Does 'playtime' reduce stimulus-seeking and other boredom-like behaviour 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

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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 sensory or cognitive stimulation (e.g. novel objects, sensory stimuli, or exploration) that are particularly relevant for combating boredom (Meehan & Mench 2007; Manteuffel *et al.* 2009; Wells 2009; Meagher 2019). Opportunity to play generally could be effective, as play has been suggested as a mechanism for countering boredom (e.g. Burghardt 1984; Held & Špinka 2011; Burghardt 2014; Ahloy-Dallaire *et al.* 2018). However, until recently, objective indicators of boredom were lacking, so it was difficult to assess whether stimulating EE was ever successful in tackling it.

Boredom is a negative emotion, which is caused by monotony that fails to engage attention and to maintain optimal arousal levels (Wemelsfelder 2005; Eastwood *et al.* 2012; Burn 2017). It is associated with a motivation for almost anything different or more arousing than the stimuli available (Mason & Burn 2011; Meagher & Mason 2012; Meagher 2019). The motivation for general stimulation as being key to objectively indicating boredom was identified and used by Meagher and Mason (2012) who distinguished possible reasons why environmentally unenriched farmed mink were observed to lie awake with their eyes open more than enriched mink (Meagher *et al.* 2013). They suggested that if lying awake was due to boredom, the mink without EE would voluntarily interact with diverse stimuli ranging from pleasant to unpleasant, whereas this would not be the case for the alternative explanations of apathy or anhedonia. Mink lacking EE did indeed interact with ambiguous and negative stimuli more readily than enriched mink did, indicating that they sought general stimulation – even if it was not pleasant – which is consistent with boredom. Those results were largely replicated in a follow up study (Meagher *et al.* 2017).

Motivation for general stimulation is part of a more general aversion to a monotonous situation, so manifestations of boredom can present as stimulus-seeking (as in the mink), or as restlessness, risk taking, unprovoked aggression, or escape behaviour (Burn 2017). However, this set of behaviours is not entirely unique to boredom, because some of the manifestations of it could also occur in other states, such as excitement, exploration, frustration, pain or playfulness. Therefore, it is the seemingly paradoxical juxtaposition of these highly active behaviours versus low arousal states, such as lying awake and yawning, that seems to characterise boredom (Berlyne 1960; Wemelsfelder 2005; Fahlman *et al.* 2013). This is because boredom seems to occur when stimulation is of insufficient quality to maintain optimal arousal levels, making the animal drowsy but not tired, and motivating it to raise its arousal levels by whatever means possible (Burn 2017).

It is these two classes of indicators (drowsiness and arousal-seeking behaviours) that we chose to measure when assessing whether additional playtime could help reduce potential boredom in laboratory ferrets (*Mustela putorius furo*). Not all low arousal behaviours are relevant to boredom, because different types of inactivity can have very different

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

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

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

Materials and Methods

Animal housing and husbandry

Fourteen adult female pigmented ferrets were used. They were housed long term to participate in other studies. They had been obtained from Highgate Farms (UK) from 12-16 weeks old, and weighed between 670 and 1070g (mean±SD = 891±110g) at the time of testing. Nine of the ferrets were 1 year old, and five were 2-3 years old. For the purposes of other studies (e.g. Town *et al.* 2017) unrelated to the current paper, the five older ferrets were chronically implanted for bilateral electrophysiological recording from auditory cortex (Warp-16 microdrives (Neuralynx, MT), housing 16 independently moveable tungsten microelectrodes (WPI Inc., FL)). All animals were also trained on auditory discrimination tasks which required restricted access to water in their home cage during testing, but they participated in this study during their weeks off when they had unrestricted access to water in their home cage; they had a minimum of 65h ad lib water before participating.

Ferrets were housed in a room maintained at 15-24°C, with artificial lighting switched on according to their winter cycle at 8:00 and off at 18:00h. All ferrets had access to food (VitalinTM chicken and rice pellets, Grove Pet Foods, Lincoln) and water ad libitum. Ferrets were socially housed in multi-tier cages that could be interconnected via tunnels. During the

data collection period of this study, ferrets were housed in groups of four in a single multi-level cage (175x90x74cm, four levels accessible via ramps, Tecniplast). Cages were provided with with woodshavings as bedding, paperwool, green plastic tunnels, small cardboard boxes and large paper bags.

All ferrets were allowed outside their cages to explore freely in their holding room every day at 12:30h, during cage cleaning. During this period (45-60 mins) they explored the floor of the room and could interact with conspecifics from and in other cages. The ferrets' social groups were mixed and re-formed every week. The ferrets were also regularly handled and stroked by staff members.

This study was ethically approved by the Clinical Research and Ethical Review Board (CRERB) at the Royal Veterinary College, reference number URN 2017 1755-3.

Playtime treatment

For three consecutive days (Monday-Wednesday) two of the four ferrets within the experimental cage received 1h of extra playtime. This occurred at 10:00h-11:00h, on the basis of pilot observations that revealed this as the ferrets' most active daytime period. The playtime treatment involved the ferrets being allowed out of their homecage in the holding room with two ferrets from another cage, similar to that occurring during cage-cleaning, but extra stimuli were provided, such as tunnels and balls (Table 1). The experimenter (JR) was also present to supervise and provide additional voluntary interaction with these ferrets. The remaining two cagemates stayed within the cage and acted as controls.

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).

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

Stimulus Interaction

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

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 were each tested on unique dates), with ferret ID as a random factor. When there was complete separation of data (behaviour performed in one treatment and not at all in the other treatment), a non-parametric McNemar test was used.

For the stimulus interaction test, the effect of treatment was run in a GLMM across all ferrets, with whether or not the ferret investigated the stimuli as the outcome,. The fixed predictors were treatment, stimulus type (positive, ambiguous, or negative), their two-way

interaction, ferret start position, and stimulus presentation order, with ferret ID, age/implant, and date/group as random factors. GLMMs were also run for only those ferrets who contacted the stimuli, and the measured outcomes tested were duration of, and latency to, contact. Latency was square root transformed to provide a normal distribution before running through the GLMM. The same predictors were used as with the previous GLMM. However, where insufficient degrees of freedom were observed to support the interaction, separate models were run per stimulus type (positive, ambiguous and negative stimuli). When a ferret did not contact a stimulus at all during the 2-minute observation, that data point was excluded as a missing value in the models of latency and duration of contact.

Results

Homecage Behavioural Observations

On the day after playtime, ferrets spent significantly more time sleeping (GEE: OD = 11.462; 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 when in the control condition. In turn, ferrets in the control condition spent more time lying awake with eyes open (GMM: OD = 4.126; 95% CI [2.70, 6.260]; P < 0.001) and screeching (GEE: OD = 17.407; 95% CI [17.405, 17.405], P < 0.001). The statistically significant effects are shown in Figure 1. Walking/running and sniffing the bars showed no significant treatment effects. Signs of aggression other than screeching were too rare for analysis alone, but when combined with screeching to form an 'overall aggression' frequency, this showed no statistically significant effects.

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 trend in the same direction for ambiguous stimuli (Coeff +/- S.E. = 0.948 +/- 0.482; 1.967; P = 0.055; Figure 2). There was no significant difference or trend in latency to contact the positive stimuli.

After playtime ferrets spent significantly less time interacting with the ambiguous (GLMM: Coeff +/- S.E. = -0.998 +/- 0.379; t = 2.637; P = 0.011) and negative (Coeff +/- S.E. = -1.733 +/- 0.278; t = 6.231; P < 0.001) stimuli, than under control conditions. Again, there was no significant treatment effect on duration interacting with the positive stimuli.

Discussion

The results suggest that playtime reduces behaviours consistent with boredom in laboratory ferrets, even measured 24h after the most recent play event. It seems that, just as boredom-like states sometimes appear to prompt play in animals (Burghardt 1984; Held & Špinka 2011; Ahloy-Dallaire *et al.* 2018), the inverse may also be true; play can reduce signs of boredom.

The increased interactions of the control ferrets with negative and ambiguous stimuli is entirely consistent with the aforementioned research in environmentally enriched versus standard-housed mink (Meagher & Mason 2012; Meagher *et al.* 2017), and this combined with aggressive screeching and lying awake further characterises a boredom-like state (Burn 2017). If playtime can help reduce general aggression in laboratory animals, this could be of great value for some species where aggression is a significant problem. However, in the ferrets, overall aggression was rare and showed no significant treatment effect, with only the screeching vocalisation being reduced after play. It is possible that the screeching was not truly aggressive, although it is described as occurring mainly in negative contexts (Boyce *et al.* 2001), so its reduction via playtime is consistent with improved welfare. In future, recording screeching alongside the other behaviours with which it occurs would help in interpreting its social context.

When ferrets had not had playtime in the current study, they chose to interact with even negative stimuli: scents that had made them gape, headshake and withdraw in our pilot studies. This is consistent with previous observations that animals in monotonous situations seemingly prefer even unpleasant experiences over their existing monotony, which perhaps confirms the aversive nature of boredom (Burn 2017). Examples include humans self-administering electric shocks when asked to think their own thoughts when alone for 15 min (Wilson *et al.* 2014), rats and hamsters choosing aversive food after eating solely their preferred food for several consecutive days (Galef & Whiskin 2003, 2005), and mink in barren cages choosing to interact with predator cues, handling gloves and sudden air puffs (Meagher & Mason 2012).

In the ferrets, playtime increased sleeping (i.e. lying down with eyes closed, not open), and sitting/standing stationary, neither of which we predicted. These are low arousal behaviours, but they do not suggest that arousal was sub-optimal after playtime, because they did not co-occur with obvious attempts to raise arousal. One possible explanation for these low arousal behaviours could be that the ferrets were simply tired out by the playtime. However, the fact that the ferrets responded just as readily to the positive stimuli after playtime as they did in the control condition, makes fatigue an unlikely explanation. Instead, their willingness to investigate positive stimuli, but not ambiguous or negative stimuli, suggests that they were more 'choosy' about their stimulation on the day after playtime than in the control condition.

This choosiness suggests that the increased low arousal behaviour after playtime could indicate a form of satisfaction or relaxation; the playtime may thus exemplify EE that has satisfied the motivation for general stimulation (Meagher 2019).

Our attempts to blind the observer to the treatments were unsuccessful, which means that the results require replication under blinded conditions to eliminate the possibility of expectation bias (Tuyttens *et al.* 2014). We limited the potential for bias as much as possible before the experiment began, by discussing it explicitly and encouraging an impartial attitude; for example, whilst we hypothesised that playtime would reduce boredom, we discussed the possibility that instead we could find an equally noteworthy contrast effect if playtime caused the ferrets to perceive the homecage as more, rather than less, boring (as described in Rennie & Buchanan-Smith 2006). We also discussed how to interpret non-significant results to help counter publication bias towards significant outcomes (Fanelli 2010; Dwan *et al.* 2013). If we were thus successful in avoiding expectation bias, then the results do indeed suggest that playtime reduced behavioural indications both of sub-optimal arousal (lying awake with eyes open) and of motivation for greater stimulation (agonistic screeching, and interactions with negative and ambiguous stimuli) (Burn 2017).

It is worth noting that even the control ferrets here did have EE in their homecage and explored their holding room daily, and the results should not be interpreted as showing that their standard EE was ineffective. For ethical reasons, we did not compare the control treatment against a barren cage, and it is possible that we would have found many more signs of compromised welfare in the barren environment had we done so. It is also important to remember that the purpose of different EE varies, such as refuges to provide security, so not all beneficial EE functions to provide stimulation or reduce boredom.

Conclusion and animal welfare implications

In conclusion, subject to replication, the results here suggest that offering playtime to laboratory animals may be an effective refinement to reduce potential boredom and promote a more 'relaxed' state, even outside the playtime context.

Acknowledgements

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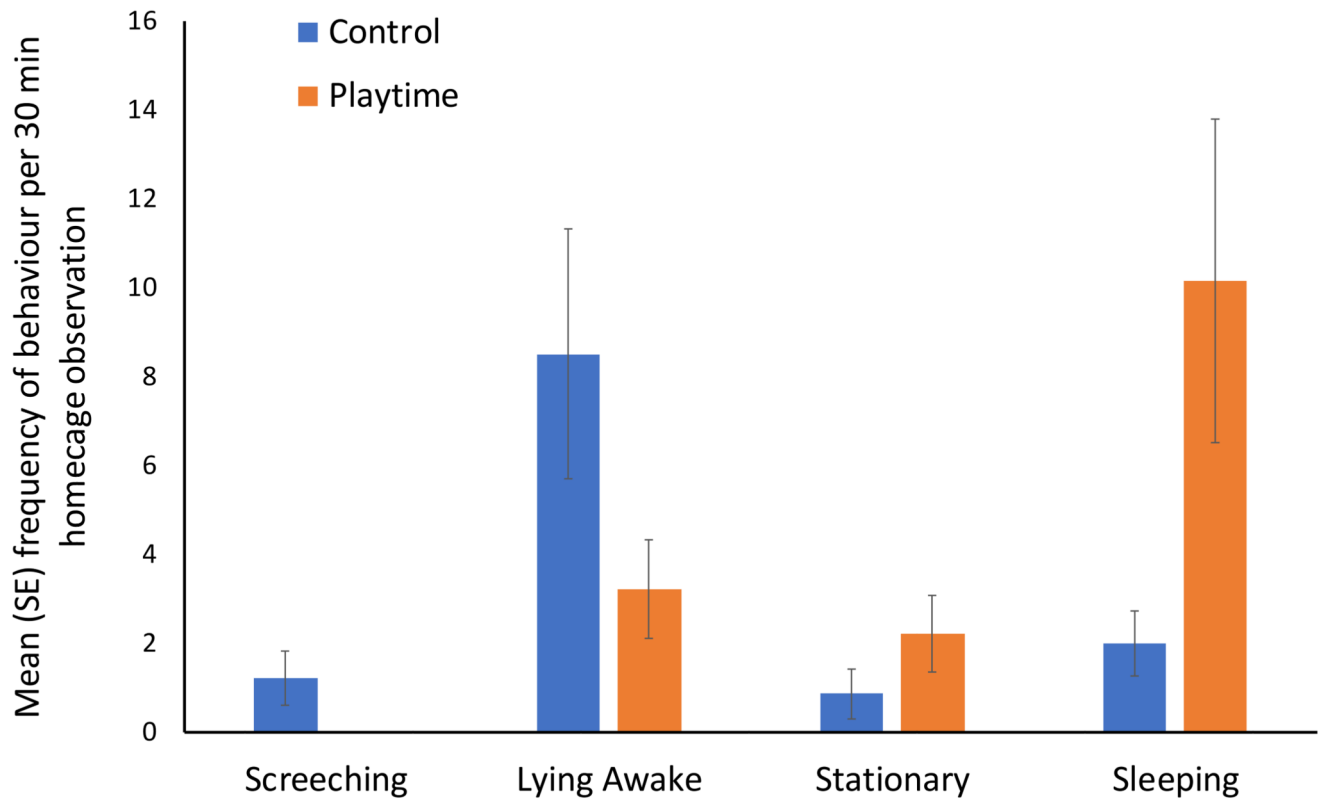
Figure 1.
Mean ± SE frequency of screeching, lying awake with eyes open, sitting or standing stationary, and sleeping in ferrets who had and had not received extra playtime. The subjects were female ferrets (n = 14) in a paired experimental design. Behaviour was recorded every 30 s over a 30 min observation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

(a)

(b)
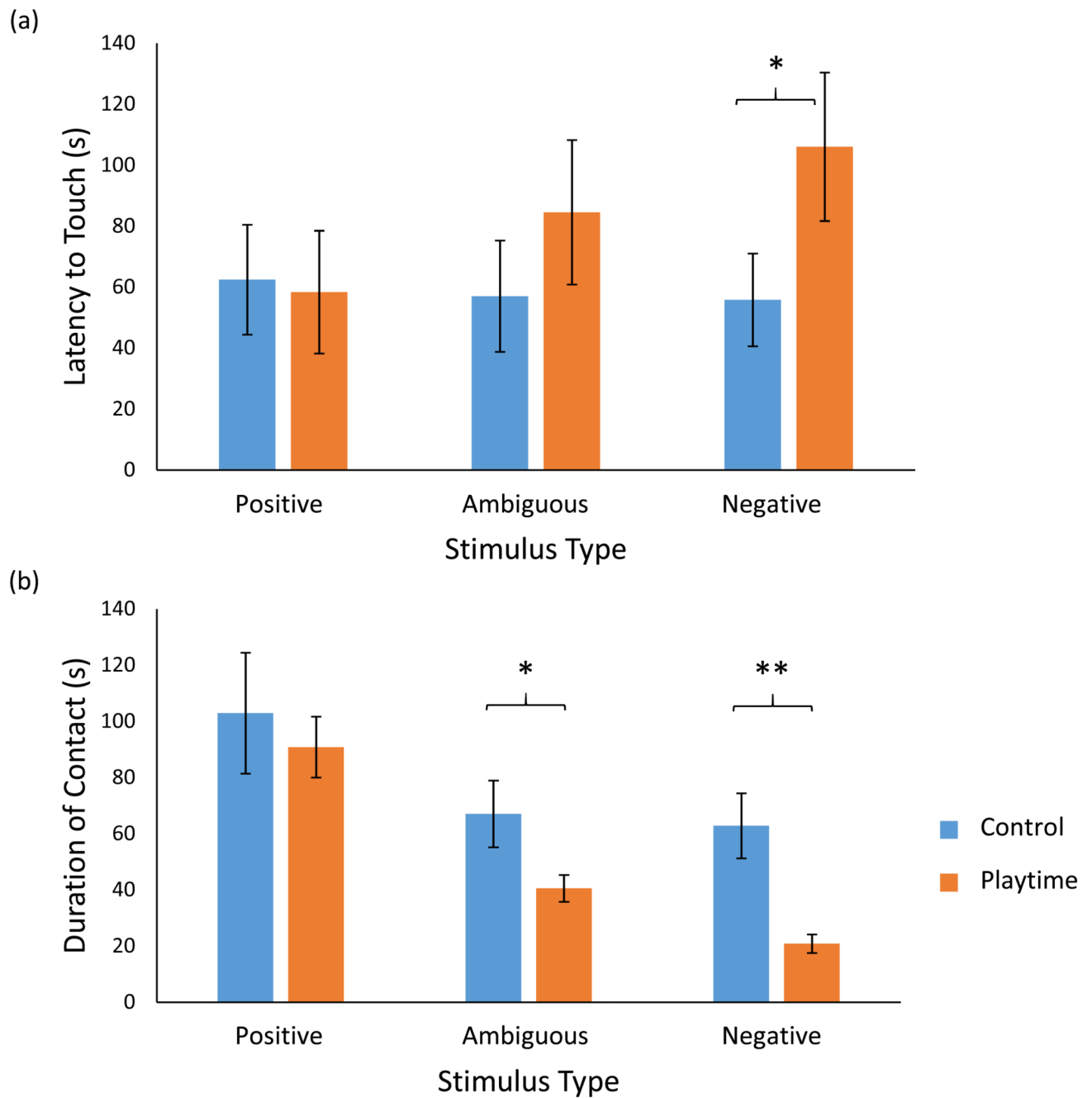
Figure 2.

Interactions with stimuli presented to ferrets who had and had not been given extra playtime. The mean ± SE latency to contact the stimuli is shown in (a), and the mean ± SE duration of contact with stimuli is shown in (b). Positive stimuli = ball with bell and mouse bedding; ambiguous = empty plastic bottle and empty tea-leaf strainer; and negative = bitter apple spray and peppermint oil. The subjects were female ferrets (n = 14) in a paired experimental design, with control data in blue and extra playtime data in orange.

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).

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

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

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.