The effect of different types of enrichment on the exploratory behaviour of the laboratory ferret (*Mustela putorius furo*).

Master thesis assigned by the Faculty of Veterinary Medicine, Department of Animals in Science and Society.

Abstract

The Domestic or European ferret (*Mustela putorius furo*) is increasing in number as a laboratory animal and as a pet. Knowledge on the ferrets' ethological needs is essential to guarantee a good welfare and health for those ferrets. Unfortunately, up until now only little is known about ferret behaviour in general. This study aims to determine whether different types of enrichment affects the exploratory behaviour of ferrets. Six groups of six female neutered ferrets (N=36) were used in this study. One cage was housed under standard conditions (SC, N=6), three cages were housed with preferred enrichment (EC: two hammocks, a foraging ball and a water bowl, N=18) and two cages were housed with non-preferred enrichment (npEC: two ferret balls, a golf ball and an extra food bowl, N=12). All three types of conditions contained a sawdust bedding, a bucket, a water nipple and a food bowl. Recordings of two full days of each cage were observed. The first day was the baseline measurement in which all ferrets were housed under standard conditions. The second day was a month later when the ferrets were housed with or without enrichment, according to their grouping. As regards the duration, the three different groups did not differ significantly in the mean time spent on exploratory behaviour in period 2 compared to period 1: npEC (0,42 min ± 4,97 min, P=0,777), EC (2,56 min ± 7,59 min, P=0,171) and SC (-1,91 min ±3,44 min, P=0,233), nor did the differences in the Δ values of the mean time spent on exploration significantly differ between the three groups (-1,24 min ±6,60 min , P=0,277). Only the ferrets in cage 2 (one cage of the EC group), showed a significant decrease in the mean time spent on exploratory behaviour from period 1 to period 2 (-6,59 min ±6,17 min, P=0,047). As regards the frequency, the EC group differed significantly in the mean frequency of exploration in period 2 from period 1 (-34,56 times/day ± 41,69 times/day, P= 0,003), whereas SC (-12,00 times/day ± 19,21 times/day, P= 0,187) and npEC (0,50 times/day ± 27,22 times/day, P= 0,95) did not. The differences in the Δ values of the mean frequency of exploratory behaviour did not differ significantly between the three groups, but there was a trend for a difference between the EC and SC group (P=0,022). The results show that it does not matter if you house female neutered ferrets with non-preferred enrichment or under standard housing conditions. Additionally, in this study some results indicate that preferred enrichments may have had an effect on the exploratory behaviour and welfare of female neutered ferrets.

Keywords: ferret, *Mustela putorius furo*, exploration, exploratory behaviour, enrichment, behaviour, animal welfare.

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1. INTRODUCTION

The Domestic or European ferret (*Mustela putorius furo*) is used in relatively small numbers as a laboratory animal, however, their number is increasing due to their unique application for human influenza studies (1). Furthermore, ferrets are increasingly popular as pets, in 2015 200.000 ferrets were kept as pet in the Netherlands (2), which is four times more than was estimated in 2004 (3). Knowledge on the ferrets' ethological needs is essential to guarantee a good welfare and health for pet and laboratory ferrets. Therefore, more information is needed on e.g. foraging strategies, food intake, social behaviour, play behaviour, need for resting places, exploration etcetera (1,4,5). Moreover, the interesting question can be raised if different types of enrichment can fulfil ferrets' species specific ethological needs, and if so, which enrichment is the most optimal to be used in captivity for which ethological need?

All animals have species specific behavioural needs (6) and ferrets are no exception (5). In general, ten behavioural systems are described in literature, each being equally important with continuous switches from one to another. Whether the motivation for one behavioural system is greater than another depends on previous experiences, contexts, circumstances, the environment and whether the behaviour is self-rewarding (6). The behavioural systems described in literature are as follows:

1. Social behaviour (including sexual-, caring- and play behaviour);
2. Locomotor behaviour;
3. (object) Manipulative behaviour;
4. Flight behaviour;
5. Hide/rest/sleep behaviour;
6. Maintenance behaviour;
7. Temperature regulation (and other) behaviour;
8. Excretion behaviour (including marking);
9. Foraging behaviour;
10. Eating and drinking behaviour.

Enrichment is defined in literature as: 'specific items placed in the cage with the animals, or, more broadly, to describe a process to improve animal welfare' (7) and as: 'an animal husbandry principle that seeks to enhance the quality of captive animal care by identifying and providing the environmental stimuli necessary for optimal psychological and physiological well-being'(8) (9). Similar to other animal species, environmental enrichment is believed to be beneficial for the welfare and overall health of ferrets (10,11) as enrichments may satisfy some species specific behavioural needs. Different enrichments satisfy different behavioural systems and some even satisfy multiple behavioural systems (6,12). It is broadly believed that if animals are unable to perform their behavioural needs, this can result in chronic stress.

So far little is known about ferret behaviour in general and even less is known about their preference for enrichments (13) and if these enrichments can fulfil the ferrets' ethological needs in a captive environment. Previous research showed that ferrets prefer an enriched cage over a barren cage and that enriched ferrets were more curious and active than ferrets kept in barren cages, the last group even became lethargic and showed signs of stereotypic behaviour (14).

Up until now, one consumer demand study investigated the preference and motivation for enrichments of seven neutered female ferrets (13). The ferrets were housed individually in a seven-chamber consumer demand set up with one corridor with *ad libitum* food (food bowl) and water (water bottle with nipple) supply. The control chamber had nothing in it except for a sawdust bedding. The other six chambers were enriched chambers in a random order: social contact, tunnels, foraging toys, sleeping enrichment, balls and water bowls. To enter one of the chambers from the

corridor the ferrets had to pass through a weighed door. To return to the corridor, however, they only had to pass through an unweighted cat flap. In this study, it was shown that ferrets worked significantly harder to reach water bowls, foraging enrichment, social enrichment and tunnels than an empty chamber. Besides, it was shown that the ferrets had the highest motivation to gain access for sleeping enrichments, and more specifically within this category, the hammock. The ferrets in this study did not show a higher motivation to reach balls than an empty chamber (13). Based on this study, the hammock, the foraging ball and the water bowl were regarded as preferred enrichment, whereas the golf ball, the ferret ball and an extra food bowl were regarded as non-preferred enrichment in the present study.

One assumed essential ethological need of animals, which may be stimulated and fulfilled by enrichments, is exploratory behaviour. In farmed mink, a close relative of the ferret, exploration is mentioned as the most important behavioural pattern inducing stress (15,16). Exploration can be seen in many species, including ferrets, and involves manipulation of items and increased locomotor activity (11,17). Thus exploratory behaviour includes both manipulative and partly locomotor behaviour of the behavioural systems (6). Exploration is mostly seen in strange or new environments or environments with strange or new objects or subjects with the function to gain information and familiarity of its environment with all possible sensory equipment. For ferrets the nose is a very important tool to explore using its keen olfactory sense, visual and tactile equipment are important as well (11,17).

It is described that exploration can be classified as a high priority behaviour for ferrets (5,11). This is based on the registration of 'the dook' sound: a vocalization that is commonly heard in exploring ferrets and which is assumed to be a sound specifically expressed in positive and/or rewarding contexts. (11). Besides, it has been described that the reason that exploratory behaviour can still be seen in captive animals may be explained by the concept of self-rewarding behaviours. The performance of exploratory behaviour in captive animals may not have a real functional benefit anymore, but due to the fact that exploratory behaviour is self-rewarding, animals still have this behaviour in their behavioural repertoire (6,15,18).

Animals in barren cages are often assumed to be bored, since they are less active and have a negative state caused by a lack of stimulation (19). Animals that do not have the opportunity to explore will thus become bored with serious consequences for their welfare and health, like stress and stereotypes (15,17,19,20). Bored animals seek for novel stimulation, since there is a lack of stimulation in their environment. Exploratory behaviour is regarded as a remedy for boredom, since animals can seek for novel stimulation by exploring their environment. For animals housed in cages this also includes exploratory behaviour towards the outside of the home cage. For this reason it has been described that the reason why animals show exploratory behaviour may be explained by the concept of boredom (17,19,20). Furthermore, environmental enrichment is regarded as a remedy for boredom and therefore an improvement for animal welfare. In a study done in caged minks it was tested if minks in enriched cages showed less signs of boredom than those housed in non-enriched cages. This was done by exposing them to different stimuli and measure their behavioural response and interest towards the stimuli. Non-enriched minks showed more interest in all kinds of stimuli and investigated more, which is consistent with boredom. Thus the conclusion was that environmental enrichment reduces boredom (19). For this reason, to guarantee a good welfare and health of ferrets the housing conditions should provide opportunities, like enrichments, to satisfy this behavioural need to explore. Hence, a behavioural pattern worthwhile to be studied in closer detail.

The mink studies that have been described before, study the exploratory behaviour of mink outside their (enriched or non-enriched) home cage (19). The effect of enrichments on the exploratory behaviour of ferrets within their home cage towards the outside of the home cage has not been studied yet. In other laboratory animals, some information on exploratory behaviour towards the

outside of the home cage has been found. A study done in laboratory rats investigated long-term effects of environmental enrichments on their behaviour. For this study, 72 rats were housed together in groups of four in either enriched or non-enriched cages for six weeks. In the second, fourth and sixth week the rats were behaviourally sampled in the dark and light phase of the day. Exploratory behaviour was one of many behaviours observed. In this study it was shown that unenriched rats showed significantly more exploratory behaviour (sniffing cage lid, cage wall and sniffing air outside the cage) and bedding-directed activities (pushing or pulling and digging bedding material) than enriched rats. Besides, the total amount of movement (movement and/or climbing the cage lid) was significantly increased as well in unenriched rats, in the dark phase and in the second week. According to this study, these results may simply be due to the fact that unenriched rats do not have cage objects to interact with, in contrast to the enriched rats. After adjustment of the data for time spent interacting directly with enrichments however, the significant differences between unenriched and enriched rats remained as they were, which does not support this theory. Therefore, it is considered that perhaps a more fundamental behavioural change underlies. Besides, indirect interaction with objects was not taken into account in this adjustment, so this could still be an explanation for these differences as well (21). In their next study, rats were put in a cage with either one object or multiple objects. They found that rats in a cage with multiple objects engage less time in bedding-directed behaviours and exploratory behaviour than rats housed with one object (22).

Another study done with 79 male and 71 female mice, also showed that enriched female and male mice explored less than those housed in standard housing conditions. The mice were observed for 5 days, for 10 minutes each day. On day one a similar total time of exploratory behaviour was found in both groups in both genders. Subsequent trials, however, showed a decrease in exploratory behaviour in both groups and genders, but in mice housed in an enriched environment this decrease was bigger. The findings were explained by the reasoning that exploratory behaviour does not provide a lot of novelty for enriched mice, the mice therefore show less exploratory behaviour. For the non-enriched mice however, exploratory behaviour provides more novelty and thus non-enriched mice explore more (23).

The present study aims to determine how different types of enrichment affect the ferrets' explorative behaviour. This study will focus on exploratory behaviour that is directed towards the outside of the home cage: rearing, scratching, tunnelling and sniffing. This behaviour is hypothesised to represent a lack of stimulation within the enclosure, with the ferret looking for stimulation outside of their home cage.

Following this line of reasoning, ferrets that are provided with preferred enrichments will spend more time on their enrichments and may therefore have their need for exploration fulfilled by the enrichment. For this reason, it is expected that both the ferrets housed with preferred enrichment and those housed with non-preferred enrichment will show a decrease in exploratory behaviour from period 1 to period 2. Additionally, it is expected that the ferrets housed with preferred enrichment will show a greater decrease in exploratory behaviour than the ferrets that are provided with non-preferred enrichments. The ferrets housed under standard housing conditions are expected to show an increase in exploratory behaviour from period 1 to period 2, as they have the least stimulation within their enclosure.

2. Animals, material and methods

2.1 Ethical note

This study was ethically approved by the Animal Care and Use Committee of Intravacc in Bilthoven, The Netherlands (PO201300161).

2.2 Animals, housing and husbandry

36 neutered female ferrets were used in this study. The ferrets were 7 months old at the start of this experiment and weighed 823 ± 83 grams (mean ± SD). The ferrets were randomly divided over 6 cages of 6 animals, since this is the standard group housing condition at Intravacc. One cage was housed under standard conditions (N=6), three cages were housed with preferred enrichment (N=18) and two cages were housed with non-preferred enrichment (N=12). The enrichment items provided in each condition are as follows and illustrated in figure 1:

- Standard condition (SC): a cage with sawdust bedding (JRS LIGNOCEL® Hygienic animal bedding), a water bottle (1 litre), a food bowl (Adori® stoneware food bowl, ø18 cm, 5 cm high) with *ad libitum* commercially ferret feed (Hope Farms Ferret Balance®) and a flexible plastic bucket where the ferrets could sleep in.
- Preferred condition (EC): two hammocks (Adori® hammock, 50 × 45 cm), a foraging ball (Happy Pet® tumble 'n treat, Ø 6 cm) and a water bowl (Adori® stoneware food bowl filled with water, ø18 cm, 5 cm high). These items are added to the standard condition.
- Non-preferred condition (npEC): two ferret balls (Ferret ball: Ø 25 cm with 4 holes: Ø 10.2 cm), a golf ball (Ø 4cm) and an extra food bowl (Adori® stoneware food bowl filled with water, ø18 cm, 5 cm high). These items are added to the standard condition.

Note that all groups contain the enrichment of the standard group.

The preferred and non-preferred enrichment were based on the seven chamber consumer demand study done in ferrets that has been described in the introduction (13).

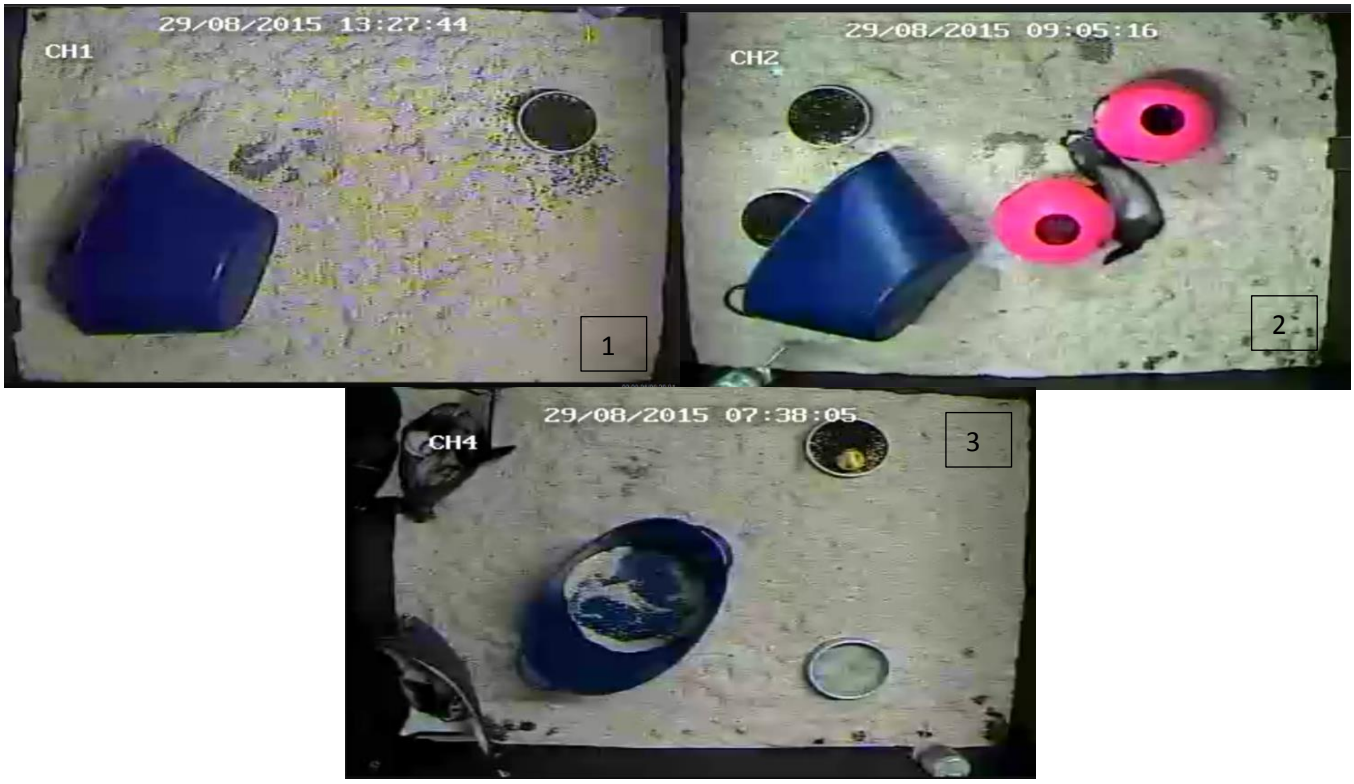

Figure 1. Pictures of the three different housing conditions of the ferrets: 1 is the standard housing condition; 2 is the non-preferred enrichment; and 3 is the preferred enrichment.

In the table below an overview of the different enrichments used in this study and the behavioural systems that are satisfied by these enrichments is displayed. This to show that the different enrichments used in this study may satisfy different behavioural systems.

	Social behaviour	Locomotor behaviour	(object) Manipulative behaviour	Flight behaviour	Hide/ rest/ sleep behaviour	Temperature regulation (and other) behaviour	Excretion behaviour (including marking)	Foraging behaviour	Eating and drinking behaviour	Maintenance behaviour
Hammock		X	X	X	X					
Bucket		X	X	X	X					
Sawdust			X		X		X	X	X	
Water bowl			X			X			X	
Food bowl								X	X	
Foraging ball		X	X					X	X	
Ferret ball		X	X	X	X					
Golf ball		X	X							
Other ferrets	X	X			X	X				

Table 1. An overview of which behavioural system(s) are satisfied by which enrichment(s) used in this study. Note that the order is random and that each behavioural system is equally important (5,6,12).

The ferrets were acclimatized in the standard housing conditions for 10 days. After that the baseline, which is period 1 (P1), was filmed. Subsequently, 4 days later the groups of ferrets obtained the additional enrichments concurrent to their condition. 24 days after obtaining the additional enrichments, period 2 (P2) was filmed. The timeline of this study is visualised in table 2.

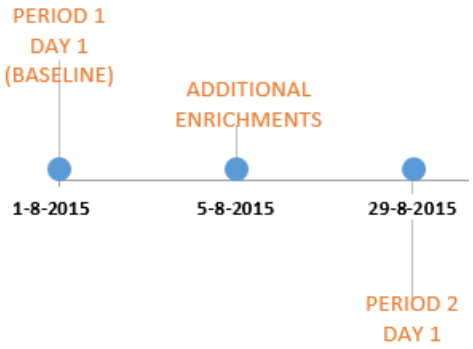

Table 2. Timeline of this study.

The ferrets were housed indoors in phenolic faced plywood floor pens of 1.8 m^2 (150 x 120 cm) covered with 3-5 cm of sawdust (JRS LIGNOCEL® Hygienic animal bedding). The walls of the enclosure were 70 cm high phenolic faced plywood which contained no bars or gaps. The water in the drinking bottle (1 litre) and the commercially ferret feed (Hope Farms Ferret Balance®) were refreshed daily, to ensure that the ferrets could eat and drink *ad libitum*. Feces and sawdust were removed daily, and if necessary, sawdust was added to ensure a constant thickness of the sawdust layer. The health and body condition of the ferrets were regularly monitored and once a week the ferrets were weighed on a Mettler PM11 scale in a bin. The room with the cages had a constant temperature between the ranges of 20°C-22°C, a humidity range of 50-70% and contained a light-dark schedule of 8:16 hours using artificial TL lightening. Auditory stimulation was available in the form of a radio, this to mask noises from outside the home cage.

2.3 Data collection

The experiment lasted for 4 weeks, resulting in two measurements (baseline or P1 and P2). In both periods the ferrets were filmed for one full day (24 hours) using security cameras (BASCOM B03P CCTV) with 30 m infrared sight and motion detection, which were linked to a DVR-recorder. The time the ferrets engaged in exploratory behaviour (in hh:mm:ss per day) was scored for 23 hours per day, excluding the time spend on and around the care of the ferrets of one hour (nourishing, cleaning etc.). This hour has been left out, because the ferrets show different behaviour in the presence of the caretakers and is therefore not representative.

2.4 Behavioural observations

The videotapes of the ferrets were observed using an ethogram as shown in table 3. Individual ferrets were distinguished by shaving a specific pattern (the different patterns are displayed in figure 2). As soon as an individual showed explorative behaviour the start- and end time and code from the ethogram was noted in Excel 2013. It was noted as well whether the exploratory behaviour was performed on sawdust, the wall, an object (bucket, ferret ball, hammock, and water bottle) or unsupported (rearing).

Behaviour	**Description of the behaviour**	**Code**
Tunnelling	Putting head down in the sawdust and then walk, moving the sawdust and creating some sort of a 'tunnel'	T
Scratching	Quickly alternating movement of the front paws in a bucket, in sawdust or against the wall	Sc
Rearing	Standing upright on the hind legs unsupported or by leaning on the wall, drinking bottle or enrichment objects (bucket, ferret ball)	R
Sniffing	Draw air repeatedly into the nose close to sawdust or the wall as though the ferret is smelling at something	Sn

Table 3. Ethogram of the explorative behaviours of the ferrets as scored in this study

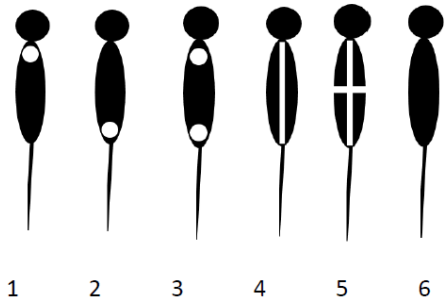

Figure 2. Different shaving patterns to distinguish the individual ferrets.

2.5 Data analysis and statistics

Data were analysed using Excel 2013 and SPSS24 for windows. $P < 0,05$ was accepted as the probability level for statistical significance and data are noted as mean ± SD, if not otherwise stated. The analysis and statistics were the same for both the duration and frequency. Firstly, an one-sample Kolmogorov-Smirnov Test was performed to analyse whether the data were distributed normally. Subsequently, an one-way ANOVA was performed on the data of the baseline measurement to determine whether the groups differentiated from each other significantly in the duration or frequency of exploration when housed under equal housing conditions. Since this difference was nearly significant for the duration (8,01 min± 4,73 min, P=0,066) and was significant for the frequency (51 times/day± 33,66 times/day, P= 0,003), the delta values (Δ = period 2-period 1) were used in the next test. The delta values (Δ) of the mean time spent on exploration (duration) and of the mean frequency of exploration of the three groups (EC, npEC and SC) was analysed using a one-way ANOVA. If this was significant (P<0,05), Post Hoc comparisons using the Bonferroni correction were performed. Lastly, a paired T-Test was performed to test whether the animals in one cage or group showed a difference in the duration or frequency of exploration from period 1 to period 2.

3. Results

3.1 Normality of the data

Data of the duration were distributed normally (8,01 min± 4,73 min, P=0,949). Data of the frequency were distributed normally as well (51 times/day ±33,66 times/day, P=0,291).

3.2 Duration

3.2.1 Exploratory behaviour between the three groups

The mean time spent on exploration by the three groups (npEC, EC and SC) in period 1 and 2 is shown in figure 3. The three groups did not significantly differ in the mean time spent on exploration in period 2 from period 1: npEC (0,42 min ± 4,97 min, P=0,777), EC (2,56 min ± 7,59 min, P=0,171) and SC (-1,91 min ±3,44 min, P=0,233). Besides, the Δ values (Δ = period 2-period 1) of the mean time spent on exploration were not significantly different between the three groups (P=0,277: npEC (-0,41 min ±4,96 min), EC (-2,84 min ± 7,97 min) and SC (1,91 min ± 3,44 min)). These results also show a high variety within and between the groups, which is shown in figure 4 (Δ). This will be discussed later on.

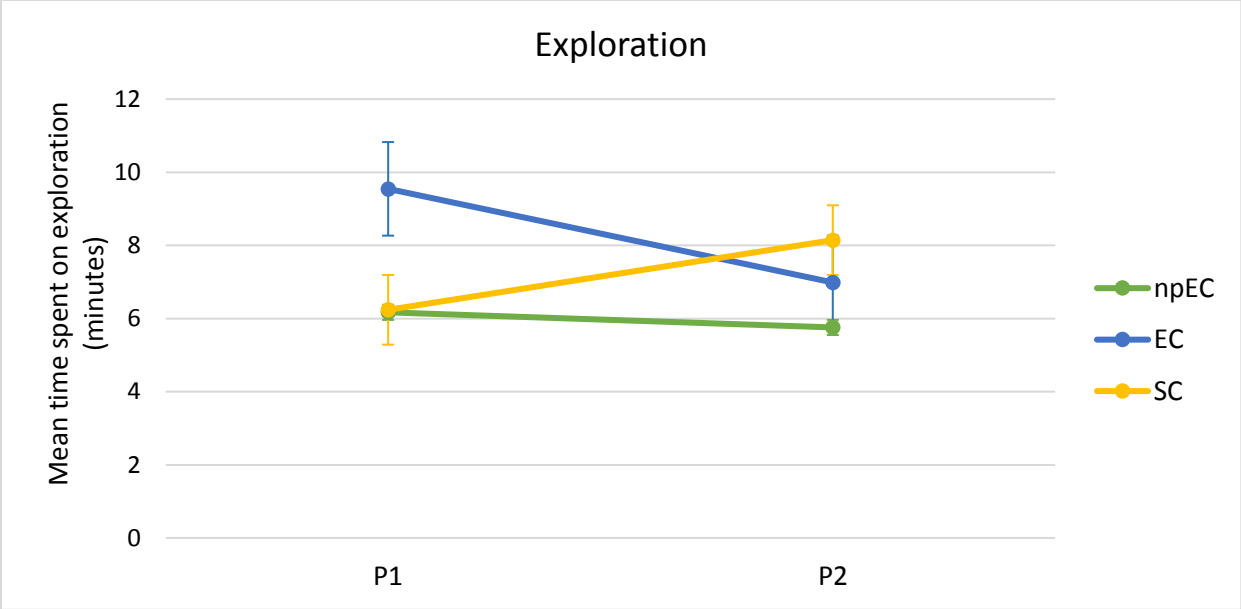

Figure 3. Mean time spent on exploratory behaviour in period 1 (P1, baseline) and period 2 (P2) by the three different groups: npEC=non-preferred Enriched Condition, EC=preferred Enriched Condition and SC=Standard Condition. Error bars represent the standard error.

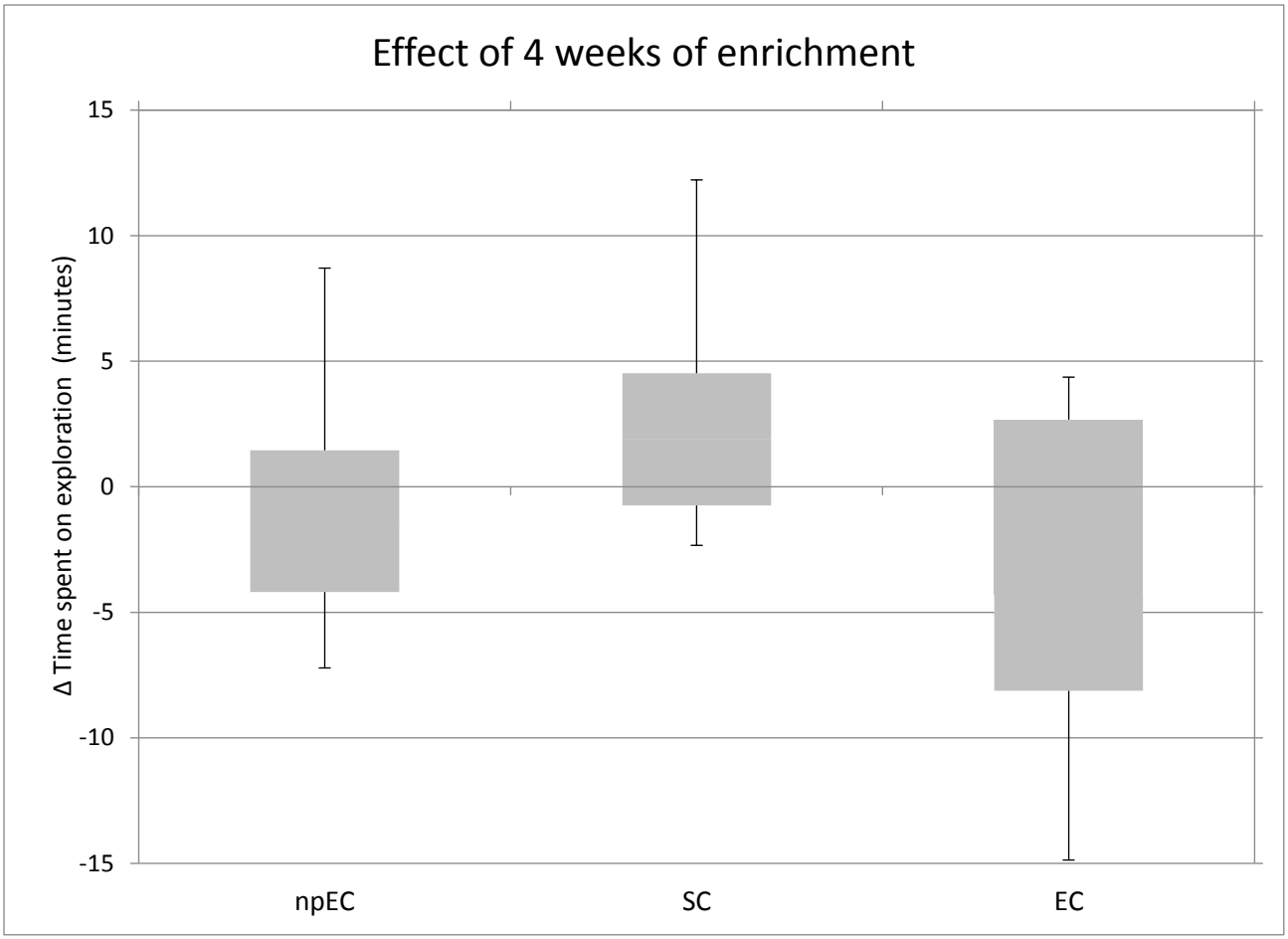

Figure 4. Boxplot of the difference in time (Δ or P2-P1) spent on exploratory behaviour by each group after 4 weeks of enrichment. npEC=non-preferred Enriched Condition, EC=preferred Enriched Condition and SC=Standard Condition. Error bars represent median± minimum and maximum.

3.2.2 Differences in exploratory behaviour within the cages

In figure 5, the mean time spent on exploratory behaviour in period 1 and 2 is shown per cage. For cage 1 (0,43 min ± 4,30 min, P= 0,818), cage 3 (-1,91 min ±3,44 min, P=0,233), cage 4 (-2,31 min ±8.00 min, P=0,511), cage 5 (0,40 min ±5,98 min, P=0,877) and cage 6 (4,26 min ±7,91 min, P=0,245) the mean time spent on exploratory behaviour did not significantly differ in period 2 from period 1. The ferrets in cage 2 however, explored significantly less in period 2 than in period 1 (-6,59 min ±6,17 min, P=0,047). These results also show a high variety within the cages and this is shown in figure 6 (Δ).

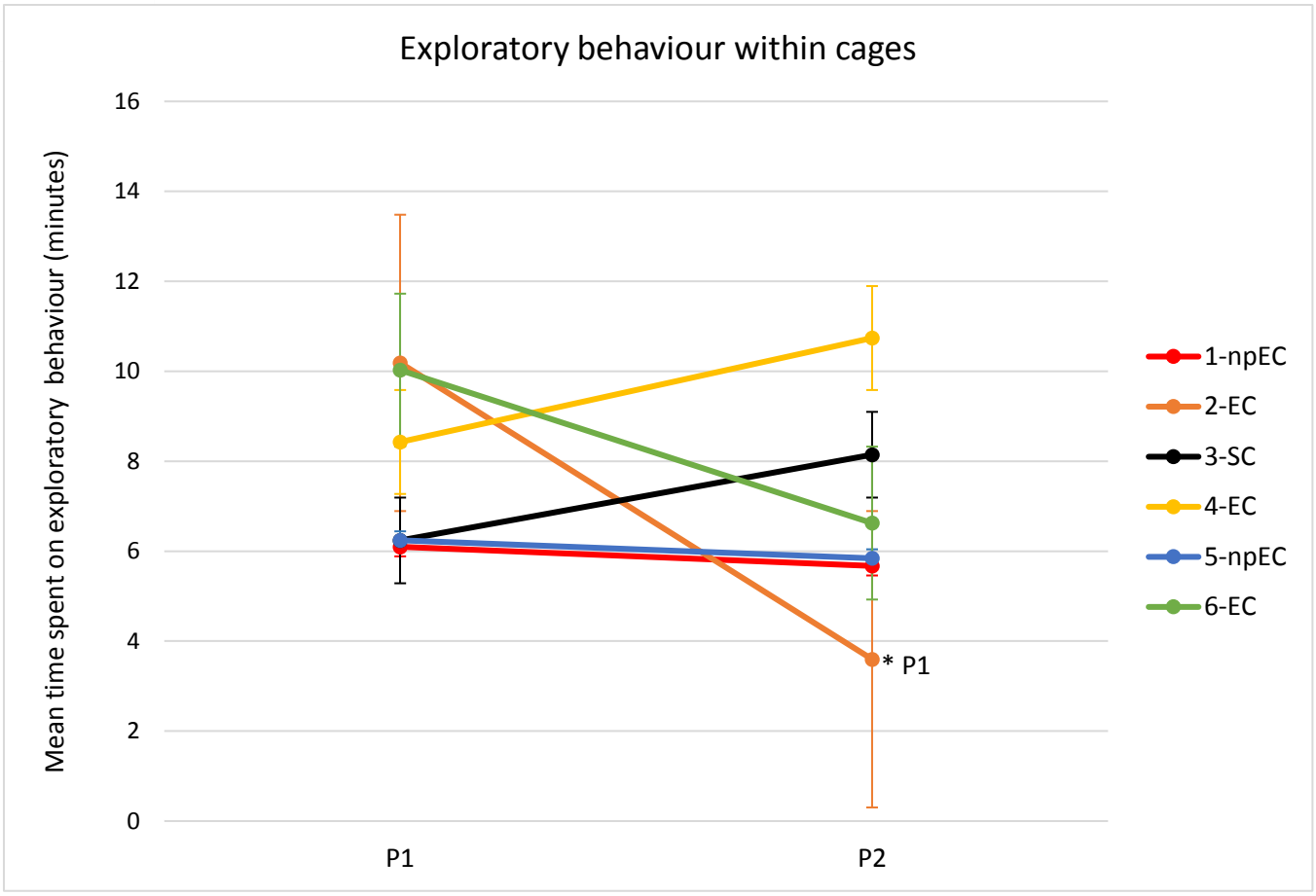

Figure 5. Mean time spent on exploratory behaviour in period 1 (P1, baseline) and period 2 (P2) by ferrets housed within the same cage. npEC=non-preferred Enriched Condition, EC=preferred Enriched Condition and SC=Standard Condition. Error bars represent the standard error. * P=<0,05.

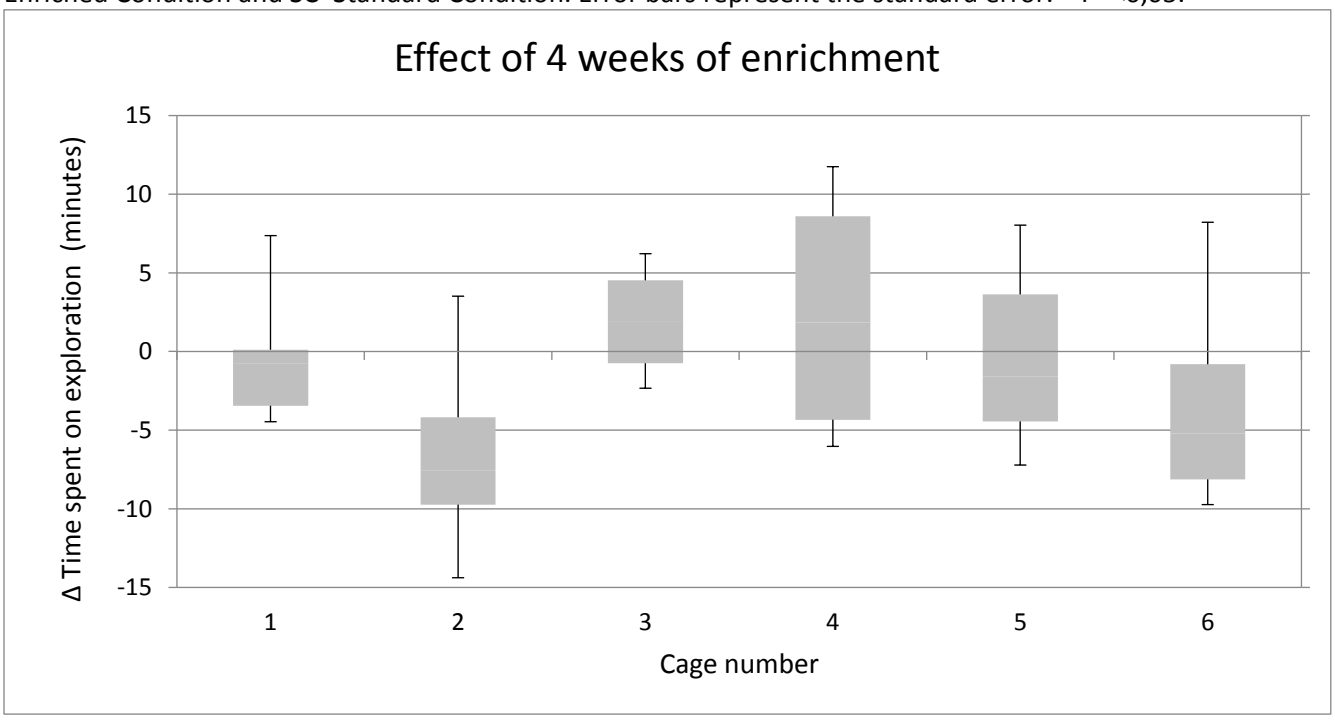

Figure 6. Boxplot of the difference in time (Δ or P2-P1) spent on exploratory behaviour by each cage after 4 weeks of enrichment. Cage number 1 and 5 are npEC (non-preferred Enriched Condition), cage number 3 is SC (Standard Condition) and cage number 2, 4 and 6 are EC (preferred Enriched Condition). Error bars represent median± minimum and maximum.

3.3 Frequency

3.3.1 Exploratory behaviour between the groups

The mean frequency of exploratory behaviour of the three groups (npEC, EC and SC) in period 1 and 2 is shown in figure 7. Firstly, EC was significantly different in the frequency of exploration in period 2 from period 1 (-34,56 times/day ± 41,69 times/day, P= 0,003), whereas SC (-12,00 times/day ± 19,21 times/day, P= 0,187) and npEC (0,50 times/day ± 27,22 times/day, P= 0,95) were not. Thus, the ferrets housed with preferred enrichment showed a significant decrease in the frequency of exploration from period 1 to period 2. Besides, the Δ values (Δ = period 2-period 1) of the frequency of the exploratory behaviour were significantly different between the three groups (P=0,007: npEC (-0,50 times/day ± 27,22 times/day), EC (-34,56 times/day ± 41,69 times/day), SC (-12,00 times/day ± 19,21 times/day). Post Hoc comparisons using the Bonferroni correction (adjusted α=0,0167) however, showed that the EC group was not significantly different from the npEC (P= 0,038) group but there was a trend for a difference between the EC and SC group (P=0,022). The SC and npEC group were mutually not significant (P= 1,000). These results also show a high variety within and between the groups, which is shown in figure 8 (Δ). This will be discussed later on.

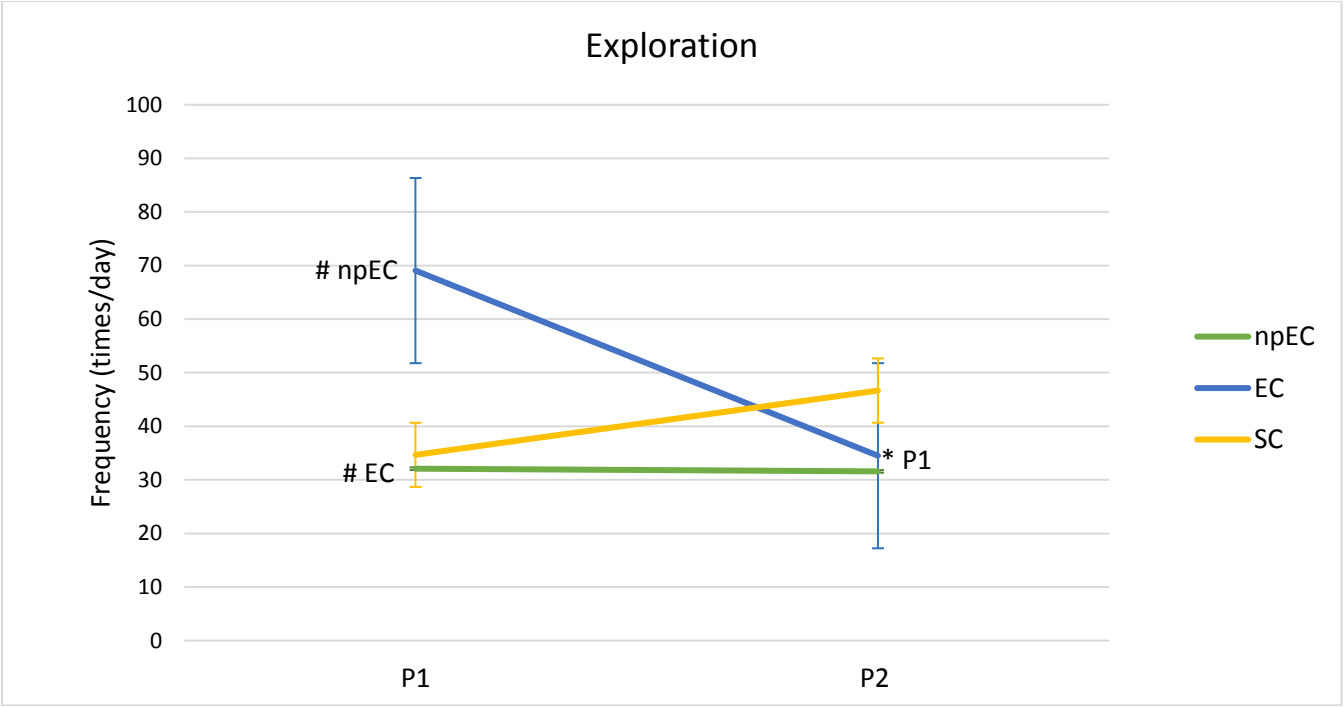

Figure 7. Frequency of exploratory behaviour in period 1 (P1, baseline) and period 2 (P2) by the three different groups: npEC=non-preferred Enriched Condition, EC=preferred Enriched Condition and SC=Standard Condition. Error bars represent the standard error. * P=<0,05, # α=<0,0167 (adjusted α)

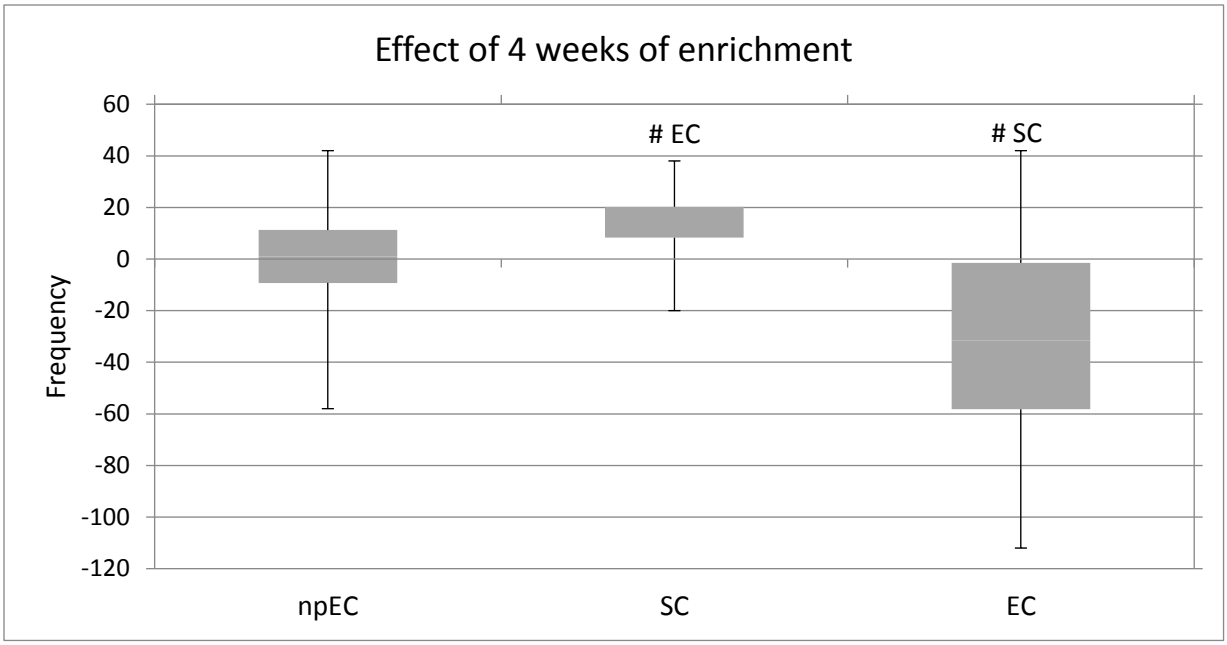

Figure 8. Boxplot of difference in frequency (Δ or P2-P1) of exploratory behaviour by each group after 4 weeks of enrichment. npEC=non-preferred Enriched Condition, EC=preferred Enriched Condition and SC=Standard Condition. Error bars represent median± minimum and maximum. * α=<0,0167 (adjusted α), # α=< 0,033 (trend).

4. Discussion

The aim of this study was to determine the effect of different types of enrichment on the exploratory behaviour of laboratory ferrets. Firstly, it was expected that both the ferrets housed with preferred enrichment (two hammocks, a water bowl and a foraging ball) and those housed with non-preferred enrichment (two ferret balls, an extra food bowl and a golf ball) would show a decrease in exploratory behaviour towards the outside of the home cage from period 1 to period 2. Additionally, it was expected that ferrets with preferred enrichment would show a greater decrease in exploratory behaviour than ferrets housed with non-preferred enrichments. Besides, it was expected that ferrets housed under standard housing conditions would show an increase in exploratory behaviour from period 1 to period 2, in contrast to the other two groups. In the present study, as regards the duration, only the ferrets in cage 2 (EC) showed a significant decrease in the mean time spent on exploratory behaviour (duration) from period 1 to period 2. However, the total group of ferrets housed with preferred enrichment (3 cages, N=18) did not differ significantly in period 2 from period 1 in the mean time spent on exploration. Besides, the ferrets housed with preferred enrichment did not significantly differ in the delta mean time spent on exploratory behaviour from the ferrets housed with non-preferred enrichment and those housed under standard conditions. The results of the delta frequency of exploration, showed a trend for a difference between ferrets housed with preferred enrichment and the ferrets housed under standard conditions. Additionally, the frequency of exploration of the ferrets housed with preferred enrichment decreased significantly from period 1 to period 2, whereas for the other two groups (SC and npEC) the frequency was not significantly different in period 2 from period 1. Thus the ferrets housed with preferred enrichment showed a significant decrease in the frequency of exploratory behaviour and the ferrets of cage 2, which was one of the cages housed with preferred enrichment, showed a significant decrease in the mean time spent on exploration. These two results are both in line with the expectations. The rest of the results are not in line with the expectations.

First, the differences in the mean time spent on exploratory behaviour (duration) and the differences in the frequency of exploration from period 1 to period 2 will be discussed for each group (SC, EC, npEC). The mean time spent on exploratory behaviour (duration) will also be discussed for each cage (1-6). Thereafter, the differences in the delta values (Δ) of the mean time spent on exploratory behaviour and the differences in the delta values (Δ) of the frequency of exploration between the three groups will be discussed.

4.1 Differences in the exploratory behaviour from period 1 to period 2 of each group (SC, EC, npEC)

4.1.1 Standard Condition (SC)

It was expected that the ferrets of the standard condition would show an increase in exploratory behaviour from period 1 to period 2. The reason for this is that it is assumed that animals in barren cages are bored (19). Since the ferrets in the standard condition are only housed with a sawdust bedding, a bucket, a food bowl and a water nipple it is conceivable that these ferrets became bored in period 2. Exploratory behaviour can be a remedy for boredom, since animals seek for novel stimulation by exploring their environment (17,19,20). Thus the expectation was that due to a lack of stimulation within their cage, the ferrets of the standard condition would be bored in period 2 and therefore explore more in period 2, compared to period 1. In this study, the mean time spent on exploratory behaviour (duration) by the ferrets of the standard group did not differ in period 2 from period 1. Moreover, the frequency of exploration was not significantly different in period 2 from period 1 as well. This is both not in accordance with the expectation. The explanation for this is probably the wide variation within the group. As regards the duration two of the six ferrets spent less time on exploratory behaviour (-2,33 min and -1,13 min), while the other four spent more time on exploratory behaviour (+0,42 min, +3,35 min, +4,92 min and +6,22 min). As regards the frequency one of the six ferrets showed a lower frequency of exploration (-20 times/day), while the other five

showed a higher frequency of exploration (+7 times/day, +12 times/day, +23 times/day, +12 times/day and +38 times/day). Additionally, the total time spent on exploratory behaviour differs between individuals of the standard condition as well.

4.1.2 Enriched Condition: preferred enrichment (EC)

It was expected that ferrets housed with preferred enrichment would spend more time on enrichments and therefore less time on exploratory behaviour in period 2 in comparison to period 1 (baseline). The ferrets of the three cages with preferred enrichment (N=18) together did not show a significant difference in the mean time spent on exploratory behaviour (duration) between period 1 and 2. This result is therefore not in line with the expectation. This may be explained by the variation within this group. Within cage 4, three ferrets showed an increase in exploratory behaviour (+11,75 min; +8,83 min and +7,90 min), the other three on the other hand showed a decrease (-6,03 min; -4,38 min and -4,20 min). Within cage 6 two ferrets showed an increase in exploratory behaviour, of which one is only a slight increase (+8,22 min and +0,15 min respectively), the rest of the ferrets showed a decrease in exploratory behaviour (-9,73 min; -8,58 min; -6,77 min and -3,68 min). Additionally, the total time spent on exploratory behaviour differs between individuals of the EC group as well. To conclude, probably due to a wide variation within and between the ferrets of the different cages of the EC group, the differences in the mean time spent on exploratory behaviour for two of the three cages (cage 4 and 6) and for the three cages together are not significant.
The decrease in the mean time spent on exploratory behaviour was significant for cage 2 alone (N=6). The reason for this could be that the bucket in this cage was standing upright the whole day in period 2, whereas the bucket was laying down all day in period 1. If the bucket was standing upright, rearing against the bucket was not noted, since it was not clear whether the ferret wanted to go into the bucket or if it was exploring. In period 1 some ferrets of this cage showed rearing against the bucket quite often. Since this is not noted in period 2, this may be an explanation for the fact that this decrease was significant for cage 2 and not for the other two cages. Besides, five ferrets in cage 2 showed a decrease in exploratory behaviour (-14,38 min, -9,90 min, -9,25 min, -5,92 min and -3,60 min), only one showed an increase in exploratory behaviour (3,52 min). Thus it seemed that within this group less variation was occurring, which could also be an explanation for the fact that cage 2 is the only cage of the three EC cages with a significant decrease in exploration. Another explanation for the significant decrease in exploratory behaviour could also be that the ferrets in cage 2 spent more time interacting with their preferred enrichment and therefore less time on exploratory behaviour, as we hypothesized. However, if this is the case, it is remarkable that the two other groups housed with preferred enrichment did not show a significant decrease in the mean time spent on exploratory behaviour.

The frequency of exploration decreased significantly from period 1 to period 2 in the ferrets housed with preferred enrichment. Thus this finding is in line with our expectation. This may simply be due to the fact that the ferrets spent more time interacting with their preferred enrichment and therefore less time on exploratory behaviour, as we hypothesized. However, if this is the case, it is remarkable that the duration is not significant, except for cage 2. Another explanation may be that the ferrets of the EC group showed a high frequency in comparison to the other two groups (SC and npEC) at baseline: the difference in frequency was even significant between EC and npEC. In period 2 the three groups show similar frequencies of exploration, thus this finding may just have been an outlier in period 1, since we only observed one day.

4.1.3 Enriched Condition: non-preferred enrichment (npEC)

It was expected that ferrets housed with non-preferred enrichment would spend less time with their enrichments than ferrets housed with preferred enrichment. Additionally, it was expected that the npEC group would show a decrease in exploratory behaviour. This because even though the group obtained non-preferred enrichments, the ferrets still have enrichments, in contrast to those housed under standard housing conditions. Moreover, even non-preferred enrichment does provide the

ferrets with choice, and offering choices to animals may improve the welfare of animals, since certainly in a restricted cage area they can exert control over their environment (24). The mean time spent on exploration (duration) by the ferrets of the npEC group did not significantly differ in period 2 from period 1. Moreover, the frequency of exploratory behaviour of the ferrets of the npEC group was not significantly different in period 2 from period 1 as well. Therefore, the results in this study are not in line with the expectations. As for the SC and the EC groups, a wide variation has been found within the npEC group as well. In cage 1, as regards the duration, one ferret explored just as long in period 2 as in period 1, three ferrets spent less time on exploration (-4,47 min, -4,10 min and -1,50 min) and two ferrets spent more time on exploration (+7,37 min and +0,15 min) in period 2 than in period 1. As regards the frequency of exploration of cage 1, two ferrets explored less frequently (-58 times/day and -13 times/day), while the other four explored more frequently (+ 2 times/day, +6 times/day, + 18 times/day and +35 times/day) in period 2 than in period 1. In cage 5, as regards the duration, four ferrets spent less time on exploration (-7,22 min, -5,38 min, -1,63 min and -1,57 min), whereas the other two ferrets spent more time on exploring (+8,03 min and +5,37 min) in period 2 than in period 1. A regards the frequency of exploration of cage 5, for one ferret the frequency was the same for both periods, three ferrets explored less frequently (-33 times/day, -8 times/day and -6 times/day) and two ferrets explored more frequently (+9 times/day and 42 times/day). Additionally, like the SC and EC group, differences in the total time spent on exploratory behaviour per individual ferret were found for the npEC group as well. This variation is probably the reason why the difference in exploration from period 1 to period 2 per cage and of the two cages together (the group) is not significant.

4.2 Delta values (Δ) compared between the three groups (SC, EC and npEC)

It was expected that ferrets housed with enrichment would spend more time on enrichments and therefore less on exploration. In specific, it was expected that ferrets would spend more time interacting with preferred enrichment than with non-preferred enrichment. Therefore, it was expected that ferrets housed with preferred enrichment would show a greater decrease in exploratory behaviour towards the outside of their enclosure than ferrets housed with non-preferred enrichments. Furthermore, it was expected that ferrets housed under standard housing conditions would show an increase in exploratory behaviour, in contrast to the other two groups. The delta values (Δ = Period 2- Period 1) of the mean time spent on exploratory behaviour were not significantly different between the three groups (SC, EC and npEC). Moreover, the delta values of the frequency of exploration were not significantly different between the groups as well, but there was a trend for a difference between EC and SC. Thus the results are not in accordance with the expectations. As discussed before, a wide variety in exploratory behaviour is observed between individuals, this may be an explanation for the fact that these results are not significant as well. Other general explanations for the fact that some results were not significant in this study will be discussed in the next section 4.3.

4.3 Explanations for the results:

Three possible explanations for the fact that some results were not significant in this study will be discussed in this section.

First, a type II error might have occurred, because in this study a small number of animals was used: 36 in total. A power analysis was performed to determine how many animals would be needed per group to ensure statistical judgments that are reliable and accurate. The outcome of this power analysis was that one hundred animals per group would be needed, which is not practically achievable for ferrets. This again shows the wide variation in the exploratory behaviour of the ferrets in this study. Thus individual differences play a major role. These individual differences may be a result from e.g. individual preference for certain enrichment and/or for certain exploration types, which is important since some exploratory behaviour types are more time-consuming than others

(e.g. rearing vs. sniffing). Other examples of individual differences are personality traits and coping styles.
Personality is described in literature as: ''individual behavioural differences that are consistent over time and across situations'' (25). A very important personality trait is exploratory behaviour (26). In general two exploration styles are described: some individuals explore their environment fast and superficially, thereby putting more focus on current reproduction, whereas others explore their environment slowly and thoroughly, thus by this investing in future reproductive success (27). Interestingly, animals that explore fast and superficially behave more boldly and aggressive, whereas animal that explore slow and thoroughly seem to be more shy and less aggressive (27). This correlation is often described in literature as a behavioural syndrome: ''suite of correlated behaviours expressed either within a given behavioural context or across different contexts'' (28). A coping style is defined in literature as: ''a coherent set of behavioural and physiological stress responses which is consistent over time and which is characteristic to a certain group of individuals'' (29). Two coping styles can be distinguished: proactive and reactive. Proactive individuals control or manipulate their environment (bold), therefore they explore fast and are more aggressive. Reactive individuals in contrast respond more passively towards their environment (shy), explore thoroughly and slowly and are less aggressive (28). Thus, these different coping styles are in line with the behavioural syndromes that have been described before. Personality, behavioural syndromes and coping styles should be taken into account, since this can influence the results regarding exploratory behaviour a lot.

Second, in many animal species it has been found that habituation effects affect the effectiveness of environmental enrichment (30-34). In a study done in bush dogs, which were housed in an environment containing food enrichment, exploratory and searching behaviour towards the enrichment increased at onset of the experiment. After approximately 20 days, occurrence of this behaviour decreased gradually (33). Another study done in kennelled dogs found as well that the interest in different toys waned over time (the observations were 5 days in total), but the speed of habituation depended on the toy (30). In a study done in weaned pigs it has been found that interaction with enrichments decreased in two weeks. Additionally, they showed that novelty was increased by the rotation of enrichment (32). In our study the enrichment was given on the fifth of august. The ferrets were observed 24 days later on the 29th of august. It is conceivable that the ferrets may be habituated to their enrichments after 24 days. If this is the case, the effectiveness of the enrichment is decreased. Therefore the observed effect of enrichment may not be as clear as it could have been and therefore this study may have missed out on the maximal effect of the enrichment on exploratory behaviour. However, since it was assumed in the introduction that preferred enrichment would fulfil in the behavioural needs of ferrets, it was expected that the ferrets in this study would not lose interest in the enrichments. If habituation effects did occur in this study, this may be explained by the fact that the preferred enrichment did not fulfil in the behavioural needs of the ferrets in this study. This, however seems unlikely, since the preferred enrichment was based on a seven chamber consumer demand study in which the ferrets worked significantly harder to reach the preferred enrichments used in this study (hammock, foraging ball and water bowl) than to reach the non-preferred enrichments (golf ball, ferret ball and an extra food bowl) and an empty chamber (13). An alternative explanation could be that the ferrets housed under standard conditions were sufficiently fulfilled in their behavioural needs. This explanation is more likely, since the ferrets housed under standard conditions were able to sleep (bucket or sawdust), forage (sawdust), drink (water nipple), flee/hide (bucket) and show object manipulative and locomotor behaviour (bucket), as shown in table 1. The preferred enrichment may have fulfilled more in these behavioural needs, but not significantly more.

Third, providing multiple preferred items as enrichment within one cage does not necessarily mean that this forms the perfect environment for ferrets (24).

4.4 limitations of the study

There are seven limitations of this study that should be taken into account.

First, is observer bias, also known in literature as observer-expectancy effect or expectancy bias. In a recent study observer bias among veterinary students was tested (the observer in this study was a veterinary student). The students had to score positive and negative interactions in pigs. They observed the same recordings twice, but with different contextual information. Evidence for observer bias was found, since the students scored more positive when they were told that the pigs were selected for high social breeding value and reverse (35). In the present study the observer knew which group it was observing, since the objects in the cage determine the housing condition, on which the grouping was based. For this reason observer bias may have been of influence on the results of this study as well.

Second, the hour that is left out due to cleaning of the cages was different in period 1 from period 2. In period 1 the cages were cleaned at 09:45-10:45 am, in period 2 however, the cleaning was at 11:15-12:15 am. The ferrets were usually awoken by the cleaning and used to be very active around cleaning time. In general, the ferrets seemed to be awaking earlier than expected in period 2. It seemed like the ferrets awoke around the cleaning time of period 1, which may be caused by the formation of a biorhythm in period 1. This may have been of influence on their activity level and therefore also on their exploratory behaviour.

Third, it should be taken into account that the motivation of animals for enrichments may depend on their housing conditions: socially housed in groups versus social isolation. This has been found in studies done in pigs (36,37) and mice (38) and this may also be true for ferrets. The preferred and non-preferred enrichment were based on a seven chamber consumer demand study done in ferrets that were housed solitary (13). The preference of solitary ferrets for certain types of enrichment found in the consumer demand study can possibly not be generalized to ferrets that are housed in groups. If this is the case, this may have had an effect on this study as well, since the ferrets in the present study are housed in groups of six. Interestingly, in the seven chamber consumer demand study the ferrets did not sleep in the ferret ball, but in the present study they did, thus this may indicate that there is a difference in the preference for enrichment between solitary housed ferrets and ferrets housed in groups. Moreover, in rats it has been found that the motivation for exploration can also be influenced by housing conditions (social isolation vs. social group housing) (39). Thus the effects of enrichment on the exploratory behaviour and the exploratory behaviour alone may have been different when the ferrets would have been housed and observed solitary.

Fourth, it was expected that the duration and frequency of exploration in the baseline measurement (period 1) would be similar for all ferrets, since they were housed under equal housing conditions. However, the results of the baseline measurement showed a wide variation in the mean time spent on exploratory behaviour (duration) and frequency of exploration. As regards the frequency the EC group even differed significantly from the npEC group. Therefore, this may have been of influence on the results.

Fifth, enrichments may increase or decrease the amount of space within the cage. For example the hammock serves as a second floor, which therefore provides more surface. The ferret ball and the water bowl on the contrary lead to a decrease in the amount of space available to the animals. Less space could frustrate the diversity and quality of behaviours (21), therefore this should be taken into account as well.

Sixth, male ferrets are less commonly used in animal studies, therefore only female ferrets were used in this study. The advantage of this is less variability due to sex, a consequence and disadvantage of this is that the results cannot be generalized to male ferrets.

Seventh, there is a possibility that not all the exploratory behaviour is noted, since a camera with a 2D top view was used to observe the ferrets. If the ferret was underneath a hammock, in a bucket or in a dark corner of the box for example, it was not always clear what the ferret was doing. In this case the behaviour was not scored at all to prevent errors in the data.

4.5 recommendations

For future studies, I would recommend using a top view together with a side view instead of a top view alone, which may give more reliable data. Another improvement for a future study would be to determine per individual animal which enrichment(s) is preferred. This to minimize the effect of individual differences. Moreover, it would be recommendable to observe more days per period, since in this study data of only one day per period is collected. Data of more days, for instance for the baseline measurement would be more reliable. Besides, whether a habituation effect occurred can then be determined as well.

5. Conclusion

Ferrets housed with enrichment were expected to spend more time on enrichments and be more fulfilled in their need for exploration. In specific, it was expected that ferrets would spend more time on preferred enrichment than non-preferred enrichment. Therefore, it was expected that ferrets housed with preferred enrichment would show a greater decrease in exploratory behaviour towards the outside of their enclosure than ferrets housed with non-preferred enrichments. Furthermore, it was expected that ferrets housed under standard housing conditions would show an increase in exploratory behaviour. Besides, it was assumed that ferrets housed with preferred enrichment would be more fulfilled in their behavioural needs than those housed under standard conditions or with non-preferred enrichment. Therefore it was assumed that the welfare of the ferrets housed in preferred enrichment would improve. Except for the significant decrease in the mean time spent on exploratory behaviour (duration) by cage 2 (one cage of the EC group) and the significant decrease in the frequency of exploration of the ferrets housed with preferred enrichment (EC), none of the results of this study support these the expectations. Based on the results in this study it may be concluded that with regards to the exploratory behaviour of female neutered ferrets it does not matter if you house them in a cage with non-preferred enrichment or under standard housing conditions. Besides, it may be concluded that preferred enrichment may have an effect on the exploratory behaviour of female neutered ferrets, since the frequency of exploration for the EC group and the mean time spent on exploratory behaviour (duration) for cage 2 were significantly lower in period 2 than in period 1. Thus, the welfare was probably similar for the ferrets housed with non-preferred enrichment and for those housed under standard housing conditions, whereas the welfare of the ferrets housed with preferred enrichment may have been improved. The fact that some results with regards to exploratory behaviour were not significant in this study may be explained by several possible reasons, as discussed in previous section (4. Discussion). Among those are: a wide variation within and between the groups and cages, individual differences in personality and coping styles, and the habituation effect.

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