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THE CAUSES AND CONSEQUENCES OF STEREOTYPIC BEHAVIOUR IN THE STRIPED MOUSE, *RHABDOMYS*

by

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Submitted in fulfilment

of the requirements for the degree of

DOCTOR OF PHILOSOPHY

in the **FACULTY OF SCIENCE**

at the **UNIVERSITY OF THE WITWATERSRAND**

Supervisors: Profs Neville Pillay and Georgia Mason

May 2012

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. It has not been submitted for any degree or examination at any other University.



……...

Megan Anne Jones

18 May 2012

ABSTRACT

Environmentally-induced stereotypic behaviour (SB) results from the chronic impact of captivity on brain development and function and, consequently, on behaviour. My study sought to determine group- and individual-level predictors and correlates of SB in the striped mouse, *Rhabdomys dilectus*, and to identify the mechanisms underlying the stereotypic phenotype. This aim was addressed by collecting cross-sectional and longitudinal behavioural and physiological data from a combination of wild-caught (WC) and captive-born (CB) striped mice born and reared in different social and environmental conditions. First, I examined the group-level effects of rearing conditions. Results confirmed the genetic contribution to SB performance in striped mice, and furthermore indicated that (1) striped mice weaned at or later than their natural weaning age are less likely to develop SB than striped mice weaned prematurely; (2) striped mice reared biparentally showed significantly less SB as adults than striped mice reared by their mothers alone; (3) striped mice raised from weaning in enriched conditions were four times less likely than standard-housed individuals to develop SB, an effect which endured after enriched-housed striped mice were transferred to standard housing; and (4) birth origin predicts the emergence of SB in striped mice, with those WC individuals trapped as adults being relatively protected from the development of SB compared with both WC individuals trapped as juveniles and CB striped mice. I also showed that (1) WC striped mice were more fearful and less active than CB animals, but that these traits did not covary with SB, and (2) WC striped mice were less perseverative and behaviourally more flexible than CB animals, traits that did covary with SB. Second, I characterized the developmental trajectory of SB in a large group of CB, standard-housed striped mice, and then investigated potential individual-level predictors, mediators, and correlates of SB in these animals. Measures of perseveration, activity, and anxiety/fearfulness assessed in juveniles before the development of SB did not predict which animals later developed SB, but stereotypic adults were more active and more perseverative than non-stereotypic individuals. Whilst preweaning developmental maturity did not predict which striped mice later developed SB, striped mice showing accelerated development in the preweaning period were likely to show SB at an earlier age, at a higher frequency, and potentially with reduced variability, effects which persisted into adulthood. In conclusion, my study shows that more naturalistic rearing environments reduce the incidence of SB, an effect mediated by genetic factors and possibly also by experience-dependent alterations in

forebrain function. However, further work is necessary to explore whether the association between forebrain function and SB expression in striped mice is causal because, whilst environments which decrease perseverative tendencies also reduce SB, I found no evidence that such tendencies predict which striped mice later develop SB.

DEDICATION

I dedicate this thesis to the memories of Xylo, JJ, and the many striped mice who started this journey with me, but are sadly not here to share in the joy of its completion.

ACKNOWLEDGEMENTS

Grateful thanks to my supervisors, Neville Pillay and Georgia Mason, who have guided my development as a scientist, a science writer, and as a person, and whose support, guidance, and encouragement have allowed me to realise this dream. Without your expertise in the fields of ethology, stereotypic behaviour, and human psychology (!), this thesis would not have been possible. Appreciation also to the many academic, administrative, and support staff members in the School of Animal, Plant and Environmental Sciences with whom I have interacted during my undergraduate and postgraduate studies, and to Mark Lewis and Carsten Schradin who kindly allowed me to visit their labs during the course of my PhD.

This journey could also not have been accomplished without my family, friends, peers, and four-legged companions. They have provided me with the requisite material and emotional support to sustain me throughout my long and meandering academic adventure. I am grateful to the many people who, since my childhood, have believed in me when I have doubted myself. These include my parents, my godmother Gwyn, Mrs Goldstein, Di van Zyl, Jackie Hull, Daneen Wade, Adi Miles, Warwick Allan, Frank Bergmann, Viki Vorster, Leonora, Italia, Cindy, Rosie, Anne-Marie Lydall, and Lindy Karpelowsky. My appreciation goes to Pete, and his amazing capacity to soothe and reassure me when I panic, and to tease me when I fall into despair ("What? You want to die? Isn't that overkill?"); to my many wonderful friends (Heath, Francesca, Heather, Angie, Kimmy, Sharon) and South African and Canadian academic siblings (Mathew, Tasmin, Luke, Sneha, Helen, Megan, Jenny, Tracy, Becky, Maria, Jamie, Laura) who provide me with love, laughter, companionship, and practical support; and to my animal family who daily remind me to delight in the present. A toast also to my parents, Alan and Lis, for giving me not just life, but a life in which I have always known that I am dearly loved, cherished, and valued: a doctorate is a family's, not an individual's, achievement. We have accomplished this together.

Finally, I wish to thank the multiple funders from whom I received generous financial support during my PhD: the National Research Foundation; the University of the Witwatersrand; the School of Animal, Plant and Environmental Sciences; the International Society for Applied Ethology; the Association for the Study of Animal Behaviour; the Humane Society of the United States; and the Universities Federation for Animal Welfare.

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CHAPTER ONE
General Introduction

STEREOTYPIC BEHAVIOUR

Stereotypic behaviour (SB), traditionally defined as behaviour that is repetitive, invariant, and without any obvious function (Mason 1991), is the most common form of abnormal behaviour in captive animals (Mason & Latham 2004). Worldwide, it is estimated that over 85 million laboratory and farm animals perform SBs (Mason & Latham 2004), as do approximately half a million captive wild animals held in zoos or conservation centres (Mason et al. 2007). Because of their undeniable association with impoverished and restricted rearing environments and housing conditions, and their phenomenological and aetiological similarity to the abnormal repetitive behaviours observed in human neuropsychiatric conditions, Mason (2006) suggests that SBs are caused by frustration, repeated attempts to cope, and/or environmentally-induced brain dysfunction. The diverse mechanisms putatively involved in the performance of environmentally-induced SBs contribute to the multiplicity of forms of SB observed within and among species, e.g. somersaulting, digging, and bar-biting in laboratory housed rodents (Würbel 2006), box-walking and cribbing in stabled horses (McBride & Hemmings 2009), pacing and weaving in captive carnivores (Clubb & Vickery 2006), and self-clasping, rocking, and digit-sucking in maternally-deprived primates (Novak et al. 2006).

Ethological and neuroscience explanatory models

Two study areas have contributed much to our understanding of SBs, viz., ethology and neuroscience (Latham & Mason 2010), although the field still awaits a truly comprehensive model of SB development (Rushen & Mason 2006; Lanovaz 2011) which integrates the actions of more distal (e.g. social and environmental) and proximal (e.g. genetic inheritance, individual-level differences in motivation, and behavioural proxies of brain function) causal and mediating factors. Ethological explanations of SB view it as a 'normal' behavioural response to an 'abnormal' environment. These models seek to understand the motivational basis for SB performance based on the confluence of internal (e.g. energy deficit) and external factors (e.g. restricted foraging opportunities) within the constraints of adaptive, species-typical behaviour patterns (Clubb & Vickery 2006). Captive environments, which are

generally lacking in space, physical complexity, and/or social stimulation, induce SB by repeatedly frustrating highly motivated appetitive or consummatory behaviours (Rushen 1993; Toates 2001), by preventing animals from meeting their needs (Hughes & Duncan 1988); and/or by providing little behavioural competition (Mason & Turner 1993). Evidence from a number of studies supports an ethological explanation of SB. For example, bar-biting in laboratory mice, *Mus musculus*, arises from repeated escape attempts (Nevison et al. 1999; Lewis & Hurst 2004); stereotypic digging in gerbils, *Meriones unguiculatus*, results from the need to access shelter (Wiedenmayer 1997); and, in captive carnivores, motivation to roam, quantified by species' home range size and daily travel distances, predicts frequency of pacing (Clubb and Mason 2003).

Ethological explanations of SB dominated early work on captive animals (e.g. Mason 1991; Lawrence & Rushen 1993). However, over the past decade, ethologists have increasingly drawn on explanatory models of SBs from the fields of neuroscience, clinical psychology, and psychiatry to inform their understanding of SB. There has been an attendant shift in focus from the more distal causes of SB, i.e. the types of environments which cause frustration, to the more proximal structural and functional brain changes which are directly responsible for generating the behaviours (Rushen & Mason 2006). Neuroscientists explain SBs in terms of forebrain dysfunction similar to that which underpins SB in human conditions such as schizophrenia, autism, and mental retardation. SBs and, more generally, Abnormal Repetitive Behaviours (ARBs; see Box 1) are hypothesised to arise secondarily to deficits in inhibitory control mechanisms in various corticostriatal circuits (Langen et al. 2011a). The primary function of these neural circuits, which relay information from various processing areas of the cortex to the basal ganglia and then back to behaviour-generating areas of the cortex via the thalamus (Graybiel 2008), is the selection and control of goal-directed motor, cognitive, and motivational behaviour (Langen et al. 2011b) and the maintenance of behavioural flexibility (Garner 2006). Deficits in inhibitory control mechanisms are believed to underpin both SBs (and other ARBs) and the phenomenon of perseveration on experimental tasks, viz., 'the continuation or recurrence of an … activity without the appropriate stimulus' (Sandson & Albert 1987, p. 1736). In humans, numerous studies have reported positive correlations between measures of perseveration and levels of repetitive behaviours in non-clinical as well as clinical populations: in a sample of normal adults, perseveration on a rule-changing task was positively associated with scores on an obsessive-compulsive inventory (Zohar et al. 1995); autistic children who performed more poorly on a two-choice guessing task, a measure

of recurrent perseveration, also showed higher rates of SBs (Turner 1997), as did patients with schizophrenia (Frith & Done 1983). Using measures adapted from human paradigms, an increasing number of studies of captive animals suggest that non-human SB is also associated with deficits in behavioural control generally and with difficulties in recurrent perseveration specifically. In various species, a relationship has been shown between individual levels of SB and recurrent perseveration (e.g. blue and marsh tits, *Parus caeruleus* and *P. palustris*, Garner et al. 2003; bears, *Ursus thibetanus* and *Helarctos malayanus*, Vickery & Mason 2003; deer mice, *Peromyscus maniculatus*, Tanimura et al. 2008; American mink, *Neovison vison*, Dallaire et al. 2011). Moreover, conditions or treatments that elicit SB such as deprivation-rearing (rhesus monkeys, *Macaca mulatta*, Gluck & Sackett 1974) or high dose amphetamine administration (laboratory rat, *Rattus rattus*, Evenden & Robbins 1983) have also been shown to induce recurrent perseveration in genetically manipulated animals such as DAT knockout mice; repetitive locomotion is associated with altered dopaminergic activity in the striatum and nucleus accumbens (Ralph et al. 2001); and, in deer mice, a series of experiments has shown correlations between individual levels of SB and regional activity of implicated forebrain areas, both of which covary with housing conditions (reviewed in Lewis et al. 2006).

Toward an integrated model of SB development and performance

In summary, the ethological and neuroscience models of SB focus on different levels of explanation for phenomenologically, and potentially also aetiologically, similar patterns of invariant and repetitive behaviours. They thus provide complementary, rather than mutually exclusive, accounts of SB development, and together contribute to our understanding of different aspects of the behaviour as it emerges in different forms, in different species, and in different contexts. These two models also provide strong theoretical support for the idea that SB represents a spectrum of responses, which ranges from the transient maladaptive responses of a normal animal to an abnormal environment to the symptoms of profound and permanent brain dysfunction (Mason 2006).

Currently, we lack an integrated theoretical model of SB development. Ethological, but not always neuroscience, explanations account well for certain features of SBs. These include: (1) the forms that SBs assume based on their emergence from ethologically relevant source behaviours (e.g. bar-biting in laboratory mice derives from their attempts to escape the cage; Nevison et al. 1999); (2) the repetitiveness of SBs which may arise from sustained elicitation

of behaviour by motivationally-salient internal or external stimuli and/or from potentially reinforcing consequences of performing SBs (Mason 2006); and (3) the unvarying nature of SBs which may develop secondarily to the repeated performance of a behaviour (e.g. via procedural learning, habit formation, or 'automation' of the behaviour c.f. 'central control'), or be determined by the unvarying captive environment (Mason 2006). Neuroscience explanations, however, account equally well or better for other aspects of SB performance, such as: (1) the persistence of SBs even after animals are removed from SB-eliciting environments (emancipation from original causes; e.g. Cooper et al. 1996); (2) the greater susceptibility of younger animals, whose brains are still developing, to environmental and social impoverishment (Novak et al. 2006), and the relative protection enjoyed by older animals which have been previously enriched (e.g. Lewis et al. 2006), or which were born in the wild and only brought into captivity as adults (Mason 2006a); (3) the within-bout repetitiveness of SBs and their association with high activity levels, which can both be accounted for by behavioural disinhibition (note, however, that the invariance of the behaviour over longer time scales, as well as the variability seen between-individuals and between-species, is poorly explained by deficits in behavioural disinhibition; Garner 2006); and (4) the association of SBs with situations which induce chronic stress as well as their exacerbation by acute stressors (Mason 1991), which links neatly with the known stress-sensitivity of the corticostriatal pathways (Cabib 2006) and their association with anxiety states (Judge et al. 2011).

Various recent experimental findings are also inadequately accounted for by either the ethological or the neuroscience explanations of SB. For example, whilst the early work of Garner and Mason (2002) in bank voles provides compelling evidence that a single process, consistent with suppression of indirect pathway activity, underpins all observed behavioural changes in stereotypic bank voles, subsequent work in other species, such as marsh tits and blue tits (Garner et al. 2003) and sun bears and Asiatic bears (Vickery & Mason 2004), indicates that at least one other, unknown, process is involved in the relationship between recurrent perseveration and SB. Also, more recently, findings from two studies in ICR mice (Latham & Mason 2010; Gross et al. 2011) and one in young (but not adult) mink (Dallaire et al. 2011) suggest that variation in recurrent perseveration might not always underpin variation in levels of SB. These results highlight some of the limitations of the neuroscience model, and suggest that whilst variation in recurrent perseveration explains most of the variation in SB performance in certain species and/or contexts, it may have a less important

role in other instances. In these cases, the variation in SB performance might better be accounted for by ethological explanations (e.g. motivational variables; Wiedenmayer 1997), or by altered functioning in neurocircuitry typically associated with different forms of ARBs such as the associative (Garner 2006) or limbic (Cabib 2006) loops (see Box 1).

Almost two decades ago, Ödberg (1993) highlighted the need for a comprehensive theoretical model of environmentally-induced SBs which integrates neuroscientific and ethological approaches. Although research over the past decade has advanced our understanding of the neuroscience of captive animal SB in particular, researchers have generally used different research approaches and techniques in different species. We thus do not yet have a good understanding of the motivational, developmental, and neurophysiological and neuroanatomical causes and correlates of any *single* species (Rushen & Mason 2006).

Why study stereotypic behaviour?

A better understanding of the mechanisms driving the development of SB is important for at least four reasons. First, understanding what goes 'wrong' can tell us much about the 'normal' motivation for and control of behaviour. Multiple studies now also indicate a continuity of functioning which can explain variation in normal behaviour as well as in pathology (Judge et al. 2011). A more complete and integrated model of SB development and performance may therefore also contribute to our understanding of individual and species differences in a range of associated domains such as learning, routine formation, and/or stress responsivity, and help us predict the capacity of species, and individuals within a species, to respond to a wide range of developmental disruptions or environmental changes.

Second, the performance of SB has important welfare implications. SBs have long been associated with conditions linked to poor welfare (Mason 1991b; Lawrence & Rushen 1993; Appleby 1999). Although there is a not a simple link between SB development and welfare, a substantial body of empirical evidence links environments which induce SB to compromised welfare although, within these environments, stereotypic animals may fare better than their non-stereotypic counterparts (Mason & Latham 2004). In instances where SBs arise from frustrated attempts to perform species-typical behaviours or from chronic stress, their association with poor welfare is more clear cut, and they are likely associated with aversive emotional states (Mason 2006). However, less obvious are the welfare correlates of SBs induced by brain dysfunction. If one accepts, for example, Duncan and Fraser's (1997)

definition of welfare as compromised physiological integrity, then dysfunction-induced SBs would, by definition, compromise welfare. However, the link between brain dysfunction and the subjective state of suffering (e.g. Duncan's (1993) definition of welfare – 'welfare is what animals feel' (p. 8)) – is hazy. Garner and Mason (2002) have, for example, suggested that the performance of SB in animals may be accompanied by an animal's frustration with the inability to control its behaviour, as has been reported in humans with various ARBs. In some cases, it can also be argued that the environments which induce SB also cause long-lasting changes in brain systems involved in stress reactivity, thus likely compromising welfare throughout the lifespan and independently of the current environment (Mason & Latham 2004). Nonetheless, in other instances, it is also possible that brain dysfunction is induced by factors unrelated to welfare (e.g. genetic manipulations; anoxia at birth), or that such dysfunction remains long after the welfare-compromising experience as a behavioural 'scar' (e.g. Mason 1991b). In these situations, animals might be dysfunctional and stereotypic but may not necessarily be experiencing current poor welfare. There is also compelling evidence that, in certain contexts and for certain individuals, the non-performance of SB highlights significant welfare concerns (e.g. extreme fearfulness; see Meagher 2011). An enhanced understanding of the developmental causes and consequences of SB performance (and non-performance) will assist our assessment of the welfare correlates of the behaviour.

Third, independent of welfare concerns, SBs are sometimes considered problematic for practical or aesthetic reasons, and effective strategies are thus sought to prevent their performance. In horses, for example, cribbing can lead to extensive wear of the incisors, result in loss of condition, and also predispose spasmodic colic (McBride & Hemmings 2009); in farm-housed mink, stereotypic animals grow more slowly and have smaller, less valuable pelts (de Jonge 1999); and, in many zoo animals, the performance of SBs can reduce the educational and conservational value of the exhibits (Swaisgood & Shepherdson 2006). Increased insight into the developmental origins of SBs will help in preventing their development and/or in diminishing their severity in ways which do not further compromise welfare, as might certain pharmacological 'treatments' which reduce SB performance but do not address the underlying causes of the behaviour (Mills & Luescher 2006).

Fourth, if the performance of SBs indicates underlying brain dysfunction, the validity of certain types of research using stereotypic animals is brought into question (Würbel 2001; Würbel & Garner 2007). Specifically, the validity and reliability of behavioural research that

uses tests which could show treatment interactions with perseveration, impulsivity, activity level, stress reactivity, or other behavioural effects of brain dysfunction, may well be compromised if subjects are stereotypic (Garner 2006), although, to my knowledge, this hypothesis has not been tested directly. Until we know the answer, however, it seems advisable to follow the 'good welfare is good science' approach to laboratory animal housing and husbandry (Garner 2006).

BOX 1. ABNORMAL REPETITIVE BEHAVIOURS

Although the focus of this thesis is on SB, it is useful to step back and consider the broader grouping of behaviours to which SB belongs, the Abnormal Repetitive Behaviours (ARBs), and to briefly review the neuroanatomical, neurophysiological, and neurocognitive research into ARBs that has informed our understanding of SB.

ARBs describe a wide range of behaviours observable across many non-human animal species, and in a range of human neurodevelopmental and neuropsychiatric disorders (Garner 2006; Garner et al. 2011). Whilst the performance of repetitive behaviour is normal in early development (e.g. in young children; Evans et al. 1997) and is an adaptive component of normal behaviour in many animals (e.g. fixed action patters, Mason 1991; repetitive but highly skilled actions acquired through practice, Toates 2001), some forms of repetitive behaviours may be contextually inappropriate or odd, cause impairment in functioning, and be performed at high frequency in invariant ways (Turner 1997; Garner 2006; Langen et al. 2011a). Repetitive behaviours of this subclass are considered abnormal, and hence termed ARBs. Examples of ARBs in captive animals other than SBs include barbering (fur and whisker plucking; Garner et al. 2011) and self-injurious behaviour (SIB; Novak et al. 2006).

Loosely, ARBs are conceptually grouped into two categories: (1) 'lower-order' repetitive motor actions (e.g. SBs, motor tics, repetitive manipulation of objects), and (2) 'higher-order' repetitive behaviours, which have a distinct cognitive component and are characterized by adherence to specific rules or orientation towards specific goals (e.g. compulsions, rituals, insistence on sameness, circumscribed interests) (Turner 1997). Although, historically, lower- and higher-order repetitive behaviours were considered distinct and separable phenomena (Langen et al. 2011), more recent work in humans and non-human animals indicates their phenomenological and

aetiological similarities (Garner 2006). According to Turner's (1997) behavioural disinhibition hypothesis, all ARBs represent the day-to-day expression of a deficit of behavioural control (i.e. failure to suppress out-of-context behavioural elements or to terminate behavioural elements; Garner et al. 2011) within the basal ganglia, striatal, and forebrain structures. These same deficits, in a diagnostic setting or on an experimental task, also cause perseveration, i.e. the inappropriate repetition of behaviour (Garner 2006). Because the corticostriatal loops are not unitary, but comprise multiple, parallel, segregated circuits which pass from various cortical targets to the striatum, through output nuclei in the basal ganglia, to the thalamus, and then from there back to a cortical area (Langen et al. 2011a, b), behavioural control deficits may arise from disruption of coordinated functioning within and between any of these pathways, and manifest as different forms of ARBs (Garner 2006; Garner et al. 2011; Figure 1). As summarised in Figure 1, different forms of ARBs are accordingly roughly associated with different forms of perseveration: (1) continuous perseveration, the inappropriate repetition of individual movement patterns (Garner 2006), results from dysfunction of the primary motor branch of the motor loop (Garner et al. 2011); (2) recurrent perseveration, the inappropriate repetition of responses or complex motor patterns (Garner 2006), manifests secondary to disruption of the sensorimotor loop (Langen et al. 2011a), and is specifically implicated in the performance of SBs; (3) stuck-in-set perseveration, the inappropriate repetition of goals or abstract rules (Garner 2006), arises from alerted functioning in the associative loop (Langen et al. 2011a); and (4) affective perseveration, the failure to inhibit emotionally motivated responses to reward cues (Hauser 1999), is associated with dysregulation of the limbic loop (Langen et al. 2011a). Each corticostriatal loop is further subdivided into two pathways, the inhibitory indirect pathway (which inhibits unwanted actions) and the excitatory direct pathway (which elicits desired behaviours). These pathways function in concert to fine-tune behavioural output. Reduced activity in the indirect pathway specifically is thought to be linked to ARBs (Lewis et al. 2006) via a failure to suppress unwanted behaviours.

Langen et al. (2011) provide a detailed review of the corticostriatal anatomy and the mediation of the various repetitive behaviours through dysregulation of direct versus indirect pathway activity, dorsal versus ventral striatum imbalances, and/or via an altered balance of activity between striatal striosomes and matrix.

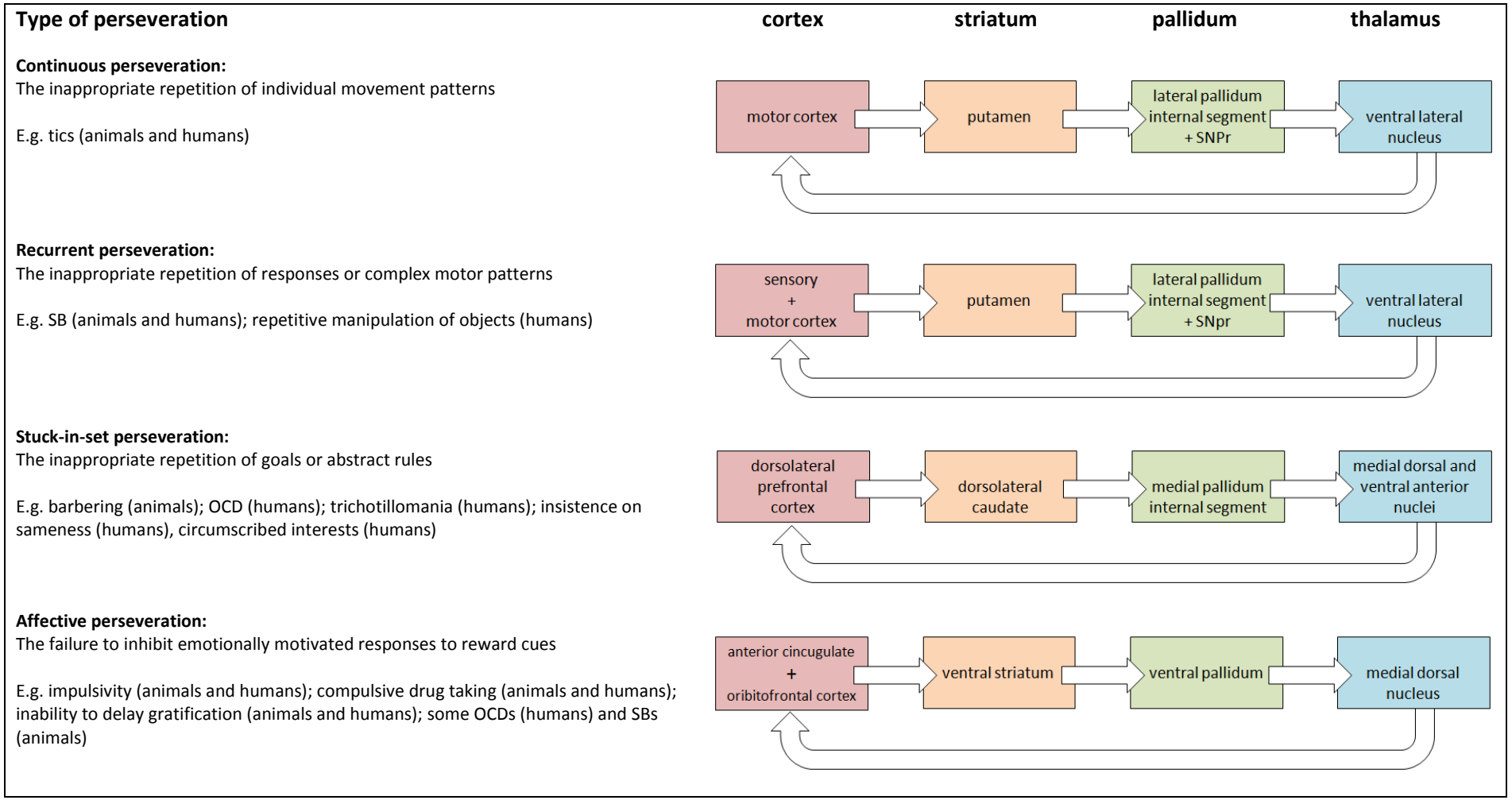

Figure 1. Definitions and examples of four forms of perseveration and the main input, relay, and output regions of the associated corticostriatal macrocircuits. The form of ARB shown in humans and/or animals is believed to be determined by the location of the damage to corticostriatal circuitry. SB = stereotypic behaviour; SNpr = substantia nigra pars reticulata; OCD = obsessive compulsive disorder. Adapted from Langen et al. 2011a, p. 348.

MOTIVATION FOR THE STUDY

The aims of this thesis were to determine group- and individual-level predictors and correlates of SB in a wild rodent species, the striped mouse, *Rhabdomys dilectus*, to identify the mechanisms through which these factors bring about the stereotypic phenotype, and thus, ultimately, to enhance our theoretical understanding of the ethological and neurobiological processes underpinning the development and performance of SB in captive wild animals. I addressed these aims by collecting cross-sectional and longitudinal behavioural and physiological data from a combination of wild-caught (WC) and captive-born (CB) striped mice which were born and reared in different social and environmental conditions, and then by testing three main hypotheses. First, building on previous work in striped mice (Schwaibold & Pillay 2001), I investigated the genetic contribution to individual variation in SB performance in CB striped mice (Chapter 2, Jones et al. 2008), and explored the relationship between SB and reproductive output (Chapter 3, Jones et al. 2010a). Second, I hypothesised that on a group level, more naturalistic rearing conditions would reduce the incidence of SB, and tested this by comparing the SB incidence of striped mice with differential rearing and developmental histories, viz., biparental v. uniparental care (Chapter 4, Jones et al. 2010b); premature v. natural weaning age v. delayed weaning (Chapter 4, Jones et al. 2010b); birth origin (Chapter 5, Jones et al. 2011a); and standard housing v. environmental enrichment (Chapter 6, Jones et al. 2011b). I also investigated correlates of the birth origin effect in order to begin exploring potential ethological and/or neurobiological mechanisms underpinning the diminished incidence of SB in WC striped mice (Chapter 5, Jones et al. 2011a). Third, based on the findings from the studies investigating group-level predictors and correlates of SB, I characterized the developmental trajectory of SB in a large group of CB, standard-housed striped mice, and then investigated potential individual-level predictors of SB in these animals to assess the relative contribution and interaction of ethological and neurobiological causal factors (Chapter 7, unpublished).

STUDY SPECIES

Population biology and socio-ecology

The striped mouse, genus *Rhabdomys*, is a small (40-50g) muroid rodent that is widespread and abundant in the southern African subregion (De Graaff 1981; Willan & Meester 1989; Pillay 2000a, b, c; Skinner & Chimimba 2005; Schradin & Pillay 2005; Ganem at al. in

press). It has a brown pelage with a lighter underbelly, and is characterized by four dark stripes on its dorsal surface running from head to tail (Skinner & Chimimba 2005). There is some regional (and potentially species and subspecies; see below) variation in morphology. Striped mice from the southwestern regions of southern Africa are slightly larger than those from the more northern regions, and those from the xeric western areas have a paler coat and longer tails than do striped mice from the mesic eastern regions, which also have a more yellow-brown colouration (Pillay 2000a, c). There is no distinct sexual dimorphism in the genus (Skinner & Chimimba 2005).

Unlike most rodents, the striped mouse exhibits a diurnal, bimodal activity pattern, with activity concentrated around mornings and evenings, and reduced during the midday period (Schumann et al. 2005; MacKay 2011). Its omnivorous diet, ability to survive without water provided its food has a minimum water content of 15% (Willan 1982), and extreme plasticity in habitat preference are likely reasons for its wide (if discontinuous; Brooks 1982) distribution throughout southern Africa (Rambau et al. 2003; Ganem at al. in press).

Striped mice are seasonal breeders, and are reproductively active from spring to autumn in moist eastern grasslands (Willan & Meester 1989) and in spring in arid western regions (Schradin & Pillay 2003) of South Africa. After a gestation period of 22-23 days, free-living females give birth to approximately five pups; captive females have slightly larger litters (e.g. 7.2 ±1.8; Pillay 2000). Pups begin to consume solid food at 10 days, leave the nest from 12 days, and weaning occurs around 16 days of age (Brooks 1982). Sexual maturity is reached between approximately five to six weeks (range 34-90 days; Brooks 1982). Females have an inter-litter interval of approximately 23-30 days (Pillay 2000).

Striped mice in the arid western regions of southern Africa have a flexible social organisation and mating system that appears to be shaped primarily by population density, and secondarily by resource (particularly food and cover) availability and thermoregulatory requirements (Scantlebury et al. 2006; Schradin et al. 2011). In arid habitats (Succulent karoo; Schradin & Pillay 2005a, Schradin et al. 2011), striped mice can be described as territorial, group-living, solitary foragers that display biparental care (Schradin & Pillay 2004). In mesic grassland habitats (e.g. Kwa-Zulu Natal Midlands; Wirminghaus & Perrin 1993; Pretoria Highveld; Brooks 1974; Zimbabwe; Choate 1972) and semi-succulent thorny scrub (e.g. Eastern Cape; Perrin 1980a, b) striped mice are solitary, with females rearing their litters on their own. Both

sexes maintain territories that overlap the territories of the opposite, but not the same, sex (Schradin & Pillay 2005). However, males from both mesic and xeric populations display parental care in captivity (Schradin & Pillay 2005), suggesting a plesiomorphic occurrence in the mesic populations, since the desert-living form represents the putative ancestral form (Rambau et al. 2003).

Taxonomy and phylogeny

Striped mice have two different karyotic forms (2n = 28 and 2n = 46; Taylor 2000). Based on this finding and on the analysis of mitochondrial DNA (Rambau et al. 2003), as well as evidence of divergent behavioural repertoires among populations (e.g. courtship behaviours; Pillay 2000b; Pillay et al. 2006), Rambau and colleagues (2003) suggested that *Rhabdomys,* previously considered a monospecific genus containing the single species *R. pumilio*, be reclassified as two species: *R. pumilio* (the social form that occurs in xeric habitats; 2n = 48), and *R. dilectus* (the solitary form, found in mesic areas, that comprises two subspecies *R. d. dilectus*, 2n = 46, and *R. d. chakae,* 2n = 48); this taxonomic grouping has been corroborated by studies of the mate recognition system of the taxon (Pillay et al. 2006) as well as by their different environmental niches (Ganem at al. in press). *R. dilectus* (subspecies unknown) from the Highveld grasslands are used in the studies presented here. To avoid cross-breeding of the subspecies, all breeding pairs were established between males and females originating from the same trapping site.

Suitability of striped mice as a study animal

Striped mice, like most captive rodents (Würbel 2006), exhibit a number of different SBs, with most stereotypic individuals displaying more than one form of SB (Schwaibold & Pillay 2001; van Lierop 2005). SBs emerge early in development, sometimes as early as weaning, and persist throughout the lifespan. Striped mice are suitable research subjects because, as with other small rodent species (Latham & Mason 2004), they have short generation times, breed successfully in captivity (Pillay 2000b), and are easy to house and handle. Additionally, they are non-endangered and, being diurnal, are easy to observe. Striped mice are moreover a suitable model for investigating the development of SB in captive wild animals for, whilst few wild-caught adult striped mice develop SB in captivity (Chapter 3; Mason 2006a), approximately 50% of captive-born individuals become stereotypic as a consequence of housing in standard laboratory cages, without drug challenge, and without a specific eliciting stimulus (e.g. Schwaibold & Pillay 2001; van Lierop 2005). This CB incidence of SB is also

comparable to that reported in a number of zoo species (e.g. brown bears, *Ursus arctos*, 48%; clouded leopards, *Neofelis nebulosa*, 49%), as is the proportion of time that stereotypic striped mice engage in SB (~50%; Nel 2003), which is similar to that engaged in by lions, *Leo Panthera* (48%), and spectacled bears, *Tremarctos ornatus* (52%) (reviewed in Clubb & Mason 2007).

FORMAT OF THE THESIS

This thesis comprises an introductory chapter (Chapter 1), six experimental chapters (Chapters 2-7), and a general discussion chapter (Chapter 8). Five of the six experimental chapters have already been published: the pdf versions of these publications are inserted into the thesis. Chapter 7, the only unpublished experimental chapter, is formatted similarly to the published chapters. Each of the eight chapters thus has its own reference list, with consequent repetition of references (and introduction and discussion material) among chapters. Tables and figures are numbered sequentially within each chapter and not for the thesis as a whole. The pages for the entire thesis, however, are numbered in sequence.

Note: In the multi-author work already published, I was the primary designer and executor of the experiments and was responsible for the data analysis, the initial write-up, and the preparation of the manuscripts for publication. My supervisors provided standard supervisory guidance throughout. All experimental work was approved by the University of the Witwatersrand's Animal Ethics Screening Committee: 2005/46/3; 2005/61/1; 2006/94/03.

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CHAPTER TWO

All a mother's fault? Transmission of stereotypy in striped mice *Rhabdomys*

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Applied Animal Behaviour Science, **115**, 82-89

Available online at www.sciencedirect.com
ScienceDirect
Applied Animal Behaviour Science 115 (2008) 82–89
APPLIED ANIMAL BEHAVIOUR SCIENCE
www.elsevier.com/locate/applanim

All a mother's fault? Transmission of stereotypy in striped mice *Rhabdomys*

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Accepted 28 May 2008
Available online 7 July 2008

Abstract

Environmentally induced stereotypy is the most common abnormal behaviour in captive animals. However, not all animals housed in identically impoverished environments develop stereotypy, possibly because of differences in genetic predisposition. To investigate the transmission of stereotypy in striped mice, *Rhabdomys*, we established four breeding treatments (non-stereotypic parents; stereotypic mother and non-stereotypic father; non-stereotypic mother and stereotypic father; stereotypic parents), and recorded offspring stereotypy prevalence. The prevalence of stereotypy was five times greater in the offspring of stereotypic than those of non-stereotypic females, regardless of whether the sire was stereotypic, and three times greater in offspring sired by stereotypic males paired with non-stereotypic females than in offspring from non-stereotypic parents. Our data show that stereotypy has a strong genetic component. However, the greater maternal than paternal contribution to stereotypy prevalence in offspring indicates that genetics alone cannot explain the observed transmission pattern. As previous work has excluded social influences on the development of stereotypy, we suggest that maternally mediated prenatal factors (e.g. gestational stress) might also predispose the stereotypic phenotype in striped mice.
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Keywords: Stereotypic behaviour; Genetics; Epigenetic factors; Prenatal stress

1. Introduction

Environmentally induced stereotypy, the most common abnormal repetitive behaviour in captive animals (Garner and Mason, 2002; Mason and Latham, 2004), results from the chronic

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0168-1591/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.applanim.2008.05.007

impact of captivity on brain development and function (Garner, 2007; Lewis et al., 2007; Mason, 2007). Stereotypies develop in sub-optimal captive environments which lack necessary stimuli for normal development, induce chronic stress, repeatedly expose animals to uncontrollable and unpredictable aversive situations, are barren or restrictive, and/or cause frustration by preventing an animal from meeting its basic needs (Mason, 1991a,b). Consequently, many laboratory, zoo, and farm species, whether domesticated or not, display stereotypic behaviour (see Mason and Latham, 2004, for prevalence data) but, when free-ranging or wild, show only semi-related behavioural topographies which lack the defining invariance and repetitiveness of stereotypy.

However, not all individuals that are housed in sub-optimal environments develop stereotypy (Mason and Latham, 2004). This indicates that factors other than the environment, such as genetic predisposition, contribute to the ontogeny of stereotypies. To date, studies investigating the transmission of stereotypy support the genetic predisposition hypothesis. For example, offspring of stereotypic bank voles *Clethrionomys glareolus* are more likely to be stereotypic than offspring from non-stereotypic parents (Ödberg, 1986; Schoenecker and Heller, 2000), and similar patterns have been observed in domestic horses *Equus caballus* (Kiley, 1977; Smith, 1984), mink *Mustela vison* (Jeppesen et al., 2004; Svendsen et al., 2007), and striped mice *Rhabdomys* (Schwaibold and Pillay, 2001).

As behaviour can be transmitted from parents to offspring via genetic and/or non-genetic means, such as social learning (Galef, 1996) and developmental factors (Bateson, 2003; Stamps, 2003), studies exploring vertical transmission of behaviour must tease apart genetic from epigenetic or learned responses. Previous research in *Rhabdomys* has used cross-fostering experiments to understand the mechanisms for stereotypy transmission from mothers to their offspring (Schwaibold and Pillay, 2001). The development of stereotypy was found to be strongly related to the stereotypy status of the biological mother, and it was thus concluded that stereotypies are genetically rather than socially transmitted (Schwaibold and Pillay, 2001). However, the contribution of the father to the development of stereotypies was not assessed.

In the present study, we assessed the genetic transmission of stereotypies from both the mother and the father in *Rhabdomys*, a small, diurnal muroid rodent that is widespread and abundant in southern Africa (De Graaff, 1981). At least 50% of captive-bred striped mice (Schwaibold and Pillay, 2001; Jones, unpublished data) develop at least one form of locomotor stereotypy (sensu Würbel, 2007), the most common of which are circuit-running, somersaulting, cage-climbing, jack-hammering, and windscreen wiping.

Four treatment groups were established, comprising breeding pairs of combinations of stereotypic (S) and non-stereotypic (NS) females and males. Based on the evidence of previous studies, and because the genetic contribution from each parent is expected to be equal and additive (e.g. Schoenecker and Heller, 2000), we predicted that litters with two stereotypic parents would show the highest prevalence of stereotypy, followed by litters with only one stereotypic parent, and litters from non-stereotypic parents having the lowest stereotypy prevalence.

2. Materials and methods

In order to obtain an appropriate sample size to test the aims of the study, our approach was to breed wild caught striped mice from a grassland locality (25°40′S; 28°30′E) in South Africa, identify and breed combinations of stereotypic and non-stereotypic F1 offspring, and test the transmission of stereotypy to F2 offspring. In captivity, striped mice were housed in standard lab-o-tec cages (300 mm × 200 mm × 150 mm). Cages contained ∼2 cm woodshavings as bedding and a handful of hay as nesting material.

Epol™ mouse cubes and water were provided *ad libitum*. We supplemented the diet twice weekly with a small amount (±5 g) of parrot seed mix.

F1 offspring were weaned at 20 days, marked with hair dye for individual identification (Schradin and Pillay, 2004), and housed in same-sex sibling groups. From 30 days of age, we video-recorded the behaviour of these individuals for 15 min twice weekly for 7 weeks, so as to identify which individuals were stereotypic, using the criteria provided in Tables 1 and 2; stereotypic individuals displayed at least 10 bouts of stereotypy in the observation session, each with three or more repetitions (Table 2). Recordings took place between 08:00 and 12:00 h when *Rhabdomys* is most active (Pillay, 2000). No human observers were present in the room during taping. Individuals which consistently displayed stereotypies (i.e. the behaviour was observed in ≥60% of the observation sessions) were regarded as stereotypic, whereas those that displayed no stereotypies were classed as non-stereotypic; we recorded the absence/presence rather than the duration of stereotypy, since stereotypic behaviour is an ''all or nothing'' occurrence in *Rhabdomys*, so that non-stereotypic individuals never displayed stereotypies in our study (see also Schwaibold and Pillay, 2001). In our experiments, stereotypic individuals always displayed locomotory stereotypy in ≥60% of observation sessions (i.e. stereotypic animals never performed stereotypies in less than 60% of observations sessions; equally, non-stereotypic individuals never displayed stereotypy).

When these F1 individuals reached sexual maturity (±100 days), a subset was selected and pseudo-randomly assigned to one of four breeding treatments, comprising 15 pairs each: (1) stereotypic female and stereotypic male (S♀–S♂); (2) stereotypic female and non-stereotypic male (S♀–NS♂); (3) non-stereotypic female and stereotypic male (NS♀–S♂); (4) non-stereotypic female and non-stereotypic male (NS♀–NS♂). Pairs were kept together for at least 100 days, and then separated only after the weaning of their final litter. Both the mother and the father were thus always present with the pups until weaning. Male *Rhabdomys*, sometimes in the field and always in captivity, contribute equally to parental care and display similar behaviours to the mother, with the exception of suckling behaviour (Schradin and Pillay, 2004).

For each treatment, we recorded the number of pairs that reproduced (reproductive success) and, for these successful pairs, the number of litters and the total number of F2 offspring produced. F2 offspring were weaned at 20 days, marked with hair dye for individual recognition, and housed in sibling groups in lab-o-tec cages. Between days 30 and 50, these groups were video-recorded on 10 non-consecutive days, for between 10 and 15 min per day, to determine the absence or presence of stereotypy (as described above) in individuals. For logistical reasons, the period for recordings could not be standardised to 15 min, but one

Table 1
Ethogram of *Rhabdomys* behaviour

Behaviour	Definition
Inactive	The individual is motionless, lying on the substrate, usually in the nest; changes of resting position, provided the mouse remains in the same resting place, are regarded as inactivity
General locomotion	Non-stereotypic walking or running around the cage floor or lid, including digging in the wood shavings, sniffing, rearing, nesting, and object manipulation (e.g. of a cardboard tube). These behaviours are not usually performed more than once in succession (compared to stereotypic behaviour; below)
Eating	Manipulating or chewing of mouse cubes or seeds
Drinking	Drinking from the water bottle
Grooming	Squatting on the hind legs, grooming head, body, tail, and/or genitals
Amicable (if animals are group housed)	Sniffing, allogrooming, huddling
Aggression (if animals are group housed)	Chasing, biting, boxing
Stereotypy	An invariant behaviour (which may be qualitatively similar to some other behaviours in this table) but which consists of three or more repetitions in succession (Mason, 1993; Vickery and Mason, 2004); see Table 2 for descriptions of stereotypy types

Table 2
Type, definition, and prevalence (in the current study) of stereotypies in *Rhabdomys*

Stereotypy type	Definition	Prevalence[a]
Circuit-running	Running in the cage along a fixed route (frequently in a figure-of-eight pattern)	27.93% ($n = 93$)
Somersaulting	Backward flipping, with or without touching the cage lid	26.73% ($n = 89$)
Cage-climbing	Climbing on the cage lid, using either fore, or fore and hind limbs	21.32% ($n = 71$)
Jack-hammering	Jumping up and down on the hind limbs, usually in the corner of the cage	24.02% ($n = 80$)

A behaviour was regarded as a stereotypy if it comprised of at least three repetitions in succession (Mason, 1993; Vickery and Mason, 2004).

[a] Prevalence data are provided for F2 offspring. Data from all four breeding treatments (stereotypic female and stereotypic male; stereotypic female and non-stereotypic male; non-stereotypic female and stereotypic male; non-stereotypic female and non-stereotypic male) were pooled because of a lack of statistical significance (see text).

group was not favoured over another because of the different time sampling periods, which ranged from 125 to 135 min for all groups. We recorded the type of stereotypy shown by individuals, the proportion of offspring per litter which were stereotypic and the sex ratio of stereotypic offspring per breeding pair. In addition, we calculated the number of pairs per treatment that produced stereotypic offspring to ascertain if there was a tendency for some pairs to produce stereotypic offspring.

2.1. Data analysis

All analyses were performed using Statistica 7.1 (StatSoft Inc., 2006). Logistic regression analyses were used to analyse the proportion data (i.e. pairs that reproduced, number of pairs producing stereotypic young, number of stereotypic females and males) among the treatments (categorical predictor; see Kinahan and Pillay, 2008).

A generalised linear model (GLZ) with an ordinal multinomial distribution and a probit link function was used to analyse the prevalence of the different types of stereotypy in the stereotypic F2 offspring in all treatments. For both the logistic and GLZ analyses, significance was determined using Wald statistics, and estimate coefficients (β) were used to assess the strength of the each independent factor on the dependent variable when the overall Wald statistic was significant.

General linear models (GLM) were used to analyse the total number of litters and total offspring produced per pair (dependent variable) in each treatment (categorical predictor). A GLM was also used to compare the proportion of stereotypic offspring for each pair (dependent variable) in each of the four treatments (categorical predictor). For this analysis, we calculated the proportion of stereotypic offspring produced by each pair, and not the proportion of stereotypic offspring produced per litter, because not all pairs produced similar number of litters (see Section 3) and because offspring in a related group are not statistically independent of each other (Boonstra and Hochachka, 1997). Since proportion data violate the assumptions of normality, the data set was arcsine square root transformed prior to analysis (Zar, 1996). We also included the total number of offspring produced by each pair as a continuous predictor (covariate). Fisher's *post hoc* tests were used to identify specific differences in the GLM analyses when $\alpha \leq 0.05$.

3. Results

Of the 15 pairs established per treatment, 93% each of the S♀–S♂ and S♀–NS♂ treatments, 80% of the NS♀–NS♂ treatment, and 73% of the NS♀–S♂ produced litters (Table 3). Treatment was not a significant predictor of reproductive success (Wald $\chi^2_3 = 3.13$, $p = 0.372$) or of the number of litters produced ($F_{3,47} = 1.04$, $p = 0.384$). However, treatment significantly influenced the total number of offspring produced ($F_{3,47} = 8.82$, $p < 0.001$): treatments in which the mother

Table 3
The reproductive performance of *Rhabdomys* pairs assigned to one of four treatments: (1) stereotypic female and stereotypic male (S♀–S♂); (2) stereotypic female and non-stereotypic male (S♀–NS♂); (3) non-stereotypic female and stereotypic male (NS♀–S♂); (4) non-stereotypic female and non-stereotypic male (NS♀–NS♂)

Treatment	Number of pairs reproducing	Number of pairs producing stereotypic young	Total number litters	Mean number of litters (S.E.)	Total number of offspring	Number of stereotypic offspring (proportion)	Number of stereotypic females, males
S♀–S♂	14 of 15	14	39	2.79 (0.09)	293	160 (0.54)	90, 70
S♀–NS♂	14 of 15	14	35	2.50 (0.14)	240	123 (0.51)	64, 59
NS♀–S♂	11 of 15	11	27	2.25 (0.20)	121	38 (0.31)	21, 17
NS♀–NS♂	12 of 15	7	27	2.25 (0.17)	123	12 (0.10)	7, 5

was stereotypic (S♀–S♂, S♀–NS♂) produced about twice the number of offspring than treatments in which the mother was non-stereotypic (NS♀–S♂, NS♀–NS♂; Table 3).

Treatment was also a significant predictor of whether successful pairs produced stereotypic offspring (Wald $\chi^2_2 = 6.81$, $p = 0.033$; note the regression was forced through the origin because of the 100% occurrence of stereotypy in some treatments, resulting in a lower than expected degrees of freedom) and, whereas all of the reproductively successful pairs in the S♀–S♂, S♀–NS♂ and NS♀–S♂ treatments produced stereotypic offspring, proportionally significantly fewer (7 of the 11) NS♀–NS♂ pairs produced stereotypic offspring ($\beta = -1.52$, S.E. = 0.60, Wald $\chi^2_1 = 6.37$, $p = 0.012$; Table 3).

There was no sex effect in the prevalence of stereotypy among the treatments (Wald $\chi^2_3 = 0.58$, $p = 0.902$; Table 1).

Only four types of stereotypies were recorded in our study, all which were locomotor stereotypies (Table 2). Interestingly, there was no evidence of windscreening wiping behaviour, which was common in another population of striped mice (Schwaibold and Pillay, 2001). The distribution of the four types of stereotypies did not differ significantly among the F2 offspring in the treatments (Wald $\chi^2_3 = 6.32$, $p = 0.100$).

The prevalence of stereotypy in F2 offspring was strongly related to its occurrence in the parents ($F_{1,46} = 22.52$, $p < 0.001$). *Post hoc* tests revealed four patterns. (1) Offspring with two stereotypic parents (S♀–S♂) were not at a greater risk of developing stereotypy than offspring with only a stereotypic mother (S♀–NS♂; $p = 0.567$), with about 50% of the offspring in both these treatments displaying stereotypy (Fig. 1). (2) In contrast, significantly fewer ($p < 0.001$) stereotypic offspring were produced in treatments in which only the mother (NS♀–S♂; 30% stereotypic offspring) or both parents were non-stereotypic (NS♀–NS♂; 10% stereotypic offspring; Fig. 1). (3) Offspring with at least one stereotypic parent (S♀–NS♂, NS♀–S♂) were significantly more likely to develop stereotypy than offspring from non-stereotypic parents (NS♀–NS♂; $p < 0.001$). (4) Maternal stereotypy (S♀–NS♂) was a better predictor of offspring stereotypy than paternal stereotypy (NS♀–S♂; $p = 0.039$). The total of number of offspring produced by a pair (covariate) was not a significant predictor of the stereotypy prevalence ($F_{1,46} = 0.01$, $p = 0.973$).

4. Discussion

The prevalence of stereotypy was five times greater in the offspring of stereotypic than non-stereotypic females, regardless of whether the male was stereotypic, and three times greater in

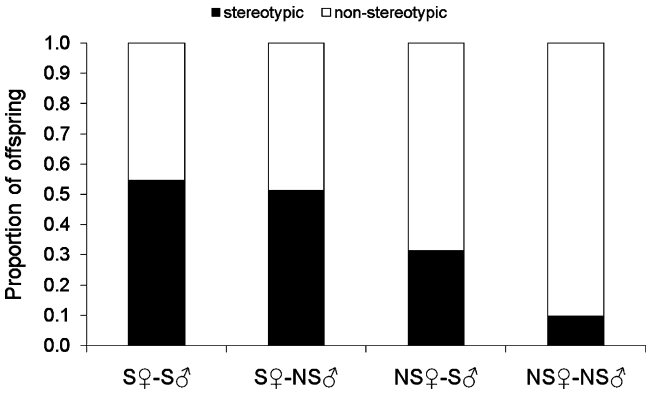

Fig. 1. Stereotypy prevalence in offspring produced in four breeding treatments of *Rhabdomys*: (1) stereotypic female and stereotypic male (S♀–S♂); (2) stereotypic female and non-stereotypic male (S♀–NS♂); (3) non-stereotypic female and stereotypic male (NS♀–S♂); (4) non-stereotypic female and non-stereotypic male (NS♀–NS♂). Bars represent the proportion of stereotypic: non-stereotypic offspring produced in each treatment.

offspring sired by stereotypic males paired with non-stereotypic females than in offspring from non-stereotypic parents. Therefore, our prediction that mothers and fathers contribute equally and additively to stereotypy in their offspring (e.g. Schoenecker and Heller, 2000), so that stereotypy prevalence would be highest in offspring from S♀–S♂ treatment, was not met.

Our findings, in combination with previous work in *Rhabdomys* showing that the tendency to develop stereotypy is related to the occurrence of stereotypy in the biological mother (Schwaibold and Pillay, 2001), point towards a genetic basis for stereotypy as well as a positive relationship between stereotypy and reproductive output. However, the larger maternal than paternal contribution to the development of stereotypic behaviour in the S♀–NS♂ compared with the NS♀–S♂ treatment, as well as the lack of difference in stereotypy transmission between the S♀–NS♂ and S♀–S♂ treatments, indicates that genetics alone cannot explain the observed transmission patterns. Below, we consider two non-genetic mechanisms which potentially can account for our findings.

First, pups might associate more strongly with their mothers than their fathers postnatally. If the amount of time spent with a stereotypic parent influences the development of stereotypy via, for example, social facilitation or learning (Galef, 1996), offspring of stereotypic mothers would be more likely to display stereotypy than offspring with only stereotypic fathers. This seems unlikely, however, as it has previously been shown (1) in cross-fostering experiments that the stereotypic status of the mother does not influence the development of stereotypy in fostered offspring (Schwaibold and Pillay, 2001), and (2) that *Rhabdomys* parents, with the exception of suckling, contribute equally to parental care (Schradin and Pillay, 2004).

Second, gestational effects between conception and birth might have enduring effects on neurobehavioural development (Chapillon et al., 2002; Kofman, 2002; Patin et al., 2004), and so predispose a stereotypic phenotype. For example, numerous studies in rodents have shown that the offspring of mothers that are psychologically stressed during pregnancy (e.g. because of changes to the social environmental, housing conditions) are more anxious as adults than offspring whose mothers were not stressed (e.g. Macri and Würbel, 2006), most probably as a result of an abnormally up-regulated hypothalamic-pituitary-adrenal axis (HPA; Kofman, 2002). Evidence is mixed for stereotypic animals having higher baseline stress hormone (corticosterone) levels than non-stereotypic animals (reviewed by Mason and Latham, 2004), and is untested in

Rhabdomys. However, if stereotypic mothers do show HPA axis hyperactivity (as occurs in animals that are prenatally stressed), it is likely that their offspring would be more stress sensitive and impulsive, the latter of which is known to predispose stereotypy in *Rhabdomys* (Jones, unpublished data). Studies examining the effects of prenatal stress on postnatal stereotypy development would elucidate the viability of this hypothesis.

5. Conclusion

Our study revealed two outcomes. First, the data extend the findings of Schwaibold and Pillay (2001) that stereotypy has a strong genetic component and is transmitted from both mothers and fathers, and that social transmission of stereotypy is unlikely. Secondly, our study is unique in that we showed that maternal stereotypy, rather than paternal stereotypy, is a better predictor of stereotypy prevalence in *Rhabdomys*, indicating that epigenetic factors, such as gestational stress, might also predispose the ontogeny of stereotypy. Future research should investigate if and how the prenatal environment affects the expression of stereotypic behaviour in genetically susceptible animals. Moreover, studies are needed to systematically investigate the heritability of the different types of stereotypy.

Acknowledgements

We are grateful to Georgia Mason for much helpful discussion, and for the comments from two referees on earlier versions of this paper. Many thanks to Jacobeth Maloka, Bongi Gwala, and Fred Mkhize for technical assistance. Funding was provided by the National Research Foundation and the University of the Witwatersrand. Approval for this study was granted by the Animal Ethics Screening Committee of the University of Witwatersrand (AESC: 2003/23/2A).

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CHAPTER THREE

Increased reproductive output in stereotypic captive *Rhabdomys* females: Potential implications for captive breeding

Megan Anne Jones, Mathew van Lierop, Georgia Mason, Neville Pillay (2010)

Applied Animal Behaviour Science, **123**, 63-69

Applied Animal Behaviour Science 123 (2010) 63–69

Contents lists available at ScienceDirect

Applied Animal Behaviour Science

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

Increased reproductive output in stereotypic captive *Rhabdomys* females: Potential implications for captive breeding

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ARTICLE INFO

Article history:
Accepted 22 December 2009
Available online 22 January 2010

Keywords:
Conservation
External validity
Fitness
Reproduction
Stereotypic behaviour
Striped mice

ABSTRACT

Captive animal populations can diverge considerably from populations in the wild, despite the animals not being deliberately domesticated. If the phenotypes which are of benefit in captivity are heritable, the genotypes of captive-stock can diverge swiftly and substantially from wild-stock. Using striped mice, *Rhabdomys*, we tested the relationship between reproductive output and stereotypic behaviour, a heritable repetitive abnormal behaviour common in captive wild animals. Individuals ($n = 120$; 60♂, 60♀) were assigned to pairs in one of four treatment groups formed from combinations of non-stereotypic and stereotypic mothers and fathers, and various measures of reproductive output were recorded. Reproductive output (e.g. total number of offspring) for stereotypic females (but not stereotypic males) was significantly greater than for non-stereotypic striped mice. We suggest that, overall, unintended selection is likely to increase the incidence of stereotypic behaviour in a captive striped mouse population because (1) stereotypic females breed more successfully than non-stereotypic striped mice, and (2) genetic variance underlies the trait. The potential implications of these findings for the validity of behavioural studies using captive-bred wild animals and for conservation breeding programmes are discussed.
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1. Introduction

Stereotypic behaviour in captive animals is caused by frustration and/or brain dysfunction (Mason, 2006a). Manifesting only in captivity, it affects at least 85 million domestic animals (Mason and Latham, 2004), as well as a significant proportion of captive wild animals housed in zoos (at least 10,000; reviewed in Mason et al., 2007) and laboratories (e.g. primates, Novak et al., 2006; roof rats, *Rattus rattus*, 100%, Callard et al., 2000; bank voles, *Clethrionomys glareolus*, 30–50%, Cooper et al., 1996; Ödberg, 1987; Schoenecker and Heller, 2000; striped mice, *Rhabdomys*, 57%, Jones et al., in preparation). Whilst stereotypies typify sub-optimal environments (Mason, 1991; Mason and Latham, 2004), highly stereotypic individuals often show better welfare than less stereotypic conspecifics housed in similar sub-optimal conditions (Mason and Latham, 2004). Apparent benefits linked with stereotypic behaviour including reductions in heart rate (e.g. calves; Seo et al., 1998), and elevated reproductive success. In farmed mink (*Mustela vison*), for example, stereotypic females are sometimes more fertile and experience lower pup mortality than non-stereotypic females (Jeppesen et al., 2004; cf. Svendsen et al., 2007) and, in caged bank voles, stereotypic behaviour has recently been shown to be associated with better survival, fecundity, and therefore increased lifetime reproductive success (Schønecker, 2009). The mechanisms for such beneficial effects of stereotypic behaviour are unclear. It could be (1) that both stereotypy (Mason and Latham,

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0168-1591/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.applanim.2009.12.013

2004) and increased reproductive success (Broom and Johnson, 1993; Wingfield and Sapolsky, 2003) reflect relatively low stress in sub-optimal cages; (2) that stereotypic behaviour has an indirect pleiotropic effect on reproductive success through its association with the directly selected trait of activity level or body condition, as is sometimes the case in mink (Jeppesen et al., 2004); or (3) that being of a fecund genotype predisposes animals to stereotypic behaviour, through genetic or epigenetic means, the latter perhaps influenced by litter-size mediated variations in the maternal care received in infancy (Priestnall, 1972).

Whatever the mechanisms behind this relationship, because stereotypic behaviour typically has a genetic component (bank voles, Ödberg, 1986; Schoenecker and Heller, 2000; mink, Jeppesen et al., 2004; Svendsen et al., 2007; domestic horses, *Equus caballus*, Kiley, 1977; Smith, 1984; striped mice, Jones et al., 2008; Schwaibold and Pillay, 2001), such findings strongly suggest that stereotypic genotypes may be selected for in captive conditions. Circumstantial evidence for this is that across a host of species, the F1 captive generation is more stereotypic than the wild-caught generation, although it is currently unknown whether this difference has a genetic, developmental, or environmental basis (Mason, 2006b); and that, in our randomly bred captive colony of *Rhabdomys* a greater proportion of F2 mice (56%) than F1 mice (41%) showed this behaviour (Fisher's Exact Test, $p = 0.0011$; unpublished results).

Such effects are potentially important because when phenotypes which are favoured (selected for) in captivity are heritable, the genetic characteristics of captive animals can diverge swiftly and substantially from wild-stock (Lynch and Walsh, 1998; McDougall et al., 2006; McPhee, 2003). For example, in coonstripe shrimp, *Pandalus danae* (Marliave et al., 1993), just 10 generations of captive breeding caused changes in morphology, physiology, genotype, and ease with which they could be caught, despite there being no deliberate domestication; whilst in oldfield mice, *Peromyscus polionotus subgriseus*, generations of captive breeding resulted in diminished predator response behaviours, most likely because of relaxed natural selection pressures (McPhee, 2003). Such changes are undesirable if animals are part of conservation breeding programmes of which the central aim is to preserve the wild-type genetic and phenotypic characteristics of the species (Kleiman, 1996; McDougall et al., 2006; Snyder et al., 1996). For instance, it is suspected that bolder swift foxes, *Vulpes velox*, breed better than more fearful individuals in captivity but, upon reintroduction to their natural habitat, boldness predicts higher mortality (Bremner-Harrison et al., 2004). Such changes are also undesirable if captive-bred animals are being used to investigate phenomena occurring in wild individuals since these changes compromise the external validity of research findings.

Using *Rhabdomys* as a model, we assessed the relative reproductive success of stereotypic and non-stereotypic individuals. We set up four treatment groups, formed from combinations of non-stereotypic and stereotypic mothers and fathers. This design makes our study unique since we could assess the relative contribution of maternal and paternal stereotypic behaviours to reproductive output (cf. the recent work in bank voles examining only the maternal contribution; Schønecker, 2009), and thus test the prediction that both male and female stereotypic striped mice would reproduce more successfully than non-stereotypic mice.

2. Methods

2.1. Model species

Rhabdomys, the African striped mouse, is a small (40–70 g), diurnal, muroid rodent that is widespread and abundant in southern African (Skinner and Chimimba, 2005). When captive-bred individuals are housed in standard laboratory cages, approximately half F1 mice develop locomotor stereotypic behaviour (e.g. circuit running; somersaulting; jack-hammering; and cage-climbing; see Jones et al. (2008) for detailed definitions of these behaviours). Our previous work in *Rhabdomys* has shown that this behaviour is genetically rather than socially transmitted (Schwaibold and Pillay, 2001), but with a greater maternal than paternal contribution to stereotypy development suggesting that maternally mediated epigenetic factors also influence the expression of the behaviour (Jones et al., 2008).

2.2. Housing and husbandry

All mice used in this study were housed in the Milner Park Animal Unit at the University of the Witwatersrand, under partially controlled environmental conditions (light regime of 14L:10D, lights on at 05:00 h; 20–24 °C; 30–60% relative humidity). Striped mice were kept in standard Labotec cages (300 mm × 200 mm × 150 mm) containing ~2 cm woodshavings as bedding and a handful of hay as nesting material. Epol® mouse cubes (Epol, Pretoria West, South Africa) and water were provided ad libitum. We supplemented the diet twice weekly with a small amount (±5 g) of a parrot seed mix.

2.3. Procedure

To obtain an appropriate sample size, we bred 40 wild-caught striped mice from a South African Highveld grassland locality (25°40′S; 28°30′E). The resultant F1 juveniles were weaned at 20 days, housed in same-sex sibling groups, and marked with hair dye for individual identification (Schradin and Pillay, 2004). From 30 days of age, we video-recorded the behaviour of the striped mice for 15 min twice weekly, for 7 weeks, to identify stereotypic and non-stereotypic individuals. Recordings took place between 08:00 h and 12:00 h, during which time *Rhabdomys* is most active (Pillay, 2000). No human observers were present in the room during taping. Because of the bimodal distribution of stereotypic behaviour in captive *Rhabdomys* populations (Fig. 1), we regarded animals that were consistently stereotypic (i.e. performed at least one bout in more than 60% (≥9 of 14) of the independent observation sessions) as stereotypic, whereas

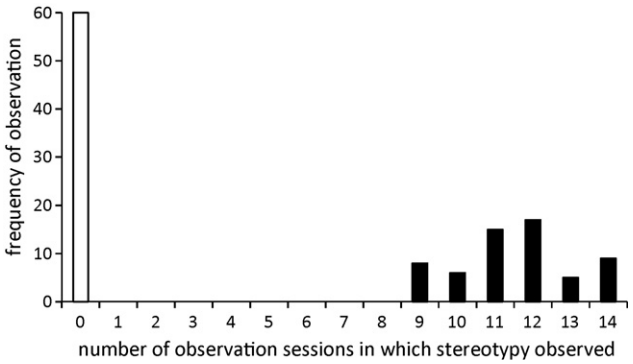

Fig. 1. Number of striped mice (total $n = 120$) observed for stereotypic behaviour over a total of 14 independent observation sessions. We regarded animals that performed at least one bout of stereotypy in more than 60% (≥9 of 14) of the observation sessions as stereotypic (black bars), whereas those that displayed none as non-stereotypic (white bar).

those that displayed none (during video recording, or in ad lib observations of the colony) were classed as non-stereotypic. We never recorded individuals with stereotypic behaviour in less than 60% of the observation sessions (see Jones et al. (2008) for the detailed scoring procedure and justification thereof).

When F1 mice were between 80 and 120 days old, we established 60 unrelated breeding pairs by assigning individuals to one of four treatments. Litters were equally represented in each of the four treatment groups. Treatment groups (15 pairs each), based on whether mice were stereotypic or non-stereotypic, were comprised as follows: (1) stereotypic female and stereotypic male (S♀–S♂); (2) stereotypic female and non-stereotypic male (S♀–NS♂); (3) non-stereotypic female and stereotypic male (NS♀–S♂) and (4) non-stereotypic female and non-stereotypic male (NS♀–NS♂). Pairs were kept together for at least 100 days (in which time a maximum of three litters could be produced), and then separated only after the weaning of their final litter. Both the mother and the father were thus always present with the pups until weaning. Male striped mice, sometimes in the field and always in captivity, contribute equally to parental care and display similar behaviours to the mother, with the exception of suckling behaviour (Schradin and Pillay, 2004). Offspring were separated from their parents on day 20 (at least 3 days before another litter of pups would be born to the female), and then housed in same-sex sibling groups.

We recorded the number of successfully breeding pairs (pairs producing at least one litter) in each treatment and, for the successfully breeding pairs, the following measures: interval between pairing and birth of the first litter; inter-litter interval; number of litters; litter size at birth and at weaning; and the proportion of the litter surviving to weaning. We weighed the mothers and their litters on the day of birth (day 0) and again on day 20 (weaning), and calculated the pre-weaning average pup growth rates of litters using the formula [ln(mass time 2) − ln(mass time 1)]/(time 2 − time 1)].

2.4. Ethical note

Wild-caught mice were trapped using PVC live traps (290 mm × 60 mm × 70 mm). The traps were set for four nights, covered with grass to buffer temperature extremes, and baited with an excess (half a handful) mixture of rolled oats, raisins, salt, and sunflower oil. Moistened cotton wool was provided for water, and dry cotton wool as bedding. Traps were checked twice daily in the morning and late afternoon, immediately after activity peaks of *Rhabdomys*. This ensured that trapped individuals would be unlikely to spend more than 2 h in the traps. No trap deaths were recorded. Following capture, mice were transferred from traps into individual holding cages (250 mm × 250 mm × 120 mm, containing woodshavings for bedding and a handful of hay as nesting material, and provisioned with mouse cubes and a water bottle), and then transported to the University of the Witwatersrand—an approximately 60-min road trip.

After completion of this study, wild-caught mice were retained as breeding stock, whilst captive-born mice ($n = 822$) were used as subjects in other behavioural studies or euthanized ($n = 257$) using gradual-fill carbon dioxide euthanasia.

Approval for this study was granted by the Animal Ethics Screening Committee of the University of Witwatersrand (AESC: 2003/23/2A).

2.5. Data analysis

All analyses were performed using Statistica 7.1 (Statsoft Inc., Southern Africa). We compared the number of successfully breeding pairs in each treatment using logistic regression. General Linear Models (GLM), with maternal and paternal stereotypic status (and their interaction) as categorical predictors, were used to analyse the following variables: latency to produce a first litter, inter-litter interval, total number of litters produced, litter size at birth and at weaning (using a repeated measures design), total number of offspring born and total number of offspring surviving to weaning (using a repeated measures design), average pup growth rate (litter size was included as a continuous predictor to account for the effect of litter size on growth rate), and the proportion of offspring in each litter surviving to weaning (data were arcsine square root transformed; Zar, 1996). For pairs that produced more than one litter, we used average values for the litters produced per pair to avoid pseudoreplication. Tukey *post*

hoc tests were used to identify specific differences when $\alpha \leq 0.05$.

3. Results

Table 1 provides the reproductive values measured in this study together with the statistical results. Significant findings are graphically represented in Fig. 2. The number of successfully reproducing pairs did not differ among the treatments. Maternal, but not paternal or maternal × paternal, stereotypic status was a significant predictor of the following variables: latency to first litter; litter size at birth and weaning; total offspring born and weaned; and percentage offspring survival to weaning. *Post hoc* tests revealed that stereotypic females had greater reproductive success (i.e. they produced more offspring, which grew faster and had better survival) than non-stereotypic females. The interaction between maternal and paternal stereotypies was a significant predictor of pup growth rate, with pups of stereotypic mothers (irrespective of paternal stereotypy) growing faster than pups in the NS♀–S♂ group, followed by pups in the NS♀–NS♂ treatment. Parental stereotypic status did not affect the total number of litters produced or inter-litter interval.

Since maternal mass is known to sometimes differ between stereotypic and non-stereotypic mink females, and to affect reproductive success (e.g. Jeppesen et al., 2004), we investigated this in these *Rhabdomys*. However, we found no mass difference between stereotypic and non-stereotypic mothers (Table 1) and controlling for dam mass in supplementary analyses did not change any of the results.

4. Discussion

Stereotypic behaviour in dams was associated with an increased reproductive output in striped mice. Compared to non-stereotypic individuals, stereotypic *Rhabdomys* mothers birthed (and weaned) nearly twice as many young within a 100-day period than did non-stereotypic females. Recording lifetime reproductive success could perhaps have yielded a more accurate estimate of the relative fitness of striped mice. However, such a protocol would have resulted in many surplus animals requiring euthanasia (see Section 4), and we suspect that our conclusions would not have changed since captive female striped mice show a sharp decline in reproduction after their third litter (Pillay, personal observation). In general, paternal phenotype did not affect reproductive success, with one exception: irrespective of paternal stereotypy, pups from stereotypic mothers grew faster than pups from non-stereotypic mothers but, in young from non-stereotypic dams, paternal stereotypy predicted a faster growth rate. These results therefore show, as predicted from other species, that stereotypic behaviour in *Rhabdomys* is associated with enhanced reproductive success.

The improved reproduction of stereotypic *Rhabdomys* shows both similarities to and differences from the similar findings reported for mink and bank voles (see Section 1) and is, to our knowledge, the first to measure the contribution of paternal stereotypy on reproductive

Table 1
Data (mean ± SEM) and statistical results of analyses for reproductive variables measured in breeding pairs comprising stereotypic (S) and non-stereotypic (NS) striped mice. Specific differences are provided for significant predictors (Tukey *post hoc* tests), and are indicated in bold type.

Variable	NS♀–NS♂	NS♀–S♂	S♀–NS♂	S♀–S♂	Maternal stereotypic status	Paternal stereotypic status	Matenal × paternal interaction
Number of successfully breeding pairs	12/15	11/15	14/15	14/15	Wald $\chi^2_3 = 3.13$, $p = 0.372$		
Latency to first litter	32.00 ± 2.86	35.32 ± 5.00	30.08 ± 2.75	27.67 ± 1.11	**$F_{1,47} = 6.05$, $p = 0.018$ (S♀ > NS♀)**	$F_{1,47} = 0.47$, $p = 0.496$	$F_{1,47} = 2.69$, $p = 0.108$
Inter-litter interval	28.22 ± 2.08	26.89 ± 4.21	24.78 ± 0.42	28.05 ± 0.99	$F_{1,47} = 2.23$, $p = 0.144$	$F_{1,47} = 1.12$, $p = 0.297$	$F_{1,47} = 0.07$, $p = 0.796$
Total number of litters	2.25 ± 0.25	2.27 ± 0.31	2.58 ± 0.21	2.75 ± 0.17	$F_{1,47} = 2.92$, $p = 0.094$	$F_{1,47} = 0.67$, $p = 0.416$	$F_{1,47} = 0.02$, $p = 0.885$
Litter size at birth/weaning[a]	4.24 ± 0.42	4.74 ± 0.45	6.71 ± 0.34	7.36 ± 0.40	**$F_{1,47} = 35.88$, $p < 0.001$** (S♀ > NS♀)	$F_{1,47} = 1.19$, $p = 0.280$	$F_{1,47} = 0.08$, $p = 0.781$
	3.66 ± 0.34	4.52 ± 0.45	6.63 ± 0.33	7.26 ± 0.39			
Total offspring born/weaned[a]	10.25 ± 1.72	10.25 ± 1.37	16.83 ± 2.01	21.33 ± 1.90	**$F_{1,47} = 20.70$, $p < 0.001$ (S♀ > NS♀)**	$F_{1,47} = 0.91$, $p = 0.344$	$F_{1,47} = 0.98$, $p = 0.327$
	8.25 ± 1.23	9.66 ± 1.28	16.67 ± 1.98	21.00 ± 1.87			
Pup growth (g/day)	0.060 ± 0.006	0.073 ± 0.007	0.095 ± 0.003	0.101 ± 0.002	**$F_{1,47} = 63.71$, $p < 0.001$ (S♀ > NS♀)**	**$F_{1,47} = 10.63$, $p < 0.01$ (S♂ > NS♂)**	**$F_{1,47} = 4.78$, $p = 0.03$ (S♀–S♂ = S♀–S♂ > NS♀–S♂ > NS♀–NS♂)**
Offspring survival (%)	88.53 ± 3.58	95.45 ± 2.09	98.90 ± 0.65	98.76 ± 0.75	**$F_{1,47} = 8.46$, $p = 0.006$ (S♀ > NS♀)**	$F_{1,47} = 1.49$, $p = 0.229$	$F_{1,47} = 1.63$, $p = 0.208$
Maternal mass (g)	59.38 ± 5.28	64.52 ± 7.78	69.21 ± 3.92	73.55 ± 4.26	$t_{49} = -1.306$; $p = 0.196$	n/a	n/a

[a] A repeated measures design was used to analyse litter size at birth and weaning and total offspring at birth and weaning. The repeated measures variable and the various interactions were not significant ($p > 0.05$).

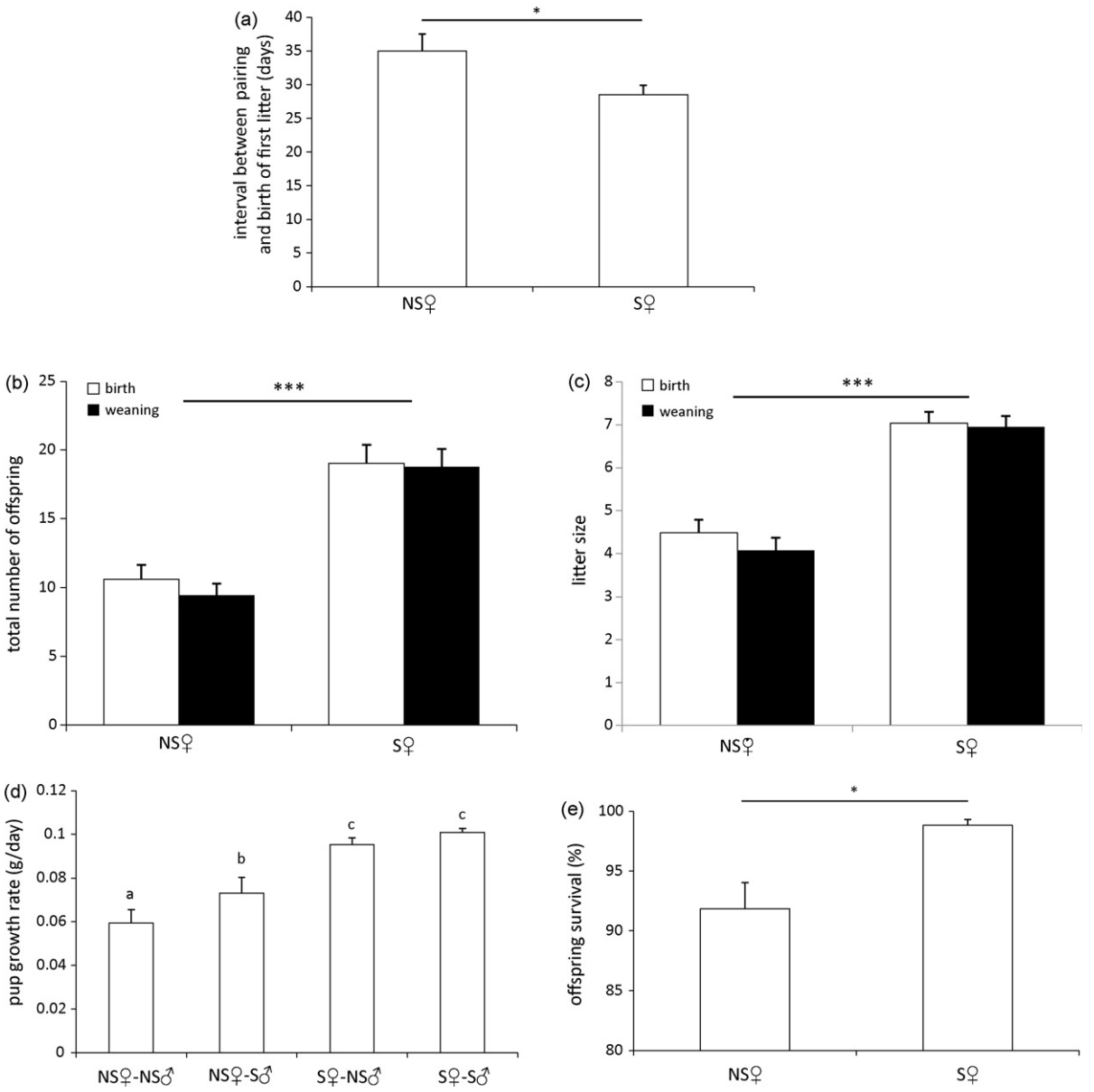

Fig. 2. (a–e) Five measurements (mean + SE) of differential reproductive output in stereotypic (S) and non-stereotypic (NS) mice. If paternal or maternal × paternal stereotypic status did not predict the outcome, pooled mean + SE values are provided for the maternal effect only. Asterisks indicate significant differences between treatments ($^{*}p < 0.05$; $^{***}p < 0.001$). Alphabets show homogenous groups (from Tukey *post hoc* tests) when the maternal × paternal stereotypic status was a significant predictor of outcome.

parameters. In all three species, maternal stereotypic behaviour has been associated with improvement in at least some measures of fecundity. However, in mink, this relationship is not consistent across farms – Svendsen et al. (2007) found no differences in reproduction between high and low stereotyping lines of animals – and Jeppesen et al. (2004) study showed that the increased fertility and reduced pup mortality observed in stereotypic mothers could be better explained by the typically lower body weight of stereotypic dams, a mediating effect not observed in this study in *Rhabdomys*. Additionally, compared with *Rhabdomys*, the young of stereotypic mink females may grow more slowly in consequence of reduced levels of maternal care and the tendency of stereotypic dams to build less well insulated nests (Mason et al., 1995). The recent retrospective analysis of Schønecker (2009) showed that stereotypic bank vole mothers, similar to striped mice, have reduced latencies between pairing and the birth of their first litter but, in contrast to *Rhabdomys*, Schønecker (2009) found no difference in litter size or number of weanlings between stereotypic and non-stereotypic dams. Moreover, the study showed that the pups from stereotypic females sometimes experience higher pre-weaning pup mortality—replicating an effect observed in previous work on bank voles (Ödberg, 1987; Sørensen and Randrup, 1986).

The consistent findings of increased fecundity across the three species discussed above suggest a widespread link between stereotypic behaviour and reproductive success. In Section 1, we suggested three possible mechanisms through which this effect might occur. First, there might be a direct link between the variables if, for example, stereotypy and increased reproductive success both reflect better "coping" in sub-optimal caging. However, this seems unlikely in striped mice given that we have found no relationship between stereotypy and physiological (faecal corticosterone) or behavioural (e.g. behaviour in a light-dark box) measures of stress/anxiety (Jones et al., 2009). Second, the effect might be mediated via the pleiotropic promotion of stereotypic behaviour because it is indirectly related to a directly selected trait such as body condition (e.g. mink; Jeppesen et al., 2004), activity, or promiscuity. We have excluded the effect of body weight *per se* in *Rhabdomys* (although not of body composition), and our data argue against the pleiotropic selection for promiscuity because, whilst latency to first litter is shorter in stereotypic females, there is no difference in inter-litter interval, as would be expected if stereotypic striped mice were more likely to engage in impulsive sexual activity than non-stereotypic mice. Nevertheless, with our current data, we cannot examine whether body composition or activity (which is higher in stereotypic than non-stereotypic *Rhabdomys*; Jones et al., 2006) is a better predictor of reproductive success than the absence/presence of stereotypy. The third suggested mechanism was a potential direct link between a fecund and stereotypic genotype: again, we are unable to distinguish whether, for example, offspring born in larger litters are more prone to develop stereotypic behaviour (litter size also being a heritable trait/large litter size resulting in earlier weaning, which is known to predispose stereotypy in *Rhabdomys* as well as other species (Jones et al., in review), or whether the stereotypic phenotype directly/indirectly causes an increase in litter size.

A point of interest is why only female stereotypic *Rhabdomys* (and not males, with the exception of the effect of paternal stereotypic status on growth rate) showed higher reproductive output. This finding argues against a direct genetic link between fecundity and stereotypy being the sole mediator of the findings in this study, instead adding support to the second hypothesis that maternally mediated pleiotropic influences contribute to the observed differences in reproductive success. Future work should investigate whether differences between stereotypic and non-stereotypic dams in terms of activity level, body composition, and consequently hormonal profiles might have favoured reproduction in the stereotypic animals. Additionally/alternatively, the increased food consumption of stereotypic striped mouse females (±3 g per day/41% extra) suggests that the high activity levels of stereotypic *Rhabdomys* females confer the ability to maintain a reproductively favourable body weight and to concentrate dietary protein (Jones et al., 2006). As protein is critical for the initiation and success of reproduction in *Rhabdomys* (Nel, 2003; Perrin, 1980), differences in protein intake require direct testing in future studies.

Because stereotypic behaviour has a genetic basis in *Rhabdomys*, as in many other species (see Section 1), and since it is associated with increased reproductive success, stereotypy may thus increase in prevalence over successive generations of captive breeding—for which we already have preliminary evidence in striped mice (see Section 1). If confirmed, and found to be a widespread effect in captive wild animals, there could be considerable practical implications. First, genetic changes predisposing a high incidence of stereotypic behaviour could well be associated with selection for a correlated suite of behavioural traits which, whilst neutral or even adaptive in captivity, may be maladaptive in the wild. Whilst such variation in behaviour/temperament profiles is often maintained in populations in a frequency dependent manner (Gosling, 2001), shifts in the nature or strength of selection pressures, as will occur if stereotypic behaviour influences reproductive success, can move populations away from their adaptive optima and thus reduce the post-release survivorship of captive-bred individuals in conservation breeding programmes (e.g. Bremner-Harrison et al., 2004; Jule et al., 2008). A second potential problem is if stereotypic behaviour *per se*, and its underlying neurological causes (Garner and Mason, 2002; Lewis et al., 2006), compromises the abilities of released animals to behave appropriately (Vickery and Mason, 2003, 2004). Both this first and second problem will also raise doubt about the validity and replicability of laboratory based behavioural work in which the underlying assumption is that behavioural processes observed in the laboratory reflect those occurring in wild-type individuals in nature (Würbel and Garner, 2007). A third serious caveat is that if captive species are likely to become increasingly stereotypic, more ambitious attempts will be needed to enrich their environments to reduce this aesthetically undesirable behaviour (e.g. Swaisgood, 2007; Swaisgood and Shepherdson, 2005, 2006).

5. Conclusion

Overall, the consistently and dramatically improved reproductive output of stereotypic female *Rhabdomys* (and higher growth rates conferred by the genotypes of both parents) suggests that stereotypic behaviour, manifested only in captivity, has positive fitness correlates in captive conditions. It also suggests that stereotypic behaviour could possibly signal a captive environment that is placing animals under very different selection pressures from those occurring in the wild, with unintended selection likely to further increase the incidence of stereotypic behaviour in captive populations. This has potential implications for the validity of behavioural studies using captive-bred wild animals and for conservation breeding programmes.

Acknowledgements

We are grateful to Jacobeth Maloka and the staff of the Johannesburg Zoo for technical assistance, María Díez León for much help with references, Lis Jones for her proofreading the manuscript, and two anonymous referees for their helpful comments on an earlier draft of the paper.

Funding was provided by the National Research Foundation (grant number: 2069110), the University of the Witwatersrand (Anderson Capelli Fund), and the Johannesburg Zoo.

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CHAPTER FOUR

Early social experience influences the development of stereotypic behaviour in captive-born striped mice *Rhabdomys*

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Applied Animal Behaviour Science, **123**, 70-75

Applied Animal Behaviour Science 123 (2010) 70–75

Contents lists available at ScienceDirect

Applied Animal Behaviour Science

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

Early social experience influences the development of stereotypic behaviour in captive-born striped mice *Rhabdomys*

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ARTICLE INFO

Article history:
Accepted 17 December 2009
Available online 21 January 2010

Keywords:
Biparental care
Early experience
Paternal care
Social enrichment
Stereotypic behaviour
Striped mouse
Weaning age

ABSTRACT

The early life experience of captive animals, in combination with their genetic inheritance, can predispose or promote the development of stereotypic behaviour in later life. To investigate the early social effects of weaning age and the presence/absence of the father on the development of stereotypic behaviour in adulthood, we retrospectively analysed data from our captive colony of striped mice, *Rhabdomys*. In the first analysis, pairs of young from each litter were respectively weaned on postnatal days 12 (4 days before natural weaning), 16, or 20, and the incidence of stereotypic behaviour recorded between days 50 and 60. Early weaning (day 12) was associated with a significantly higher incidence of stereotypic behaviour than later weaning (day 16 or 20). In the second analysis, pups were raised with either the father present (biparental care) or absent (uniparental care). Biparentally reared animals showed significantly less stereotypic behaviour as adults than individuals reared by their mother alone. In both analyses, young from stereotypic mothers showed a higher incidence of stereotypic behaviour than offspring from non-stereotypic dams, with experiential and genetic effects combining to influence the adult phenotype. Together, these data indicate that the early social environment influences the development of stereotypic behaviour in adulthood, and suggest directions for future research into the mechanisms of the epigenetic effects of early social experience on the development of stereotypic behaviour, such as whether amounts of parental care received per pup cause these effects, and whether changes in stereotypic behaviour are paralleled by changes in stress responsiveness.

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1. Introduction

Early life experience, in concert with genetic inheritance, has profound and enduring effects on the organisation of the central nervous system (CNS) and thus an animal's behavioural, psychological, and physiological responses to the environment (Laviola and Terranova, 1998; Caldji et al., 2000; Gilmer and McKinney, 2003). Whereas free-ranging young mammals usually have extended physical and social contact with their mother, father and/or family group, captivity is often associated with both parental and social deprivation (Latham and Mason, 2008; Newberry and Swanson, 2008): individuals typically experience an earlier and more abrupt weaning than is natural, and are often not raised in their species-typical social groupings which might include the father (e.g. biparental care in some rodent species; Dewsbury, 1985) or the larger family unit (e.g. communal nesting in mice, *Mus musculus*; Hayes, 2002). Because the epigenetic effects of early social deprivation modify stress responsiveness in later life (Caldji et al., 2000) these effects can, in the long-term, affect the adjustment of animals to the captive environment.

The adverse effects of premature weaning on general behaviour are well characterised in rodents (Laviola and

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0168-1591/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.applanim.2009.12.009

Terranova, 1998; Kikusui et al., 2008). Early weaning results in both deprivation of maternal care and separation from the mother before adequate developmental maturity. This deprivation induces immediate neuroendocrine and behavioural stress responses, causing long-lasting effects on brain and behaviour, in particular stress reactivity (Kikusui et al., 2008). In the few existing studies investigating the association between weaning age and the development of abnormal behaviour, premature weaning has been linked to the later development of stereotypic behaviour, repetitive behaviour that is caused by frustrated motivation to perform (or receive, in the case of infants) species-typical behaviour and/or from the chronic impact of deprived conditions on CNS development and function (Latham and Mason, 2008). For instance, laboratory mice weaned at 17 days showed increased rearing (upright standing on the hind legs, often associated with single vertical jumps at the cage wall) shortly after weaning, and performed more stereotypic behaviour (bar-biting) in adulthood than mice weaned 4 days later at 21 days (Würbel and Stauffacher, 1997). In farmed mink (*Mustela vison*), whilst there were no observable effects of weaning age on activity immediately post-weaning, the early weaned mink in one study showed increased tail-biting several months later (Mason, 1995) and, in another study, higher levels of stereotypic behaviour (Jeppesen et al., 2000). These, often initially latent, effects of early weaning suggest early but pervasive changes in motivational state or in the control of behaviour (Latham and Mason, 2008), although these changes have yet to be assessed directly. In contrast with the effects of premature weaning, delayed weaning is associated with greater exploration in adulthood (Adriani and Laviola, 2002) and, in certain rearing environments, with reduced anxiety and abnormal behaviour (stereotypic behaviour and barbering—self-removal of hair, or removal of hair by a cagemate; Bechard et al., submitted for publication).

Naturally occurring variations in maternal care (e.g. the amount of licking and grooming provided to pups) influence neuroendocrine development, and consequently adult behaviour and effects mediated through epigenetic modification of gene expression in the brain (Caldji et al., 2000; Champagne and Curley, 2009). However, little is known about the effects of paternal care in biparental species (Bredy et al., 2007). The best characterised model thus far of biparental care is the California mouse (*Peromyscus californicus*), in which biparental rearing results in pups receiving more licking and grooming than if only the mother were present (Wright and Brown, 2002; Bredy et al., 2004). In this species, it has also been shown that the presence of the father affects the development of aggression (Frazier et al., 2006; Bester-Meredith and Marler, 2007) and cognition (novel object recognition; Bredy et al., 2004). To our knowledge, however, the effect of biparental care on stress responsiveness has not been tested, nor the link assessed between variations in maternal or paternal care and the development of stereotypic behaviour.

In this study, we investigated the roles of (1) weaning age and (2) the absence/presence of the father (uni- or biparental care) on the development of stereotypic behaviour by retrospectively analysing data from our captive colony of striped mice, *Rhabdomys*, a species showing highly developed paternal care (Schradin and Pillay, 2003). In the first analysis, we assessed the effect of weaning age (days 12, 16, or 20) on expression of stereotypic behaviour in adult striped mice (50–60 days). In the second analysis, we compared the incidence of stereotypic behaviour in adult striped mice that were raised by the mother alone (uniparental care) or by both parents (biparental care). In both analyses, because stereotypic behaviour is known to be genetically (but not socially) transmitted in striped mice (Schwaibold and Pillay, 2001; Jones et al., 2008), we controlled for the stereotypic status (stereotypic or non-stereotypic) of the parents, and also investigated the potential interaction of genotype with the respective early social experiences. We predicted that early weaning would predispose young to the development of stereotypic behaviour, whereas biparental care would protect against its development.

2. General approach

2.1. Housing

All striped mice were housed in the Milner Park Animal Unit at the University of the Witwatersrand, under partially controlled environmental conditions (14L:10D, lights on at 05:00 h; 20–24 °C; 30–60% relative humidity). Individuals were kept singly, in pairs, or in same-sex sibling groups in standard Labotec cages (300 mm × 200 mm × 150 mm), containing ~2 cm woodshavings as bedding and a handful of hay as nesting material. Epol® mouse cubes and water were provided *ad libitum*. The diet was supplemented twice weekly with a small amount (±5 g) of parrot seed mix.

2.2. Approach to analysis

This study is based on retrospective analyses of data collected from NP's striped mouse colonies between 1997 and 2005. Whilst care has been taken to exclude potential biases through careful *a priori* selection of data and/or to account for biases in the data sets using statistical methods (e.g. we factor in the stereotypic status of the parents), some minor confounds remain in the analyses, which we acknowledge, and discuss in the respective discussion sections for each analysis (below).

2.3. Ethical note

Animal use and care, in the relatively barren conditions described above, conformed to our institution's ethical guidelines at the time when the research was conducted. We have subsequently modified our animal care protocols to mandate basic enrichment in order to enhance the welfare of the striped mice and also to reduce the incidence of stereotypic behaviour.

3. Weaning age

3.1. Methods and materials

3.1.1. Procedure

Juvenile offspring from G1 to G4 breeding pairs that produced six pups (the mean litter size for captive

Rhabdomys is 7.2 ± 1.8; Pillay, 2000) were included in a socio-ecological study investigating the effect of weaning age on life history strategy (Pillay, unpublished data). Founder stock was from a Highveld grassland locality (25°40′S; 28°30′E). Fathers were separated from the mothers before parturition to prevent post-partum insemination and a second pregnancy. In total, 20 litters were used in the analysis: in 10 of these litters both parents were non-stereotypic, and in 10 litters the mother was stereotypic and the father non-stereotypic; Jones et al. (2008) describe in detail how the stereotypic status of the striped mice was established. In each litter, pairs of offspring were randomly selected for weaning (defined here as physical separation from the mother) at days 12 (2/litter), 16 (2/litter), or 20 (2/litter), with weaning at day 16 corresponding with the typical weaning age for striped mice in the field (Brooks, 1982). Within litters, sex ratio was not always equally balanced but, across litters, males and females were equally represented in each treatment group. After weaning, striped mice were housed in sibling pairs until day 60. Stereotypic status (stereotypic; non-stereotypic) was assessed between days 50 and 60 using daily direct observations, for 15 min, over five non-consecutive days (Jones et al., 2008), and the proportion calculated of offspring in each sibling pair developing stereotypic behaviour. Stereotypic behaviours observed were all locomotor, and included circuit-running, somersaulting, cage-climbing, and jack-hammering. These forms of abnormal behaviour were regarded as stereotypic if the behaviour comprised at least three repetitions in succession (see Jones et al., 2008). Mass at weaning was also recorded but, because striped mice in each sibling pair were not individually marked at weaning, we cannot link individual weaning mass to stereotypic status in adulthood.

3.1.2. Data analysis

To assess the effect of weaning age on the development of stereotypic behaviour, we used a general linear model (GLM) with maternal stereotypic status as a categorical predictor, weaning age as the repeated measure, and the proportion of offspring (in each of the three paired sibling pairs, from each of 20 litters) developing stereotypic behaviour as the dependent variable. Proportion data were arcsine square root transformed prior to analysis (Zar, 1996). Tukey *post hoc* tests were used to identify specific differences when $\alpha \leq 0.05$. All analyses were performed using Statistica 8.0 (Statsoft Inc. 2007). Because mass at weaning can influence the development of stereotypic behaviour (Würbel and Stauffacher, 1997), we also present summary data (mean + SEM) for the averaged mass of individuals within each sibling pair at weaning on days 12, 16, and 20 in relation to the range of weaning mass observed in free-living striped mice.

3.2. Results

Weaning age predicted stereotypic behaviour development ($F_{2,36} = 11.26$, $P < 0.001$), with striped mice weaned at 12 days far more likely to develop stereotypic behaviour as mice weaned at either 16 or 20 days ($P < 0.001$) (Fig. 1). Maternal stereotypic status, too, was a significant predictor of offspring stereotypic behaviour development

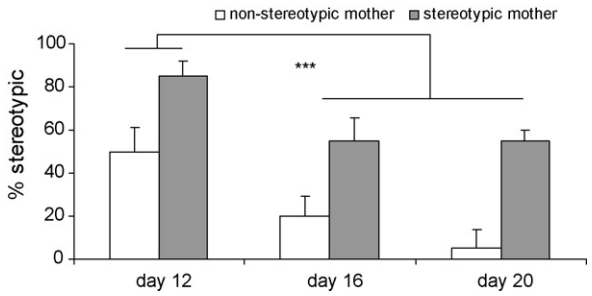

Fig. 1. The incidence of stereotypic behaviour (mean + SEM) in offspring pairs weaned at days 12, 16, or 20 from non-stereotypic (white bars) and stereotypic (grey bars) mothers. At each weaning age, offspring from non-stereotypic mothers were less likely to be stereotypic than were offspring from stereotypic mothers. $^{***}P < 0.001$.

($F_{1,18} = 24.22$, $P < 0.001$) with offspring of stereotypic mothers more likely to develop stereotypic behaviour than offspring of non-stereotypic mothers (Fig. 1). The interaction between maternal stereotypic status and weaning age was not a significant predictor of the incidence of stereotypic behaviour. As would be expected, mass at weaning was higher in older animals and, for mice weaned at days 16 or 20, was at least as high as is typically recorded in free-living animals (Fig. 2).

4. Uniparental v. biparental care

4.1. Methods and materials

4.1.1. Procedure

Subjects used in this analysis were offspring from a separate group of G1–G4 captive-born breeding pairs (32 pairs) used as part of various socio-ecological studies investigating, *inter alia*, the effect of the father on offspring physical development (Schradin and Pillay, 2003; Nel, 2003). The founder stock also originated from a Highveld grassland. Breeding pairs had produced at least one previous litter. Fathers were all non-stereotypic, whereas half of the mothers were stereotypic, and half non-stereotypic. The first litter used for study was raised by both parents (biparental care). Since *Rhabdomys* show post-partum oestrus, females were inseminated soon after the birth of their first litter. Fathers were separated from the females when the first litter was weaned at 16 days and thus, for the second litter (born 7–11 days after the first litter was weaned), young were raised by the mother alone (uniparental care). After weaning at 16 days, offspring were housed in same-sex sibling groups. Stereotypic status of the offspring (stereotypic; non-stereotypic) was assessed between days 50 and 60, as described for the first analysis, and the proportion calculated of offspring in each litter (male and female combined) developing stereotypic behaviour.

4.1.2. Data analysis

To assess the effect of the presence of the father on the development of stereotypic behaviour, we used a GLM with maternal stereotypic status as a categorical predictor, parental care type (uni- v. biparental) as a repeated measure, and the proportion of offspring in each litter

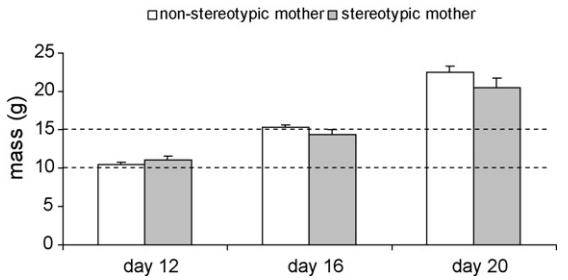

Fig. 2. Mean mass at weaning (+SEM) of offspring pairs weaned at days 12, 16, or 20 from non-stereotypic (white bars) and stereotypic (grey bars) mothers. The dashed lines indicate weaning mass range for free-living striped mice (~day 16; Brooks, 1982).

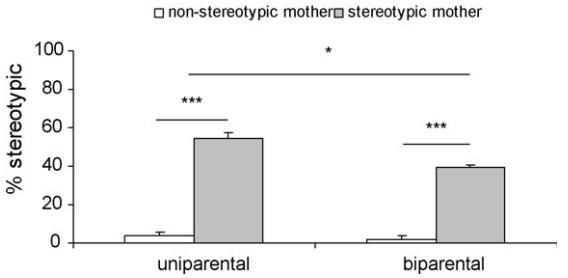

Fig. 3. The incidence of stereotypic behaviour (mean + SEM) in litters from a stereotypic or non-stereotypic mother receiving either uniparental or biparental care. In each parental care type, offspring from non-stereotypic mothers were less likely to be stereotypic than were offspring from stereotypic mothers. $^{*}P < 0.05$; $^{***}P < 0.001$.

($n = 32$ paired litters in each parental care type) developing stereotypic behaviour as the dependent variable. Data were arcsine square root transformed prior to analysis (Zar, 1996). Tukey *post hoc* tests were used to identify specific differences when $\alpha \leq 0.05$. All analyses were performed using Statistica 8.0 (Statsoft Inc. 2007).

4.2. Results

Parental care type significantly predicted stereotypic behaviour development ($F_{1,30} = 5.14$, $P = 0.03$), with young raised by both parents showing a lower incidence of stereotypic behaviour than young raised by mothers alone (Fig. 3). Maternal stereotypic status was again a significant predictor of offspring stereotypic behaviour development ($F_{1,30} = 326.23$, $P < 0.001$) with offspring of stereotypic mothers more likely to develop stereotypic behaviour than offspring of non-stereotypic mothers. As was the case with weaning age, there was no significant interaction between maternal stereotypic status and parental care type.

5. Discussion

The results from our retrospective analyses support our prediction that early social experiences, specifically those relating to weaning age and the absence or presence of the father, have significant effects on the development of stereotypic behaviour in later life. They also show that in striped mice, experiential and genetic effects seem to combine in an apparently additive way to produce the stereotypic phenotype.

5.1. Weaning age

As predicted, early weaning at day 12 predisposed striped mice to the development of stereotypic behaviour. Although these early weaned striped mice were able to feed on solid food and move independently from their mother, in both the field and in captivity they would have continued to suckle and be in close contact with their mothers until at least 16 days (Brooks, 1982). We had expected that slightly delayed weaning (days 20 v. 16) would protect against stereotypy development, given that pups in nature usually experience some interaction with their mothers (if not suckling) after their initial dispersal at 16 days (Willan and Meester, 1989), but this was not supported in our analysis. Future work should investigate whether (1) a more extended period of contact between mothers and pups is necessary (which more closely approximates the 25-day contact period in the wild before the next litter is born, after which contact is minimal despite juveniles remaining in the natal territory; Willan and Meester, 1989) to protect striped mice from the development of stereotypic behaviour; and/or (2) delayed weaning might be of less benefit to offspring when it extends beyond the natural weaning period for the species (c.f. delaying the weaning age in a standard mouse facility in which separation is prior to natural weaning and dispersal age; Bechard et al., submitted for publication).

In laboratory mice, Würbel and Stauffacher (1997) demonstrated that not only weaning age, but also developmental stage at weaning, is a predictor of stereotypic behaviour development, with mice of lighter mass more likely to develop stereotypic behaviour than heavier animals—potentially because the stress involved in breaking the maternal bond is increased in those individuals more likely to experience a greater fitness cost. Indeed, young striped mice in our study on day 12 were, as expected, lighter than at day 16, and lighter at day 16 than at day 20. Whilst it is possible that in striped mice maternal deprivation *per se* may play a greater role in stereotypic behaviour development than developmental immaturity (c.f. Latham and Mason, 2008), especially because laboratory conditions promote more rapid weight gain than in the wild (Pillay, 2000), further work is needed to tease apart the correlated effects of treatment (age at weaning) and body mass by looking at individual differences in mass at weaning and the subsequent development of stereotypic behaviour. In fact, the large mass difference in striped mice weaned at days 16 and 20, yet their similar susceptibility to the development of stereotypic behaviour, suggests that weaning mass does not determine vulnerability to stereotypic behaviour in a simple linear way.

A confound in this analysis is that striped mice from the same litter were weaned at different ages (see also Cook, 1999; Kikusui et al., 2007). Whilst such a method is desirable because it reduces the number of animals used, it confounds weaning age with a reduction in litter size. Future work is thus also required to replicate this finding

when isolating the effect of weaning age from that of reduced litter size and the loss of siblings—especially since there is a known link in this species between stereotypic behaviour and large litter sizes (Jones et al., in press).

5.2. Uniparental v. biparental care

To our knowledge, this is the first study to suggest an effect of biparental care on the development of stereotypic behaviour. Whilst subjects used in this study originated from a population (Highveld grasslands) in which paternal care does not occur in nature, this population shows highly developed paternal care in the laboratory (Schradin and Pillay, 2003). Biparentally reared striped mouse pups thus likely receive greater parental contact, including licking and grooming, than pups would receive from their mothers alone, although they do not, in the laboratory, show more rapid growth or development (Schradin and Pillay, 2003; Rymer, 2009). Since greater maternal investment is associated with reduced stress responsiveness in offspring (Caldji et al., 2000), the provision of higher levels of total parental care thus seems a likely mechanism through which the incidence of stereotypic behaviour in striped mice is reduced in litters raised by both parents.

Two confounding variables, because of the unbalanced nature of the experimental design, weaken our interpretation of these results: (1) Females were pregnant whilst rearing the first, but not second litter: pregnancy whilst lactating can sometimes reduce maternal care (e.g. in mice, König and Markl, 1987) which we would expect to increase the incidence of stereotypic behaviour, and which would thus have reduced, and not inflated, the significance of our result; and (2) mothers in their second parity were subject to removal of the father before birth, which could potentially induce stress (although this is less likely to occur in striped mice than in monogamous species as free-ranging individuals do not establish pair bonds). Nonetheless, future work should attempt to confirm the effect of paternal care whilst controlling for pregnancy status and the potential stress of separation.

More generally, we need now to investigate the ways in which life experiences either predispose or promote stereotypic behaviour in later life. Candidate mechanisms for such epigenetic influences include (1) dysregulation of behavioural control as a consequence of adverse events during early experience canalising dysfunctional brain development (e.g. Lewis et al., 2006), and/or (2) increasing stress responsiveness and/or the performance of source behaviours as a result of the lack of paternal input/early separation from the mother (e.g. Würbel and Stauffacher, 1997), mechanisms potentially mediated by the quality or quantity of parental contact received by pups. The stress-related or welfare correlates of these early social experiences particularly merit future research since the effects of these events do not necessarily co-vary with the expression of stereotypic behaviour (Mason and Latham, 2004) but, similarly to stereotypic behaviour, have bearings on animal well-being and the validity of research (Jones et al., in preparation).

6. Conclusion

Our findings add to evidence that early social experience can have lasting effects on adult abnormal behaviour, and suggest that *Rhabdomys* provide a promising model for investigating the effects of weaning age and paternal care on the development of stereotypic behaviour. In this species, genetic predisposition combines with early social experience. We suggest that future prospective studies control for the confounding issues discussed above and further investigate the age when captive striped mice would chose voluntarily to leave the nest, in the presence and the absence of the father, and in relation to developmental stage, and whether the presence of the father would be protective against the development of stereotypic behaviour in early weaned striped mice. Future work should also investigate the mechanisms through which weaning age and paternal care (and/or their correlates in the experiments reported here) modify the development of stereotypic behaviour, including whether amounts of parental care received per pup mediate these effects, and whether increases in stereotypic behaviour are paralleled by increases in stress responsiveness and/or brain dysfunction.

Acknowledgements

Funding was provided by the National Research Foundation (NRF; grant number: 2069110) and the University of the Witwatersrand. MJ was partially supported whilst writing this paper by an NRF Scarce Skills Scholarship. GM was funded by an NSERC Canada Research Chair. Appreciative thanks to Lis Jones for her helpful proofreading and to two anonymous reviewers for their valuable comments on an earlier draft of this manuscript. Approval for these studies was provided by the Animal Ethics Screening Committee of the University of the Witwatersrand.

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CHAPTER FIVE

Correlates of birth origin effects on the development of stereotypic behaviour in striped mice, *Rhabdomys*

Megan A. Jones, Georgia J. Mason, Neville Pillay (2011)

Animal Behaviour, **82**, 149-159

Animal Behaviour 82 (2011) 149–159

Contents lists available at ScienceDirect

Animal Behaviour

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

Correlates of birth origin effects on the development of stereotypic behaviour in striped mice, *Rhabdomys*

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ARTICLE INFO

Article history:
Received 6 October 2010
Initial acceptance 16 November 2010
Final acceptance 15 April 2011
Available online 17 May 2011
MS. number: 10-00681R

Keywords:
animal welfare
birth origin
captive breeding
Rhabdomys
stereotypic behaviour
striped mouse
wild animal

The behaviour and physiology of wild animals born in zoos, laboratories and breeding centres can differ substantially from that of their wild-caught (WC) conspecifics. For instance, captive-born (CB) animals are typically more prone to developing abnormal repetitive behaviours. In captive striped mice, *Rhabdomys*, we first confirmed that birth origin predicted the emergence of stereotypic behaviour (SB), with CB mice being most at risk. Second, to investigate correlates of this birth origin effect, we tested WC and CB striped mice in behavioural tasks to quantify fear/anxiety, activity and perseveration, and measured faecal corticosterone to assess physiological stress. WC mice proved more fearful and less active than CB animals, and had higher levels of faecal corticosterone metabolites. These effects, however, were unrelated to SB. WC mice were also less perseverative and more behaviourally flexible than CB animals, traits that covary with SB. Third, a retrospective analysis of laboratory records showed that SB incidence was significantly lower in adult-caught than juvenile-caught striped mice, with juvenile males being the most severely affected by early removal from the wild. In conclusion, our results indicate that adult, but not juvenile WC striped mice are typically protected against SB development in captivity, despite having poorer welfare than their CB conspecifics. They also reveal profound behavioural changes in CB mice, changes suggestive of altered forebrain function, a hypothesis now needing direct testing.
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Wild animals are often bred in research laboratories, zoos and conservation centres. Several recent studies show that captive-born (CB) animals may differ behaviourally and physiologically from their wild counterparts, a potential problem when the aim of captive breeding is to conserve or study wild phenotypes. Some observed differences are unsurprising: CB animals may lack the experience to perform certain natural behaviour patterns competently (e.g. golden-lion tamarins, *Leontopithecus rosalia*: Kleiman et al. 1990; black-footed ferrets, *Mustela nigripes*: Biggins et al. 1999; bank voles, *Clethrionomys glareolus*: Mathews et al. 2005), and they are also typically less scared of humans than captive wild-caught (WC) conspecifics (e.g. black rhinoceros, *Diceros bicornis*: Carlstead et al. 1999; capybaras, *Hydrochoerus hydrochaeris*: Nogueira et al. 2004; starlings, *Sturnis vulgaris*: Feenders & Bateson 2011). Probably as a consequence of their reduced fear, CB animals also generally appear to have better welfare in captivity: for example, compared with WC conspecifics, CB pigtailed macaques, *Macaca nemestrina*, show reduced mortality after a stressor (Ha et al. 2000). Other differences between WC and CB animals, however, are somewhat counterintuitive: compared with captive WC conspecifics, the offspring of CB mongoose lemurs, *Lemur mongoz*, have greater mortality (Perry et al. 1992); female CB white rhinoceroses, *Ceratotherium simum simum*, often fail to conceive (Swaisgood et al. 2006); and zoo-housed CB Asian elephants, *Elephas maximus*, are likely to die prematurely (Clubb et al. 2008). These examples indicate that birth origin can have dramatic effects, both positive and/or negative, on the phenotypes of captive animals.

Birth origin also has a striking influence on the development of highly repetitive stereotypic behaviours (SBs) such as pacing or body rocking. Although SBs afflict at least 10 000 captive zoo animals worldwide (Mason et al. 2007), in eight of the 11 species studied to date they are rare or absent in WC individuals, and more common in conspecifics born in captivity (Mason 2006; Latham & Mason 2008). The hypothesized causes of SBs are two-fold. First, SBs may arise from poor adjustment to impoverished captive conditions, resulting in the sustained elicitation of highly motivated, but ultimately thwarted (i.e. frustrated), natural behaviour patterns (hereafter 'source behaviours'; frustrated motivation

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doi:10.1016/j.anbehav.2011.04.010

hypothesis). For example, bar chewing in laboratory mice, *Mus musculus*, derives from repeated attempts to escape (Nevison et al. 1999), whereas stereotypic digging in gerbils, *Meriones unguiculatus*, is triggered by the lack of a species-typical tunnel-shaped entrance to a nesting chamber (Wiedenmayer 1997). Second, SBs can arise secondarily to changes in areas of the forebrain, especially the neural pathways between the cortex and the basal ganglia, responsible for behavioural flexibility and for the inhibition of inappropriate and unsuccessful responses (abnormal behavioural control hypothesis; e.g. Garner 2006; Lewis et al. 2006; Graybiel 2008; Latham & Mason 2010). Thus in a number of species, a relationship has been found between individual levels of SB and increased 'perseveration' ('the continuation or recurrence of an…activity without the appropriate stimulus'; Sandson & Albert 1987, page 1736) and reduced behavioural flexibility (e.g. Garner & Mason 2002). Furthermore, treatments that induce SB (e.g. deprivation rearing or dosing with psychostimulants) correspondingly induce both perseveration and changes to these brain regions (e.g. Robbins et al. 1990; Lewis et al. 2006; Latham & Mason 2008). The likely causes of low/absent SB are therefore low motivational frustration, leading to the lack of repetition of source behaviours, and/or a well-functioning, species-typical forebrain that permits the ready inhibition of inappropriate behaviours. Both of these have been suggested to explain why SB is rarer in complex, naturalistic captive environments than it is in small barren cages (e.g. Latham & Mason 2010). However, a third reason has also been proposed to explain why some animals do not stereotype, even when they are kept in impoverished cages. Within these impoverished, SB-motivating environments, nonstereotypic individuals are generally atypically inactive (Meyer-Holzapfel 1968; Altman 1999), and also often seem to have poorer welfare than their stereotypic cagemates (Mason & Latham 2004). This suggests that in adverse captive conditions, inactivity is an alternative response to SB, perhaps because it represents hiding as a result of fear or excessive resting/sleeping secondary to 'apathy' (defined here as a lack of interest or motivation; Marin 1990).

How birth origin influences the development of SBs is unknown. However, the hypothesized reasons for low SB performance suggest that this birth origin-induced variation is mediated by experience-dependent changes in (1) forebrain structure and function (affecting abilities to inhibit inappropriate behaviours); (2) motivational systems (affecting the degree to which natural behaviours are frustrated and/or animals are fearful or apathetic); and/or (3) the extent of animals' fearfulness or apathy (affecting the level of hiding behaviour and inactivity). This gap in our knowledge reflects the type of previous investigation into CB–WC differences in SB. Previous reports have been either serendipitous findings from studies in which the main focus was not SB, or have comprised findings from the retrospective analysis of multizoo data of animals spread over numerous sites (Mason 2006). As a result, none have allowed investigation into the precursors or correlates of the influence of birth origin on SB in a way that could shed light on underlying mechanisms. In this study, using the striped mouse *Rhabdomys*, we had the unique opportunity to compare WC and CB conspecifics kept as study subjects in the same laboratory. These small, diurnal murid rodents are abundant in many southern African habitats (Skinner & Chimimba 2005); they are not endangered, they offer the typical advantages of a rodent species (e.g. small body size, successful reproduction in captivity and short generation times; Schradin & Pillay 2003) and, because they are diurnal (Schradin 2006), are easy to observe and are not prone to sleep disruption when tested during the day. The striped mouse is a particularly good model for studying birth origin effects on SB as about half of all CB striped mice reared in standard cages develop locomotor SBs (Schwaibold & Pillay 2001; Jones et al. 2008, 2010a, b). This incidence of SB is also similar to that reported in a number of zoo species (e.g. brown bears, *Ursus arctos*, 48%; clouded leopards, *Neofelis nebulosa*, 49%; reviewed in Mason et al. 2007), as is the proportion of time that stereotypic striped mice engage in SBs (about 50% of active time in striped mice [Nel 2003] compared with 48% in lions, *Leo panthera*, and 52% in spectacled bears, *Tremarctos ornatus* [reviewed in Clubb & Mason 2007]). The current study comprised three experiments in which we assessed whether WC striped mice are less stereotypic than CB animals (experiment 1), explored correlates of birth origin and SBs as preliminary investigations into potential mechanisms (experiment 2), and analysed historical data to identify any constraints on the protective effects of being WC (experiment 3).

GENERAL METHODS

All wild striped mice were trapped using PVC live traps (290 × 60 mm and 70 mm high) which were covered with grass for insulation, and set for 4 consecutive days. Traps were baited with half a handful of a mixture of oats, raisins, salt, peanut butter and vegetable oil, and contained water-moistened cotton wool for hydration and dry cotton wool for bedding. Traps were checked both early morning and late afternoon, immediately after the peak activity of *Rhabdomys*, ensuring that caught individuals were unlikely to spend more than 2 h in the traps. No trap deaths were recorded. Following capture, individuals were transferred into holding cages (200 × 150 mm and 150 mm high, containing wood shavings for bedding, a handful of hay for nesting material, and provisioned with mouse cubes and water bottles), and then transported by road to the University of the Witwatersrand. Thereafter, adults were housed individually (except during breeding) as wild grassland striped mice are naturally solitary living (Schradin & Pillay 2005), and the tendency to fight often precludes the caging of same-sex groups after weaning age (M. Jones, personal observations). Striped mice used in these studies were ultimately euthanized using either an overdose of an inhalant anaesthetic (Isoflurane or Halothane) or via carbon dioxide asphyxiation. Approval for all studies was provided by the University of the Witwatersrand's Animal Ethics Screening Committee.

EXPERIMENT 1: WC VERSUS CB MICE

In this first experiment, we compared the incidence of SB in a cohort of WC striped mice to a randomly chosen subsample of their first-generation CB offspring. Because previous work in striped mice has suggested that SB is genetically based, and also may be selected for over generations in captivity (Jones et al. 2010b), we used only F1 CB individuals to maximize the genetic similarity between WC and CB animals (so allowing us to distinguish between environmental and genetic effects). We predicted that WC striped mice would be less likely to develop SBs than their CB offspring.

Methods

Study subjects

WC striped mice (males: $N = 11$; females: $N = 15$; all adults at the time of capture) were trapped in a grassland locality (Honeydew; Gauteng; 27°55′S, 26°4′E) between July 2006 and May 2007 as part of ongoing studies into SB in captive wild animals. The comparison group of CB striped mice was a randomly selected group of the F1 offspring bred from 15 different WC breeding pairs (males: $N = 36$; females: $N = 34$) for use in other behavioural studies.

Housing and husbandry

Striped mice were housed in the Milner Park Animal Unit at the University of the Witwatersrand, under partially controlled environmental conditions (14:10 h light:dark, lights on at 0500 hours; 20–24 °C; 30–60% relative humidity). All striped mice were kept singly in standard Labotec cages (300 × 200 mm and 150 mm high) from the time of capture (WC) or from shortly after weaning (CB). The cages contained about 2 cm of wood shavings as bedding, a PVC tubing nestbox (10 × 10 cm and 15 cm high, open at both ends) provisioned with hay and paper towel as nesting material, and one cardboard toilet roll. Mouse cubes and water were provided ad libitum. A small amount (±3 g) of seed mix (sunflower, millet) was sprinkled in the cage daily, together with fresh fruit and/or vegetables (±10 g).

Procedure

The behaviour of the WC mice was assessed twice, 1 month and 5 months after capture, using direct behavioural observations. Each assessment covered 5 days, and involved one 15 min observation session per day. We defined SB as a repetitive behaviour comprising at least three successive repetitions (see Table 1 for definitions of the various forms of SBs observed; also Jones et al. 2010a). Striped mice consistently displaying SB (i.e. the behaviour was observed in at least three of the five observation sessions) were classed as stereotypic, whereas those displaying no SB were classed as nonstereotypic (see Jones et al. 2010a for a rationale of this dichotomous scoring method, which was the standard protocol in this laboratory). CB striped mice were similarly observed for SB over two 5-day periods, 1 month after being housed individually in Labotec cages (i.e. at 2 months of age; young adulthood), and again when 7 months old.

Statistical analysis

We examined the effect of birth origin on SB using a logistic regression with a binomial logit function (Statistica 8.0, Statsoft Inc., Tulsa, OK, U.S.A.). We included birth origin and sex as categorical predictors, and SB status (stereotypic or nonstereotypic) as the dependent variable. In this and subsequent analyses, all tests were two tailed, and differences were considered significant when $\alpha \leq 0.05$.

Results

All striped mice that showed SB in the first observation period also showed SB during the second observation session. Similarly, all nonstereotypic striped mice identified as such in the first observation period remained nonstereotypic when observed for a second time. SB was thus stable over the 5-month period of data collection, validating our scoring system. Birth origin was a significant predictor of the incidence of SB (Wald $\chi^2_1 = 11.633$, $P < 0.001$), with WC striped mice showing substantially less SB (1/26; 4% stereotypic) than their CB counterparts (40/70; 57%). We found no effect of sex on the incidence of SB (Wald $\chi^2_1 = 0.221$, $P = 0.638$).

Discussion

As predicted, first-generation CB striped mice were substantially more likely to be stereotypic than their WC counterparts, confirming that striped mice are a suitable model for studying the influence of birth origin on SB development. In addition, the observed consistency of SB over time (i.e. after 1 month and 5 months of individual housing in Labotec cages) in both CB and WC individuals indicates that the manifestation of SB during the first observation session was not simply a transient differential reaction to a recent change in housing conditions, but a stable trait. Next, in experiment 2, to explore the association between birth origin and SB, we studied a subsample of these CB and WC subjects in more detail, investigating (1) group differences in behaviour and physiology and (2) individual correlates of SB performance.

EXPERIMENT 2: BIRTH ORIGIN CORRELATES OF SB

The suggested biological causes of low SB (see Introduction) indicate three main mechanisms that could underpin the diminished incidence of SB in WC striped mice. In turn, these potential mechanisms predict different correlates of the low SB of WC animals. Here, we therefore collected various cross-sectional behavioural and physiological data to investigate the correlates suggested by the following three hypotheses: WC animals seldom stereotype because (1) subjects maturing in the wild have more flexible behaviour and are less perseverative; (2) WC individuals adjust better to captivity than CB conspecifics and are less frustrated by its confines; and/or (3) WC individuals adjust poorly to captivity, but express this as inactivity.

Over the last decade, noninvasive behavioural paradigms have been used with good success to quantify behavioural repetition in stereotypic subjects, and in groups of animals subjected to SB-enhancing treatments such as nonenriched housing (e.g. bank voles: Garner & Mason 2002; songbirds: Garner et al. 2003; black bears, *Ursus americanus*, and sun bears, *Ursus malayanus*: Vickery & Mason 2003; parrots: Garner et al. 2003; and deer mice, *Peromyscus maniculatus*: Tanimura et al. 2008). These methods have included 'gambling' (guessing) tasks, and reversal and extinction learning procedures. However, many of these procedures require subjects to undergo extensive operant training, to which striped mice are poorly suited: a large proportion of animals (especially nonstereotypic or WC individuals) fail to complete sufficient trials for initial learning to occur (N. Pillay, personal observations, unpublished data), and furthermore, human handling and contact may well be a differential stressor for WC and CB animals, thus acting as a confound. To quantify behavioural flexibility/perseveration, we therefore used the four-arm maze (e.g. Lalonde 2002), a behavioural test that does not depend on previous training. Analysis of arm entry sequence in a four-arm maze (or a T-maze) is typically measured by scoring spontaneous alternation behaviour (SAB; defined fully in Methods), a commonly used index of perseveration in assessing obsessive compulsive disorder (OCD)-like phenotypes

Table 1
Form and definition of stereotypic behaviours observed in striped mice

Form of stereotypic behaviour	Definition
Cage climbing	Climbing on the cage lid, using either forelimbs or fore- and hindlimbs
Circuit running	Running in the cage along a fixed route (frequently in a figure-of-eight pattern)
Jack hammering	Jumping up and down on the hindlimbs, usually in a corner of the cage or, if a nestbox is present, on and off the nestbox
Prelooping	Climbing upside down on the cage lid with forelimbs and then hindlimbs, moving backwards along lid, then dropping down by releasing the hindlimbs first
Somersaulting	Backward flipping, with or without touching the cage lid
Windscreen wiping	With hindpaws stationary or moving only slightly on the cage floor, the forelimbs oscillate to-and-fro in a large arc against the cage walls

(e.g. Yadin et al. 1991; Ulloa et al. 2004) that are characterized by perseverative deficits in inhibitory control (Joel et al. 2008). SAB is also sensitive to the effects of lesions in corticostriatal circuitry (Divac et al. 1975; Delatour & Gisquet-Verrier 1996) and to the administration of drugs (e.g. Quinpirole) that are standard pharmacological models of OCD in rodents (Joel et al. 2008). In addition to scoring SAB, we used sequential analysis (SA) to assess the pattern of arm entries in the maze because, although SAB detects whether individuals perseverate in their arm choice rather than alternating among the different arms, it does not assess whether an animal predictably enters a particular arm in a regularly repeated sequence.

We made use of a second behavioural test, the light–dark box, to assess fear/anxiety. Here, the proportion of time spent in the dark compartment and the latency to emerge from the dark compartment after first entry into it are commonly used indexes of rodent anxiety (Belzung & Griebel 2001; Bourin & Hascoët 2003), correlating closely with scores from other behavioural measures of anxiety (e.g. open field, Kim et al. 2002). Pharmacological studies show that the tendency to prefer the dark compartment and the latency to emerge from it are reduced by anxiolytic drugs and potentiated by anxiogenic drugs (Imaizumi et al. 1994). We also collected faecal samples to measure circulating levels of the stress hormone corticosterone while striped mice were in their home cages. Faecal corticosterone levels are an accepted measure of hypothalamic – pituitary–adrenal (HPA) axis activity, with high levels often reflecting increased stress/fear (Touma & Palme 2005). Faecal sampling has fewer welfare concerns than blood sampling because it is noninvasive (Touma et al. 2003) and, in striped mice, faecal pellets are easier to collect than urine samples. Faecal sampling also gives a picture of the levels of free, unbound hormone over a period of several hours (e.g. Touma et al. 2003, Touma & Palme 2005). Finally, we assessed levels of activity in the home cage, as well as in the four-arm maze (centre crossings; e.g. Hliňák & Krejči 2006). Previous studies have reported a link between hyperactivity and SB in bank voles (Garner & Mason 2002), while hypoactivity can potentially reflect hiding behaviour and/or apathy (Meyer-Holzapfel 1968; Broom & Johnson 1993).

We made three predictions. (1) If forebrain function were altered in CB individuals in the same way as in deprivation-reared primates and nonenriched-reared deer mice (see Introduction), we would predict (1a) higher SAB scores and (1b) lower SA scores in WC compared with CB animals (both measures reflecting lower perseverative tendencies). Furthermore, if reduced perseveration and low SB were to share a common underlying mechanism, we would predict higher SAB scores and lower SA scores in nonstereotypic striped mice, for these traits to covary, and for any birth origin effect on SA and SAB to vanish if SB were controlled for in the model. (2) If WC striped mice were better adjusted to captivity than CB animals, we would expect lower levels of fear/anxiety and hiding: individuals would (2a) spend more time in the light compartment of the light–dark box, (2b) show shorter latencies to re-enter the light compartment after their first entry into the dark compartment, (2c) have reduced corticosteroid levels and (2d) spend less time in their nestboxes during peak activity times. Furthermore, if these effects were related to the lower incidence of SB in WC mice, we would expect reduced fear/anxiety in all nonstereotypic individuals. (3) In contrast, if aversive emotional states were suppressing SB by inducing inactivity, we would expect WC animals to be more fearful/anxious and less active (corollaries of predictions 2a–c above) and, again, if such responses were related to the low SB of WC mice, we would also predict nonstereotypic animals in general to have higher anxiety, lower activity and to spend more time hiding/being inactive. Similar to prediction (1), if either predictions (2) or (3) were correct, we would also expect that any birth origin effects on these variables would disappear if we included SB as a categorical predictor in the statistical model.

Methods

Study subjects and housing

First, to assess group differences in behaviour and physiology between WC and CB striped mice, we used all 26 WC striped mice from experiment 1, and a random subsample of 14 of their F1 CB offspring (eight stereotypic, six nonstereotypic; data set 1). This data set, however, did not allow us to tease apart the relative correlates of birth origin and SB because only one WC mouse was stereotypic. We therefore created a second data set, for which we generated a larger sample of WC individuals that included all striped mice trapped subsequent to the first cohort and that developed SB ($N = 4$). This data set, 2, thus comprised five stereotypic and 25 nonstereotypic WC animals. Sample sizes were ultimately unbalanced as, while we sought to use all available animals, we were also constrained by the number of individuals that were available to keep for prolonged periods in captivity and/or not being used in other projects, as well as the number of suitable WC animals trapped after experiment 1.

We collected all data when WC mice were about 15 months old (based on their body size, we assumed that WC individuals were on average 3 months old when captured), and when the age of CB mice ranged between 13 and 16 months. Housing conditions were identical to those described in experiment 1.

Procedure

Behavioural data were collected from testing in two apparatuses (four-arm maze and light–dark box) and from home cage observations. Test sessions took place between 0700 and 1100 hours, during which time *Rhabdomys* is most active (Pillay 2000). A minimum of 24 h was allowed between sessions. All apparatuses were illuminated from above with fluorescent lighting, and the sessions were recorded by a video camera positioned directly above the apparatuses or to the side of the home cage. The apparatuses were cleaned between tests, using soap and water. Video recordings were scored using Observer 5.0 (Noldus Information Technology, Wageningen, The Netherlands).

Four-arm maze. The four-arm maze consisted of four enclosed arms (7.5×7.5 cm and 15 cm high), constructed from clear PVC, connected to a central area (10×10 cm and 20 cm high). A subject was placed into the central area, and its behaviour recorded for 10 min. The frequency of arm entries was recorded as a measure of locomotor activity. We followed the methods of Hliňák & Krejči (2006) to calculate spontaneous alternation scores, defining an SAB score for a series of four-arm entries (a tetrad) as the ratio of actual arms entered to the possible number of arms that could have been entered. Thus, in the arm entry sequence 12342…234, an alternation score for each tetrad was calculated as follows: for the first tetrad (**1234**2…234), the mouse entered four different arms out of a possible four, giving an alternation score of $4/4 = 1$; for the second tetrad (1**2342**…234), it entered three arms out of a possible four, and hence scored $3/4 = 0.75$, with the last three entries of the sequence (12342…**234**) not being considered because these did not constitute a complete tetrad. Total SAB scores for the trial were calculated by averaging SAB scores across all tetrads in a sequence, with low overall scores representing a tendency to enter a more restricted number of arms and to make more repeat visits of the same arm.

We used sequential analysis to assess the predictability of a striped mouse entering a particular arm following entry into another particular arm. For example, a striped mouse might have

the sequence 1234123412341234, repeating the same pattern of arm entries, yet would be judged, using spontaneous alternation, as not perseverating (all tetrads would receive a score of 1). Sequential analysis, however, can detect this form of perseveration by assessing whether one behavioural element is more or less likely than chance to follow another behavioural element (see Van Hooff 1982). For each individual, the sequence of arm entries was coded into transition matrices with the current behavioural element (an entry into one of the arms) represented in the columns and the preceding arm entered represented in the rows. Using the software Matman (Noldus Information Technology), we calculated the adjusted residuals (i.e. differences between observed and expected values for each transition frequency) for each matrix and then used the generated χ^2 value for each matrix as an index of routine formation (the higher the χ^2 value, the more predictable a mouse's pattern of arm entry; thus unlike the SAB score, here high scores mean more predictable). One individual (WC, non-stereotypic female) was excluded from SAB and SA analysis because she remained in one arm for the entire trial, precluding score calculation.

Light–dark box. The light–dark box comprised a glass tank divided in half (each half 30 × 22.5 cm and 30 cm high with three ventilation holes in the Perspex lid); the two halves were connected by a 6 × 6 cm opening on the floor of the tank. One half of the tank, the dark compartment, had black walls and lid; the other half, the light compartment, had clear walls and lid. A subject was placed in the light compartment facing away from the opening into the dark compartment, and its behaviour recorded for 5 min. We scored the proportion of time in the dark compartment and the latency to return to the light compartment after first entry into the dark compartment.

Home cage observations. Subjects were videorecorded in their home cage for 40 min per day for 3 consecutive days. From the recordings, we observed the behaviour of subjects over two 10 min periods (at minutes 5–15 and 25–35), scoring the following behaviours: time spent in the nest (inside the nestbox, usually inactive, resting or sleeping); time spent outside the nest (activity outside the nestbox, excluding SB); and also total duration of SB (SB being defined in experiment 1). We did not consider inactivity outside the nestbox because this was very rarely seen. We also assessed the forms of SB shown by WC and CB striped mice since previous work in passerine birds has shown this to differ between CB and WC individuals (Keiper 1969).

Faecal corticosterone metabolites. Individual faecal samples were collected between 0800 and 1000 hours (timing of collection was standardized to minimize variation caused by fluctuation in circadian corticosterone production; Touma & Palme 2005). To collect a sample, a striped mouse was transferred from its home cage into a clean plastic container where it remained until five faecal pellets had been collected or 1 h had elapsed, whichever occurred sooner. The sampling period was time limited to avoid the stress of the procedure contaminating the sample (e.g. Touma et al. 2003, 2004; Latham & Mason 2010); rodent faecal samples usually represent plasma levels of unbound hormone at least 4 h earlier (Touma et al. 2003). The procedure was repeated the following week if insufficient pellets were collected. Samples were frozen immediately after collection, and later sent for analysis to Gordon Laboratories (Sedgefield, Stockton-on-Tees, U.K.). In total, samples were analysed for all 14 CB mice (eight stereotypic, six nonstereotypic) and 17 WC mice (five stereotypic, 12 nonstereotypic; samples were unfortunately not collected from the remaining 13 WC individuals since these animals had been euthanized prior to the approval of this part of the experiment).

Data analysis

Data were analysed in general linear models (GLMs) or generalized linear models (GLZ). For data set 1, we compared group differences in behaviour and physiology using logistic models with birth origin and sex as categorical predictors. To investigate individual correlates of SB (data set 2), we similarly used logistic models, but this time included birth origin, sex and SB status as the categorical predictors. For the quantitative SB data on home cage time budgets, further analyses were run to compare how time consuming SBs were in WC and CB striped mice (we included birth origin as the categorical predictor and how time-consuming SBs were in stereotypic striped mice as the dependent variable; non-stereotypic individuals were excluded) and to assess the relationship between our two measures of perseveration (SAB and SA) and how time consuming SBs were.

Results

Birth origin effects on behaviour and physiology

Summary data and results from statistical analyses of the two groups, WC and CB, in data set 1 are given in Table 2. Overall, CB striped mice were more perseverative than WC individuals: in the four-arm maze, birth origin was a significant predictor of SA scores, with CB individuals entering the arms in a more predictable sequence than WC animals (thus having higher scores). CB striped mice also showed a strong trend towards having lower SAB scores, indicating more repeated entries into a limited number of maze arms. We found no effect of sex on either SA or SAB scores.

Compared with CB striped mice, WC individuals spent more of the trial in the dark compartment of the light–dark box, and took longer to emerge from the dark compartment after first entry into it. Sex did not predict the proportion of time spent in the dark or of the latency to emerge therefrom. Corticosterone metabolite levels

Table 2
Behavioural and physiological results and analyses for CB and WC striped mice from data set 1

	Results: median value (1st and 3rd quartiles)		Analyses: Wald χ^2_1 (P)	
	CB (N=14)	WC (N=26)	Birth origin	Sex
SAB score	0.83 (0.79, 0.86)	0.87 (0.84, 0.88)	3.485 (0.062)	0.095 (0.758)
SA chi-square	25.00 (19.30, 29.90)	18.50 (16.30, 22.10)	**8.469 (0.004)**	0.738 (0.390)
Time in dark compartment (%)	47.70 (39.56, 52.67)	64.62 (52.21, 75.31)	**13.840 (<0.001)**	0.330 (0.565)
Latency to emerge (s)	14.78 (8.35, 21.81)	36.03 (24.53, 62.51)	**377.986 (<0.001)**	0.027 (0.869)
Faecal corticosterone (μg/ml)	7.52 (6.76, 8.08)	8.51 (7.95, 9.82)	**5.480 (0.019)**	3.710 (0.054)
Frequency of arm entries	75.50 (59.50, 111.75)	45.50 (32.00, 66.75)	**7.892 (0.005)**	0.050 (0.823)
Time in nestbox (%)	67.62 (35.62, 83.63)	98.40 (83.83, 100.00)	**7.434 (0.006)**	1.380 (0.240)
Home cage activity (%)	32.39 (16.37, 64.38)	1.56 (0.00, 16.16)	**7.434 (0.006)**	0.349 (0.560)

A low SAB score means more perseverative behaviour, whereas a low SA chi-square means less perseverative behaviour. Significant differences are indicated in bold type.

were significantly higher in WC than CB mice, and tended to be higher in females than in males. In the home cage, WC striped mice, irrespective of sex, spent more time in the nestbox. In both the home cage and the four-arm maze, WC individuals were also less active than CB individuals. Neither of the activity measures was influenced by sex, but time in the nest was significantly predicted by the proportion of time in the dark compartment of the light–dark box.

Individual correlates of SB

In the analyses of data set 2, we tested whether these between-group differences in perseveration, anxiety/fear and home cage activity were related to individual susceptibilities to stereotype. When we examined the concurrent effects of birth origin and stereotypy status on perseveration, the main effect of birth origin disappeared, and stereotypic status was a strong predictor of both SA (Wald $\chi^2_1 = 9.45$, $P = 0.002$) and SAB scores (Wald $\chi^2_1 = 10.99$, $P < 0.001$; Fig. 1a, b). In both cases, stereotypic individuals were more perseverative than nonstereotypic animals. The analysis of SA scores also revealed a significant interaction of birth origin and stereotypic status (Wald $\chi^2_1 = 4.25$, $P = 0.039$): while stereotypic mice always showed more predictable sequences of arm entry, this effect was particularly pronounced in WC stereotypic mice.

When quantitative SB time budget data were analysed (non-stereotypic striped mice being excluded from these analyses), birth origin was a significant predictor of the severity of SBs (Wald $\chi^2_1 = 8.01$, $P = 0.005$): the few stereotypic WC mice actually had more time-consuming SBs than WC conspecifics, spending around 76.32% (45.40, 77.26%; median, 1st and 3rd quartiles) of their time engaged in SB, compared with 23.93% (13.05, 31.51%) of stereotypic CB striped mice. SB forms observed in WC striped mice were also less diverse than in CB animals: all five WC individuals were circuit-runners, whereas stereotypic CB striped mice showed a greater intra- and interindividual diversity of SBs such as windscreen wiping and prelooping in addition to circuit running. The time spent stereotyping, like the incidence of SB, was predicted by SA scores (Wald $\chi^2_1 = 3.918$, $P = 0.048$) but not, however, by SAB (Wald $\chi^2_1 = 1.531$, $P = 0.216$). That is, the striped mice that had the most time-consuming SBs also had the highest SA scores, thus showing greater perseveration in the four-arm maze.

For measures of fear/anxiety, however, the main effect of birth origin persisted in data set 2 and with SB status as a categorical predictor: WC individuals thus spent a greater proportion of the trial in the dark compartment (Wald $\chi^2_1 = 7.88$, $P = 0.005$; Fig. 2a), took longer to emerge from the dark compartment after first entry into it (Wald $\chi^2_1 = 10.71$, $P = 0.001$; Fig. 2b) and, regardless of stereotypy status, had higher levels of faecal corticosterone metabolites (Wald $\chi^2_1 = 4.78$, $P = 0.029$; Fig. 3). Correspondingly, the time devoted to SB in stereotypic mice was also unrelated to these measures of anxiety/stress.

In contrast, activity level in the four-arm maze was significantly predicted by SB status in analyses of data set 2, with stereotypic animals, irrespective of birth origin, having a higher frequency of arm entries than nonstereotypic striped mice (Wald $\chi^2_1 = 9.12$, $P = 0.003$; Fig. 4a). Home cage activity was also similarly related to stereotypy status: both CB and WC stereotypic striped mice spent more time active than their nonstereotypic counterparts (Wald $\chi^2_1 = 18.00$, $P < 0.001$; Fig. 4b). In terms of nestbox use, stereotypic WC striped mice also spent the least amount of time in the nestbox, followed by stereotypic CB striped mice, nonstereotypic CB striped mice and, finally, nonstereotypic WC mice (birth origin*stereotypy status: Wald $\chi^2_1 = 7.73$, $P = 0.005$; Fig. 4c).

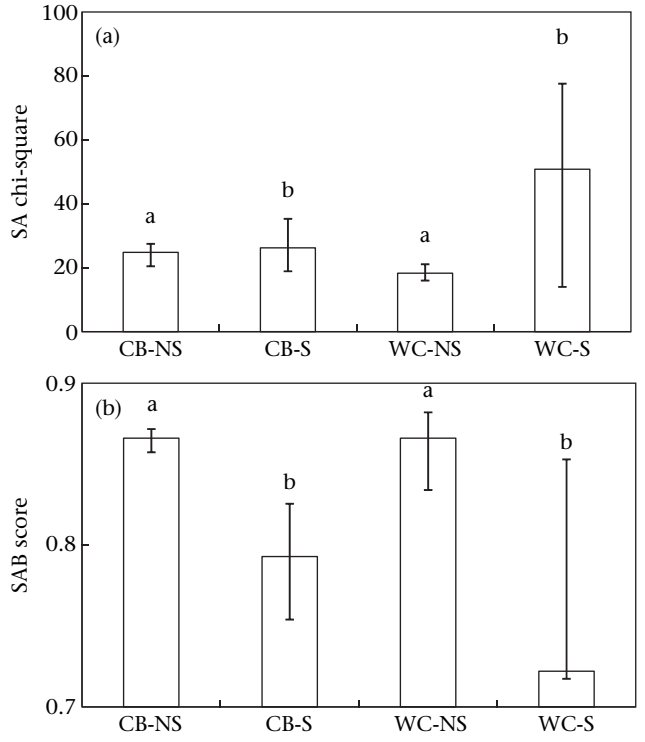

Figure 1. Perseveration in the four-arm maze. (a) Sequential analysis chi-square. (b) Spontaneous alternation score. CB = captive-born; WC = wild-caught; NS = nonstereotypic; S = stereotypic. The same alphabet letters denote groups that are not significantly different. Bars = median; whiskers = 1st and 3rd quartiles.

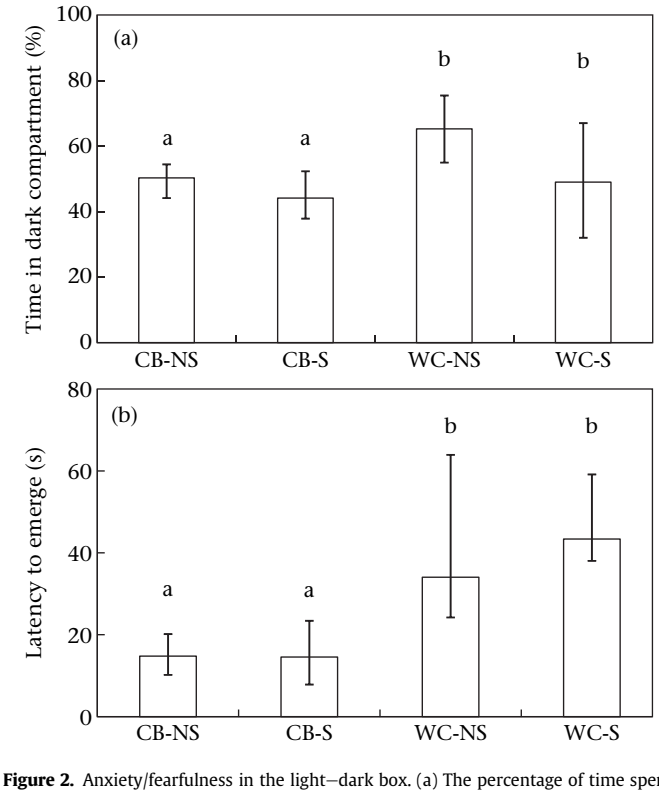

Figure 2. Anxiety/fearfulness in the light–dark box. (a) The percentage of time spent in the dark compartment. (b) Latency to first emergence from the dark compartment. CB = captive-born; WC = wild-caught; NS = nonstereotypic; S = stereotypic. The same alphabet letters denote groups that are not significantly different. Bars = median; whiskers = 1st and 3rd quartiles.

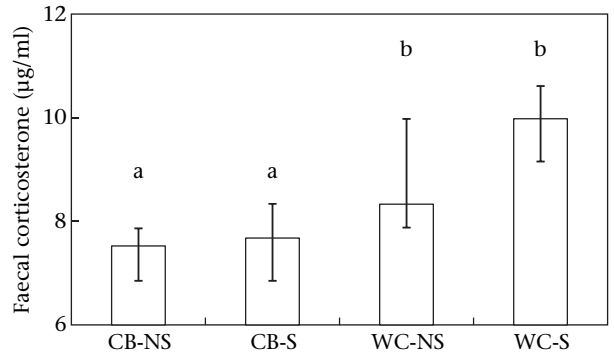

Figure 3. Faecal corticosterone. CB = captive-born; WC = wild-caught; NS = nonstereotypic; S = stereotypic. The same alphabet letters denote groups that are not significantly different. Bars = median; whiskers = 1st and 3rd quartiles.

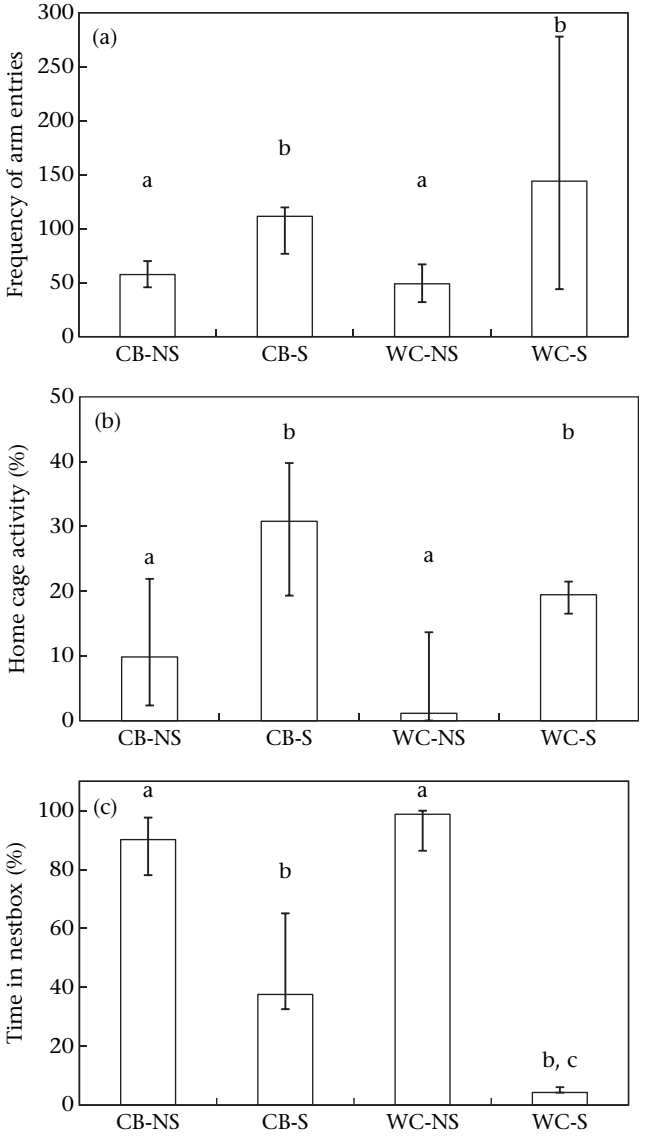

Figure 4. Measures of activity. (a) Frequency of arm entries in the four-arm maze. (b) The percentage of active time in the home cage (median, 1st and 3rd quartiles). (c) The percentage of home cage time in the nestbox (median, 1st and 3rd quartiles). CB = captive-born; WC = wild-caught; NS = nonstereotypic; S = stereotypic. The same alphabet letters denote groups that are not significantly different. Bars = median; whiskers = 1st and 3rd quartiles.

Discussion

On a group level, the behavioural and physiological profiles of WC and CB randomly selected striped mice differed in several ways. WC striped mice were less perseverative in the four-arm maze, more fearful/anxious in the light–dark box, had higher circulating levels of the unbound (thus active) stress hormone corticosterone, as inferred from faecal metabolites of the hormone, and were less active in both the four-arm maze and in the home cage. Because time hidden in the nestbox during the peak activity period was also significantly predicted by the proportion of time spent in the dark compartment of the light–dark box, we suggest that the extreme inactivity of WC striped mice is a result of fear. When we assessed individual susceptibility to stereotype by accounting for SB status (and using our second data set, which oversampled stereotypic WC mice to permit statistical analysis), birth origin remained a significant predictor of anxiety/fear, but differences between CB and WC striped mice in terms of activity and perseveration disappeared, with these variables better accounted for by the stereotypic status of the animals. Thus the low activity levels and low perseveration of WC animals were statistically associated with their low SB, while their higher stress levels were associated with their birth origin but not stereotypy status. Furthermore, when we assessed the severity of SB from time budget data, similar patterns emerged: striped mice with the most time-consuming SBs were also the most perservative under test (as measured using SA).

Our findings hence support our first prediction that subjects maturing in the wild have greater behavioural flexibility and are less likely to perseverate than CB conspecifics, and that such effects are indeed related to low/absent SB. Thus, although all stereotypic subjects, irrespective of birth origin, were more inclined to form routines than nonstereotypic subjects, the markedly lower incidence of SB in WC striped mice was accounted for by the much higher proportion of WC than CB striped mice benefitting from enhanced behavioural flexibility. Our results did not, in contrast, support our second prediction that WC striped mice would adjust better to captivity than CB animals. Instead, WC striped mice were generally more anxious/fearful than their CB counterparts. While it can be argued that anxiety/fearfulness is an adaptive trait under natural conditions, in captivity, where there is no danger of predation, WC animals continually experiencing their environment as threatening (because of their early experience) indicates a problematic animal–environment misfit and can thus be considered a maladjustment. Overall, our results confirm the pattern outlined in the Introduction in which WC animals have poorer welfare than CB conspecifics. There did not appear to be a direct statistical link between this heightened anxiety/fear and the prevention of SB evident in WC animals (in contrast to prediction 3). The elevated SB of those few WC mice that performed this behaviour was an interesting, but unexpected result, and is considered further in the General discussion.

In summary, our results identify specific correlates of the birth origin effect on SB development, which in turn hint at the mechanisms possibly underpinning these birth origin effects on SB, an issue we return to in the General discussion. However, they do not indicate approximately when during development birth origin exerts its enduring influence. Is it sufficient simply to be conceived or gestated in the wild, or do animals additionally need to grow up in the complexity of natural conditions? For example, some studies of environmental enrichment suggest that the brain needs to mature in a complex environment to reap its benefits fully (e.g. Lewis et al. 2006). In experiment 3, we therefore retrospectively analysed historical data to tackle this final question.

EXPERIMENT 3: AGE AT CAPTURE

To characterize further the birth origin effects and test whether the effects of being WC on SB were acquired prenatally or postnatally, we retrospectively analysed past laboratory records to investigate effects of age at capture on the development of SB. This approach allowed us to obtain data from a large sample size of WC striped mice without any extra trapping. We hypothesized that age at capture would influence the susceptibility of striped mice to SB development for two interrelated reasons. First, social and physical deprivation early in life is known to affect an organism's brain and behavioural development more adversely than deprivation later in life (Max et al. 2010) and, second, an increased duration of exposure to environmental complexity is thought to confer greater and more lasting protection against later adversity (Nithianantharajah & Hannan 2006; cf. Lewis et al. 2006). We accordingly predicted that juvenile-caught striped mice would show a higher incidence of SB than adult-caught animals.

Methods

Study subjects and housing

We collated and analysed data from WC striped mice trapped and observed in N.P.'s laboratory over the past two decades ($N = 204$), dividing these data into animals trapped as juveniles ($N = 103$) and those trapped as adults ($N = 101$). These striped mice were trapped in four grassland localities in South Africa as part of various socioecological studies between 1993 and 2001: Alice (Eastern Cape; 32°46′S, 26°52′E); Kamberg (KwaZulu-Natal; 29°23′S, 29°42′E); Suikerbosrand (Gauteng; 26°31′S, 28°18′E); and Irene (Gauteng; 25°20′S, 28°9′E). Striped mice used in this study, in accordance with husbandry protocols during this period, were housed in standard Labotec cages (see experiment 1) provisioned with a grass mixture as nesting material.

Procedure

Immediately after trapping, striped mice were sexed and weighed, and their age was assessed by examining the appearance and/or size of the testes or vaginal opening. Using these parameters, we divided our sample into juvenile-caught mice (<30 g; nonscrotal/nonperforate) or adult-caught mice (>40 g; scrotal/perforate; De Graaff 1981). Striped mice weighing between 30 and 40 g were excluded to eliminate individuals whose age class was uncertain (about 10% of the sample). Four weeks after capture (by which time all juvenile-caught striped mice were adults), individuals were assessed using this laboratory's standard protocol at that time for the absence/presence of SB over a 5-day period (one 15 min observation session per day) as described in experiment 1. Although the WC mice used in this study were not monitored for the development of SB after this observation phase, we have only once incidentally observed a WC striped mouse developing SB later than 4 weeks after capture (unpublished data; also see experiment 1), and so this protocol should classify age class accurately.

Data analysis

We analysed these categorical data using logistic regression with a binomial logit function, including sex, trapping locality, age class and sex*age class as categorical predictors, and SB status as the dependent variable.

Results

There were no overall effects of sex (Wald $\chi^2_1 = 0.37$, $P = 0.54$) or trapping locality (Wald $\chi^2_1 = 3.95$, $P = 0.27$) on SB. However, age at capture significantly predicted the development of SB (Wald $\chi^2_1 = 26.51$, $P < 0.001$): 55% of juveniles developed SB compared with only 19% of adults, but there was a significant age*sex interaction (Wald $\chi^2_1 = 5.71$, $P = 0.017$) because males were more affected by age at capture than females (Fig. 5). When we reanalysed the data separately for males and females, age class at capture remained a significant predictor of SB development in both sexes (males: Wald $\chi^2_1 = 25.58$, $P < 0.001$; females: Wald $\chi^2_1 = 4.28$, $P = 0.039$).

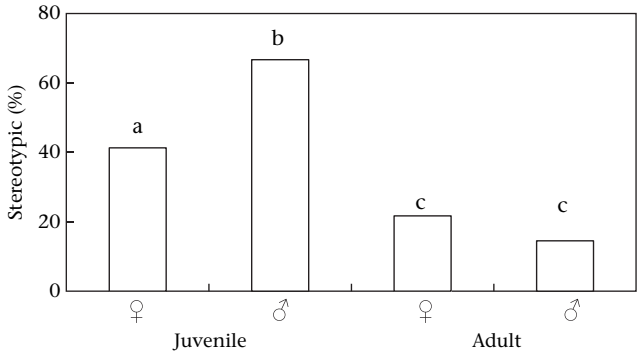

Figure 5. The percentage of juvenile and adult female and male WC striped mice that developed stereotypic behaviour after 4 weeks of captive housing.

Discussion

As predicted, birth origin effects were mediated by age at capture: the incidence of SB was significantly lower in adult-caught striped mice than juvenile-caught individuals (19% versus 55%), with juvenile-caught striped mice displaying an incidence of SB similar to that of CB individuals (55% versus 57%). It appears that an extended period of early environmental complexity is thus necessary to protect striped mice against SB development, with the beneficial effects of birth origin accruing predominantly postnatally. Unexpectedly, male juvenile-caught striped mice were more severely affected by early removal from the wild than female juvenile-caught individuals, possibly reflecting a differential impact of captivity on juvenile males and females.

GENERAL DISCUSSION

As in most other species of wild animals studied to date (see Introduction), our findings showed that WC striped mice were less likely to develop SB than CB conspecifics. Unique to this study, however, was our use of CB and WC striped mice that had been kept in the same laboratory which allowed us to investigate the associated correlates of birth origin on SB, with potential implications for all captive wild species kept and bred in laboratories, zoos and breeding centres. The better understanding of these birth origin effects is important for a number of reasons. First, SB and its correlates reflect profound alterations in behaviour, and perhaps even indicate atypical CNS development in CB wild animals compared to their WC counterparts. This has implications for the validity of ethological studies using CB animals to model behavioural processes in WC animals and, moreover, could impact on the success (or otherwise) of captive-breeding and release programmes. Second, although the absence of SB typically indicates conditions associated with good welfare (e.g. Mason & Latham 2004), the low levels of SB seen in WC animals seem to represent an important exception to this general 'low SB–good welfare' link.

Our analyses showed that striped mice had to reach adulthood in the wild to achieve maximal protection against SB development. Here, sex played an interactive role, with juvenile males four times

as likely to develop SB as WC adult males, while females were only twice as likely to become stereotypic if caught as juveniles rather than as adults. In consequence, juvenile-caught males were more stereotypic than both juvenile-caught females and wild-caught adults of either sex, and were even as prone to becoming stereotypic as CB animals. These findings suggest that both age at capture and sex may be important determinants of the behavioural phenotype of WC animals in laboratories, zoos and conservation breeding centres. Furthermore, since all the striped mice, even juvenile-caught, were captured post weaning age (after which time grassland striped mice are solitary; Schradin & Pillay 2005), this suggests that the physical aspects of environmental space and complexity, and/or an extended period of autonomous decision making, may underlie the effects of birth origin and not, for instance, that of the premature loss of parental care (a common confound in other CB–WC comparisons; Mason 2006; Latham & Mason 2008).

Instead of being stereotypic, WC striped mice were extremely inactive within their home cages, spending most of their time in their nestboxes when they otherwise would be expected to be active. Similar high levels of inactivity or being out of sight within an enclosure are commonly reported in many species kept in zoos (e.g. Meyer-Holzapfel 1968; Altman 1999), and our findings now suggest this may be a particular problem in WC animals. Because WC mice were also more stressed and fearful, and because there was a significant association between time in the nestbox and separate behavioural measures of fear, these results also suggest that this extreme inactivity may well be a manifestation of poor welfare In addition, the few WC striped mice to develop SBs showed two notable characteristics. First, they developed only one form of SB, circuit running, while CB animals displayed a greater variety of SBs. Second, the stereotypic WC striped mice devoted significantly more time to SB than their stereotypic CB peers. Thus, when WC striped mice stereotyped, the SBs appeared to be more severe and perhaps more frenetic, a phenomenon now needing investigating in other captive wild species. The exclusive use of circuit running by WC striped mice was unexpected, but could reflect a greater a priori physical fitness than in CB animals and/or frustrated ranging; free-ranging grassland striped mice have home ranges of about 1000 m^2 (Schradin & Pillay 2005), some 16 000 times bigger than the laboratory cages, and frustrated ranging opportunities have been shown to underpin locomotor stereotypies in the Carnivora (Clubb & Mason 2003, 2007). In passerine bird species, WC individuals displayed a similar preference for route-tracing SBs over other forms of SB such as 'spot picking' (Keiper 1969).

Our investigations of perseveration, fear/anxiety and activity revealed substantial effects of birth origin, with some, but not all variables correlating with SB and so reflecting potential mediators of the link between birth origin and of SB development. The clearest correlate of SB that might account for the effect of birth origin was perseveration. Thus on a group level, WC striped mice showed more flexible patterns of arm entry in the four-arm maze but, when we controlled for SB, the effect of birth origin disappeared: stereotypic striped mice, regardless of birth origin, were more perseverative than their nonstereotypic counterparts. Scores on one of our measures of perseveration, SA, also predicted the duration of time engaged in SB. These behavioural results thus suggest that, like deprivation-reared primates, and like deer mice developing in standard versus enriched housing conditions (Lewis et al. 2006; Latham & Mason 2008), forebrain development is altered in CB animals in a manner that decreases both behavioural flexibility and the ability to inhibit inappropriate behavioural repetitions, with perseveration and SB being two outcomes of such a change. A second factor associated with SB was activity. Stereotypic striped mice were, as discussed, more active than nonstereotypic individuals, an observation agreeing with findings in other animals (e.g. bank voles) in which enhanced rates of behavioural initiation are thought to predispose both hyperactivity and SB (Garner & Mason 2002).

The regional activity of specific forebrain areas (candidates include the motor cortex, striatum, nucleus accumbens and thalamus) should now be identified using such techniques as pharmacological probes (e.g. Schoenecker & Heller 2000; Presti et al. 2003) or post mortem histology, for instance for dendritic branching or metabolic activity (via cytochrome oxidase staining; e.g. McBride & Hemmings 2005; Lewis et al. 2006). Our results suggest altered striatal function in CB mice compared to WC mice; they predict changes in such regions that predate the emergence of SB and also predict correlational associations between the magnitude of such effects and perseveration and SB scores. Such studies might similarly help explain the way the age-at-capture effect interacted with sex, since the trajectories of brain maturation in human children and adolescents are influenced by sex, with maltreatment-induced abnormalities in brain development more common in males, possibly because of hormonally driven differences in dendritic arborization, pruning and myelination (De Bellis et al. 2001). One remaining question here, however, is whether the better predictor of SB development is (1) the effect of age at capture (and the developmental stage of the CNS), (2) the duration of time spent in the wild or (3) both of these. Additional further studies would be necessary to tease apart these confounding variables and thus the effects of sex, developmental stage, age and early environmental risk factors on the predisposition to stereotype.

Despite SB usually indicating group-level poor welfare (see Introduction), the fact that WC striped mice were less stereotypic than CB animals did not necessarily mean that they had better welfare. In fact, as summarized above, WC individuals (irrespective of SB status) were more anxious/fearful than CB striped mice in behavioural tests, and they had higher circulating levels of corticosterone. Thus, the virtual absence of SB in WC animals might be an important exception to the 'low SB–good welfare' rule of thumb. The very low activity levels of WC striped mice seemed to be an alternative behavioural response to adverse captive conditions. Future work should investigate more fully whether this inactivity reflects (1) fear-mediated hiding in the nestbox (for instance using tests in which fear cannot be confounded with activity, or nestboxes that vary in the degree of cover they offer) or (2) depression-related 'apathy', a hypothesis that could be assessed by running tests for anhedonia (e.g. Willner et al. 1996) in CB versus WC animals and investigating its links with home cage inactivity. A third sign of reduced welfare in WC animals is that the few WC striped mice that did stereotype did so at significantly higher levels than seen in CB mice. This suggests that they may be more frustrated by the confines of captivity than animals born into it and that, for those few individuals susceptible to SB development because of their perseverative tendencies, this frustration exacerbates the performance of SBs. Any subsequent work should therefore also test directly for differences in motivational frustration between WC and CB individuals (as has been done between previously enriched laboratory mice and their standard-housed counterparts; Latham & Mason 2010), and for the relationships between SB and frustration within these two groups.

In conclusion, our results show that WC striped mice, provided they are trapped as adults, are typically protected from the development of SB when captive housed and are also less perseverative under test, suggesting intact forebrain function. WC striped mice (whether stereotypic or not) none the less adjust more poorly to captivity than those animals born into it. These results suggest that

striped mice are a promising species in which to investigate the neurological mechanisms underpinning the pervasive effects of birth origin, and indicate the need for further investigations in other species to test, for instance, whether and how birth origin and age at capture, inactivity, welfare and captive conditions interact to shape phenotype. Finally, given the likely role of physical environmental complexity in mediating behavioural flexibility via influencing forebrain development, it may well also be worth considering on ethical grounds whether enriched-reared CB striped mice would be better suited subjects for such experiments, thus minimizing the need for future trapping and use of highly anxious/fearful WC animals.

Acknowledgments

Funding was provided by the National Research Foundation (NRF; grant number: 2069110) and the University of the Witwatersrand to N.P. M.J. was partly supported during her Ph.D. by an NRF Scarce Skills Scholarship and, for this work, by a grant from the Universities Federation of Animal Welfare (UFAW). G.M. was supported by an NSERC Canada Research Chair and Discovery Grant. M.J. thanks Tasmin Rymer, Sneha Joshi and Alessia Fowler for help with data collection, Rebecca Meagher for her insightful comments and assistance with references, Jamie Dallaire for his generous after-hours' discussion of data, Lis Jones for skilfully proofreading the manuscript and two anonymous referees for their helpful comments.

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CHAPTER SIX

Early environmental enrichment protects captive-born striped mice against the later development of stereotypic behaviour

Megan Anne Jones, Georgia Mason, Neville Pillay (2011)

Applied Animal Behaviour Science, **135**, 138-145

Applied Animal Behaviour Science 135 (2011) 138–145

Contents lists available at SciVerse ScienceDirect

Applied Animal Behaviour Science

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

Early environmental enrichment protects captive-born striped mice against the later development of stereotypic behaviour

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ARTICLE INFO

Article history:
Accepted 26 August 2011
Available online 1 October 2011

Keywords:
Birth origin
Enrichment
Frustration
Neuroprotection
Rhabdomys
Stereotypic behaviour
Welfare

ABSTRACT

Understanding how birth origin (whether born in the wild or captivity) influences behavioural development is important for fundamental and applied ethology, especially when captive-bred (CB) individuals from wild species are used in research or conservation. CB animals are typically much more prone to stereotypic behaviour (SB) than are wild caught (WC) conspecifics, an effect which in striped mice is accompanied by increased tendencies to form behavioural routines. However, WC mice, if stereotypic, are far more severely affected than CB mice and are also more physiologically stressed and inactive than CB mice, regardless of their stereotypy status. Capturing subjects from the wild to further study these latter effects raises serious practical, ethical and potentially conservation concerns. Here, we therefore tested whether rearing CB striped mice in enriched conditions and then placing them in standard cages could provide a more suitable model for investigating how wild-caught (WC) conspecifics respond to the reduced environmental complexity they experience after capture. Compared with striped mice which were always standard-housed ($n = 36$), enriched striped mice ($n = 24$) were four times less likely to develop SB and, similarly to the benefits of being wild born, early environmental enrichment (30–170 days) successfully protected CB striped mice from the emergence of SB after transfer to standard caging (171–240 days). However, unlike WC mice, previously enriched CB striped mice which then became stereotypic did not develop markedly more severe SBs; they showed diverse forms, unlike stereotypic WC mice which exclusively circuit ran; and they were not more inactive once SB levels were controlled for. These findings thus show that early environmental complexity can have lasting suppressive effects on SB, suggesting that early enrichment protocols could indeed provide a practicable, potentially more ethical model for investigating the causal mechanisms underpinning birth origin effects on SB. Furthermore, they add to a corpus of data for this species showing that more naturalistic early experiences, both social and physical, lastingly protect against SB development. These results also highlight how unpredictable and poorly understood early enrichment effects are (since in some species early enrichment exacerbates later SB); and moreover reveal that early environmental complexity is not the sole factor shaping behavioural phenotypes in WC animals, with differential social experience and human contact being other likely causes of developmental divergence.

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1. Introduction

Stereotypic behaviours (SBs) are rife in animals housed in restricted and impoverished environments (Mason and Latham, 2004), but typically less prevalent and

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doi:10.1016/j.applanim.2011.08.015

time-consuming in more complex, enriched conditions (Mason et al., 2007). In general, situations inducing or exacerbating SBs also decrease welfare (Mason and Latham, 2004). The performance of SBs suggests the thwarting of highly motivated behaviours (Latham and Mason, 2010) and/or neurodevelopmental disruption to pathways of the forebrain and basal ganglia which subserve the control of normal, flexible behaviour (Garner, 2006; Lewis et al., 2006; Latham and Mason, 2010). In captive wild animals (vs. captive domestic animals), SBs are especially common in those individuals born and reared in captivity, most likely because captive-born (CB) animals experience earlier and/or more prolonged deprivation than do conspecifics captured from the wild (i.e. wild-caught [WC] individuals; Mason, 2006). This effect of birth origin on SB development is evident in the behaviour of captive striped mice, *Rhabdomys* (Jones et al., 2011): striped mice which spend a prolonged period of time in the wild before capture are substantially less likely to stereotype than CB individuals. Their low SB is linked to increased behavioural flexibility under test compared to that of CB animals, indicating that non-stereotypic WC mice have relatively normal behavioural control. However, as a group, regardless of whether or not they are stereotypic, WC mice are more fearful than CB individuals, have higher corticosteroid outputs, and are concerningly far more inactive (Jones et al., 2011).

In contrast to WC animals, in which previous experiences in complex, natural environments typically protect against later SB development, the barren housing of formerly environmentally enriched CB animals has inconsistent long-term influences on SB performance. In some environmental enrichment (EE) studies, previously enriched animals (e.g. bank voles, *Clethrionomys glareolus*; Ödberg, 1987; deer mice, *Peromyscus maniculatus*; Lewis et al., 2006) remain, like WC individuals, less stereotypic after being transferred to barren cages than standard-housed controls—an effect which correlates, in deer mice, with altered dendritic morphology in the motor cortex and striatum (Turner et al., 2003), reduced striatal enkephalin, a marker for indirect pathway activity (Presti and Lewis, 2005), and high cytochrome oxidase activity in the substantia nigra in the motor cortex and basal ganglia, an index of activity-dependent plasticity and neuronal metabolic activity (Turner et al., 2002).

In contrast, other EE studies have found the opposite effect: for instance, SB incidence and severity is higher in laboratory mice (*Mus musculus*) from which EE is removed than in mice which have always been deprived, and its exacerbation in previously enriched mice is statistically associated with evidence of heightened frustration (Latham and Mason, 2010). The unpredictable response of CB enriched-reared animals to an environmental downshift (i.e. transfer from rich, complex conditions to comparatively impoverished housing) likely reflects the relative influences of motivational and neurodevelopmental factors. When an environmental downshift is associated with continuing low levels/incidences of SB, the motivational sequelae of the downshift (i.e. negative contrast/frustrative non-reward) are arguably weaker determinants of behaviour than is intact forebrain function, which protects against SB development ('Protection Hypothesis': Latham and Mason, 2010). In contrast, when an environmental downshift exacerbates SB performance, it presumably does so by means of frustration, which motivates SB performance in individuals for which the previous environmental complexity was insufficient to normalize forebrain development ('Frustration Hypothesis': Latham and Mason, 2010).

Understanding how and why previous experience in complex environments influences SB development is of central concern to fundamental and applied ethologists. For example, in captive wild-derived animals, better understanding of the influences of early experience on adult phenotype is necessary for multiple reasons: practically, birth origin differences in SB incidence indicate an undesirable divergence of behavioural phenotype between WC and CB individuals. This divergence raises concerns about the validity of ethological studies using CB animals to model behavioural processes in WC animals as well as the current management of wild animals in captive-breeding and release programmes. Understanding birth origin effects is the first step towards ameliorating these concerns. However, capturing animals from the wild to investigate birth origin effects, raises other practical, ethical and, for many species, conservation concerns. Retrospective analysis of historical data sets (e.g. Jones et al., 2011), which avoids these issues, provides some insights into the correlates of birth origin effects, but the results from such analyses do not necessarily provide causal insights and are limited by the variables recorded. Furthermore, catching new animals from the wild precludes the investigation of some research questions principally because the experiences of WC animals before capture are unknown and cannot easily be manipulated, making it difficult or impossible to tease apart the relative contributions of early social environment, physical environment, and/or prior lack of exposure to humans on their subsequent responses to captivity. Rearing CB animals in enriched and complex captive environments and then placing them in standard barren cages might provide an alternative opportunity to assess how WC conspecifics respond to reduced environmental complexity, mimicking the transfer of WC animals from complex natural environments to barren captive environments. Such experiments could also allow for the experimental manipulation of candidate variables and thus, ultimately, elucidation of the mechanisms underpinning the effects of birth origin and early experience on SB.

In this study, we therefore investigated the outcomes of an EE paradigm on the emergence of SB in F1 CB striped mice. This research was needed because, as reviewed above, early enrichment cannot always be assumed *a priori* to have effects similar to those of being WC. We compared the incidence of SB in CB striped mice housed from shortly after weaning (30 days) until late adulthood in standard laboratory cages with the incidence of SB in CB striped mice housed in larger and more complex cages (Phase 1). Thereafter, we transferred the previously enriched striped mice to standard caging and, 10 weeks later, again compared the incidence of SB between the two treatment groups (Phase 2). Because little is known about how cage characteristics influence the form of SB (Würbel, 2006), and because WC mice that stereotype exclusively circuit run, we also



Fig. 1. The standard Labotec™ cage in which breeding pairs and standard-housed striped mice were kept. The cage contained woodshavings, a PVC tubing nest box provisioned with hay and paper towelling, and one cardboard roll.



Fig. 2. The enriched tank connected by a PVC tube to a small Labotec™ cage, in which enriched-housed striped mice were kept. The tank contained ~5 cm woodshavings as bedding, another ~15 cm of a hay mixture, a PVC tubing nest box provisioned with hay and paper towelling, between 3 and 5 cardboard rolls, and a 1 m long flexible plastic pipe. The small cage contained ~2 cm of woodshavings.

recorded preferred SB form both before and after transfer to standard caging. Finally, given that non-stereotypic WC striped mice are very inactive, and that stereotypic WC striped mice have markedly time-consuming SBs (Jones et al., 2011; responses suggestive of fear and frustration respectively), during Phase 2 we additionally recorded the proportion of time that striped mice spent engaged in non-stereotypic activity in the home cage, or performing SB in the nest.

2. Methods and materials

2.1. Subjects

CB striped mice (males: $n = 32$; females: $n = 28$) used in this study were a randomly selected group of F1 offspring from 10 WC breeding pairs. The WC founder stock, which originated from a Highveld grassland locality (Gauteng, South Africa; 27°55′S, 26°4′E), were trapped for use in other behavioural studies (see Jones et al., 2011).

2.2. Housing and husbandry

Striped mice were housed in either the Milner Park Animal Unit at the University of the Witwatersrand, under partially controlled environmental conditions (14L:10D, lights on at 05:00 h; 20–24 °C; 30–60% relative humidity) or, because of the extra space required for the enriched housing, in a room in the adjacent Biology Building (14L:10D, lights on at 05:00 h; ambient temperature and humidity). Breeding pairs and standard-housed experimental striped mice (all located in the Milner Park Animal Unit) were kept in Labotec™ cages (300 mm × 200 mm × 150 mm) containing ~2 cm woodshavings as bedding; a PVC tubing nest box (10 cm × 10 cm × 15 cm, open at both ends) provisioned with a grass mix (*Eragrostis* grass, oat hay, and/or lucerne) and paper towelling as nesting material; and one cardboard roll (Fig. 1). Enriched-housed striped mice (about half of which were housed in the Milner Park Animal Unit and half in the Biology Building) were kept in large tanks (460 mm × 300 mm × 320 mm high) with three metal sides, a clear perspex front, and a wire mesh lid; the tanks were connected by a 20 cm long white PVC tube (5 cm diameter) to a small cage (20 mm × 150 mm × 150 mm) in which food and water were provided (Fig. 2). The large tank contained a deep layer of woodshavings as bedding (~5 cm) covered by an additional ~15 cm layer of a grass mix. The tank was also provisioned with a PVC tubing nest box containing paper towelling as bedding; a 1 m long, 5 cm diameter, flexible plastic pipe; and between 3 and 5 cardboard rolls. Both standard-housed and enriched-housed striped mice had access *ad libitum* to Epol mouse cubes and water. Each day, a small amount (±3 g) of seed mix (sunflower, millet, canary) was sprinkled in the cages/tanks to encourage foraging, and fresh fruit and/or vegetables (±10 g) were provided.

2.3. Procedure

Captive-born F1 striped mice were separated from their dams at 22 days of age and housed until 30 days old in their sibling groups in large tanks (the same as for the enriched-housing, but not connected to a small cage; Fig. 3). Thereafter, striped mice were pseudo-randomly assigned to individual housing in one of two treatment groups (balancing animals across treatment groups for litter and for sex): (1) enriched-housed/standard-housed (E/S; $n = 24$) or (2) standard-housed/standard-housed (S/S; $n = 36$). Striped mice in the E/S treatment were first housed in enriched tanks for 140 days (between 30 and 170 days of age; Phase 1) after which they were transferred to standard cages for another 70 days (171–240 days; Phase 2). Individuals in the S/S treatment were housed in standard cages for the entire 210-day experimental period (i.e. during both Phases 1 and 2).

2.3.1. SB incidence and form

We defined SB as a repetitive behaviour comprising at least three successive repetitions (Mason, 1993; Vickery and Mason, 2004; see Table 1 for definitions of the various forms of SBs observed). During Phase 1 (30–170 days), individual striped mice were assessed every day for 140 days for SB in their home cages using *ad libitum* sampling (when a striped mouse was seen performing SB, the presence and the form of the SB displayed was recorded on its cage tag by MJ). These direct observations were corroborated using video-recording of home cage behaviour (no observer present), for 20 min/day, on three non-consecutive days, between days 161 and 170. Thereafter, during Phase 2 (171–240 days), the home cage behaviour of striped mice



Fig. 3. Timeline indicating age (days) at which striped mice were weaned, assigned to either the S/S or E/S treatment group, and kept in Phase 1 or 2 housing.

was observed for 5 days/week, for 10 weeks, for 15 min/day, using direct behavioural observations (by MJ) and, on three randomly chosen days between days 231 and 240, their behaviour was video-recorded for 20 min/day. Striped mice which never displayed SB during Phases 1 or 2 of the experiment were classified as non-stereotypic (NS) for that phase, whereas those animals displaying SB during that time period were classified as stereotypic (S; see Jones et al., 2010a,b, 2011; for a justification of this dichotomous classification method).

An individual's preferred form of SB during Phase 1 was roughly evaluated (c.f. Phase 2) based on the written recordings made on each striped mouse's cage tag: if only a single form of SB had been documented, that SB form was designated as the preferred form. If, however, more than one form of SB had been displayed (irrespective of how frequently each form was performed), we designated the preferred form of SB as 'mixed'. In Phase 2, using a more rigorous method, we determined the preferred form of SB for stereotypic mice by dividing the total number of times a particular SB was shown by the total number of days on which any SB was observed (more than one form of SB could be recorded within one observation session). If a particular SB was observed in more than 70% of the observation sessions (provided the sum of the second and third most common forms did not exceed 70% of the observation sessions), that form of SB was regarded as the preferred form. If no SB form was performed in more than 70% of observation sessions, or if the sum of the second and third most common forms exceeded 70%, then the preferred form of SB was designated as 'mixed'.

2.3.2. Phase 2 home cage behaviour

During Phase 2 only, we calculated the proportion of observation days on which striped mice either (1) did not leave their nest box; (2) were active outside the nest box (locomotion, grooming, drinking, eating, but excluding SB); or (3) performed SB (i.e. mutually exclusive categories).

2.4. Data analysis

We ran analyses using either SPSS (Version 18) or Statistica (Version 8.0).

2.4.1. SB incidence

To assess the effects of treatment on the incidence of SB between the two treatment groups, we used a Generalized Linear Model (GLZ) with a repeated measures design, followed by pair-wise planned contrasts (SPSS). In the model, we included treatment and sex as categorical predictors; phase (Phase 1 or 2) as the repeated measure; and the stereotypic status (S or NS) of each striped mouse as the dependent variable. We nested housing location (Milner Park Animal Unit/Biology Building) within treatment to account for the potential effects of differential housing location during Phase 1.

2.4.2. SB form

For Phases 1 and 2 separately (since categorization of preferred form was not directly comparable), we assessed the preferred form of SB between treatment groups using a GLZ with treatment group as the categorical predictor, and the frequency of each form of SB as the response variable (Statistica).

2.4.3. Phase 2 home cage behaviour

First, to examine between-group differences in home cage behaviour, we compared the proportion of observation sessions (arcsine square root transformed; Zar, 1996) in which striped mice did not leave the nest box; were

Table 1
Descriptions of the four main forms of SB observed.

Stereotypic behaviour	Description
Circuit running	Running in the cage along a fixed route.
Prelooping	Climbing upside down on the cage lid with forelimbs and then hindlimbs, moving backwards along lid, followed by dropping down through releasing the hindlimbs first.
Somersaulting	Backward flipping, with or without touching the cage.
Windscreen wiping	Hindlimbs remain stationary on the cage floor, or move far less than do forelimbs. Forelimbs oscillate to-and-fro in a large arc against the front/side of the cage, or the food hopper.

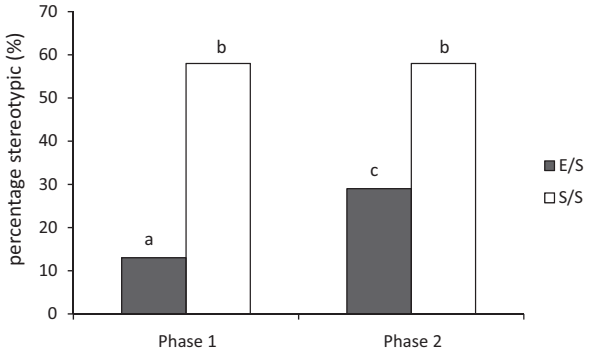

Fig. 4. Prevalence of stereotypic behaviour in striped mice reared in enriched and standard housing (Phase 1), and after transfer of enriched-housed animals to standard housing (Phase 2). Alphabet letters indicate homogenous groups.

active outside the nest; and in which individuals performed SB, using separate General Linear Models (GLM), including sex, treatment group, and previous housing location, which was nested within treatment group, as the categorical predictors (Statistica). Second, to assess whether previous housing type (enriched or standard) or current SB status was a better predictor of the proportion of observation sessions in the nest box and of non-stereotypic activity outside the nest, we re-ran the above GLMs, but included SB status as a categorical predictor. Third, we compared the severity of the SBs of stereotypic striped in the E/S and S/S groups using a GLM with sex, treatment group, and previous housing location nested within treatment group, as the categorical predictors, and the proportion of observation days on which stereotypic striped mice displayed SBs (arcsine square root transformed) as the dependent variable. Statistical findings were considered significant if $P \leq 0.05$.

2.5. Ethical note

Individual housing is ethically acceptable for this species, unlike many other rodents, because grassland-derived animals are naturally solitary. Striped mice used in this study were ultimately either euthanized with an overdose of an inhalant anaesthetic (Isoflurane or Halothane) or returned to breeding stock. Approval for this study was provided by the Animal Ethics Screening Committee of the University of the Witwatersrand (2006/94/03).

3. Results

3.1. SB incidence

Treatment (Wald $\chi^2 = 9.261$; $P = 0.002$), phase (Wald $\chi^2 = 4.400$; $P = 0.036$), and their interaction (Wald $\chi^2 = 4.400$; $P = 0.036$) were all significant predictors of the incidence of SB. At the end of Phase 1, whilst still in initially different housing conditions, striped mice from the E/S group (thus still in enriched cages) were significantly less likely to perform SB (3 out of 24; 13% stereotypic) than individuals housed in standard cages (21 out of 36; 58% stereotypic) (pairwise comparison; $P < 0.001$; Fig. 4). During Phase 2, when all striped mice were standard-housed, individuals from both treatments that had displayed SB in Phase 1 continued to stereotype. In the S/S treatment group, no further animals developed SBs (thus 42% remained non-stereotypic) but, in the E/S group, an additional four mice became stereotypic (bringing the total to 7 out of 24; 29%). This increase in the incidence of SB from Phase 1 to 2 in the E/S group was statistically significant ($P = 0.032$) but, nonetheless, the overall incidence in the E/S group at the end of Phase 2 was still significantly lower than in the S/S group ($P = 0.017$). The effects of sex (Wald $\chi^2 = 0.119$; $P = 0.730$) and housing location (Wald $\chi^2 = 0.011$; $P = 0.916$) on the incidence of SB were not significant.

3.2. SB form

During Phase 1, the three stereotypic enriched-housed striped mice all showed circuit running (and no other forms of SB), whereas the 21 stereotypic S/S striped mice showed considerable intra- and inter-individual variation in their SB form: 62% circuit running ($n = 13$), 10% prelooping ($n = 2$), 5% somersaulting ($n = 1$), 5% windscreen wiping ($n = 1$), and 19% mixed ($n = 4$) (between-group differences in form; Wald $\chi^2_4 = 10.342$; $P = 0.035$). During Phase 2, S/S striped mice who were stereotypic during Phase 1 continued thus, and did not deviate from their established preferred forms of SB. In contrast, in the E/S group, two of the three previously identified circuit runners continued to display a preference for circuit running as did two of the four 'new' stereotypers (bringing the group total for circuit running to 57%; $n = 4$); one of the previous circuit runners switched to somersaulting (14%; $n = 1$); and there was one new case of prelooping and one of mixed SB (14%; $n = 1$ respectively). Accordingly, during Phase 2, and in contrast to Phase 1, there were no differences between treatment groups in the preferred form of SB (Wald $\chi^2_4 = 0.775$; $P = 0.942$).

3.3. Phase 2 home cage behaviour

At a group level, E/S striped mice spent more time in the nest ($F_{1,56} = 5.711$; $P = 0.020$) and performed less SB ($F_{1,56} = 6.165$; $P = 0.016$) than did individuals in the S/S treatment group (Fig. 5). E/S and S/S striped mice, however, showed comparable levels of non-stereotypic activity ($F_{1,56} = 0.111$; $P = 0.740$). When we examined the concurrent effects of treatment group and stereotypy status on home cage behaviour, the main effects of treatment group disappeared (all $P > 0.1$), and stereotypic status was the sole predictor of behavioural outcome (Fig. 6): stereotypic striped mice, irrespective of treatment group, spent less time in the nest ($F_{1,55} = 196.087$; $P < 0.001$), and showed higher levels of non-stereotypic activity ($F_{1,55} = 9.114$; $P = 0.004$). There were no differences between treatment groups in the SB severity of stereotypic striped mice ($F_{1,24} = 1.481$; $P = 0.236$): thus, in both groups, stereotypic individuals performed similar levels of SB. In none of the above analyses was the response variable predicted by sex or Phase 1 housing location (all $P > 0.1$).

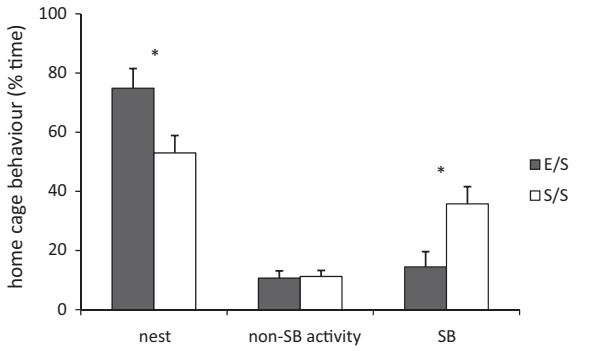

Fig. 5. Percentage of total observation sessions during Phase 2 in which striped mice from the E/S and S/S treatment groups were in the nest, engaged in non-stereotypic activity, or performing stereotypic behaviour. *$P < 0.001$.

4. Discussion

In this study, we aimed to investigate the outcomes of an EE paradigm on the emergence of SB in CB striped mice, and thus whether an enrichment protocol could provide a practical, ethical means for investigating the mechanisms underpinning the protective effects of birth origin on SB development in WC animals. When striped mice were housed in enriched tanks, individuals were over four times less likely to develop SB than standard-housed controls (Phase 1). Thus the enrichment proved highly effective at reducing the prevalence of SB. Moreover, after these previously enriched striped mice were subsequently kept in standard caging for just over 3 months, only about one-quarter of formerly non-stereotypic individuals developed SB; in consequence, the overall incidence of SB in the E/S treatment group at the end of Phase 2 remained significantly lower than in the S/S comparison group. These results are consistent with our first prediction that early enrichment confers striped mice with long-lasting protection against the emergence of SB in later life, and thus indicate that a CB EE-based model of birth origin effects does merit further investigation. They also show that the early physical environment alone can have lasting effects, even when social experiences and human contact are standardized, unlike the case in the WC vs. CB comparisons (Jones et al., 2011), so implicating early exposure to complex environments as one of the major causal factors underpinning the onset (or lack of onset) of SB.

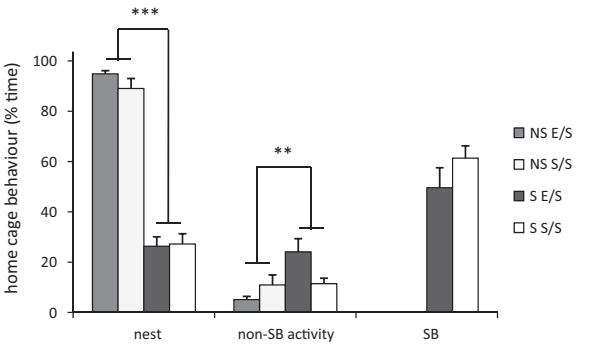

Fig. 6. Percentage of total observation sessions during Phase 2 in which striped mice were in the nest, engaged in non-stereotypic activity, or performing stereotypic behaviour in non-stereotypic (NS) and stereotypic (S) striped mice from the E/S and S/S treatment groups. **$P < 0.01$; ***$P < 0.001$.

However, early enrichment and its removal did not completely model the effects of being WC on SBs. For one, although enriched tanks greatly reduced the prevalence of SB whilst animals were living in such conditions, they did not abolish it entirely—a presumed difference in behaviour from life in the wild: SBs have not been observed in free-living striped mice, and it is currently assumed SBs do not occur in the wild. Second, although early EE moderately successfully protected against the emergence of later SB in non-enriched cages, the prevalence of SB in such animals (7/24, 29% stereotypic) was still significantly higher than the prevalence of SB in a random sample of WC adults (1/26; 4% stereotypic; Jones et al., 2011). Third, once moved to standard cages, the previously enriched striped mice and S/S individuals showed comparable inter-individual variation in their predominant SB form; this contrasts with our previous work showing that WC striped mice developed only one form: circuit-running (Jones et al., 2011). The fourth and final difference is that those previously enriched striped mice that developed SB did not develop more time-consuming (severe) forms than did S/S individuals. Furthermore, early enrichment also did not completely model the effects of being WC on other aspects of behaviour. In particular, E/S animals were not more inactive than S/S striped mice, once levels of SB were statistically controlled for.

We hypothesise that the reduced incidence of SB in the E/S striped mice in the present study stems from environmentally mediated increases in behavioural flexibility, and thus that E/S striped mice, like their WC counterparts, will be less perseverative under test as a result of presumed intact forebrain function. Direct test of this hypothesis in future studies would further validate the use of an enrichment paradigm to model birth origin effects. We also suspect that these animals' greater SB prevalences than those of truly WC conspecifics reflect, at least in part, the limited physical complexity that is offered by enriched tanks compared to life in the wild. Tests of this hypothesis could include comparisons of CB mice raised in tanks vs. those reared even more naturalistically, for example in large outdoor enclosures. For the other main difference from WC mice—the lack of increased inactivity once SB was statistically controlled for—we hypothesise that this reflects that E/S individuals are not motivated by fear to hide in the nest boxes, unlike WC animals, where inactivity correlates with raised levels of faecal corticosterone metabolites and more fearful behaviour in the light–dark box (proportion of time in the dark compartment; latency to emerge from the dark compartment; Jones et al., 2011). If this hypothesis is correct, we would expect E/S mice to be no more fearful in, for example, light–dark emergence tests than S/S mice, and for E/S mice to be likewise less fearful under test than WC mice. Since the high fearfulness of WC mice is likely to arise from a lack of habituation or even socialisation to humans, this hypothesis would also predict that raising CB mice in some automated system precluding

human contact would yield adult subjects with the same fearful and inactive phenotypes as WC conspecifics.

As emphasised in the Introduction, removing environmental enrichments from animals has inconsistent effects on SB, with enrichment sometimes protecting them from later SB development, as seen here, whilst at other times dramatically increasing the incidence and severity of SB compared with animals that always have been barren-housed. Currently there is insufficient knowledge to predict what effect will be seen with any given species or type of early enrichment: an intellectually unsatisfying situation. For future studies, we now propose two mediating variables as particularly likely to explain these discrepant findings:

(1) Differences in developmental stage when enrichment is both introduced and removed, in conjunction with the duration of exposure to the enrichment. The animal's developmental stage when enrichment is introduced/removed might influence whether animals (a) are motivated to interact with, and thus derive benefit therefrom (c.f. Tilly et al., 2010), and/or (b) whether environmentally induced changes to CNS structure and function are possible, since the benefits of enrichment on SB may be limited to, or be more effective, during 'sensitive periods' which typically occur earlier rather than later in ontogeny (Cooper et al., 1996; Hadley et al., 2006);
(2) The nature and degree of enrichment provided, which to date has varied from specific or modest additions to standard cages, through to ambitious "everything but the kitchen-sink" additions of multiple enrichments or even attempts to approximate natural conditions. In our study and the studies of Lewis and colleagues (e.g. Lewis et al., 2006)—where enrichment had an enduring effect on SB performance—enriched animals were provided with substantially larger cages than standard-housed controls (but c.f. Ödberg, 1987). These cages were also structurally complex, and contained a variety of natural and manufactured objects which could be explored, manipulated, and/or used for hiding. In comparison, in two of the studies (Vinke, 2004; Latham and Mason, 2010) where enrichment removal exacerbated the performance of SB, the complexity of enrichment was considerably less. As suggested by Latham and Mason (2010), enrichments might vary in their ability to normalize forebrain function and in their motivational salience, and thence the ability of enrichment to induce negative contrast and frustration at removal thereof.

The data reported here do support what is emerging as a *prima facie* general rule for striped mice, viz. the more relatively naturalistic their early rearing environment, the lower the incidence of SB in later life. This has been shown by manipulating the quality and quantity of parental care, as well as the complexity and duration of exposure to physically enriched environments (Table 2). In consequence, it is important for future studies to investigate the welfare correlates of low SB in all these subjects, including the E/S animals of this experiment. Although 'low SB, good welfare' is a useful general rule of thumb, the low levels of SB in WC striped mice correlate with poor adjustment to captive conditions (Jones et al., 2011), and therefore the presence of higher levels of motivational frustration (c.f. S/S) is an *a priori* prediction for E/S subjects: thus even if this motivational frustration is not expressed through the performance of SB, it may impact negatively on welfare-relevant emotional states. In lab. animals, where rearing conditions in commercial production units might differ from housing in research labs., consideration should certainly be given, *inter alia*, to the sustainability of introduced enrichments to pre-empt the welfare problems likely induced by enrichment removal (Latham and Mason, 2010).

Overall, our new EE-based model of birth origin effects in striped mice would thus (1) permit the systematic manipulation of the confounding variables of duration of exposure and developmental age; (2) allow for the investigation of untested hypotheses such as whether environmental complexity confers cumulative benefits, or whether animals simply need to receive a certain threshold "dose"; and (3) enable more accurate investigation of if and when during ontogeny sensitive periods may exist for the prevention and/or development of SB in WC animals. If previously enriched striped mice were, as we predict, found to

Table 2
Incidence of SB of striped mice reared under less and more naturalistic conditions (less naturalistic; more naturalistic). Levels of SB are consistently and significantly lower in the more naturalistic treatment conditions.

	Incidence of SB	
	Less naturalistic	More naturalistic
Weaning age (12 days vs. 16 or 20 days old)[a]	~68% (n = 20)	~34% (n = 20)
Parental care (only mother vs. both parents)[a]	~29% (n = 32)	~21% (n = 32)
Birth origin (captive-born vs. wild-caught)[b]	57% (40/70)	16% (5/32)
Age at capture (juvenile vs. adult)[b]	55% (57/103)	19% (19/101)
Enrichment (standard vs. enriched)[c]	58% (21/36)	13% (3/24) (enrichment present)
	58% (21/36)	29% (7/24) (after enrichment removal)

[a] The incidence reported is the proportion of each litter of striped mice which developed SB, averaged across litters from each treatment group. The number of litters used in the analyses is provided in parentheses. All fathers were non-stereotypic, and half of the mothers were stereotypic and half non-stereotypic. For ease of the current comparison, data for litters from stereotypic and non-stereotypic dams have been combined. The absence/presence of SB was scored when offspring were between 50 and 60 days old (Jones et al., 2010a,b).
[b] The number of striped mice showing SB in relation to the total number of animals observed is given in parentheses. Birth origin data: the absence/presence of SB in WC striped mice (all captured as adults) was scored thrice – at 4 weeks, 5 months, and 1 year after capture (scores were stable over time). In CB striped mice, SB was scored twice – at 1 month and 5 months after weaning (scores were similarly stable over time). Age at capture date: SB status was scored 4 weeks after capture, by which time all juvenile-caught striped mice were adults (Jones et al., 2011).
[c] Data from current paper.

be no more fearful once in standard cages than individuals which had always been barren-housed, then a CB model of birth origin effect would have the additional fortuitous consequence of improving the overall welfare of study subjects. Findings from such studies would help inform how captive environments need to be modified to facilitate the development of WC phenotypes in CB animals—so improving the validity of findings from laboratory studies which model behavioural processes observed in the wild, as well as promoting the success of captive-breeding and reintroduction programmes. Findings should also contribute to the development of housing protocols which provide sustainable welfare benefits to wild as well as domestic captive animals.

5. Conclusion

Our data showed that enriched-housing from immediately after weaning substantially reduced the incidence of SB in striped mice, and that these beneficial effects persisted (although to a slightly lesser degree) when animals were moved as adults to standard cages. Whilst additional studies are needed to further extend and validate this model, this suggests that an early enrichment protocol could provide a practicable and potentially more ethical means to investigate the causal mechanisms underpinning birth origin effects whereby CB and WC animals differ in behavioural phenotype, especially in terms of SB.

Acknowledgements

Funding was provided by the National Research Foundation (NRF; grant number: 2069110) and the University of the Witwatersrand. MJ was partially supported whilst collecting data by an NRF Scarce Skills Scholarship. GM was funded by an NSERC Canada Research Chair. Appreciative thanks to Margaret Quinton and Becky Meagher for assistance with the statistical analysis, to Heath Lancaster for having taking the photographs, to Lis Jones and Anne-Marie Lydall for their helpful proofreading, and to two anonymous referees for their constructive input.

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CHAPTER SEVEN

Characterization of the striped mouse model of stereotypic behaviour: predictors, mediators, and correlates of development

This manuscript is in preparation for Developmental Psychobiology

ABSTRACT

Stereotypic behaviours (SBs), repetitive behaviours induced by frustration, repeated attempts to cope, and/or forebrain dysfunction, are rife in captive animals. Our longitudinal study characterized the developmental trajectory of striped mouse SBs and, on an individual level, investigated potential life history (postnatal development) and behavioural factors (activity, perseveration, anxiety/fearfulness) which might predict the development of SB and/or mediate, or correlate with, its performance. Just over half of all standard-housed captive-born striped mice developed SB, with stereotypic striped mice showing substantial intra- and inter-individual variation in SB form. Over development, SB performance increased in frequency but decreased in variability. Measures of perseveration, activity, and anxiety/fearfulness assessed in juveniles before the onset of SB neither predicted which individuals later developed SB nor mediated the frequency or variability of later SB performance in stereotypic individuals. However, increases in perseverative tendencies and activity over development paralleled the development of SB, occurring only in stereotypic individuals. Thus, as adults, stereotypic striped mice were more active and more perseverative than non-stereotypic individuals although, within the sample of stereotypic adult striped mice, measures of SB frequency and variability did not correlate with levels of perseveration. Whilst preweaning developmental maturity did not predict which striped mice later developed SB, striped mice that were more developmentally mature in the preweaning period were likely to show SB at an earlier age, and to perform it with higher frequency, and possibly also with reduced diversity, effects which persisted into adulthood. In conclusion, our results characterize the general developmental trajectory of SB in striped mice, suggest that SB is associated with altered forebrain functioning but is not causally associated with the type of perseveration measured here, and indicate that different factors, some neurobiological and some others motivational, account for different aspects of the stereotypic phenotype.

Keywords:
Behavioural development
Behavioural variability
Perseveration
Rhabdomys
Stereotypic behaviour
Striped mice

Stereotypic behaviours (SB), repetitive behaviours induced by frustration, repeated attempts to cope, and/or forebrain dysfunction (Mason 2006), are observed in a variety of human psychiatric and neurodevelopmental disorders (e.g. schizophrenia, autism, mental retardation; Lewis & Kim 2009), and are also performed by at least 85 million captive animals worldwide (Latham & Mason 2004). Animal examples include bar chewing in barren-housed laboratory rodents (Würbel 2006), body rocking in isolated primates (Novak et al. 2006), sham chewing in hungry farm animals (Bergeron et al. 2006), and pacing in caged carnivores (Clubb & Vickery 2006). SBs characteristically develop in impoverished and restricted environments which lack the necessary sensory, motor, and social stimulation to facilitate normal development, and which thus routinely frustrate species-typical behaviours (Hughes & Duncan 1988). In contrast, SBs are only very rarely, and then but transiently, observed in free-ranging wild animals (Mason 2006). Because of the association of SB with barren housing conditions and inadequate husbandry, high SB incidences within a population of animals raise welfare concerns even though, on an individual level, SB performance is an imperfect indicator of current or even historical distress (Latham & Mason 2004).

The hypothesised causes of SBs are two-fold, explaining the development of the behaviour on two complementary levels, motivational and neurobiological. First, ethologists understand SBs in terms of their motivational link to species-typical behaviours (Latham & Mason 2010). In captivity, many of these behaviours are thwarted, resulting in the sustained elicitation of natural behaviour patterns (hereafter 'source behaviours'). A number of empirical findings support this hypothesis which, in general, focuses on the more distal (group level) causes of SB. For example, bar chewing in laboratory mice, *Mus musculus*, arises from repeated escape attempts (Nevison et al. 1999; Lewis & Hurst 2004), stereotypic digging in gerbils, *Meriones unguiculatus*, is motivated by the need to access shelter (Wiedenmayer 1997), and, in captive carnivores, motivation to roam, quantified by species

home range size and daily travel distances, predicted frequency of pacing (Clubb and Mason 2003). Second, in contrast, neuroscientists explain the more proximal, individual-level causes of SBs in terms of forebrain dysfunction similar to that which underpins SB in human conditions (Lewis & Kim 2009). SBs are thus considered to arise secondarily to changes in inhibitory control mechanisms located primarily in neural pathways connecting the frontal cortex and the dorsal basal ganglia, brain areas responsible for the inhibition of inappropriate and unsuccessful behaviours and for the maintenance of behavioural flexibility (e.g. Garner 2006; Lewis et al. 2006; Graybiel 2008). In humans, relationships have repeatedly been found between measures of perseveration ('the continuation or recurrence of an … activity without the appropriate stimulus'; Sandson & Albert 1987, page 1736) and levels of repetitive behaviours in both clinical and non-clinical populations. For instance, in autistic children, individuals who performed poorly on a two-choice guessing task, a measure of recurrent perseveration, also showed higher rates of SBs (Frith 1970) and, in a sample of normal adults, perseveration on a rule-changing task correlated positively with scores on an obsessive-compulsive inventory (Zohar et al. 1995). Results from an increasing number of studies of captive animals suggest three reasons that animal SBs might likewise be associated with impaired behavioural control: (1) In a number of species, a relationship has been shown between individual levels of SB and recurrent perseveration (e.g. blue and marsh tits, *Parus caeruleus* and *P. palustris*, Garner & Mason 2002; bears, *Ursus thibetanus* and *Helarctos malayanus*, Vickery & Mason 2003; deer mice, *Peromyscus maniculatus*, Tanimura et al. 2008; American mink, *Neovison vison*, Dallaire et al. 2011). (2) Conditions or treatments that elicit SB such as deprivation-rearing (rhesus monkeys, *Macaca mulatta*, Gluck & Sackett 1976) or high dose amphetamine administration (laboratory rat, *Rattus rattus*, Evenden & Robbins 1983) also induce recurrent perseveration. (3) In deer mice, a series of experiments has shown correlations between individual levels of SB and regional activity of implicated forebrain areas (reviewed in Lewis et al. 2006).

The past decade has witnessed an increased scientific interest in the underlying and interlinked distal and proximal causes of SB in animals (Mason & Rushen 2006; Langen et al. 2011), and a number of studies have examined the impact of the early developmental environment on the elicitation of the behaviour and, on a group level, tracked associated brain and behavioural changes (e.g. Lewis et al. 2006; Novak et al. 2006). However, very few longitudinal studies focus on the development trajectories of SB both in human clinical disorders (Symons et al. 2005) and in captive animals (Mason & Rushen 2006). This paucity

of work in human and non-human individuals over time is surprising for at least four reasons, since such studies would: (1) produce data that could facilitate the developing and testing of hypotheses about the causes of SB by establishing temporal precedence (e.g. Cronin et al. 1985); (2) contribute to the knowledge base necessary for designing prevention and intervention strategies (Latham & Mason 2004; Tanimura et al. 2010); (3) enhance our core definitional understanding of the behaviour (e.g. multiple authors have argued that the key to understanding the emergence of SBs is not through analysis of their static and partly subjective features, but through understanding their dynamic developmental changes; Newell 1996; Würbel 2006); and (4) allow us to quantify and empirically test poorly verified ideas about developmental changes in SB (Mason 2006), and quantify attendant changes in neurostructural (e.g. altered dendritic morphology in the dorsolateral striatum) and neurobehavioural factors (e.g. recurrent perseveration) which might correlate or causally drive changes in SB over the course of its development (Mason 2006). Candidate developmental changes warranting investigation (and also sometimes used as indicators of SB severity; e.g. Dallaire et al. 2011) include: increased frequency and duration (e.g. Ödberg 1986; Würbel et al. 1996; Wiedenmayer 1997; Meehan et al. 2004; Tanimura et al. 2010); reduced variability in form and organization (e.g. Cronin 1985; Mason 1993; Vickery 2003; Tanimura et al. 2010); reduced dependence on original eliciting factors (establishment; e.g. Würbel et al 1996; Wiedenmayer 1997); and persistence in situations which would not originally have elicited SB (emancipation; e.g. Cooper et al. 1996).

Our current study was designed to build on and comprehensively extend our knowledge of striped mouse stereotypic behaviour. By using a longitudinal design, we sought to characterize in detail the forms of SB shown in striped mice, *Rhabdomys*; describe the developmental trajectory of striped mouse SBs over 21 weeks from birth to adulthood; and, on an individual level, investigate potential life history and behavioural risk factors for the development of SB, as well as track developmental changes in these variables to better understand their contribution to the stereotypic phenotype. Striped mice are small, non-endangered, murid rodents which are widely distributed across a number of habitats in the southern African subregion (Skinner & Chimimba 2005). They offer the typical advantages of a rodent species (small body size, successful reproduction in captivity, short generation times; Schradin & Pillay 2003) and, because they are diurnal (Schradin 2006), are easy to observe. Furthermore, since they are wild-derived (v. domesticated) captive animals, they provide a suitable model of the behavioural processes involved in the development of SB in

wild-caught (WC) animals (Jones et al. 2011a, b). Similar to the trend shown in animals from many WC species (reviewed by Mason 2006), few WC striped mice develop SB, although about half of all captive-born (CB) striped mice reared in standard cages do so (Jones et al. 2008, 2011a). The heterogeneity of form and frequency of SB among CB striped mouse individuals additionally facilitates the investigation of individual variation and the predictors thereof.

For ease of reporting and reading, we have divided this paper into two sections. In Section 1, we characterise the development of SB in striped mice, and then test two oft-cited but seldom tested hypotheses about developmental changes in SB performance, viz., increased frequency and decreased variability. In Section 2, we then explore various life history (e.g. weaning mass) and behavioural (e.g. perseveration, anxiety/fearfulness) variables which are associated with SB development and performance to gain insight into the causal mechanisms underlying its expression. Specifically, we sought to identify factors that: (1) predict which striped mice develop SB (predictors or risk factors); (2) influence the expression of the behaviour in SB individuals (mediators or mediating factors); and/or (3) covary with SB, but are not necessarily causally related to it (correlates).

GENERAL METHODS

Subjects, housing, and husbandry

Striped mice used in this study (n = 68; female = 34; male = 34) were first generation (F1) offspring of 17 breeding pairs established from wild-caught animals trapped in a grassland locality (Honeydew, Gauteng, South Africa; 27°55'S, 26°4'E). All striped mice were housed in the Milner Park Animal Unit at the University of the Witwatersrand, under partially-controlled environmental conditions (14L: 10D, lights on at 05h00 hours; 20 – 24 ºC; 30 – 60% relative humidity). Breeding pairs, their litters, and adult offspring were kept in standard Labotec™ cages (300 x 200 x 150 mm) containing ~2cm woodshavings as bedding, a PVC tubing nest box (10 x 10 x 15 cm, open at both ends) provisioned with hay and paper towel as nesting material, and one cardboard toilet roll. Pups were separated from their parents on PN22 (day of birth was designated as PN0), and housed in mixed sex sibling groups in large tanks until PN30. The tanks (460 x 300 x 320 mm high), which had three metal sides, a clear perspex front, and a wire mesh lid, contained ~5 cm of woodshavings as bedding, ~20 cm of a mixture of *Eragrostis* grass, oat hay and/or lucerne, a PVC tubing nest box containing paper

towelling as nesting material, and between 3 to 5 cardboard rolls. From PN31 onwards, striped mice were individually housed in standard Labotec™ cages (described above). All striped mice were provided with ad libitum Epol mouse cubes. A small amount (± 3g) of seed mix (sunflower, millet) was sprinkled in the cages/tanks daily, together with fresh fruit and/or vegetables (±10g).

Procedure

Longitudinal data were collected for individual striped mice from two days postpartum (PN2) until striped mice were five months old (~PN160). We recorded developmental data from PN2 until weaning at PN22, tested juvenile striped mice in two behavioural apparatuses between PN22 and PN30 (four-arm maze; light-dark box; details provided below), scan sampled home cage behaviour of striped mice five times per week, for 18 weeks, between PN31 and PN156 (for analysis purposes, these data were divided into two time periods: Phase I [weeks 1 to 9, corresponding to early adulthood] and Phase II [weeks 10 to 18, corresponding to adulthood]; Brooks 1982), and retested adult striped mice in the four-arm maze and light-dark box when they were ~PN160 days old. We also video-recorded the home-cage behaviour of adult striped mice when animals were around five months old (Figure 1).

Preweaning developmental testing

On the last four days before expected parturition, we performed daily checks of the nest. We designated the day on which pups were found as PN1 and the previous day (PN0) as the day of birth. On PN2, we sexed, marked individually, and weighed all pups (to the nearest 0.1g). On PN22 (weaning), pups were again weighed. Between days PN2 and PN10, we examined pups daily to determine age at eye opening and incisor eruption (both markers of physical development; Brooks, 1982).

Postweaning and adulthood behavioural testing

Testing sessions took place between 0700 and 1100, during which time striped mice are most active (Pillay 2000). We allowed a minimum of 24 hours between testing in the four-arm maze and in the light-dark box. Both these apparatuses were illuminated from above with fluorescent lighting, and the sessions were recorded by a video camera positioned directly above them. The apparatuses were cleaned between tests using soap and water. Recordings were scored by MJ using Observer 5.0 (Noldus, the Netherlands).

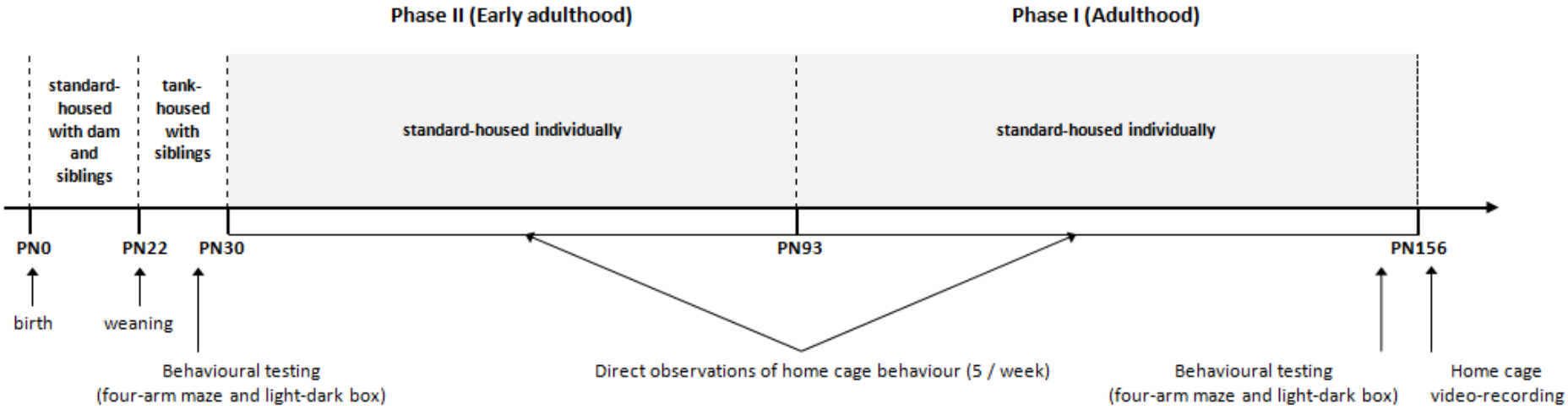


Figure 1. Timeline indicating housing condition and experimental procedure for striped mice over the 5-month data collection period (from birth until ~160 days old).

Four-arm maze. The four-arm maze consisted of four enclosed arms (7.5 x 7.5 x 15 cm), constructed from clear PVC, connected to a central area (10 x 10 x 20 cm). A subject was placed into the central area, and its behaviour recorded for 10 minutes. The frequency of arm entries was recorded as a measure of locomotor activity. We followed the methods of Hliňák and Krejči (2006) to calculate Spontaneous Alternation Behaviour (SAB) scores, defining a SAB score for a series of four arm entries (a tetrad) as the ratio of actual arms entered to the number of arms that could have been entered. Thus, in the arm entry sequence 12342…234, an alternation score for each tetrad was calculated as follows: for the first tetrad (**1234**2…234), the mouse entered four different arms out of a possible four, giving an alternation score of 4/4=1; for the second tetrad (1**2342**…234), it entered three arms out of a possible four, and hence scored 3/4=0.75; with the last three entries of the sequence (12342…**234**) not being considered because these did not constitute a complete tetrad. Total SAB scores for the trial were calculated by averaging SAB scores across all tetrads in a sequence, with low overall scores representing a tendency to enter fewer arms, and to conduct more repeat visits of the same arm (Jones et al. 2011).

We used sequential analysis to assess the predictability of a striped mouse, once having entered a particular arm, then entering another particular arm. For example, a striped mouse might have the sequence, 1234123412341234, repeating the same pattern of arm entries, yet would be judged, using spontaneous alternation, as not perseverating (all tetrads would receive a score of 1). Sequential analysis can, however, detect this form of perseveration by assessing whether one behavioural element is more or less likely than chance to follow another behavioural element (see Van Hooff 1982). For each individual, the sequence of arm entries was coded into transition matrices with the current behavioural element (an entry into one of the arms) represented in the columns and the preceding arm entered represented in the rows. Using the software Matman™ (Noldus, the Netherlands), we calculated the adjusted residuals (i.e. differences between observed and expected values for each transition frequency) for each matrix and then used the generated χ^2 value for each matrix as an index of routine formation (the higher the χ^2 value – the Sequential Analysis (SA) score – the more predictable a mouse's pattern of arm entry; thus, unlike the SAB score, here high scores mean greater predictability) (Jones et al. 2011).

Light-dark box. The light-dark box comprised a glass tank divided into two equal-sized compartments (30 x 22.5 x 30 cm each), each with three ventilation holes in a Perspex lid,

connected by a 6 x 6 cm opening on the floor of the tank. One half of the tank, the dark compartment, had black walls and lid. The other half, the light compartment, had clear walls and lid. A subject was placed in the light compartment facing away from the opening into the dark compartment, and its behaviour recorded for 5 minutes. We scored the proportion of time in the dark compartment (entire body in the dark compartment) and the latency to return to the light compartment after first entry into the dark compartment.

Direct behavioural observations

Between PN31 and ~PN156, for 18 weeks in total, MJ directly observed striped mice five times per week, for one 15-minute observation session per day, and scored the absence/presence of SB; the absence/presence of activity outside the nest, and the form(s) of SB displayed by stereotypic striped mice. We defined SB as a repetitive behaviour comprising at least three successive repetitions (Jones et al. 2008). The various forms of SBs observed are defined in Table 1.

Assessment of home cage behaviour

Between PN150 and PN160, subjects were video-recorded in their home cage for 40 minutes per day for three consecutive days. From the recordings, MJ observed the behaviour of subjects over two 10-minute periods (5-14 min and 25-34 minutes), scoring the following behaviours: time spent in the nest (inside the next box, usually inactive, resting, or sleeping); time spent outside the nest (activity outside the nest box, excluding SB); and also total duration of SB. We did not consider inactivity outside the nest box because this was very rarely seen.

Ethical note

Individual housing is ethically acceptable in grassland-derived striped mice because free-ranging animals, unlike their desert-derived counterparts, are naturally solitary; hence individual housing in enriched cages is linked with negligible SB (Jones et al. 2011b). Striped mice used in this study were ultimately euthanized with an overdose of inhalant anaesthetic (Isoflurane or Halothane) or returned to stock for use in other behavioural projects. Approval for this study was granted by the Animal Ethics Screening Committee of the University of the Witwatersrand (2005/46/3).

SECTION 1: CHARACTERIZATION OF THE DEVELOPMENT OF STEREOTYPIC BEHAVIOUR

In this section, our primary aim was to track the development of SB in striped mice from when they were individually housed shortly after weaning (PN30) until mid-adulthood (~PN156). Previous work has demonstrated that: SB emerges post weaning in striped mice (Schwaibold & Pillay 2001; Jones et al. 2008; only once has it been observed pre weaning; MJ, personal observations); about half of all CB striped mice develop SB (Schwaibold & Pillay 2001, Jones et al. 2011a, b); and after striped mice first manifest SB, they seldom cease displaying the behaviour (only once has this been observed to stop after developing; MJ, personal observations). Furthermore, it is known that there is substantial intra- and inter-individual variation in the forms of SBs shown (Schwaibold & Pillay 2001, Jones et al. 2011a, b). Little, however, is known about the developmental course of SB in striped mice, viz., the upper and lower limits of its initial expression, the changes in the frequency of SB performance during development, the predominant forms of SB early and later in development, and the intra- and inter-individual variability of SB forms over time.

A secondary aim of this section was to test two of the four hypothesised behavioural changes believed to characterize SB (see Introduction). First, we sought to investigate whether striped mouse SBs become less variable and more predictable over time and, second, we examined whether striped mouse SBs become more time-consuming over the course of their development. Although invariability and predictability are assumedly important defining characteristics of SB, few studies have quantified these aspects of the behaviour, and all those that have, have used different methodologies. For example, Cronin (1985), Vickery (2003), and Mason (1993) examined features of within bout rigidity and flexibility quantifying the number of repetitions of the behavioural elements comprising the SB. In contrast, Tanimura et al. (2010) examined the temporal organization of deer mouse SB to assess its relative flexibility or predictability. Because striped mice show a variety of different forms of SB which appear to change in frequency over development, we assessed between-bout variability of SB performance in two ways. First, we quantified whether individuals had a preferred form of SB at different points in development. Second, to provide a more sophisticated measure of form variability, we adapted diversity measures from ecology (e.g. Colwell 2009) to obtain measures of form variability which are sensitive to the total number of forms of SB in a striped mouse's behavioural repertoire, and the relative abundance of these different

forms (described in detail in the procedure section, below). Finally, because the association between variability and frequency of SB has previously been shown to be negatively correlated in adult mink (Mason 1993), and might be expected, *a priori,* to correlate negatively if changes in frequency and variability over development share a similar cause, we additionally sought to examine the interplay between SB variability and frequency.

Procedure

Using the data collected from the 18-week period of direct behavioural observations and from the video-recordings of home cage behaviour, we classed striped mice as either stereotypic or non-stereotypic. Striped mice which consistently displayed SB during Phase II of observations (i.e. the behaviour was observed most weeks, several times per week, and was present when home cage behaviour was video-recorded) were regarded as stereotypic (S), whereas those striped mice that displayed no stereotypic behaviour were classed as non-stereotypic (NS) (see Jones et al. 2010a for a justification of this scoring method). For each stereotypic striped mouse, we also recorded (1) age of onset of SB (number of weeks after individual housing); (2) the frequency of SB (proportion of observation days on which SB was shown during Phases I and II and, from the video-recordings, the proportion of home cage time engaged in SB); (3) the preferred forms of SB shown during Phases I and II (see below); and (4) the variability of SB form during Phases I and II (see below).

Determination of preferred form of SB. For Phases I and II separately, we determined the preferred form of SB according to the methods of Jones et al. (2011). For each individual striped mouse, we divided the total number of times a particular SB was shown by the total number of observation sessions in which any SB was observed. More than one form of SB could be recorded within one observation session, and thus the sum of the percentage observations could exceed 100%. If a particular SB was observed in more than 70% of the observation sessions, provided the sum of the second and third most preferred forms did not exceed 70%, that form of SB was regarded as the preferred form. If no form of SB was performed in more than 70% of observation sessions, or if the sum of the second and third most preferred forms exceeded 70%, then the preferred form of SB was designated as "mixed".

Determination of variability of SB. For Phases I and II separately, we calculated two measures of the variability of the forms of SB for individual striped mice, Evenness and

Diversity, using the freeware application EstimateS Version 8.2.0 (Colwell 2009). Although these indices are typically used to calculate species diversity in ecological studies, they have a broad scope of application, and have previously been used in behavioural studies (e.g. Vickery 2003). We first calculated "Form Evenness" (Shannon J') to quantify the unequal representation of the forms of SB against a hypothetical sample in which all forms of SB are equally likely, i.e. the ratio of observed diversity to maximum possible diversity. A low Form Evenness (tending towards 0) indicates a high dominance of one or a few forms of SB, whereas a high Form Evenness (tending towards 1) indicates that all forms of SB are equally likely. Second, we calculated "Form Diversity" (Shannon-Weiner H'), a measure which incorporates both richness (i.e. the total number of forms of SB observed) and evenness into a single value. The Shannon-Weiner H' measures the average degree of 'uncertainty' in predicting which form of SB will be chosen if selecting randomly from the total pool of SBs shown. Larger H' values indicate greater diversity of SB forms, whereas smaller values (tending to 0) indicate lower diversity. We also recorded the total number of different forms of SB observed for each individual striped mouse in each phase.

Statistical analyses

Analyses were run using SPSS (Version 20) and Graphpad Instat (Version 3). In these and subsequent analyses, all tests were two tailed. Differences were considered significant when $\alpha \leq 0.05$.

In both Sections 1 and 2, we first analysed the data sets using the Restricted Maximum Likelihood (REML) method of variance components analysis to assess the effects of random factors (litter number, breeding pair identity, maternal identity, paternal identity) on the categorical and continuous outcome variables. As these random factors had minimal impact on the relevant variables, they were excluded from further analyses.

We compared the difference in incidence of SB in male and female striped mice using Fisher's Exact Test, and examined the effect of sex on the age of SB development using a Generalized Linear Model (GLZ) with sex as the categorical predictor and age of SB development as the dependent variable. We compared the difference in incidence of striped mice having a preferred "mixed" or "single" form of SB in Phases I and II using a logistic regression with a binomial logit function. For SB frequency and the various measures of form

variability, we compared the differences between Phases I and II using separate Generalized Linear Models (GLZ) with a repeated measures design, followed by pair-wise planned contrasts. In these models, we included sex and sex*phase as the categorical predictors, phase (Phase I or II) as the repeated measure, and the scores for SB frequency or form variability as the dependent variable. To assess the association between SB frequency and the various measures of form variability, we first parsed the data for male and female striped mice, and then analysed the data using Spearman Rank Correlation.

Results

Incidence of SB. Striped mice began showing SB as early as the first week after individual housing (PN30-37; 18% of subjects in the first week), whilst some developed it as late as the 12th week (PN114-121) (Figure 2). In total, 59% (40/68) of the sample of striped mice developed SB. According to the best fit polynomial for all the data, the equation

$$y = (0.256 + 21.612x) / (1 + 0.258x + 0.003x^2)$$

where y = % stereotypic and x = weeks post individual housing, indicates that half of all striped mice which develop SB (i.e. 29.5%) did so by 2.105 weeks post individual housing (~PN45), and 90% did so by 7.747 weeks post individual housing (~PN84). There was no effect of sex on the development of SB (21/34 male, 19/34 female; Fisher's Exact Test, P = 0.806) or on the age of development of SB (Wald $\chi^2_1 = 0.154$, $P = 0.695$).

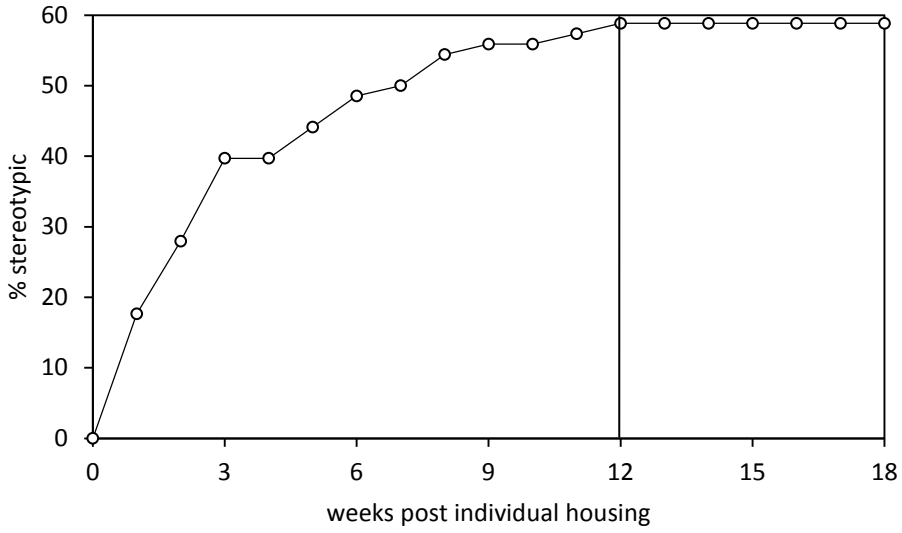


Figure 2. The cumulative percentage of striped mice showing stereotypic behaviour post individual housing (PN30). No further striped mice developed stereotypic behaviour after week 12.

Frequency of SB. As shown in Figure 3, there was an increase in the weekly frequency of SB over time; at each time point, only the cumulative scores of those striped mice which had developed SB were included in the calculations of average frequency. When the average SB frequencies for Phases I and II were compared for striped mice which showed SB in both time periods, frequency of SB was significantly higher in Phase II than in Phase I (Wald χ^2_1 = 16.680, $P < 0.001$; Figure 4). There was no effect of sex or sex*phase on SB frequency ($P > 0.10$). Home cage video-recording of stereotypic striped mice adults indicated that individuals spent around 27% of their active time engaged in SB (Median 26.70%, First Quartile 12.12%, Third Quartile 53.91%). There was no effect of sex on the proportion of home cage time engaged in SB (Wald $\chi^2_1 = 0.601$, $P = 0.438$).

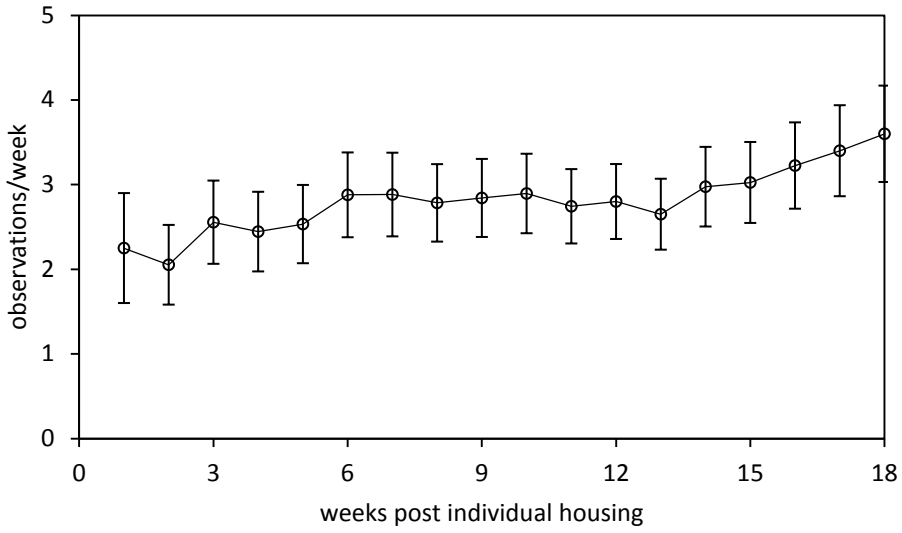


Figure 3. Average (± SEM) frequency of stereotypic behaviour (observations per week) in striped mice post individual housing. Only the scores of those striped mice which had developed SB at each time point are included in the calculations of the average weekly frequency.

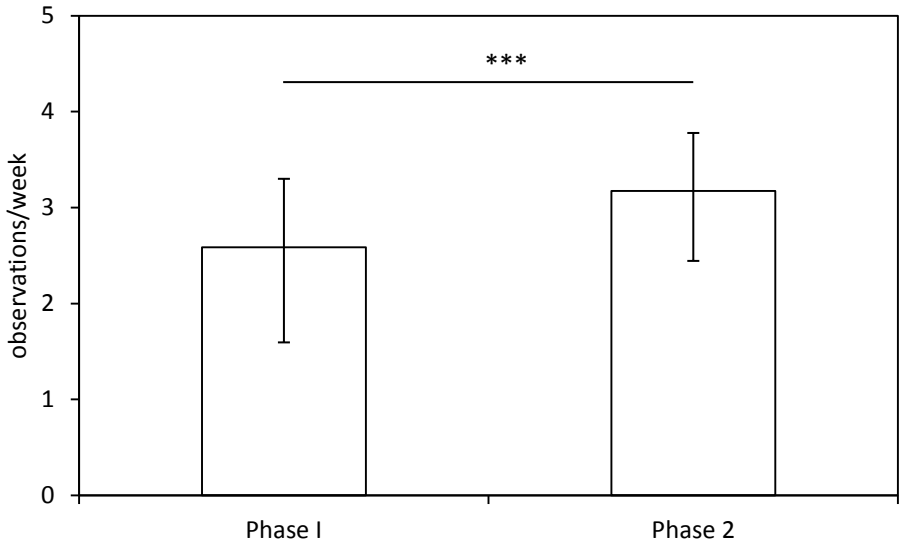


Figure 4. Frequency of stereotypic behaviour (average observations per nine-week period) in striped mice during Phase I and Phase II. Bars = median; whiskers = 1st and 3rd quartiles. *** indicates $P < 0.001$.

Forms of SB. In total, we observed 11 different forms of SB (Table 1). Seven of these were performed significantly less frequently in the Phase II than in Phase I (cage climbing, digging, hopper looping, looping, prelooping, rearing, twirling). Five of the 11 different forms observed (circuit running, prelooping, somersaulting, twirling, windscreen wiping) were a preferred form for one or more striped mouse mice in either Phases I or II (Table 2). When the preferred forms of SB were compared between Phases I and II, a "mixed" form of SB was more common in Phase I than in Phase II in only male striped mice (sex*phase Wald $\chi^2_1 = 3.861$, $P = 0.049$; Figure 5).

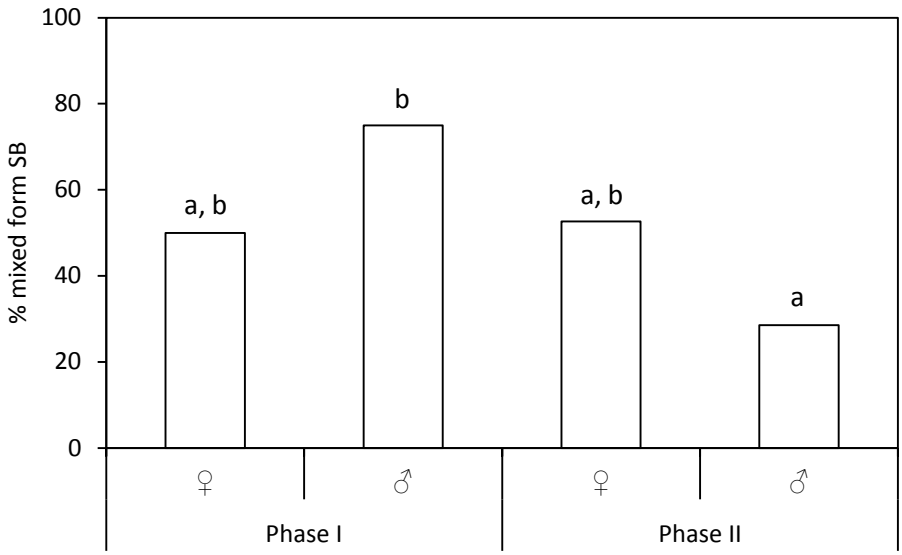


Figure 5. Percentage of male and female striped mice in Phases I and II preferring a "mixed" form of stereotypic behaviour. Alphabet letters denote homogenous groups.

Table 1. Descriptions of the forms of SB observed in striped mice, and the percentage of observation days on which the SB was shown in Phases I and II. The incidence of SB in each phase was compared using Chi Square Tests. Bolded values indicate $P \leq 0.05$.

Stereotypic behaviour	**Description**	**% of 'SB days' on which SB shown (Phase I)**	**% of 'SB days' on which SB shown (Phase II)**	**Difference in incidence between Phase I and II**
Cage climbing	Climbing on the cage lid, using either forelimbs or fore- and hindlimbs	20	10	↓ ***P* < 0.0001**
Circuit running	Running in the cage along a fixed route	54	61	$P = 0.1713$
Digging	Digging in the wood shavings	14	3	↓ ***P* = 0.0006**
Hopper looping	With hind limbs remaining virtually stationary on the floor of the cage in the section between the hopper and the Perspex front, animals rotate their upper body from an upright position in which they face the front of the cage, to a horizontal position underneath the hopper in which they face the cage lid, and then back to an upright position	41	20	↓ ***P* < 0.0001**
Jack hammering	Jumping up and down on the hindlimbs, usually in a corner of the cage or on and off the nest box	27	26	$P = 0.1168$
Looping	Climbing upside down on the cage lid using forelimbs and then hindlimbs, and then moving backwards along lid, followed by dropping down through releasing the forelimbs first	42	16	↓ ***P* < 0.0001**
Prelooping	Climbing upside down on the cage lid using forelimbs and then hindlimbs, and then moving backwards along lid, followed by dropping down through releasing the hindlimbs first	31	25	↓ ***P* = 0.0307**
Rearing	Rearing, usually in the corner of the cage, maintaining hindpaws on the floor and raising forelimbs off the floor	29	22	↓ ***P* = 0.0195**
Somersaulting	Backward flipping, with or without touching the cage lid	13	10	$P = 0.2534$
Twirling	Hanging from the cage lid using forelimbs, and spinning around by alternately removing one forelimb and replacing it with the other	12	4	↓ ***P* < 0.0001**
Windscreen wiping	Oscillating forelimbs to-and-fro in a large arc against the front side of the cage or the food hopper, with hindlimbs remaining stationary on the cage floor, or moving far less than the forelimbs.	18	21	$P = 0.2369$

Table 2. Preferred forms of SB in striped mice in Phases I and II.

Stereotypic behaviour	Preferred form (%) Phase I N = 38	Preferred form (%) Phase II N = 40
Mixed	63.16	40.00
Circuit running	15.80	37.50
Prelooping	7.89	7.50
Somersaulting	7.89	7.50
Twirling	2.63	0.00
Windscreen wiping	2.63	7.50

Variability of SB form. Because at least four weeks of SB performance were required to calculate the Form Evenness and Form Diversity indices, the data from only 35 of the 40 stereotypic striped mice could be included in these analyses. Significantly fewer forms of SB were shown by striped mice in Phase II than in Phase I (Wald $\chi^2_1 = 9.938$, $P = 0.002$; Figure 6). There was no effect of sex or sex*phase on the number of forms of SB shown ($P > 0.1$). There were, however, significant sex*phase effects on the differences in the variability of SB form between Phases I and II measured using either Form Evenness (Wald $\chi^2_1 = 8.575$, $P = 0.003$; Figure 7) or Form Diversity (Wald $\chi^2_1 = 3.882$, $P = 0.049$; Figure 8). For both measures, Phase II SBs showed reduced variability compared with Phase I SBs, especially in male striped mice.

In Phase I, SB frequency was positively correlated with the number of different forms of SB displayed in both female (Spearman r = 0.664; $P = 0.003$) and male (Spearman r = 0.680; $P = 0.001$) striped mice, and with Form Diversity in both female (Spearman r = 0.555; $P = 0.017$) and male (Spearman r = 0.510; $P = 0.022$) individuals. Form Evenness was significantly negatively correlated with SB frequency in male (Spearman r = -0.522; $P = 0.022$) but not female striped mice (Spearman r = -0.197; $P = 0.465$). In Phase II, there was no association between SB frequency and the number of different forms of SB displayed or SB Form Diversity in female or male striped mice, nor between SB frequency and the Form Evenness of SB in males (all $P > 0.1$). There was, however, a negative correlation between SB frequency and Form Evenness in female striped mice (Spearman r = -0.550; $P = 0.015$).

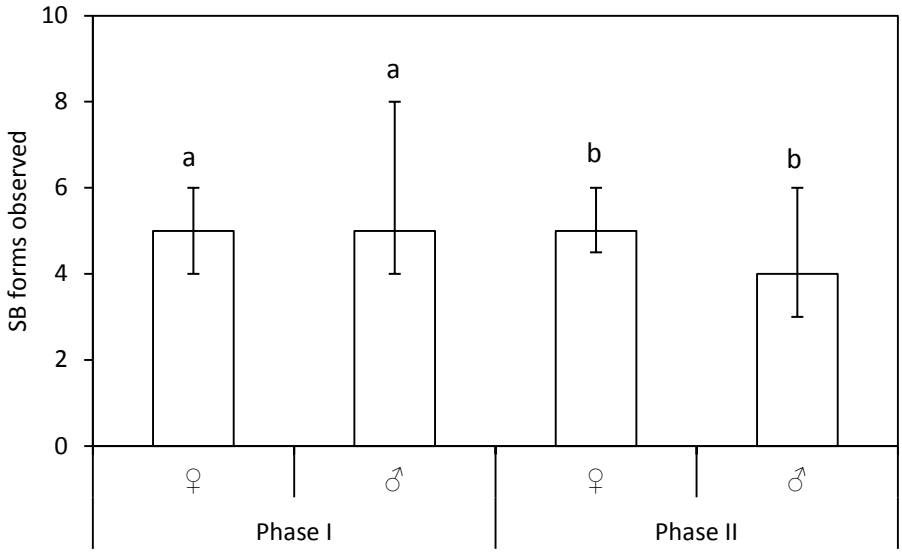


Figure 6. The number of different forms of SB shown during Phases I and II in female and male striped mice. Bars = median; whiskers = 1st and 3rd quartiles. Alphabet letters denote homogenous groups.

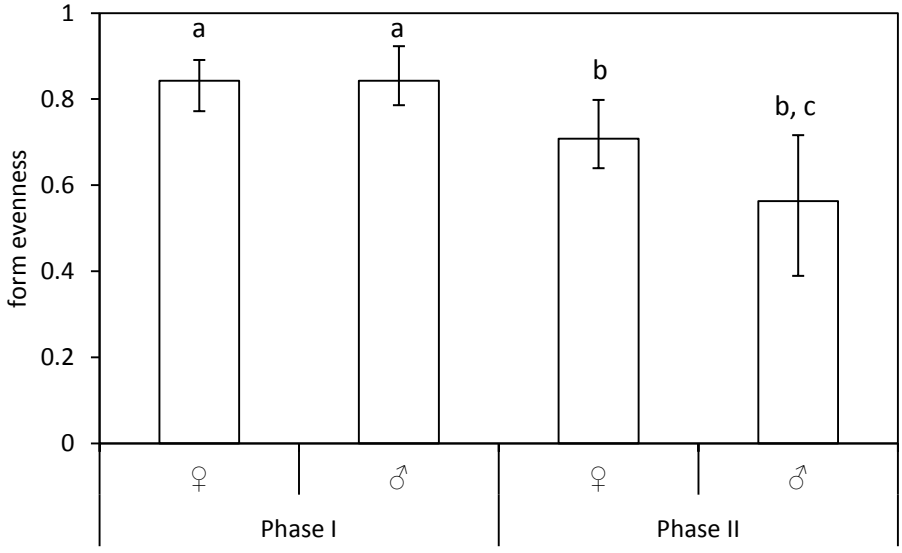


Figure 7. SB Form Evenness during Phases I and II in female and male striped mice. Bars = median; whiskers = 1st and 3rd quartiles. Alphabet letters denote homogenous groups.

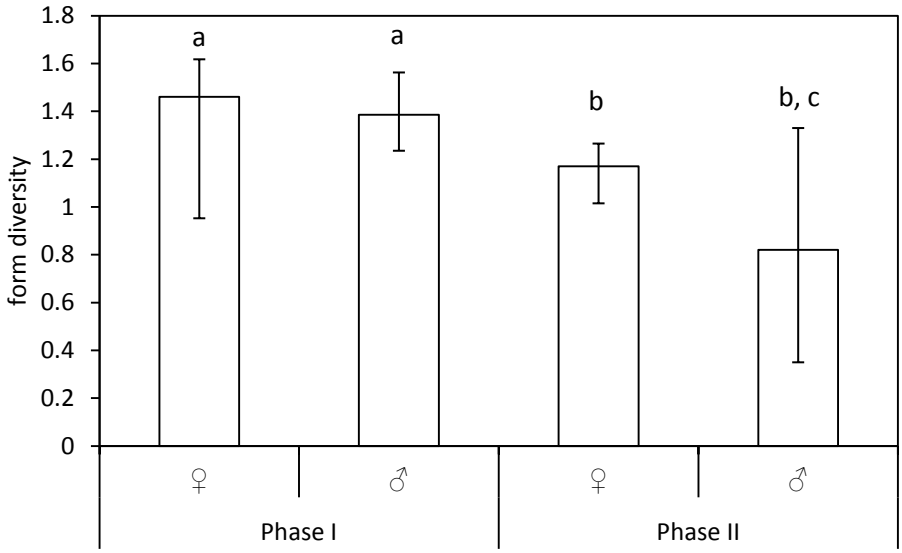


Figure 8. SB Form Diversity during Phases I and II in female and male striped mice. Bars = median; whiskers = 1st and 3rd quartiles. Alphabet letters denote homogenous groups.

Discussion

The studies in the current section characterized the developmental trajectory of SB in striped mice from shortly after weaning until mid-adulthood, and compared differences in two of the performance characteristics of SB, frequency and variability, over behavioural development. Consistent with previous work (e.g. Schwaibold & Pillay 2001; Jones et al. 2008), we showed that SB developed in just over half of all CB striped mice, and was equally prevalent in males and females. Although 50% of stereotypic striped mice had developed the behaviour by just over two weeks after individual housing, and 90% by just under eight weeks, some subjects only manifested it as late as the twelfth week. However, no new occurrences of SB were observed after this point. Over the 18-week period of observations, the frequency of SB gradually increased and, by adulthood, stereotypic striped mice spent about a quarter of their active time engaged in SB. This level of SB is consistent with one of our previous studies of SB levels in CB striped mice (23.93%; Jones et al. 2011), although is substantially lower than the levels of ~50% reported in an earlier study by Nel (2003). A potential explanation for this difference is the variation in the provision of shelter within the home cage. In the two recent studies, striped mice were provided with nest boxes and nesting material, facilitating hiding behaviour, whereas in Nel's work only nesting material was provided.

As previously reported in striped mice and in other species (see Introduction), we observed substantial intra- and inter-individual variation in the SB form. Of the 11 different forms of

SB observed, all were locomotor. Seven of these forms appeared to represent less developmentally mature forms of SB (cage climbing, digging, hopper looping, looping, prelooping, rearing, twirling): these forms occurred at a greater frequency in Phase I than in Phase II, and qualitative assessment suggests a closer association between these forms of SB and species-typical behaviours, and thus also between the potential source behaviours from which more complex SBs might develop. For instance, activity directed towards the cage lid, such as climbing on the under surface of the cage lid, is believed to reflect attempts to explore or to escape from the cage (Würbel 2006). In striped mice, cage climbing, looping, and prelooping, SB forms occurring at a significantly higher frequency in Phase I, were topographically similar to the behavioural exploration of the cage lid that a number of striped mice performed shortly after individual housing. During early development, this source behaviour appeared to develop into cage climbing, looping, and prelooping SBs. Later on, these SBs typically developed into somersaulting. Between Phases I and II, there was also a notable reduction in the form variability of SB, and this effect was particularly strong in male striped mice, an unexpected sex difference which we return to in the General Discussion.

Finally, similar to Mason (1993), we investigated the interrelationship between SB frequency and variability both during the early stages of SB development and later, when the behaviour was presumably more established. Contrary to our prediction, there was a positive relationship postweaning between SB frequency and the number of different forms of SB observed as well as with SB form diversity, suggesting that early in development striped mice which spend more time engaged in SB are also more likely to experiment with different forms of the behaviour. As predicted, there was a negative association between SB form evenness and SB frequency, but in male striped mice only. These discrepant results likely reflect a characteristic of how these indices are calculated (form diversity is calculated by dividing form evenness by the number of different forms of SB observed), together with the unexpected finding that in Phase I the number of forms of SB observed correlates negatively with form evenness (i.e. early in development, striped mice that have one very dominant form of SB, are also likely to show a greater number of different forms of SB, although at very low rates). In adulthood, contrary to our predictions and the findings of Mason (1993), we found virtually no association between SB frequency and SB variability, indicating that whilst, on a group level, both these variables changed over time, they did not covary with each other. An exception to this finding was the result for the relationship between form evenness and SB frequency in female striped mice. Here, similar to the results from Mason (1993), there was a

moderate negative correlation between SB frequency and variability indicating that striped mice females which performed higher levels of SB also showed reduced form variability of their SBs.

SECTION 2: PREDICTORS AND CORRELATES OF THE DEVELOPMENT AND PERFORMANCE OF STEREOTYPIC BEHAVIOUR

On a group level, a large body of epidemiological work identifies the environmental (e.g. barren, spatially restricted) and developmental (e.g. maternal deprivation, social isolation, premature weaning) factors which predispose the development of SB in captive animals, and also the multifactorial mechanisms through which this occurs (see Introduction). However, on an individual level, within SB-inducing contexts, not all animals develop SB, and not all animals that develop SB manifest it equally severely (Mason 1993). Some of the observed inter-individual variation reflects heritable genetic contributions (e.g. Schoenecker & Heller 2000; Schwaibold & Pillay 2001), whereas other identified risk factors for SB are epigentically based and include temperament, stress reactivity, and developmental maturity at critical life stages. In the few studies which have investigated these individual-level risk factors that could plausibly influence the development of SB via influencing motivational priorities (e.g. escape behaviour v. hiding) and/or through their indirect influence (e.g. via corticosterone) on brain development, the following have been found: stereotypic farmed mink are typically less fearful than non-stereotypic animals (Hansen & Jeppesen 2006); stereotypic animals of a number of species (e.g. mink, Hansen & Jeppesen 2006; black and sun bears, Vickery & Mason 2004) have higher activity levels than non-stereotypic animals; some studies have shown lower levels of anxiety in crib-biting horses than in non-cribbers (Nagy et al. 2010), although others have found the opposite (Hausberger et al. 2007; Minero et al. 1999); in ICR mice, individuals which have a heightened corticosterone response after enrichment removal (Latham & Mason 2010) or post weaning (Würbel & Stauffacher 1997) go on to develop higher levels of SB (Latham & Mason 2010); and, also in ICR mice, poorer physical condition at standard weaning age has been shown to predict higher levels of SB performance in adults (Würbel & Stauffacher 1998).

A problem of many, although not all, of the studies investigating group- and individual-level causes of SB development is that data are often correlational, and the putative causal variables are assessed only after SB has developed, hence providing no means to assess

temporal precedence, a necessary criterion of causality. In striped mice, we have previously shown group-level differences in perseverative tendencies between WC and CB individuals, differences which do covary with SB performance, and which suggest that both perseveration and SB reflect dysfunctional changes induced by inadequate housing environments (Jones et al. 2011a). However, to investigate with more certainty the causal relationship between perseveration and the development of SB, it needs to be shown that perseveration changes together with SBs as the latter develop, and that individual differences in perseverative tendencies, measured before the development of SB, predict which individuals will develop SB.

In this section, we collected various developmental and behavioural data to investigate potential predictors of the development of SB (viz., developmental maturity; perseverative tendencies; activity levels; anxiety/fear), and then tracked changes in perseveration and anxiety/fear after the development of SB. We made three predictions. (1) If developmental immaturity predisposes striped mice to developing SB, similarly to the effect of low weaning weight in ICR mice, we would predict that retarded postnatal development (i.e. low birth mass, low weaning mass, delayed eye opening, and/or delayed incisor eruption, all markers of delayed development) would be linked to the later development of SB and, in stereotypic striped mice, would be positively associated with later SB frequency and invariability. (2) If forebrain dysfunction plays a causal role in the development of SB, we would predict that striped mice at risk of developing SB will have higher perseverative tendencies as juveniles, and that scores for perseveration will increase over the course of development only in stereotypic striped mice. Moreover, we would expect that perseveration scores would covary with SB frequency and invariability. (3) Similarly, if high activity levels predispose SB, we would expect that highly active juveniles would develop SB later, and that activity levels for only stereotypic striped mice would increase over the course of development and correlate positively with the frequency and invariance of SB in stereotypic striped mice. We made no *a priori* predictions about the association of fear/anxiety and the development of SB since high levels of fear/anxiety have been associated with both high (e.g. Hansen & Jeppesen 2006) and low (e.g. Nagy et al. 2010) levels of SB, both of which have not been shown to covary previously in striped mice with SB status (Jones et al. 2011a).

Procedure

Data collection methods for the preweaning developmental variables (PN2 and PN22 mass; age of eye opening; age of incisor eruption), postweaning and adulthood behavioural variables (frequency of arm entries; SAB score; SA chi-square; time in dark compartment; latency to emerge), and adulthood home cage behavioural variables (time spent in the nest; time spent outside the nest; total duration of SB) are described in the General Methods section. The frequency and variability of SB in Phases I and II were calculated according to the procedure outlined in Section 1. We also calculated the average weekly frequency with which striped mice were observed to leave the nest box using the raw data we collected during Phase II's direct behavioural observations.

Due to logistical problems, only 36 of the 68 striped mice were tested postweaning in the four-arm maze (18 female, 18 male; 15 non-stereotypic, 21 stereotypic). Of these, nine individuals (5 female, 4 male; 4 non-stereotypic, 5 stereotypic) were excluded from SAB and SA analyses because they made three or fewer transitions during the entire trial, precluding score calculation. During behavioural testing at adulthood, all 68 striped mice were tested in all apparatuses. However, seven individuals were excluded from adult SAB and SA analysis: four because they made three or fewer transitions during the entire trial in the four-arm maze, precluding score calculation (1 female, 3 male; 1 non-stereotypic, 3 stereotypic), and three because of technical malfunction of the recording equipment, precluding scoring of the trials (3 female; 1 non-stereotypic, 2 stereotypic). One individual (stereotypic female) was excluded from the analysis of light-dark box variables (technical malfunction of recording equipment), and two individuals (both non-stereotypic females) were excluded from analyses of home cage data (technical malfunction of recording equipment).

Statistical analyses

First, to investigate potential risk factors for the development of SB, we examined the effect of preweaning and postweaning variables on SB development using separate logistic regressions with binomial logit functions, followed by pairwise planned contrasts. We included sex as a categorical predictor and SB status (stereotypic or nonstereotypic) as the dependent variable as it was the predicted outcome in the model. For stereotypic striped mice only, we then tested for the influence of these preweaning and postweaning variables on the age of onset of SB, and the frequency and variability of SB using a logistic regression with sex as a categorical predictor. As mentioned in the previous section, because at least four

weeks of SB performance were required to calculate the Form Evenness and Form Diversity indices, the data from only 35 of the 40 stereotypic striped mice could be included in calculation of scores for Phase I. Second, to investigate whether behavioural measures of fear/anxiety, activity, and perseveration changed in parallel with the development of SB, we compared group differences between stereotypic and non-stereotypic striped mice using GLZs with sex, SB status, repeat (postweaning or adulthood) and SB status*repeat as the categorical predictors, and the behavioural scores as the dependent variables. Thereafter, we used planned contrasts to identify specific differences in the data set. Third, to compare group differences between stereotypic and non-stereotypic adult striped mice on variables which were measured only in adulthood, we used GLZs with sex, SB status, and sex*SB status as the categorical predictors, and the behavioural scores (time spent in the test; time engaged in non-stereotypic activity; observations per week) as the dependent variables. Finally, for adult stereotypic striped mice only, we ran further GLZ analyses to assess the relationship between adulthood behavioural scores for measures of fear/anxiety, actitivy, and perseveration (continuous predictors) and Phase II SB frequency and diversity scores (dependent variables). In these models, we also included sex as a categorical predictor.

Results

Predictors of SB development and performance characteristics. The results from the statistical analyses are given in Table 3. Unless otherwise indicated, sex was not a significant predictor of any variables associated with SB development or performance characteristics ($P > 0.05$). Contrary to predictions, none of the recorded preweaning or postweaning variables was a significant predictor of which striped mice developed SB. However, within the subset of striped mice which became stereotypic, we identified a number of preweaning and postweaning predictors of SB development. Individuals with a lower birth mass (PN2) had a later age of SB onset, and later eye opening predicted earlier SB onset. Mass at weaning (PN22), but not birth mass (PN2), was also positively associated with SB levels during Phases I and II and with the levels observed when striped mice were video-recorded in their home cages during adulthood. In addition, age of incisor eruption was negatively associated with SB levels during Phases I and II, but not with the measure of SB frequency derived from home cage video-recordings. In the analyses of the association between preweaning variables and SB variability, female striped mice showed increased variability in SB form compared to male striped mice in Phase II but not Phase I and, in a number of these instances, sex was a significant predictor of SB variability. Whilst there was no association between birth mass

(PN2), weaning mass (PN22), or age of incisor eruption and measures of SB Evenness or Diversity in Phases I or II, age of eye opening was a significant predictor of SB variability in Phase II (though not Phase I), with earlier eye opening associated with reduced Evenness and Diversity scores (i.e. less variable SBs).

In terms of postweaning predictors of SB performance, we found no predictors of age of development of SB or of the frequency of performance of SB in Phase II or during adulthood home cage video-recording. However, Phase I SB frequency was negatively associated with low SAB scores in the four-arm maze (increased perseverative tendencies) and with time spent in the dark compartment (increased anxiety/fearfulness). Similarly to the analyses of preweaning variables, female striped mice once again showed more variable SBs and, in a number of analyses of postweaning variables, sex was a significant predictor of Evenness and Diversity scores. None of the recorded postweaning variables was, however, a significant predictor of Evenness or Diversity scores in either Phase I or Phase II.

Table 3. Preweaning and postweaning predictors of SB status, age of onset of SB, and SB frequency and variability for stereotypic striped mice only. Values given are Wald χ^2_1 (P). Bolded values indicate $P \leq 0.05$. * indicate that, in the given model, sex was a significant predictor of the variable of interest.

			Frequency of SB			Variability of SB			
	SB status	Age of onset of SB	Phase I	Phase II	Home cage video-recording	Phase I Form Evenness	Phase I Form Diversity	Phase II Form Evenness	Phase II Form Diversity
Preweaning variables									
PN2 mass (g)	1.179 (0.277)	**5.427 (0.020)** ↑ mass → ↓ age onset	0.295 (0.587)	0.988 (0.320)	3.039 (0.081)	0.000 (0.999)	0.322 (0.570)	0.824(0.364)*	0.345 (0.557)
PN22 mass (g)	2.117 (0.146)	0.184 (0.668)	**7.237 (0.007)** ↑ mass → ↑ frequency	**4.650 (0.031)** ↑ mass → ↑ frequency	**7.907 (0.005)** ↑ mass → ↑ frequency	0.269 (0.604)	0.364 (0.546)	0.085 (0.770)	0.149 (0.700)
Age of eye opening	0.057 (0.812)	**5.450 (0.020)** later eye opening → ↓ age onset	3.001 (0.083)	0.121 (0.728)	0.031 (0.861)	0.019 (0.891)	1.864 (0.172)	**4.169 (0.041)*** later eye opening → ↑ variability	**5.327 (0.021)*** later eye opening → ↑ variability
Age of incisor eruption	0.344 (0.557)	0.716 (0.398)	**4.315 (0.038)** earlier eruption → ↑ frequency	**3.562 (0.059)** earlier eruption → ↑ frequency	2.475 (0.115)	0.202 (0.653)	2.273 (0.131)	0.012 (0.913)*	0.112 (0.738)
Postweaning variables									
Frequency of arm entries	0.011 (0.916)	2.672 (0.102)	0.325 (0.569)	0.273 (0.601)	0.468 (0.494)	0.177 (0.674)*	0.750 (0.386)	0.007 (0.935)	0.000 (0.985)
SAB score	0.387 (0.534)	0.339 (0.561)	**6.718 (0.010)** ↑ perseverative → ↓ frequency	1.448 (0.229)	0.002 (0.963)	3.824 (0.051)*	0.007 (0.931)*	0.000 (0.996)	0.195 (0.659)
SA chi-square	2.103 (0.147)	3.365 (0.067)	0.016 (0.900)	0.256 (0.613)	0.055 (0.814)	0.637 (0.425)*	2.926 (0.087)*	0.374 (0.541)	0.007 (0.935)
Time in dark compartment (%)	0.227 (0.633)	0.027 (0.870)	**6.391 (0.012)** ↑ % in dark → ↓ frequency	0.044 (0.833)	2.191 (0.139)	3.413 (0.065)	0.553 (0.457)	1.635 (0.201)*	1.848 (0.174)*
Latency to emerge (s)	0.402 (0.526)	0.648 (0.421)	1.405 (0.236)	2.175 (0.140)	0.543 (0.461)	0.014 (0.907)	0.744 (3.882)	1.118 (0.290)*	0.017 (0.896)

Effects of stereotypy status on behaviour over development. There was no effect of sex in any of the analyses (all $P > 0.05$). In the postweaning period, all striped mice showed equivalent levels of activity in the four-arm maze. However, between the postweaning period and adulthood, there was a significant increase in activity level in the four-arm maze in stereotypic striped mice only, whilst activity levels in nonstereotypic individuals remained similar to those shown in the postweaning period (stereotypy status*repeat Wald $\chi^2_1 = 5.233$, $P = 0.022$; Figure 9).

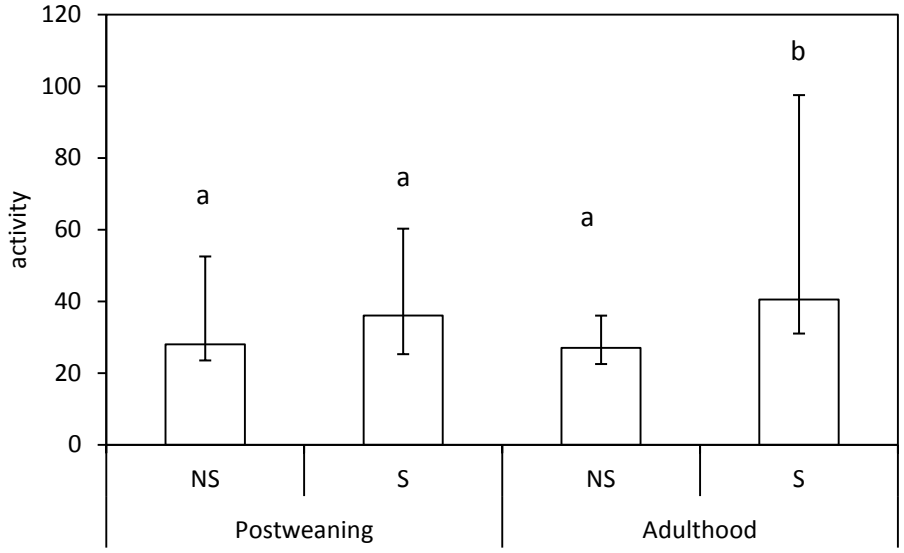


Figure 9. Frequency of arm entries in the four-arm maze in non-stereotypic and stereotypic striped mice postweaning and in adulthood. Bars = median; whiskers = 1st and 3rd quartiles. NS = non-stereotypic; S = stereotypic. Alphabet letters denote homogenous groups.

Similar to activity level in the four-arm maze, all striped mice showed similar levels of perseveration in the postweaning period, and there was again a significant stereotypy status*repeat interaction, for both SA (Wald $\chi^2_1 = 11.949$, $P = 0.001$; Figure 10a) and SAB (Wald $\chi^2_1 = 3.913$, $P = 0.048$; Figure 10b) scores. Over the course of development, stereotypic striped mice started to perform more predictable sequences of arm entry on both measures of perseveration (pairwise comparisons $P < 0.05$) whilst, in contrast, non-stereotypic striped mice showed increased variability in their sequences of arm entry over development, as measured using SAB ($P = 0.014$) although not SA scores ($P = 0.896$).

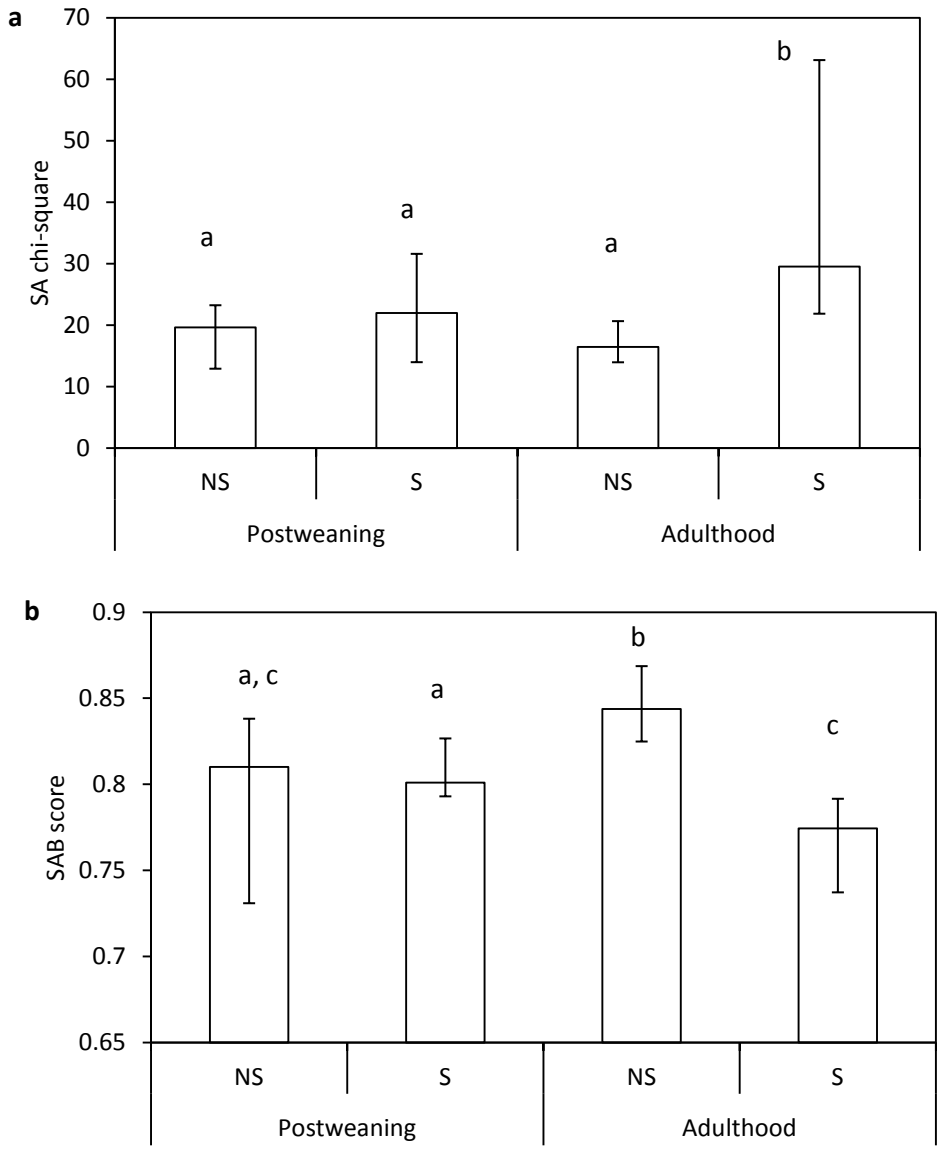


Figure 10. Perseveration of striped mice in the four-arm maze in non-stereotypic and stereotypic striped mice postweaning and in adulthood. (a) Sequential analysis chi-square. (b) Spontaneous alternation behaviour score. Bars = median; whiskers = 1st and 3rd quartiles. NS = non-stereotypic; S = stereotypic. Alphabet letters denote homogenous groups.

There was no influence of SB status on levels of fear/anxiety measured in the light-dark box either postweaning or in adulthood (time in the dark compartment, Wald $\chi^2_1 = 0.190$, $P = 0.663$, Figure 11a; latency to emerge, Wald $\chi^2_1 = 0.049$, $P = 0.825$, Figure 11b). There was, however, a significant effect of developmental stage on the levels of fear/anxiety measured in the light-dark box for both stereotypic and non-stereotypic striped mice, with scores for both time in the dark compartment (Wald $\chi^2_1 = 16.164$, $P < 0.001$) and latency to emerge (Wald $\chi^2_1 = 6.428$, $P = 0.011$) decreasing over the course of development.

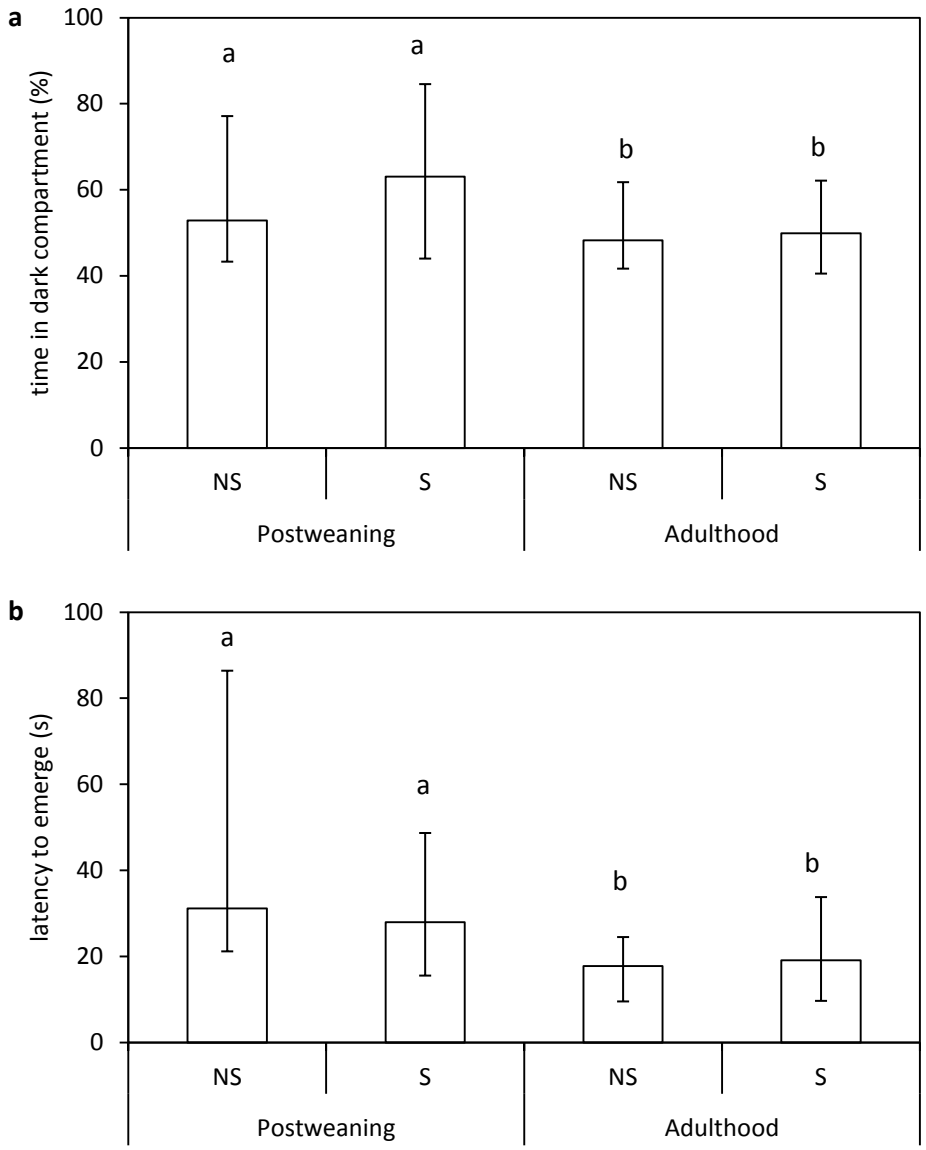
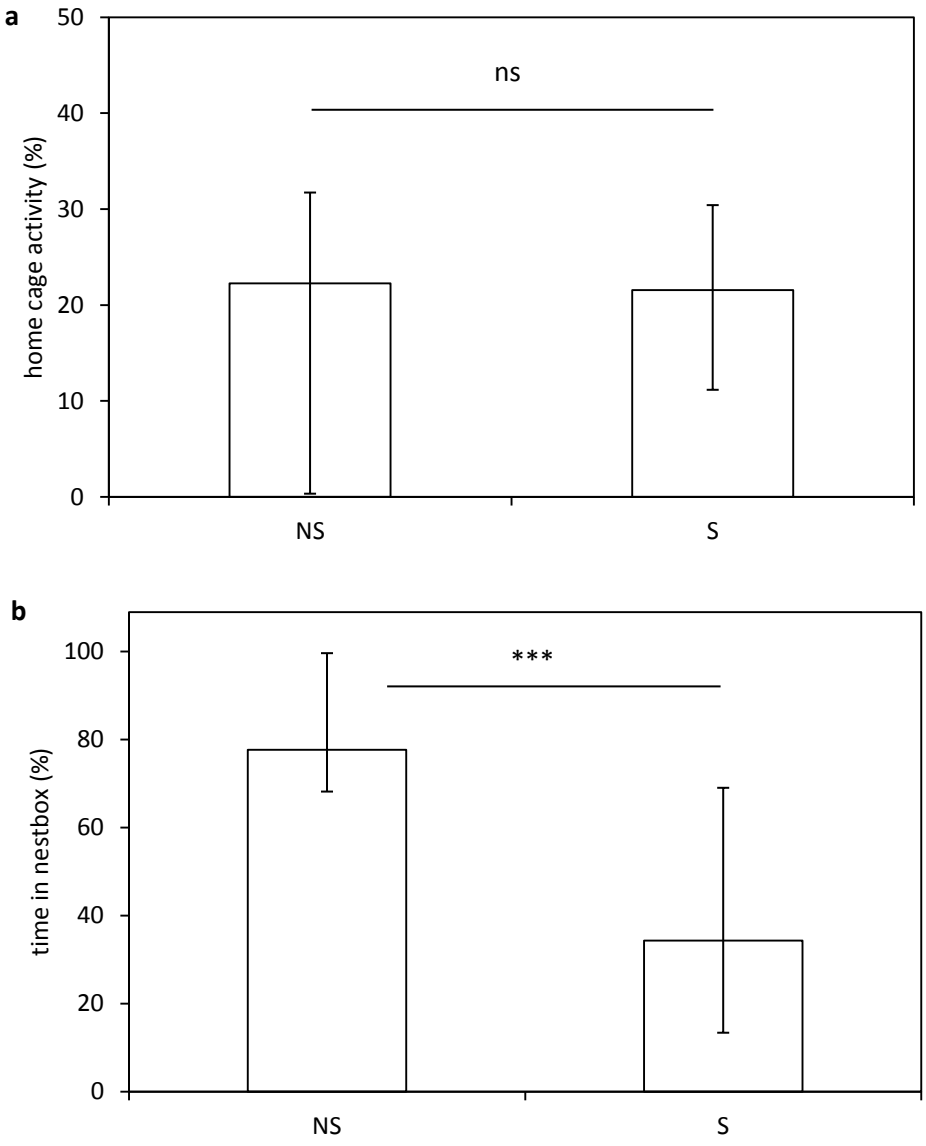


Figure 11. Anxiety/fearfulness in the light-dark box in non-stereotypic and stereotypic striped mice postweaning and in adulthood. (a) The percentage of time spent in the dark compartment. (b) Latency to first emergence from the dark compartment. Bars = median; whiskers = 1st and 3rd quartiles. NS = non-stereotypic; S = stereotypic. Alphabet letters indicate homogenous groups.

Effects of stereotypy status on behaviour in adulthood. Stereotypic striped mice were observed more frequently during direct behavioural observations (Wald $\chi^2_1 = 42.100$, $P < 0.001$; Figure 12c) than non-stereotypic individuals, and spent less time in their nest boxes (Wald $\chi^2_1 = 29.458$, $P < 0.001$; Figure 12b). However, the time engaged in non-stereotypic activity outside the nest box was comparable between stereotypic and non-stereotypic

individuals (Wald $\chi^2_1 = 1.516$, $P = 0.218$; Figure 12a). In no analyses was sex or sex*SB status a significant predictor of behavioural outcome (all $P > 0.10$).



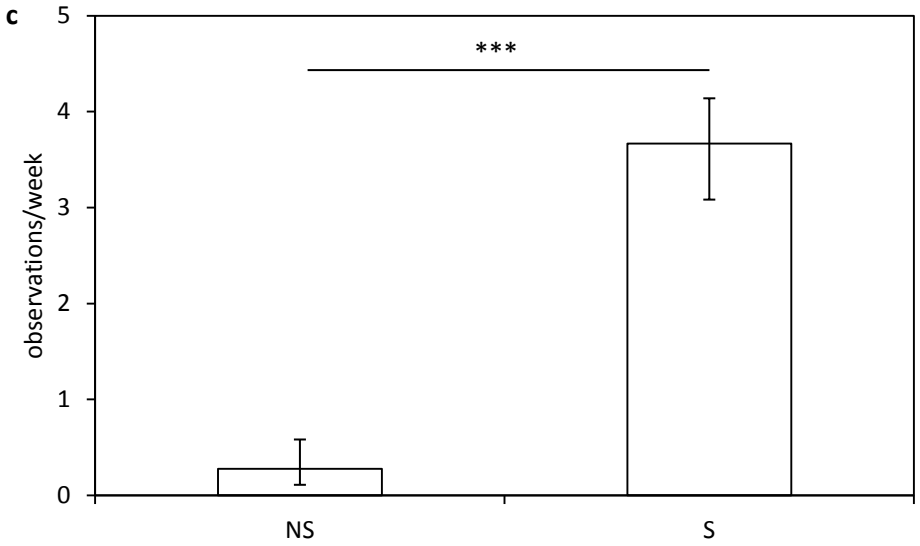


Figure 12. Measures of activity of non-stereotypic and stereotypic striped mouse adults. (a) The percentage of active time in the home cage. (b) The percentage of home cage time in the nest box. (c) The weekly frequency with which striped mice left the nest box during Phase II. Bars = median; whiskers = 1st and 3rd quartiles. NS = non-stereotypic; S = stereotypic. ns = not significant; *** = $P \leq 0.001$.

Correlates of the performance characteristics of SB in adulthood. Summary data and results from the statistical analyses are given in Table 4. We found no association between measures of perseveration, activity, and fear/anxiety and the frequency or diversity of adulthood SB. In no analyses was sex a significant predictor of outcome (all $P > 0.05$).

Table 4. The association between behavioural results from Part II and the frequency and diversity of adult SB in striped mice. Values given are Wald χ^2_1 (P).

Parameter	Frequency of SB		Diversity of SB	
	Part II	Home cage video-recording	Part II Form Evenness	Part II Form Diversity
SAB score	0.326 (0.627)	3.642 (0.056)	0.681 (0.409)	0.871 (0.351)
SA chi-square	0.455 (0.500)	2.354 (0.125)	3.010 (0.083)	1.991 (0.158)
Frequency of arm entries	0.011 (0.917)	2.372 (0.124)	2.720 (0.099)	1.125 (0.289)
Time in dark compartment (%)	2.267 (0.132)	0.050 (0.823)	0.507 (0.476)	0.093 (0.761)
Latency to emerge (s)	0.092 (0.762)	0.025 (0.875)	1.077 (0.299)	0.885 (0.347)

Discussion

By tracking the development of SB in individual striped mice over time, we sought to identify predictors, mediators, and correlates of SB performance over its developmental course. None of our recorded variables from the preweaning or postweaning periods predicted which striped mice would develop SB. This lack of temporal precedence either indicates that the identified correlates of SB observed later in development (discussed next) are unlikely causal factors in the development of SB or, alternatively, that the tasks used here to measure these variables are insufficiently sensitive for use in juvenile, but not adult, striped mice. We return to this issue in the General Discussion.

We did, however, identify several premorbid factors which mediated the performance characteristics of SB in stereotypic striped mice. Accelerated development, in particular mass at either birth or weaning and age of incisor eruption, was associated with an earlier age of development of SB, as well as a higher frequency of SB performance both in juvenile and adult striped mice. There was also some indication that developmental maturity, assessed using age of eye opening, predicted the variability of SB in adulthood only, although birth or weaning mass had no effect. We identified but one result which was contradictory to the general pattern of findings: for unknown reasons, delayed eye opening (i.e. delayed development) was associated with an earlier age of SB onset. Considered in their totality, however, these findings do not support our prediction that retarded postnatal development predisposes the development of SB, at least within the range of variation measured in this current study (but see Jones et al. 2010 where early weaning, and thus also low weaning weight, was associated with an increased incidence of SB). However, the data do support the hypothesis that physical competence or strength facilitates the performance of SB, an idea previously suggested by Würbel et al. (1996) to explain differences in source behaviours and consequent predominant forms of SB in ICR mice of different weaning weights.

In general, but with two exceptions, postweaning behavioural measures did not predict the later performance characteristics of SB in stereotypic striped mice. There was, however, some suggestion that higher levels of fear/anxiety postweaning, as measured by the proportion of time spent in the dark compartment but not by the latency to emerge from it, mediated the frequency of SB performance early on in its development, although not in adulthood – i.e. in juvenile mice, high levels of fearfulness might inhibit the performance of SB. A second finding, which was contrary to our predictions, was that postweaning

perseverative tendencies, assessed using the SAB but not SA scores, predicted lower frequency of SB, but again only early in the development of SB.

Similar to previous work in striped mice, we found group differences in perseverative tendencies between adult stereotypic and non-stereotypic striped mice on both our measures of perseveration. Dissimilar, however, to the majority of findings in other species (see Introduction) and our previous findings in striped mice (Jones et al. 2011a), we found no association between frequency of SB and levels of perseveration. We also found no association between SB variability and levels of perseveration in adult striped mice. Similar to our previous findings (Jones et al. 2011a) which showed that stereotypic striped mouse adults are more active than non-stereotypic individuals, but that both groups show comparable levels of anxiety/fearfulness, we again demonstrated that neither activity level nor anxiety/fearfulness mediates the frequency or variability of SB performance in adult striped mice.

Finally, we also tracked changes in behavioural measures of perseveration, activity, and fear/anxiety over the course of SB development. These findings indicate that stereotypic, but not non-stereotypic, striped mice become more perseverative and more active over the course of development, and thus the development of perseverative tendencies and heightened activity levels covary with (though do not precede) the development of SB. There was no influence of stereotypy status on levels of anxiety/fearfulness during development, but there was a significant decrease from postweaning to adulthood in both measures of anxiety/fearfulness in non-stereotypic as well as stereotypic striped mice. A similar decrease in anxiety-like behaviours over development is seen in other rodent species (e.g. Hefner & Holmes 2007).

GENERAL DISCUSSION

The aims of this study were two-fold. First, we sought to characterize the developmental trajectory of SB in striped mice from birth to adulthood. Second, we aimed to investigate individual level predictors, mediators, and correlates of the development and performance of this behaviour across the lifespan, with a specific interest in testing hypotheses about the roles of its putative causal factors.

Unlike many other rodent models of SB in which nearly all individuals are stereotypic (e.g. deer mice, Lewis et al. 2006; bank voles, Garner & Mason 2002; lab mice, Gross et al. 2011), only just over half of the striped mice in this study developed SB, an incidence similar to that in non-rodent species of CB wild animals (e.g. brown bears, *Ursus arctos*, 48%; clouded leopards, *Neofelis nebulosa*, 49%; Mason et al. 2007). Typically, SBs appeared shortly after weaning, a finding consistent with observations in other rodents (Ödberg 1987; Powell et al. 1999). There was, however, substantial variation in age of onset of striped mouse SB, possibly mediated in part by developmental maturity (discussed later), with some individuals developing SB as late as 12 weeks after individual housing. As in previous studies of striped mice (Schwaibold & Pillay 2001; Jones et al. 2008; Jones et al. 2010a, b; Jones et al. 2011a, b), we observed a number of different forms of locomotor SBs, as well as substantial intra- and inter-individual variation in form. We also tested two previously poorly investigated hypotheses about the behavioural changes believed to characterize the developmental course of SB, as well as quantifying the severity thereof (see Introduction). Our data supported both of the tested hypotheses, viz., that (1) SBs increase in frequency over the course of development, and (2) SBs decrease in variability. Interestingly, however, although on a group level SB frequency and invariability increased over time, these two measures did not correlate positively with each other during earlier or later stages of SB development, as would be expected if the same causal mechanisms underpinned the observed developmental changes. In particular, as perseveration did not correlate with form variability in striped mice, it would be interesting to systematically test whether the developmental changes in the form variability of SB covary with measures of 'automation' of the behaviour (Mason 2006). 'Automation', or 'central control', which refers to forms of motor or procedural learning (Mason 2006) in which the central nervous system provides behavioural output with decreasing reliance on external feedback (Mason 1993), has long been implicated as the cause of the establishment of SBs (e.g. Fentress 1986), but remains poorly investigated. Mason (2006) provides a thorough review of the type of experimental evidence which would support the 'central control' hypothesis.

An unexpected finding was the consistently observed reduced form variability of SB in male striped mice. Although sex was not a significant predictor of any other variable associated with SB, this finding is interesting for at least two reasons. First, it emphasises that the processes mediating supposed indices of severity of SBs are diverse and currently poorly understood. Second, if form invariability is indeed a valid index of SB severity, it would add

to a previous finding in striped mice that male animals are in some ways more severely affected by the constraints of captivity than females (male juvenile-caught striped mice were far more likely to develop SB in captivity than female juvenile-caught striped mice; Jones et al. 2011a). In the phenotypically closely-related human impulsive-compulsive spectrum disorders (e.g. obsessive compulsive disorder), females, conversely, are typically worse affected than males (Sadock & Sadock 2007). However, sex differences are seldom observed in SB incidence in other mouse or captive species (but see horses where stallions are more at risk; Wickens & Heleski 2010). These two recent findings of subtle sex-differences in striped mice might, however, be explained by similar mechanisms to those investigated in human studies where it has been shown that the higher incidence of maltreatment-induced abnormalities in brain development in males is possibly related to hormonal differences between males and females, differentially altering the trajectory of brain maturation and hence periods of developmental susceptibility (De Bellis et al. 2001). Further studies are needed to investigate this hypothesis in striped mice as well as in other species.

An association has been found between perseveration and SB in a number of different species of captive animals (e.g. Garner & Mason 2002; Garner et al. 2003; Vickery & Mason 2003; Tanimura et al. 2008; Dallaire et al. 2011; but see Latham & Mason 2010; Gross et al. 2011). Although analyses from the current study in striped mice do not show the previously found correlation between SB *level* and perseveration (in this study, levels of SB are not correlated with scores for perseveration), findings in the previous (Jones et al. 2011a) and current study do indicate a group level difference in perseveration scores (and activity level) between stereotypic and non-stereotypic adult striped mice, and show that increases in perseverative tendencies (and in activity) over development parallel the development of SB and occur only in stereotypic individuals. However, as argued by Garner and colleagues (e.g. Garner 1999; Garner 2006; Gross et al. 2011) and in the Introduction, such correlational evidence, however intuitively appealing, does not imply that SB and perseveration, the focus here, are causally related, or that SBs and perseveration both reflect environmentally-mediated alterations to corticostriatal circuitry. To our knowledge, ours is the first study to test the prediction that individual differences in perseveration temporally precede and thus potentially cause individual differences in the development and performance of SB. Our data do not support this prediction: perseveration scores measured in juveniles prior to the development of SB did not predict which striped mice developed SB, nor did they predict its age of onset or later frequency or variability in stereotypic individuals. Instead, the data suggest that alternative or

additional mechanisms, not measured in the current study, underlie the development and performance of SB in striped mice, and possibly also in other species. We did, however, identify a mediating variable of the performance characteristics of SB in stereotypic striped mice, although not which individuals were more susceptible to developing SB: striped mice that were more developmentally mature in the preweaning period were likely to show SB at an earlier age, and to perform it with higher frequency, and possibly also with reduced diversity, effects which persisted into adulthood. In contrast, though, to some other studies (e.g. Würbel & Stauffacher 1996; Latham & Mason 2010), we found no evidence for a causal or mediating effect of fear/anxiety on the development or performance of SB in striped mice.

Three differing lines of explanation could explain the above findings. First, it is possible that SBs are caused by a deficit in inhibitory control and thus causally linked to perseveration on formal tasks, but that the measure of perseveration used here was inadequate to assess this in juvenile striped mice. The clear group differences between stereotypic and non-stereotypic adult striped mice, however, suggests otherwise, as does the lack of correlation between scores for perseveration and levels of SB. Future work could further validate SA and SAB in the four-arm-maze as measures of recurrent perseveration by correlating the performance of individual animals on this test with performance on more common tests of recurrent perseveration such as the bias-corrected two-choice gambling task (Garner et al. 2003). A second line of reasoning is that whilst SBs might be caused, in part, by deficits in inhibitory control, perseveration might explain only a small amount of variation in SB performance and thus only be weakly correlated with it. Whilst the early work of Garner and Mason (2002) in bank voles provides compelling evidence that a single process, consistent with suppression of indirect pathway activity, underpinned all observed behavioural changes in stereotypic animals, subsequent work in other species, such as marsh tits and blue tits (Garner et al. 2003) and sun bears and Asiatic bears (Vickery & Mason 2004), indicates that at least one other, unknown process, was involved in the relationship between recurrent perseveration and SB. Also, more recently, findings from two studies in ICR mice (Latham & Mason 2010; Gross et al. 2011) and one in young (but not adult) mink (Dallaire et al. 2011) suggest that variation in recurrent perseveration might not always underpin variation in levels of SB. It is therefore possible that whilst variation in recurrent perseveration explains the majority of variation in SB performance in certain species and/or contexts, it may have a less important role in other instances. In these cases, the variation in SB performance might better be explained by motivational variables (e.g. Wiedenmayer 1997; see Introduction), or by

systems-level changes in related, but dissociable, neurocircuitry such as the frontal (Garner 2006) or ventral striatal limbic (Cabib 2006) loops, loops more commonly associated with stuck-in-set and affective perseveration respectively. A third possible explanation for the current findings is that SBs, in striped mice at least, are not causally related to recurrent perseveration or to deficits in inhibitory control in the indirect corticostriatal pathways. Instead, SBs might develop consequent to individual variation in, or dysregulation of, closely related pathways, such as those suggested in the preceding paragraph, which are influenced by genetic and/or environmental factors (e.g. birth origin; Jones at al. 2011a) in similar, but not identical, ways to the indirect corticostriatal pathways. Neuro-anatomically and -physiologically, a substantial volume of evidence implicates the involvement of brain regions closely associated with the corticostriatal circuits and basal ganglia in the performance of SB (e.g. Lewis et al. 2006; Lanovaz 2011), and brain development in these areas specifically seems very susceptible to the effects of restricted or impoverished rearing conditions (Lewis et al. 2006) and/or to the effects of chronic or acute stress (Judge et al. 2011). To test the hypothesis that SB is not causally related to variation in indirect pathway activity and thus recurrent perseveration, but instead to individual differences in related pathways, future research must investigate not just recurrent perseveration, but also stuck-in-set perseveration (e.g. via modified IntraDimensional-ExtraDimensional set-shifting tasks; Garner et al. 2004) and affective perseveration (e.g. via modified detour reaching task; Dallaire et al. 2011), and also complement and validate these behavioural investigations with neuro-anatomical or -physiological studies of the implicated forebrain regions (e.g. Schoenecker & Heller 2000; McBride & Hemmings 2005; Lewis et al. 2006).

When investigating all three of the above hypothesized explanations for the current results, it will also be necessary to reduce the 'noise' in the data sets by ensuring that all the behaviours classed as SBs are phenotypically similar or comparable, and that potentially distinct forms of SBs observed are not crudely or mistakenly classed together. Given the well-known bewildering phenomenological and aetiological diversity of SB in both humans and captive animals (Langen et al. 2011), delineation of more homogenous subgroups of SB will be necessary to facilitate the understanding of, and empirical investigation into, the causes of SB and its developmental progression (Cowan et al. 2002). Further characterization of the developmental trajectory of SB in striped mice, as is being done in deer mice (Tanimura et al. 2010), should facilitate such subgrouping, and thus permit more nuanced investigation of the neurobiological basis of SB as suggested above.

Acknowledgements

Funding was provided by the National Research Foundation (NRF; grant number: 2069110) and the University of the Witwatersrand to NP. MJ was partly supported during her PhD by an NRF Scarce Skills Scholarship. GM was supported by an NSERC Canada Research Chair and Discovery Grant. MJ thanks Tasmin Rymer, Sneha Joshi, and Alessia Fowler for help with data collection; Rebecca Meagher, Jamie Dallaire, Maria Diez-Leon, and Luke Duncan for their insightful comments and assistance with references; Viviene Williams and Heather van der Burg for their help with the data analysis; and Lis Jones for proofreading this and other MSs.

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CHAPTER EIGHT
General Discussion

Stereotypic behaviour (SB) results from the chronic impact of captivity on brain development and function, and consequently on behaviour. It affects over 85 million animals worldwide, and reflects a spectrum of responses, which range from the transient maladaptive responses of a normal animal to an abnormal environment to the symptoms of profound and permanent brain dysfunction. SB development and performance has warranted increasing research attention because of its strong association with poor welfare and also because of more recently raised concerns about the validity of scientific experiments using stereotypic animals as study subjects. However, despite the high incidence of SB in captive populations and its worrying implications for welfare and for science, we still lack a comprehensive explanatory model of SB development and performance which integrates ethological and neuroscience accounts of SB, and one which preferably does so within a single species.

My study aimed to characterize group- and individual-level predictors and correlates of SB in the striped mouse, *Rhabdomys dilectus*, in order to identify the mechanisms through which these factors bring about and maintain the stereotypic phenotype and so contribute toward an integrated theoretical understanding of SB development and performance. This involved testing several hypotheses about more distal putative causes of SB (e.g. social and environmental factors), as well as more proximal causes (e.g. genetic inheritance; individual-level differences in motivation and behavioural proxies of brain function). In this section, I review the main hypotheses tested, and the evidence for and against these; discuss inconsistencies between the results from my studies in striped mice and the work of others in other species; review the contribution of striped mice towards a more integrated theoretical model of SB; suggest directions for future research; and, finally, outline the practical implications and applications of the findings from this study.

SUMMARY OF OBJECTIVES AND FINDINGS

This study had three broad objectives. First, building on previous work in striped mice and in other species (see General Introduction), I investigated the genetic transmission of SBs from both the mother and the father, and also the relative reproductive success of non-stereotypic

and stereotypic individuals. The study design used in these experiments was unique and differed from those in previous studies in striped mice and other species: I set up four treatment groups, formed from combinations of non-stereotypic and stereotypic mothers and fathers, and so could assess the relative contribution of maternal and paternal stereotypic status to offspring stereotypic status and to reproductive output. I showed that SB has a strong genetic component, but that maternally mediated prenatal factors might also predispose the SB phenotype (Jones et al. 2008, Chapter 2; previously it has been shown that there is negligible postnatal social transmission of SB from dams to their pups; Schwaibold & Pillay 2001); that the reproductive output for stereotypic females, but not stereotypic males, is significantly greater than for non-stereotypic striped mice of both sexes (Jones et al. 2010a, Chapter 3); and that there is likely unintended selection for SB in captivity because the phenotype is associated with greater reproductive output underpinned by apparent genetic variance (Jones et al. 2010a, Chapter 3).

Second, I hypothesised that, on a group level, more naturalistic rearing conditions would reduce the incidence of SB (by reducing the motivation to stereotype and/or by normalizing brain development), and tested this by comparing the SB incidence of striped mice reared in different social and physical environments, whilst also accounting for genetic influence (stereotypic status of the mother and father) where possible. A large body of previous work from multiple disciplines (e.g. neuroscience, Rosenzweig & Bennett 1996; applied ethology, Swaisgood & Shepherdson 2006; developmental psychology, Laviola & Terranova 1998, Caldji et al. 2000) indicates that early life experience, in concert with genetic inheritance, has profound and enduring effects on brain development and organization, and thus on behavioural, psychological, and physiological responses to both the current and the future environment. Almost two centuries ago, Holmes (1839) wrote in the Proceedings of the Veterinary Medical Association: “I believe, then crib-biting, to be a habit which takes place in consequence of the change that is produced in the animal when brought from a state approaching that of nature into an artificial one – a state of domestication” (cited in McBride & Hemmings 2009). Subsequent studies into the distal causes of SB have almost unequivocally supported Holmes’ contention that the less naturalistic, less complex, and less variable the rearing environment, the more likely the development of SB. Furthermore, evidence from environmental enrichment paradigms supports the corollary of this statement, i.e. that more naturalistic, more complex, and more variable environments ameliorate or prevent the development of SB (e.g. Swaisgood & Shepherdson 2006). In striped mice, I

assessed the impact of a number of different factors related to the "naturalness" of the early rearing environment on the development of SB. Some of these factors had been previously tested in other species (weaning age, environmental enrichment). However, other factors (birth origin, biparental care) had not been previous systematically investigated, but were nonetheless amenable to study in striped mice, being a wild-derived rodent species which shows paternal care in captivity (Schradin & Pillay 2003).

Results from my studies investigating the impact of more and less natural rearing environments indicated that striped mice weaned at or later than their natural weaning age are less likely to develop SB than striped mice that are weaned prematurely (Jones et al. 2010b, Chapter 4); that striped mice reared biparentally show significantly less SB as adults than striped mice reared by their mothers alone (Jones et al. 2010b, Chapter 4); that individuals raised from weaning in enriched conditions are four times less likely than standard-housed individuals to develop SB, an effect which endures after enriched-housed striped mice are transferred to standard housing (Jones et al. 2011a, Chapter 6); and that birth origin predicts the emergence of SB (birth origin effect), with those WC individuals trapped as adults being relatively protected from the development of SB compared with both WC individuals trapped as juveniles and captive-born (CB) striped mice (Jones et al. 2011b, Chapter 5). In the analyses of weaning age and biparental care, experiential and genetic effects combined additively to influence the adult phenotype. These findings also suggest, more generally, that adult striped mice are less susceptible to the development of SB than younger individuals: adult-caught, but not juvenile-caught, striped mice were relatively immune to developing SB; and early environmental enrichment protected young CB striped mice against the later development of SB when barren housed as adults (whereas previous work in striped mice (van Lierop 2005) and in other species (e.g. Cooper & Nicol 1996) has shown the reduced efficacy of environmental enrichment in older animals).

The third objective of this study was to identify potential proximal causes of SB and to further our understanding of how more distal, group-level causes of SB, such as birth origin, might bring about individual differences in the development and performance of SB through their influence on the motivation to stereotype and/or on neurodevelopment. I approached this task in two ways. First, in Jones and colleagues 2011b (Chapter 5), I explored correlates of the birth origin effect. Whilst I showed that WC striped mice were more fearful and less active than CB individuals, these traits did not covary with SB, and thus were unlikely to

account for the diminished incidence of SB in these striped mice. However, I did find that WC striped mice were less perseverative and behaviourally more flexible than CB individuals, and showed that these traits covaried with SB, suggesting a potential relationship between perseveration and the development of SB. Collectively, these results suggest that whilst WC striped mice are typically protected against SB development in captivity, they nonetheless have poorer welfare than their CB conspecifics – despite the absence of SB in these WC mice, which absence usually indicates group-level good welfare (Mason & Latham 2004). Second, I characterized the developmental trajectory of SB in a large group of CB, standard-housed striped mice, and then investigated potential individual-level predictors of SB in these animals to explore the proximal causes of SB (Chapter 7). As in my previous experiments, I showed that stereotypic striped mice were more active and more perseverative than non-stereotypic animals. However, within the sample of stereotypic adult striped mice, measures of SB frequency and variability did not correlate with perseveration, as would be expected if they were underpinned by the same causal factors. Moreover, measures of perseveration, activity, and anxiety/fearfulness assessed in juveniles before the development of SB did not predict which animals later developed SB, nor did these measures predict the later frequency or variability of SB in stereotypic striped mice. There was, however, some indication that accelerated development predicted which juvenile striped mice would be predisposed to developing SB as adults, suggesting that physical competence is a necessary cause of SB in striped mice.

UNEXPECTED FINDINGS AND INCONSISTENT OR CONTRADICTORY RESULTS

For the most part, as summarised above, findings from the six experiments were in agreement with each other, and, taken together, my results were in general keeping with the prevailing knowledge base about environmentally-induced SBs. Below I note and briefly discuss some exceptions.

Two findings were unexpected, but usefully suggest avenues for future work. First, in Chapter 2, I showed that there is a greater maternal than paternal contribution to the observed transmission pattern of SB. Previous work in striped mice has likewise shown that SB is strongly related to the SB status of the mother, and is genetically but not socially transmitted (Schwaibold & Pillay 2001). Here, I also examined the contribution of the father, confirming

the predicted maternal and paternal genetic transmission of the behaviour. Surprisingly, however, I found that the prevalence of SB was five times greater in the offspring of stereotypic as opposed to non-stereotypic females (irrespective of the stereotypic status of the father), although only three times greater in offspring sired by stereotypic males paired with non-stereotypic females as opposed to offspring from non-stereotypic parents. Is it all a mother's fault? This finding does suggest that mothers have greater influence than fathers on their offspring's susceptibility to stereotype, and that gestational effects between conception and birth have enduring effects on neurobehavioural development (Chapillon et al. 2001; Patin et al. 2004). For example, prenatal circulating levels of corticosteroids might differ between foetuses of stereotypic and non-stereotypic mothers, so influencing offspring HPA axis activity in later life (Macri & Würbel 2006; Macri et al. 2011), and thus possibly predisposing SB (Latham & Mason 2010). Studies examining the effects of prenatal stress on postnatal SB development would test the viability of this intriguing hypothesis, with potentially important implications for the conditions in which captive breeding animals are housed and for our theoretical understanding of the complex causes of SB.

The second unexpected finding points to subtle sex-differences in the vulnerability to stereotype in striped mice, with male mice potentially being more severely affected than females (Chapters 5 and 7). Typically, although contrary to research in closely associated animal Abnormal Repetitive Behaviours (ARBs) such as barbering (Garner et al. 2004) and human conditions such as OCD (Sadock & Sadock 2007), sex differences in SB incidence are seldom noted (but see horses where stallions are more at risk; Wickens & Heleski 2010). In Chapter 5, I showed that juvenile-caught male striped mice were at a much higher risk of developing SB than juvenile-caught females and, in Chapter 7, that CB male striped mice show less variable SBs than females (invariability of SB is a proposed indicator of its severity; Dallaire et al. 2011). Human studies show that developing male brains are more sensitive to maltreatment than female brains (De Bellis et al. 2001), and I accordingly suggest that hormonal differences between male and female striped mice might interact with rearing conditions to influence brain development and predispose SB. Further work is needed to investigate this hypothesis in striped mice in particular, as well as to investigate any subtle sex differences in the development and performance of SB in other species.

One finding was inconsistent across two experiments (Chapters 5 and 7), and also with some (but not all) findings in other species. Typically, scores for recurrent perseveration have been

found to correlate with levels of SB (see General Introduction). In Chapter 5, in a combined sample of WC and CB striped mice, scores on one of my measures of perseveration, Sequential Analysis (SA) but not Spontaneous Alternation Behaviour (SAB) scores, predicted the duration of time engaged in SB. However, in Chapter 7, on a larger sample of CB striped mice, I found no association between level of SB and SA or SAB scores for perseveration, despite finding significant group differences between stereotypic and non-stereotypic individuals. This between-study variation may at least in part be explained by age differences in the study populations in Chapters 5 and 7: striped mice in Chapter 7 were substantially younger than the individuals tested in Chapter 5. Previous work by Dallaire and colleagues (2011) has similarly found no association between perseveration and SB levels in young mink, although did so in an older sample of different animals. This somewhat contradictory finding of no correlation between perseveration and SB levels in striped mice in Chapter 7 may also be partly an artefact of the different type of distribution of SB in the striped mouse population. Whereas SB shows a continuous distribution in all of the other captive species studied to date, striped mouse SB has a bimodal distribution, with approximately half of CB individuals remaining non-stereotypic (Jones et al. 2008). This is the first research to show differences in perseveration scores between groups of stereotypic and non-stereotypic animals. Alternatively, as discussed in detail in Chapter 7, and in accordance with the negative findings by Latham and Mason (2010) and Gross and colleagues (2011), perseveration scores and levels of SB may correlate imperfectly, or not at all, if in certain instances and species recurrent perseveration reflects only a small proportion (or none) of the variation in SB performance. It is also possible that recurrent perseveration may explain the variation in some characteristics of SB (e.g. the behaviour's repetitiveness, or whether individuals are predisposed to SB or not), but not necessarily the variation in the level (or variability) of SB. In the next section, I return to the idea that different causal factors may predict different aspects of SB, and possibly differentially across development.

STRIPED MOUSE CONTRIBUTIONS TO THEORETICAL UNDERSTANDING OF STEREOTYPIC BEHAVIOUR

To date, few studies have adopted an interdisciplinary neuroscientific and ethological framework to investigate the multifactorial causes of SB development and performance (c.f. Latham & Mason 2010). The field has consequently lacked an integrated understanding of the motivational, developmental, neurophysiological, and neuroanatomical causes and

correlates of SB in a single species (Rushen & Mason 2006). Next, I address the contributions that the work in striped mice has made to our theoretical understanding of SB, and highlight some of the important knowledge gaps necessitating future study.

The observations and manipulations of early social and environmental conditions have added to our knowledge of the more distal cause of SB, viz., the types of environment which, on a group level, induce SB in genetically vulnerable individuals. Some protocols have rendered results consistent with both the ethological and neuroscience explanations of SB (e.g. weaning age, uni- v. bi-parental care, early enrichment), whereas others have allowed us to test competing predictions *apropos* the primacy of the ethological or neuroscience explanatory framework. In two separate studies examining the effects of environmental downshifts (enriched housing → standard housing; wild (free-ranging) → standard housing), I showed that previous experience of a complex environment protects adult striped mice against the later development of SB. These results provide group-level support for the neuroscience hypothesis, and suggest that the variation in SB levels between treatment groups is induced by experience-dependent changes in forebrain structure and function. However, they do not support the ethological hypothesis which predicts higher levels of SB after animals are moved to SB-eliciting environments.

Studies on an individual level (Chapters 5 and 7) have provided a finer-grained understanding of the proximal causal processes involved in the development and performance of SB. In the comparison of WC and CB striped mice, I found group differences in perseveration and anxiety/fearfulness, but showed that only perseveration co-varied with SB performance, thus again supporting the neuroscience explanation of SB development. In a longitudinal study of SB development in CB striped mice, I similarly found an association between SB and perseveration in adult animals, although whether perseveration was causally related to the development of SB was less clear since reduced behavioural flexibility did not appear to precede the onset of SB. Indeed, none of the investigated preweaning or postweaning variables distinguished between individuals that would go on to develop SB and those that would not, hence leaving these risk factors unidentified. However, within the group of CB striped mice which did proceed to develop SB, ethologically-linked factors (e.g. physical competence) appeared to play a significant role in determining age of onset as well as levels of SB expression. Motivational factors most likely also determined SB form.

More broadly, my striped mouse work highlights a number of philosophical and practical factors which demand consideration when investigating the causes of SB. First, the work questions the validity and usefulness of one-dimensional quantifications of SB by challenging (1) the implicit assumption that recording the level of SB is a sufficient means to characterize intra- and inter-individual variation among stereotypic individuals, and (2) the notion that *either* a neurological *or* an ethological explanation can adequately account for this phenotypically diverse group of abnormal behaviours. In striped mice, different factors, which do not necessarily covary, appear to account for different aspects of the stereotypic phenotype such as whether individuals are stereotypic or not, the levels of SB shown, the variability of the SB, and possibly the form of the SB. As put forward by Mason (2006), these causal factors may also vary in relative importance over the course of the individual's lifetime, and suggest areas for future longitudinal research in striped mice. Research investigating the potential interplay between and/or co-occurrence of neurological and ethological factors should be particularly informative, for instance where neurological deficits become salient only when controlling for motivational influences (e.g. Latham 2005).

Second, the imperfect association between recurrent perseveration and SB levels in striped mice, also now shown in a few other recent studies (Latham & Mason 2010; Dallaire et al. 2011; Gross et al. 2011), underscores the need for further longitudinal studies into the relationship between recurrent perseveration and SB, which studies should additionally investigate other putative proximal causal or mediating neurocognitive processes (e.g. stuck-in-set perseveration, affective perseveration; see Figure 1 in the General Introduction) as well as their neuroanatomical and neurophysiological correlates. For instance, future work in striped mice could look at the regional activity of specific forebrain areas (candidates include the motor cortex, striatum, nucleus accumbens, and thalamus) using pharmacological probes (e.g. Schoenecker & Heller 2000; Presti et al. 2003) or post mortem histology (e.g. McBride & Hemmings 2005; Lewis et al. 2006), and investigate the group-level and individual-level correlates of the findings, specifically the impact of identified distal causes (e.g. early weaning, uni-parental care, environmental deprivation) on potential proximal factors.

Third, given the phenomenological and aetiological diversity of SB in striped mice and other animals, delineation of more homogenous subgroups of SB will be necessary to facilitate the understanding of, and empirical investigation into, the causes of SB and its developmental progression. A similar approach, endophenotyping, is increasingly used in human psychiatric

research, with fruitful results, to understand how specific genes mediate neurobiological development, endophenotype expression, and ultimately the disease state of interest (Amann et al. 2010; Skuse 2001). Initially, a subgrouping approach might be based on more easily observable physical characteristics of the behaviour such as form (e.g. somersaulting, cage climbing). This, however, runs the risk of crudely or mistakenly classing together behaviours which are superficially similar but aetiologically or prognostically distinct. At a later stage, subgrouping of SBs may be more meaningfully informed by results from neurocognitive, neuroanatomical, or neurophysiological testing. In due course, this should allow the delineation of dissociable forms of SBs within and/or between individuals. Further characterization of the developmental trajectory of SB and its varied correlates in striped mice, and in other species, should facilitate such subgrouping, and thus permit more nuanced investigation of the multifactorial ethological and neurobiological basis of SB.

Ultimately, an integrated explanatory model of SB development and performance does not simply demand that we find evidence consistent with both ethological and neuroscience explanations within the same model species, and then appeal *ad hoc* to these different theories. Instead, as with integrated theoretical models from other fields (e.g. integrative psychology; Palmer & Woolfe 1999), a more complete explanatory framework requires that we synthesise potentially disparate elements of various theories to specify the 'common currency' (c.f. Cabanac 1971) through which distal and proximal ethological and neurobiological factors effect their influences and are coordinated. Moreover, such an explanatory model should allow us to explain general patterns (e.g. why WC striped mice seldom stereotype) and exceptions thereto (e.g. why some WC striped mice do stereotype), and should concurrently account for independent variation in different aspects of the stereotypic phenotype (e.g. instances where SB frequency and scores for recurrent perseveration covary, and situations where they do not). Because of the many shared characteristics of SB in striped mice and in other animals, as well as the advantages of the model outlined in the General Introduction, striped mice represent a promising model species around which a more integrated theory of SB development could be constructed.

CROSS-SPECIES IMPLICATIONS AND APPLICATIONS

If applied with discretion, findings in striped mice can likely be generalized to other species of captive wild animals. On a fundamental level, as argued previously, a better understanding of the mechanisms driving the development of SB in striped mice would inform our understanding of the causal process in other species less amenable, for practical or conservation reasons, to direct investigation. Practically, this would provide an *a priori* framework for predicting the effects of different forms and timings of environmental and social disruptions, as well as for determining the potential efficacy of intervention strategies to prevent the development of the behaviour or ameliorate its performance, in welfare-friendly ways (Mason et al. 2007).

The finding in striped mice that low levels of SB are not necessarily associated with good welfare challenges the 'low-SB – good-welfare' rule of thumb, and has practical bearing on the generic use of SB as a welfare indicator (e.g. Mason & Latham 2004). Specifically, it suggests that the virtual absence of SB in many WC animal species might reflect poor rather than good welfare; more generally, it highlights the welfare significance of the (potentially independent) *causes* of the absence or presence of the SB rather than simply the absence or presence of SB *per se*. From a neurobiological perspective, it is unlikely that dysfunction of the corticostriatal circuits is causally linked to suffering (levels of the stress hormone, corticosterone, did not differ between stereotypic and non-stereotypic CB striped mice; Chapter 5) whilst, conversely, results in striped mice indicate that intact functioning does not necessarily prevent suffering (as a group, WC striped mice were less perseverative but had higher circulating levels of corticosterone than CB animals). Ethological explanations, however, for SB do appear to be more tightly linked to welfare, at least on a group level: SB-inducing environments tend also to compromise welfare, although their influence on welfare may be independent from their influence on SB. Ethological explanations may also account for the non-performance of SB and very low activity levels in some animals if, for instance, fear or depressive-like states suppress the motivation to stereotype (Meagher 2011). I did not, though, find direct evidence for the suppression of SB by fear/anxiety in striped mice, although the impact of depressive-like states remains untested.

Finally, the work in striped mice hints towards an answer to the question: does the relationship between SB and perseveration reflect abnormal, dysfunctional changes in

behavioural control, or is this simply normal variation? Because WC striped mice are less perseverative and are less likely to stereotype, differences appear to reflect environmentally-induced dysfunction, with WC and CB populations having different means and variances. However, in Chapter 5, WC and CB populations also showed similar statistical ranges of perseveration scores (albeit with scores differentially distributed within these ranges). This similarity of range argues against the dysfunction hypothesis and in favour of the idea that differences in perseveration reflect normal variation and merely predispose SB under certain conditions. Further studies are needed to test, for instance, the impact of perseveration and associated neurobiological changes on other behaviours since dysfunction should induce a wide range of behavioural abnormalities, whereas individual variation should not (Garner 2005).

CONCLUSIONS

Stereotypic behaviours are a phenomenologically and aetiologically diverse group of abnormal, repetitive behaviours which are rife in captivity, but virtually absent in wild or free-ranging animals. They are believed to result from the chronic impact of captivity on brain development and function and, consequently, on behaviour. My study has shown that more naturalistic rearing environments reduce the incidence of SB in striped mice, an effect mediated by genetic factors and possibly also by experience-dependent alterations in forebrain function, reflected behaviourally as perseveration. Results furthermore suggest that a confluence of neurobiological and ethological factors cause and mediate the development and performance of SB, with different factors accounting for different aspects of the stereotypic phenotype. Compared with SB research in other animals, striped mice are currently one of the better understood species and, for a wild animal, are remarkably amenable to study. They thus provide a promising and practicable model through which an integrative explanatory framework of SB in captive wild animals may be developed.

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