

1 Does 'playtime' reduce stimulus-seeking  
2 and other boredom-like behaviour in  
3 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)  
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).

## 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 al.*  
118 *et 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 (Vitalin™  
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 Homecage Behavioural Observations

163 On the fourth day (Thursday, after three treatment days), an observation of homecage  
 164 behaviour was conducted by the experimenter (JR), who stood quietly 1m away from the homecage.  
 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 homecage, 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.

<b>Behaviour</b>	<b>Definition</b>	<b>Hypothetical relevance</b>
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.

## 192 Stimulus Interaction

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 <i>et al.</i> 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.

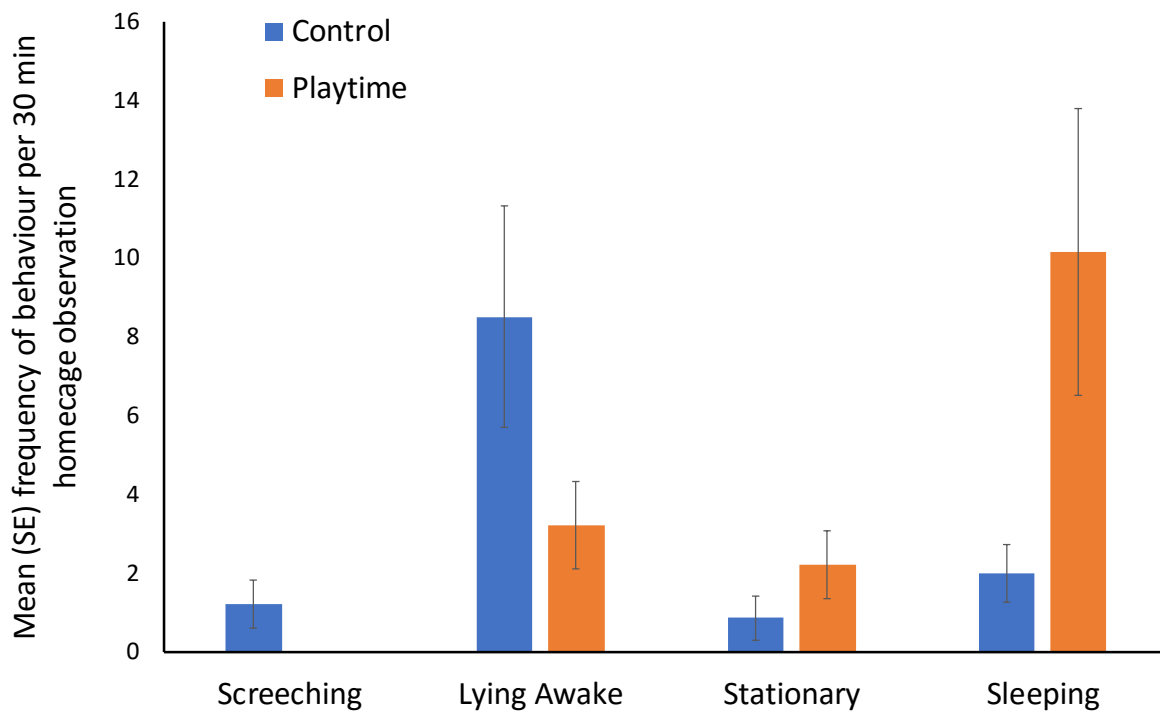
## 239 Results

### 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.

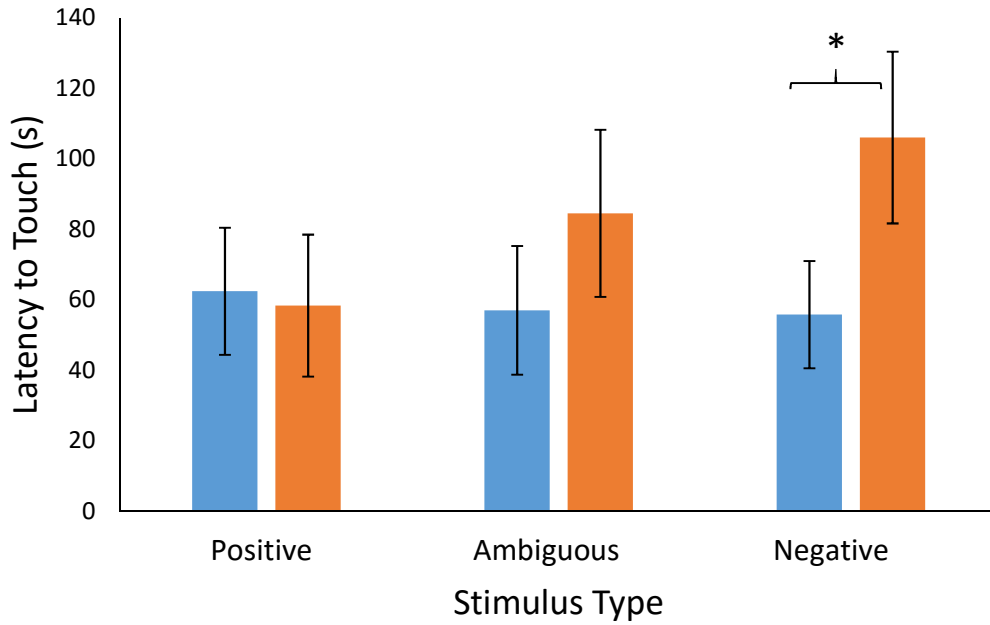
### 262 Stimulus Interaction

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.

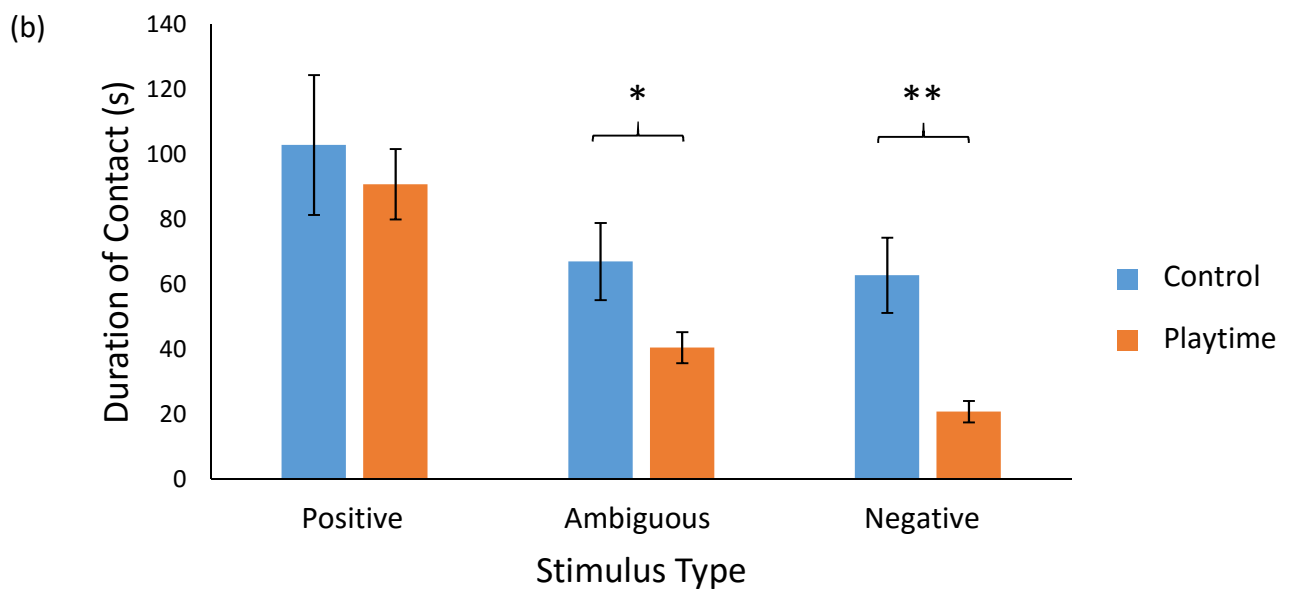
269 Of ferrets who did contact stimuli, playtime significantly increased latencies to contact the  
 270 negative stimuli (GLMM: Coeff  $\pm$  S.E. = 0.974  $\pm$  0.376,  $t = 2.592$ ,  $P = 0.012$ ), with a non-significant

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)



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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  
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 (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.

## 345 Acknowledgements

346 We are grateful for funding for this project: JR was supported by a BBSRC funded London  
347 Interdisciplinary Doctoral Training Studentship; JKB was supported by Wellcome Trust (Grant Ref  
348 098418/Z/12/Z). We would also like to thank Dr Yu-Mei Ruby Chang for statistical advice and  
349 Stephen 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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