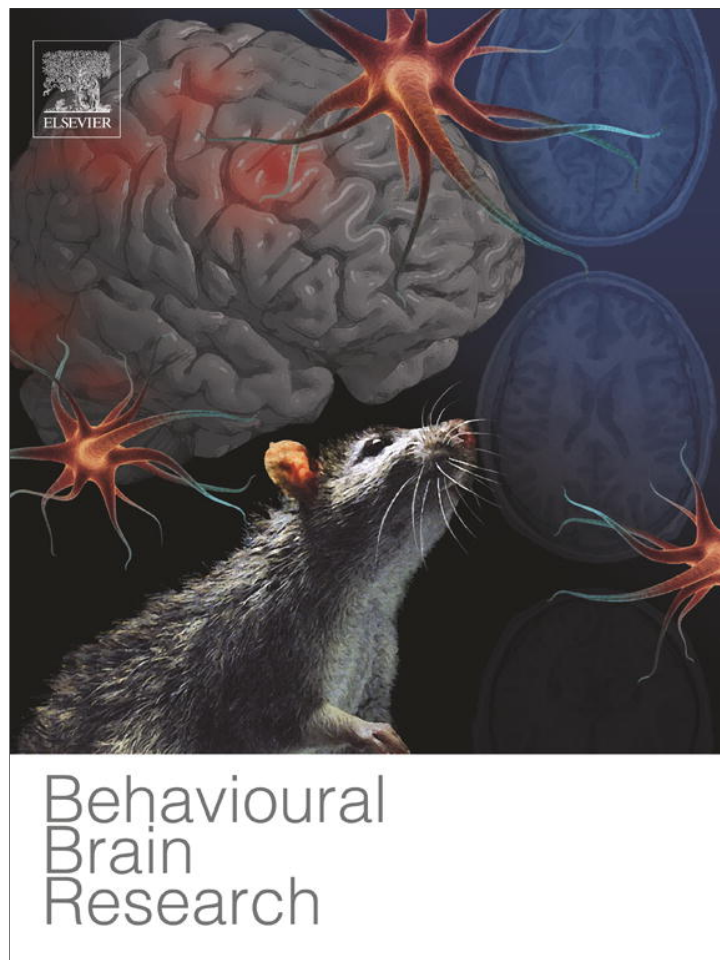


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## Behavioural Brain Research

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## Research report

## Environmentally enriched rearing environments reduce repetitive perseveration in caged mink, but increase spontaneous alternation

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## HIGHLIGHTS

- ▶ Enrichment during rearing decreased stereotypy and perseveration in mink.
- ▶ Environmental enrichment increased spontaneous alternation behaviour in mink.
- ▶ Mink with more spontaneous alternation also gained more rewards in our two-choice guessing task.
- ▶ Unexpectedly, stereotypy and perseveration did not co-vary in these young adult mink.

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## ABSTRACT

Studies spanning 15 species (including American mink, *Neovison vison*) demonstrate that within similarly-housed populations, individuals displaying high levels of stereotypic behaviour (SB) typically show perseverative responding (e.g. during set-shifting, or reversal/extinction learning). Similar correlations in autism and schizophrenia suggest this indicates captivity-induced cortico-striatal circuit dysfunction. However, this pattern does not prove developmental impairment: SB, perseveration and their inter-correlations also occur in normal humans. We therefore differentially-reared enriched versus non-enriched mink to investigate whether treatments that exacerbate SB correspondingly increase perseveration (Study 1). Enriched-rearing did reduce SB and perseverative response repetition (in two-choice guessing tasks), while increasing spontaneous alternation: a strategy yielding more rewards, and suggesting enhanced hippocampal development. This complements previous research demonstrating cortical/hippocampal impairments and reduced behavioural flexibility in non-enriched animals, with implications for research animals and wild animals captive-raised for reintroduction into nature. Consistent with previous data, highly stereotypic subjects repeated guessing task responses most rapidly, suggesting disinhibition during repetition. However, unexpectedly, SB and perseveration did not co-vary across individuals. We therefore suggest that behavioural changes manifest as increased perseveration are important but do not fully explain captive animals' SBs, possible reasons including the contributory role of differential motivations for underlying source behaviours. Re-analyses of old data (Study 2) confirmed that spontaneous alternation is profitable; and demonstrated that the precise methods used for quantifying perseveration and SB can modify the strength of apparent relationships between them, as can statistically controlling for feeding motivation: as predicted, partialling out motivational effects increased the variance in SB predicted by perseveration.

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## 1. Introduction

Stereotypies, traditionally defined as repetitive, highly unvarying behaviours without apparent goal or function [1], are widespread in captive animals, being performed by over 85 million worldwide [2] and statistically the norm in some populations (e.g. single-housed laboratory primates; zoo-housed giraffes [3]).

These behaviours are part of a broader spectrum of abnormal, repetitive activities including self-biting and fur- or feather-plucking [4,5]. Here we use “stereotypic behaviour” (SB) to cover this range of activities, defined broadly as “repetitive behaviour induced by frustration, repeated attempts to cope, and/or CNS dysfunction” [5]. Although diverse in aetiology, captive animals' SBs generally typify unnatural husbandry: abnormal rearing environments (e.g. early weaning [6]), or sub-optimal current environments (e.g. barren, non-enriched cages [7,8]). Ethologists traditionally propose that these SBs represent sustained attempts to perform specific normal activity patterns, triggered by correspondingly specific

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motivational states that are frustrated by captivity. Evidence for this includes that feather-plucking by hens (*Gallus gallus*) involves pecks morphologically identical to normal foraging pecks [9] and is often followed by feather-eating [10] which seems to aid gut function [11]; and that bar-chewing by mice (*Mus musculus*) in laboratory cages is focused at locations sometimes permitting escape [12], and elevated in previously-enriched mice: subjects displaying higher breakpoints to leave non-enriched cages and gain enrichments [13]. Recently, however, this ethological perspective on SBs has been supplemented by explanations derived from neuroscience and human clinical psychology, focusing on forebrain pathology.

Human SBs, ranging from predictable repeats of individual actions to recurring expressions of restricted interests, are common in schizophrenia [14], autism and other pervasive developmental disorders [15–17]; Alzheimer's and other dementias [18]; people who have over-used stimulants such as amphetamine [19]; and children raised in severely deprived environments [20,21]. These SBs reflect underlying forebrain changes affecting multiple aspects of behaviour, both subtle and conspicuous, generally involving compromised abilities to inhibit inappropriate responses. These SBs are therefore often accompanied by, and even co-vary with, other forms of reduced behavioural flexibility including perseveration: the inappropriate repetition of responses or activities, or "continuation or recurrence of an activity without the appropriate stimulus" [22,23]. Perseveration, tested for via laboratory assessments involving card sorting, object naming or word list generation [22], is prominent in schizophrenia, dementia, autism, and children raised in extreme privation [4,23,24]. Furthermore, when stereotypic schizophrenics or autistics participate in simple guessing or sequence-generation games, individuals with the most repetitive motor actions and/or most circumscribed interests [14,25], also most frequently repeat answers or response sequences in a perseverative way. Corticostriatal systems are often implicated here [e.g. 26], with much evidence for this coming from pharmacologically- or genetically-modified animal models. In rats, for instance, repetitive movements induced by apomorphine, amphetamine or cocaine correlate with over-activation of corticostriatal striosome-based circuits relative to matrix-based circuits [27]; in DAT knock-out mice, repetitive locomotion is accompanied by increased dopaminergic activity, especially in the dorsal striatum and nucleus accumbens [28,29]; while in deer mice, striatal infusion of dopamine receptor antagonists selectively decreases repetitive jumping, but not other activity [30], and dysfunction of the basal ganglia's 'indirect', inhibitory pathways is implicated by reduced striatal leu-enkephalin [31] and attenuation of stereotypy by adenosine receptor agonists [32].

Correspondingly, growing evidence suggests that captive animals' SBs have similar neurobiological bases [4,26,33]. Aside from several impressive papers on non-enriched-raised deer mice [16,30–32], most evidence is behavioural, based on tests for perseveration akin to those applied to human subjects, or assessments of behavioural flexibility during reversal/extinction learning. These studies, currently spanning 16 species, typically utilise individual differences across similarly-housed and -raised subjects, either contrasting stereotypic with non-stereotypic individuals or comparing subjects that spontaneously vary in SB levels. The vast majority find positive relationships between SB and behavioural inflexibility or perseveration. Populations in which positive correlations occur include captive-bred bank voles (*Clethrionomys glareolus*) [36]; wild-caught and captive-bred striped mice (*Rhabdomys*) [37]; captive-bred laboratory mice (*M. musculus*) [38]; captive-bred adult American mink (*Neovison vison*) performing pacing and other locomotor SBs [45]; wild-caught caged blue tits (*Parus caeruleus*) and marsh tits (*P. palustris*) [34]; and captive-bred orange-winged Amazon parrots (*Amazona amazonica*) [35].

Other studies similarly exploit individual differences between similarly housed subjects, but use animals with uncertain, varying past life histories that could have shaped their current phenotypes; these reveal positive relationships between SBs and perseveration in horses (*Equus caballus*) [39,40], captive-bred rhesus macaques (*Macaca mulatta*) [41,43], lion-tailed macaques (*Macaca silenus*), squirrel monkeys (*Siamiri sceurius*), and capuchin monkeys (*Cebus apella*) [42]; and caged Asiatic black bears (*Ursus thibetanus*) and Malayan sun bears (*Helarctos malayanus*) [44]. Finally, in hens [10], genetically high and low feather-peckers may correspondingly differ in extinction learning. Exceptions not displaying such patterns are as follows: repetitive scrabbling with the forelimbs in caged mature adult mink [45]; and bar-mouthing and similar behaviours in both ICR-CD1 laboratory mice [13,47] and deer mice (*Peromyscus maniculatus*) [46], although, as we review below, these rodent behaviours are affected by differential rearing.

This substantial body of data does not, however, conclusively demonstrate captivity-induced dysfunction. First, not all repetitive behaviours indicate impairment: while the specific forms and frequencies of captive animals' SBs rarely occur in the wild [33; although see 48,49], repetitive or ritualistic behaviours do occur naturally [reviewed in 50; also see 5,49]. Likewise, SBs can occur in normal humans: in normal children, for instance, ritualistic and repetitive behaviours are part of daily life [51–53]. Second, perseveration also occurs to varying degrees in normal adults and children [54,55]. Third, this perseveration may statistically co-vary with SB in non-clinical subjects, reflecting normal, stable aspects of individual variation: thus positive correlations occur between SBs and tests of response inhibition and/or set shifting in normal children and adults [55–58]. Without controlled, well-documented differences in ontogeny, we thus cannot determine whether captive animals' responses represent differential pathology caused by detrimental housing, or instead similar normal individual variation. To assess any causal role played by captivity, we need experimental comparisons of animals from different, known developmental backgrounds.

Only a few studies have both manipulated animals' rearing or adult housing environments, and assessed SB and perseveration. Decades ago, severely deprivation-reared rhesus monkeys were found to be both perseverative [59] and more stereotypic than mother-reared controls [e.g. 60]. Similarly, capuchins captive-bred as pets seem both more stereotypic and poorer at extinction learning than mother-raised animals (although they lived at different facilities, making location a confound: [61,62]). However, in neither of these studies were perseveration and SB statistically compared. African striped mice maturing to adulthood in the wild were less perseverative in a 4-arm maze, and less stereotypic, than captive-raised conspecifics [37] (as mentioned above, perseveration and SBs also significantly co-varied at the individual level). In deer mice, enriched rearing environments were similarly found to reduce both SB and reversal deficits compared with mice reared in standard non-enriched cages [46], despite the two traits *not* co-varying within each group. Likewise, in laboratory ICR CD-1 mice, one study found that enriched rearing environments reduced perseveration and SB, but again independently: the two aspects of behaviour did not co-vary [47]; while another study found no effects of enriched versus non-enriched past rearing conditions in adults currently housed in standard cages [13]. Finally, Dallaire et al. [45] differentially-raised juvenile mink until early adulthood in either enriched or non-enriched housing: enriched rearing significantly reduced locomotor SB, but had no effect on perseveration in two-choice guessing tasks. In addition, these authors provided mature, non-enriched-raised adult females with enrichments. Enrichments again reduced SB but not perseveration; they also weakened the covariance between perseveration and SB evident in the animals' original, non-enriched housing.

Such findings suggest that elevated perseveration and SB may represent common responses to sub-optimal developmental environments, but they are far from conclusive. We therefore re-tested the hypotheses that environments exacerbating SB also exacerbate perseveration, and in a correlative manner at the individual level (Study 1). This type of research is important not just for understanding the aetiology of SB, but for revealing whether types of captivity associated with SB render animals generally behaviourally inflexible: evidence of neuropathology and compromised behavioural functioning that would be problematic in cases where behavioural normality is desirable (such as in wild animals in conservation captive-breeding programmes destined for release [63; see also 4]). We used mink as subjects, since they provide an ideal model study system especially for wild Carnivora (which often stereotypically pace in zoos and captive breeding centres [3]); are sensitive to environmental enrichment [45,64]; and display readily quantifiable “recurrent perseveration” (repetitions of previous motor responses when new responses are required: [22]) in two-choice guessing tasks using random reward schedules [45]. In Study 1, we also controlled for the strong genetic effect on SB in this species [65], by using 16 sibling pairs that were differentially raised, from natural dispersal age [66] through to adulthood, in either enriched or non-enriched environments. Our main predictions were that enriched rearing would reduce both the frequencies of locomotor SB and levels of recurrent perseveration, with a positive co-variation evident between the two. In Study 2, we re-analysed data from our previously published study of differentially-reared young mink [45], to investigate differences in results between this and the newer study, and to test new hypotheses raised by Study 1.

## 2. Study 1: methods

### 2.1. Subjects, housing and husbandry

Subjects were 16 brother pairs of adult American mink, from Michigan State University's experimental fur farm, that were singly housed from 3 months of age (juvenile), with one male from each pair randomly assigned to a Non-Enriched Environment (NEE) or an Enriched Environment (EE) cage. The indoor housing facility had artificial fluorescent lighting, on a cycle corresponding to natural daylight hours, with outside airflow via vents and an extractor fan. Heaters maintained the room at ~10–15 °C during winter months. All mink were fed each afternoon (exact time depended on the timing of behavioural observations or perseveration trials) with c. 250 g of mink feed: a meat-based paste. For these mink, the noise of the feed-cart rather than time of day was the key predictor of meal-times [cf. 67,68], inducing anticipatory SB. Each mink had a wire mesh home cage (W60 cm × D75 cm × H45 cm) with an exterior nestbox (W21 cm × D25 cm × H30 cm) and an *ad libitum* water drinking nipple. The EE treatment also had two wire mesh towers with ramps (W55 cm × D15 cm × H120 cm) and a 3 m long connecting tunnel leading to an additional, larger, enriched compartment (W120 cm × D75 cm × H45 cm) containing a nestbox, a 120 cm long running water trough (5–10 cm deep) for wading and a selection of structural or manipulable novel objects (e.g. balls, bells, brushes, baskets, tubes, chains and shelves) that were restocked on a monthly basis until animals were 10 months old (young adulthood) ([64] shows a diagram). Mink were previously shown to be highly motivated to utilise these enriched cages [64]. Consistent with this, on average, the EE mink in this study spent 33% (±2.24SE) of their total observed active time budget interacting with the enrichments – both structural and manipulable (excluding additional time spent resting or sleeping in the tunnels, towers or EE compartment). Each male was housed between one male and one female neighbour, from which they were visually isolated by plastic cage dividers. Subjects were experimentally naive at time of perseveration testing (February 2012). All housing conditions and procedures were approved by the University of Guelph's Animal Care Committee and Michigan State University's Institutional Animal Care and Use Committee.

### 2.2. Behavioural observations

Scanning observations were conducted by one observer (DLMC) for 5 days in each of the months of November 2011, December 2011, January 2012 and April 2012, respectively 4, 5, 6 and 9 months after differential housing. Data were collected from approximately 08:30–14:00, always pre-feeding; this was the animals' ‘active period’, during which most locomotor SB occurs [67,68]. Each mink was observed for two separate 4-min periods on each day and behaviour noted every 15 s. This method minimised disturbance: mink rapidly habituated (determined by the mink no longer watching the observer) after an approximately 30-s period of standing still

outside their cages (1 m distance). An individual's observation times were balanced across days. Direct observation has been validated against data collected from video [69]. All observations were subsequently categorised into four behavioural groups: ‘Activity’ – all normal active behaviour including feeding, drinking, grooming, walking, interacting with enrichments, urinating, defecating, and also ‘borderline SBs’ (SB-like activities not meeting the criterion of 3+ consecutive sequences) since these were previously shown not to co-vary with perseveration [45]; ‘Inactivity’ – lying down resting (eyes open) or sleeping (eyes closed); and two forms of SB. One was ‘Locomotor Stereotypy’ – 3+ consecutive sequences of bobbing, pacing, head twirling or route tracing (we term this specifically a “stereotypy” because it correlated with recurrent perseveration, following [4]); and stereotypic ‘Scrabbling’ – scratching with forepaws at plastic cage dividers or nestbox walls. We recorded this scrabbling behaviour separately since it is often directed at neighbouring mink, leading to disagreement over its classification as an abnormal behaviour [70,71]; it is not widely reported across Carnivora; and Dallaire et al. [45] found no relationship between scrabbling and recurrent perseveration.

### 2.3. Recurrent perseveration test

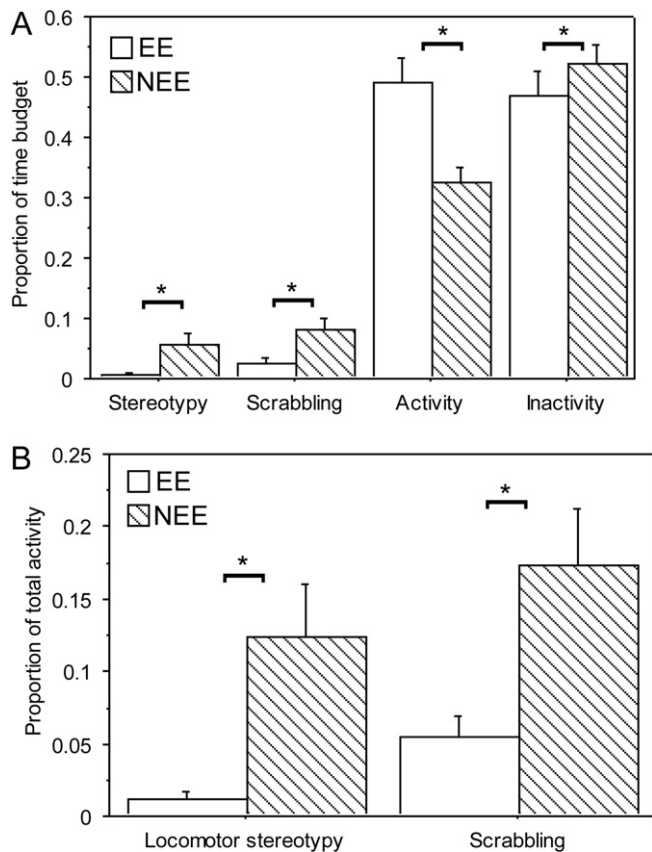
Recurrent perseveration in animals can be measured by ‘gambling’ or guessing tasks that present an individual with two options, each randomly rewarded 50% of the time [33]. Our testing apparatus was previously successfully used with mink (see Fig. 1 in [45]); it comprised a wire mesh and plastic cage that attached to the nestbox hole of the home cage, and presented the mink with a two-choice guessing task from a central holding vestibule, wherein a plastic divider was raised and the subject could push up, with its head, one of two metal doors on either its right or left side to receive a food treat from a hole at the end of a short tunnel approximately 3/4 mink body length. Mink voluntarily entered this apparatus, and were then locked in for the duration of their trials; no handling was required. To avoid behavioural cues from the tester, the apparatus was designed to visually occlude the tester from the mink during trials. The food treat was either ~0.75 g of hot dog or tinned sardines, depending on individual preferences determined during prior training sessions. This training occurred over 2 days, using ‘forced choice’ where only one door could be pushed open, on a random basis, and this was always rewarded. Each mink experienced approximately 30 training trials across the 2 days, by the end of which 28 mink were voluntarily pushing the doors to access rewards. Four did not respond well to training and were excluded from further testing, leaving 12 sibling pairs and 4 unrelated singles (1EE, 3NEE).

All testing was conducted during February 2012, when mink were young, 10 month-old adults, between 8:00 and 17:00, by one tester (DLMC) blind to SB levels (although not to housing). Daily feeding occurred after testing to maximise reward motivation. Individuals were tested across 6 consecutive days with 20 trials each per day (120 trials/mink in total); testing order was randomised across individuals and treatment. Each trial commenced when the plastic slide was raised and concluded when the mink chose a door and its nose touched the food hole. If a mink did not choose within 3 min it was deemed a mis-trial and the plastic lowered (this occurred 1–4 times/120 for 7 mink). Either the right or left door was randomly rewarded, with a weighted correction for side biases (using the formula ‘probability that left door is correct = (1 – number of left responses in the last 20 trials)/20’ [45]) designed to make equal choices of both sides (but avoiding long repetitive series) the best strategy [35,45]. The door chosen was recorded, as was the latency in seconds from slide-raising until choice (nose to the food hole).

To quantify recurrent perseveration we used the three indices successfully used by Dallaire et al. [45]. Excess Repetition measures how frequently each individual repeats its previous response. The other indices (Third-order Markov Model [34,35,37] and Detrended Fluctuation Analysis, DFA [72]) measure longer sequential dependency or patterning. In all indices, higher scores represent more repetition or patterning (though note that for Excess Repetition, positive scores indicate repetition and negative scores indicate alternation). We also calculated average response latencies for both repeating and alternating responses, predicting that more stereotypic individuals would have poorer response inhibition and therefore shorter response latencies, with SB frequency correlating negatively with average repeating, but not alternating, response latencies [cf. 35,36,45]. Additionally, to explore whether certain behavioural strategies might be more successful, we calculated the percentage of ‘correct’ (i.e. rewarded) responses for each individual over the whole experiment.

### 2.4. Statistical analyses

Behavioural data were combined across the four observational periods to provide an average proportion of the scanned time budget that each individual spent in ‘locomotor stereotypy’, ‘scrabbling’, ‘active’, or ‘inactive’ behaviours. (Note that exploratory analyses run using only the two most recent observational periods, i.e. those temporally closest to the guessing task, gave similar results.) We calculated locomotor stereotypy both as a proportion of the total time budget and as a proportion of total activity (i.e. the sum of all behaviours other than inactivity) to control for individual activity levels [cf. 36]. Proportional data were arcsin-square root transformed to normalise residuals, while response latencies were log-transformed ( $\log_{10} X$ ). We used General Linear Models (GLMs) to test for treatment effects on the four behavioural categories and three measures of perseveration; and to investigate



**Fig. 1.** Bar plots showing (A) the proportion of total time budget spent performing the four observed behavioural categories, 'Locomotor stereotypy', 'Scrabbling', 'Activity', 'Inactivity' and (B) proportion of total activity spent performing 'Locomotor stereotypy' and 'Scrabbling' for enriched (EE) and non-enriched (NEE) males. The mean (+SE) of the raw observational data are depicted, "\*" indicate significant differences.

relationships between locomotor stereotypy or scrabbling and each separate index of perseveration. 'Family' was a random effect in all models, to control for heritable co-variation between the NEE and EE brothers, using restricted maximum likelihood (REML) estimation methods as recommended for mixed models [73]. All analyses were conducted in JMP 10.0 (SAS Institute Inc., Cary, NC, USA) with  $\alpha$  set at 0.05 using Type III SS (Type 1 SS gave similar results);  $p$  values were two-tailed despite largely directional predictions.

We also compared the percentage of correct responses (i.e. those yielding a food reward) versus the percentage of alternations (i.e. the proportion of responses involving switches between R and L door). This was to explore whether responses that were similarly predictable (one component of perseveration) were similarly inappropriate (the other necessary component of perseveration) – an analysis triggered by the finding that alternation, rather than random responding, proved to be favoured by EE mink (see Section 3). These data could not be transformed to meet the assumptions of parametric statistics, and so we used Spearman's rank correlations. These analyses controlled for treatment by calculating ranks and computing  $p$  values separately for each block of subjects (EE versus NE). These values were then combined by taking a weighted average (by number of subjects in each block, minus one) to yield an overall  $\rho$  [74].

### 3. Study 1: results

Treatment significantly affected all behavioural categories. EE males performed less locomotor stereotypy (proportion of time budget:  $F_{1,15} = 10.73, p = 0.0051$ ; proportion of total activity  $F_{1,15} = 11.28, p = 0.0043$ ) and less scrabbling (proportion of time budget:  $F_{1,15} = 9.08, p = 0.0087$ ; proportion of total activity  $F_{1,12.63} = 10.1, p = 0.0075$ ) (Fig. 1A and B). They also spent less time inactive ( $F_{1,15} = 4.50, p = 0.05$ ), but displayed more normal activity ( $F_{1,15} = 25.09, p = 0.0002$ ) (Fig. 1A). In terms of perseveration under test, treatment significantly affected two indices in the expected direction, with EE mink showing less repetitive (cf. alternating)

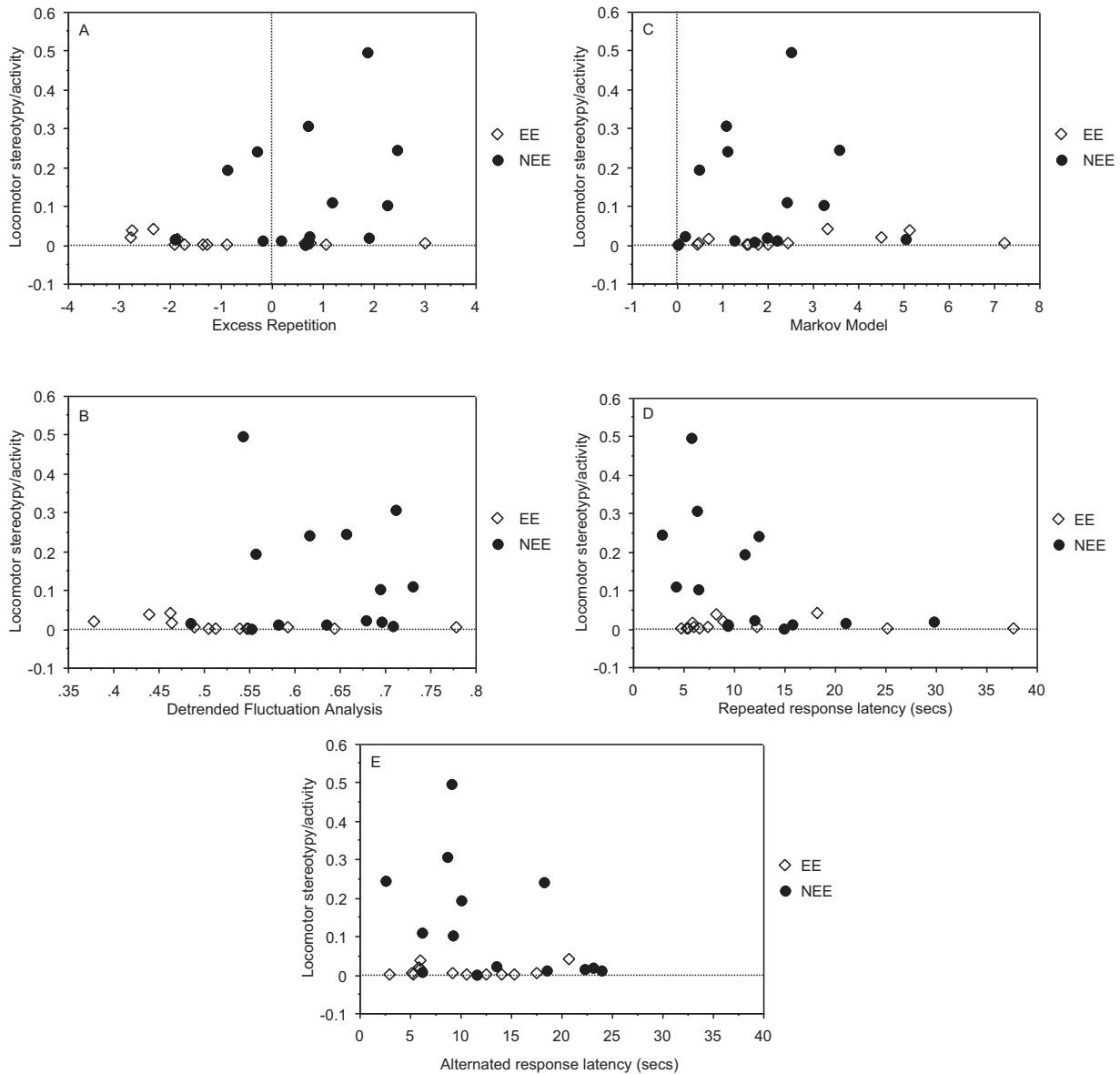
perseveration: Excess Repetition ( $F_{1,14.22} = 12.15, p = 0.003$ ) and DFA ( $F_{1,14.87} = 11.55, p = 0.004$ ) (Fig. 2A and B). Treatment did not, however, significantly affect Markov Model indices ( $F_{1,9.53} = 0.20, p = 0.67$ ) (Fig. 2C) – although these Markov analyses excluded one extreme outlier: a NEE male who responded in long strings of repeated rights except for 1 day of responding in a string of repeated lefts (patterns so statistically unlikely that the software crashed during analysis). Note that a high Markov score indicates a highly patterned series of responses, regardless of whether patterns are alternated or repeating. We therefore tested whether an effect of treatment on the Markov index would emerge once the degree of response alternation (already shown to be higher in EE mink) was statistically controlled for: it did not ( $F_{1,12.16} = 0.36, p = 0.56$ ). Spearman's rank correlations between reward rate and the alternating response styles, favoured by EE mink (quantified as the proportion of responses involving alternations), further showed that alternating was the most effective strategy at gaining rewards: mink who alternated more received more treats over the course of the experiment ( $\rho = 2.31, p = 0.011$ ) (Fig. 3).

Contrary to predictions, however, locomotor stereotypy did not correlate with any index of recurrent perseveration (all indices versus locomotor stereotypy/time budget:  $F_{1,20.17-22.63} < 0.66, p > 0.60$ ; and versus locomotor stereotypy/total activity  $F_{1,20.08-22.58} < 0.83, p > 0.44$ ), even when Markov indices were statistically corrected for degree of alternation; and data inspection suggested that these absent relationships were not artefacts of low power or unusual outliers: see Fig. 2A–C. The same was true (less unexpectedly) for scrabbling ( $F_{1,19.56-22.08} < 0.46, p > 0.33$ ).

During guessing task trials, treatment did not significantly affect the average repeated ( $F_{1,13.61} = 0.037, p = 0.85$ ) or alternated ( $F_{1,13.49} = 0.44, p = 0.52$ ) latencies to respond. However, as predicted, highly stereotypic mink specifically performed repeated responses more rapidly under test. While there was no overall significant relationship between locomotor stereotypy/time budget and repeated response latency ( $F_{1,23.17} = 2.61, p = 0.12$ ), there was a significant interaction with treatment ( $F_{1,23.33} = 4.58, p = 0.04$ ). The relationship with SB was significant when repeated response latencies were compared with locomotor stereotypy/activity ( $F_{1,23.08} = 4.39, p = 0.05$ ), and the interaction with treatment was stronger ( $F_{1,23.24} = 5.92, p = 0.02$ ) (Fig. 2D). This interaction proved to be because locomotor stereotypy negatively co-varied with latencies to repeat in NEE mink only (due to virtually no variance in locomotor stereotypy in EE animals) (Fig. 2D). In contrast, alternated response latencies never co-varied with this SB (locomotor stereotypy/time budget:  $F_{1,20.68} = 0.35, p = 0.56$ ; locomotor stereotypy/total activity:  $F_{1,20.7} = 0.87, p = 0.36$ ), nor showed an interaction between it and treatment (locomotor stereotypy/time budget:  $F_{1,23.89} = 0.94, p = 0.34$ ; locomotor stereotypy/total activity:  $F_{1,23.94} = 0.92, p = 0.35$ ) (Fig. 2E). Scrabbling showed a different pattern of relationships with response latencies: an overall significant positive relationship between repeated response latencies and scrabbling (scrabbling/time budget:  $F_{1,22.55} = 7.81, p = 0.01$ ; scrabbling/total activity:  $F_{1,20.11} = 6.51, p = 0.019$ ) (Fig. 4A) although no co-variation between alternated response latencies and scrabbling (scrabbling/time budget:  $F_{1,19.4} = 1.99, p = 0.17$ ; scrabbling/total activity:  $F_{1,16.31} = 1.36, p = 0.26$ ) (Fig. 4B). Thus, mink prone to high levels of scrabbling were slower to respond in guessing tasks when making repeats; while mink prone to high levels of locomotor stereotypy showed significantly faster reactions whenever repeating the last response they had made.

### 4. Study 2: methods

Study 2 re-analysed data from Dallaire et al. [45], both alone and combined with Study 1's data, to investigate possible reasons for differences and test new research questions raised by Study 1, as follows. First, to try and understand why SB and



**Fig. 2.** Scatterplots showing the relationship between locomotor stereotypy as a proportion of total activity and Excess Repetition (A), Detrended Fluctuation Analysis (B), and Markov Model values (outlier excluded) (C), repeated response latency (s) (D) and alternated response latency (s) (E) for enriched (EE) and non-enriched (NEE) males.

perseveration co-varied in our older females [45] but not Study 1's subjects, we used non-parametric Mann-Whitney *U* tests (Statview 5.0.1) to compare levels of locomotor stereotypy and perseveration in Study 1's animals and these former mink. This was because two findings from Dallaire et al. [45] suggested the possibility of threshold effects: the relationship between Excess Repetition and locomotor stereotypy was non-linear, flattening at higher levels of both variables such that increasing levels of perseveration were not reflected in greater locomotor stereotypy; and, somewhat in contradiction, when mature mink were given enrichment late in life this reduced SB but not perseveration, and weakened the relationships between them such that two perseveration indicators (Excess Repetition and Markov Model) no longer co-varied with locomotor stereotypy. We therefore compared the NEE female mink (i.e. prior to enrichment-provision) of Dallaire et al. to our NEE male mink, and the Dallaire et al. females after enrichment-provision to our EE males; similarly, we compared all indices of perseveration between the studies using two-sample *t*-tests (two-tailed) (Statview 5.0.1). Second, to boost statistical power, we combined Study 1's data with those from the similarly differentially-reared young adults of Dallaire et al. [Experiment 3]. SB had not co-varied with perseveration in either dataset on its own. Combining them assessed whether with a larger sample size, co-variation between SB and perseveration would become detectable. Because we did not have 120 trials for all of Study 1's mink (see Section 2.3) this required the re-calculation of the DFA index for the young Dallaire et al. subjects (where exactly 120 trials for all mink had enabled comparisons between larger paired sections of data) to match Study 1's calculation methods. The GLMs for investigating this combined dataset blocked by housing treatment (enriched/non-enriched), as well as by

a three-level factor termed 'group' which combined sex and study (thus "Study 1 males", "Dallaire young males", and "Dallaire young females").

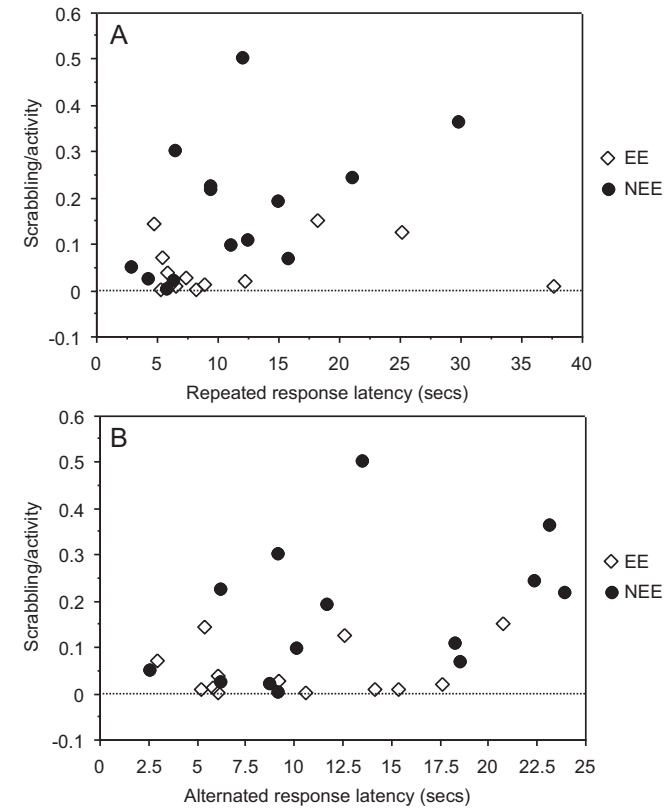
Our remaining tests re-analysed the old data alone. Since Study 1 revealed that alternators gained more rewards, we re-analysed Dallaire et al.'s data on mature females to see if this was a general phenomenon. In addition, to test the hypothesis that motivational effects on SB can act as confounds potentially obscuring links between SB and perseveration, we also reanalysed the mature females' links between SB and perseveration, statistically controlling for feed leftovers (the % of observation days with food left from the day before): indicators of feeding motivation (leftovers indicating relatively low hunger) that are likely to co-vary with SB [67,75,76], and had been recorded during the subject's enriched housing phase as part of another experiment [77]. Finally, Study 1 had suggested little difference between locomotor stereotypy/time budget and locomotor stereotypy/activity: neither co-varied with perseveration. However, published studies vary as to whether they correct for overall activity, and we therefore investigated whether, in data that do reveal relationships between SB and perseveration, controlling for activity makes these relationships stronger or weaker.

## 5. Study 2: results

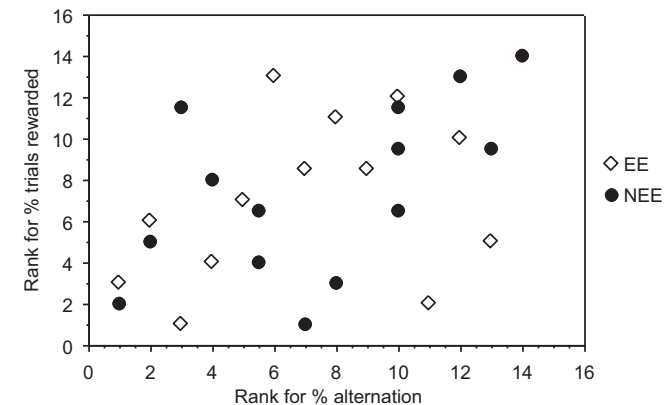
Mann-Whitney *U* comparisons between Study 1's males and the older adult females [45] showed a trend in NEE subjects for

**Table 1**  
The mean  $\pm$ SE values for locomotor stereotypy and the three indices of perseveration for non-enriched [NEE] and environmentally-enriched [EE] adult females [45] and our young males. The values from Dallaire et al. are from older adult females provided with enrichment later in life; these subjects had showed the expected relationships between perseveration and SB.

	Locomotor stereotypy/time budget	Locomotor stereotypy/activity	Markov Model	DFA	Excess Repetition
NEE adult females [45]	0.10 [ $\pm$ 0.02]	0.29 [ $\pm$ 0.047]	-0.093 [ $\pm$ 0.13]	0.58 [ $\pm$ 0.027]	0.29 [ $\pm$ 0.22]
NEE males [Current study]	0.057 [ $\pm$ 0.018]	0.115 [ $\pm$ 0.036]	1.93 [ $\pm$ 0.38]	0.65 [ $\pm$ 0.024]	0.86 [ $\pm$ 0.35]
Comparison	Z = -1.69, p = 0.09	Z = -2.41, p = 0.016	t <sub>16</sub> = -5.09, p = 0.0001]	t <sub>30</sub> = -1.80, p = 0.08	t <sub>24</sub> = -1.38, p = 0.18
EE adult females [45]	0.039 [ $\pm$ 0.021]	0.078 [ $\pm$ 0.037]	0.18 [ $\pm$ 0.15]	0.59 [ $\pm$ 0.025]	0.44 [ $\pm$ 0.45]
EE males [Current study]	0.006 [ $\pm$ 0.003]	0.013 [ $\pm$ 0.005]	2.42 [ $\pm$ 0.59]	0.53 [ $\pm$ 0.028]	-0.85 [ $\pm$ 0.48]
Comparison	Z = -0.54, p = 0.59	Z = -0.68, p = 0.49	t <sub>14</sub> = -3.70, p = 0.002]	t <sub>25</sub> = 1.22, p = 0.24	t <sub>25</sub> = 1.98, p = 0.06



**Fig. 4.** Scattergrams showing the relationship between scrabbling as a proportion of total activity and repeated response latency (s) (A) and alternated response latency (s) (B) for enriched (EE) and non-enriched (NEE) males.



**Fig. 3.** A scatterplot showing the ranks of enriched (EE) and non-enriched (NEE) males within each treatment group for percentage of trials that were rewarded (rank within group) versus percentage alternation (rank within group) displayed.

Dallaire's adult females to be more stereotypic than Study 1's males (see Table 1). Despite this, Study 1's males were more perseverative than the similarly-housed Dallaire adult females, at least according to Markov indices. (However, as mentioned previously, this measure does not differentiate between alternation and repetition). A similar trend was seen for DFA in NEE subjects only and EE males trended towards more alternation. However, our EE males showed more alternation than the EE adult females of the previous experiment (see Table 1).

Combining Study 1's data with the Dallaire data from similarly differentially-reared young mink still failed to reveal any overall correlation between locomotor stereotypy and any index of perseveration, despite the greater power and even using locomotor stereotypy/activity ( $F_{1,29} = 1.69, p = 0.206$  for Markov measures;  $F_{1,30} < 0.40, p > 0.316$  in all other models). However, SB was predicted by an interaction between DFA and 'group' (Study 1 males versus Dallaire young males versus Dallaire young females):  $F_{2,30} = 3.86, p = 0.032$  for locomotor stereotypy/time budget, and  $F_{2,30} = 2.53, p = 0.097$  for locomotor stereotypy/total activity. Splitting by group revealed that locomotor stereotypy did not co-vary with DFA in the Dallaire females, just as it did not in Study 1's males; however Dallaire males now showed a new positive correlation between DFA and locomotor stereotypy ( $F_{1,3} = 14.18, p = 0.033$  for locomotor stereotypy/time budget;  $F_{1,3} = 9.15, p = 0.057$  for locomotor stereotypy/total activity).

New analyses of mature females' data [45] investigated whether performing alternation predicted gaining more treats in the task. As in Study 1, there was a highly significant positive relationship between the two (in NEE conditions:  $F_{1,13} = 40.07, p < 0.001$ ;  $F_{1,10} = 24.46, p = 0.001$ ). The last analyses investigated factors that might affect strengths of relationships detected between SB and perseveration. In the mature mink [45] in NEE conditions, locomotor stereotypy co-varied with perseveration regardless of whether expressed as a proportion of observations or active time, but relationships were stronger and more variance explained using the latter (Table 2). Their scrabbling still did not co-vary with perseveration, however, even when corrected for overall activity. Finally, data collected on food-leaving when these females were enriched-housed [77] allowed investigation of whether controlling for this motivational variable would similarly strengthen relationships between perseveration and SB (originally weak compared to the relationships observed in NEE housing): it did (Table 2). Scrabbling still did not co-vary with perseveration even when corrected for feed leftovers ( $F_{1,9} < 0.28, p > 0.611$ ).

## 6. Discussion

As predicted, enriched-rearing and -housing reduced SB levels in Study 1: both the Carnivora-typical locomotor stereotypies (e.g. pacing and body-twirling) formerly correlated with recurrent perseveration, and repetitive scrabbling with the forepaws at the cages' walls and floors. Differential housing also affected measures of recurrent perseveration, particularly those sensitive

**Table 2**

The relationship between locomotor stereotypy/time budget or locomotor stereotypy/total activity and the three perseveration measures (DFA, Markov Model and Excess Repetition) for the mature mink of Dallaire et al. [45] in Non-Enriched housing (NEE) and Enriched (EE) housing; including corrections for food leftovers. Partial *r* is the correlation coefficient corrected for the two cohorts used in the study [45].

	In NEE housing		In EE housing		In EE housing: corrected for food leftovers	
	Locomotor stereotypy/time budget	Locomotor stereotypy/total activity	Locomotor stereotypy/time budget	Locomotor stereotypy/total activity	Locomotor stereotypy/time budget	Locomotor stereotypy/total activity
DFA	$F_{1,13} = 4.19$ $p = 0.061$ Partial $r = +0.484$	$F_{1,13} = 4.83$ $p = 0.047$ Partial $r = +0.519$	$F_{1,10} = 5.81$ $p = 0.0037$ Partial $r = +0.588$	$F_{1,10} = 7.67$ $p = 0.020$ Partial $r = +0.604$	$F_{1,9} = 7.36$ $p = 0.024$ Partial $r = +0.657$	$F_{1,9} = 7.53$ $p = 0.023$ Partial $r = +0.658$
Markov Model	$F_{1,13} = 4.73$ $p = 0.049$ Partial $r = +0.487$	$F_{1,13} = 5.69$ $p = 0.033$ Partial $r = +0.533$	$F_{1,10} = 0.54$ $p = 0.478$ Partial $r = +0.216$	$F_{1,10} = 0.68$ $p = 0.429$ Partial $r = +0.409$	$F_{1,9} = 1.66$ $p = 0.229$ Partial $r = +0.385$	$F_{1,9} = 1.76$ $p = 0.217$ Partial $r = +0.393$
Excess Repetition	$F_{1,13} = 7.91$ $p = 0.015$ Partial $r = +0.612$	$F_{1,13} = 11.12$ $p = 0.005$ Partial $r = +0.678$	$F_{1,10} = 1.56$ $p = 0.240$ Partial $r = +0.344$	$F_{1,10} = 1.93$ $p = 0.195$ Partial $r = +0.371$	$F_{1,9} = 4.62$ $p = 0.060$ Partial $r = +0.582$	$F_{1,9} = 4.73$ $p = 0.058$ Partial $r = +0.586$

to the repetition of responses and long sequences rather than patterning (recurring combinations of L and R group sequences). As predicted, enriched-raised mink had lower scores than standard-caged subjects in Detrended Fluctuation Analyses, indicating less perseveration: specifically fewer long sequences of repeats. Furthermore, this was the only index that had predicted SB even after enrichment in the older females of our earlier work [45]. According to another metric, enriched-raised mink also had significantly lower Excess Repetition values (and of all three measures of perseveration, this Excess Repetition measure had been the strongest correlate of SB in NEE-raised and -housed old adults in our former work [45]). In contrast, differential rearing had no effect on third-order Markov measures – an index sensitive to higher order patterning (repeated sequences or chains of four responses – although one NEE male was excluded from this analysis for having incalculably high values). This measure had also yielded paradoxical results in our past work (in differentially-reared young mink, it suggested that NEE subjects were more perseverative than EE in males, yet less perseverative than EE subjects in females [45]). Interestingly, that non-enriched-rearing enhances perseverative response repetition but not *sequence* repetition was also found in mice by Gross et al. [47]. Overall, housing conditions that induce higher SB in mink thus also induce more perseveration, especially of a type involving multiple immediate repetitions of a response.

This broadly agrees with most former investigations of differential rearing effects on similar variables, resembling previous findings in rhesus monkeys, capuchins, deer mice, African striped mice, ICR-CD-1 laboratory mice and deprived human children (see Section 1). Together, these studies thus suggest that individual differences in the quality of past rearing conditions could well have contributed to the relationships between SB and perseveration found in horses, bears and primates: long-lived animals whose pasts were undocumented and/or unknown when studied, yet likely to vary between subjects (see Section 1). Study 1's treatment effects also suggest that our former failure to find differential rearing outcomes in similar young mink [45] was a Type II error, perhaps resulting from that work's small sample sizes and/or uncontrolled variance between families. Finally, this finding suggests that non-enriched housing in mink may have similar effects on cortico-striatal circuits to those identified in deer mice (see Section 1): a hypothesis now needing direct test. The practical implications of such effects are considered further below.

Not all treatment effects on responses in the guessing task were as anticipated, however: enriched mink showed a marked tendency to alternate responses (R-L-R-L...). Superficially this might seem perseverative, contradicting our other findings. However, 'perseveration' specifically refers to *inappropriate* repetition. In a set-up where repeated choices of one arm were punished as side

biases (see Section 2.3), alternation was in principle an appropriate strategy, and in practice a successful one: high alternation was demonstrably associated with higher rates of reward gain in Study 1, and retrospectively shown to have been a profitable strategy in former subjects too (Study 2). Similar systematic alternations in T-mazes have been widely studied in rodents [78,79] (as well as other species, including another mustelid: the ferret [80]). Termed 'spontaneous alternation behaviour' (SAB), it is hypothesised to represent either an adaptive foraging strategy labelled 'win-shift' [81], or exploration, curiosity and information-gain [82,83]. Furthermore, in rodents, just as in our enriched-reared young mink (although not NEE-reared mink given EE late in adult life: Table 1), SAB can be enhanced by enrichment [84–86]. In one wild rodent it also appeared to be enhanced by being raised in nature rather than captivity [37]. SAB depends on the integrity of both limbic and non-limbic forebrain pathways [79]. Normal SAB can thus be disrupted by pre-frontal and dorsal striatal lesions: behavioural effects thought mediated by disinhibition and perseveration [79], and highly relevant to current views of SB. Compromised hippocampal functioning also reduces SAB [79]: important in the context of this experiment because numerous rodent studies show that environmental enrichment increases hippocampal neurogenesis and volume [87–91]. Together this suggests another neurobiological hypothesis to now test in mink: that enriched-rearing boosts hippocampal development (research made feasible by recent innovative studies of the hippocampus in this species [92]).

Despite strong treatment effects on perseveration and SB in expected directions, we unexpectedly found no relationships between locomotor stereotypy and recurrent perseveration at the individual level in Study 1. The only evidence that locomotor stereotypy related to the disinhibition of repeated responses was that mink with high levels of this SB also made particularly rapid repeats of recurring choices, an effect previously seen in stereotypic parrots as well as mink [35,36,45]. Repeat response latency was not, however, affected by housing-type, suggesting that it may reflect intrinsic normal individual variation in behavioural control rather than differential dysfunction. Study 2's re-analyses did suggest a hitherto un-noticed link between DFA (recalculated for this work) and locomotor stereotypy in our former study's young males [45], but revealed no such effect in its females nor for any other measure of perseveration. Overall, in mink, there would thus seem to be no clear relationship between locomotor stereotypy and recurrent perseveration in young animals. This adds to similarly absent relationships between perseveration and SB for scrabbling and borderline SB in adult mink [45]. It also resembles the absent relationships reported within similarly-housed ICR-CD 1 mice and deer mice (see Section 1). So does this mean that individual differences in behavioural flexibility or inhibition play no obligate role in



individual variation in SB? Could similar conclusions be drawn at the group level, in cases where group differences in SB are unrelated to group differences in perseveration (e.g. differentially-reared but then standard-housed ICR-CD-1 mice [13])? This is plausible, as discussed below. However, it is also possible that at least some of these non-significant findings are Type II errors resulting from small sample sizes or methodological issues that add noise: topics that we discuss next.

One possible source of noise leading to Type II errors could be differential activity, which is not always controlled for in published studies. Stereotypers tend to be more generally active [e.g. 36,93], and controlling for this does seem advisable: doing so strengthened relationships between locomotor stereotypy and repeat response latencies in Study 1, and between locomotor stereotypy and perseveration in Study 2. However, this cannot be a general problem as many relevant studies (our own included) included such controls, yet still found no relationships between SB and perseveration. A second possible source of noise could arise from inappropriate assessments of altered behavioural control. Behavioural disinhibition and inflexibility are manifest in diverse forms, from impulsive responding to poor abilities to shift between cognitive sets or decision rules [e.g. 94–96; see also 22,97], reflecting the involvement of differing forebrain regions [e.g. 98,99], receptor sub-types [e.g. 94] and/or degrees of progression of underlying disease states [e.g. 100]. These diverse forms of altered behavioural control also differentially predict different SB types [e.g. 25,38], raising the possibility that if the wrong form is tested for, then inter-relationships between a given SB and altered behavioural control may be missed. Indeed this may happen even if the right form of perseveration is simply quantified in a particular way, as revealed by the differing assessments of recurrent perseveration tried by Gross and colleagues [47], and the different outcomes that emerged for Dallaire's differentially-raised young males [45] when DFA was recalculated for Study 2. Finally, that SBs differ in type, even when superficially phenotypically similar, may mean that pooling forms together can add noise to datasets; for example, in deer mice, splitting animals by form of SB suggested that backward somersaulters are more impaired at reversal learning than repetitive jumpers; while in mink, had we pooled locomotor stereotypies, borderline SBs and scrabbling together, it is probable that relationships between locomotor stereotypies and recurrent perseveration would not have been detected in our previous work [45], nor relationships with short latency repeats (see [45] and Study 1).

Type II errors are unlikely to be the whole explanation however, as both Study 1 and 2 illustrate. Dallaire et al. [45] found strong correlations between locomotor SB and perseveration in adult female mink. Our new analyses (Study 2) also show similar effects in their young males. To add to the picture, other findings from our lab further indicate that in mature adult male mink, locomotor stereotypies have measurable detrimental behavioural correlates, in this case compromising courtship with conspecifics: in two-year old males, highly stereotypic individuals perform less well when given opportunities to mate, gaining fewer copulations despite never overtly showing SB in front of females [63]. In contrast, Study 1 surprisingly did not reveal even hints of any such patterns – despite using the same room, the same colour-type of mink, the same NEE cages, the same SB assessments, and, compared with [45], the same testing apparatus and statistical techniques for assessing perseveration. If methodological issues likely cannot explain the different results, then nor can power: in Study 1 we used more animals, and statistically controlled for 'family effects'; compared to former subjects [45], Study 1's males showed a similar or even wider range of perseveration (see Study 2), although admittedly they ranged less widely in SB (having a lower mean and variance); and furthermore, pooling our data with older data from similarly-raised young mink still revealed no overall main effects (Study 2). One issue explored

in Study 2 was the possibility of threshold effects. Previous results [45] suggested that relationships between locomotor SB and Excess Repetition break down at high values of both, but analyses indicated this could not explain Study 1's lack of effects: its subjects had rather low, not high, SB values; nor were their Excess Repetition scores higher than in previous work. Previous results also indirectly suggested that SB may not co-vary with some measures of perseveration (Excess Repetition and Markov Model) at low values of SB, at least when these low values result from adding enrichments [45]. Study 1's young subjects did have low SB values, making this a plausible hypothesis for future test. Overall, we therefore echo our previous suggestion [45] that clear relationships between locomotor SB and perseveration exist in older adult mink, but that this pattern may only develop over time [55], perhaps as stereotypy progressively comes to dominate more of the active time budget: a hypothesis now needing direct test via longitudinal data.

More broadly, we agree with Gross et al. [47] that generalised behavioural changes, manifest as increased perseveration, are neither necessary nor sufficient to fully explain all aspects of captive animals' SBs. Thus while clearly a common correlate of individual SB levels (see Section 1), they do not explain individual variation in all cases, as we both reviewed and demonstrated empirically in Study 1. Furthermore, although commonly altered by rearing conditions that exacerbate SB (see Section 1, and Study 1's findings), again it is clear from other studies that environmental enrichment can also modulate SB without concomitant, lasting changes in perseveration (for instance if it is provided but then removed, enhancing SB and frustration [13], or provided to mature animals late in life [45]). So what else might explain individual or group differences in SB? We hypothesise that motivational differences (or "individual differences in the animals' normal responses to an abnormal environment" [47]) – the aspects of behaviour traditionally studied by ethologists – are major contributors to variance in SB [cf. 13]. In mice, testing this hypothesis would involve assessing whether motivations to escape from non-enriched cages [cf. 12,13] are better predictors of SB than are degrees of perseveration, or at least explain variance in the data not accounted for by perseveration. In young mink, fully testing this hypothesis could involve similar experiments, since carnivores' locomotor SBs have likewise been hypothesised to develop from repeated escape attempts [101]; and/or assessing differential feeding motivations, since hunger is known to exacerbate mink SB [67,75,76]. Study 2 confirmed this as worthwhile hypothesis for future test in mink: our opportunistic controls for feed-leaving in enriched females (where low feed-leaving reflects low hunger) revealed that statistically factoring out the effects of hunger on locomotor stereotypy usually strengthened the previously weak relationships between this SB and perseveration.

While individual differences in SB may thus be influenced by differential motivations to perform frustrated natural behaviours, our data and other studies suggest that they interact with a developmental background of neurological dysfunction that is induced by impoverished rearing. For laboratory rodents, especially rats, non-enriched environments' adverse effects on learning, cognition and the neurobiology of relevant brain areas have long been appreciated [87–91,102]. Furthermore, as data accumulate on *M. musculus* and other stereotypic mouse species (see Section 1), the way that non-enriched environments can also increase SB, reduce behavioural flexibility, and alter the balance of activity between direct and indirect cortico-striatal circuits, is becoming evident. We agree that this "evidence for an underlying dysfunction of behavioural control mechanisms (has) ... implications for the use of laboratory rodents as valid research models" [47]. Such effects may be useful for researchers interested in symptoms of autism [e.g. 16,31–32]), but they are problematic for those assuming that control subjects are normal, or that surgical, genetic and pharmacological

manipulations are effected against a normal background. Our findings for mink, a semi-wild carnivore, raise further practical and ethical implications, since pacing and similar SBs [3,103] are common in wild carnivores in zoos and conservation breeding centres. For wild animals in general, developing in captivity is known to reduce brain size [e.g. 104,105], hippocampal volume [106,107] and problem-solving abilities [e.g. 108]. For Carnivora, including several endangered mustelids, captive-bred animals also have markedly low survivorship after release into the wild, often due to apparent behavioural impairments [109–112]. This problem can be somewhat mitigated by enriching the captive rearing environments, but to-date these have proved only semi-effective [111,113,114]. We therefore suggest that simple, non-invasive, voluntary tests like those used here on mink, might prove useful in captive breeding centres as pre-release behavioural screens. They could be used to evaluate the effectiveness of different enrichment regimes at improving captive-bred carnivores' behavioural flexibility and SAB, and thence perhaps even help ensure that these animals have the cognitive abilities needed to cope with life in the wild.

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