

1 Does 'playtime' reduce stimulus-seeking
2 and other boredom-like behaviour in
3 laboratory ferrets?
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18 Running title: Does playtime reduce boredom in laboratory ferrets?
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23 Abstract

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

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

45 Introduction

46 Many environmental enrichment (EE) attempts are intended at least partly to relieve
47 boredom, either stated explicitly or implicitly, such as when the aim is to increase ‘stimulation’,
48 ‘exploration’ or ‘cognitive challenge’ (e.g. Anderson & Wood 2001; Celli et al. 2003; Wells 2004;
49 Meehan & Mench 2007; Puppe et al. 2007; Langbein et al. 2009). EE has been well defined before,
50 and can encompass any environmental or husbandry modification that increases the welfare or
51 biological functioning of a captive animal (e.g. Chamove 1989; Newberry 1995; Patterson-Kane 2001;
52 Swaisgood & Shepherdson 2005). In the case of laboratory animals, EE is additionally important for
53 increasing animal normalcy, to maximise the external validity of research (Bayne & Würbel 2014).
54 The specific aims can vary, such as reducing fear (e.g. providing secluded shelters) or satisfying
55 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) (but
95 see Bennett et al. 2006) and encourage general exploration (Widman & Rosellini 1990) over the long
96 term. On the other hand, in primates at least, EE within the homecage appeared more effective in
97 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).

111 **Materials and Methods**

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113 **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 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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152 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)

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

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

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162 Homeage Behavioural Observations

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

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

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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 differences
 183 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.

192 Stimulus Interaction

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

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207 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

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

210

211 [Statistical Analysis](#)

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

218 For the observations of unprovoked behaviour, many behaviours were too rare for statistical
 219 analysis, so the outcomes that could be tested were sleeping, lying with eyes open, sitting or
 220 standing stationary (sitting and standing summed together), walking/running, sniffing the bars,
 221 screeching, and aggression (screeching, biting and chasing summed together). The fixed factor
 222 predictors were treatment, time points, age/implant (considered together because animals with
 223 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.

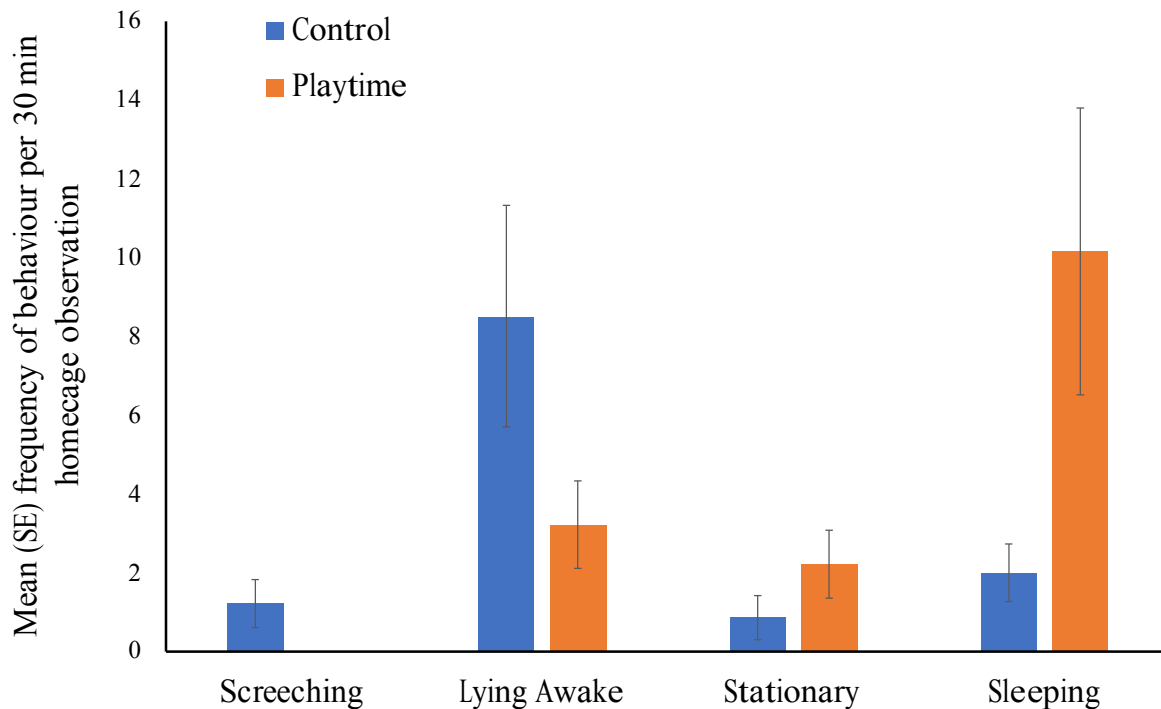
239 Results

240 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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252 Figure 1. Mean \pm SE frequency of screeching, lying awake with eyes open, sitting or standing stationary, and
 253 sleeping in ferrets who had and had not received extra playtime. The subjects were female ferrets ($n = 14$) in a
 254 paired experimental design. Behaviour was recorded every 30 s over a 30 min observation per ferret per
 255 treatment.

256 Younger ferrets without an implant spent more time screeching (GEE: OD = 3.427; 95% CI
 257 [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
 258 sleeping (GEE: OD = 0.356; 95% CI [0.209, 0.606], $P < 0.001$) than older ferrets. They also exhibited
 259 increased frequencies of sniffing bars (GEE for SF: EO = 3.307; 95% CI [1.085, 10.086]; $P = 0.035$) and
 260 walking/running (GEE for WR: EO = 2.309; 95% CI [1.247, 4.275]; $P = 0.008$) than older animals. Time
 261 point and date/group showed no significant effect on behaviour.

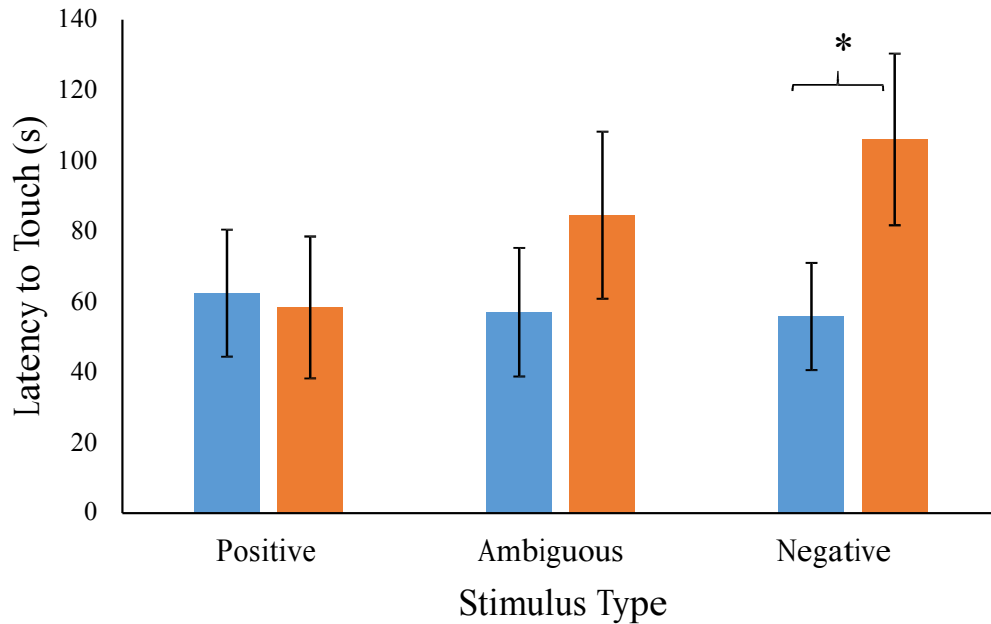
262 Stimulus Interaction

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

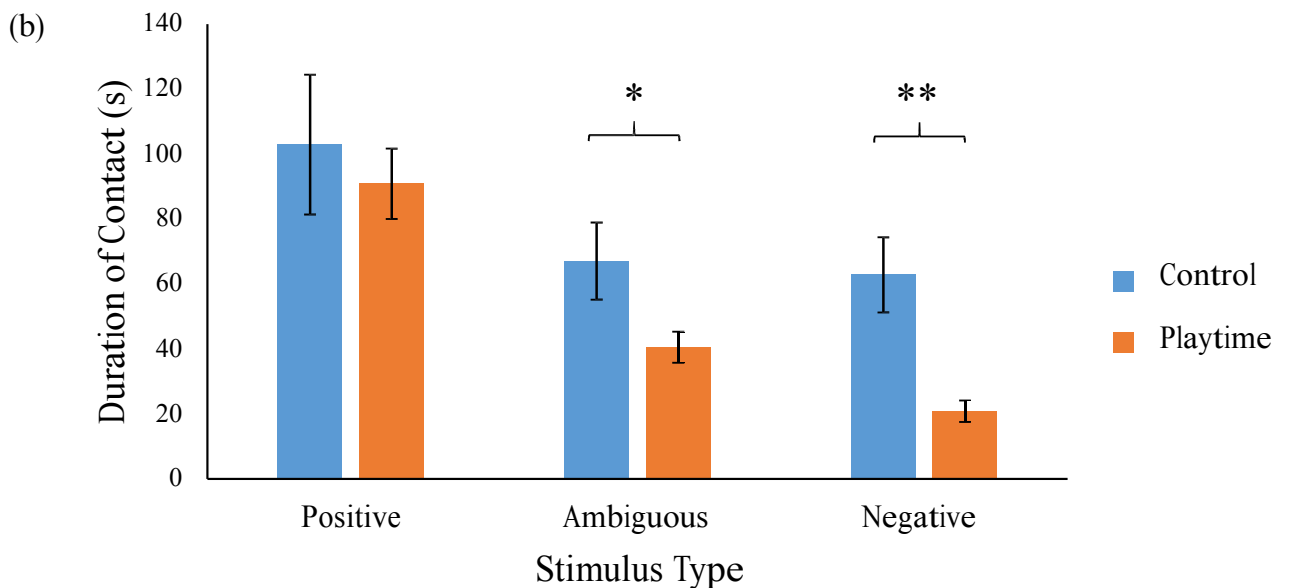
269 Of ferrets who did contact stimuli, playtime significantly increased latencies to contact the
 270 negative stimuli (GLMM: Coeff \pm S.E. = 0.974 \pm 0.376, $t = 2.592$, $P = 0.012$), with a non-significant

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.

273 (a)



274



275

276 Figure 2. Interactions with stimuli presented to ferrets who had and had not been given extra playtime. The
277 mean \pm SE latency to contact the stimuli is shown in (a), and the mean \pm 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.

286 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 even
305 unpleasant experiences over their existing monotony, which perhaps confirms the aversive nature of
306 boredom (Burn 2017). Examples include humans self-administering electric shocks when asked to
307 think their own thoughts when alone for 15 min (Wilson et al. 2014), rats and hamsters choosing
308 aversive food after eating solely their preferred food for several consecutive days (Galef & Whiskin
309 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 (Tuytens 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.

341 **Conclusion and animal welfare implications**

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.

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349 Town and Joseph Sollini for support in testing the ferrets. The manuscript was approved for
350 submission by RVC (manuscript number: PPS_01949).

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