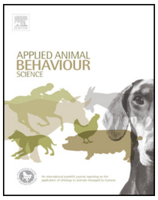
Applied Animal Behaviour Science 177 (2016) 59–69

Contents lists available at ScienceDirect

Applied Animal Behaviour Science

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



# Environmentally enriching American mink (*Neovison vison*) increases lymphoid organ weight and skeletal symmetry, and reveals differences between two sub-types of stereotypic behaviour

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## ARTICLE INFO

*Article history:*
Received 6 February 2015
Received in revised form 3 December 2015
Accepted 23 December 2015
Available online 29 December 2015

*Keywords:*
Stereotypic behaviour
Animal welfare
American mink
Immune system
Corticosteroids
Fluctuating asymmetry
Enrichment
Carnivores

## ABSTRACT

Enrichment studies for wild carnivores (e.g., in zoos) are often short-term, use enrichments of unknown motivational significance, and focus on glucocorticoids and stereotypic behaviour (SB), ignoring other stress-relevant variables. Our study assessed the broad behavioural and physiological effects of enriching American mink—a model carnivore—with preferred stimuli long-term, and investigated the welfare implications of individual differences in SB. We raised 64 male-female pairs with or without enrichment. At 7 months, pairs were split and mink individually housed (adults being solitary), first by being temporarily moved to identical non-enriched cages (permitting observation blind to rearing condition). Two weeks later, one mink per original pair (half female, half male) was returned to his/her rearing cage for re-observation, sample collection for faecal cortisol metabolite (FCM) analysis, and additional research for 1.5 years before being humanely killed. Stress-sensitive variables were then measured *post-mortem*. Enriched-raised mink in their rearing conditions excreted less FCM ($F_{1,29} = 8.33$, $p = 0.003$), and performed less SB than non-enriched mink. Two SB sub-types occurred: (1) 'loco' stereotypies: locomotor, whole body and head stereotypies (e.g., pacing, nodding), previously shown to correlate with recurrent perseveration; and (2) repetitive scrabbling with the forepaws. Enriched housing reduced both (at 7 months: loco stereotypies: $F_{1,60} = 25.3$, $p < 0.0001$; scrabbling: $F_{1,60} = 24.0$, $p < 0.001$; effects still trends 1.5 years later). However, the sub-types responded differently to the current availability and/or usage of enrichment. Thus enrichment-use (which was stable) tended to negatively correlate with scrabbling but not loco stereotypies. Furthermore, after the relocation to identical non-enriched cages, loco stereotypies remained lower in enriched-reared than non-enriched-reared mink ($F_{1,58} = 31.33$, $p < 0.0001$), but scrabbling rapidly increased (such that within two weeks, enriched- and non-enriched-reared mink were indistinguishable). *Post-mortem*, enriched-reared mink showed less skeletal fluctuating asymmetry ($F_{1,42} = 2.87$, $p = 0.048$) and had heavier lymphoid organs (thymus: $F_{1,41} = 3.43$, $p = 0.035$; spleen: $F_{1,45} = 13.11$, $p = 0.010$). However, *within* treatment groups, neither these measures nor FCM covaried with SB. In conclusion, long-term housing with preferred enrichments not only reduced SB and FCM, but also induced anatomical changes consistent with better cell-mediated immunity and reduced developmental stress. In addition, these results should refine the use of SB and its sub-types in welfare assessment, since scrabbling seemed to reflect the prevailing presence/absence and utilisation of enrichment, while motor, whole body and head SBs appeared to reflect more stable, long-term effects of differential rearing; and furthermore, within each housing type, individual differences in SB appeared to reflect response styles rather than differential welfare.

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http://dx.doi.org/10.1016/j.applanim.2015.12.002
0168-1591/© 2016 Published by Elsevier B.V.

## 1. Introduction

Environmental enrichment refers to the provision of stimuli to improve animals' welfare in captivity (Newberry, 1995; Shepherdson, 1998). Enriched environments are thus generally preferred by animals: they voluntarily interact with them, and may even work to access them, indicating high motivation (e.g., American mink *Neovison vison*, Mason et al., 2001; Hansen and Jensen, 2006; pigs *Sus scrofa*, Elmore et al., 2011). Enrichments also influence an array of stress-sensitive physiological and behavioural variables. For example, in diverse wild and domesticated species, environmental enrichment decreases the performance of stereotypic behaviour (reviewed by Swaisgood and Shepherdson, 2005); and reduces activity of the hypothalamic pituitary adrenal (HPA) axis, decreasing adrenal gland weight (Beattie et al., 2000; Abou-Ismail and Mahboub, 2011) and lowering glucocorticoid levels (Carlstead et al., 1993; Belz et al., 2003). In laboratory rodents, two lymphoid organs essential for cell-mediated immunity are also often heavier in enriched animals: the thymus (Van Loo et al., 2004) and spleen (Abou-Ismail, 2011), both of which involute with chronic stress (e.g., Mitchell et al., 2006; Corbin et al., 2008). Finally, in laboratory rats and farmed rabbits, environmental enrichment can reduce levels of fluctuating asymmetry (Sorensen et al., 2005; Tuyttens et al., 2005): an anatomical measure of developmental instability (Polak, 2003) that is associated with early stress (Møller and Swaddle, 1997), and therefore used by females as part of mate choice in many species (e.g., Møller, 1993; Waitt and Little, 2006).

Our research had two main aims. The first was to gather for the first time basic information on all the behavioural, anatomical and physiological effects of environmental enrichment described above in a model carnivore, the American mink. Although captive wild carnivores have attracted many enrichment studies (Shepherdson et al., 1998; Swaisgood and Shepherdson, 2006), their scope has been rather limited, typically focusing on stereotypic behaviour or cortisol output, and often being rather short-term and using putative enrichments of unknown motivational significance to the animals (e.g., Poessel et al., 2011). Perhaps as a result, enrichment only halves the performance of stereotypic behaviour in such studies, instead of abolishing/preventing it (Shyne, 2006; Swaisgood and Shepherdson, 2006), suggesting it was inadequate or offered too late in development (Mason et al., 2007). American mink represent the single species of carnivore attracting the most enrichment and stereotypic behaviour research, because they are widely farmed for their fur (e.g., Hansen and Jeppesen, 2001; Mason et al., 2001; Mononen et al., 2008; Axelsson et al., 2009; Meagher et al., 2013). However, no mink study has yet explored the long-term effects of preferred enrichments on variables sensitive to developmental stress (e.g., fluctuating asymmetry) or aspects of stress physiology relevant to immune function, even though these could have implications for health and productivity on fur farms. More detailed research could also help promote the more effective use of enrichment to enhance breeding and health in carnivores in zoos and captive breeding centres (especially other mustelids, such as European mink *Mustela lutreola*, and black-footed ferrets *Mustela putorius*). Therefore we predicted that being raised and housed long-term with preferred enrichments would not only reduce cortisol output and adrenal weight, and reduce or even prevent stereotypic behaviour, but also reduce fluctuating asymmetry, and increase spleen and thymus weights.

Our second main aim was to investigate why individuals vary in their reactions to both enriched and non-enriched housing conditions, and the welfare implications of such variation. These individual differences are most clearly manifested, and best studied, in stereotypic behaviour. In non-enriched housing conditions, individuals typically vary greatly and consistently in how much of it they display. For example, some fur-farmed mink spend most of their active time performing this behaviour, while other, identically-housed, conspecifics show none at all (e.g., Hansen, 1993; Mason, 1993; Meagher et al., 2012b). Similar effects occur in other species (e.g., pigs, Terlow et al., 1991; African striped mice *Rhabdomys pumillio*, Jones et al., 2010). Paradoxically, given the links between stereotypic behaviour and poor environments, the more highly stereotypic individuals generally seem to have better welfare than less stereotypic conspecifics within these sub-optimal conditions. For example, highly stereotypic mink and African striped mice produce more offspring than less stereotypic, identically-caged peers (Jeppesen et al., 2004; Jones et al., 2010), and bouts of oral stereotypic behaviour correlate with decreased heart rate in both sows (Schouten et al., 2000) and horses *Equus caballus* (Minero et al., 1999). Meta-analyses suggest that such individual-level (i.e. within-population) patterns are typical (Mason and Latham, 2004). This has led to speculation that stereotypic behaviour either helps captive animals cope with sub-optimal conditions (e.g. Wiepkema et al., 1987), or that non-stereotypic animals in such conditions are instead very inactive due to additional welfare problems, such as pain, sickness, or depression-induced apathy (Mason, 2006; Meagher and Mason, 2012; Fureix et al. in press)

Similar individual variation in behaviour is also seen in enriched conditions. Stereotypic behaviour is far less common here, but certain individuals may still display it. This has been observed in several rodent enrichment studies (e.g., Ödberg, 1987; Turner et al., 2003; Latham and Mason, 2010; Bechard et al., 2011; Jones et al., 2011) as well as two parrot species (orange-winged Amazon parrots *Amazonica amazonica*, Meehan et al., 2004; African grey parrots *Psittacus erithacus*, Lumeij and Hommers, 2008), and mink (e.g., Hansen et al., 2007; Dallaire et al., 2012; Díez-León et al., 2013). These findings suggest that for some individuals, the enrichments provided are insufficient at reducing the psychological stress or central nervous system changes generally implicated in stereotypic behaviour. If true, this hypothesis predicts positive correlations between stereotypic behaviour and physiological signs of stress across enriched-housed animals. Furthermore, one potential explanation for individual differences in apparent enrichment-effectiveness is individual variation in enrichment-use. In mink (Dallaire et al., 2012), plus species as diverse as mice, bears (Asiatic black bears, *Ursus thibetanus*, and Malayan sun bears, *Ursus malayanus*), rhesus monkeys *Macaca mulatta*, and African grey parrots, individuals housed with identical enrichments show stable differences in the degree to which they interact with them (Line et al., 1991; Vickery, 2003; Lumeij and Hommers, 2008; Walker and Mason, 2011); and when feather-plucking parrots were given enrichments, the individuals who voluntarily used them the most, showed the greatest reductions in this stereotypic behaviour (Lumeij and Hommers, 2008).

We therefore predicted that in non-enriched conditions, physiological and anatomical signs of stress would be lower in more stereotypic individuals. In contrast, in enriched environments, we predicted that highly stereotypic individuals would show more physiological and anatomical indicators of stress than less-/non-stereotypic identically-housed conspecifics, and relatively low enrichment use.

To maximise our chances of success, we chose environmental enrichments shown in other studies to be motivating for mink. We also raised subjects from infancy with or without enrichment, not collecting our first data until young adulthood after 5–6 months of differential exposure, and collecting the rest after one or two breeding seasons and up to 2 years of differential housing. Data belonging