Enhancing cage space utilization for laboratory rats *(Rattus norvegicus)*

Evaluation of two enrichment structures

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Utvärdering av två berikningskonstruktioner

Linnéa Särén

Uppsala 2018

Ethology and Animal Welfare – Bachelor's programme

Photo: Ann-Christin Nordkam, 2018

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Abstract

The rearing environment of laboratory rats has a tremendous effect on their behaviour and cognitive skills. Rats housed in an enriched environment have been shown to be more resilient and better able to cope with challenges in the future. Their problem solving skills are enhanced, and rats will even exert a certain degree of physical strength to access an enriched environment. The majority of rats at AstraZeneca, Gothenburg, are group housed in the Enriched Rat Cage (ERC) system, with a very good size and enrichment standard compared to large parts of the laboratory animal science community. However, the height and overall large space, is not fully utilized. The aim of this study was to evaluate two enrichment structures with a potential to improve the cage space utilization.

A shelf and a PVC tunnel was mounted to the back wall of the ERC. Six female rats were placed in pairs in one ERC rack, containing three cages. A surveillance system was set up with one camera for each cage. Recordings were performed in four 24-hour cycles, with the first cycle on day 1, starting directly after introducing the rats to the modified ERC. The following recording cycles were on day 8, 15 and 22. Analysis of behaviour was performed during 5 minutes, every other hour, totalling for 240 minutes, using The Observer XT 11.5. The behaviour and in cage location was recorded for each cage, containing two rats, along with duration for each behaviour (n=3).

The results show a more frequent use of the tunnel than the shelf. The duration of each interaction was also longer for the tunnel than for the shelf. The mean duration for tunnel increased from 0.6 ± 0.3 seconds on day 1, to 287.0 ± 43.1 seconds on day 22. Mean duration on shelf did not differ much over the four recording cycles; results were between 1.9 ± 1.2 seconds and 7.5 ± 1.6 seconds. Both structures were used throughout the course of the day, where most interactions with the shelf occurred during the night, the rat's active period, and the tunnel was used more during the day, the more inactive part of a rat's day. The tunnel, as well as the shelf, fulfil the goal of better cage space utilization. This serves to enhance cage complexity, which is argued to be of more importance for rat well-being than a larger cage. The conclusion is that both the tunnel the shelf was used by the rats and should be studied and developed further, and, in the future, implemented in all ERC racks at AstraZeneca, Gothenburg.

1. Introduction

1.1 Research animals

According to the Swedish Animal Protection Welfare Act (1988:534), an animal is regarded a research animal if it is used for scientific research, disease diagnosis, development and manufacture of pharmaceuticals or chemical products, teaching, if the use means killing the animal, being subjected to surgery, injection or blood loss or if the animal is caused or threatened to be caused suffering or other comparative purposes.

Mice are the most common species used for laboratory research, followed by rats being the second most common species. In Sweden during 2015, the total number of research animals reported, according to the EU definition of research animals, which excludes test fishing, was 258 403. Of these, mice represented about 68%, whereas rats only constituted about 8.5% (Användningen av försöksdjur i Sverige under 2015, 2017).

Animals in captivity should be held in a way that promotes health and wellbeing, as well as being able to perform species specific behaviours (§4 Djurskyddslagen, 1988:534). There should be a sufficient amount of attractive resting and sleeping places, complemented with good bedding material. The cage should also be appropriately complex so that the animals can perform a wide range of species-specific behaviours. The animals should be given some sort of control over their environment, to be able to decrease stress behaviours. All animals should also be provided with species specific and individual enrichment, to promote natural behaviours like foraging, movement, social contact, resting, hiding and physical and mental activity (Statens jordbruksverks föreskrifter och allmänna råd (SJVFS 2012:26) om försöksdjur, senast omtryckt genom SJVFS 2015:38, saknr L150).

1.2 The wild rat

Rattus norvegicus, the brown rat, common rat or Norway rat is an active, nocturnal, gregarious and omnivorous species (Greenman & Duhring, 1931). They normally have a home range of up to 20-30 meters in diameter (Barnett, 1975), where they move around, foraging and collecting nesting material. However, they also move around without any particular stimuli affecting them, such as threats or hunger, due to the rat being a highly exploratory and inquisitive species.

They live in colonies, usually with a group of related females sharing a burrow and raising their offspring together. At low population densities, one male can occupy a burrow of females, mating with them, defending the territory and keeping other males away, making them territorial and polygynous. However, at high population densities, the rats are despotic and polygynandrous. In those situations, there are too many males making it impossible for any male to defend their own females. Hence, one male becomes socially dominant with subordinate males surrounding him. When a female is in heat, she mates with several different males, in the same way as the males mate with several different females (Hanson, 2006).

The wild rat dig extensive burrow systems that keeps growing and changing. The system comprises of entrance/-s, tunnel segments, cavities/chambers and nests. Tunnels are usually wide enough for one rat to pass through, whereas cavities can accommodate around seven rats. A typical burrow system has 16 tunnel segments, 6.8 entrances/exits, 4.5 cavities, including nest chambers and houses 5.5 adult rats (Calhoun, 1963).

Studies where laboratory rats have been released into the wild show that they quickly adapt and start show more natural behaviours, like patrolling the home range, digging and inhabiting burrows (Boice, 1977; Berdoy, 2002).When given the possibility, rats reared in a laboratory environment immediately start digging burrows, although they are generally less complex (Stryjek *et al.*, 2012).

1.3 The laboratory rat

Albino forms of the Norway rat was introduced to the research laboratory during the 1840s, which led to the rat being the first mammalian species to be domesticated and bred for scientific reasons (Richter, 1959). Good rat husbandry started to evolve at The Wistar Institute of Anatomy and Biology in Philadelphia around 1910. Research was conducted to find out what factors are important to make the laboratory rat "contented and happy", developing caging and research apparatus that was much better than the older methods. For example, rats could be housed in cages with a substrate that allowed them to burrow and a large running wheel (Greenman & Duhring, 1931).

Since then, cages for laboratory animals have most often been designed with focus on aspects regarding the human, and not from an animal perspective. Things like costs, handling of cages, observability of animals as well as the use of space has been more important than the welfare of the animal (Baumans, 2005). This has led to cages being small and relatively barren, often without nesting material or hiding places, or the possibility for the animals to perform their natural behavioural repertoire. Without the possibility to exert sufficient physical activity, laboratory rats often have a rapid weight gain and become overweight, following their sedentary lifestyle (Spangenberg *et al.*, 2005).

1.4 Environmental enrichment

Environmental enrichment can be defined as "an improvement in the biological functioning of captive animals resulting from modifications to their environment" (Newberry, 1995).
When enriching the environment of an animal, there should always be a clear purpose of why and a goal to fulfil. Goals of enrichment can be to reduce abnormal behaviour, to provide animals with more sense of control over its environment and/or to increase the frequency and diversity of species specific behaviours (Baumans, 2005). When performing enrichment studies, there are usually two clear purposes; the first one is to improve the living environment of the studied animal and the second to evaluate how important a specific object or resource is to the animal's behaviour (Chamove, 1989).

The foundation of environmental enrichment was laid in the 1920s when primate researcher Robert Yerkes observed that providing captive primates with an apparatus which they could interact with, improved their well-being (Yerkes, 1925; Shepherdson, 2003). In 1947, psychologist Donald Hebb brought home a group of rats for his children to keep as pets. He kept a control group of rats in his laboratory, housed in standard, barren cages. When the rats later on were tested for their problem solving skills, the pet rats achieved better scores than the laboratory rats. This led Hebb to conclude that "the richer experience of the pet group during development made them better able to profit by new experiences at maturity" (Hebb, 1949).

Today, numerous studies are made to evaluate how the home cage can be improved for the laboratory animals. Studies show that rats given a cage with a larger floor area are engaging more in active behaviours, like running and climbing. This leads to an increase of physical fitness, with lower weight gain, a higher oxidative capacity, better performance in inclined plane tests, as well as more diversity in behaviours (Spangenberg *et al.*, 2005). The larger floor area also gives the opportunity to provide the rats with valued resources like more hiding places in the form of nest boxes (Lidfors *et al.*, 2014) as well as other structures and objects the rats might appreciate. Other studies claim that the larger floor area is not what is most important, but that a more complex cage environment is more beneficial to the animal (Baumans & Van Loo, 2013).

Rats are known to be thigmotactic, meaning that they tend to move while close to or in contact with a vertical surface (Barnett, 1975). Hence, when providing a cage with a larger floor area, it is important to furnish the cage with structures for different purposes, not leaving a large open area. However, rats in enriched cages with several different structures, both more permanent objects as well as loose manipulative objects, tend to move around in the open space more frequently than rats in unenriched cages (Abou-Ismail *et al.*, 2010; Abou-Ismail, 2011). They also tend to occupy more sheltered parts of the cage less frequently, such as under the food hopper or the waterspout (Abou-Ismail *et al.*, 2010; Abou-Ismail, 2011). Studies also show that with an increasing number of vertical surfaces in a certain space, the number of behaviours occurring in rats, in that place also increases (Lamprea *et al.*, 2008).

1.5 The 3Rs

The concept of the 3Rs was founded by W. M. S. Russell and R. L. Burch in their book, The Principles of Humane Experimental Technique (1959). They wrote the book as a foundational framework with the hopes that others would adapt their mind-set and continue to build on the concept. Today the 3Rs is a fundamental part of animal experimentation.

The 3Rs stands for *Replace, Reduce* and *Refine*. Replace is about replacing animal experimentation when possible, with computer models, *in vitro* assays, using lower standing species etc. Reduce is focused on decreasing the number of animals used in studies. This can be achieved through better planning of experiments, assurance of statistic accuracy, standardisation of strain etc. (Tornqvist *et al.*, 2014). Refine means improving the conditions for animals before, during and after experimentation. This can include the proper use of anaesthesia and analgesia, enrichment of housing conditions, training of both humans and animals before procedures, post-operative care and euthanasia.

Today NC3Rs, National Centre for the Replacement, Refinement & Reduction of Animals in Research (United Kingdom, www.nc3rs.org.uk), is a big part in driving the 3Rs research forward in Europe.

1.6 Choice of study

I currently work as an *in vivo* scientist at the Laboratory Animal Science Department at AstraZeneca, Gothenburg. I started to think about how to improve the environment for the rats that are housed at our animal facility for a long period of time, like the sentinel animals. AstraZeneca have a good rat-housing standard, in terms of group housing our rats in large cages as compared to most of the other laboratory animal community.

The majority of rats at AstraZeneca, Gothenburg, are group housed in the Enriched Rat Cage (ERC) system. These cages were developed with rebuilt rabbit cages as a model (Lidfors *et al.*, 2014), which were used at AstraZeneca before the ERC racks. However, even though these cages have a large floor area and a good cage bottom to ceiling height of 38 cm, the extra space is not utilized at all. I decided to see if I could improve the cages for my technical training rats. In lack of shelves that I could easily mount on the walls, I attached cardboard tunnels to the back wall with cable ties. These tunnels are usually placed on the floor of the cages. It turned out that, after a few days, the rats started to spend a lot of time in them, mostly sleeping but also playing.

However, there were two big problems with this setup. Firstly – hygiene. The tunnels were made of cardboard and could therefore not be washed, and had to be discarded after usage. Secondly – mounting and dismounting the tunnels was difficult and time consuming. Therefore, it was not possible to continue doing this on a regular basis. However, if there were scientific proof of the rats appreciating the tunnels, then it might be possible to develop a better product that we could use in our standard cages.

2. Aim and Questions

The aim of this study was to evaluate two new enrichment structures, to better utilize the height and space of the cage. If one or both structures proves to be frequently used by the rats, any or both of them may be developed further, put in production and implemented in all rat cages at our facility.

The questions that will help in evaluating this are:

- Which enrichment structure do the rats spend most time with?
- Which enrichment structure do the rats interact most with?
- When are each enrichment structure mostly used throughout the 24 h?
- How does the usage of each enrichment structure change over time?

3. Material and Methods

3.1 Animals and housing

This study was carried out at the Laboratory Animal Science Department at AstraZeneca, Gothenburg. Six female Wistar rats (Charles River, Germany) were included in the study, and arrived at AstraZeneca, Gothenburg, at the age of 9 weeks. They were placed in the AstraZeneca Gothenburg's standard rat housing, the Enriched Rat Cage (ERC) (Scanbur A/S (Karlslunde, Denmark)), randomised into pairs of two rats in each cage. There are three cages in one ERC rack, where one cage measures 82 cm in length, 66 cm in depth and 38 cm in height. This equals a floor space of 5412 cm^2. The rats were placed in a room that can hold up to 10 ERC racks, but were alone in the room for the bigger part of the study. For a couple of days at a time during the study, there were also two female, pregnant rats. They were removed from the room the day after delivery.

A standard cage is equipped with a long, removable shelf along the left side wall, where the rats can hide underneath, as well as using the top as a viewpoint. It is placed 13 cm above the cage floor. They also have a small ladder to climb, on the right hand wall.

The rats received non-autoclaved tap water in a water bottle, and ad libitum standard feed (R70, Lantmännen Lantbruk, Sweden) in a food hopper, hanging on the outside of the cage door. The floor was lined with hardwood chip bedding (J Rettenmaier and Sönhe, Germany), each cage was enriched with cotton rolls (J Rettenmaier and Sönhe, Germany), shredded paper (Papyrus, Sweden), and gnawing sticks (Tapvei, Estonia). Cage change was carried out once a week. The room had 20 air changes per hour, a temperature of 20-23 °C and a relative humidity of 40-60%. A 12:12 hour light:dark cycle was kept with dawn at 06.00, with full daylight at 06.30 and dusk at 17.30, with full darkness and a soft night light at 18.00.

3.2 The enrichment structures

A grey PVC tunnel (Ahlsell), 21 cm long with an inner diameter of 10 cm, was fitted on the left hand side of the back wall, at a height of 23 cm from the floor. Three holes, approximately 2.5 cm in diameter, were drilled in the tunnel to make sure that it was possible to see if any rat occupied the tunnel.

On the right side of the back wall, a shelf made of acetal co-polymer (TICONA), 21 cm long and 10 cm wide, was mounted on the same height as the tunnel's floor level, spaced 12 cm apart (Fig. 1). The shelf was fitted permanently and was therefore washed with the rack. The tunnel was extractable and washed by hand.

Figure 1. Cage interior of the Enriched Rat Cage (ERC) with the tested two enrichment structures, a tunnel and a shelf, mounted at the back wall. Photo: Ann-Christin Nordkam, 2018

3.3 Study design

The rats were placed in the modified ERC at study start, when they were 13 weeks old. The rats' behaviour was recorded using a surveillance system from Nexuscctv, for 20 minutes per hour for 24 hours, on day 1, 8, 15 and 22. Recording started one hour after the transfer into the modified ERC. Three cameras (Nexus 233DB) were placed about 50 cm in front of the ERC, each filming one cage, in an angle to obtain the best visual view of the rats' movements. The cameras had a 3.6 mm lens, 720 p resolution and a 1.0 MP sensor. The cameras were also equipped with 26 built-in infrared diodes that ensured a good view during night time. The recordings were stored on a four channel, 500GB DVR (Nexus 2804AS-S). Cage change was always carried out the day after recording, i.e. on day 9, 16 and 23.

3.4 Behaviour analysis

The video files were imported into The Observer XT (version 11.5), a program for management and analysis of observational data (Noldus Technology, The Netherlands). A study design was set up and behavioural parameters were defined, see Table 1. Analysis was carried out on 5 minutes of recording for every other hour, starting with 5 minutes at 13.00 and ending with 5 minutes at 11.00 the following day. This gives 12 five minute intervals per day, a total of 60 minutes observation per day, and 240 minutes in total, during the study.

Table 1. *Definitions of behaviours recorded in The Observer XT*

Behaviour	**Definition**
In tunnel	More than half of the rat is located inside the tunnel
In contact with tunnel	In contact with the tunnel structure (tunnel and wall mount) with one or two front paws
Sniffing tunnel	Holding the nose within 2 cm of the tunnel for some time
On shelf	More than half of the rat is located on the shelf
In contact with shelf	In contact with the shelf structure (shelf and wall mount) with one or two front paws
Sniffing shelf	Holding the nose within 2 cm of the shelf for some time
On ladder	On and in contact with the ladder with one or more paws
On long shelf	On the long shelf, making contact with at least one hind paw
Under long shelf	More than half of the rat is located under the long shelf
In contact with long shelf	In contact with the long shelf with one or two front paws
Sniffing long shelf	Holding the nose within 2 cm of the long shelf for some time
Other behaviour	All other behaviours in the open area including, but not limited to, eating, drinking, moving and climbing the wire bar door

All behaviours were analysed as mutually exclusive, where the start of one behaviour automatically stopped the previous behaviour. The rat could be standing on the long shelf when it started sniffing the tunnel. In this case, the behaviour of being on the long shelf was stopped while sniffing, even though it was in fact still on the long shelf. When the sniffing stopped and the rat was still on the long shelf, it was then recorded as on the long shelf again.

3.5 Statistics

Each rat cage is considered a statistical unit, hence, the data material (n=3) is too small to analyse statistically. Results are presented descriptively with means ± standard error around the mean (SEM).

4. Results

4.1 Time spent with each enrichment structure

The mean total of time spent in the tunnel (9.4 ± 1.3 minutes) was higher than time spent on the shelf (0.2 ± 0.04 minutes), for all three cages over the four recorded days in the experiment (Fig. 2). Due to the fact that the behaviours of *sniffing tunnel, in contact with tunnel, sniffing shelf* and *in contact with shelf* were behaviours with very short duration, they were excluded from this analysis.

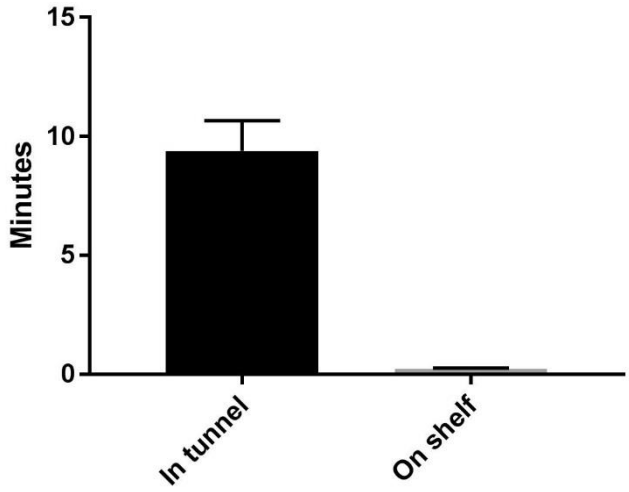

Figure 2. Mean ± SEM of total of time (minutes) spent in the tunnel and on the shelf, over four 24 h cycles and 240 minutes observation time, for all rats in all cages (n=3).

4.2 Number of interactions with each enrichment structure

When calculating the number of interactions in connection with each enrichment structure, the behaviours of *sniffing tunnel, in contact with tunnel, sniffing shelf* and *in contact with shelf* have been included.

The mean number of interactions with the tunnel was quite stable over all four days, varying between 22.0 ± 3.5 and 29.0 ± 7 interactions per day, whereas the interactions with the shelf decreased steadily, from 20.7 ± 8.0 interactions on day 1 to 4.3 ± 0.9 interactions on day 22 (Fig. 3).

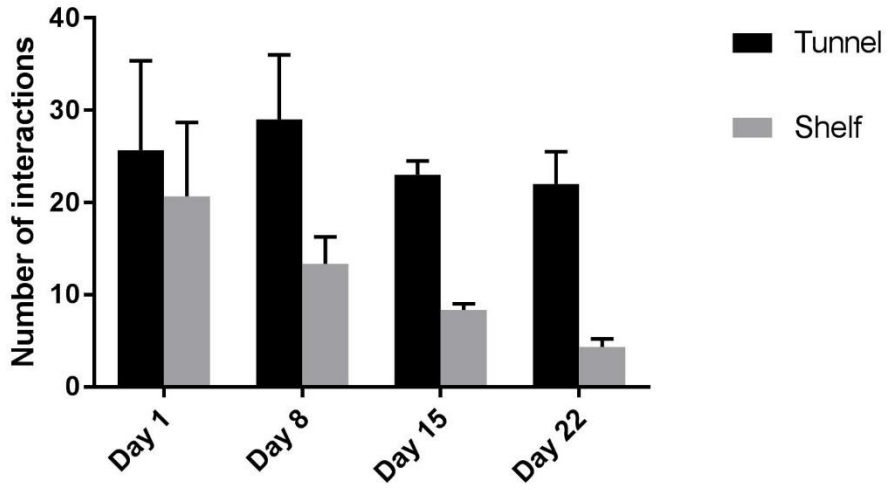

Figure 3. Mean ± SEM number of interactions per day and per enrichment structure, performed by all rats in all cages (n=3). Behaviours included are *in tunnel, sniffing tunnel, in contact with tunnel, on shelf, sniffing shelf* and *in contact with shelf.*

When excluding the investigative behaviours, *sniffing tunnel, in contact with tunnel, sniffing shelf* and *in contact with shelf,* the trend is very similar. The biggest difference was on day 1, which mostly included investigative behaviour in general, 3.7 ± 1.8 interactions with the tunnel and 9.0 ± 4.9 interactions with the shelf (Fig. 4). The tunnel was used more frequently and at a quite stable level, with only a small variation between 18.9 ± 3.5 interactions on day 8 and 20 ± 1.5 interactions on day 15. The interactions with the shelf were decreasing, from 10.7 ± 0.9 interactions on day 8 to 3.3 ± 0.9 interactions on day 22. During day 15 and 22, the majority of interactions were non-investigative behaviours, meaning in tunnel and on shelf.

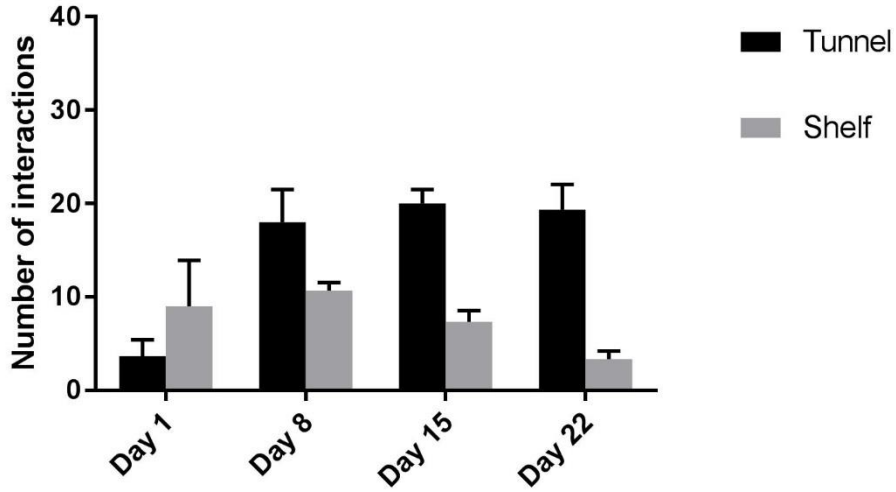

Figure 4. Mean ± SEM number of times per day rats were recorded either in the tunnel or on the shelf by all rats in all cages (n=3).

4.3 Usage of each enrichment structure throughout the day

When compiling the time spent in the tunnel by all rats on all four days, it is clear that the rats spent most time in the tunnel during their normal inactive phase, which is during the light hours of the day, and less time was spent during the night, when the rats are more active (Fig. 5).

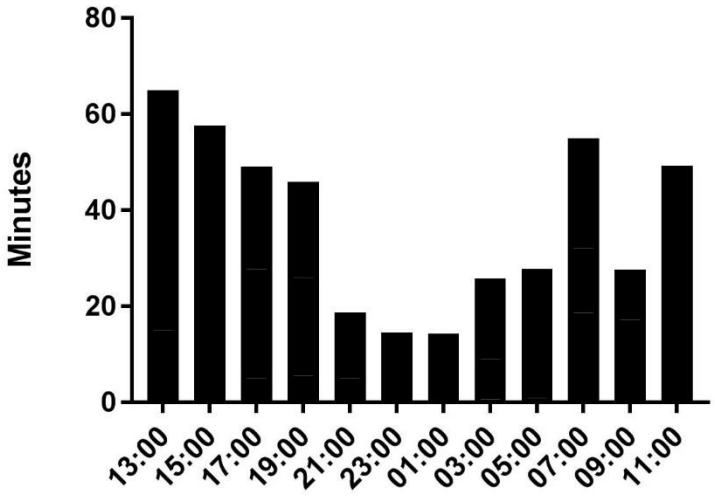

Figure 5. Distribution over the 24 h of time spent by all rats in the tunnel over all four days.

Time spent on the shelf by all rats on all four days was mostly during their awake phase, at night time (Fig. 6).

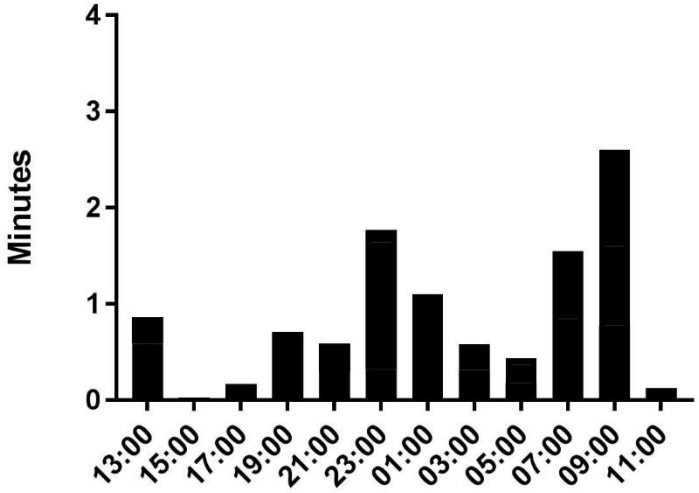

Figure 6. Distribution over the 24 h of time spent by all rats on the shelf over all four days.

These data (Fig. 5 and 6) correlate quite clearly. Between 07.00 and 19.00, the rats were more inactive, whereas during the dark hours between 19.00 and 07.00 the rats were more active. Dusk occurs for 30 minutes between 17.30 and 18.00, but the rats remained somewhat inactive for a while until somewhere between 19.00 and 21.00, even though the room is dark.

An animal technician usually perform a daily check on the animals sometime between 07.30 and 09.00 which could explain the peak in activity on the shelf at the 09.00 recordings, where time spent in the tunnel was lower at the same time.

4.4 Change over time

On the first day of recording (Fig. 7), the animals were inquisitive and performed more sniffing and quick touches. They only ran through the tunnel a few times. The rats spent most of their time under the long shelf (59.2 %), as well as on the long shelf (10.6 %). The tunnel was occupied for only 0.1 % of the time, and the rats were on the shelf for 0.8 % of the time.

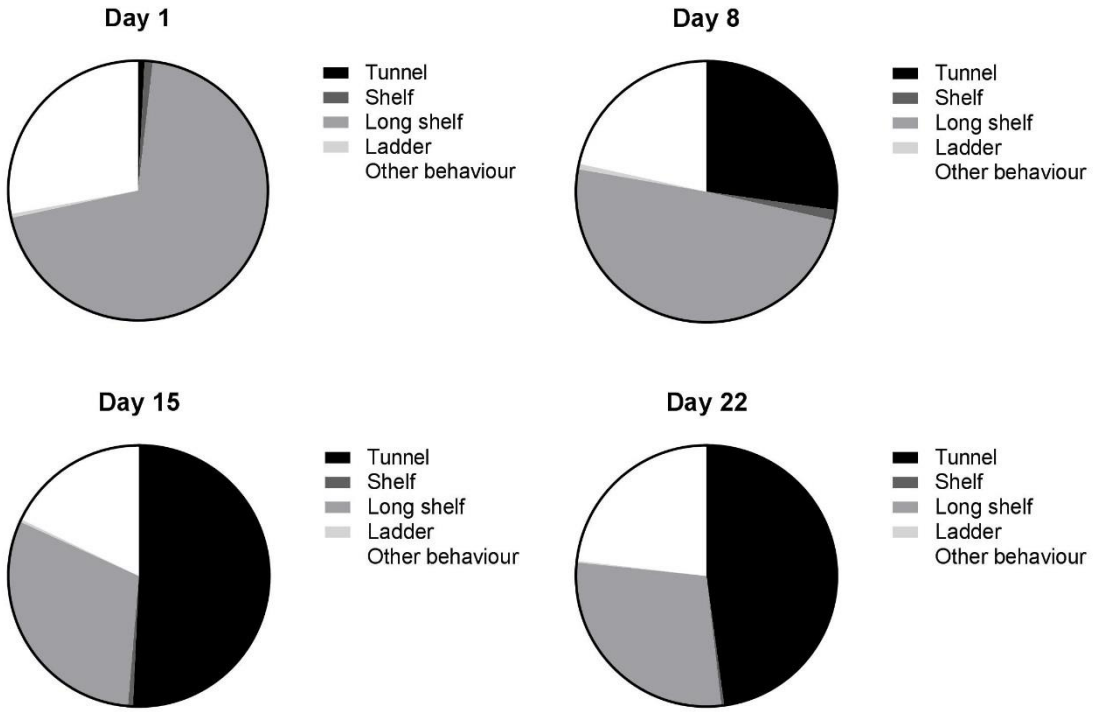

Figure 7. The distribution of time spent by all rats in all cages (n=3) performing all behaviours, over four 24 h cycles and 240 minutes observation time. Tunnel includes *in tunnel, sniffing tunnel, in contact with tunnel.* Shelf includes *on shelf, sniffing shelf, in contact with shelf.* Long shelf includes *on long shelf, under long shelf, sniffing long shelf, in contact with long shelf*.

Over the following recorded days (Fig. 7) a large part of the behaviour was shifted from interacting with the long shelf to spending time in and with the tunnel. On day 8, 26.7 % of the rats' time was spent inside the tunnel, and 35.4 % under the long shelf. During the last two observation cycles of the study, the rats spent approximately 50 % of their total time inside the tunnel (50.6 % on day 15 and 47.8 % on day 22). Time spent under the long shelf decreased to 25.2 % on day 15 and 22.2 % on day 22. Time spent on other behaviour was fairly constant throughout the study.

The mean time ±SEM spent in the tunnel (Fig. 8) by all rats in all cages (n=3), increased from the start of the study (0.6 ± 0.3 seconds on day 1) to the last observation cycle (287.0 ± 43.1 seconds on day 22).

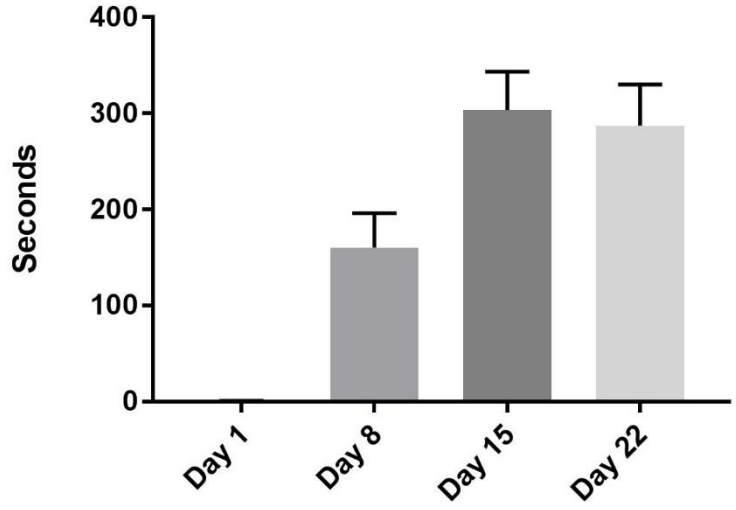

Figure 8. Mean time (± SEM) in seconds, spent in the tunnel, by all cages (n=3) over the four days of experiment.

The mean time ± SEM spent on the shelf did not differ much over the four days of experiment (Fig. 9). The longest recorded mean duration occurred on day 8 (7.5 ± 1.6 seconds) and the shortest mean duration was recorded on day 22 (1.9 ± 1.2 seconds).

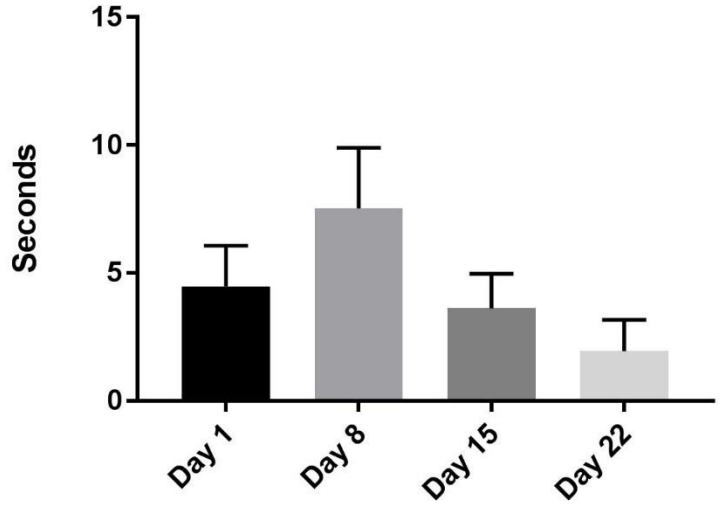

Figure 9. Mean time (± SEM) in seconds, spent on the shelf, by all cages (n=3) over the four days of experiment.

The average time per interaction (Table 2) was calculated by dividing the total amount of time in seconds spent by all rats with each of the enrichment structures on each day, with the total number of interactions with each of the enrichment structures on each day. The average time spent in the tunnel increases for every day of recording, whereas time spent on the shelf peaks at day 8 and then decreases.

Table 2. *The average time per interaction in the tunnel and on the shelf on day 1, 8, 15 and 22 of recording for 6 female rats housed in pairs in the Enriched Rat Cage system. Data presented in seconds*

	In tunnel (seconds)	On shelf (seconds)
Day 1	2.9	6.0
Day 8	106.9	8.5
Day 15	182.3	5.9
Day 22	178.3	7.0
Average for all days	148.0	6.9

5. Discussion

5.1 Related studies

There are few studies performed where rats are housed in anything other than the standard laboratory rat housing. Therefore, it is hard to find information on rats' preference to wall suspended tunnels and shelves. In fact, not a single article can be found on this subject. A few articles, where rats have been housed in larger cages similar or identical to the ones used at AstraZeneca, Gothenburg, have been identified, but these studies have been evaluating the effect of the bigger cage and the possibility to group house the rats (Remes, 2007; Lidfors et al., 2014). From that perspective, this study can be considered as quite unique.

Interestingly, other studies, that has in one way or another, evaluated the rats' preference for tunnels, have all come to the same conclusion; rats do not have any preference for tunnels when compared to other objects, or even an empty cage. For example, 10 adult Long-Evans male rats, with a weight span of 476 to 750 g, meaning that the rats were quite old, were placed individually in a specially built cage, designed to tip slightly to the part of the cage where the rat was located. The cage had a mesh floor and was empty, except for the test object placed in one or the other end of the cage. Each object was placed in the cage with each rat for 8 days. A computer monitored on which side the rat was. No significant difference in the time on each cage side was detected when a tunnel was the object of testing i.e. no preference could be concluded (Chmiel Jr and Noonan, 1996). A similar study also concluded that rats show no preference towards tunnels as enrichment (Bradshaw and Poling, 1991). However, these studies used old rats that had been single housed in empty cages with wire mesh floor during their entire life, until the start of the study. Sudden subjection to new objects might have had a stressful impact on the rats. In this study, the rats were quite young and females.

A more recent study evaluated rats' preference for different resources; larger caging, social housing, toys and cage complexity (Patterson-Kane et al., 2001). The rats were pair housed in standard plastic cages, and had an average age of 18 months. The rats were tested in both a T-maze as well as in a continuous access box with a hole between two cages. One side of each test arena had an empty standard cage and the other one hade the test item. The testing was run during the active hours, under red light, and each rat was tested 60 times for each test item and in each test arena. The study showed no preference for any enrichment option, over an empty

cage, including the tunnel. These results contrasts to the observations of our study, showing a clear preference for the tunnel. However, the strongest preference for the tunnel in this study was noted during the light, inactive period, and this time period was not assessed by Patterson-Kane et al. (2001). This difference in study design may well be a reason for the difference in study outcomes. The period of night is probably not the optimal time to test the rats' interest for a tunnel, since they are more active during the night and will therefore not actively seek shelter unless they are very anxious.

Many of the preference studies are relatively short term and it is likely very stressful for rats to be placed in an unfamiliar barren cage by themselves, with new test objects in short intervals. Overall, very few studies on environmental enrichment have been performed over longer periods of time. Hence, limited information exist on how enrichment objects effect the animals in the long run (Abou-Ismail, 2011).

5.2 Cage complexity

Rat cages, as well as housing for other laboratory animal species, have often been designed with focus on affordability, cleaning routines and ergonomics, and not with focus on animal welfare and well-being (Baumans, 2005). The available space in rat cages is often not used to its full extent. By better utilisation of walls, floor and ceiling, as well as providing partitions to divide the cage into different areas would serve to increase the psychological space for rats (Chamove, 1989).

In addition to taking another step towards fully using the three-dimensional space, the tunnel and the shelf also contributes to enhancing the complexity of the cage. Many studies argue that increasing the complexity of the cage would improve the housing standard and the well-being of the rats, if the possibility to provide them with larger cages does not exist (Denny, 1975; Abou-Ismail et al., 2010; Abou-Ismail, 2011; Baumans & Van Loo, 2013).

Still, the combination of a larger cage and increased cage complexity, together with social housing (Pinelli et al., 2017) would be the optimal approach, which is also demonstrated in a study using a new type of two level cages (Wheeler et al., 2015).

5.3 Shelter

The tunnel can be seen as a shelter, or a form of nest box, and it has been shown that a nest box is a highly coveted resource for laboratory rats (Manser et al., 1998a; Manser et al., 1998b; Patterson-Kane et al., 2001; Patterson-Kane, 2003). Patterson-Kane et al. (2001) demonstrated that when given the choice between an empty cage and one furnished with a nest box, rats displayed a significant preference for the cage containing the nest box.

It has also been shown that rats will exert a certain degree of physical strength by lifting a weighted door to gain access to a cage containing a nest box (Manser et al., 1998b). A nest box can have several purposes for a rat. It can serve as a secure resting place, shelter from predation and in-group fighting, a way to escape bright light and control temperature, and

therefore giving the rat some sort of control over their own environment (Chmiel Jr & Noonan, 1996; Manser et al., 1998a).

Before the start of the study, the rats included in the study usually sought shelter under the long shelf. Even though studies has shown that rats prefer opaque nest boxes with closed ends (Chmiel Jr & Noonan, 1996; Manser et al., 1998a), the rats were observed to use the tunnel frequently after being introduced to the modified cage, despite the open ends and drilled holes along the side. A big part of their time spent in a sheltered place was shifted to the tunnel. This can be due to different reasons, for example, the location above the cage bottom can feel safer and the dim lighting directly underneath the cage ceiling makes it quite dark, both in and directly outside, the tunnel. It might also be due to the fact that the tunnel is smaller in size, where the tight fit appeal more to the thigmotactic tendencies of rats (Lamprea et al., 2008).

5.4 Design of the enrichment structures

When setting up this study, the main goal was to enhance the housing conditions of the rats. However, the people who are working with the animals on a daily basis were also kept in mind. The shelf and the tunnel needs to be easy to clean. The shelf can be cleaned with the rest of the cage rack, but the tunnel must be extractable, so it can be washed manually or put through the sectioned tunnel washer. The washing of the shelf worked properly, but the design of attaching the tunnel to the wall could be improved. As the tunnel was attached to the wall-mounted holder by cable ties, it was difficult both to remove for washing, as well as to attach in a clean cage after washing.

One idea for a better design would be to manufacture a holder similar to the water bottle holder, but it was not possible to do in-house for this study. In regards to working with the animals, one concern was that the rats would be hard to catch if they decided to hide in the tunnel. However, this was not a problem in this study.

5.5 Use of the enrichment structures

Four 24-hour recording cycles were performed, with the first on day 1, when the enrichment was introduced, and the others on day 8, 15 and 22. The data show that the tunnel was used frequently throughout the study. On the first day of recording, the rats investigated the tunnel thoroughly and later the rats spent an increasing amount of time inside the tunnel. The shelf was also used throughout the study, but very sparsely, mostly just as a passage from the right side of the cage into the tunnel. Potentially, the shelf could be used as an escape route, if "in-cage fighting" would occur, or to get a good overview of the cage and the rest of the animal holding room. The tunnel and shelf were used together when the rats were playing and chasing each other, and when they played on their own.

The pattern of usage of the tunnel and the shelf throughout the day matches the expectations, as well as the circadian sleep rhythm in rats (Borbély & Neuhaus, 1978). The tunnel was mostly used during the light, more inactive, phase, whereas the shelf was used during the dark, active,

phase. There was an observed peak in activity on the shelf at 09.00, when also a drop in the use of the tunnel was noted. This shift in activity was most likely due to an animal technician being in the room in close connection to the time of filming.

The results show that the total amount of time spent in any of the two sheltered places, in the tunnel or under the long shelf, increased over the study period. The mean time per interaction with both being in tunnel or under the long shelf also increased. We did not note the behaviours occurring in the tunnel or on the shelf. Hence, we do not know if the observed increased time in the tunnel reflects an increased time of sleep. For future studies it would be relevant to investigate this further as sleep can be used as an indicator of good animal welfare (Abou-Ismail *et al.*, 2007)

5.6 Study implications due to enrichment

Many scientists, using rats in investigative models, argue that the increase in behavioural variation, that might happen when providing rats with environmental enrichment, larger cages and/or social housing, can compromise the accuracy of their studies and are therefore reluctant towards using enrichment for their study animals. This is something that has been shown to be wrong by many studies. They have clearly demonstrated good effects of enrichment without compromising the derived study data (Wolfer *et al.*, 2004; Würbel & Garner, 2007). It has also been proven that the variability between animals within a study does not increase, even though variation in behaviour increases with environmental enrichment (Marashi *et al.*, 2004; Van de Weerd *et al.*, 2010), nor does enrichment disrupt the standardisation of project setup (Würbel, 2007; Baumans *et al.*, 2010).

Another study concludes that rearing rats in small, barren cages, without the ability to perform natural behaviours inhibits the brains development, leading to altered brain functions (Würbel, 2001). From that perspective, housing conditions are highly relevant, especially when it comes to research in behavioural neuroscience.

5.7 Limitations of the study

The current study included six animals placed in pairs in three cages, which is quite few. Hence, there is a risk that the outcome of the study is a result of individual differences, or in this case similarities, in preference, rather than being a representative of the general rat population. A larger group of animals would provide a more reliable statistical result.

However, the study was planned to serve as a pilot study for a following, larger study. To conduct a pilot study also makes sense from a 3R perspective, reducing the number of animals used, but still provide sufficient data for study improvement and power calculations.

The filming could be improved. For this study, one camera was used per cage. This resulted in the entire cage not being completely visible on the recordings. It did not make any difference for the current study design, but in order to monitor additional parameters more cameras per cage or different angles would be necessary to gain full visibility of the rats and their behaviours. Moreover, on the filming of day 22, the angle was not correct, so a larger part of

the cage was out of the picture. Luckily, this did not affect the outcome of this particular evaluation as the locations needed for evaluation, according to the study set up, was still in view.

The rats were not individually marked, therefor it was impossible to follow the activity pattern of each rat, such as the entering and leaving of the tunnel. In order to follow each rat, 24 hours continuous recording would be necessary but would require extensive data handling.

5.8 Contribution of the study and future research

There are limited studies done on rats housed in anything other than the small, standard rat cages. More studies using large enriched cages would contribute to increase awareness of this housing and this may lead to other facilities starting to re-evaluate their rat housing systems.

Hopefully, there will be a continuation of this project, especially to further evaluate the effect of the tunnel on the rats. If free reins were given to plan a new study using the tunnels, both sexes and an additional strain would have been included, to evaluate if there are any gender or strain bias preferences. A larger number of animals, based on this pilot study, would be used for the statistical accuracy, as well as including a group of control animals to compare with the test subjects would be preferable. A longer study, following the rats once weekly over a period 6 months, would be good to evaluate if the interest in the tunnel is due to it being a novelty, or if the behaviour is consistent over time. Behavioural, as well as location recordings would be included to clarify what the rats do in each place, and for how long each behaviour is performed.

Well-being can be defined as "the ability of the animal to cope successfully with its environment" (Broom, 1986) and indicators of good animal welfare can be increased sleep, increased activity, increased body weight, increased muscle mass and physical strength, decreased antagonistic behaviour and more variation in behaviour (Abou-Ismail *et al.*, 2010; Abou-Ismail, 2011; Abou-Ismail & Mahboub, 2011). Therefore, recording of body weights and food intake, to detect differences in growth ratio depending on housing would be valuable to do. Additionally, stress levels could be compared through corticosterone measurements in blood or urine.

It would also be interesting to see if the tunnel still would be as attractive, if a different type of shelter was provided, or if the shelf was used more if the design or placement was altered.

6. Conclusion

The results of this study demonstrated that both the tunnel and the shelf were used continuously throughout the study by the rats.
Time spent in the tunnel increased over the study, where approximately half the rats' day was spent inside the tunnel at day 15 and 22.

Time spent on the shelf was quite stable throughout the study, with a slight decrease at the end of the study. However, the level of use was low compared to the use of the tunnel. Continued development of design and placement of the shelf might lead to increased usage.
The number of interactions with the tunnel increased over the study period, whereas the number of interactions with the shelf decreased.
The tunnel was most frequently used during the day, and the rats used the shelf mostly during the night.
Both structures do meet the goal of increasing the utilized cage space, but in different ways.
Further development and studies are needed before implementing either enrichment structure in all cages at AstraZeneca, Gothenburg.

7. Summary

The rat is the second most common species used in research in Sweden. It is an inquisitive species that dig and live in burrow systems, and is mostly active during the night. Studies have shown that rats who are born and raised in a laboratory environment, quickly adapts to the wild if released. Therefore, it is important to create a living environment for laboratory rats that allows the rats to perform their natural behaviours.
AstraZeneca, Gothenburg, has a very good housing standard compared to a large part of the laboratory community, with group housing in large cages, called the Enriched Rat Cage (ERC). Unfortunately, the space in these large cages are not fully used.
The focus of this study was to evaluate two structures that could lead to more of the cage being used. A PVC tunnel and an acetal co-polymer shelf was mounted to the back wall of the ERC. Six female rats were placed in pairs in three ERC. They were recorded for four 24-hour cycles, the first one after being introduced to the modified ERC, and the following on 8, 15 and 22 days after introduction. Analysis were done on 5 minutes of recording every other hour, 240 minutes in total, where the location of the rats was observed.
The result show that the rats used the tunnel frequently. The rats were a bit hesitant, but very interested in the tunnel during the first day, sniffing it and running through it, while playing with the cage mate. After the initial days, they started to spend more time inside the tunnel during the day, instead of their normal shelter, under a long shelf on the bottom of the cage. The tunnel was not used as much during the night, but the rats were observed using it then as well.
The shelf was not used as much as expected, it was mainly used when entering or exiting the tunnel. However, there was a noticeable increase of use during the night, when the rats are more active.
Together, the shelf and the tunnel increase the complexity of the cage, they create a new passageway through the cage that serves as a way of exercise as well as a way to escape if they feel scared or threatened. They also meet the goal of using the extra cage space in a better way. Hopefully, the tunnel will be developed further and applied in all ERC racks at AstraZeneca in the future.

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