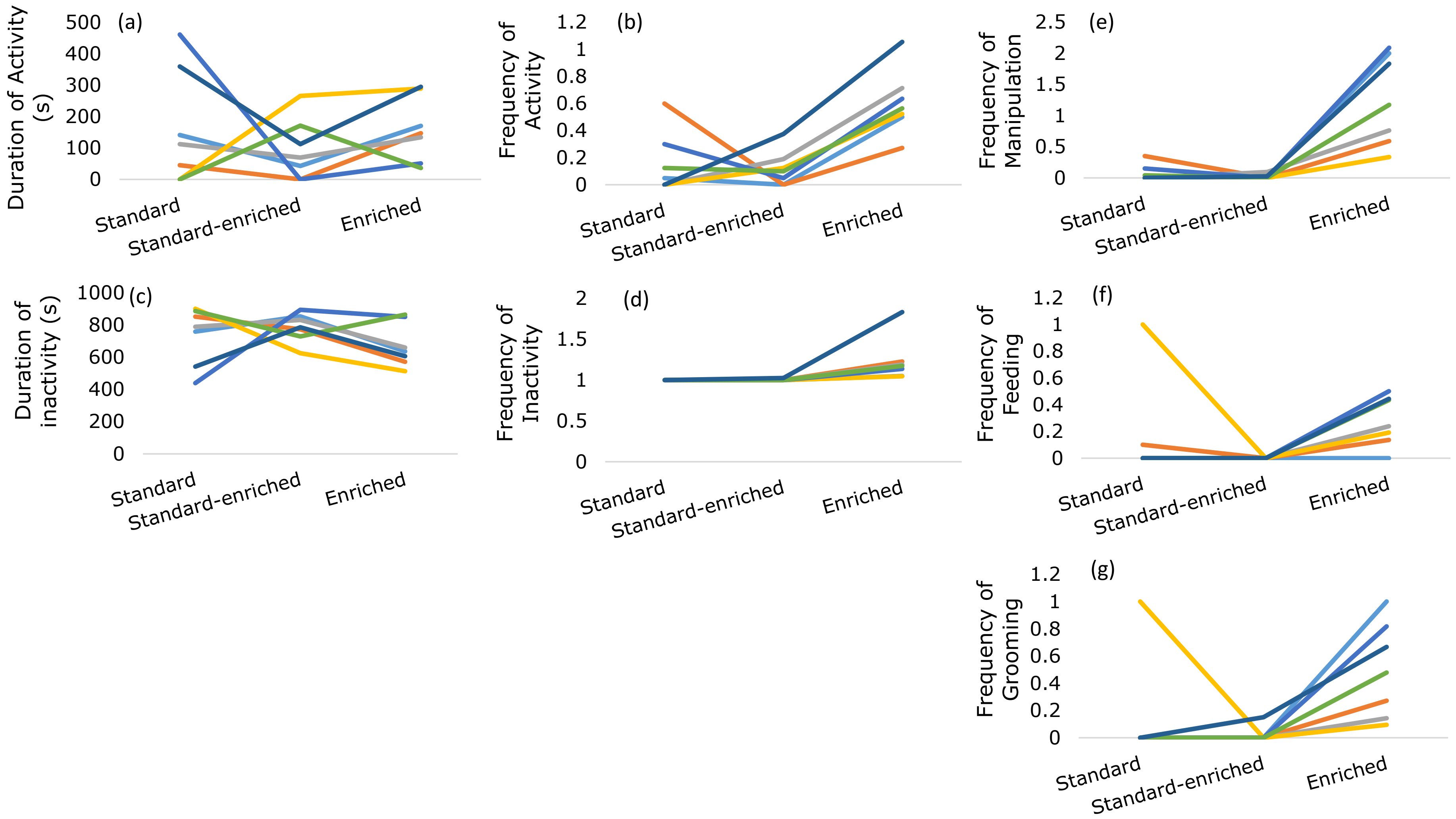

Figure 7. Changes in behaviour in individual non-stereotypic striped mice in the Standard, Standard-enriched and Enriched treatments. The data for each individual for each treatment is connected by a different coloured line. (a)- (d) show the duration and frequency data and (e) - (g) show the frequency data of behaviours.

While most individuals showed a decrease in activity in the Standard-enriched treatment, 2 individuals showed an increase in the same treatment. The frequency of activity increased in most individuals from the standard to Enriched treatments (Figure 7b). Duration of inactivity decreased in most individuals from the Standard-enriched to the Enriched treatment, with an exception of two individuals in which inactivity increased (Figure 7c). The frequency of inactivity plateaued in all individuals from the Standard to Standard-enriched treatments and then increased slightly in the Enriched treatment (Figure 7d). Frequency of object manipulation was low in the Standard and Standard-enriched treatment and increased in the Enriched treatment in all individuals (Figure 7e). The frequency of both feeding (Figure 7f) and grooming (Figure 7g) were low in the Standard treatment in most individuals and increased in the Enriched treatment.

In summary, stereotypic striped mice showed differing levels of stereotypic behaviours in the three treatments. Eight out of 15 mice showed lower levels of stereotypic behaviours in the Enriched treatment. Stereotypic behaviours were significantly high in the Standard-enriched treatment, with 10 individuals showing increased levels of stereotypic behaviours. Furthermore, 10 stereotypic mice showed lower levels of stereotypic behaviours in the Standard treatment (Table 8).

Discussion

My aim was to ascertain whether stereotypic and non-stereotypic striped mice responded differently to cages of varying complexities and whether this response was influenced by the personality of the striped mice tested. I predicted that at the group level, stereotypic striped mice would show reduced levels of stereotypic behaviours in enriched conditions, while non-stereotypic mice would show reduced activity. At the individual level, I expected both stereotypic and non-stereotypic striped mice to change their behaviours depending on the housing conditions.

Group level effects

As expected, in stereotypic mice, the levels of stereotypic behaviours were significantly reduced, while activity was significantly high in the Enriched treatment. Similarly, several studies indicate that increasing cage complexity and the addition of environmental enrichments reduce stereotypic behaviours (e.g. bank voles, *Clethrionomys glareolus*, Ödberg, 1987; deer mice, *Peromyscus Maniculatus*, Powell et al., 1999, 2000; lion-tailed macaques, *Macaca silensus*, Mallapur et al., 2005; dairy cattle, Redbo, 1990).

Table 8. Personality scores (time spent in the light compartment and latency to approach novel object) and changes in stereotypic behaviours in stereotypic individuals in the Standard, Standard-enriched and Enriched treatments

Individual number	Time spent in light compartment (s)	Latency to approach novel object (s)	Enriched	Standard-enriched	Standard
1	19.97	0.00	HIGHER	LOW	HIGH
2	147.1	36.35	LOW	HIGH	HIGHER
3	110.35	150.36	HIGHER	LOW	LOW
4	187.41	0.00	LOW	HIGHER	LOW
5	40.87	0.00	HIGHER	HIGH	LOW
6	2.33	381.7	LOW	HIGH	HIGHER
7	2.33	381.7	LOW	HIGHER	HIGH
8	216.65	0.00	LOW	LOW	LOW
9	179.69	169.78	HIGH	HIGHER	LOW
10	207.26	0.00	LOW	HIGHEST	LOW
11	0.00	155.29	HIGH	HIGHER	LOW
12	0.00	332.87	LOW	LOW	LOW
13	0.00	81.75	HIGHER	HIGH	LOW
14	105.21	29.58	LOW	HIGHER	HIGH
15	104.98	127.17	HIGHER	LOW	LOW

Stereotypic striped mice, which display locomotor stereotypic behaviours, require space to perform these behaviours, yet with an increase in size and space in the Enriched treatment, they still reduced the performance of stereotypic behaviours. This finding therefore implies that cage complexity rather than the availability of space results in the reduction of stereotypic behaviours, similar to the findings in mink, *Neovison vison* (Hansen et al., 1994; Hansen et al., 2007). Overall, general activity, which comprised mainly of wheel running, also increased in the stereotypic mice, which might also have led to the mitigation of stereotypic behaviours in the Enriched treatment, concurring with results found on laboratory mice (Sherwin, 1996; Sherwin and Nicol, 1996; Howerton et al., 2008). Nevertheless, the lower levels of object manipulation in stereotypic than non-stereotypic striped mice in the Enriched treatment was unexpected. Therefore, it appears that once non-stereotypic striped mice became habituated to a novel environment, they started exploring the environments thoroughly.

The duration of stereotypic behaviours was greater in the Standard-enriched treatment than the Standard or Enriched treatments. Environmental enrichment usually reduces or prevents abnormal behaviours (Young, 2003; Swaisgood and Shepherdson, 2006; Abou-Ismail, 2011), and these findings could be due to the placement of environmental enrichment in the already restricted standard laboratory housing. Standard laboratory housing is associated with reduced welfare as well as frustration since it prevents the occurrence of natural behaviours (Balcombe, 2006). Locomotor stereotypic behaviours require space for their performance, so the addition of environmental enrichment in such small cages and a further reduction in the available space aggravated stereotypic behaviours in striped mice. Similarly, blue jays, *Cyanocitta cristata* and domesticated budgerigars, *Melopsittacus undulates,* which were housed in cages with reduced space which increased their stress levels, displayed higher levels of abnormal behaviours than those housed in larger cages (Keiper, 1969; Gebhardt-Henrich and Steiger, 2006). Interestingly, despite an increase in space in the Enriched treatment, stereotypic behaviours were reduced, which implies that space acts in synergy with the environmental enrichment in reducing stereotypic behaviours.

Female stereotypic striped mice showed increased levels of object manipulation compared to males, while males showed greater levels of inactivity. Males and females have different life history strategies (Nevison et al., 1999), patterns of hormone secretion (Quiñones-Jenab et al., 1999; Beatty, 1979) and differences in genetic predisposition (de Visser et al., 2007), all of which could have contributed to the sex differences observed in terms of object manipulation. Female Sprague–Dawley rats showed higher levels of activity and enrichment use than males (Peňa et al., 2006), as did female Japanese quail, *Coturnix coturnix japonica* (Miller et al., 2006). These sex differences in response to enrichment use suggest that males and females may benefit differently from enrichments.

Individual level effects

The multivariate regression analyses used to assess whether personality influenced how individual striped mice interacted with different cage complexities showed individual differences in response to the different cage complexities. While individual stereotypic striped mice that spent a longer time in the light compartment (i.e. bolder), showed a linear increase in stereotypic behaviour in the Standard and Standard-enriched treatments, they also showed a non-random decrease in stereotypic behaviours in the Enriched treatment. In particular, stereotypic individuals did not show consistent behavioural responses in the three treatments, since there was little consistency between behaviours in the personality tests and in response to the cages of varying cage complexities. Even though stereotypic behaviours were generally attenuated in the Enriched treatment, individual stereotypic mice responded differently to the enriched conditions. A few stereotypic mice actually showed an increase in stereotypic behaviours in the same treatment. Furthermore, while most stereotypic individuals showed an increase in activity and object manipulation in the Enriched treatment, a few also decreased the levels of activity and object manipulation. In contrast, except for two individuals, the majority of non-stereotypic striped mice showed consistency in behaviours across the three treatments: most individuals increased activity and object manipulation in the Enriched treatment.

Taken together, these results imply that stereotypic striped mice modify their behavioural responses depending on the environmental context, and therefore demonstrate a flexible response. It is also possible that the stereotypic behaviours were not fully established and may not have passed the critical period after which environmental enrichment would not have a protective effect on the development of stereotypic behaviours (Lewis et al., 2006). However, this might not be the case in *Rhabdomys* because stereotypic behaviours are fixed at 45 days of age in this taxon (Jones et al., 2011). In contrast, while non-stereotypic mice showed more or less consistent behaviours in these contexts, there was an indication of flexible behavioural responses in two individuals; a larger sample is required to thoroughly investigate the individual variation. As in my study, both bold and shy trout, *Onchorhyncus mykiss*, varied their responses when exposed to different environmental situations (Frost et al., 2013). Non-stereotypic striped mice, showed a negative relationship between the latency to approach the novel object in personality tests and inactivity in the standard housing. It is possible that less bold non-stereotypic animals assess the situation before displaying behaviour and are therefore less predictable in their behaviours. Similarly, less bold rainbow trout, *Onchorhyncus mykiss*, change their behaviour when their relative competitive ability may be similar or less than that of conspecifics (Frost et al., 2007).

Several personality studies have showed behavioural flexibility (e.g. pumpkinseed sunfish, *Lepomis gibbosus* (Coleman and Wilson, 1998), squid, *Euprymna tasmanica* (Sinn et al., 2008), great tits, *Parus major* (Jacobs et al., 2013) and mice (Benus et al., 1987; Sluyter et al., 1996). However, all these studies show the ability to alter behavioural responses in less bold individuals and not bold individuals. While my findings contradict the literature, a non-rodent study provides partial support for my findings: Thomson et al. (2012) showed that bold trout, *Oncorhynchus mykiss*, were comparatively more flexible in terms of activity and altering levels of neophobia relevant to a behavioural challenge, while less bold trout were more fixed in their behavioural responses and remained shy.

Coppens et al. (2010) and Koolhaas et al. (2010) maintain that the mechanisms underpinning behavioural flexibility, i.e. the ability of individuals to vary their behaviours in response to different environmental stimuli, can be explained to arise proximally from changes in the prefrontal cortex, responsible for behavioural flexibility and inhibition of inappropriate behaviours. In particular, individual variation in the serotonergic and dopaminergic input to the medial prefrontal cortex may explain individual variation in coping styles, since serotonin is involved in behavioural flexibility. Interestingly, in both stereotypic animals and proactive copers, the levels of serotonin, which is crucial for behavioural flexibility, are also low. Nevertheless, it has been shown that with exposure to enrichment, the levels of serotonin are elevated (Brenes et al., 2009), which may have caused the behavioural flexibility evident in the stereotypic individuals. Moreover, behavioural flexibility is an integral component of the coping style an individual uses when responding to environmental cues, and proactive individuals should be more rigid in their behaviours and reactive individuals more flexible (Koolhaas et al., 1999).

Conclusions

My study is one of the first to test the involvement of individual differences (personality) to explain variation in responses to environmental stimuli in stereotypic mice, and generated novel outcomes. My study shows that the personality of the stereotypic individuals does not predict behavioural variation in different environmental conditions and contradicts the literature that bold/proactive individuals show fixed, rigid behavioural routines and reduced behavioural inhibition and less bold/reactive individuals show behaviours guided by environmental cues (Benus et al., 1991; Koolhaas et al., 1999; Coppens et al., 2010; Ijichi et al., 2013). Stereotypic striped mice were generally bold but both stereotypic and non-stereotypic individuals altered their behavioural responses depending on the context. While personality does not influence the behaviour of striped mice under different cage complexity, the randomised exposure to treatments in my study in sub-adult and later when they became adults, might have been confounded by the age of exposure to treatments (see Tilly et al., 2010). In future studies, I will examine the relationship between stereotypic status, age, personality and environmental enrichment.

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Supplementary material

Table S1. Multivariate regression analysis of the frequency of stereotypic behaviour, activity, inactivity, object manipulation, feeding and grooming and the duration of stereotypic behaviour, activity and inactivity with latency to approach the novel object (novel object test) and time spent in the light compartment (startle test) as the predictor variables for stereotypic mice in the Standard, Standard-enriched and Enriched treatments. Linear and polynomial decomposition are reported. Values in bold are significant.

FREQUENCY							
Personality test	**Behaviour**	**Parameter Estimates**	**P**	**β**	**R²**	**F**	***df***
Time spent in light compartment	Stereotypy	0.002	0.359	0.51	0.12	0.68	6,39
	Active	0.000	0.933	-0.04	0.21		
	Inactive	0.001	0.584	0.30	0.16		
	Manipulate	0.012	0.120	0.72	0.41		
	Feed	0.004	0.294	0.47	0.43		
	Groom	0.004	0.428	0.35	0.46		
Latency to approach novel object	Stereotypy	0.001	0.376	0.39	0.12	1.28	6,39
	Active	0.000	0.920	-0.04	0.21		
	Inactive	0.002	0.082	0.76	0.16		
	Manipulate	-0.001	0.839	-0.07	0.41		
	Feed	-0.002	0.394	-0.31	0.43		
	Groom	0.000	0.873	-0.06	0.46		
Time spent in light compartment^2	Stereotypy	0.001	0.376	0.39	0.12	1.07	6,39
	Active	0.000	0.838	0.10	0.21		
	Inactive	0.000	0.962	-0.03	0.16		
	Manipulate	0.000	0.074	-0.81	0.41		
	Feed	0.000	0.126	-0.68	0.43		
	Groom	0.000	0.334	-0.41	0.46		
Latency to approach novel object^2	Stereotypy	0.000	0.658	-0.19	0.12	0.75	6,39
	Active	0.000	0.921	0.04	0.21		
	Inactive	0.000	0.199	-0.56	0.16		
	Manipulate	0.000	0.587	0.20	0.41		
	Feed	0.000	0.549	0.21	0.43		
	Groom	0.000	0.637	0.16	0.46		
DURATION							
Time spent in light compartment	**Stereotypy**	**2.875**	**0.042**	**1.07**	**0.41**	1.74	3,42
	Active	0.728	0.546	0.31	0.37		
	Inactive	-3.148	0.074	-0.98	0.29		
Latency to approach novel object	Stereotypy	0.657	0.375	0.39	0.41	0.79	3,42
	Active	-0.291	0.652	-0.20	0.37		
	Inactive	0.283	0.759	0.14	0.29		
Time spent in light compartment^2	**Stereotypy**	**-0.015**	**0.036**	**-1.07**	**0.41**	1.56	3,42
	Active	0.000	0.973	-0.02	0.37		
	Inactive	0.014	0.109	0.85	0.29		
Latency to approach novel object^2	Stereotypy	-0.001	0.714	-0.16	0.41	0.49	3,42
	Active	0.001	0.662	0.19	0.37		
	Inactive	-0.002	0.546	-0.27	0.29		

Table S2. Multivariate regression analysis of the frequency of activity, inactivity, object manipulation, feeding and grooming and the duration of activity and inactivity with latency to approach the novel object and time spent in the light compartment (startle test) as the predictor variables in non-stereotypic striped mice in the Standard, Standard-enriched and Enriched treatments. Linear and polynomial decomposition are reported. Values in bold are significant.

FREQUENCY							
Personality test	**Behaviour**	**Parameter Estimates**	**P**	**β**	**R²**	**F**	***df***
Time spent in light compartment	Active	0.005	0.241	1.56	0.67	0.43	5,10
	Inactive	0.006	0.278	1.60	0.59		
	Manipulate	0.009	0.344	1.12	0.73		
	Feed	0.000	0.931	0.16	0.34		
	Groom	0.005	0.510	1.16	0.40		
Latency to approach novel object	Active	0.000	0.971	0.02	0.67	1.19	5,10
	Inactive	0.001	0.234	0.62	0.59		
	Manipulate	-0.002	0.241	-0.50	0.73		
	Feed	0.001	0.246	0.77	0.34		
	Groom	0.000	0.862	0.11	0.40		
Time spent in light compartment^2	Active	0.000	0.279	-1.43	0.67	0.37	5,10
	Inactive	0.000	0.295	-1.54	0.59		
	Manipulate	0.000	0.393	-1.01	0.73		
	Feed	0.000	0.803	-0.46	0.34		
	Groom	0.000	0.484	-1.23	0.40		
Latency to approach novel object^2	Active	0.000	0.568	-0.26	0.67	1.99	5,10
	Inactive	**0.000**	**0.047**	**-1.07**	**0.59**		
	Manipulate	0.000	0.250	0.48	0.73		
	Feed	0.000	0.260	-0.73	0.34		
	Groom	0.000	0.883	0.09	0.40		
DURATION							
Time spent in light compartment	Active	0.565	0.851	0.37	0.21	0.13	2,13
	Inactive	-2.397	0.621	-0.97	0.24		
Latency to approach novel object	Active	0.859	0.152	1.05	0.21	1.86	2,13
	Inactive	0.058	0.950	0.04	0.24		
Time spent in light compartment^2	Active	-0.003	0.844	-0.39	0.21	0.10	2,13
	Inactive	0.012	0.663	0.86	0.24		
Latency to approach novel object^2	Active	-0.002	0.126	-1.11	0.21	3.41	2,13
	Inactive	-0.001	0.520	-0.44	0.24		

CHAPTER FOUR

Effects of age on the use of environmental enrichment in stereotypic African striped mice, *Rhabdomys dilectus*

Abstract

Although environmental enrichment has generally been shown to enhance captive animals' welfare and reduce stereotypic behaviours, it can be questioned why enrichment is not always successful in its intended purpose? I addressed this question by investigating whether the age at which an enrichment protocol was implemented influenced the behavioural responses to cages of varying complexity in African striped mice, *Rhabdomys dilectus*. I also considered whether the personality (i.e. consistent individual variation) modulated this response. The personality of the striped mice was first tested, using three conventional personality tests (i.e. novel-object, light-dark and startle-response tests) as sub-adults at 43 days of age. Thereafter, they were individually exposed to decreasing (Enriched to Standard-enriched to Standard) and increasing (Standard to Standard-enriched to Enriched) levels of complexity, so that they would experience differing levels of complexities at different ages. The age at which environmental enrichment was introduced did not influence behavioural responses to cages of different complexity. The behavioural responses were also not influenced by the personality of the stereotypic individuals. Stereotypic behaviours were low in the Enriched treatment as expected, but not all stereotypic striped mice responded to the treatments in a consistent manner. Stereotypic individuals that spent a longer time in the light compartment (bolder) in personality tests showed varying levels of stereotypic behaviours and inactivity in the three treatments. Similarly, the levels of stereotypic behaviours, inactivity and feeding also varied in stereotypic mice that took a longer time to approach the novel object (less bold) in personality tests. There were no age-related differences in the behavioural responses of non-stereotypic striped mice. My results reveal that age may not be an important factor in influencing behavioural responses to varying environments and that stereotypic striped mice show flexible behavioural responses to cages of varying complexity irrespective of their personality.

Keywords: Age, Personality, Environmental enrichment, Striped mice, Stereotypic behaviour

Introduction

Environmental enrichment exposes animals to physical, cognitive and social stimulation, greater than what they would receive under standard housing (Van Praag et al., 2000; Simpson and Kelly, 2011). Provision of environmental enrichment enhances learning abilities, spatial memory (Leggio et al., 2005; Petrosini et al., 2009), decreases the occurrence of abnormal behaviours, such as stereotypic behaviours (Würbel et al., 1998; Powell et al., 2000; Turner et al., 2003), and importantly increases the ability of animals to cope with stressors (Newberry, 1995; Young, 2003). Many factors can influence the efficacy of environmental enrichments, for example, the variability of enrichment protocol (Simpson and Kelly, 2011) as well as personality (i.e. individual differences). However, in a previous study (Chapter 3), I showed that personality did not influence how striped mice interacted with environmental enrichment and that both stereotypic and non-stereotypic striped mice displayed flexible behavioural responses, depending on context or cage complexity.

Factors such as the age at which the environmental enrichment is introduced can also explain individual variation in the responses to enrichments, which could potentially affect the efficacy of environmental enrichment (Walker and Mason, 2012). For example, studies conducted on bank voles, *Clethrionomys glareolus* and deer mice, *Peromyscus maniculatus,* show that age influences the impact of enrichment on stereotypic behaviour. In these studies, although older animals displayed a reduction of stereotypic behaviours when subjected to environmental enrichment, younger animals exposed to the same conditions had a greater chance of reducing or eliminating stereotypic behaviours (Cooper et al., 1996; Hadley et al., 2006).

The aim of my study was to investigate whether the age of stereotypic and non-stereotypic African striped mice, *Rhabdomys dilectus,* influenced their behavioural responses to cages of varying complexities and whether personality modulated this response. Striped mice readily display stereotypical behaviours in captivity, with approximately half of captive born individuals exhibiting stereotypic behaviours as a result of being housed in standard laboratory cages (Schwaibold and Pillay, 2001). Individuals underwent three personality tests after which they were exposed to three treatments in a decreasing (Enriched to Standard-enriched to Standard) and increasing (Standard to Standard-enriched to Enriched) order of complexity. Such a design accounted for the longitudinal effects of aging by exposing striped mice of different ages to differing levels of enrichment. I predicted that 1) individual stereotypic striped mice exposed to treatments in an increasing complexity would show higher levels of stereotypic behaviours in the Standard treatment and lower levels of stereotypic behaviours in the enriched (Standard-enriched and Enriched) treatments, and 2) individual stereotypic striped mice exposed to treatments in a decreasing complexity would show lower levels of stereotypic behaviours in the enriched treatments (Standard-enriched and Enriched), which would then increase in the Standard treatment.

Materials and methods

Striped mice used in this study were captive born F1 and F2 individuals, obtained from a population in Pretoria (25º 40ʺ S; 28º 30ʺ E), South Africa. They were housed in the Milner Park Animal Unit, University of the Witwatersrand under partially controlled environmental conditions: 14L: 10D light: dark cycle (lights on at 05h00); 22ºC-24ºC and 30-60% rH. Subjects were housed singly in clear Lab-o-tec ™ cages (L × H × W: 300 mm × 200 mm × 150 mm). Wood shavings (± 3 cm) were provided as bedding and a handful of *Eragrostis* grass (± 20 g) and ± 5 g of shredded tissue paper were provided as nesting material. PVC nest-boxes (L × H × W: 100 mm × 100 mm × 150 mm, open at both ends) were also provided in each cage. Epol® mouse cubes and water were available *ad libitum*. About 10 g of fresh fruit (apples, pears) or vegetables (lettuce, carrots, broccoli) and ± 5 g of mixed seed were provided daily per individual.

Given that the transmission of stereotypic behaviours has a genetic basis in striped mice (Jones et al., 2008) and therefore to increase the chance of producing stereotypic and non-stereotypic individuals, seven stereotypic and seven non-stereotypic pairs (i.e. both male and female were either stereotypic or not) were established under standard laboratory conditions (as described above) of which there were only five stereotypic and three non-stereotypic successful breeding. The young were separated from the mother at 22 days and housed singly in Lab-o-tec™ cages (described above). These offspring were used in experiments (Figure 1).

Observations for stereotypy and personality tests

From day 22, observations were made daily on the young, twice a day for a total of half an hour, in order to establish the absence/presence of stereotypic behaviour. Stereotypic individuals were those that displayed 10 or more bouts of stereotypy per observation session, each with three or more repetitions (after Jones et al., 2008). Only individuals that exhibited locomotor stereotypic behaviours were used in this study. Individuals that did not exhibit any stereotypic behaviour were classified as non-stereotypic and were used as a comparison with the stereotypic individuals. The absence/ presence rather than the duration of stereotypy was recorded because stereotypy is an ‘all or nothing’ occurrence in striped mice (Jones et al., 2008). At 43 days of age stereotypic and non-stereotypic striped mice underwent three conventional personality tests (see Miller et al., 2006), namely Light-dark, Startle and Novel-object tests to establish their personality types, using the procedure described in Chapter 2.

Environmental enrichment and stereotypy

Following the personality tests, stereotypic and non-stereotypic striped mice were then subjected to three treatments in an increasing (stereotypic: male, n= 4: female, n= 7; non-stereotypic: male, n= 4: female, n= 5) and decreasing (stereotypic: male, n= 4: female, n= 6; non-stereotypic: male, n= 3: female, n= 3) order of complexity (Figure 1). The starting ages (45 days) and the duration (45 days) of the treatments were fixed.

Treatment 1. (Standard housing/ baseline) - test subjects were housed individually in their original Lab-o-tec™ cages (See above).

Treatment 2. (Standard-enriched housing) - test subjects, were housed individually in their original Lab-o-tec™ cages as above but with the addition of one to two small cardboard tubes (± 50 mm diameter) for enrichment.

Treatment 3. (Enriched housing) - test subjects were housed individually in a larger tank (L × H × W: 600 mm × 410 mm × 300 mm), provided with a nest box with the same dimensions as in Standard housing), a deep layer of wood shavings as bedding (± 40 g) and *Eragrostis* grass (± 20 g). In addition to cardboard tubes, several enrichment devices, i.e. a running wheel (± 15 cm diameter), Habitrail™ PVC tunnels and balls were provided.

The behaviour of all animals was video-recorded in all treatments every second day (i.e. 22 days of recording) from 09h00 to 12h00 as striped mice are most active between these time (Pillay, 2000). Before commencing video-recording of behaviours in each treatment, all individuals were allowed to acclimatize to the new treatment for 24 hours. Using Observer software (version 5.0; Noldus Information Technology), the frequency of six behaviours (see Table 1) were scored using continuous sampling: inactive; active; feeding/ drinking; manipulating objects in a cage; grooming; and stereotypic behaviour (if applicable).

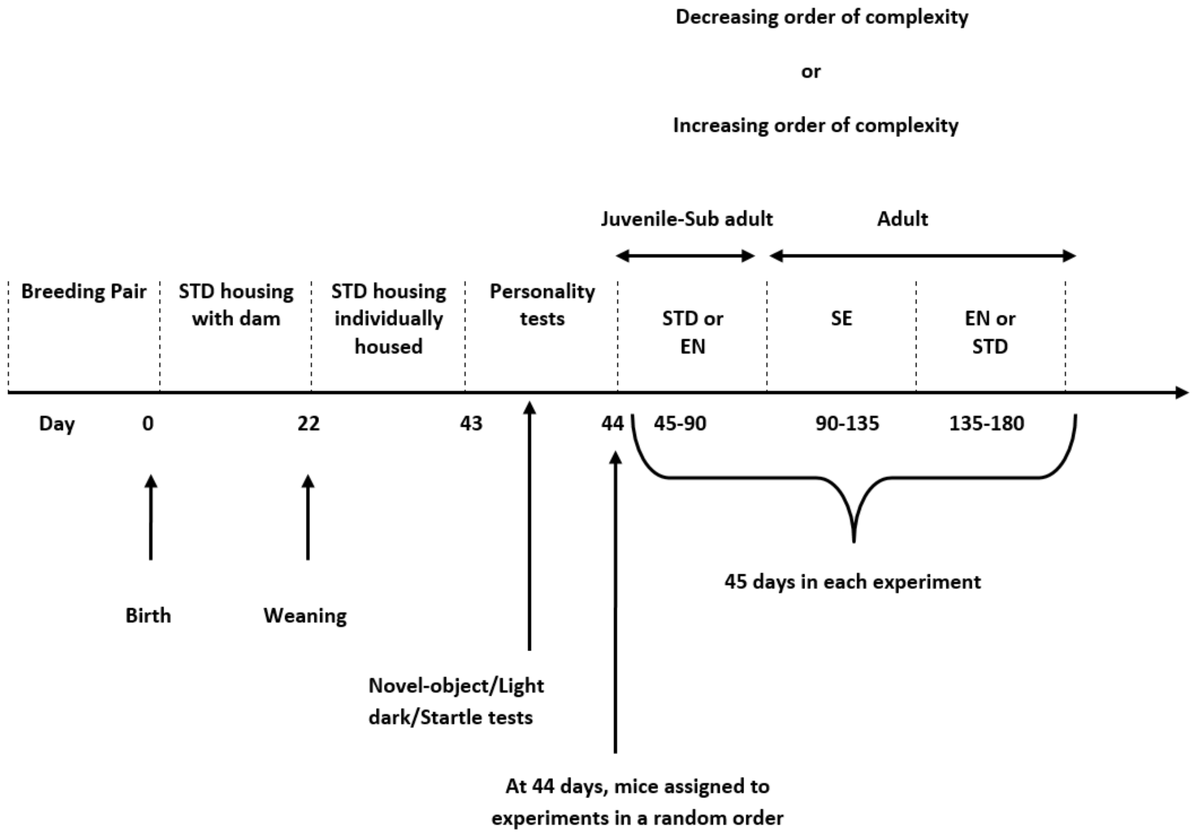

Figure 1. Timeline showing the ages (days) at which striped mice were weaned, exposed to personality tests and assigned in a decreasing and increasing order of complexity to Standard (STD), Standard-enriched (SE) and Enriched (EN) treatments.

Table 1. Ethogram of striped mice behaviours scored in three treatments.

Behaviour	Definition
Inactive	Individual motionless and resting or out of sight
Active	Non-stereotypic movement or running on the cage floor usually digging in the wood shavings + wheel running in Treatment 3
Object manipulation	Manipulating enrichments (e.g. biting or nudging of cardboard tubes and wheels)
Feeding/Drinking	Manipulating or chewing of mouse cubes, seeds or vegetables/fruits and drinking from a water bottle
Stereotypic behaviour	A repetitive and invariant behaviour > 3 times in succession
Grooming	Squatting on hind legs, grooming head, body, tail, and/or genitals

In addition, I also recorded the frequency of the wheel running behaviour displayed by test subjects in the Enriched treatment (i.e. Treatment 3). Every two weeks, the cages/tanks, PVC tunnels and all the contents were cleaned and the cardboard tubes replaced.

Data analyses

Data were checked for normality and homogeneity of variance using the Shapiro-Wilk and Levene's tests. All the statistical analyses were two-tailed with statistical significance accepted at $p \leq 0.05$ and were analysed using R (Ver. 2.13.0; R Development

Core Team, 2011) and Statistica (version 7 Statsoft, USA) software. Tukey post hoc tests were used to identify specific trends. Using, the pwr.chisq.test function in the *pwr* package (Blomberg, 2014), a power analyses was used to calculate effect size.

Previously (Chapter 2 and Chapter 3), I showed that at group level, stereotypic striped mice were bolder than non-stereotypic mice, while at an individual level, stereotypic mice were flexible in their behaviours. Based on my previous findings, I considered just the individual level differences because these were instructive of differences between individuals than the group level differences. The relationship between personality and the behaviours of individual stereotypic and non-stereotypic striped mice in the Standard, Standard-enriched and Enriched treatments for both increasing and decreasing order of complexity were analysed using a polynomial multivariate regression for multiple dependents in the same model. The frequency and the duration of behaviours were included as dependent variables. The startle and novel object personality test scores were used as the continuous predictors, and the treatments (Standard, Standard-enriched and Enriched) and age were used as categorical predictors. Since non-stereotypic individuals do not exhibit stereotypic behaviours, separate tests were conducted for both stereotypic and non-stereotypic mice. For all dependent variables, I first examined the homogeneity of slopes of the continuous predictors and their interaction to determine whether a single test (multivariate regression) or separate regressions were required. For both tests, I report the coefficient of determination, F- and P- values and parameter estimates for linear and polynomial decomposition. I also examined the beta coefficient, obtained when all variables are standardized to a mean of 0 and a standard deviation of 1. The polynomial variables are reported as linear and quadratic functions indicated with a "^{2}".

Results

I found a low power of 0.24, indicating that the small number of test animals affected the analysis. Age was not a significant predictor of the behaviours ($F_{3, 51}$= 0.528, p=0.665). Furthermore, Tukey post hoc tests revealed that age was not a significant predictor of behaviours in all treatments.

Stereotypic mice

An examination of the beta coefficients allows an assessment of the relative contribution of each independent variable on the dependent variable. There was a significant association between the time spent in the light compartment and the frequency of stereotypic behaviours. With an increase in the time spent in the light compartment, there was a linear increase in stereotypic behaviours but a non-random polynomial decrease in this behaviour (**Supplementary material:** S1). Furthermore, the time spent in the light compartment^2 was positively associated with the duration of stereotypic behaviours: with an increase in the time spent in the light compartment, there was a non-random polynomial increase in stereotypic behaviours. Figure 2a shows that 6 out of 11 individuals showed a decrease in the duration of stereotypic behaviours from the Standard to Standard-enriched treatment. Stereotypic behaviours were low in 7 individuals and high in 4 individuals in the Enriched treatment. The frequency of stereotypic behaviours followed a similar pattern to that of the duration, with some individuals showing an increase in stereotypic behaviour and others showing a decrease in the Enriched treatment (Figure 2b). Duration and frequency of stereotypic behaviours were low in the Enriched treatment and increased in the Standard-enriched treatment (Figures 2c and 2d).

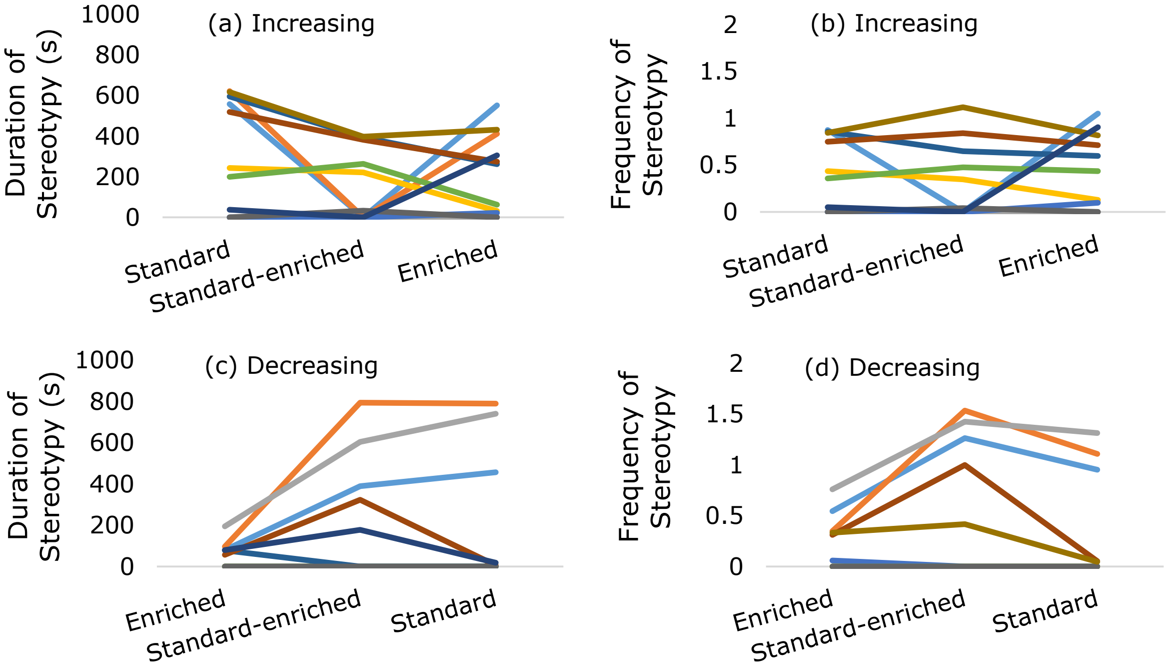

Figure 2. Changes in duration and frequency of stereotypic behaviour of individual stereotypic striped mice exposed to treatments (Standard, Standard-enriched and Enriched) in an increasing (a, b) and decreasing (c, d) order. The data for each individual for each treatment are connected by a different coloured line.

Figure (3a) showed that activity was high in the Standard treatment, decreased in the Standard-enriched treatment and increased in the Enriched treatment in 10 of 11 individuals.

The frequency of activity plateaued from the Standard to Standard-enriched treatment in all individuals and increased in the Enriched treatment (Figure 3b).

While duration of activity was high in 5 of the individuals in the Enriched treatment, it was low in 6 individuals in the same treatment (Figure 3c). Figure 3d shows that frequency of activity was high in the Enriched treatment and decreased from the Standard-enriched to Standard treatment.

There was a significant association between the time spent in the light compartment and the frequency of inactivity. With an increase in the time spent in the light compartment, there was a linear increase inactivity but a non-random polynomial decrease in inactivity. There was also an association between the duration and frequency of inactivity and the latency to approach the novel object: with an increase in latency to approach the novel object, inactivity increased in some treatments while it decreased in other treatments. Furthermore, the time spent in the light compartment^2 was negatively associated with duration of inactivity, with a non-random polynomial decrease in inactivity.

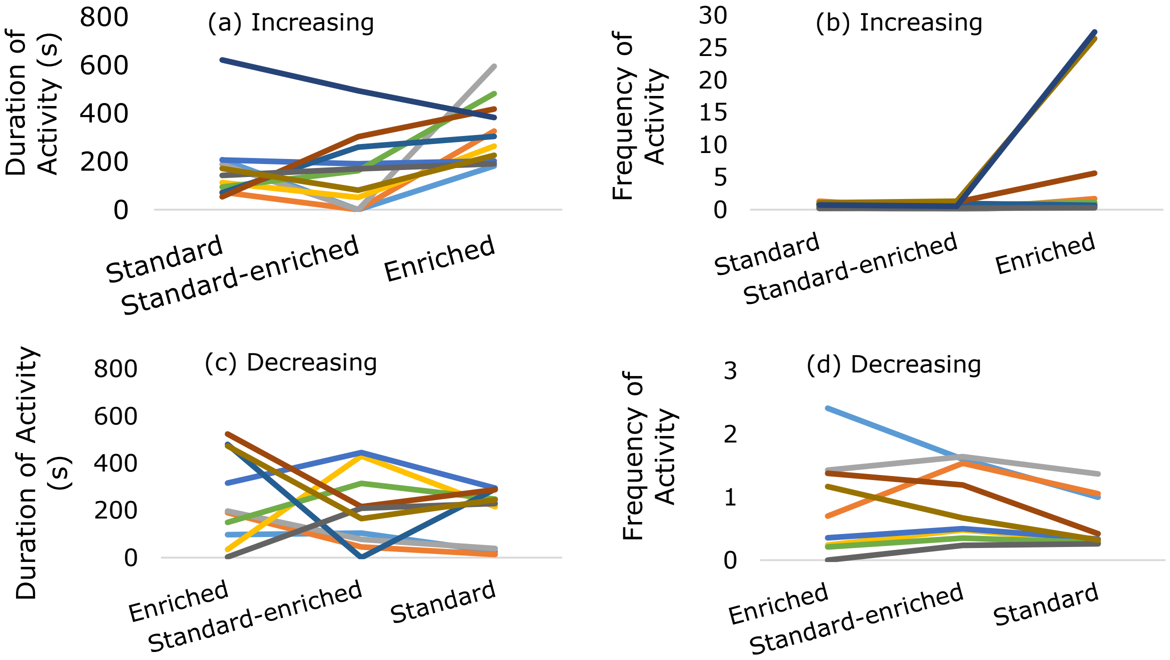

Figure 3. Changes in duration and frequency of activity of individual stereotypic striped mice exposed to treatments (Standard, Standard-enriched and Enriched) in an increasing (a, b) and decreasing (c, d) order. The data for each individual for each treatment are connected by a different coloured line.

In 6 of 11 individuals, duration of inactivity decreased from the Standard-enriched to Enriched treatment, while for 5 individuals, it increased in the Enriched treatment (Figure 4a). The frequency of inactivity was more or less constant in the three treatments (Figure 4b). Duration of inactivity was high in the Enriched treatment in 7 individuals and low in 3

(Figure 4c). It decreased in 7 individuals from the Standard-enriched to Standard treatments. Frequency of inactivity was constant across treatments with an exception of a few individuals (Figure 4d).

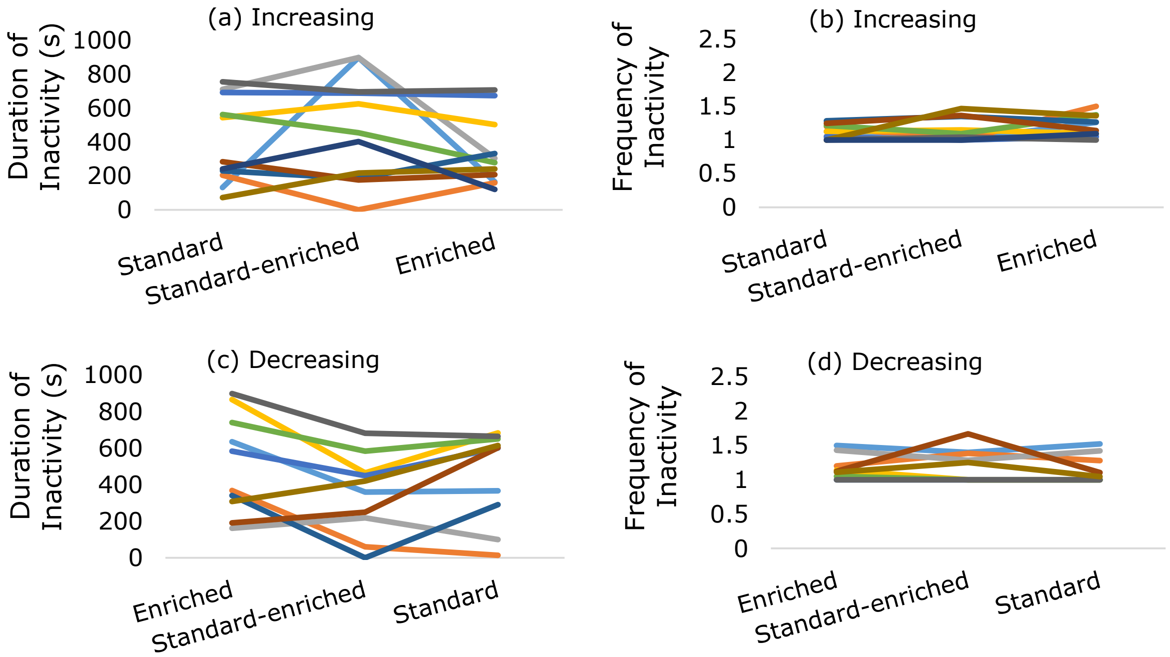

Figure 4. Changes in duration and frequency of inactivity of individual stereotypic striped mice exposed to treatments (Standard, Standard-enriched and Enriched) in an increasing (a, b) and decreasing (c, d) order. The data for each individual for each treatment are connected by a different coloured line.

There was a significant association between the time spent in the light compartment^2 and object manipulation and grooming. Although the linear component was not significant, there was a polynomial decrease in these behaviours with an increase in the time spent in the light compartment. Frequency of object manipulation decreased in 5 individuals from the Standard to Standard-enriched treatment and increased in 5 individuals in the Enriched treatment (Figure 5a). Similarly, frequency of object manipulation was high in the Enriched treatment and decreased in the Standard treatment (Figure 5b).

Furthermore, there was a significant association between the latency to approach a novel object and feeding, with feeding decreasing with an increase in the latency to approach the novel object. Frequency of feeding decreased from the Standard-enriched to the Enriched treatment in most individuals (Figure 5c), while it decreased from the Standard-enriched to Standard treatment (Figure 5d). Patterns of grooming differed in the individuals in all treatments: while it increased in some in the Enriched treatment, in others it decreased (Figure 5e and 5f).

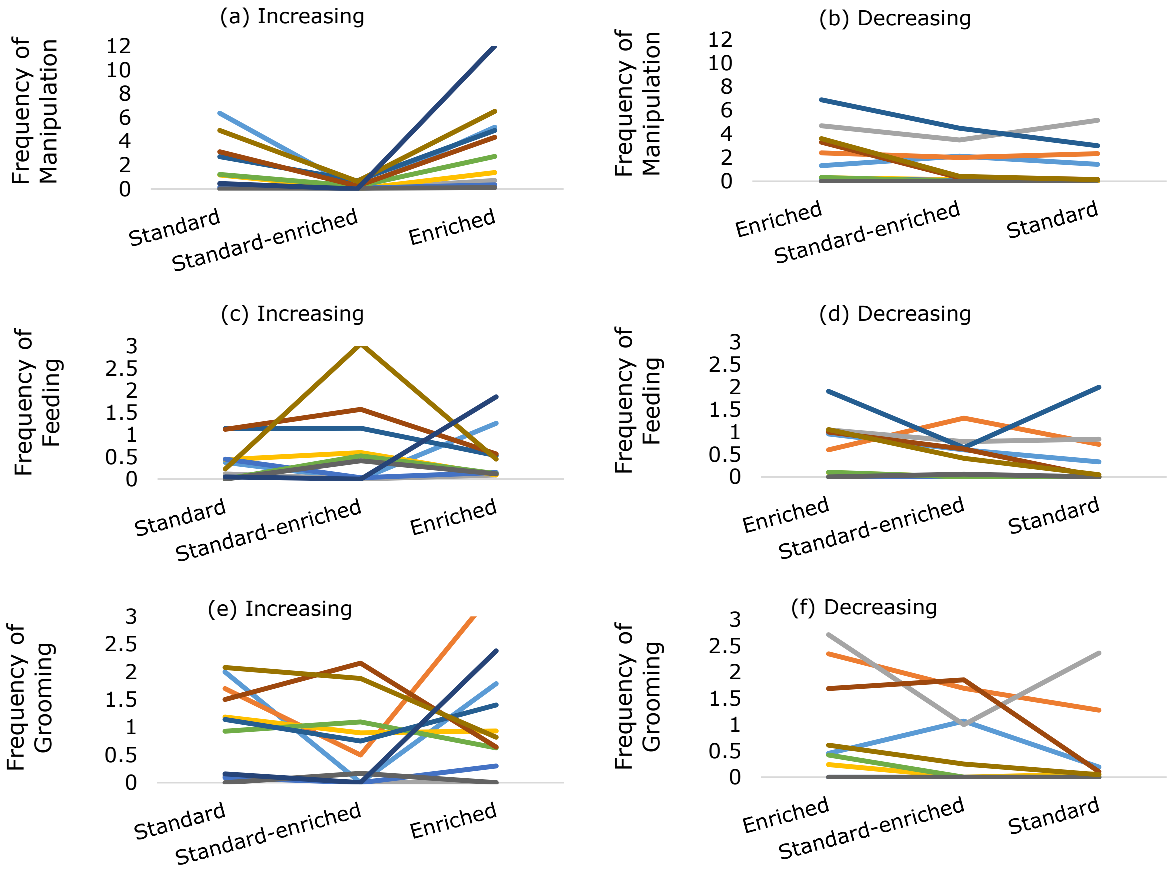

Figure 5. Changes in frequency of behaviours of individual stereotypic striped mice exposed to treatments (Standard, Standard-enriched and Enriched) in an increasing and decreasing order. The data for each individual for each treatment are connected by a different coloured line.

<u>Non-stereotypic mice</u>

Although there were no significant associations between the personality test scores and the frequency and duration of behaviours in non-stereotypic mice, there were 7 positive and 13 negative and 4 positive and 4 negative non-significant associations between the frequency and duration of behaviours and the personality scores respectively (**Supplementary material**: S2). Nevertheless, Figure 6a shows that duration of activity increased from Standard to Standard-enriched treatment in 5 of 9 individuals and decreased in 4 individuals from Standard-enriched to the Enriched treatment. The frequency of activity followed a similar pattern as the duration, increasing in most individuals in the Enriched treatment (Figure 6b).

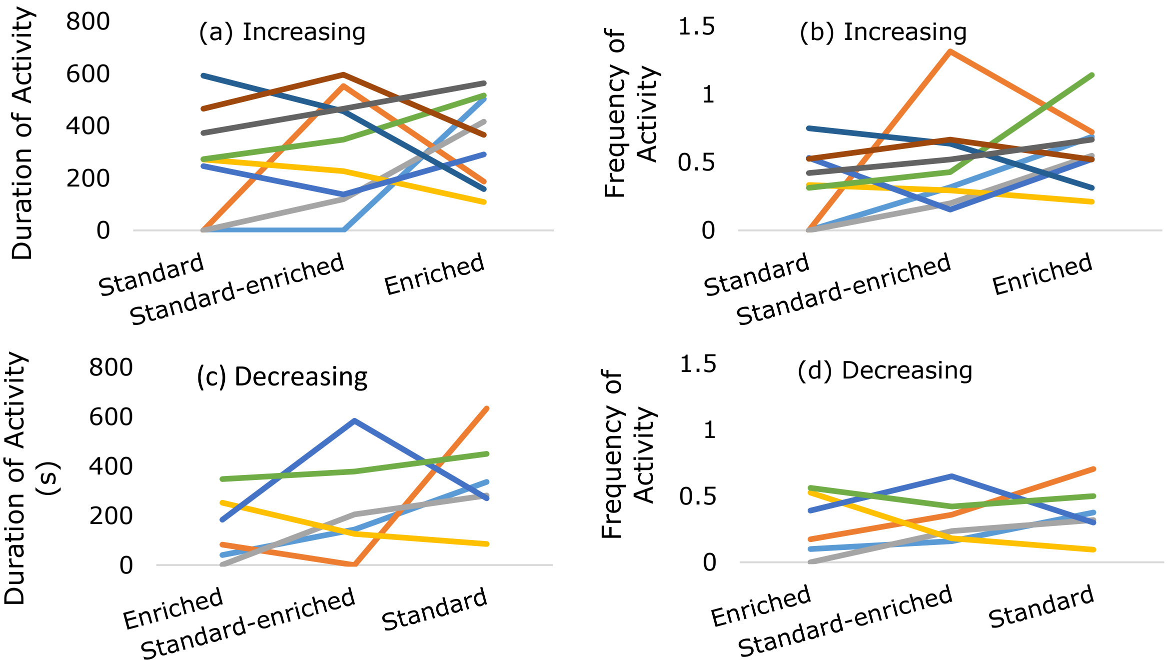

Figure 6. Changes in duration and frequency of activity of individual non-stereotypic striped mice exposed to treatments (Standard, Standard-enriched and Enriched) in an increasing (a, b) and decreasing order (c, d). The data for each individual for each treatment are connected by a different coloured line.

The duration of activity varied in individuals in the Enriched treatment, but increased in the Standard treatment in 4 individuals (Figure 6c), while the frequency of activity was low in most individuals in the Enriched treatment and increased from the Standard-enriched to Standard treatment (Figure 6d).

While some individuals showed an increase in the duration of inactivity from Standard to Standard-enriched treatment and a decrease in the Enriched treatment; others showed a decrease in inactivity from Standard to Standard-enriched treatment and an increase in the Enriched treatment (Figure 7a). The frequency of inactivity was constant in all three treatments with an exception of 1 individual that showed an increase in the Standard-enriched treatment (Figure 7b).

Duration of inactivity was high in most individuals in the Enriched treatment (Figure 7c), while the frequency of inactivity was slightly high in three individuals in the Enriched treatment and plateaued from the Standard-enriched to the Enriched treatments (Figure 7d).

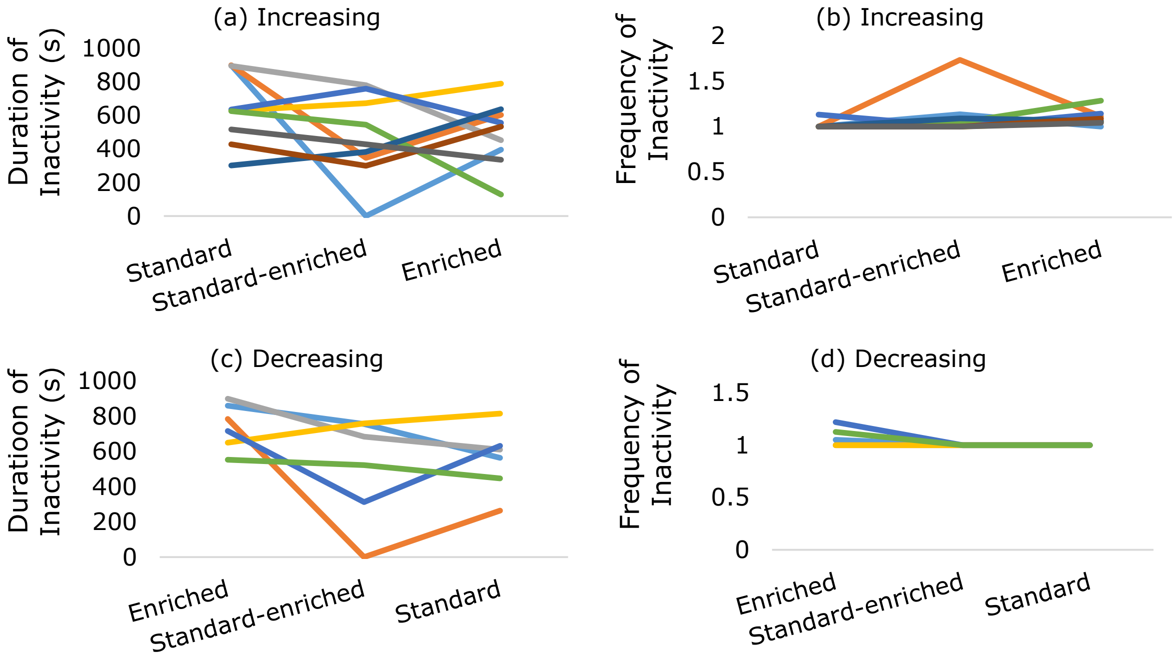

Figure 7. Changes in duration and frequency of inactivity of individual non-stereotypic striped mice exposed to treatments (Standard, Standard-enriched and Enriched) in an increasing (a, b) and decreasing (c, d) order. The data for each individual for each treatment are connected by a different coloured line.

Frequency of object manipulation increased in most individuals in the Enriched treatment (Figure 8a). Similarly, Figure 8b shows that frequency of object manipulation was high in the Enriched treatment and plateaued from the Standard-enriched to Standard treatments. Frequency of feeding was variable across treatments (Figure 8c) while there was less feeding in the three treatments (Figure 8d). Frequency of grooming increased in most individuals in the Enriched treatment (Figure 8e), while it was slightly high in the Enriched treatments for most individuals and decreased from the Standard-enriched to Standard treatments (Figure 8f).

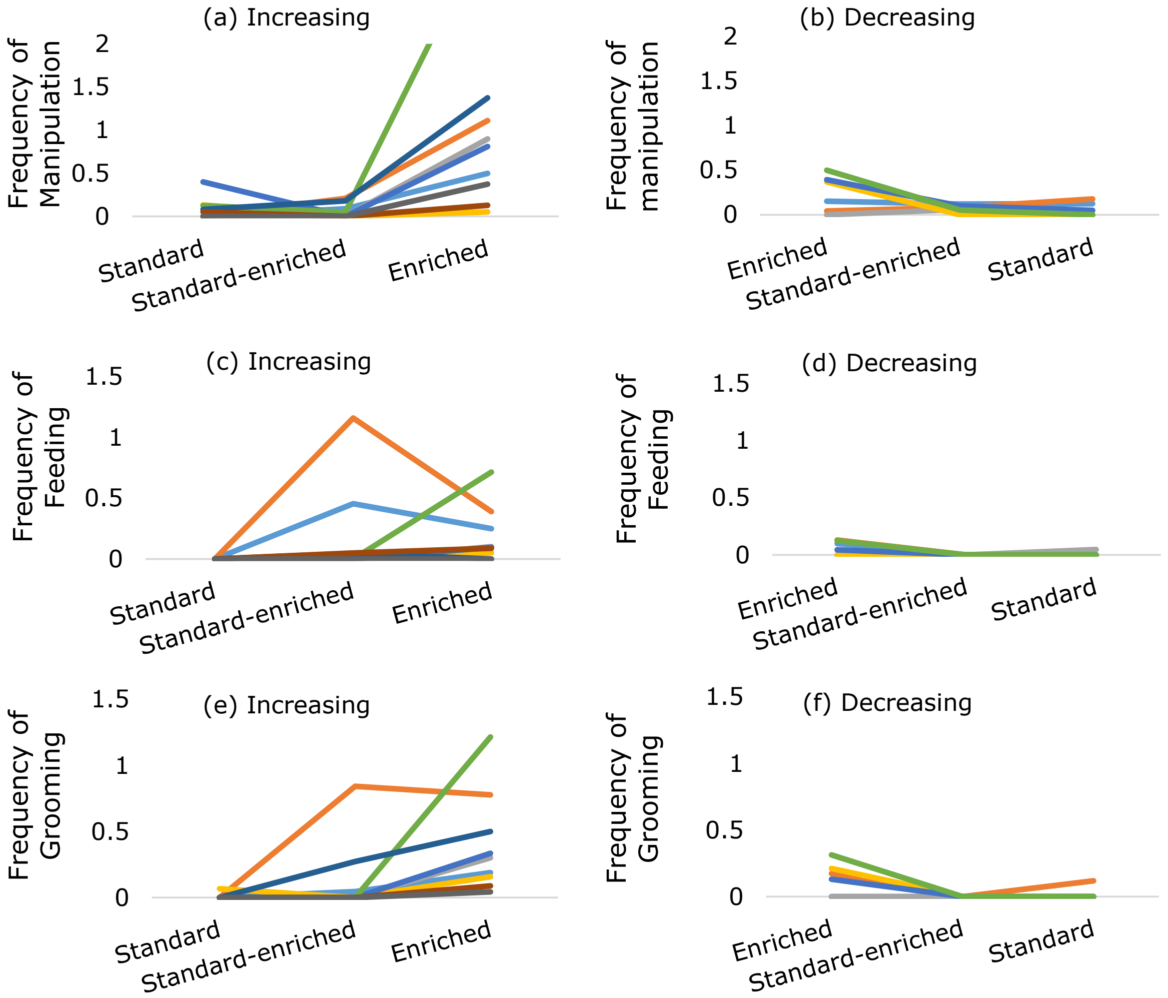

Figure 8. Changes in frequency of behaviours of individual non-stereotypic striped mice exposed to treatments (Standard, Standard-enriched and Enriched) in an increasing and decreasing order. The data for each individual for each treatment are connected by a different coloured line.

In summary, stereotypic behaviours were low in 7 individuals and high in 4 individuals in the Enriched treatment (Table 2) in individuals exposed in an increasing order of complexity. Similarly, stereotypic behaviours were low in 7 individuals and high in 3 in individuals exposed to a decreasing order of complexity (Table 3).

Table 2. Personality scores (duration in light compartment and latency to approach the novel object) and the relative performance of stereotypic behaviours of individual stereotypic striped mice exposed to treatments (Standard, Standard-enriched and Enriched) in an increasing order of complexity.

Individual number	Time spent in light compartment (s)	Latency to approach novel object (s)	Standard	Standard-enriched	Enriched
1	60.53	63.55	HIGHER	LOW	HIGH
2	216.65	0.00	HIGHER	LOW	HIGH
3	0.00	0.00	LOW	LOW	LOW
4	0.00	49.57	HIGHER	HIGH	LOW
5	0.00	0.00	LOW	LOW	LOW
6	71.74	28.17	HIGH	HIGHER	LOW
7	128.63	20.83	HIGHER	HIGH	LOW
8	10.42	12.86	HIGHER	HIGH	LOW
9	5.82	23.61	LOW	LOW	LOW
10	6.65	21.74	HIGHER	LOW	HIGH
11	64.93	19.43	HIGH	LOW	HIGHER

Table 3. Personality scores (duration in light compartment and latency to approach the novel object) and changes in stereotypic behaviours in stereotypic individuals exposed to treatments (Enriched, Standard-enriched and Enriched) in a decreasing order of complexity.

Individual number	Time spent in light compartment (s)	Latency to approach novel object (s)	Enriched	Standard-enriched	Standard
1	107.09	0.00	LOW	HIGH	HIGHER
2	216.65	0.00	LOW	HIGHER	HIGHER
3	0.00	0.00	LOW	HIGH	HIGHER
4	52.91	180.11	LOW	LOW	LOW
5	237.29	349.33	LOW	LOW	LOW
6	12.92	432.19	LOW	LOW	LOW
7	78.33	0.00	HIGH	LOW	LOW
8	128.37	0.00	HIGH	HIGHER	LOW
9	23.88	109.42	LOW	LOW	LOW
10	0.00	50.57	HIGH	HIGHER	LOW

Discussion

I aimed to ascertain whether the age at which striped mice were exposed to treatments of varying environmental complexity influenced their behavioural responses and whether personality modulated this response. I expected stereotypic behaviours to be reduced in response to enriched conditions, irrespective of age of exposure, yet there was no influence of age on the behavioural responses. As expected, however, only the Enriched treatment and not the Standard-enriched treatment reduced the levels of stereotypic behaviours. Overall, both stereotypic and non-stereotypic individuals varied their behaviours in response to the cage complexities.

The lack of an age effect in my study differed from a previous study conducted on the same species, *Rhabdomys dilectus*, in which enrichment provided at an earlier age was more beneficial in reducing stereotypic behaviours than when provided at a later age (Jones et al., 2011). However, the discrepancies in the results could be attributed to the ages at which the striped mice were exposed to environmental enrichment, individual differences as well as the duration they were housed under enriched conditions. The striped mice in my study were housed in enriched conditions from 45 days of age (sub-adults) and tested for 45 days, by which time they had probably already developed stereotypic behaviours (Jones et al., 2011). Striped mice were 30 days of age (juveniles) when they started the experiments and were kept under enriched conditions for a prolonged period of 140 days in Jones et al. (2011). Longer duration of exposure to environmental enrichment helps to reduce stereotypic behaviour (Nithianantharajah and Hannan, 2006; Lewis et al., 2006).

However, an interesting finding is that the striped mice that were exposed to environmental enrichment at a later age (increasing complexity) also showed a decrease in stereotypic behaviours similar to the striped mice introduced to environmental enrichment at a younger age (decreasing complexity). The striped mice in the former treatment were housed under standard conditions for far longer than the latter treatment. It has been shown that the provision of enrichment is more beneficial when presented earlier in development, when the central nervous system is relatively plastic, and are more advantageous with a longer duration of exposure (Nithianantharajah and Hannan, 2006; Lewis et al., 2006). Nonetheless, my results show that exposure to environmental enrichment at an earlier age (45 days) and later on in development (>135 days) were equally beneficial in mitigating the levels of stereotypic behaviours. Similarly, Powell et al. (2000) showed that both early (at weaning) and late (60 days after weaning) environmental enrichment reduced the incidences of stereotypic behaviours in deer mice, *Peromyscus maniculatus bairdii*.

The mechanisms that could explain the lack of age effect could be attributed to neural plasticity (i.e. the ability of the brain to alter its structure and function due to stimulation from enriched conditions; Mora et al., 2007). While it has been shown that aging causes a decrease in neurogenesis in the hippocampus and the neurotransmitter systems, provision of enrichment at any age promotes neural plasticity (Mora et al., 2007). Many studies have shown that the brain structure and neurotransmitter systems are often associated with the individual differences in behaviour in humans (Macdonald et al., 2006; Most et al., 2006; Gardini et al., 2009) and other mammals (e.g., Aston-Jones et al., 1999; Hariri, 2006; Lebreton et al., 2009). For example, environmental enrichment alters the serotonergic system (Rasmuson et al., 1998; Brenes et al., 2009) and glucocorticoid receptors, which mediate the negative feedback loop on the Hypothalamic-Pituitary-Adrenal-axis (HPA axis), thereby facilitating efficient coping responses to novel environments (Leggio et al., 2005; Brenes et al., 2008; Sampedro-Piquero et al., 2014).

As in my previous study (Chapter 3), personality was not associated with the behavioural responses to cages of varying complexity in both stereotypic and non-stereotypic striped mice. Irrespective of the order in which stereotypic striped mice were exposed to the treatments, multivariate regression showed significant associations between the personality scores and the behaviours, but these responses were not consistent across the different treatments, similar to my previous findings (Chapter 3). For example, stereotypic striped mice that spent a longer time in the light compartment (i.e. bold mice) showed varying levels of stereotypic behaviours and inactivity in the three treatments. Similarly, stereotypic mice, which took longer to approach the novel object (i.e. less bold mice), also showed varying behavioural responses. Individuals varied the levels of stereotypic behaviours, inactivity and feeding. While previous studies on rodents have only shown flexible behavioural responses in less bold mice (Benus et al., 1987; Sluyter et al., 1996), both bold and less bold stereotypic striped mice show flexible behavioural responses depending on the environment (i.e. the ability to alter behavioural responses to different environmental stimuli; Coppens et al., 2010). Nonetheless, these findings have been shown in other non-rodent species, for example, both bold and less bold rainbow trout, *Onchorhyncus mykiss*, altered their behavioural responses based on their relative competitive ability (Frost et al., 2007).

The causal mechanism underpinning behavioural flexibility is related to alterations in the prefrontal cortex, responsible for behavioural flexibility and inhibition of inappropriate behaviours. Specifically, individual variation in the serotonergic and dopaminergic input into the medial prefrontal cortex may explain individual variation in coping styles, since serotonin is implicated in behavioural flexibility (Coppens et al., 2010; Koolhaas et al., 2010). Behavioural flexibility is well known in the *Rhabdomys* spp. which shows flexible social organisation (i.e. social flexibility; Schradin et al., 2010; Schradin et al., 2012), flexible mating strategies (Schradin, 2008) and flexible development of exploration (Rymer and Pillay, 2012).

Conclusions

The age at which environmental enrichment was introduced did not influence the behavioural responses of striped mice. While this could be attributed to a low sample size, my findings indicate that the behavioural responses of stereotypic striped mice were not influenced by the personality of the individuals. Such flexibility in responses was also seen in the reduction of stereotypy in striped mice exposed to enrichment in both younger and older striped mice. Interestingly, flexibility in individual and age responses is governed by similar neurobiological mechanisms (e.g. serotonergic systems). Future studies must experimentally isolate age and individual effects to demonstrate whether they are related mechanistically.

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Supplementary material

Table S1. Multivariate regression analysis of the frequency of stereotypic behaviour, activity, inactivity, object manipulation, feeding and grooming and the duration of stereotypic behaviour, activity and inactivity with latency to approach the novel object (novel object test) and time spent in the light compartment (startle test) as the predictor variables for stereotypic mice exposed to treatments (Standard, Standard-enriched and Enriched) in an increasing and decreasing order of complexity. Linear and polynomial decomposition are reported. Values bold are significant.

FREQUENCY							
Personality test	**Behaviour**	**Parameter Estimates**	**P**	**β**	**R²**	**F**	***df***
Time spent in light compartment	**Stereotypy**	**0.004**	**0.043**	**0.69**	**0.21**	1.51	6,50
	Active	0.004	0.855	0.06	0.10		
	Inactive	**0.002**	**0.030**	**0.73**	**0.23**		
	Manipulate	0.018	0.061	0.58	0.34		
	Feed	0.003	0.284	0.36	0.23		
	Groom	0.007	0.066	0.60	0.26		
Latency to approach novel object	Stereotypy	-0.002	0.306	-0.47	0.21	0.90	6,50
	Active	-0.010	0.608	-0.25	0.10		
	Inactive	-0.001	0.080	-0.80	0.23		
	Manipulate	-0.013	0.126	-0.64	0.34		
	Feed	**-0.005**	**0.050**	**-0.90**	**0.23**		
	Groom	-0.005	0.203	-0.57	0.26		
Time spent in light compartment ^{2}	**Stereotypy**	**0.000**	**0.017**	**-0.82**	**0.21**	1.78	6,50
	Active	0.000	0.776	-0.10	0.10		
	Inactive	**0.000**	**0.014**	**-0.84**	**0.23**		
	Manipulate	**0.000**	**0.038**	**-0.65**	**0.34**		
	Feed	0.000	0.117	-0.52	0.23		
	Groom	**0.000**	**0.021**	**-0.77**	**0.26**		
Latency to approach novel object ^{2}	Stereotypy	0.000	0.677	0.19	0.21	0.46	6,50
	Active	0.000	0.769	0.14	0.10		
	Inactive	0.000	0.241	0.53	0.23		
	Manipulate	0.000	0.326	0.41	0.34		
	Feed	0.000	0.184	0.61	0.23		
	Groom	0.000	0.495	0.30	0.26		
DURATION							
Time spent in the light compartment	Stereotypy	0.013	0.992	0.00	0.28	0.61	3,52
	Active	1.043	0.239	0.51	0.13		
	Inactive	-1.219	0.331	-0.38	0.29		
Latency to approach novel object	**Stereotypy**	**-1.929**	**0.044**	**-0.93**	**0.28**	2.95	3,52
	Active	-0.028	0.966	-0.02	0.13		
	Inactive	**2.504**	**0.009**	**1.20**	**0.29**		
Time spent in the light compartment^2	Stereotypy	0.004	0.475	0.28	0.28	0.46	3,52
	Active	-0.005	0.262	-0.49	0.13		
	Inactive	0.001	0.880	0.06	0.29		
Latency to approach novel object^2	Stereotypy	0.003	0.252	0.53	0.28	1.38	3,52
	Active	0.001	0.724	0.18	0.13		
	Inactive	-0.004	0.069	-0.84	0.29		

Table S2. Multivariate regression analysis of the frequency of activity, inactivity, object manipulation, feeding and grooming and the duration of activity and inactivity with latency to approach the novel object and time spent in the light compartment (startle test) as the predictor variables in non-stereotypic striped mice exposed to treatments (Standard, Standard-enriched and Enriched) in an increasing and decreasing order of complexity. Linear and polynomial decomposition are reported. Values in bold are significant.

FREQUENCY							
Personality test	**Behaviour**	**Parameter Estimates**	**P**	**β**	**R^2**	**F**	***df***
Time in light compartment	Active	-0.001	0.602	-0.33	0.12	0.30	5,34
	Manipulate	0.001	0.522	0.39	0.18		
	Inactive	0.000	0.912	-0.07	0.11		
	Feed	-0.001	0.644	-0.29	0.12		
	Groom	0.000	0.871	-0.11	0.09		
Latency to approach novel object	Active	0.001	0.408	0.48	0.12	0.64	5,34
	Manipulate	0.000	0.819	-0.13	0.18		
	Inactive	0.000	0.706	-0.22	0.11		
	Feed	0.000	0.775	-0.16	0.12		
	Groom	0.000	0.746	0.19	0.09		
Time in light compartment ^{2}	Active	0.000	0.555	0.38	0.12	0.50	5,34
	Manipulate	0.000	0.360	-0.57	0.18		
	Inactive	0.000	0.925	-0.06	0.11		
	Feed	0.000	0.784	0.17	0.12		
	Groom	0.000	0.923	-0.06	0.09		
Latency to approach novel object ^{2}	Active	0.000	0.262	-0.66	0.12	0.99	5,34
	Manipulate	0.000	0.818	-0.13	0.18		
	Inactive	0.000	0.529	0.37	0.11		
	Feed	0.000	0.595	0.31	0.12		
	Groom	0.000	0.881	-0.09	0.09		
DURATION							
Time spent in the light compartment	Active	2.949	0.117	1.08	0.11	1.26	2,35
	Inactive	-3.141	0.118	-1.09	0.09		
Latency to approach novel object	Active	0.433	0.614	0.26	0.11	1.69	2,35
	Inactive	-0.780	0.396	-0.44	0.09		
Time spent in the light compartment^2	Active	-0.019	0.110	-1.11	0.11	1.32	2,35
	Inactive	0.021	0.109	1.13	0.09		
Latency to approach novel object^2	Active	-0.002	0.489	-0.35	0.11	0.88	2,35
	Inactive	0.003	0.366	0.46	0.09		

CHAPTER FIVE

Wheel running: an enrichment or a re-directed stereotypic behaviour in striped mice, *Rhabdomys dilectus*

Abstract

When given the opportunity, many captive animal species make use of a running wheel. Wheel running is often used as an environmental enrichment to increase general locomotor activities. However, it is still debated whether wheel running is an enrichment or a stereotypic behaviour. To test for the role of wheel running, I exposed a group of stereotypic and non-stereotypic striped mice individually to three different, two compartment housing conditions, based on the amount of spatial restriction to perform stereotypic behaviour: 1) No Restriction; 2) Restricted Home Tank with reduced space in home tank but access to a running wheel tank; and 3) Restricted Wheel Tank with reduced space in wheel tank but access to space in home tank. Each individual spent 15 days in each treatment, during which I recorded their behaviours every second day. I also accounted for personality differences in the use of running wheels. Results showed that 52 % of the stereotypic individuals incorporated the running wheel in their stereotypic routine, implying that it was a re-directed stereotypic behaviour, and the remaining 48 % used the running wheel as enrichment. Furthermore, while wheel running may have reduced stereotypic behaviours in the striped mice that used it as enrichment, it was not solely responsible for the reduction. The combined effect of cage complexity and the running wheel acted in synergy in reducing stereotypic behaviours, as shown by an increase in activity and object manipulation. Stereotypic individuals showed flexible behavioural responses, which does not concur with personality level differences. Thus, the underlying motivation for the use of the running wheel differs between individual stereotypic mice, implying that enrichment must be tailored for individuals to address their welfare concerns.

Keywords: Striped mice, wheel running, stereotypic behaviour, re-directed behaviour, environmental enrichment.

Introduction

To enhance the welfare of captive animals often involves physical environmental enrichment, using larger and complex housing with increased opportunities for exploration, hiding and nesting (Pawlowicz et al., 2010). Running wheels are frequently used to increase the environmental complexity as well as to encourage physical activity (Pham et al., 2005; Richter et al., 2014), which in turn has been shown to enhance learning abilities and memory (Van Praag et al., 2000), increase stress resistance, have antidepressant and anxiolytic effects (Greenwood et al., 2005) and reduce the levels of stereotypic behaviours (Richter et al., 2008; Hansen and Damgaard, 2009). The use of the running wheel by a wide range of laboratory animals has captured the interests of many scientists because when given the opportunity, many animal species are highly motivated to make use of a running wheel (Sherwin, 1998a; Hansen and Jensen, 2006). For example, even when costs are imposed to gain access to a running wheel, laboratory mice readily press a lever continually in order to gain access to the wheel (Sherwin, 1998b), which is therefore perceived as a valuable resource (Howerton and Mench, 2014).

The spontaneous and incessant use of the running wheel has given rise to the assumption that animals derive pleasure from this activity (Hansen and Damgaard, 2009). However, there are several competing hypotheses of wheel running, such as the desire of an animal to perform exploratory behaviours, replacement of general locomotor activities, stereotypic behaviour or merely a form of general activity (Hansen and Damgaard, 2009; Pietropaolo et al., 2004; Sherwin, 1998b). Other studies suggest that rodents perceive running as a reward or a self-reinforcing behaviour (Latham and Würbel, 2006; Sherwin, 1998b) or is an incentive-induced behaviour which seems to activate many of the similar neural pathways stimulated by the intake of addictive drugs (Werme et al., 2000, 2002 a, b; de Visser et al., 2005, Brene et al., 2007). Despite its frequent use in laboratory rodents, it is still unclear whether wheel running is hedonistic and thus a form of enrichment or is merely a re-directed stereotypic behaviour (i.e. a behaviour incorporated in the normal behavioural routine of stereotypy performance; Richter et al., 2014), since it has the central defining characteristics of a stereotypic behaviour being a repetitive, monotonous pattern of movement, without any apparent goal or function (Mason, 1991 a, b).

In addition to enhancing cage complexities with running wheels and additional cage objects, cage size or available space is also an important factor in determining welfare of animals (Fischer et al., 2007) as well modulating the use of running wheels (Kunhen, 2002). Studies show that when given a choice, Hooded Norway rats, *Rattus norvegicus*, preferred larger cages (Patterson-Kane et al., 2001) wherein stereotypic behaviours were reduced (Gebhardt-Henrich and Steiger, 2006; Kunhen, 2002). Locomotor stereotypies are thought to arise from frustrated locomotor behaviour, which are thwarted due to limited space (Carlstead, 1998; Clubb and Mason, 2007). The inability to perform goal-directed behaviours, such as exploration, in such environments can elicit displacement activities or re-directed behaviours, from which stereotypic behaviours normally arise (Würbel et al., 1996; Würbel, 2006). For example, bar biting and jumping in mice may be considered as re-directed behaviours since the underlying motivation arises from the need to explore or escape from the cage (Würbel et al., 1996; Nevison et al., 1999).

My aim was to investigate whether wheel running acts as an environmental enrichment or is simply a re-directed behaviour in the African striped mouse, *Rhabdomys dilectus*. I exposed individuals displaying locomotor stereotypic behaviours to three enriched treatments, namely No Restriction, Restricted Home Tank, and Restricted Wheel Tank. These treatments were designed to test the importance of wheel running, by allowing access to different kinds of enrichment including a running wheel. My design allowed striped mice access to space for performing stereotypic behaviours and several enrichments including a running wheel. Non-stereotypic mice were used as a comparison. I made 3 predictions.

1) If in the No Restriction treatment, a) stereotypic behaviours are high in the home tank, low in the wheel tank and wheel running is also low, wheel running is an enrichment, since striped mice are still showing stereotypic behaviours and use the wheel as part of the cage furnishing (i.e. enrichment). However, b) if striped mice use the running wheel and decrease stereotypy, wheel running is re-directed stereotypy, as there is space to perform stereotypic behaviours.

2) If in the Restricted Home Tank treatment, a) stereotypic behaviours are low in the home tank, high in wheel tank and wheel running is low, wheel running is an enrichment, since stereotypic behaviours would be displaced to the wheel tank and wheel running becomes an enrichment activity. However, b) if striped mice use the running wheel, it is re-directed as stereotypic behaviours are re-directed to wheel running, despite available space around the wheel.

3) If in the Restricted Wheel Tank treatment, a) stereotypic behaviours are high in the home tank, low in the wheel tank and wheel running is low, wheel running is an enrichment, as stereotypic behaviours would be displaced to the home tank and wheel running would be an enrichment activity. However, b) if striped mice use the wheel, it would be a re-directed stereotypic behaviour, since due to restriction in space in the wheel tank, stereotypic behaviours would have to be displaced to wheel running.

4) Previous studies (Chapters 2-4) showed that personality did not influence behavioural responses to cages of different complexities, and I, therefore, expect that personality would not influence the behavioural responses to wheel running.

Materials and Methods

Striped mice used in this study were captive born F1 and F2 individuals, originating from a population in Pretoria (25º 40” S; 28º 30” E), South Africa. They were housed in the Milner Park Animal Unit, University of the Witwatersrand, under partially controlled environmental conditions: 14L: 10D light: dark cycle (lights on at 05h00); 22ºC-24ºC and 30-60% rH. Subjects were housed in clear Lab-o-tec™ cages (L × H × W: 300 mm × 200 mm × 150 mm). Wood shavings (± 2 cm) were provided as bedding and a handful of *Eragrostis* grass (± 20 g) and ± 5g of shredded paper towel were provided as nesting material. Individuals were also provided with a PVC nest-box (L × H × W: 100 mm × 100 mm × 150 mm). Epol® mouse cubes and water were available *ad libitum*. Approximately ±10 g of fresh fruit (apples, pears) or vegetables (lettuce, carrots, broccoli) and ± 5 g of mixed seed were provided daily per individual.

Stereotypic behaviours are genetically transmitted in striped mice (Jones et al., 2008), so to increase the chance of producing stereotypic and non-stereotypic individuals for study, eight stereotypic and nine non-stereotypic pairs (i.e. both male and female were either stereotypic or not; as described below) were established under standard laboratory conditions. The male was separated from the female prior to parturition. The young were separated from the mother at 22 days, housed singly in Lab-o-tec™ cages (described above). The offspring were used in experiments (Figure 1).

Observations of stereotypic behaviours

Observations were made twice daily on the young starting from day 22 after birth, for a total of half an hour, in order to establish the absence/presence of locomotor (e.g. circuit running) stereotypic behaviour. Stereotypic individuals were those that exhibited 10 or more bouts of stereotypy per observation session, each with three or more repetitions (for a detailed scoring method and justification thereof see Jones et al., 2008). Individuals that did not exhibit any stereotypic behaviour were categorised as non-stereotypic. Stereotypic behaviour is an ‘all or nothing’ occurrence in striped mice (i.e. an individual either displayed or did not display stereotypies). Only the absence or presence of stereotypic behaviours was recorded (see Jones et al., 2008); non-stereotypic mice never displayed stereotypic behaviours.

Once the three personality tests i.e. Light-dark/Startle and Novel-object tests were conducted (Refer to Chapter 2 for protocol), stereotypic (male, n = 12: female, n = 15) and non-stereotypic (male, n = 8: female, n = 9) striped mice were exposed individually to three treatments in a sequential order, i.e. No Restriction, Restricted Home Tank and Restricted Wheel Tank, for 15 days in each treatment. The experiments were designed to create various spatial restrictions for displaying locomotor stereotypical behaviours. In each treatment, animals were housed in a home tank (L × H × W: 460 mm × 300 mm × 320 mm high, Figure 1), attached with a PVC pipe (20 cm long and 5 cm in diameter) to a second tank of the same size, which contained a running wheel (15 cm in diameter). Food and water were provided only in the home tank.

Treatment 1. **No Restriction.** The home tank contained a nest box with nesting material as in the Lab-o-tec™ cage above, a deep layer of ± 2 cm wood shavings (± 40 g), Habitrail™ PVC tunnels and small cardboard tubes (± 50 mm diameter). The wheel tank contained a running wheel and wood shavings. These tanks provide space for a striped mouse to display stereotypic behaviours (Figure 1a).

Treatment 2. **Restricted Home Tank**. In this treatment, the home tank was partitioned vertically using a Perspex sheet (L × H × W: 350 mm × 300 mm × 5 mm), to reduce the space available to perform locomotor stereotypic behaviour in the home tank. Striped mice had access to space in the running wheel tank (Figure 1b).

Treatment 3. **Restricted Wheel Tank**. The wheel tank was partitioned using a Perspex sheet to reduce the space available to perform stereotypic behaviour in the wheel tank by allowing access only to the running wheel. There was no restriction in the home tank (Figure 1c).

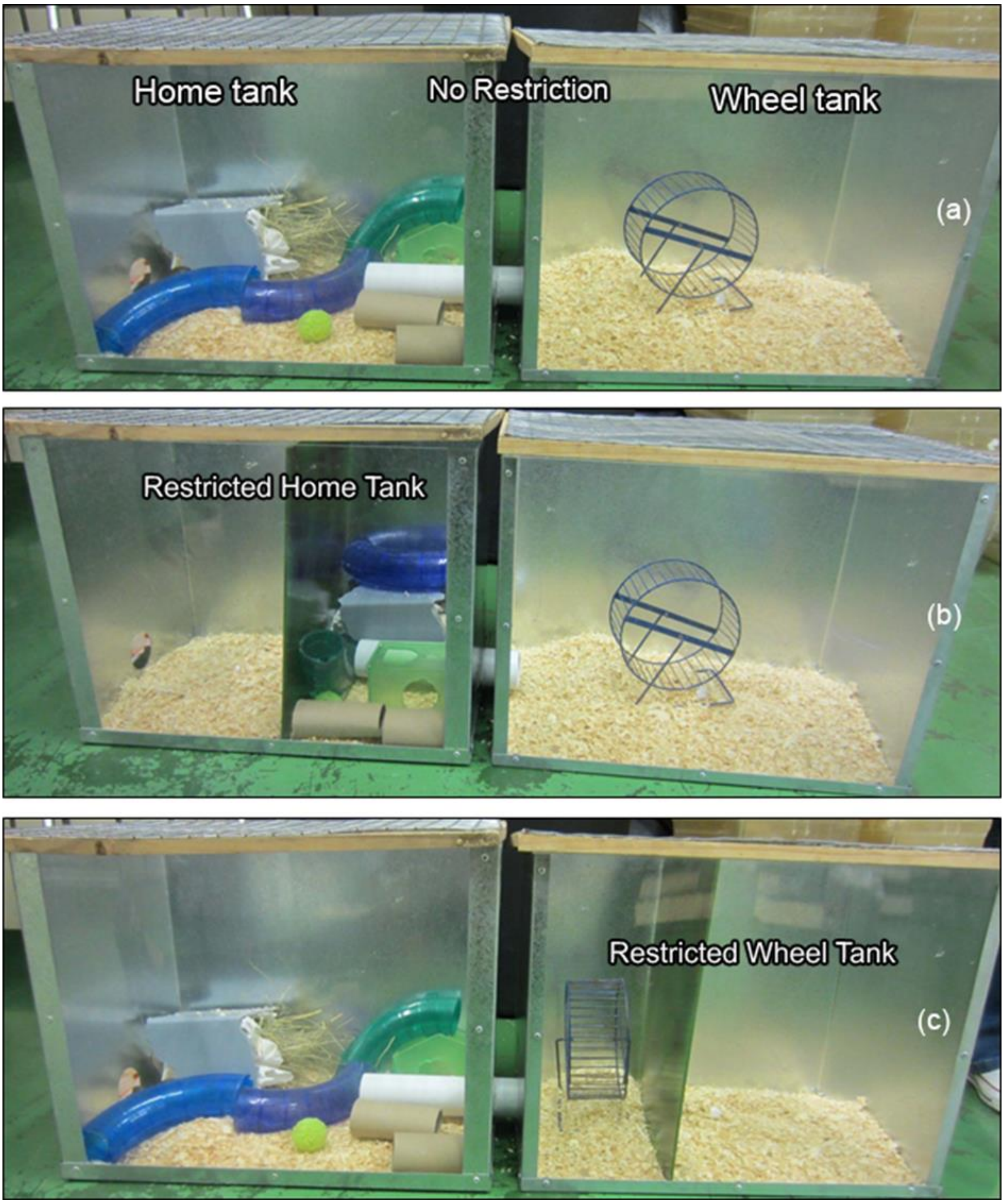


Figure 1. The experimental tanks used in the three treatments. The home tank was connected to a wheel tank by a PVC tube. The home tank contained ± 20 g wood shavings as bedding, a PVC tubing nest box, provisioned with ± 20g hay and ± 5 g paper towelling mixture as nesting material, 1-2 Habitrail™ tunnels, cardboard rolls and plastic balls. The wheel tank contained ± 20 g of wood shavings and a running wheel. (a) No Restriction treatment, (b) Restricted Home Tank and (c) Restricted Wheel Tank.

Following a 24 hour acclimatisation period to each treatment, the behaviour of individuals was recorded every second day (i.e. 8 days of recording) between 08h00-12h00, as striped mice are most active during these times (Pillay, 2000) and no human observers were present in the room during filming. Using Observer software (version 5.0; Noldus Information Technology), the frequency of five behaviours (Table 1) was scored using continuous sampling in both the home and wheel tanks. The duration was scored for four behaviours (Inactive, Active, Wheel running and stereotypic behaviour; Table 1), except object manipulation that occurred infrequently and of a short duration, and so was recorded as frequency only. Behaviours such as resting in nest box or out of sight were grouped under 'Inactive' as it was not possible to establish what the mice were doing in the nest box. Similarly, behaviours such as non-stereotypic movement or running on cage floor were grouped under 'Active' as they did not occur often. Every 15 days, once the experiments were completed, the tanks, PVC tunnels and all the contents were cleaned and the cardboard tubes replaced.

Table 1. Ethogram of Striped mice behaviours scored in the three treatments

Behaviour	Definition
Inactive	Individual motionless and resting in nest box or out of sight
Active	Non-stereotypic movement or running on the cage floor usually digging in the wood shavings
Stereotypic behaviour	A repetitive and invariant behaviour > 3 times in succession
Object manipulation	Manipulating enrichments (e.g. biting or nudging of cardboard tubes and wheels; only frequency)
Wheel running	Individual running inside or outside of the wheel

Data Analyses

The data were averaged for each behaviour for the 8 days in each treatment per individual and analysed using R (Ver. 2.13.0; R Development Core Team, 2011) and Statistica (version 7 Statsoft, USA). Data were checked for normality and homogeneity of variance using the Shapiro-Wilk and Levene's tests. All the statistical tests were two-tailed with statistical significance accepted at $p \leq 0.05$. Using the pwr.chisq.test function in the *pwr* package (Blomberg, 2014), a power analyses was used to assess effect size. To analyse whether there was a correlation between wheel running and stereotypic behaviours, a linear regression was run on the combined data of the stereotypic behaviours in the home and wheel tank.

Since non-stereotypic individuals obviously do not exhibit stereotypic behaviours and therefore cannot re-direct stereotypic behaviours, comparisons between stereotypic and non-stereotypic were made for their use of wheel running and only at the group level. At a group level, the data set was log transformed to approximate normality and the behaviours (activity, inactivity and wheel running) in the home and wheel tanks were compared using a general linear model (GLM) for multiple dependents. In the GLM, stereotypic status (stereotypic/non-stereotypic) was the independent factor, treatments (No Restriction, Restricted Home Tank, Restricted Wheel Tank) were the repeated measures and the behaviours were the multivariate dependents.

At an individual level, I focused on stereotypic striped mice only since the aim was to consider personality influences on the purpose of wheel running for stereotypic striped mice. The relationship between personality and the behaviours of individual stereotypic striped mice in the No Restriction, Restricted Home tank and Restricted Wheel tank treatments were analysed using a polynomial multivariate regression for multiple dependents. The frequency and the duration of behaviours were included as dependent variables. The startle response and novel object personality test scores were used as the continuous predictors, while the treatments (No space restriction, Restriction in Home tank and Restriction in Wheel tank) were categorical predictors. For all dependent variables, the homogeneity of slopes of the continuous predictors and their interaction were examined first to determine whether a single test (multivariate regression) or separate regressions were required. For both tests, the coefficient of determination, F- and P- values and parameter estimates for linear and polynomial decomposition are reported. The beta coefficient, obtained when all variables are standardized to a mean of 0 and a standard deviation of 1, were also examined. Polynomial variables are reported as linear and quadratic functions indicated with a “^{2}”.

Results

Results revealed a strong power of 0.73 indicating that the sample size of the stereotypic and non-stereotypic striped mice was adequate to test the aims of the study. The results of the GLM analysis indicated that stereotypy status was a significant predictor of behaviour in the three treatments ($F_{5, 118} = 5.722$, $P<0.001$). Post hoc tests revealed that non-stereotypic mice showed greater inactivity in the home tank, lower levels of activity in wheel tank and reduced wheel running than stereotypic mice (Figure 2). GLM results also showed that there was a significant influence of treatment on behaviour ($F_{10, 236} = 2.034$, $P= 0.031$).

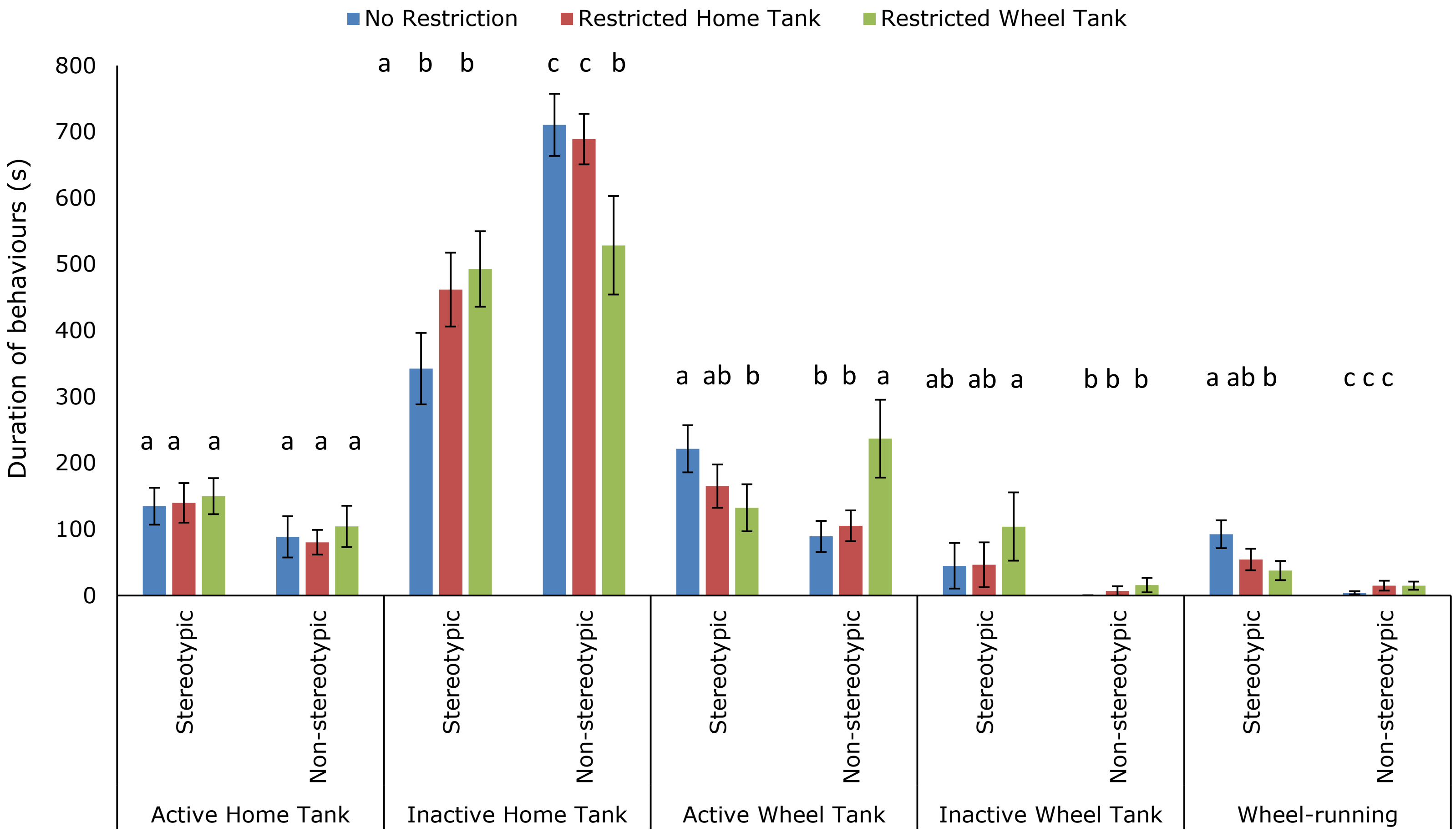


Figure 2. Mean (± SE) duration of activity, inactivity and wheel running behaviours in the Home and Wheel tanks displayed by stereotypic and non-stereotypic striped mice for three treatments (No Restriction, Restricted Home Tank, Restricted Wheel Tank). Letters above bars denote differences in particular behaviours between stereotypic and non-stereotypic mice ($p < 0.05$; post hoc tests).

Post hoc tests revealed that there were no differences in the levels of activity in the home tank of all three treatments between stereotypic and non-stereotypic mice (Figure 2). Post hoc tests further revealed that duration of inactivity was higher in non-stereotypic than stereotypic striped mice in the home tank of No Restriction and Restricted Home Tank treatments. There was no difference in inactivity in the Restricted Wheel Tank treatment. Activity was high in the wheel tank of the No Restriction and Restricted Home Tank in the stereotypic than non-stereotypic mice. However, activity was greater in non-stereotypic than stereotypic mice in the Restricted Wheel Tank treatment. Furthermore, post hoc tests indicated that inactivity was greater in the Restricted Wheel Tank treatment in stereotypic than non-stereotypic mice. There was no difference in inactivity in the No Restriction and Restricted Home Tank treatments (Figure 2). Wheel running was greater in stereotypic rather than non-stereotypic striped mice in all three treatments (Figure 2). Stereotypy status* Treatment ($F_{10, 236} = 1.565$, P=0.118) were not significant predictors of behaviour.

Linear regression analyses showed that stereotypic behaviours were weakly positively correlated with wheel running: with an increase in stereotypic behaviours, there was a slight increase in wheel running only in Restricted Home tank ($R^2 = 0.176$, $F_{1, 26} = 5.56$, p=0.026). There was no correlation between stereotypic behaviours and wheel running in the No Restriction ($R^2 = 0.053$, $F_{1, 25} = 1.42$, p=0.245) and Restricted Wheel tank ($R^2 = 0.002$, $F_{1, 22}$ =0.042, p=0.84) treatments.

Stereotypic mice

Behaviours in the Home and Wheel tanks

An examination of the beta coefficients allows an assessment of the relative contribution of each independent variable on the dependent variable (**Supplementary material**: S1). Although there were no significant associations between personality tests and the frequency of stereotypic behaviours in the home tank, there were 7 positive and 7 negative non-significant associations between the personality scores and frequency of behaviours. Frequency of stereotypic behaviour was high in 7 and low in 20 individuals in the home tank of No Restriction treatment. It reduced in 26 individuals in the Restricted Home Tank, increased in 7, and remained low in 20 individuals in Restricted Wheel Tank treatment (Figure 3a).

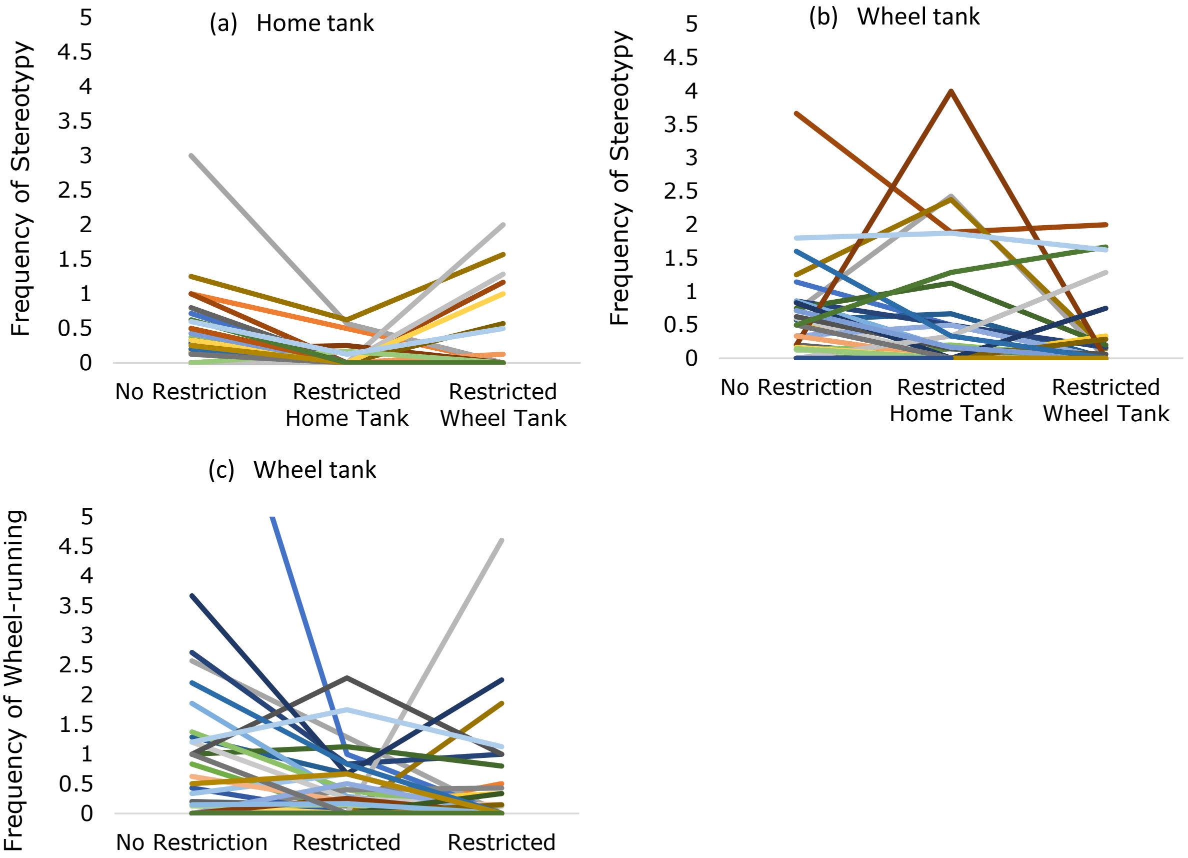
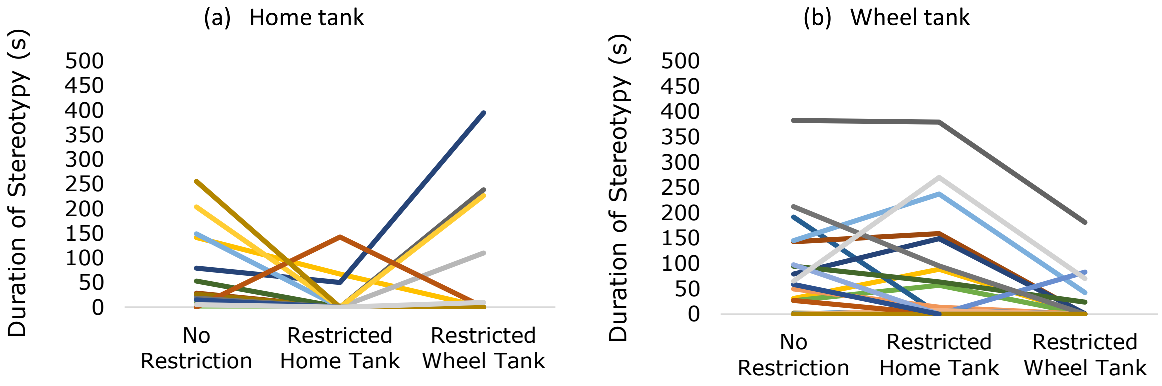


Figure 3. Changes in the frequency of stereotypic behaviour (a, b) and wheel running (c) in individual stereotypic striped mice in the home and wheel tanks in three treatments (No Restriction, Restricted Home Tank and Restricted Wheel Tank). The data for each individual for each treatment is connected by a different coloured line.

There was a positive association between time spent in the light compartment (i.e. in personality tests) and frequency of stereotypic behaviour. Figure 3b shows that in the wheel tank, the frequency of stereotypic behaviours increased with an increase in the time spent in the light compartment. Frequency of stereotypic behaviours were high in 9 individuals, low in 7 and remained at constant levels in 10 individuals from No Restriction to Restricted Home Tank treatments.

Wheel running frequency decreased in 16 individuals and increased in 11 from No Restriction to Restricted Home Tank treatment. It increased in 6 individuals and remained low in 21 individuals in the Restricted Wheel Tank treatment (Figure 3c). There were 9 negative and 10 positive non-significant associations between the personality scores and frequency of behaviours in the wheel tank.

There were no significant associations between the personality scores and duration of behaviours in the home tank. However, there were 6 positive and 6 negative non-significant associations between the personality scores and behaviours (**Supplementary material**: S2). Duration of stereotypic behaviours followed the same pattern as the frequency in the home tank, with stereotypic behaviours high in 11 individuals in the No Restriction treatment and low in 16. It increased in 5, decreased in 2 and was little in 20 from Restricted Home Tank to Restricted Wheel tank (Figure 4a).



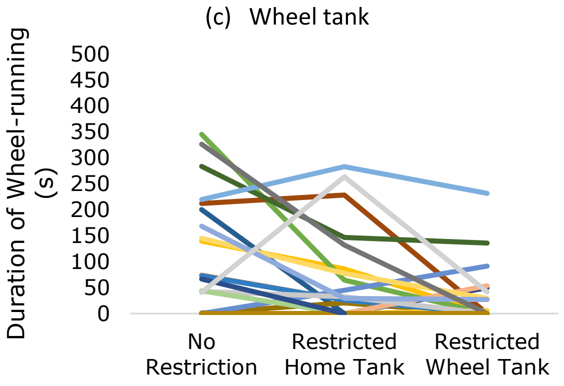
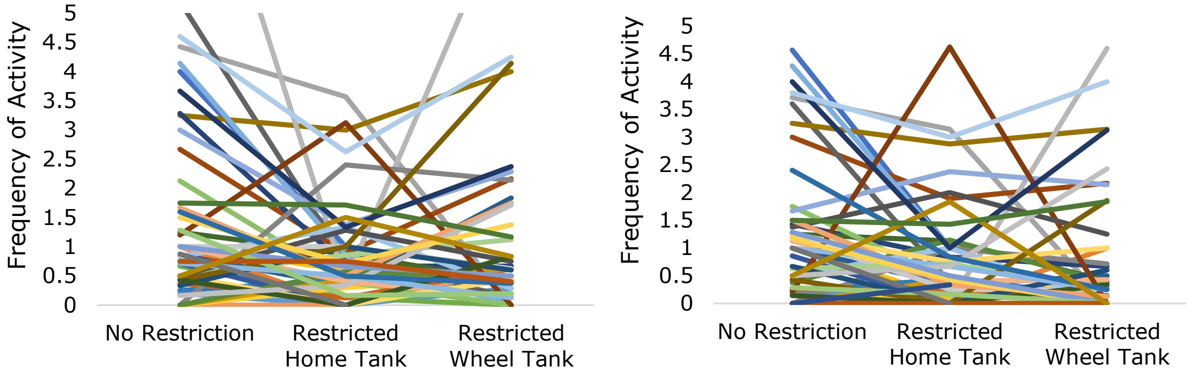


Figure 4. Changes in the duration of stereotypic behaviour (a, b) and wheel running (c) in individual stereotypic striped mice in home and wheel tanks in three treatments (No Restriction, Restricted Home Tank and Restricted Wheel Tank). The data for each individual for each treatment is connected by a different coloured line.

There was an association between time spent in the light compartment and duration of stereotypy: with an increase in the time spent in the light compartment, there was a linear increase in the duration of stereotypy but a non-random decrease in the wheel tank. Furthermore, there were associations between the latency to approach the novel object and stereotypic behaviours and wheel running. There was a linear decrease in stereotypy and wheel running but a non-random increase in these behaviours, with an increase in the latency to approach the novel object in the wheel tank. In support, duration of stereotypic behaviours varied in treatments: they were low in 17 individuals high in 10 in No Restriction treatment. It increased in 8 individuals and remained low in 19 in the Restricted Home Tank treatment. Furthermore, it decreased in 12 from Restricted Home tank to Restricted Wheel Tank (Figure 4b). The duration of wheel running varied among individuals in the three treatments, it decreased from No Restriction to Restricted Home Tank in 17 individuals and was constant in 10. It increased in 12 individuals and was low in 15 in the Restricted Home Tank treatment and decreased in Restricted Wheel Tank in 19, increased in 5 and was constant in 3 (Figure 4c).

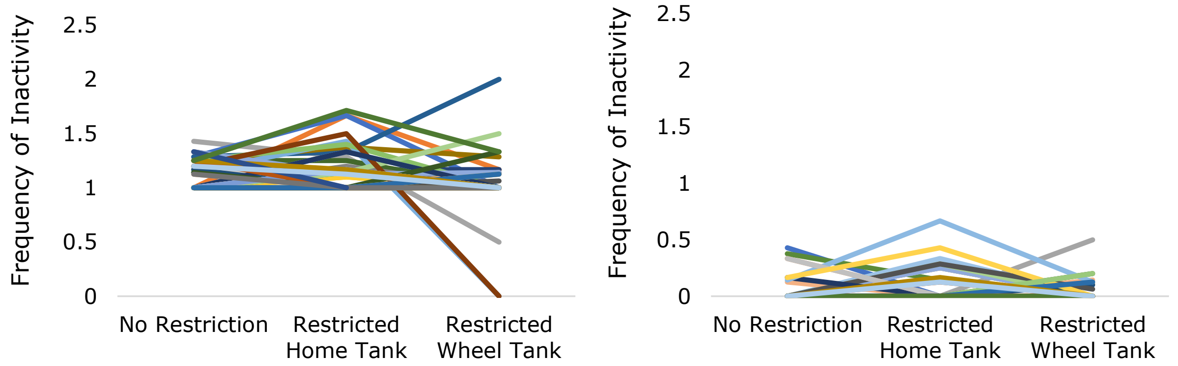
There was a positive association between the latency to approach the novel object and the frequency of activity in the home tank. Activity increased with an increase in the latency to approach the novel object in the home tank. In support, Figure 5a shows that activity decreased from No Restriction to Restricted Home Tank in 22 out of 27 individuals and increased in 5 individuals. Furthermore, 12 showed a decrease in frequency of activity in Restricted Wheel Tank treatment, while 15 showed an increase. In the wheel tank, frequency of activity was high in 15 individuals and low in 12 in No Restriction. It increased from Restricted Home Tank treatment to Restricted Wheel Tank treatment in 10 and decreased 12, while it was constant in 5 (Figure 5b).

The majority of individuals showed more or less similar trends of frequency of inactivity (Figure 5c) in the three treatments in the home tank, with an exception in 2 individuals showing a decrease from Restricted Home Tank to Restricted Wheel Tank and 1 showing an increase in the same treatment. In the wheel tank, frequency of inactivity decreased in Restricted Home tank in 3 individuals, increased in 4 and was more or less constant in the remainder of the individuals (Figure 5d).



(a) Home tank

(b) Wheel tank



(c) Home tank

(d) Wheel tank

Figure 5. Changes in the frequency of activity (a, b) and inactivity (c, d) in individual stereotypic striped mice in home and wheel tanks in three treatments (No Restriction, Restricted Home Tank and Restricted Wheel Tank). The data for each individual for each treatment is connected by a different coloured line.

There were no significant associations between the personality tests and the duration of behaviours in the home tank. However, there were 6 positive and 6 negative non-significant associations. Figure 6a shows that duration of activity decreased in 13 individuals and increased in 11 individuals from No Restriction to Restricted Home Tank, and was low in the remaining 3 individuals. It increased from Restricted Home Tank to Restricted Wheel Tank in 12 individuals and was low in 15. In the wheel tank, the duration of activity decreased from No Restriction to Restricted Home Tank in 17 individuals, increased in 8 individuals, and remained low in 2. It decreased from Restricted Home Tank to Restricted Wheel Tank in 21 individuals and increased in 6 individuals (Figure 6b).

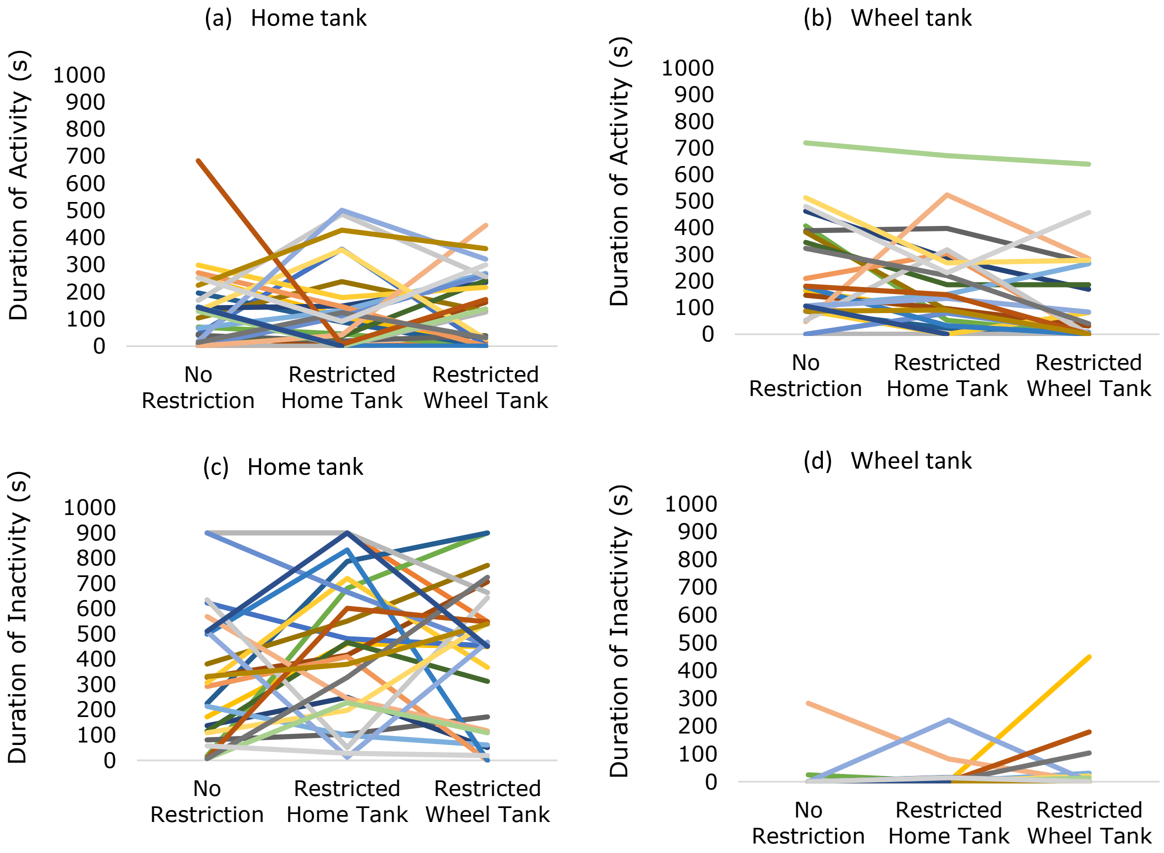


Figure 6. Changes in the duration of activity (a, b) and inactivity (c, d) in individual stereotypic striped mice in the home and wheel tanks in three treatments (No Restriction, Restricted Home Tank and Restricted Wheel Tank). The data for each individual for each treatment is connected by a different coloured line.

The duration of inactivity increased from No Restriction to Restricted Home Tank in 17 individuals, decreased in 8 and remained low in 2. It increased from Restricted Home Tank to Restricted Wheel Tank in 11 individuals and decreased in 16 individuals in the home tank (Figure 6c). In the wheel tank, individuals showed similar trends in inactivity in that there was little inactivity with an exception of a few individuals (Figure 6d).

An examination of the individual responses in the treatments showed that there was a positive association between the latency to approach the novel object and the frequency of object manipulation in the home tank. Object manipulation increased with an increase in the latency to approach the novel object in the home tank. Figure 7a illustrates that 15 individuals showed a decrease in the frequency of object manipulation from No Restriction to Restricted

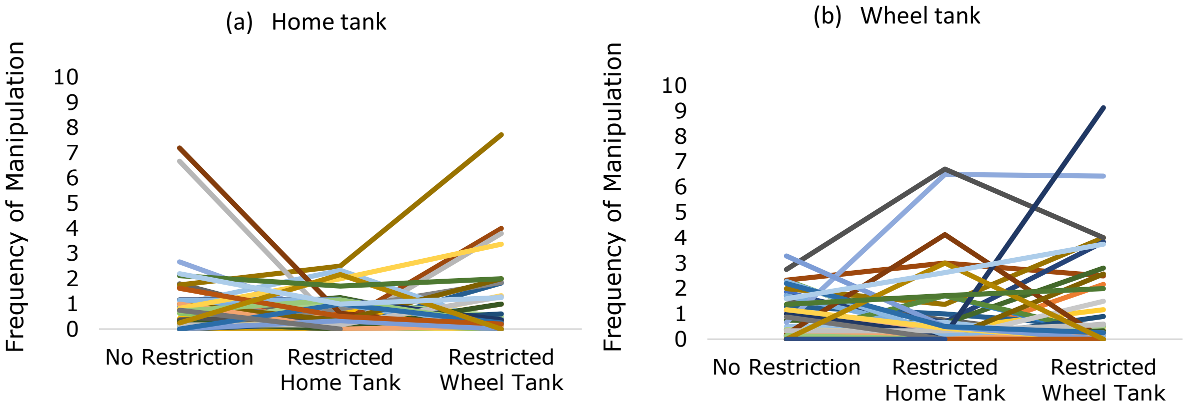


Figure 7. Changes in the frequency of object manipulation (a, b) in individual stereotypic striped mice in the home and wheel tanks in three treatments (No Restriction, Restricted Home Tank and Restricted Wheel Tank). The data for each individual for each treatment is connected by a different coloured line.

Home Tank, while 10 showed an increase in the same treatments. Seventeen of these individuals showed a decrease in object manipulation in the Restricted Wheel Tank, while 8 showed an increase in the home tank. Figure 7b shows that, in the wheel tank, frequency of object manipulation decreased from No Restriction to Restricted Home Tank in 18 individuals, increased in 6 and remained low in 3 individuals. It increased from Restricted Home Tank to Restricted Wheel Tank in 9 individuals and remained low in 18.

Interpretation of stereotypic behaviours and wheel running in three treatments in stereotypic mice

As shown in Table 2, 5 individuals consistently showed greater wheel running than stereotypic behaviours in all treatments implying re-directed behaviour. Two individuals showed higher levels of stereotypic behaviours than wheel running, meaning that wheel running was an enrichment. Six mice showed greater wheel running than stereotypic behaviours in the No Restriction and Restricted Home Tank treatments and inactivity in the Restricted Wheel Tank treatment. One individual showed increased wheel running in the Restricted Home Tank and Restricted Wheel Tank treatments. In addition, 9 individuals showed random patterns of behaviours: 4 of these showed increased stereotypic behaviours rather than wheel running in the No Restriction treatment, 2 showed increased stereotypic behaviours in Restriction in Wheel Tank treatment, 2 showed increased wheel running in the No restriction and one showed higher wheel running in the Restriction in Wheel Tank treatment.

Table 2. Interpretation of wheel running activity by individual stereotypic striped mice in the No Restriction, Restricted Home Tank and Restricted Wheel Tank treatments. A summary of the behavioural responses is provided to show whether stereotypic behaviour (SB) was greater (SB>WR) or less than (WR>SB) wheel running (WR) or when individuals were inactive or active. Individuals in bold black showed wheel running as a re-directed behaviour in two or all three treatments, individuals in bold dark blue indicate wheel running was enrichment in two or all three treatments. Individuals in light grey indicate random behavioural patterns. Dashes indicate that no interpretation could be made due to no stereotypic behaviours or wheel running in a particular treatment. Personality scores for each individual are provided.

Individual	No Restriction	Interpretation of WR and prediction conformed to	Restricted Home Tank	Interpretation of WR and prediction conformed to	Restricted Wheel Tank	Interpretation of WR and prediction conformed to	Duration in light compartment (s)	Latency to approach novel object (s)
1	WR>SB	RE-DIRECTED (1b)	WR>SB	RE-DIRECTED (2b)	WR>SB	RE-DIRECTED (3b)	2.33	381.7
2	WR>SB	RE-DIRECTED (1b)	WR>SB	RE-DIRECTED (2b)	WR>SB	RE-DIRECTED (3b)	17.01	31.9
3	WR>SB	RE-DIRECTED (1b)	WR>SB	RE-DIRECTED (2b)	WR>SB	RE-DIRECTED (3b)	0.00	49.57
4	WR>SB	RE-DIRECTED (1b)	WR>SB	RE-DIRECTED (2b)	WR>SB	RE-DIRECTED (3b)	0.00	81.75
5	WR>SB	RE-DIRECTED (1b)	WR>SB	RE-DIRECTED (2b)	WR>SB	RE-DIRECTED (3b)	141.11	19.43
6	WR>SB	RE-DIRECTED (1b)	WR>SB	RE-DIRECTED (2b)	INACTIVITY	-	187.41	0.00
7	WR>SB	RE-DIRECTED (1b)	WR>SB	RE-DIRECTED (2b)	INACTIVITY	-	40.87	0.00
8	WR>SB	RE-DIRECTED (1b)	WR>SB	RE-DIRECTED (2b)	INACTIVITY	-	216.65	0.00
9	INACTIVITY	-	WR>SB	RE-DIRECTED (2b)	WR>SB	RE-DIRECTED (3b)	139.25	0.00
10	WR>SB	RE-DIRECTED (1b)	WR>SB	RE-DIRECTED (2b)	INACTIVITY	-	0.00	332.87
11	WR>SB	RE-DIRECTED (1b)	WR>SB	RE-DIRECTED (2b)	INACTIVITY	-	52.91	180.11
12	INACTIVITY	-	WR>SB	RE-DIRECTED (2b)	WR>SB	RE-DIRECTED (3b)	0.00	155.29
13	WR>SB	RE-DIRECTED (1b)	WR>SB	RE-DIRECTED (2b)	INACTIVITY	-	54.78	12.86
14	WR>SB	RE-DIRECTED (1b)	WR>SB	RE-DIRECTED (2b)	INACTIVITY	-	108.13	23.61

15	**SB>WR**	**ENRICHMENT (1a)**	**SB>WR**	**ENRICHMENT (2a)**	**SB>WR**	**ENRICHMENT (3a)**	**60.61**	**0.00**
16	**SB>WR**	**ENRICHMENT (1a)**	**SB>WR**	**ENRICHMENT (2a)**	**SB>WR**	**ENRICHMENT (3a)**	**106.11**	**118.47**
17	**SB>WR**	**ENRICHMENT (1a)**	**SB>WR**	**ENRICHMENT (2a)**	**INACTIVITY**	**-**	**147.1**	**36.35**
18	**SB>WR**	**ENRICHMENT (1a)**	**INACTIVITY**	**-**	**SB>WR**	**ENRICHMENT (3a)**	**179.69**	**169.78**
19	INACTIVITY	-	INACTIVITY	-	WR>SB	RE-DIRECTED (3b)	19.97	0.00
20	SB>WR	ENRICHMENT (1a)	INACTIVITY	-	INACTIVITY	-	110.35	150.36
21	WR>SB	RE-DIRECTED (1b)	INACTIVITY	-	INACTIVITY	-	107.09	0.00
22	SB>WR	ENRICHMENT (1a)	WR>SB	RE-DIRECTED (2b)	INACTIVITY	-	60.53	63.55
23	INACTIVITY	-	INACTIVITY	-	SB>WR	ENRICHMENT (3a)	0.00	131.69
24	WR>SB	RE-DIRECTED (1b)	INACTIVITY	-	INACTIVITY	-	207.26	0.00
25	WR>SB	RE-DIRECTED (1b)	ACTIVITY	-	ACTIVITY	-	0.00	0.00
26	SB>WR	ENRICHMENT (1a)	ACTIVITY	-	INACTIVITY	-	105.21	29.58
27	WR>SB	RE-DIRECTED (1b)	ACTIVITY	-	INACTIVITY	-	104.98	127.17

Discussion

My aim was to establish whether wheel running was an enrichment or a re-directed stereotypic behaviour in striped mice. I provided striped mice displaying locomotor stereotypic behaviours with a choice of space to perform stereotypic behaviours and access to a running wheel. My predictions were partially supported since 52% of the stereotypic striped mice showed increased wheel running and the remaining 48% showed a decrease. Specifically, I found that 5 individuals conformed to predictions 1a, 1b and 1c (i.e. re-directed behaviour), 7 individuals conformed to predictions 1b and 2b and 1 conformed to predictions 2b and 3b (i.e. re-directed behaviour in 2 treatments). Furthermore, 2 individuals matched with predictions 1a, 2a and 3a (implying enrichment), 1 corresponded with prediction 1a and 2a, 1 corresponded with 1a and 3a and 1 corresponded to 2a and 3a, suggesting that wheel running was enrichment in 2 treatments. Nine individuals showed random behavioural patterns in the different treatments; some individuals showed an increase in inactivity and the others showed an increase in activity and object manipulation, which may imply that wheel running is enrichment as they were not diverting their entire time to wheel running but rather apportioned their time between various behaviours. As expected, individuals showed variation in the use of the running wheel: while some increased wheel running in all three treatments, others decreased wheel use, irrespective of their personality type. However, due to differences in individual responses in the use of the running wheel, it is not possible to conclude whether wheel running was an enrichment or a re-directed behaviour in stereotypic striped mice as will be discussed below.

While at first glance it appears that the reduction in stereotypic behaviours is due to wheel running acting as an environmental enrichment, it is not the case. Despite available space to perform stereotypic behaviours in the wheel tank of the Restricted Home Tank treatment, more than half of these stereotypic striped mice showed higher levels of wheel running, implying that stereotypic striped mice were incorporating the wheel in their stereotypic behavioural routine. Similarly, Hansen and Damgaard (2009) showed that mink, *Mustela vison*, which displayed pacing (i.e. a locomotor stereotypic behaviour) also, re-directed these stereotypical behaviours to wheel running, which subsequently increased the intensity of wheel running. It has been suggested that wheel running itself is a stereotypic behaviour (Kunhen, 2002). This means that reduction in stereotypic behaviours observed in my study could be interpreted as stereotypic behaviours being re-directed to wheel running. Latham and Würbel (2006) maintained that even though wheel running occurs in both stereotypic and non-stereotypic forms, there is sufficient evidence to claim that wheel running be considered a stereotypic behaviour.

However, Sherwin (1998a) argued that although wheel running is similar to stereotypic behaviour in that they are both invariant and repetitive without any obvious purpose (Mason, 1991a), these behaviours vary in their ontogeny. Whereas stereotypic behaviours develop gradually over time as a consequence of impoverished conditions lacking in motor and sensory stimulation, wheel running usually occurs spontaneously within a few minutes of the wheel being introduced and in diverse environments (Sherwin, 1998a). Nevertheless, one cannot simply use an umbrella approach and quantify wheel running as a re-directed stereotypic behaviour. Just as 52% of the individual striped mice re-directed their stereotypical behaviours to the running wheel, the other 48% of individuals seemed to show that wheel running was an enrichment and reduced stereotypic behaviours. The latter findings are consistent with several other studies (e.g. transgenic mouse model; Richter et al., 2008), CD-1 (ICR) mice; Howerton et al., 2008, mink, *Mustela vison*; Hansen and Damgaard, 2009) that showed that provision of wheel running significantly attenuated the levels of stereotypic behaviour. There are exceptions, however, such as deer mice, *Peromyscus maniculatus* (Pawlowicz et al., 2010). Nonetheless, it seems unlikely in my study that the running wheel is solely responsible for the attenuating effects. In fact, wheel running together with cage size and cage complexity could have contributed to the reduction of stereotypic behaviours. For example, despite restriction in the wheel tank or restriction in the home tank, striped mice never entirely diverted their time towards wheel running. Instead, activity and object manipulation increased, suggesting that wheel running may have rather increased general activity. Therefore, this combined effect of cage size and cage complexity in terms of extra enrichment in the home tank could have contributed to the decrease in the performance of stereotypic behaviours, as also reported in outbred ICR strain laboratory mice (Würbel et al., 1998) and bank voles, *Clethrionomys glareolus* (Ödberg, 1987). Furthermore, wheel running may have some reinforcing qualities, which the striped mice lack under captive conditions. For example, wheel running might have been associated with a reduction in stereotypic behaviours because it lowered the motivation of the striped mice to escape from the cage by providing them with an opportunity to cover relatively large distances in a small space. The striped mice used in my study cover large home ranges in natural grassland habitats (mean: 1,109 m^2: Schradin and Pillay, 2005), indicating a need to explore large areas. Clubb and Mason (2003) found that the distance travelled by carnivores in the wild was correlated to the distance travelled on the wheel in captivity, which shows that there is a common motivation behind the performance of both behaviours.

Stereotypic behaviours were positively correlated with wheel running when home tank space was restricted, implying that wheel running may not be a stereotypic behaviour but rather an enrichment or an activity in its own right. For example, Richter et al. (2008) found a negative relationship between stereotypic behaviours and wheel running in transgenic mice and suggested that wheel running may be a substitute for stereotypic behaviours. However, it is important to note that our studies differed in two important ways. First, the transgenic mice were housed in standard laboratory conditions with a running wheel that restricted the space available, whereas the striped mice were housed under enriched conditions with a separate tank for wheel running. Second, the transgenic mice exhibited a variety of stereotypical behaviours, both locomotor (circuit running/route tracing) and oral (bar-biting), while I used striped mice that exhibited exclusively locomotor stereotypic behaviours; variations in the type of stereotypic behaviour could explain how the study subjects used the running wheel. Locomotor stereotypic behaviours are usually affected by environmental variables such as the housing conditions (Bashaw et al., 2001) and by placing a running wheel in an already constrained space in the case of the transgenic mice (Richter et al., 2008), the available space required for stereotypic behaviours is reduced, which could have led to excessive use of the running wheel, either because it was a re-directed stereotypy or stress due to restricted space. In my study, a wheel was available in a separate tank when space in the home tank was reduced (Treatment 2).

The positive relationship between stereotypic behaviours and wheel running could also be interpreted as a re-directed behaviour. From personal observations, stereotypic behaviours in the striped mice were always performed in association with wheel running. For instance, the majority of individuals ran in the wheel, which was followed by circuit running, and then re-entered the wheel while it was moving and continued with the same motion over and again. Similarly, Sherwin (1998b) referenced the findings of De Kock and Rohn (1971) who showed that bank voles, *Clethrionomys glareolus,* performed some behaviours in association with wheel running, such as leaving the wheel, running a ‘figure-of-eight’ and re-entering the wheel while it was still revolving.

Nevertheless, stereotypic striped mice did not show correlations between stereotypic behaviours and wheel running in the No Restriction and Restricted Wheel Tank treatments. Instead, while wheel running decreased, object manipulation and activity increased. Such differences could be attributed to individual differences underlying the motivation of wheel running. For example, the underlying mechanism of wheel running activity appears to have a genetic constitution (Koteja et al., 1999; Lightfoot et al., 2004; de Visser et al., 2007). Neurobiologically, the mechanisms underpinning the motivation for wheel running can be explained in terms of upregulation of Fos gene expressed in mice bred for high voluntary running, particularly the lateral hypothalamus, medial frontal cortex as well as the striatum (Rhodes et al., 2003). Similar to intake of addictive drugs, wheel running seems to activate the dopamine-opiod system (Werme et al., 2000; Werme et al., 2003; Clark et al., 2014). It has been proposed that dopamine may explain the motivation to wheel run for its hedonic rewards (Knab and Lightfoot, 2010). Dopamine is high stereotypic animals (Garner, 2006; McBride and Hemmings, 2009) and wheel running may be escalating the levels of dopamine (Rhodes et al., 2003) in a positive feed-back loop. This might also be a reason for stereotypic striped mice showing more wheel running than non-stereotypic mice.

As I showed in my previous studies (Chapters 2, 3 and 4), behavioural responses to the different treatments were independent of the individual’s personality type. Individual stereotypic striped mice that spent a long time in the light compartment (i.e. more bold) varied the levels of stereotypic behaviours in the treatments: while it increased in some treatments, it decreased in the others. Furthermore, individuals that showed an increased latency to approach the novel object (i.e. less bold) also showed variable levels of stereotypic behaviours as well as wheel running, depending on the treatment. This shows that bold and less bold stereotypic mice assess the situation differently before displaying a behaviour, resulting in individuals responding differently from one situation to the other (Chapman et al., 2010; Bell and Stamps, 2004). My findings contrast those of Walker and Mason (2012), who showed that boldness, predicted enrichment use in female C57BC/6 mice. In particular, they showed that mice that were bold used more of the enrichment than those that were more fearful of novelty. However, the discrepancies in results could be attributed to the experimental protocol as all enrichments in that study were provisioned in a larger cage connected to a smaller laboratory cage, which the striped mice may have found aversive.

Conclusions

While wheel running may not reduce stereotypic behaviours on its own, it appears that individual striped mice use the wheel for different purposes. Half the individuals used it to incorporate their stereotypic behavioural routine, making it a re-directed behaviour, while the others used it as enrichment. These results have implications for animal welfare as it shows that enrichment cannot be implemented for a particular stereotypic behaviour at a group level but rather must be tailored for particular individuals.

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Supplementary material

Table S1. Regression analysis of the frequency of stereotypic behaviour, activity, inactivity, object manipulation in Home and Wheel tanks and running wheel in only the Wheel tank with latency to approach the novel object (novel object test) and time spent in the light compartment (startle test) as the predictor variables for stereotypic mice in the No space restriction, Restriction in Home tank and Restriction in Wheel treatments. Linear and polynomial decomposition are reported. Values bold are significant.

HOME							
Personality test	**Behaviour**	**Parameter Estimates**	**P**	**β**	**R²**	**F**	***df***
Time spent in light compartment	Stereotypy	0.005	0.052	0.69	0.19	2.32	6,65
	Active	0.008	0.332	0.32	0.29		
	Inactive	0.000	0.835	-0.08	0.06		
	Manipulate	0.008	0.276	0.40	0.12		
Latency to approach novel object	Stereotypy	0.003	0.086	0.54	0.19	2.10	6,65
	Active	**0.011**	**0.036**	**0.62**	**0.29**		
	Inactive	-0.001	0.236	-0.40	0.06		
	Manipulate	**0.009**	**0.030**	**0.71**	**0.12**		
Time spent in light compartment ^{2}	Stereotypy	0.000	0.210	-0.43	0.19	2.56	6,65
	Active	0.000	0.696	0.13	0.29		
	Inactive	0.000	0.768	0.11	0.06		
	Manipulate	0.000	0.665	-0.15	0.12		
Latency to approach novel object ^{2}	Stereotypy	0.000	0.147	-0.45	0.19	1.71	6,65
	Active	0.000	0.173	-0.40	0.29		
	Inactive	0.000	0.270	0.37	0.06		
	Manipulate	0.000	0.094	-0.55	0.12		
WHEEL							
Time spent in light compartment	**Stereotypy**	**0.008**	**0.036**	**0.77**	**0.15**	1.63	6,65
	Wheel running	-0.004	0.528	-0.22	0.20		
	Active	0.004	0.574	0.20	0.16		
	Inactive	-0.001	0.161	-0.54	0.04		
	Manipulate	0.006	0.260	0.42	0.10		
Latency to approach novel object	Stereotypy	-0.004	0.109	-0.51	0.15	2.13	6,65
	Wheel running	-0.002	0.587	-0.17	0.20		
	Active	0.001	0.886	0.05	0.16		
	Inactive	0.000	0.495	0.23	0.04		
	Manipulate	-0.005	0.119	-0.51	0.10		
Time spent in light compartment ^{2}	Stereotypy	0.000	0.052	-0.69	0.15	1.94	6,65
	Wheel running	0.000	0.106	0.56	0.20		
	Active	0.000	0.774	0.10	0.16		
	Inactive	0.000	0.181	0.50	0.04		
	Manipulate	0.000	0.265	-0.40	0.10		
Latency to approach novel object ^{2}	Stereotypy	0.000	0.146	0.46	0.15	2.26	6,65
	Wheel running	0.000	0.342	0.29	0.20		
	Active	0.000	0.935	-0.03	0.16		
	Inactive	0.000	0.432	-0.27	0.04		
	Manipulate	0.000	0.064	0.61	0.10		

Table S2. Regression analysis of the duration of stereotypic behaviour, activity and inactivity in Home and Wheel tanks and running wheel in only the Wheel tank with latency to approach the novel object (novel object test) and time spent in the light compartment (startle test) as the predictor variables for stereotypic mice in the No space restriction, Restriction in Home tank and Restriction in Wheel treatments. Linear and polynomial decomposition are reported. Values bold are significant.

HOME							
Personality test	**Behaviour**	**Parameter Estimates**	**P**	**β**	**R^2**	**F**	***df***
Time spent in light compartment	Stereotypy	0.673	0.104	0.61	0.13	1.36	3,66
	Active	-0.262	0.758	-0.12	0.02		
	Inactive	1.043	0.503	0.25	0.11		
Latency to approach novel object	Stereotypy	0.425	0.076	0.60	0.13	4.47	3,66
	Active	0.436	0.376	0.32	0.02		
	Inactive	1.545	0.088	0.59	0.11		
Time spent in light compartment ^{2}	Stereotypy	-0.003	0.239	-0.43	0.13	0.62	3,66
	Active	0.002	0.635	0.18	0.02		
	Inactive	-0.002	0.761	-0.11	0.11		
Latency to approach novel object ^{2}	Stereotypy	-0.001	0.144	-0.50	0.13	2.66	3,66
	Active	-0.001	0.528	-0.23	0.02		
	Inactive	-0.003	0.198	-0.44	0.11		
WHEEL							
Time spent in light compartment	**Stereotypy**	**0.934**	**0.036**	**0.74**	**0.25**	3.14	4,66
	Wheel running	-0.348	0.486	-0.25	0.20		
	Active	-1.766	0.078	-0.66	0.13		
	Inactive	-0.307	0.452	-0.29	0.04		
Latency to approach novel object	**Stereotypy**	**-0.808**	**0.002**	**-1.01**	**0.25**	4.17	4,66
	Wheel running	**-0.942**	**0.002**	**-1.07**	**0.20**		
	Active	-0.872	0.129	-0.52	0.13		
	Inactive	0.240	0.308	0.36	0.04		
Time spent in light compartment ^{2}	**Stereotypy**	**-0.006**	**0.014**	**-0.84**	**0.25**	2.87	4,66
	Wheel running	0.001	0.643	0.16	0.20		
	Active	0.006	0.231	0.43	0.13		
	Inactive	0.001	0.526	0.24	0.04		
Latency to approach novel object ^{2}	**Stereotypy**	**0.002**	**0.009**	**0.85**	**0.25**	3.26	4,66
	Wheel-running	**0.003**	**0.003**	**1.01**	**0.20**		
	Active	0.002	0.366	0.31	0.13		
	Inactive	-0.001	0.235	-0.42	0.04		

CHAPTER SIX

General Discussion

Captive animals are often exposed to aversive and impoverished conditions, which lead to the development of stereotypic behaviours (Mason, 1991 a, b; Latham and Mason, 2004). Environmental enrichment is often implemented under such conditions with the aim to reduce or abolish stereotypic behaviours. However, they are not always successful in their intended purpose, raising concerns for animal welfare. In other instances, enrichment does not benefit all individuals of a species similarly, suggesting that individual differences (personality) may underlie such discrepancies (Dallaire et al., 2012; Walker and Mason, 2012). My aim was therefore to investigate how environmental enrichment influences the expression of stereotypic behaviour in the striped mouse, and to ascertain whether enrichment is influenced by personality in my study model, the African striped mouse, *Rhabdomys dilectus*. In this discussion, I review the main findings, compare and contrast results from my studies with other available literature on striped mice and other species, highlighting inconsistencies and gaps in the literature. Finally, I suggest areas for future research.

Key findings

Following the suggestion by Ijichi et al. (2013) that personality may have a role in the development of stereotypic behaviours, in Chapter 2, I investigated whether personality predisposed the development of stereotypic behaviours. I found that stereotypic mice were bolder than non-stereotypic mice and displayed a proactive coping style. However, having a proactive coping style did not predict the onset of stereotypic behaviours for all individuals (i.e. some less bold individuals also developed stereotypic behaviour).

Having shown an association between personality and stereotypic behaviours, I next explored whether personality modulated the way stereotypic mice interacted with enriched housing (Chapter 3). Stereotypic mice were bold and proactive as a group but showed flexible behavioural responses to the cages of different environmental complexity at an individual level. The rodent literature indicates that proactive individuals are rigid and show invariant behavioural responses compared to reactive individuals, which show flexible behavioural responses (Benus et al., 1987; Benus et al., 1991; Koolhaas et al., 1999; Sluyter et al., 1996). However, both stereotypic and non-stereotypic striped mice displayed flexible behavioural responses across different housing conditions, contradicting the coping style hypothesis proposed by Koolhaas et al. (1999).

Since behavioural responses were independent of the personality type of the striped mice (Chapter 3), I then investigated whether the age at which the striped mice were exposed to the environmental enrichment influenced their responses (Chapter 4). Age was considered because of its potential confounding influence when striped mice were randomly allocated to enrichments in Chapter 3. Age of striped mice was not associated with how stereotypic striped mice interacted with the enrichment, which was not surprising because it has been shown that enrichment provided has a positive effect in reducing the levels of stereotypic behaviours, regardless of the age at which it is implemented (Powell et al., 2000). It was also evident that, as in Chapters 2 and 3, behavioural responses to different cage complexities at an individual level were independent of the personality type. There was no effect of personality or age in non-stereotypic mice in response to different cage complexities.

In Chapter 3, enrichment treatments included a running wheel and I noticed that wheel running increased the overall activity of stereotypic mice in the Enriched treatment. There are still mixed views/perceptions as to what wheel running means to rodents. Some scientists have proposed that wheel running may show the desire of an animal to perform exploratory behaviours, stereotypic behaviours, or merely a form of general activity (Hansen and Damgaard, 2009). In Chapter 5, I focussed on this particular type of cage furnishing to ascertain whether wheel running was an enrichment or stereotypic/re-directed behaviour in stereotypic striped mice. The study design was quite unique in that I gave stereotypic mice an option of space to perform stereotypic behaviours and a wheel. The results showed that wheel running seemed to be both re-directed behaviour and enrichment since individual stereotypic mice used it for different purposes. As I showed in my previous experiments, at an individual level, behavioural responses to the different treatments were independent of the personality type of the stereotypic striped mice.

Implications of my findings

Previous work on *Rhabdomys* showed that stereotypic females have increased reproductive success (Jones et al., 2010a), and the early social environment influences the development of stereotypic behaviours later in adulthood (Jones et al., 2010b). Importantly for my study, stereotypy is genetically but not socially and environmentally determined (Schwaibold and Pillay, 2001; Jones et al., 2008). Like stereotypic behaviours (Schwaibold and Pillay, 2001; Jones et al., 2008; Hemmann et al., 2014), certain aspects of personality are also heritable (Dingemanse et al., 2002, Drent et al., 2003, Van Oers et al., 2004), but may also be shaped due to environmental conditions during the early phases of ontogeny (Benus and Henkelmann, 1998; Rödel and Meyer, 2011; Guenther et al., 2015). My study presents new information to the existing literature of *Rhabdomys* species on the following aspects: 1) personality and 2) the interaction between personality, environmental enrichment and stereotypy. I address these findings in detail, below, paying particular attention to group and individual level differences.

Group level

Stereotypic striped mice were bolder, showed a proactive coping style while non-stereotypic mice were less bold, and showed a reactive coping style (Figure 1a). These findings provide a group level support for the coping style theory that suggests that proactive copers are more bold while reactive copers are less bold (Koolhaas et al., 1999). Furthermore, along with boldness, stereotypic striped mice also showed greater activity and object manipulation in the enriched treatments. These findings are in line with a number of studies that show that bold animals exhibit increased interactions with novel environments or novel objects (Wechsler, 1995; Dingemanse et al., 2002; Janczak, et al., 2003; Dingemanse et al., 2007).

My findings show that stereotypic behaviours were reduced in the enriched treatments. This is similar to a large body of previous work in the environmental enrichment literature that increasing and enhancing cage complexities ameliorates performance of stereotypic behaviours and promotes more natural behaviours (Ödberg, 1987; Powell et al., 1999, 2000; Meehan et al., 2004; Mallapur et al., 2005). However, in order to understand the utility of environmental enrichments and its impact on stereotypic behaviours, it is important to understand the development of stereotypic behaviours and the underlying neurobiological mechanisms (Figure 1a). Two putative mechanisms exist. Firstly, the dorsal striatum is a part of the basal ganglia in the forebrain that facilitates the control of behaviours, transitioning and movements between behaviours as well as the maintenance of behavioural flexibility (Garner et al., 2003; Garner, 2006). Exposure to stressful environmental conditions influences the functioning of the basal ganglia, leading to the development of stereotypic behaviours. In particular, the neural pathways utilising the neurotransmitter dopamine within the dorsal striatum are key in the initiation and control of goal-directed behaviours and alteration of these structures lead to fixed routine-like behaviours as well as stereotypic behaviours (Garner et al., 2003; Garner, 2006; Lewis et al., 2006; McBride and Hemmings, 2009; McBride and Parker, 2015).

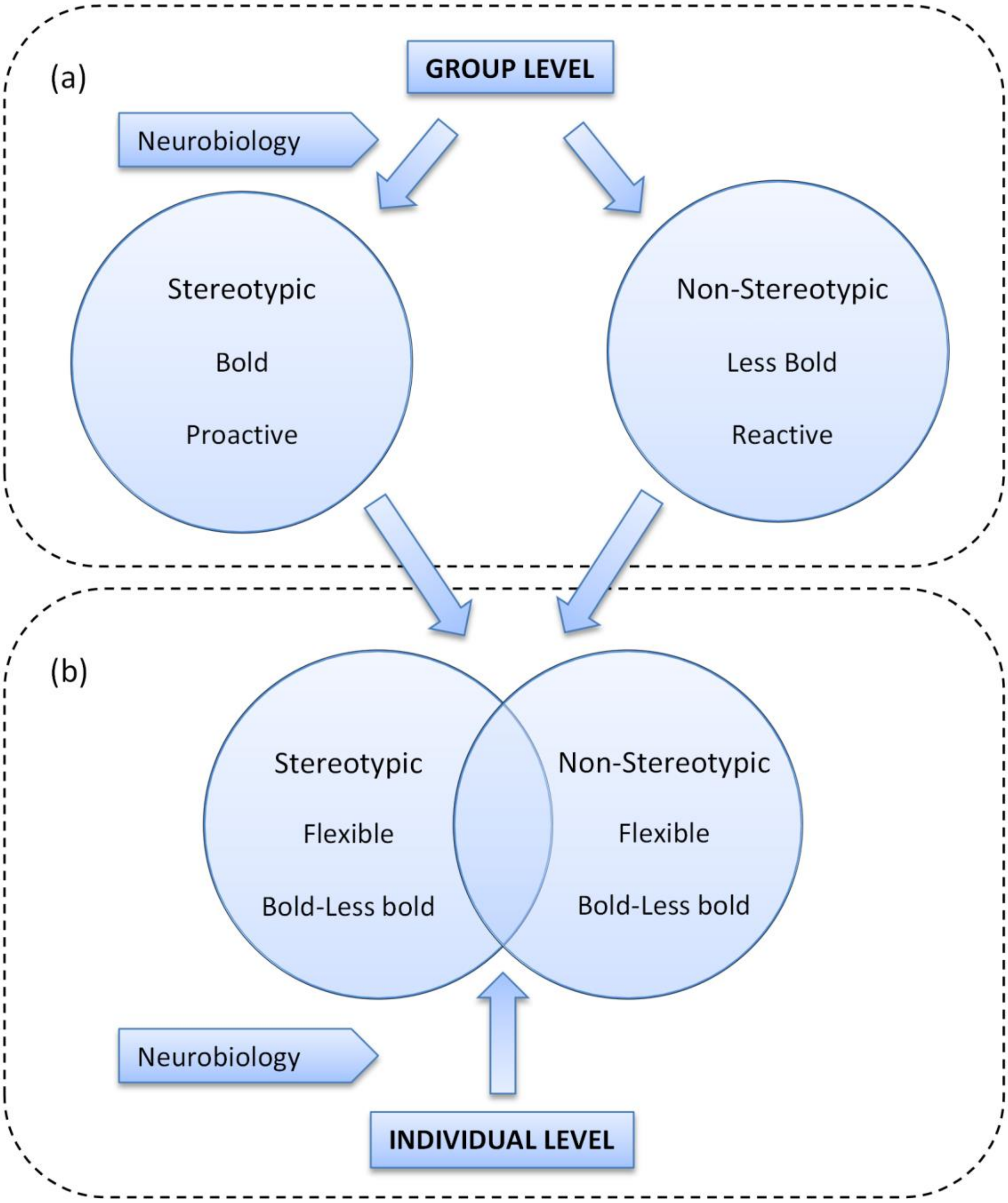

Figure 1. Venn diagram showing differences at (a) group level and similarity at (b) an individual level for stereotypic and non-stereotypic striped mice *Rhabdomys dilectus*. I hypothesise that neurobiological mechanisms underpin both group and individual level differences.

Exposure to environmental enrichment promotes behavioural, biochemical as well as structural changes, for example by increasing the number and density of neurons, synapses as well as dendritic arborisation (Van Praag et al., 2000); and increasing the Brain-Derived Neutrophic Factor in the dorsal striatum (Turner et al., 2003; Turner and Lewis, 2003).

Environmental enrichment also alters the serotonergic system (Rasmuson et al., 1998; Brenes et al., 2008) and glucocorticoid receptors which mediate the negative feedback loop on the Hypothalamic-Pituitary-Adrenal-axis (HPA axis), thereby enabling efficient coping responses to novel environments (Leggio et al., 2005; Brenes et al., 2008; Sampedro-Piquero et al., 2014).

Individual level

Despite the group level effects, an important question, which arose in my study, was why were stereotypic behaviours not always reduced in all stereotypic animals? This required an examination of individual level differences. This was achieved through multivariate regression examination of individual responses, which showed that irrespective of the personality type, behaviours were not consistent in the different treatments in stereotypic (Chapters 2, 3, 4 and 5) and non-stereotypic (Chapters 2 and 3) striped mice.

There was an overlap in personality between individual stereotypic and non-stereotypic striped mice (Figure 1b). Both individual stereotypic and non-stereotypic striped mice consistently showed flexible behavioural responses in different contexts or environments, yet individual differences (personality) were not correlated with these behavioural responses. This contradicts the personality literature, which emphasises the consistency of behaviours across contexts (Gosling, 2001). My study is partially comparable to the studies by Frost et al. (2007) and Thomson et al. (2012) on rainbow trout, *Oncorhynchus mykiss*, in which bold trout were more labile, while shy trout were relatively fixed in their responses. In fact, it appears that factors other than personality are responsible for individual flexibility. Several studies show that animals vary constantly in their behavioural response to environmental perturbations (Wilson, 1998; Koolhaas et al., 1999; Sih et al., 2004; Réale et al., 2007).

Personality traits are also often correlated, for example, individuals that are more active (i.e. faster explorers) are also more aggressive, less docile and bolder (Koolhaas et al., 2010; Réale et al., 2010). Faster explorers also use more unprotected, open areas of their environment compared to slower explorers (Koolhaas et al., 1999). However, my study showed that behavioural differences in personality are not fixed and may be dynamic depending on various extrinsic (e.g. cages of different complexities) and intrinsic (e.g. sex) factors, indicating that the coping style theory may not be true in all cases and coping is more flexible than described to date. Moreover, behaviours in the personality tests were uncoupled from behaviours in the different treatments for both stereotypic (Chapters 2, 3, 4 and 5) and non-stereotypic (Chapters 2 and 3) striped mice. Nonetheless, stereotypic animals by nature are characterised by having rigid and invariant behaviours (Latham and Mason, 2008) and therefore should have a low intra individual variability in behaviour (Japyassú and Malange, 2014). So the question that then arises is how were individual stereotypic mice able to alter their behavioural responses to cages of different complexity? Could there be some other neurobiological mechanisms that individual striped mice were using in response to different environmental complexities?

The neurobiological modulation of flexibility is not always apparent in the literature, as will become evident in the following paragraphs. One possible mechanism of flexible behavioural responses in striped mice could be related to the neurotransmitter serotonin. The Prefrontal Cortex (PFC) receives major serotonergic projections from the dorsal raphe nucleus, which regulate aggression (Blair, 2004; Siever, 2008) as well as impulsive behaviours in proactive copers (Dalley et al., 2008). Reactive copers have high levels of serotonin, which promotes behavioural flexibility. However, while serotonin has been implicated in behavioural flexibility (Koolhaas et al., 2010; Coppens et al., 2010), it would be conflicting for stereotypic mice to have higher levels of this neurotransmitter simultaneously with higher levels of dopamine, which mediate the development of stereotypic behaviours (Garner et al., 2006; Garner, 2006; McBride and Hemmings, 2009), since there is a reciprocal relationship between the serotonergic and dopaminergic systems (Daw et al., 2002). Yet, it has been suggested that the interaction between the serotonergic and dopaminergic systems may underpin impulsive aggression (reviewed in Seo et al., 2008), which is also an attribute of proactivity. For example, Van Erp and Miczek (2000) showed that during and after a confrontation, the levels of serotonin declined in the PFC of the Long-Evans rats to 80% of the baseline level, while dopamine levels peaked to 120% after the fights.

The ability of altering behavioural responses to different environments could also be attributed to the biphasic changes of dopamine in the nucleus accumbens in response to novel uncontrollable stressful conditions, thereby facilitating the adoption of either active (proactive) or passive (reactive) coping styles (Puglisi et al., 1991). Depending on the stressor, the levels of dopamine can switch from high to low. On exposure to a stressor, the levels of dopamine are high but if an animal is unable to overcome or cope with the stressor, dopamine levels drop below baseline, resulting in passive coping (Puglisi et al., 1991; Imperato et al., 1993; Pascucci et al., 2007; Cabib and Puglisi-Allegra, 2012; Sequeira-Cordero et al., 2013). The individual differences in response to enrichment also suggest that individuals have different thresholds of susceptibility that may determine when the dopamine responses take effect. Therefore, one should consider what the critical point is when striped mice switch from one coping style to the other. Understanding the underlying factors could possibly contribute to our understanding as to how striped mice in particular, or animals in general, cope with stressors in captivity.

While there is a vast array of studies on coping styles, ranging from fish (Frost et al., 2007; Silva et al., 2010; Basic et al., 2012), squid (Coleman and Wilson, 1998; Sinn and Moltschaniwskjy, 2005), pigs (Bolhuis et al., 2004) to birds (Jacobs et al., 2013), there are not many on rodents. Furthermore, there are no studies in the rodent literature showing behavioural flexibility in bolder rodents. One study, using the Short attack latency (SAL) and long attack latency (LAL), synonymous to proactive and reactive copers, showed that when shocked with an electric probe, LAL mice responded with immobility or active defence burying (Sluyter et al., 1996). While these two behavioural responses were apparent when exposed to a stressor in an unfamiliar environment, they were not present when exposed to the same stressor in a familiar environment (Sluyter et al., 1996). While my findings are not surprising because behavioural flexibility is well known in *Rhabdomys* spp. which show flexible social organisation (i.e. social flexibility; Schradin et al., 2010; Schradin et al., 2012), mating strategies (Schradin, 2008) and exploration (Rymer and Pillay, 2012), my hypotheses linking personality and stereotypy must be tested in a wider range of species to demonstrate the generalizability of my findings.

Broadly, my study highlights the importance of considering individual differences in environmental enrichment studies involving stereotypic animals. In particular, my study questions the validity of tailoring environmental enrichment at the species level. Various studies implement a kitchen-sink approach whereby everything and anything is provisioned (Rosenzweig and Bennett, 1969; reviewed in Swaisgood and Shepherdson, 2006). However, my study emphasises that due to individual differences in response to varying cage complexity, enrichment cannot be tailored for a particular target species per se. Before implementing enriching environments, and in addition to the aetiology of stereotypical behaviours, there is a need to account for the individual differences in responses, although I am mindful that such an approach can be onerous.

While there is no general consensus on the definition of animal welfare (Latham and Mason, 2004), scientists contend that the concept of welfare assessment entails a balance of both positive and negative indicators (Yeates and Main, 2008). Negative welfare include performances of abnormal or stereotypic behaviours and excessive aggression, whereas performance of species-specific behaviours is supposed to imply a positive welfare (Dawkins, 1998). My study shows that the same environmental enrichment cannot be provided for both stereotypic and non-stereotypic mice due to individual level differences. My study rather emphasises the importance of a differential implementation of environmental enrichment in stereotypic individuals, with individually tailored intervention. A case in point is the divergent responses to use of the running wheel in stereotypic striped mice, with more than half of the individuals tested showing a re-directed behaviour while the remainder showing wheel running as an enrichment (Chapter 5).

In my opinion, taking these results into consideration, performance of stereotypic behaviours does not necessarily mean that the animal’s welfare is compromised currently. I believe that stereotypic mice may actually be coping better than their non-stereotypic counterparts. More importantly, stereotypic behaviours should not be considered in isolation to determine an animal’s welfare, as factors such individual level differences (e.g. personality) are also crucial. Even in the absence of the eliciting environments triggering stereotypy, individual differences may determine the different thresholds of susceptibility to stress, which eventually affects how an individual responds to an environment.

Future studies and unexpected findings

The findings that not all stereotypic individuals respond to environments in the same manner suggests that future studies need to consider neurobiological mechanisms at an individual level in both stereotypic and non-stereotypic individuals. In addition to the serotonergic and dopaminergic systems levels that I mentioned earlier, a wider neurobiological investigation is required to include, for example, Corticotropic Releasing Factor (CRF), which also facilitate the use of active or passive coping styles by modulating the levels of serotonin in the dorsal nucleus raphe (Valentino et al., 2009; Sequiera-Cordero et al., 2013) in both striped mice and other species. Moreover, the interplay between different neurotransmitters and hormones underlying behavioural flexibility needs consideration. For example, Delville et al. (1996) showed how treatment with fluoxetine, a serotonin agonist, modulates the levels of aggression mediated by the hormone vasopressin. Like aggression, behavioural flexibility is also controlled by serotonin and it may be possible that other hormones interact with this neurotransmitter that ultimately underpins behavioural flexibility.

Veenema et al. (2004) showed that there are distinct differences in the high-aggression (proactive) and low-aggression (reactive) mice in terms of the HPA axis, which is typically associated with stress. When exposed to a stressor, low-aggression mice showed an increase in HPA response and high corticosterone levels compared to the high-aggression mice. In light of these findings, the authors concluded that low aggression seems to be indicative of a maladaptive coping style to stress. The relationship between stereotypic behaviours and corticosterone levels is untested in the *Rhabdomys* spp., although this relationship is not predictable in some other stereotypic animals (Latham and Mason, 2004). It would thus be worthwhile investigating whether the same neuroendocrinal mechanisms underlie the differences in stereotypic or non-stereotypic striped mice because of its welfare implications. For example, the greater inactivity levels in non-stereotypic mice may actually be hinting towards anxiety or depressive-like behaviours (Meagher et al., 2013; Meagher and Mason, 2012), which if true may imply that non-stereotypic mice are more affected by the suboptimal housing.

While the personality tests used in my study have been routinely performed on captive (Rymer et al., 2008; Jones et al., 2011) and free-living (Yuen et al., 2015) striped mice, there is an issue with the reliability and validity of personality tests. For example, only one personality test (i.e. the startle response test) was able to detect differences in personality in the striped mice but not the light-dark and novel-object tests. There has been some disquiet about the methodological approach of using personality tests, with Carter et al. (2013) emphasising the need for validating personality tests because personality studies usually involve measurements of different behavioural traits using multiple tests or one test measuring multiple traits. Furthermore, Weiss and Adams (2013) mention that the same kind of test may not be feasible across different species. Our lab is the process of addressing these concerns and already Yuen et al. (2015) showed strong correlation between personality traits in striped mice and that a single test can be a useful measure of personality in this taxon.

While the many aspects of an animal’s ontogeny shapes its personality e.g. litter composition (Benus and Henkelmann, 1998; Guenther and Trillmich, 2015), it would be worthwhile investigating how it also moulds behavioural flexibility across different ontogenic stages.

That age did not influence how striped mice interacted with environmental enrichment was surprising given its importance in an earlier study of striped mice (Jones et al., 2011). This finding could have been attributed to the differences in the experimental protocol across studies of striped mice or possibly due to small sample size.

In Chapter 3, my results revealed that stereotypic behaviours were unusually high in the Standard-enriched treatment rather than the Standard treatment. Provision of environmental enrichment usually reduced the occurrence of stereotypic behaviours if not alleviating these behaviours (Swaisgood and Shepherdson, 2006). One possible explanation I gave for this was that provision of environmental enrichment to the already restricted and congested standard laboratory housing (Mason, 1991 a, b; Abou-Ismail et al., 2010) may have frustrated and thus aggravated the performance of stereotypic behaviours. The question that then arises from this is that, is space also not an important factor in contributing to the mitigation of stereotypic behaviours? Furthermore, my findings contrasted those of Walker and Mason (2012) who showed that bolder mice exhibited greater enrichment use than those fearful of novelty (i.e. less bold). I suggested that this could have been due to the experimental protocol, as all the enrichments in their study were provided in a larger cage setting connected to a smaller cage. This finding further suggests that space combined with enrichment is critical and needs to be considered.

Conclusion

I studied the interaction between stereotypic behaviours, personality and environmental enrichment and assessed whether personality modulated the manner in which stereotypic and non-stereotypic animals used enrichment. This was a complex undertaking in which I designed experiments based on the theoretical models developed by Ijichi et al. (2013). My thesis contributes to science by providing the first empirical data to assess the relationship between personality and stereotypy and their combined association with environmental enrichment. My initial approach was to consider group level effects only but later it became apparent that individual level approaches are important because personality is an individual based trait, and it was surprising that other studies have not adopted this approach even though they are discussed (e.g. Dallaire et al., 2012; Walker and Mason, 2012). My study showed that group level differences might mask individual level differences. Indeed, at an individual level, stereotypic striped mice showed behavioural flexibility depending on the environment to which they were exposed, which in turn affected the efficacy of environmental enrichment. Finally, my study suggests that welfare and well-being of animals require an assessment of individual trajectories in the development of stereotypic behaviours.

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