Does 'playtime' reduce stimulus-seeking and other boredom-like behaviour in laboratory ferrets?

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Running title: Does playtime reduce boredom in laboratory ferrets?

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Abstract

Much environmental enrichment for laboratory animals is intended to enhance animal welfare and normalcy by providing stimulation to reduce 'boredom'. Behavioural manifestations of boredom include restless sensation-seeking behaviours combined with indicators of sub-optimal arousal. Here we explored whether these signs could be reduced by extra daily play opportunity in laboratory ferrets. Specifically, we hypothesised that playtime would reduce restlessness, aggression, sensation-seeking and awake drowsiness, even 24h later in the homecage. Female ferrets (n = 14) were group housed in enriched multi-level cages. Playtime involved exploring a room containing a ball pool, paper bags, balls containing bells, and a familiar interactive human for 1h. This was repeated on three consecutive mornings, and on the fourth morning, homecage behaviour was compared between ferrets who had experienced the playtime treatment versus control cagemates who had not. Their investigation of stimuli (positive = mouse odour or ball; ambiguous = empty bottle or tea-strainer; and negative = peppermint or bitter apple odour) was also recorded. We then swapped treatments, creating a paired experimental design. Ferrets under control conditions lay awake with their eyes open and screeched significantly more, but slept and sat/stood less, than following playtime. They also contacted negative and ambiguous stimuli significantly more under control conditions than they did following playtime; contact with positive stimuli showed no effects. Attempts to blind the observer to treatments were unsuccessful, so replication is required, but the findings suggest that playtime may have reduced both sub-optimal arousal and restless sensation seeking behaviour, consistent with reducing boredom.

Keywords: Animal welfare; Boredom; Environmental enrichment; Exploration; Ferrets; Laboratory animals

Introduction

Many environmental enrichment (EE) attempts are intended at least partly to relieve boredom, either stated explicitly or implicitly, such as when the aim is to increase 'stimulation', 'exploration' or 'cognitive challenge' (e.g. Anderson & Wood 2001; Celli *et al.* 2003; Wells 2004; Meehan & Mench 2007; Puppe *et al.* 2007; Langbein *et al.* 2009). EE has been well defined before, and can encompass any environmental or husbandry modification that increases the welfare or biological functioning of a captive animal (e.g. Chamove 1989; Newberry 1995; Patterson-Kane 2001; Swaisgood & Shepherdson 2005). In the case of laboratory animals, EE is additionally important for increasing animal normalcy, to maximise the external validity of research (Bayne & Würbel 2014). The specific aims can vary, such as reducing fear (e.g. providing secluded shelters) or satisfying species-specific needs (e.g. providing perches for arboreal species), but it is those aimed at providing

56 sensory or cognitive stimulation (e.g. novel objects, sensory stimuli, or exploration) that are

57 particularly relevant for combating boredom (Meehan & Mench 2007; Manteuffel *et al.* 2009; Wells

58 2009; Meagher 2019). Opportunity to play generally could be effective, as play has been suggested

59 as a mechanism for countering boredom (e.g. Burghardt 1984; Held & Špinka 2011; Burghardt 2014;

60 Ahloy-Dallaire *et al.* 2018). However, until recently, objective indicators of boredom were lacking, so

61 it was difficult to assess whether stimulating EE was ever successful in tackling it.

62   Boredom is a negative emotion, which is caused by monotony that fails to engage attention

63 and to maintain optimal arousal levels (Wemelsfelder 2005; Eastwood *et al.* 2012; Burn 2017). It is

64 associated with a motivation for almost anything different or more arousing than the stimuli

65 available (Mason & Burn 2011; Meagher & Mason 2012; Meagher 2019). The motivation for general

66 stimulation as being key to objectively indicating boredom was identified and used by Meagher and

67 Mason (2012) who distinguished possible reasons why environmentally unenriched farmed mink

68 were observed to lie awake with their eyes open more than enriched mink (Meagher *et al.* 2013).

69 They suggested that if lying awake was due to boredom, the mink without EE would voluntarily

70 interact with diverse stimuli ranging from pleasant to unpleasant, whereas this would not be the

71 case for the alternative explanations of apathy or anhedonia. Mink lacking EE did indeed interact

72 with ambiguous and negative stimuli more readily than enriched mink did, indicating that they

73 sought general stimulation – even if it was not pleasant – which is consistent with boredom. Those

74 results were largely replicated in a follow up study (Meagher *et al.* 2017).

75   Motivation for general stimulation is part of a more general aversion to a monotonous

76 situation, so manifestations of boredom can present as stimulus-seeking (as in the mink), or as

77 restlessness, risk taking, unprovoked aggression, or escape behaviour (Burn 2017). However, this set

78 of behaviours is not entirely unique to boredom, because some of the manifestations of it could also

79 occur in other states, such as excitement, exploration, frustration, pain or playfulness. Therefore, it

80 is the seemingly paradoxical juxtaposition of these highly active behaviours versus low arousal

81 states, such as lying awake and yawning, that seems to characterise boredom (Berlyne 1960;

82 Wemelsfelder 2005; Fahlman *et al.* 2013). This is because boredom seems to occur when stimulation

83 is of insufficient quality to maintain optimal arousal levels, making the animal drowsy but not tired,

84 and motivating it to raise its arousal levels by whatever means possible (Burn 2017).

85   It is these two classes of indicators (drowsiness and arousal-seeking behaviours) that we

86 chose to measure when assessing whether additional playtime could help reduce potential boredom

87 in laboratory ferrets (*Mustela putorius furo*). Not all low arousal behaviours are relevant to

88 boredom, because different types of inactivity can have very different implications for animal

89 welfare, but lying awake with eyes open is one of the most relevant to boredom (Meagher *et al.*
90 2013).

91   Playtime, in 'playrooms' outside the home environment, has been used as putative EE in
92 species including rats (Widman & Rosellini 1990), pigs (Casey *et al.* 2007), dogs (Adams *et al.* 2004),
93 cats (Wilson *et al.* 1965), and primates (reviewed in Rennie & Buchanan-Smith 2006). Playing and
94 exploration opportunities can enhance cognitive function (Wilson *et al.* 1965; Pereira *et al.* 2007)
95 (but see Bennett *et al.* 2006) and encourage general exploration (Widman & Rosellini 1990) over the
96 long term. On the other hand, in primates at least, EE within the homecage appeared more effective
97 in terms of enhancing welfare than were regular playtimes, with primates performing increased
98 abnormal behaviour upon being returned to barren cages after playtimes than without playtimes
99 (reviewed in Rennie & Buchanan-Smith 2006). There could therefore be some concern that playtime
100 benefits are only transient, and that there could even be a negative contrast effect: the playtime
101 could increase homecage restlessness if the animal learns that the homecage is insufficiently
102 stimulating compared with the playroom.

103   Playtimes have not yet been investigated in terms of their potential to reduce animal
104 boredom specifically. If they are effective in this respect, they should ideally not just reduce
105 boredom during the playtime itself, but also to some extent back in the homecage, indicating that
106 the playtime has satisfied the motivation for greater stimulation. In the current study, we therefore
107 aimed to investigate the hypothesis that, if playtime reduces boredom even back in the homecage, it
108 would decrease behaviour indicating both stimulus-seeking and suboptimal arousal. We tested this
109 in laboratory ferrets in their homecages one day after playtime. We used a playtime paradigm
110 designed to offer all types of play: locomotor, social, object, and exploratory play (Burghardt 1984).

Materials and Methods

Animal housing and husbandry

114   Fourteen adult female pigmented ferrets were used. They were housed long term to
115 participate in other studies. They had been obtained from Highgate Farms (UK) from 12-16 weeks
116 old, and weighed between 670 and 1070g (mean±SD = 891±110g) at the time of testing. Nine of the
117 ferrets were 1 year old, and five were 2-3 years old. For the purposes of other studies (e.g. Town *et*
118 *al.* 2017) unrelated to the current paper, the five older ferrets were chronically implanted for
119 bilateral electrophysiological recording from auditory cortex (Warp-16 microdrives (Neuralynx, MT),
120 housing 16 independently moveable tungsten microelectrodes (WPI Inc., FL)). All animals were also

121   trained on auditory discrimination tasks which required restricted access to water in their home

122   cage during testing, but they participated in this study during their weeks off when they had

123   unrestricted access to water in their home cage; they had a minimum of 65h ad lib water before

124   participating.

125   Ferrets were housed in a room maintained at 15-24°C, with artificial lighting switched on

126   according to their winter cycle at 8:00 and off at 18:00h. All ferrets had access to food (VitalinTM

127   chicken and rice pellets, Grove Pet Foods, Lincoln) and water ad libitum. Ferrets were socially

128   housed in multi-tier cages that could be interconnected via tunnels. During the data collection

129   period of this study, ferrets were housed in groups of four in a single multi-level cage (175x90x74cm,

130   four levels accessible via ramps, Tecniplast). Cages were provided with with woodshavings as

131   bedding, paperwool, green plastic tunnels, small cardboard boxes and large paper bags.

132   All ferrets were allowed outside their cages to explore freely in their holding room every day

133   at 12:30h, during cage cleaning. During this period (45-60 mins) they explored the floor of the room

134   and could interact with conspecifics from and in other cages. The ferrets' social groups were mixed

135   and re-formed every week. The ferrets were also regularly handled and stroked by staff members.

136   This study was ethically approved by the Clinical Research and Ethical Review Board (CRERB)

137   at the Royal Veterinary College, reference number URN 2017 1755-3.

138   Playtime treatment

139   For three consecutive days (Monday-Wednesday) two of the four ferrets within the

140   experimental cage received 1h of extra playtime. This occurred at 10:00h-11:00h, on the basis of

141   pilot observations that revealed this as the ferrets' most active daytime period. The playtime

142   treatment involved the ferrets being allowed out of their homecage in the holding room with two

143   ferrets from another cage, similar to that occurring during cage-cleaning, but extra stimuli were

144   provided, such as tunnels and balls (Table 1). The experimenter (JR) was also present to supervise

145   and provide additional voluntary interaction with these ferrets. The remaining two cagemates

146   stayed within the cage and acted as controls.

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Table 1. Overview of the stimuli available to ferrets during the 1-hour of extra playtime.

Playtime stimuli	Specifications
Rigid Tunnel	SnuggleSafe Way to Go Fun Tunnel 90cm x 15cm
Hard Brown Tube Piping	Short Plumbing Pipe
Ball with Bell (x4)	Bell Ball Cat Toys (Aimé) - Pack of 4, 10.7 x 3.6 x 15 cm
Plastic Ball (x6)	Marshall Pet Products Pop-N-Play Ball Pit Balls
Large Brown Empty Paper Sack	Previously contained ferret dry food (Vitalin pellets)

These stimuli were partly on the basis of recommendations from a 7-chamber EE study investigating motivation in ferrets for different types of EE (Reijgwart *et al.* 2017).

Each week a different pair of ferrets was allocated to the playtime treatment, while their cagemates acted as controls. By the end of the 8-week study, all ferrets had experienced both treatments. This created a paired experimental design, unbalanced across cages because of the weekly mixing of social groups. Sampling was primarily opportunistic, based on which ferrets were off-study on a given week and whether animals had previously experienced been in the playtime or control group. Seven ferrets experienced playtime first, and seven control first.

Homecage Behavioural Observations

On the fourth day (Thursday, after three treatment days), an observation of homecage behaviour was conducted by the experimenter (JR), who stood quietly 1m away from the homecage. This occurred at 10:00-10:30h, i.e. at the same time as the playtime treatment had started on the preceding days, and 24h after the start of the most recent treatment.

Live behavioural observations were developed on the basis of a pilot study, which had been conducted over 1 week preceding the study and which also served as an attempt to habituate the ferrets to the observations. The behavioural ethogram is shown in Table 2. The 30 min protocol consisted of scan sampling of behaviour on a one-zero basis every 30 s for the four ferrets within the homecage, scanning from left to right, top to bottom (Martin & Bateson 2007). When a ferret performed multiple behaviours simultaneously, only the most fleeting behaviour was recorded so as not to miss it, based on a priority list (behaviours that occasionally occurred together, listed from highest priority to lowest were: Screeching > Biting > Chasing > Walk/Run > Standing).

177 **Table 2. Ethogram of ferret behaviours** and their relevance to the hypothesis**.**

Behaviour	Definition	Hypothetical relevance
Biting	The animal bites another animal	Restlessness/stimulus-seeking
Chasing	The animal follows at a run another animal who is retreating	Restlessness/stimulus-seeking
Climbing	The animal moves along tunnel, or on a rope, cage bars or ramp	Restlessness/stimulus-seeking
Digging	The animal claws at the sawdust with paws/pushes the sawdust around with nose	Restlessness/stimulus-seeking
Drinking water	The animal is stationary consuming water	Restlessness/stimulus-seeking
Eating Food	The animal is stationary consuming food - mouth is chewing	Restlessness/stimulus-seeking
Allo-grooming	The animal strokes tongue/claws over another ferret's fur	Restlessness/stimulus-seeking
Lying with eyes open	The animal is lying down stationary with eyes open	Sub-optimal arousal
Out of Sight	The animal is out of sight for observation	Included for completeness
Screeching	The animal makes a vocal screeching noise	Restlessness/stimulus-seeking
Standing	The ferret stands stationary on all four feet for at least 2 seconds	Sub-optimal arousal
Sniffing Bars	The animal approaches the cage bars, sniffing and looking out with eyes open	Restlessness/stimulus-seeking
Self-grooming	The animal strokes tongue/claw over its fur	Restlessness/stimulus-seeking
Sitting	The animal is sitting stationary with head up and eyes open	Sub-optimal arousal
Sleeping	The animal is lying down stationary with head down and eyes closed	Sub-optimal arousal
Stretching	The animal is stretching	Sub-optimal arousal

| Walking/running | The animal uses four limbs to locomote on a horizontal surface | Restlessness/stimulus-seeking |
| Yawning | The animal opens its mouth with head tilted backwards | Sub-optimal arousal |

178 The ethogram was based upon pilot investigations of homecage ferret behaviour patterns of interest. The pilot
179 study consisted of instantaneous scans every 30s for a period of 1h starting at 10:00h and ending at 11:00h for
180 a total of 1 week prior to commencing the behavioural observation study. The behaviours are separated
181 according to whether they were hypothesised to signal restlessness/stimulus-seeking or suboptimal arousal
182 aspects of behaviour, and thus decreased following playtime. They are all normal behaviours, so any
183 differences would be relative between the two treatments rather than indicating that the behaviours always
184 indicate restlessness/stimulus-seeking or suboptimal arousal.

185 Ideally, a person other than the experimenter would have administered the treatment, allowing the
186 experimenter to remain blind to treatment during behavioural observations. However, due to
187 personnel shortage, the experimenter had to both supervise the playtime treatment and conduct
188 behavioural observations, so video recordings were taken to enable later blind scoring and testing of
189 observer reliability. Despite this attempt, the video-recordings proved excessively dark, preventing
190 identification of each ferret and observation of behaviour, so only the live-recordings could be
191 analysed.

Stimulus Interaction

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193 After completing the 30 min observation, all four ferrets were removed from their cage to explore
194 the room for 5 min to awaken any who were drowsy. They were then placed back in their cage and
195 presented with six different stimuli in a randomised order (Table 3). Each stimulus (aside from the
196 ball with bell and empty plastic bottle) was presented inside a tea-leaf strainer, and each was
197 attached to the outside of the cage for 2 min in the same position on the middle cage level, with
198 approximately 15s between each stimulus. The ferrets' interactions with the stimuli were entirely
199 voluntary. The starting location of ferrets could not be controlled, but was noted and taken into
200 account in analyses. The latency and duration of contact with the stimuli (the ferret physically
201 touching the stimuli either with their nose or paws) was recorded live by the observer for each ferret
202 for 2 min using a stopwatch. Again, video recordings were intended to provide data for later scoring,
203 but these proved too dark for analysis.

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Table 3. Overview of the stimuli presented to the ferrets.

Stimuli	Effect	Rationale
Mouse bedding contained inside a tea-leaf strainer	Positive	Attractive to ferrets due to mice being prey in the wild
Ball with bell hung	Positive	Elicits a preference and a play response in ferrets (Reijgwart *et al.* 2017).
An empty tea-leaf strainer	Ambiguous	Novel with no apparent biological relevance
An empty plastic bottle	Ambiguous	Novel with no apparent biological relevance
Cotton wool soaked with 5ml of peppermint oil (Tisserand Aromatherapy® 100% extracted peppermint oil, Sayers Common, UK) contained inside a tea-leaf strainer	Negative	An aversive scent for the ferrets (as determined by headshakes and avoidance in the pilot study)
Cotton wool soaked with 5ml of bitter apple spray (Grannick's Bitter Apple®) contained inside a tea-leaf strainer	Negative	Commercially available animal deterrent

The stimuli were chosen following (Meagher & Mason 2012) and results of our pilot studies. They were hung on the outside bars in the central section of the homecage.

Statistical Analysis

Generalized Linear Mixed Models (GLMM) were used to analyse the data in SPSS, with Generalised Estimating Equations (GEE) being used in R when there were excessive zeroes (e.g. behaviours that most ferrets did not perform at all). For binary outcomes, models were checked for inflated standard errors; for continuous outcomes, models were checked for normality of residuals and homogeneity of variance, and the outcome transformed as necessary. Statistical significance is stated with two-tailed P-values < 0.05.

For the observations of unprovoked behaviour, many behaviours were too rare for statistical analysis, so the outcomes that could be tested were sleeping, lying with eyes open, sitting or standing stationary (sitting and standing summed together), walking/running, sniffing the bars, screeching, and aggression (screeching, biting and chasing summed together). The fixed factor predictors were treatment, time points, age/implant (considered together because animals with implants were older) and date/group (considered together because the groups of any four ferrets

224   were each tested on unique dates), with ferret ID as a random factor. When there was complete

225   separation of data (behaviour performed in one treatment and not at all in the other treatment), a

226   non-parametric McNemar test was used.

227   For the stimulus interaction test, the effect of treatment was run in a GLMM across all

228   ferrets, with whether or not the ferret investigated the stimuli as the outcome,. The fixed predictors

229   were treatment, stimulus type (positive, ambiguous, or negative), their two-way interaction, ferret

230   start position, and stimulus presentation order, with ferret ID, age/implant, and date/group as

231   random factors. GLMMs were also run for only those ferrets who contacted the stimuli, and the

232   measured outcomes tested were duration of, and latency to, contact. Latency was square root

233   transformed to provide a normal distribution before running through the GLMM. The same

234   predictors were used as with the previous GLMM. However, where insufficient degrees of freedom

235   were observed to support the interaction, separate models were run per stimulus type (positive,

236   ambiguous and negative stimuli). When a ferret did not contact a stimulus at all during the 2-minute

237   observation, that data point was excluded as a missing value in the models of latency and duration
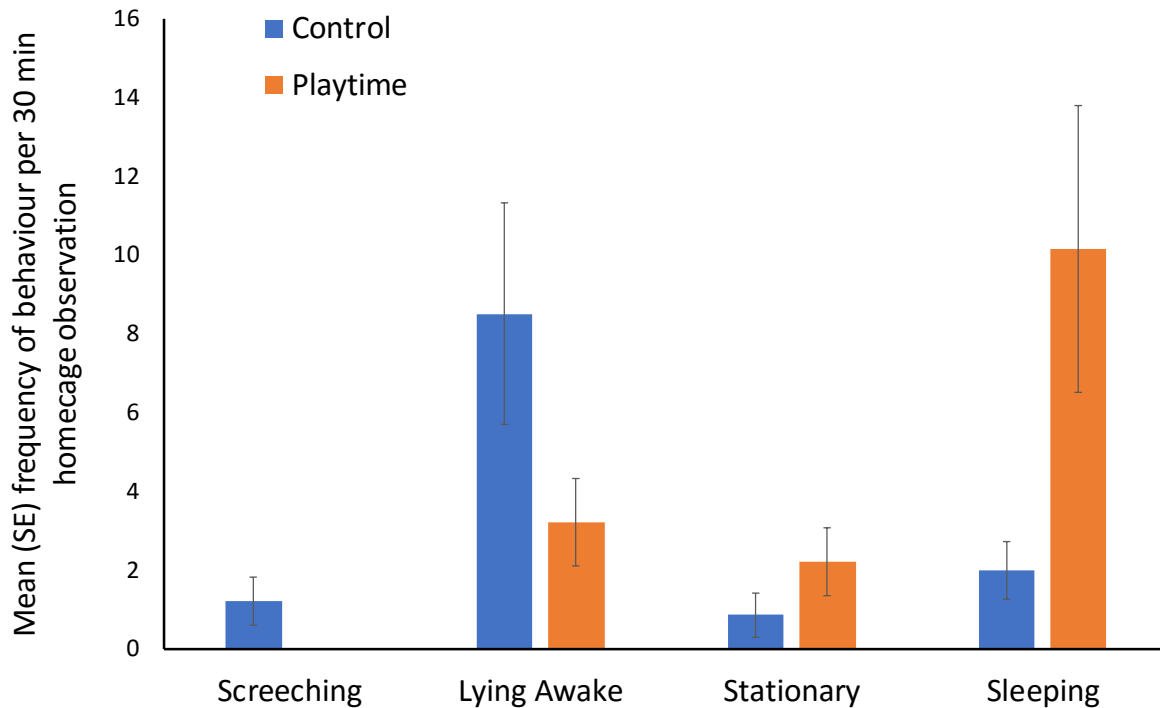
238   of contact.

Results
Homecage Behavioural Observations

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242   On the day after playtime, ferrets spent significantly more time sleeping (GEE: OD = 11.462;

243   95% CI [0.034, 0.227]; P < 0.001) and sitting (GEE: EO = 3.885; 95% CI [0.107, 0.619]; P = 0.002) than

244   when in the control condition. In turn, ferrets in the control condition spent more time lying awake

245   with eyes open (GMM: OD = 4.126; 95% CI [2.70, 6.260]; P < 0.001) and screeching (GEE: OD =

246   17.407; 95% CI [17.405, 17.405], P < 0.001). The statistically significant effects are shown in Figure 1.

247   Walking/running and sniffing the bars showed no significant treatment effects. Signs of aggression

248   other than screeching were too rare for analysis alone, but when combined with screeching to form

249   an 'overall aggression' frequency, this showed no statistically significant effects.

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Figure 1. Mean ± SE frequency of screeching, lying awake with eyes open, sitting or standing stationary, and sleeping in ferrets who had and had not received extra playtime. The subjects were female ferrets (n = 14) in a paired experimental design. Behaviour was recorded every 30 s over a 30 min observation per ferret per treatment.

Younger ferrets without an implant spent more time screeching (GEE: OD = 3.427; 95% CI [3.427, 3.427], P < 0.001) and sitting (GEE: OD = 3.665; 96% CI [1.533, 8.760], P = 0.003) and less time sleeping (GEE: OD = 0.356; 95% CI [0.209, 0.606], P < 0.001) than older ferrets. They also exhibited increased frequencies of sniffing bars (GEE for SF: EO = 3.307; 95% CI [1.085, 10.086]; P = 0.035) and walking/running (GEE for WR: EO = 2.309; 95% CI [1.247, 4.275]; P = 0.008) than older animals. Time point and date/group showed no significant effect on behaviour.
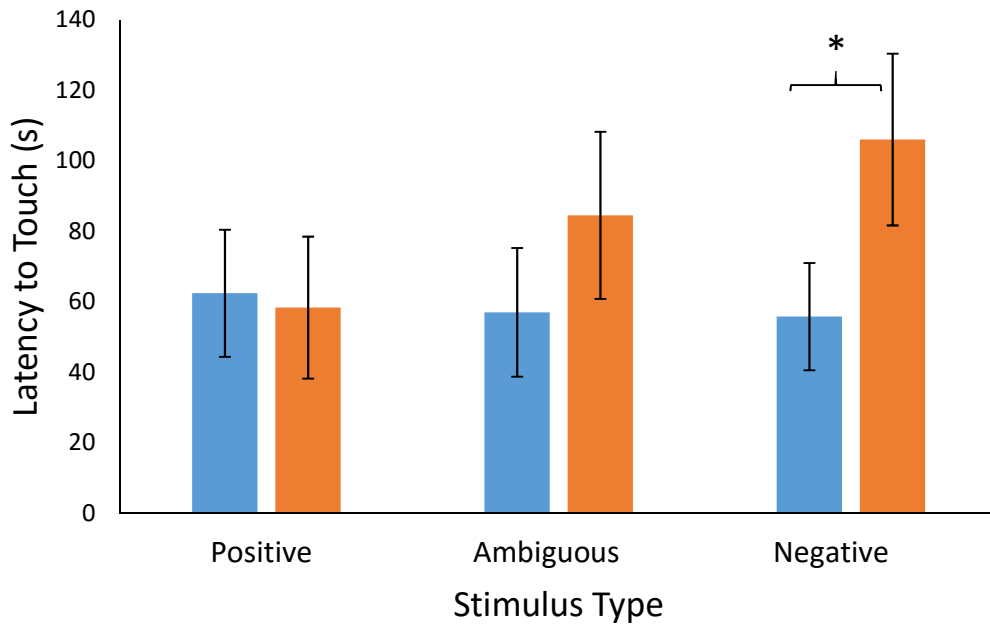
Stimulus Interaction

Ferrets in the control condition were more likely to contact stimuli than following the playtime treatment (GLMM: Odds +/- S.E. = 3.059 +/- 0.536, t = -2.217, P = 0.028). This effect was seen across stimulus types (Positive: Control = 12/14 ferrets vs Playtime = 10/14; Ambiguous: Control = 11/14 vs Playtime = 10/14; and Negative: Control = 11/14 vs Playtime = 9/14). Ferret starting position, stimulus type and order of presentation showed no effects on ferret interactions with the stimuli.

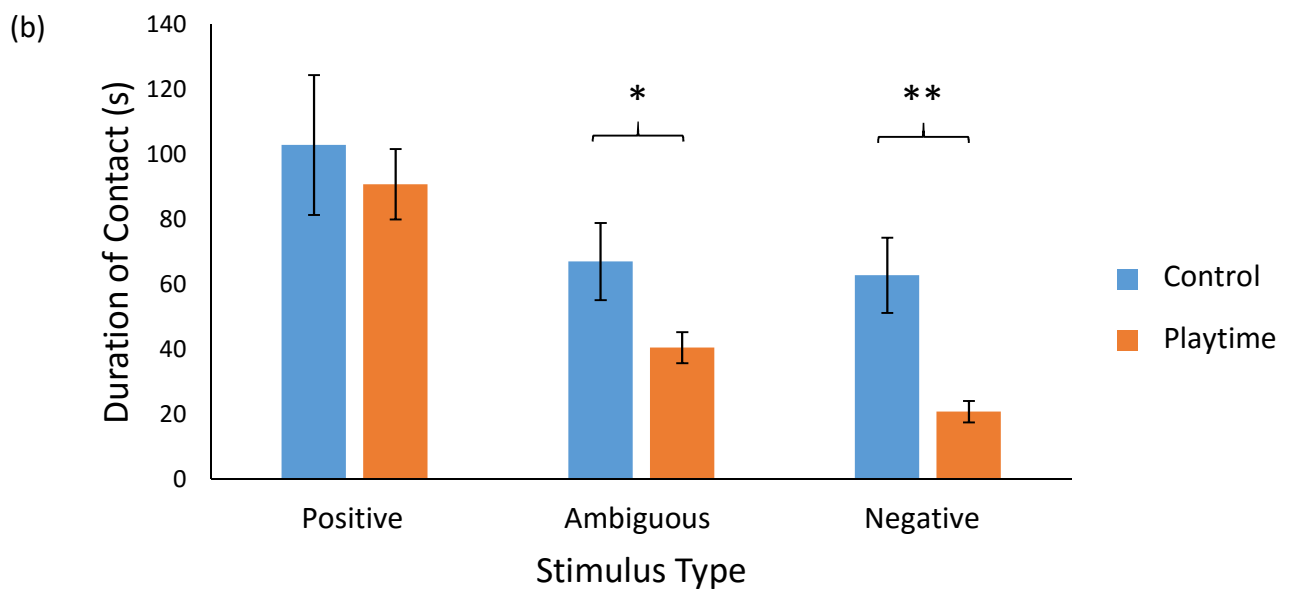Of ferrets who did contact stimuli, playtime significantly increased latencies to contact the negative stimuli (GLMM: Coeff +/- S.E. = 0.974 +/- 0.376, t = 2.592, P = 0.012), with a non-significant

271   trend in the same direction for ambiguous stimuli (Coeff +/- S.E. = 0.948 +/- 0.482; 1.967; P = 0.055;

272   Figure 2). There was no significant difference or trend in latency to contact the positive stimuli.

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(a)

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(b)

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276   Figure 2. Interactions with stimuli presented to ferrets who had and had not been given extra playtime. The

277   mean ± SE latency to contact the stimuli is shown in (a), and the mean ± SE duration of contact with stimuli is

278   shown in (b). Positive stimuli = ball with bell and mouse bedding; ambiguous = empty plastic bottle and empty

279   tea-leaf strainer; and negative = bitter apple spray and peppermint oil. The subjects were female ferrets (n =

280   14) in a paired experimental design, with control data in blue and extra playtime data in orange.

281

282   After playtime ferrets spent significantly less time interacting with the ambiguous (GLMM:

283 Coeff +/- S.E. = -0.998 +/- 0.379; t = 2.637; P = 0.011) and negative (Coeff +/- S.E. = -1.733 +/- 0.278; t

284 = 6.231; P < 0.001) stimuli, than under control conditions. Again, there was no significant treatment

285 effect on duration interacting with the positive stimuli.

Discussion

287   The results suggest that playtime reduces behaviours consistent with boredom in laboratory

288 ferrets, even measured 24h after the most recent play event. It seems that, just as boredom-like

289 states sometimes appear to prompt play in animals (Burghardt 1984; Held & Špinka 2011; Ahloy-

290 Dallaire *et al.* 2018), the inverse may also be true; play can reduce signs of boredom.

291   The increased interactions of the control ferrets with negative and ambiguous stimuli is

292 entirely consistent with the aforementioned research in environmentally enriched versus standard-

293 housed mink (Meagher & Mason 2012; Meagher *et al.* 2017), and this combined with aggressive

294 screeching and lying awake further characterises a boredom-like state (Burn 2017). If playtime can

295 help reduce general aggression in laboratory animals, this could be of great value for some species

296 where aggression is a significant problem. However, in the ferrets, overall aggression was rare and

297 showed no significant treatment effect, with only the screeching vocalisation being reduced after

298 play. It is possible that the screeching was not truly aggressive, although it is described as occurring

299 mainly in negative contexts (Boyce *et al.* 2001), so its reduction via playtime is consistent with

300 improved welfare. In future, recording screeching alongside the other behaviours with which it

301 occurs would help in interpreting its social context.

302   When ferrets had not had playtime in the current study, they chose to interact with even

303 negative stimuli: scents that had made them gape, headshake and withdraw in our pilot studies. This

304 is consistent with previous observations that animals in monotonous situations seemingly prefer

305 even unpleasant experiences over their existing monotony, which perhaps confirms the aversive

306 nature of boredom (Burn 2017). Examples include humans self-administering electric shocks when

307 asked to think their own thoughts when alone for 15 min (Wilson *et al.* 2014), rats and hamsters

308 choosing aversive food after eating solely their preferred food for several consecutive days (Galef &

309 Whiskin 2003, 2005), and mink in barren cages choosing to interact with predator cues, handling

310 gloves and sudden air puffs (Meagher & Mason 2012).

311   In the ferrets, playtime increased sleeping (i.e. lying down with eyes closed, not open), and

312 sitting/standing stationary, neither of which we predicted. These are low arousal behaviours, but

313   they do not suggest that arousal was sub-optimal after playtime, because they did not co-occur with

314   obvious attempts to raise arousal. One possible explanation for these low arousal behaviours could

315   be that the ferrets were simply tired out by the playtime. However, the fact that the ferrets

316   responded just as readily to the positive stimuli after playtime as they did in the control condition,

317   makes fatigue an unlikely explanation. Instead, their willingness to investigate positive stimuli, but

318   not ambiguous or negative stimuli, suggests that they were more 'choosy' about their stimulation on

319   the day after playtime than in the control condition. This choosiness suggests that the increased low

320   arousal behaviour after playtime could indicate a form of satisfaction or relaxation; the playtime

321   may thus exemplify EE that has satisfied the motivation for general stimulation (Meagher 2019).

322   Our attempts to blind the observer to the treatments were unsuccessful, which means that

323   the results require replication under blinded conditions to eliminate the possibility of expectation

324   bias (Tuyttens *et al.* 2014). We limited the potential for bias as much as possible before the

325   experiment began, by discussing it explicitly and encouraging an impartial attitude; for example,

326   whilst we hypothesised that playtime would reduce boredom, we discussed the possibility that

327   instead we could find an equally noteworthy contrast effect if playtime caused the ferrets to

328   perceive the homecage as more, rather than less, boring (as described in Rennie & Buchanan-Smith

329   2006). We also discussed how to interpret non-significant results to help counter publication bias

330   towards significant outcomes (Fanelli 2010; Dwan *et al.* 2013). If we were thus successful in avoiding

331   expectation bias, then the results do indeed suggest that playtime reduced behavioural indications

332   both of sub-optimal arousal (lying awake with eyes open) and of motivation for greater stimulation

333   (agonistic screeching, and interactions with negative and ambiguous stimuli) (Burn 2017).

334   It is worth noting that even the control ferrets here did have EE in their homecage and

335   explored their holding room daily, and the results should not be interpreted as showing that their

336   standard EE was ineffective. For ethical reasons, we did not compare the control treatment against a

337   barren cage, and it is possible that we would have found many more signs of compromised welfare

338   in the barren environment had we done so. It is also important to remember that the purpose of

339   different EE varies, such as refuges to provide security, so not all beneficial EE functions to provide

340   stimulation or reduce boredom.

Conclusion and animal welfare implications

341

342   In conclusion, subject to replication, the results here suggest that offering playtime to

343   laboratory animals may be an effective refinement to reduce potential boredom and promote a

344   more 'relaxed' state, even outside the playtime context.

Acknowledgements

References

Adams KM, Navarro AM, Hutchinson EK and Weed JL 2004 A Canine Socialization and Training Program at the National Institutes of Health. *Lab Animal 33*: 32-37

Ahloy-Dallaire J, Espinosa J and Mason G 2018 Play and optimal welfare: Does play indicate the presence of positive affective states? *Behavioural Processes 156*: 3-15

Anderson RC and Wood JB 2001 Enrichment for Giant Pacific Octopuses: Happy as a Clam? *Journal of Applied Animal Welfare Science 4*: 157-168

Bayne K and Würbel H 2014 The impact of environmental enrichment on the outcome variability and scientific validity of laboratory animal studies. *Revue scientifique et technique (International Office of Epizootics) 33*: 273-280

Bennett JC, McRae PA, Levy LJ and Frick KM 2006 Long-term continuous, but not daily, environmental enrichment reduces spatial memory decline in aged male mice. *Neurobiology of Learning and Memory 85*: 139-152

Berlyne DE 1960 *Conflict, arousal, and curiosity*. pp 144-192. McGraw-Hill: New York

Boyce SW, Zingg BM and Lightfoot TL 2001 Behavior of *Mustela Putorius Furo* (The Domestic Ferret). *Veterinary Clinics of North America: Exotic Animal Practice 4*: 697-712

Burghardt GM 1984 On the origins of play. In: Smith PK (ed) *Play in animals and humans* pp 5-41. Blackwell: Oxford

Burghardt GM 2014 A brief glimpse at the long evolutionary history of play. *Animal Behavior and Cognition 1*: 90-98

Burn CC 2017 Bestial boredom: a biological perspective on animal boredom and suggestions for its scientific investigation. *Animal Behaviour 130*: 141-151

Casey B, Abney D and Skoumbordis E 2007 A playroom as novel swine enrichment. *Lab Animal 36*: 32

Celli ML, Tomonaga M, Udono T, Teramoto M and Nagano K 2003 Tool use task as environmental enrichment for captive chimpanzees. *Applied Animal Behaviour Science 81*: 171-182

378   **Chamove AS** 1989 Environmental enrichment: A review. *Animal Technology 40*: 155-176

379   **Dwan K, Gamble C, Williamson PR and Kirkham JJ** 2013 Systematic Review of the Empirical Evidence

380   of Study Publication Bias and Outcome Reporting Bias — An Updated Review. *PLoS ONE 8*: e66844

381   **Eastwood JD, Frischen A, Fenske MJ and Smilek D** 2012 The Unengaged Mind: Defining Boredom in

382   Terms of Attention. *Perspectives on Psychological Science 7*: 482-495

383   **Fahlman SA, Mercer-Lynn KB, Flora DB and Eastwood JD** 2013 Development and validation of the

384   multidimensional state boredom scale. *Assessment 20*: 68-85

385   **Fanelli D** 2010 Do Pressures to Publish Increase Scientists' Bias? An Empirical Support from US States

386   Data. *PLoS ONE 5*: e10271

387   **Galef BG, Jr. and Whiskin EE** 2003 Preference for novel flavors in adult Norway rats (*Rattus

388   norvegicus*). *Journal of Comparative Psychology 117*: 96-100

389   **Galef BG, Jr. and Whiskin EE** 2005 Differences between golden hamsters (*Mesocricetus auratus*) and

390   Norway rats (*Rattus norvegicus*) in preference for the sole diet that they are eating. *Journal of

391   Comparative Psychology 119*: 8-13

392   **Held SDE and Špinka M** 2011 Animal play and animal welfare. *Animal Behaviour 81*: 891-899

393   **Langbein J, Siebert K and Nürnberg G** 2009 On the use of an automated learning device by group-

394   housed dwarf goats: Do goats seek cognitive challenges? *Applied Animal Behaviour Science 120*: 150-

395   158

396   **Manteuffel G, Langbein J and Puppe B** 2009 From operant learning to cognitive enrichment in farm

397   animal housing: bases and applicability. *Animal Welfare 18*: 87-95

398   **Martin P and Bateson P** 2007 *Measuring behaviour: an introductory guide*. pp 1-171. Cambridge

399   University Press: Cambridge

400   **Mason GJ and Burn CC** 2011 *Chapter 7: Behavioural restriction*. In: Appleby MC, Mench JA, Olsson A

401   and Hughes BO (ed) *Animal Welfare* pp 98-119. CABI Publishing

402   **Meagher RK and Mason GJ** 2012 Environmental enrichment reduces signs of boredom in caged

403   mink. *PLoS ONE 7*: e49180

404   **Meagher RK, Campbell DLM, Dallaire JA, Díez-León M, Palme R and Mason GJ** 2013 Sleeping tight

405   or hiding in fright? The welfare implications of different subtypes of inactivity in mink. *Applied

406   Animal Behaviour Science 144*: 138-146

407   **Meagher RK, Campbell DL and Mason GJ** 2017 Effects of enrichment on boredom-like states in mink

408   and their behavioural correlates: a replicate. *Applied Animal Behaviour Science 197*: 112-119

409   **Meagher RK** 2019 Is boredom an animal welfare concern? *Animal Welfare 28*: 21-32

410   **Meehan CL and Mench JA** 2007 The challenge of challenge: Can problem solving opportunities

411   enhance animal welfare? *Applied Animal Behaviour Science 102*: 246-261

412 **Newberry RC** 1995 Environmental enrichment: Increasing the biological relevance of captive

413 environments. *Applied Animal Behaviour Science 44*: 229-243

414 **Patterson-Kane EG** 2001 Environmental enrichment for laboratory rats: a review. *Animal Technology*

415 *52*: 77-84

416 **Pereira LO, Arteni NS, Petersen RC, da Rocha AP, Achaval M and Netto CA** 2007 Effects of daily

417 environmental enrichment on memory deficits and brain injury following neonatal hypoxia-ischemia

418 in the rat. *Neurobiology of Learning and Memory 87*: 101-108

419 **Puppe B, Ernst K, Schön PC and Manteuffel G** 2007 Cognitive enrichment affects behavioural

420 reactivity in domestic pigs. *Applied Animal Behaviour Science 105*: 75-86

421 **Reijgwart ML, Vinke CM, Hendriksen CFM, van der Meer M, Schoemaker NJ and van Zeeland YRA**

422 2017 Ferrets' (*Mustela putorius furo*) enrichment priorities and preferences as determined in a

423 seven-chamber consumer demand study. *Applied Animal Behaviour Science 180*: 114-121

424 **Rennie A and Buchanan-Smith H** 2006 Refinement of the use of non-human primates in scientific

425 research. Part II: housing, husbandry and acquisition. *Animal Welfare 15*: 215

426 **Swaisgood RR and Shepherdson DJ** 2005 Scientific approaches to enrichment and stereotypies in

427 zoo animals: what's been done and where should we go next? *Zoo Biology 24*: 499-518

428 **Town SM, Brimijoin WO and Bizley JK** 2017 Egocentric and allocentric representations in auditory

429 cortex. *PLoS Biology 15*: e2001878

430 **Tuyttens FAM, de Graaf S, Heerkens JLT, Jacobs L, Nalon E, Ott S, Stadig L, Van Laer E and Ampe B**

431 2014 Observer bias in animal behaviour research: can we believe what we score, if we score what

432 we believe? *Animal Behaviour 90*: 273-280

433 **Wells DL** 2004 A review of environmental enrichment for kennelled dogs, *Canis familiaris*. *Applied*

434 *Animal Behaviour Science 85*: 307-317

435 **Wells DL** 2009 Sensory stimulation as environmental enrichment for captive animals: A review.

436 *Applied Animal Behaviour Science 118*: 1-11

437 **Wemelsfelder F** 2005 Animal boredom: understanding the tedium of confined lives. In: McMillan FD

438 (ed) *Mental Health And Well-Being In Animals* pp 79-93. Blackwell Publishing: Oxford

439 **Widman DR and Rosellini RA** 1990 Restricted daily exposure to environmental enrichment increases

440 the diversity of exploration. *Physiology & Behavior 47*: 57-62

441 **Wilson M, Warren J and Abbott L** 1965 Infantile stimulation, activity, and learning by cats. *Child*

442 *Development 36*: 843-853

443 **Wilson TD, Reinhard DA, Westgate EC, Gilbert DT, Ellerbeck N, Hahn C, Brown CL and Shaked A**

444 2014 Just think: The challenges of the disengaged mind. *Science 345*: 75-77

445