Pilot consumer demand study in ferrets

Explanations for high MPP controls and recommendations for further research

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Abstract

In recent years, ferrets have become a popular animal model in biomedical research. However, they are relatively new in the laboratory environment and therefore not much is known about adequate housing and treatment of these animals in experimental environments. An important part of experimental housing is the enrichments offered to the laboratory animals. In order to determine adequate enrichments for ferrets in laboratory conditions, a pilot consumer demand study was designed by M.L.Reijgwart in 2013. In this study, ferrets had to push a weighted door in order to reach various enrichments. The amount of weight the animals were prepared to push reflects their motivation to reach the different enrichments, which could be used to determine their preferences. However, the amount of weight the ferrets pushed for food (a necessity) was not significantly different from the amount they would push for an empty room.

In this study, six different setups were designed in order to try to find an explanation for the high control results. The setups were divided in purely diagnostic and problem-solving setups. The diagnostic setups results suggested that the ability to manipulate objects is an important factor for ferrets in laboratory conditions. However, no significant differences were found in the problem-solving setups, suggesting that the used two-chamber setup may not be the right setup for a consumer demand study with ferrets. In order to determine their enrichment preferences, other methods of testing should be looked into.

Introduction

Ferrets as laboratory animals

Registration of animal experimentation in the Netherlands shows that ferrets are increasingly used as an animal model in biomedical research (NVWA, Zodoende, 2013). These ferrets are mainly used as an essential part of experiments studying influenza pathobiology, as they are the only known small mammal species in which the virus is able to sufficiently replicate and cause symptoms similar to those found in humans (Belser et al., 2011). In contrast to the more common species used in laboratory testing like mice and rats, the ferret is a relatively unknown laboratory animal. As a result, not much information is available on adequate housing and treatment of ferrets in an experimental environment. Conditions currently maintained in these settings might be deficient, and as a result alter the health and behaviour of the animals in such a way that might adversely affect both animal wellbeing and the reliability of the results of the performed experiments. The new European legislation (Directive 2010/63/EU) therefore states that all experiments using vertebrates or cephalopods should conform to the principle of the 3Rs: Replacement, Reduction and Refinement. First described by Russell and Burch in 1959, the 3Rs aim to guard the animal welfare by avoiding the use of animals when different methods are available (Replacement), minimising the number of animals used in any experiment (Reduction) and reducing the amount of suffering and stress in each individual animal (Refinement). Due to the lack of research on ferret behaviour and welfare in experimental setups, not enough data is available to correctly implement the principle of Refinement in studies using ferrets as an animal model. Further exploration of ferret behaviour and their needs is indicated in order to optimise their living conditions, improving the welfare of the animals and by extent the reliability of test outcomes.

Consumer demand studies

An important aspect of optimising living conditions, and by extent animal wellbeing, is the availability of environmental enrichment. In order to determine which types of enrichments could be suitable in ferret housing, further research is required, both using known literature to explore the natural behaviour and needs of the ferret and animal studies to test the different enrichments. For this purpose, M.L. Reijgwart conducted a pilot consumer demand study in 2013, using two populations of ferrets in two different cities in the Netherlands, Utrecht and Bilthoven.

The main goal of a consumer demand study is to quantify the motivation of an animal to gain access to specific resources, which in this case are the different environmental enrichments. This motivation is usually measured by having the animal perform a physical task in order to reach the resource. In the 2013 study the ferrets were required to push a weighted door to gain access to a compartment containing the resource. The first step in a consumer demand study is to determine the maximum effort the animal is capable of, which can be measured by having the animal work for a necessity such as food. The maximum amount of weight the ferret is willing to push in order to reach food is described as the Maximum Push Capacity (MPC). Assuming the MPC is the maximum weight the ferret is capable of pushing, the motivation of the animals to reach the different enrichments can be measured by comparing the amount of weight they are willing to push for the enrichment to the MPC. It is assumed that a higher motivation for a certain enrichment means the resource has more value to the animal and therefore would be a useful addition in laboratory ferret housing. However, before testing the different enrichments a control setup has to be created to test the motivation of the animals to reach a compartment without any resources. The amount of weight the animals are willing to push for an empty compartment is described as the Maximum Price Paid for control ($MPP_{control}$) and is assumed to be the lowest value. The $MPP_{control}$ can be used to indicate the ferrets' motivation for the empty compartment and the task itself. The $MPP_{enrichment}$ is estimated to be somewhere between the MPC and $MPP_{control}$. As a consequence, the enrichments can only be tested when a significant difference exists between the MPC and the control MPP. The ferrets in the pilot study showed a $MPP_{control}$ of 89±13% of the measured MPC. Even when also given free access to the empty compartment, by providing them with an open entrance next to the weighted door, the ferrets would still push the weighted door to 88% of their maximum push capacity. Although the $MPP_{control}$ was shown to be significantly lower than the MPC, the difference between the two values was deemed too small to test the enrichments. Since the $MPP_{enrichment}$ was estimated to be between the MPC and $MPP_{control}$, the $MPP_{enrichment}$ would be too close to either the MPC or the $MPP_{control}$ to show any significant difference. With these results, the designed setup was deemed inadequate to establish the ferrets' preference for different enrichments. In order to continue the consumer demand study, it would be necessary to determine the cause of the high MPP in the control setups. This knowledge could then be used to alter the standard cage to construct a possible setup for following experiments.

Possible explanations for high MPP controls

Although the use of ferrets in a consumer demand study was unprecedented, the setup used for the experiment was not. The study design used by Reijgwart was based on earlier, similar experiments that had been successfully performed with silver foxes (Hovland et al., 2008) and grey parrots (Zeeland et al., unpublished). This led to the assumption that one of the reasons the study was unsuccessful could be found in the species used in the experiment. Possibly, one or multiple aspects of natural ferret behaviour drove the animals to push the door up to their maximum capacity. In order to design a series of experimental setups that might be suitable for ferrets, their behaviour in the previous experiments was observed and combined with existing knowledge of ferret behaviour. Five possible explanations for the high $MPP_{control}$ were found and tested, and will be discussed in this report.

1) *Attractiveness of the test door*

The doors used in the pilot study resembled small tunnels, in which the ferrets had to go through a small space in order to get to the weighted door. Possibly these dark, tunnel-like constructions drew the ferrets' attention and influenced them to go through the door.

Comparing the results from the experiments in Utrecht and Bilthoven, a remarkable difference could be seen in the control setups. Ferrets in Utrecht would push up to 88% of their established MPC when they also had an open entrance that would allow them free access to the empty compartment. In contrast, the ferrets in Bilthoven would not, or significantly less often, push the door when free access was also granted. Attractiveness of the test door would seem like a logical explanation, but it doesn't explain why this only seems to be the case with the ferrets in Utrecht. Therefore, further investigation of this control setup was recommended to find the reason behind the seemingly unnecessary door-pushing in this situation.

2) *Manipulation of objects*

Ferrets are very active and curious animals and can often be seen exploring and manipulating objects in their environment. Possibly this behaviour is necessary for their welfare and therefore they will try to fulfil it by -for instance- pushing the test door. To test this hypothesis, two setups were created based on observations in the video-footage in earlier experiments.

2.1) Sleeping bucket

The seemingly greatest difference between the two ferret populations in Utrecht and Bilthoven was the use of different constructions provided to the animals as a sleeping place. The ferrets in Utrecht would have a heavy nightcage, while the Bilthoven ferrets were provided with a bucket made of flexible material. Video footage from Bilthoven showed that the animals often manipulated the bucket, which suggests that the ability to manipulate an object is an important enrichment for ferrets. Consequently, it could be argued that the lack of a such an object in Utrecht caused this population of ferrets to take an interest in the door.

2.2) Two-way cat flap

Video footage from both Bilthoven and Utrecht showed that the ferrets would often push through the weighted door only to immediately return by using the cat flap on the other side. This observation led to the suspicion that the manipulation of the cat flap could be a form of enrichment to the ferrets. In this case, the animals in the control group would not be pushing the door to reach the empty compartment itself but instead they would make an effort to get to the cat flap present in said room.

3) *Opportunity to see the other side*

Another possible explanation for the high MPP control is the fact that the ferrets could not see what was on the other side and therefore would continue to push the door in order to reach the empty compartment. With their curious nature, ferrets under normal circumstances have been observed to either be sleeping or exploring their surroundings (Marini, 2014). Possibly this innate curiosity drove the ferrets to push considerable amounts of weight in order to see what was on the other side of the door.

4) *Territorial behaviour*

The ability to see the empty compartment might not be enough for the ferrets. Fox & Broome (2014) stated that domestic ferrets could be seen marking their entire territory, even when said territory was equipped with a litter box. Possibly there is an aspect of territorial behaviour that motivated the animals to go into the empty compartment in order to mark or explore their entire territory. To solve this problem in an experimental setup, the ferrets would have to be able to get to the other side without pushing the door, but also without entering the space that would later be used to place the enrichments.

5) *Need to defecate in a separate room*

Video footage from earlier experiments showed that ferrets would often immediately defecate after reaching the other side, before directly returning to their home cages. Seemingly in contrast to the beforementioned territorial behaviour, these observations led to suspect that defecating in a separate room may be an essential need for ferrets.

Animals, Materials and Methods

Ethical approval

This study was ethically approved by the Institutional Animal Care and Use Committee of Intravacc (DEC 201300057) and Utrecht University (DEC 2013.I.09.073).

Animals

In order to test the different cages, 5 four year old female ferrets with a hormone implant (Suprelorin®, with 4.7 mg deslorelin, Virbac) were used. Because of limited time and a small amount of available materials, not every ferret could be tested in each setup. Therefore, most setups were tested with only three ferrets.

Housing and nutrition

In order to establish standardisation, and to rule out the influences of weather conditions, the ferrets were taken out of their usual outdoor environment and moved to the experimental setups located in an inside kennel at the Faculty of Veterinairy Medicine in Utrecht, the Netherlands. Each ferret was given a cage of 120x140 cm. Nutrition (Hill's M/D) and water (provided in bottles) remained the same as in the original outdoor kennel. The cages were cleaned daily and the ferrets were weighed weekly to monitor their health.

Experimental housing

The animals were individually housed in an experimental setup which will from now on be referred to as the "standard cage". This setup consisted of two parts: one "home compartment", in which the ferrets were provided with food, water and a nestbox intended as a sleeping facility, and one "enrichment compartment", in which different types of enrichments could be placed. These compartments were divided by a panel containing a test door, going from the home side to the enrichment compartment, and a cat flap leading back to the home side. The ferrets were initially placed on the home side and could only reach the enrichment compartment by pushing the test door. (Figure 1)

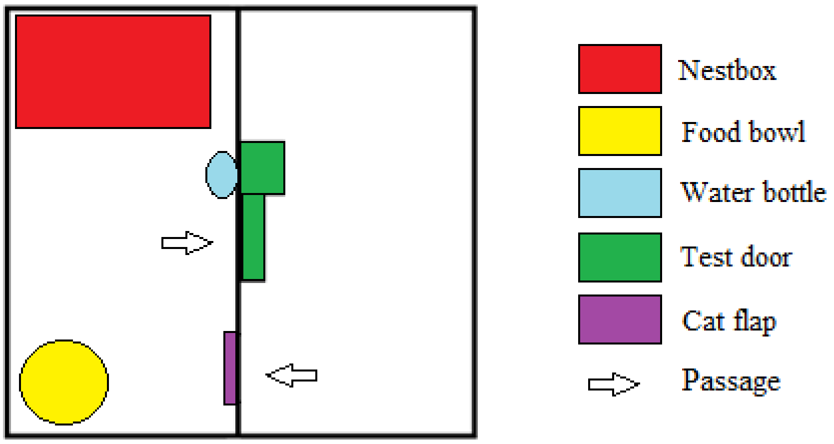

Figure 1: Standard cage setup

Acclimatization and task

Approximately one week before starting measurements, the ferrets were placed in the experimental cages to habituate to their new environment. During this acclimatization phase, the door between the two compartments was left open, allowing the ferrets to explore the entirety of their residence. The ferrets were trained on pushing the test door in the pilot study performed by M.L. Reijgwart.
At the start of the experiment, the test door was closed and recordings were started. Each day, the effort needed to open the test door was increased by adding a 250g weight to the door, starting with 0g on day 1 to a maximum of 3000g on day 13. Measures and calculations showed that the actual weight pushed could be defined as 200g plus 50% of the added weight.

Table 1: added weight and actual pushed weight for each day of the experiment

Day	Acclimatization	1	2	3	4	5	6	7	8	9	10	11	12	13
Weight added (g)	Open	0	250	500	750	1000	1250	1500	1750	2000	2250	2500	2750	3000
Actual pushed weight (g)	0	200	325	450	575	700	825	950	1075	1200	1325	1450	1575	1700

Setups

As mentioned in the introduction, five possible explanations for the high MPP controls were further explored and tested. For each of the possible explanations, at least one experimental cage was created. These were made by altering the standard cage as used in the MPC and MPP control setups. A complete overview of the used designs can be found in attachment 1. All experiments were executed in a similar way to the MPC and control tests. The ferrets were introduced to their new environment one week before measurements started in order to get used to the new situation. After this acclimatization phase, the weights on the test doors were increased daily by 250g.

It is important to make the distinction between the two types of setups that were created: the diagnostic designs and problem-solving designs. The purely diagnostic designs were created and tested only to explore the reason behind the door-pushing. These setups could not be used in further experiments. The problem-solving designs were also tested to explore the excessive door-pushing, but these designs, if deemed useful, could later be implemented in the standard test cage.

Diagnostic setups

1) Attractiveness of the test door: cat flap tunnel

As mentioned before, the tunnel-like construction of the test door might have attracted the ferrets in such a way that they would keep trying to pass through it even though there would be nothing to gain on the other side. In this case, the test door itself would be the enrichment and therefore would not be suited as the obstacle in a consumer demand study.
To test this, the cat flap normally only used to return to the home side was made to swing both ways and enclosed in a similar looking tunnel made of plastic to remove the possible extra attractiveness of the door. This created two similar entrances, the only difference being the amount of effort needed to pass through them. (Figure 2) Assuming the ferrets would prefer access to the empty compartment without effort, the cat flap should now be the preferred way to reach the other side.

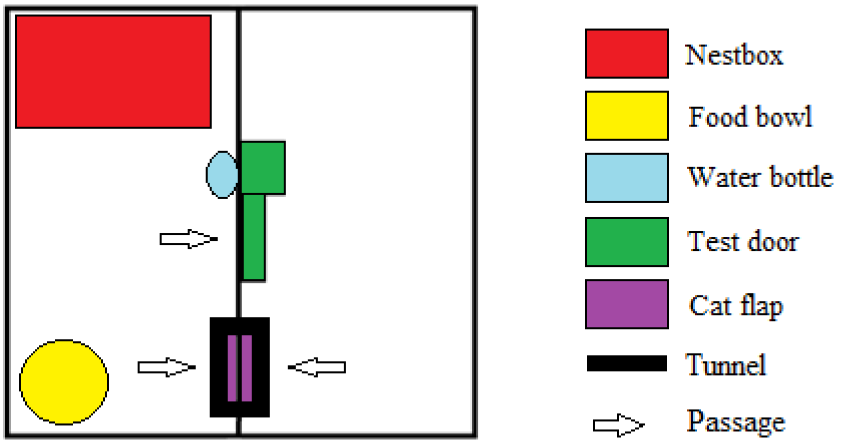

Figure 2: cat flap tunnel

2) Manipulation of objects

2.1) Sleeping bucket

The $MPP_{control}$ results showed remarkable differences between the ferrets in Utrecht and Bilthoven. To further investigate this difference, the same control experiment was repeated with the ferrets in Utrecht, but this time in the same setup that was used in Bilthoven.
The nest box used as a sleeping facility was replaced by a flexible bucket, and the animals could gain access to an empty room either by pushing the weighted door or without effort by going through a hole in the panel. In this situation the ferrets had two objects to manipulate: the sleeping bucket and the test door. (Figure 3) If manipulation of the sleeping bucket would be preferred to manipulation of the test door, this could indicate that it is a necessity for ferrets to be able to move objects in their environment.

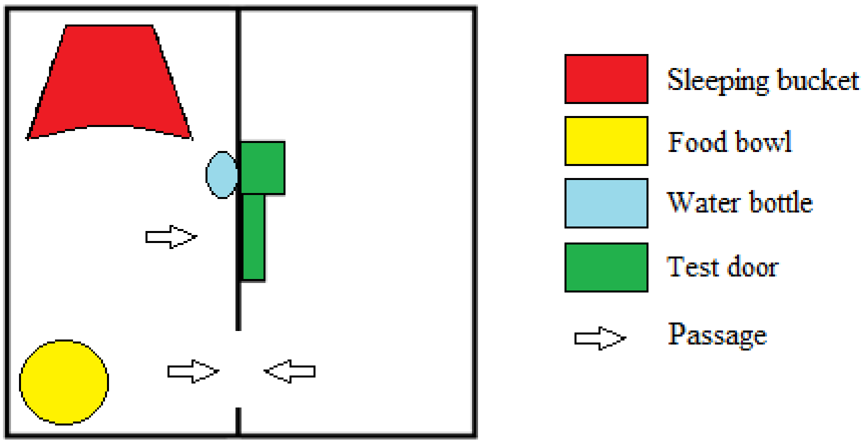

Figure 3: sleeping bucket

2.2) Two-way cat flap

Another moveable object in the previous experiments was the cat flap. In order to reach this object, the ferrets had to push through the weighted door first. It is possible that the cat flap itself was already seen as an enrichment, prompting the ferrets to push the weighted door up to their maximum push capacity. In this case, the test door itself would work adequately, but MPP controls would stay high because of the attractiveness of the cat flap. In order to investigate the cat flap as the cause of the door pushing, the existing cat flap was altered so it could be swung both ways. This would allow the ferrets to go through the cat flap without having to push the weighted door. (Figure 4)

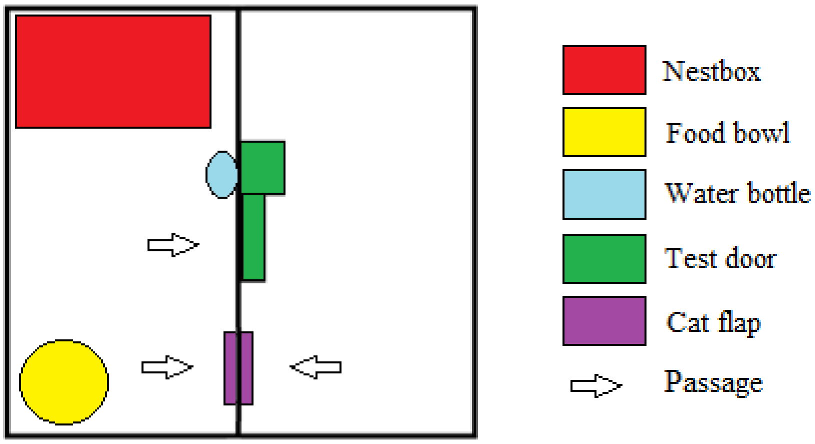

Figure 4: two-way cat flap

In all of the diagnostic setups the ferrets were also provided with the opportunity to gain access to the empty compartment without any significant labour. A consumer demand study heavily relies on this labour in order to quantify the motivation of an animal. Therefore, these setups were only used to point out potential flaws in the two-chamber setup, but could not be used as a solution to continue the consumer demand study. If only the diagnostic setups would have appeared to influence the MPP control, this could be seen as an indication that the two-chamber setup is not useful for this particular species.

Problem-solving setups

1) Opportunity to see the other side: mesh divider

To explore this option, the panel dividing the two compartments was altered so that the bottom half consisted of metal mesh that allowed the animals to see the empty space on the other side. (Figure 5) If this setup would prove to be useful in lowering the maximum pushed weight, a mesh divider could easily be implemented in the standard cage for the consumer demand study.

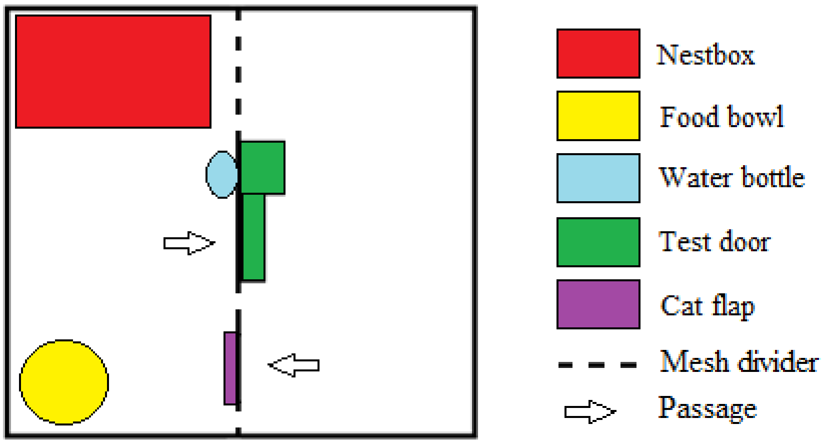

Figure 5: mesh divider

2) Territorial behaviour: mesh tunnel

To solve this problem in an experimental setup, the ferrets would have to be able to get to the other side without pushing the door, but also without entering the space that would later be used to place the enrichments. This was realised by constructing a tunnel of mesh around the borders of the empty compartment. (Figure 6) The mesh tunnel is also a construction with the potential to be implemented in the consumer demand study, because the enrichment in the room is never reached without pushing the test door first.

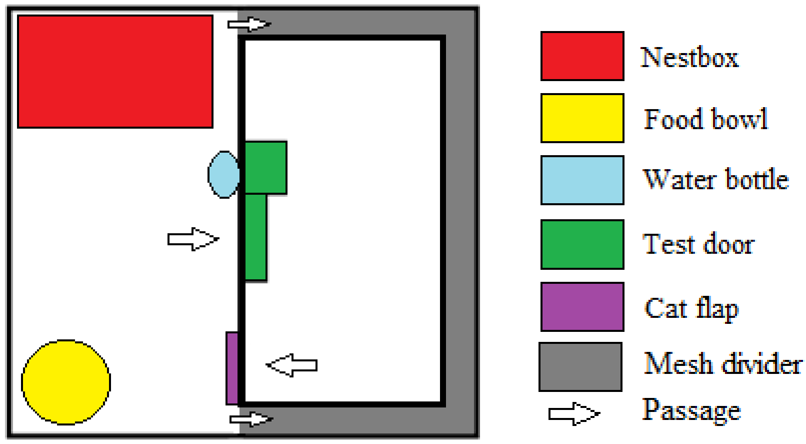

Figure 6: mesh tunnel

3) Need to defecate in a separate room: extra compartment

In order to investigate if defecating in a separate room may be an essential need for ferrets, one of the standard cages was expanded with an extra compartment attached to the home cage, so that the animals had the option to defecate in a separate room without putting in extra effort. (Figure 7) If the addition of a defecation room would prove useful in lowering the maximum paid price, it could be added to the standard cage to serve as a home cage in the consumer demand study.

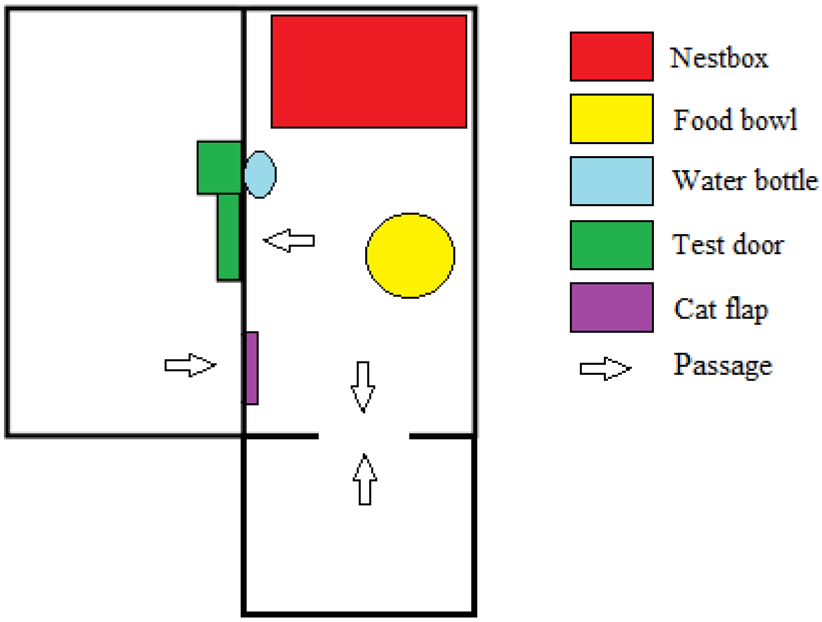

Figure 7: extra compartment

Measurements

During the experiment, the ferrets were recorded by a camera above the centre of their cage. The video footage was observed and analysed in order to gain the results. For the best comparison, the maximum price paid in each setup was regarded as a percentage of the MPC.

Statistical analysis

The maximum weight pushed in each of the tested setups was compared to the MPC of the ferrets. The most logical statistic test to perform for this would be the repeated measures ANOVA. Unfortunately, due to limited time and materials, not all ferrets in the experiment could be tested in each of the experimental setups, which led to missing values for almost every tested construction. The repeated measures ANOVA would not work in this case, therefore an unpaired t-test was used to compare the test setups with the MPC values individually.

Because of this individual testing, a correction for multiplicity had to be performed. Because of the small population size, which only consisted of 3 ferrets for most of the constructions, a Bonferroni correction would lower the p-value in such an extreme way that none of the executed test setups would seem to have any significant value. Therefore, the False Discovery Rate as suggested by Benjamini and Hochberg (1995) was used to calculate the significance of each setup.

In order to draw conclusions concerning the value of the different setups, further statistic analysis had to be performed to compare the setups not only with the MPC but also with the $MPP_{control}$ and with each other. Here, a difference had to be made once again between the purely diagnostic setups and the problem-solving setups. Since the housing setups in the problem solving setups were derived from the standard setup used in the $MPP_{control}$ tests, comparing the test setups with the $MPP_{control}$ using a repeated measures ANOVA was necessary to determine the effect of the changes applied in the used setups.

The setups used in the purely diagnostic setups showed no similarities to the setup used in the $MPP_{control}$ in the way that the ferrets did not need to push the weighted door in order to gain access to the empty compartment. Therefore these setups were compared with the control setup without the catflap.

Results

Comparing the experimental setups with the MPC

The diagnostic setups (cat flap tunnel, sleeping bucket and two way cat flap; 4, 5 and 6) in general show a considerable larger difference from the MPC than the problem-solving setups (mesh divider, mesh tunnel and extra compartment; 7, 8 and 9) (Figure 8). $MPP_{control\ (without\ cat\ flap)}$, MPP two way cat flap and MPP sleeping bucket were significantly lower than MPC (p=0.03;p=0.005;p=0.019 respectively). The average MPP for the different setups and the corresponding standard deviations are shown in table 2.

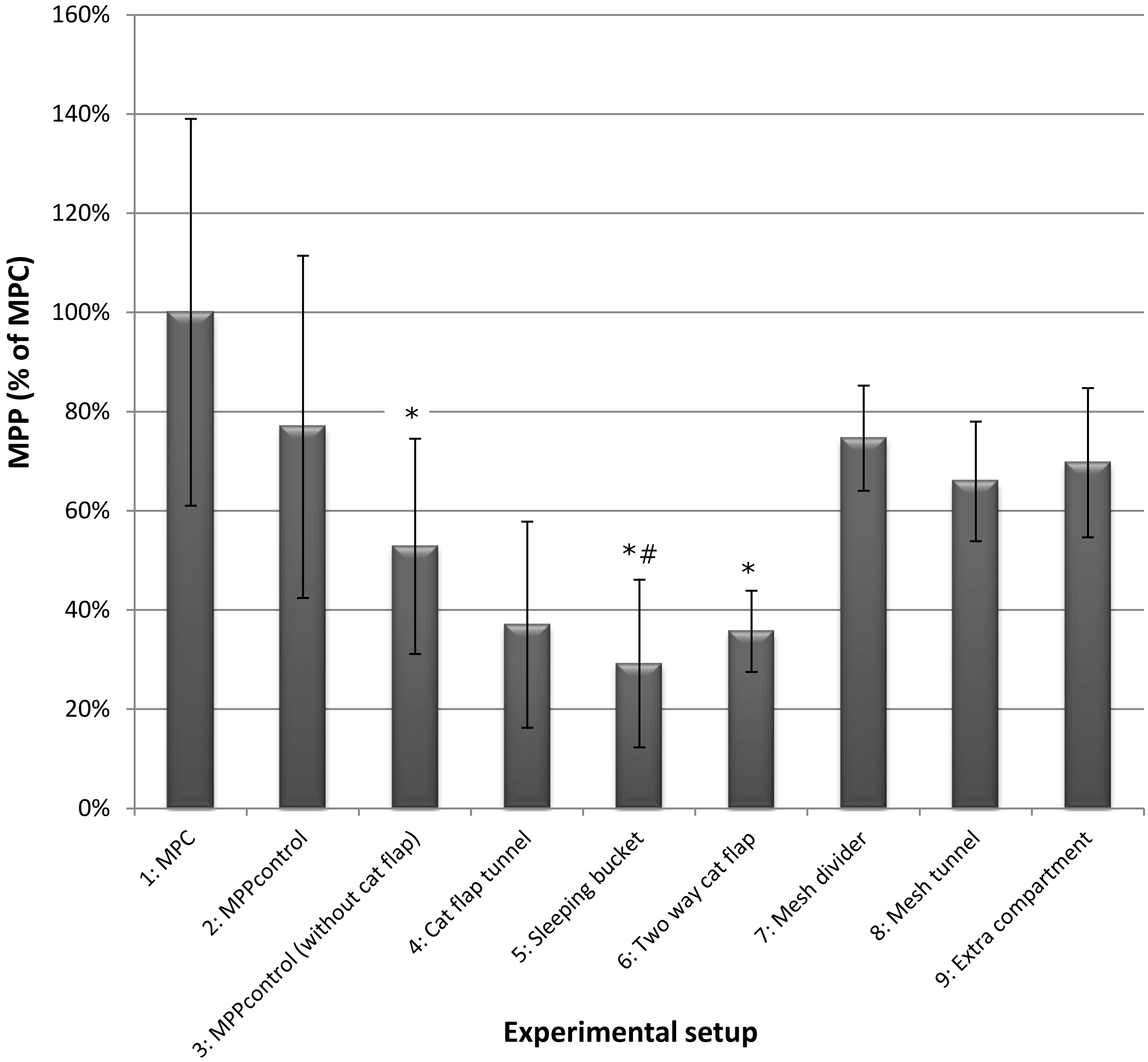

Figure 8: MPPs in each of the experimental setups, displayed as percentages of the MPC. Error bars represent standard deviations, *=significant difference with MPC, #=significant difference with $MPP_{control\ (without\ cat\ flap)}$.

Table 2: MPPs in each of the experimental setups. Significant differences show a p-value below the FDR (BH) thresholds and are highlighted using *

#	n	Setup	MPP (% of MPC)	p-values	Rank	FDR (BH) thresholds
3	6	$MPP_{control\ (without\ cat\ flap)}$	53±22%	0,003*	1	0,007
4	3	Cat flap tunnel	37±21%	0,034	4	0,029
5	3	Sleeping bucket	29±17%	0,019*	3	0,021
6	3	Two way cat flap	36±8%	0,005*	2	0,014
7	3	Mesh divider	75±11%	0,055	6	0,043
8	3	Mesh tunnel	66±12%	0,040	5	0,036
9	3	Extra compartment	70±15%	0,073	7	0,05

Diagnostic setups (4, 5, 6)
MPP in the setup containing the sleeping bucket was significantly lower than $MPP_{control(without\ cat\ flap)}$ (p=0.012). The MPP in the other diagnostic setups (cat flap tunnel and two-way cat flap) did not significantly differ from the $MPP_{control(without\ cat\ flap)}$ (p=0.227;p=0.338 respectively).

Problem-solving setups (7, 8, 9)
No significant differences were found between each of the problem-solving setups and the control setup (2), i.e. the mesh divider, mesh tunnel and extra compartment did not lower the MPP for the empty compartment (p=0.297;p=0.071;p=0.267 respectively).

Discussion

Comparing the experimental setups with the MPC
Comparing the maximum price paid in each of the experimental setups with the maximum push capacity of the ferrets showed that two of the three diagnostic setups (the sleeping bucket and the two way cat flap) led to a significant difference in the amount of weight pushed to reach the empty compartment. Both of these designs were implemented to test the ferrets' need to manipulate objects. This might mean that it is essential for ferrets to be able to interact with objects in their cage and in absence of an additional object they will use present objects (in this case the test door) as a substitute. This hypothesis is supported by Meagher & Mason (2012), who established that boredom corresponded with an increase in interaction with stimuli in caged mink, a species closely related to the ferret.

A notable observation is that, in both of these setups, in addition to the weighted test door, there was another, less strenuous way of reaching the empty compartment. The pilot consumer demand study has already demonstrated that the ferrets experienced the task of pushing the door as strenuous, as the ferrets would push the door less frequently with increasing weights. Possibly, they simply pushed less weight in these setups because it was not necessary for them in order to reach the other side.

Diagnostic setups
Although the sleeping bucket and the two way cat flap both showed a significantly lower result than the MPC, further comparisons had to be made in order to more accurately determine the value of these designs. Because both designs were derived from the control MPP without cat flap, a comparison of these setups was in order. When comparing the

diagnostic setups with the $MPP_{control(without\ catflap)}$, it was shown that only the design with the sleeping bucket significantly lowered the MPP. While this still indicates that ferrets have a need to manipulate objects, it also points out that not every object will have the same value, instead there are also preferences in this area.

Problem-solving setups

None of the tested problem-solving setups showed any significant difference in lowering the maximum price paid. Territorial behaviour and a need for a private defecation space might still play a role in the high MPP controls, however, neither of them can be seen as the only explanation and therefore neither would be useful in a follow-up consumer demand study.

Future recommendations

In order to continue the consumer demand study, a different approach will have to be used. It could still be possible to execute the consumer demand study using a two chamber setup. In this case, the task which the ferrets have to perform to get to the other side would need to change to something less attractive for the ferret. The curious nature of the ferret, as once more demonstrated in this study, is a great obstacle in designing new tasks. Their apparent need to explore and manipulate objects could drive the animals to perform any task that they are given, in which case the difference between the MPC and $MPP_{control}$ would remain too small to work with in a consumer demand study. Instead of performing a task, other obstacles could be looked into, in which the ferrets would have to undergo a negative experience, in order to reach the enrichment compartment. The most direct method of realising this would be the “foot shocks” method as formerly used by Schaap et al. (2013), administering low-intensity electrical pulses on the border between the home compartment en the enrichment compartment. This method however comes with an inevitable decrease of the animal welfare and therefore should not be considered when there are still other options available.

Another possibility could be to replace the two chamber setup as was used in this experiment with a three chamber setup. In this case, the home cage would be connected to two separate compartments that can both be reached by pushing the familiar weighted test door. One of these compartments would contain the tested enrichment, while the other would be an empty control room. The enrichment would be considered positive for the ferrets if they push a significantly higher amount of weight for the enrichment compartment than for the control room. Seeing that the ferrets in earlier experiments also pushed for empty compartments, the three chamber setup would not directly seem the right alternative.

The multi-chamber setup, as used by Mason & Cooper (2012) seems more likely to achieve significant differences between enrichments. This construction was proven successful with minks. The setup is similar to the three chamber setup, but includes multiple chambers all connected to the home cage and filled with different enrichments. This way the ferret can choose between different enrichments and a preference can be determined.

When using multiple chambers, a few assumptions will have to be made. Firstly, it has to be assumed that the ferrets are capable of remembering the contents of each compartment to show a preference for certain enrichments. Another important factor is the task that has to be performed. Although it has been established that the ferrets consider the pushing of the test door as a strenuous task, this study has also showed that a lack of manipulable objects will drive them to push through it. Therefore, it has to be assumed that the enrichment on the other side has more value to the ferrets than the test door. In order to determine suitable enrichments, it is important to study natural ferret behaviour.

Conclusion

None of the problem-solving setups could be appointed as the sole explanation for the high MPP controls. The opportunity to see the other side, the need to demonstrate territorial behaviour and the need for a separate defecation facility were not the main motivations for the ferrets to reach the empty compartment. As a result, none of the tested designs would be useful in a consumer demand study. The two-chamber setup using the weighted test door as obstacle is therefore not suitable in determining the enrichment preferences of ferrets. The most likely explanation would be the curious nature of the ferrets, which drives the animals to interact with objects in their environment. The diagnostic setups have shown that the ability to manipulate objects is important to ferrets. Placing moveable objects in the home cage would defeat the purpose of a consumer demand study and this setup can therefore not be used in future studies of this kind. However, this observation can be helpful in choosing the types of enrichments used in prospective multi-chamber studies, providing the ferrets with moveable objects in at least one of the enrichment compartments.

Acknowledgments

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