The use of enrichment by laboratory rats (*Rattus norvegicus)*

– does different types of temporary enrichment affect the behaviours of the rats?

Användning av berikning hos laboratorieråttor (Rattus norvegicus) – påverkar olika typer av tillfällig berikning råttornas beteende?

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Självständigt arbete 15 hp
Sveriges lantbruksuniversitet, SLU
Institutionen för husdjurens miljö och hälsa
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Abstract

Enrichment for laboratory animals is widely discussed and it is getting more commonly known as something that can help with increasing the animal welfare. However, more studies are needed in how enrichment is used and how it affects animal behaviour.

Seventeen male rats of the strain Lewis were given three different types of temporary enrichment types; cardboard, paper and fabric. The rats were filmed during three weeks and their behaviours were analysed. An ethogram was used to determine if there was a difference regarding interaction with enrichment types, activity levels and negative behaviours.

The rats interacted with the temporary enrichment differently during the two weeks when temporary enrichment was offered, compared to the first control week where no extra enrichment was given. During the first week with temporary enrichment the rats interacted the most with cardboard, but during the second week they interacted the most with the fabric. Resting behaviours where the highest during the week where the animals did not have access to extra enrichment. Active behaviours where the highest during the first week with extra enrichment, and the lowest the second week with enrichment.

When the rats did not have access to temporary enrichment they had more negative social behaviours than positive social behaviour. When the temporary enrichment was introduced the positive social behaviours increased whereas the negative social behaviours decreased during both weeks.

Enrichment should be used in a laboratory setting to increase the animal welfare. It is important that different types of enrichment is being offered to increase the opportunity of performing a more broad spectra of natural behaviours in the rats.

Keywords: animal welfare, laboratory rats, enrichment, *Rattus norvegicus*

Sammanfattning

Berikning för försöksdjur är ett allmänt diskussionsämne och det blir allt vanligare att det ses som ett verktyg för att öka djurens välfärd. Det behövs dock fler studier kring hur berikning används och hur det kan påverka djurens beteende.

Sjutton hanråttor av stammen Lewis fick tre olika typer av temporära berikningar; kartong, papper och tyg. Råttorna filmades under tre veckor och deras beteenden analyserades. Ett etogram användes för att avgöra om det fanns skillnad vad gäller interaktion med berikningstyperna, aktivitetsnivåer och negativt beteende.

Råttorna interagerade annorlunda med den tillfälliga berikningen under de två veckorna då den erbjöds, jämfört med den första kontrollveckan där ingen extra berikning gavs. Under den första veckan med tillfällig berikning interagerade råttorna mest med kartong, men under den andra veckan interagerade de mest med tyget. Andelen beteenden som räknades som ej aktiva var högst under veckan där djuren inte hade tillgång till extra berikning. Andelen aktiva beteenden var högst under den första veckan med extra berikning och lägst den andra veckan med extra berikning.

När råttorna inte hade tillgång till tillfällig berikning hade de mer negativa sociala beteenden än positiva sociala beteenden. När den tillfälliga berikningen infördes ökade de positiva sociala beteendena medan de negativa sociala beteendena minskade under båda veckorna.

Berikning bör därför användas i laboratoriemiljöer för att öka djurens välbefinnande. Det är viktigt att olika typer av berikning erbjuds för att öka möjligheten att utföra ett bredare spektrum av naturligt beteende hos råttorna.

Nyckelord: Försöksdjur, råtta, *Rattus norvegicus*, berikning, beteende, djurvälfärd.

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1. Introduction

1.1. The wild rat

The species of rat, which is mostly used in laboratories, is a domesticated form of the brown rat, *Rattus norvegicus* (Barnett, 2007). The brown rat shows a high adaptability and lives in many different habitats (Modlinska & Pisula, 2020). They often choose to settle in areas in close proximity to protection, water and rich vegetation (Barnett, 2007). Rats are regarded as nocturnal animals, with a higher activity level at the beginning and end of the dark period (Barnett, 2007). Since they have a large cerebral cortex which controls whiskers, nose and front paws and have quite large ears, they most likely rely on their sense of hearing, smell and touch to explore different objects and areas (Burn, 2008), whereas sight is consider less important (Modlinska & Pisula, 2020). In the wild, the rat spends most of its awake time gathering, playing or building/digging tunnels, which ends in rooms with different functions, such as food storage, nursery, toilet or a resting place (Barnett, 2007). The brown rat is quite good at climbing but they do not climb as much or as well as their relative the black rat, *Rattus rattus* (Foster *et al*., 2011).

1.2. The laboratory rat

It is considered that the laboratory rat was domesticated around the year 1840-1850 when albino rats were bred and started to be used in a research facility (Richter, 1959). Currently there are several hundred different strains and substrains, which are being used in many different areas of research (Krinke, 2000).

Some studies have been performed on how the domesticated rat and the wild rat differ. For example, a study by Modlinska *et al*. (2015) concluded that the wild rat was not more neophobic than the laboratory rat when it came to food. However, Sryjek *et al*. (2012) showed that wild rats are more neophobic and exhibit more behaviours that are exploratory before they interact with new objects than domesticated rats. Sryjek *et al*. (2012) also showed that wild rats were more

effective in digging tunnels and made more complex tunnels, which they could live in, compared to domesticated Wistar rats.

It has also been shown that wild and domesticated rats differ in their play behaviour where domesticated rats generally play more and throughout their life (Himmler *et al*., 2014). It can be difficult to distinguish between play fighting and true fighting, although it differs in a few ways. In true fighting, the contact is more directed towards the opponent's lower part of the body and there are usually more wounds, sounds and pilo-erection compared to play fighting (Pellis & Pellis, 1987). During play fighting the rats have less of an aggressive stance and the contact is more directed towards the nape of the neck, there is also less likely to be wounding during play fighting (Pellis & Pellis, 1987, 2007). Pellis & Pellis (1987) study showed that pinning, where one rat pushes down another rat either on the side or back, is more a sign of play fighting than of true fighting. They also showed that allogrooming is a sign of aggression if it is directed towards the lower part of the opponents body. Play fighting lacks the goals of true fighting, which is to harm or intimidate the opponent and to gain a resource (Pellis & Pellis, 1987).

1.3. Signs of stress and depression in rats

Decreased play fighting behaviours is sometimes used as a sign of depression in rats, and access to a play-partner can be used as a reward (Burke *et al*., 2021). Zaias *et al*. (2008) concludes that increased eating behaviours, higher weight gain and lower activity levels are signs of poor animal welfare in laboratory rats. A study by Abou-Ismail *et al*. (2007) concluded that longer periods of sleep where the rats slept deeply, instead of just sitting still with open eyes, was a sign of less depressed/stressed rats. A longer sleep period is regarded as something positive since rats that has a longer duration of sleep also show other signs of increased welfare (Abou-Ismail *et al*., 2011). The same study considered that such signs were more time spent in the open part of the cage, more activity and less aggression.

Rats who has been exposed to CMS (Chronic mild stress) showed an increased level of grooming in both frequency and duration (Kompagne *et al*., 2008) with more short stops for grooming (Kalueff & Tuohimaa, 2005). Kompagne *et al*. (2008) concluded that the grooming behaviour could be used as an indicator of anxiety and depression in rats.

1.4. Environmental enrichment

According to Bracke & Hopster (2006) good animal welfare is based on the fact that the animal is able to perform natural behaviour. They propose a definition of natural behaviours as a behaviour that the animal tends to perform under natural

conditions since these behaviours are lust-filled and promotes a biological function. They also say that behaviours such as foraging, grooming, exploration and play should be behaviours that one should want to see in animals in captivity. Bracke & Hopster (2006) also exclude behaviours that are normal but negatively motivated such as fear responses.

There is no clear definition of what animal enrichment is but Young (2003) proposes a definition of environmental enrichment as a process that aims to improve or enhance the animal's environment and care. Young (2003) also says that it is important to have the animal's behavioural biology and natural history in mind when developing environmental enrichment for the animals. It is also said that environmental enrichment is something that should be used to enhance the quality of animal care, and that it is supposed to be used to give the animal stimuli and ability to perform a range of diverse behaviours (Sheperdson *et al*., 1998). It should also be used in a way to give the animal a sense of choice over its own environment, and that it is developed towards species-specific behaviours, which then in turn enhances animal welfare (Westlund, 2014).

1.5. The effect of enrichment on laboratory rats

Belz *et al*. (2003) showed that rats in single housed cages, which have access to different types of enrichment, had a lowered base line of stress hormones. They concluded that a low and stable base line of stress hormones is important to more easily show how drugs can influence stress responses.

Rats in enriched cages has also been seen making more optimistic choices, the same types of tests have been made on humans and showed that depressed humans more often have a negative view and a negative bias (Brydges et al., 2011). They therefore concluded that animals in enriched cages are less depressed (Brydges *et al*., 2011).

In some cases group housing is seen as an enrichment since a cage mate is not static and will give different responses to different stimuli (Hosey, 2013). Group housed rats showed a lower heart rate and returned faster to a resting state after an experimental procedure indicating lower levels of stress, compared to solitary housed rats (Sharp *et al*., 2002).

Different types of enrichment could affect the animals as much as different types of handling, lights, smells, usage of wood chips etc. (Bayne, 2005). Rats that have access to different types of enrichment has been seen showing signs of good animal welfare (Abou-Ismail, 2011). Abou-Ismal (2011) also saw that aggression decreased between rats that had access to environmental enrichment, possibly because the enrichment gave the rats an opportunity to get away from each other.

2. Aim of the study and questions

2.1. Aim of the study

The aim of the study was to establish if access to temporary enrichment affects the activity levels of laboratory rats and to observe their interactions with the enrichment. Additionally, the study aimed to determine if unwanted behaviours, e.g. aggressiveness or stereotypic behaviours, increased from access to the temporary enrichment.

2.1.1. Questions

- Do activity levels of the rat and interaction with the temporary enrichment differ when exposed to different enrichment types?
- Do activity levels of the rat differ when temporary enrichment is present compared to when temporary enrichment is not present?
- Does the frequency of unwanted behaviour differ when temporary enrichment is present compared to when temporary enrichment is not present?

3. Materials and methods

3.1. Animals and housing

The study was carried out at The Laboratory for Experimental Biomedicine (EBM) at The University of Gothenburg in Gothenburg, Sweden, between the dates July 27th and August 15th, 2020.

The study was performed on 17 male rats of the strain Lewis from Janvier Labs, France, which arrived at EBM on November 7th, 2019 and lived together in groups of three or two. Five cages had three rats (Cage nr 1-2 and 4-6) and one cage had two rats (Cage 3). The animals were chosen since the rats were not part of an active experiment, and it was certain that they would not be treated during the time this study was conducted. The rats were marked with animal marker pens (Agnthos) in the colours red, blue or black with one stripe on the tail, and two dots on either side of the body. The markings were placed on the rats two days before the first observation time point for the week.

The rats were housed in Eurostandard type IV cages with a raised wire lid series -117/-120 (Techniplast). They were given regular tap water in a water bottle, which was refilled three times a week, or as needed. The feed was given in a food hopper and the feed was Teklad Global Diet 16% protein (Envigo). Both food and water were available ad libitum.

The cage had a layer of small wood chips on the floor, about 25 grams of wood wool and two wooden chewing blocks (TAPVEI). In each cage there was also a PVC-pipe with three openings. All cages were placed at the end of the rack, which meant that the cages had three sides (roof, back and either right or left side) to another cage, a wall or the roof of the rack. All cages except cage number 4 had another cage beneath it. The room had around 30-50 cages during all three weeks, with 2-4 animals in each cage. The room had a temperature of 19-21°C, a relative humidity of 40-70% and a 12:12 light/dark cycle. The lights started to dim up at 6.30 am with full light at 7 am and started to dim down at 6.30 pm with lights off and a soft moonlight at 7 pm. The cages and water bottles were changed once a week, two days before the first observation for the week. At the same time, the rats were weighed, and the markings were maintained. Throughout the day, researchers

and animal technicians could enter the room, but no experiments were carried out in the animal room. The animal technician cleaned the room and changed the cages of the other animals only when the observation session for the day was completed.

3.2. Experimental design

The experiment was conducted during a period of three weeks. Week 1 was a control, where no changes to the environment was made. During week 2 and 3, three different types of temporary enrichment were given to the rats in a balanced order (Table 1) so each cage could be its own control

Table 1. Enrichment and filming schedule. Two observations per day for every day during the control week and the day temporary enrichment was introduced. One observation per day each day after enrichment was introduced.

Week	Obs. day	Cage 1	Cage 2	Cage 3	Cage 4	Cage 5	Cage 6	Obs/ day
1	1	Control	Control	Control	Control	Control	Control	2
	2	Control	Control	Control	Control	Control	Control	2
	3	Control	Control	Control	Control	Control	Control	2
2	4	Fabric	Cardboard	Paper	Cardboard	Fabric	Paper	2
	5							1
	6	Paper	Fabric	Cardboard	Paper	Cardboard	Fabric	2
	7							1
	8	Cardboard	Paper	Fabric	Fabric	Paper	Cardboard	2
	9							1
3	10	Cardboard	Fabric	Cardboard	Paper	Paper	Fabric	2
	11							1
	12	Paper	Paper	Fabric	Fabric	Cardboard	Cardboard	2
	13							1
	14	Fabric	Cardboard	Paper	Cardboard	Fabric	Paper	2
	15							1

The temporary enrichment was inserted between the bars of the cage lid so the rats had to pull it into the cage themselves. The temporary enrichment consisted of:

- Paper – Two stripes of paper towels (Torky), 4.5cm wide, 14 cm long.
- Cardboard – Two pieces of cardboard from a toilet paper roll, 4.5 cm long, 4.5 cm in diameter.
- Fabric – Two pieces of knitted fabric, 4.5cm wide, 14 cm long.

Each rat was filmed for five minutes during each observation with a smartphone (Huawei P20 lite), which was handheld by the observer. The observer entered the

animal room ten minutes before the filming started. During the control week and the day the temporary enrichment was given, there was two observations per day. The day after the temporary enrichment was given there was one observation per day. Each observation lasted for approximately 100 minutes, 85 minutes of filming of the six cages and approximately 15 minutes of extra time. The first observation each day, started at 9.30 and the day when the temporary enrichment was given the second filming started at 15.30. The pieces of temporary enrichment was removed from the cage after the observation period the day after the enrichment was given.

The films from all observations were later analysed where each focal animals behaviours for five minutes was scored with continuous recording, the scoring was then used to determine the frequency of the behaviour. An ethogram was created from rats' behaviours in literature, from my own experience and other animal technicians from the same animal facilities experience. The ethogram was divided into two parts, one with behaviours not directly regarding enrichment (Table 2) and one with behaviours regarding enrichment (Table 3). This was done to more easily distinguish between different types of behaviours during the observations. If the animal stopped the behaviour for more than two seconds, it was considered as the end of the behaviour. The behaviours were not mutually exclusive, if the rat was standing on the pipe and at the same time grooming, it was scored one for the pipe and one for the grooming. If the animal then stopped grooming for two seconds and then started grooming again, while it was continuing standing on the pipe the pipe was not scored again.

Table 2. Ethogram with behaviours not directly regarding enrichment and their definitions.

Behaviours not directly regarding enrichment	**Definition**
Social interaction positive – instigator (SI+I)	Initiating social contact with another rat. The rat is touching or pushing another rat with either its front paws or nose/mouth. Grooming another rat. Pinning another rat. No sounds are heard and no wounds are made.
Social interaction, positive – Receiver (SI+R)	Another rat is interacting with the focal animal, either with physical contact or not. Either front paws or nose/mouth of the other rat touches the focal animal. Gets groomed. Gets pinned. No sounds are heard and no wounds are made.
Social interaction negative - Instigator (SI-I)	Initiating social contact in an aggressive way, biting toward the lower regions of the body. Pushing another rat away from furnishing.
Social interaction negative - Receiver (SI-R)	Another rat is interacting with the focal animal in an aggressive way, gets bitten on the lower regions of the body. Gets pushed away from furnishing.
Resting (R)	Sitting or lying still outside of the pipe for more than five seconds.
Exploring – with focus outside of the cage (UU)	Standing/sitting on all four or two legs, with or without support towards cage wall/furnishing, with the head toward something outside of the cage.
Grooming (G)	Scratching itself with either front- or back-paws, licking the fur, swipes the paws over its body.
Food/Water (F)	Gnawing at the hopper to get fodder, eating fodder. Licking on the water bottle cap.
Climbing (C)	Touches the lid grids, back legs do not touch the floor.
Jumping	Performs big or small sudden jumps without it being directed towards another rat or without showing any stress.

Table 3. Ethogram with behaviours regarding enrichment and their definitions.

Behaviours regarding enrichment	**Definition**
Cardboard (CB)	Head is under cardboard, manipulating and/or gnawing on cardboard
Fabric (FAB)	Head is under fabric, manipulating and/or gnawing on fabric
Paper (PAP)	Head is under paper, manipulating and/or gnawing on paper
Social interaction in connection to enrichment, positive – Instigator (SIE+I)	Performs the behaviour "Social interaction positive – instigator", where one or more rats are interacting with the enrichment.
Social interaction in connection to enrichment, positive – receiving (SIE+R)	Performs the behaviour "Social interaction positive – receiver", where one or more rats are interacting with the enrichment.
Social interaction negative in connection to the enrichment, negative – instigator (SIE-I)	Performs the behaviour "Social interaction negative – Instigator", where one or more rats are interacting with the enrichment.
Social interaction negative in connection to the enrichment, negative – receiving (SIE-R)	Performs the behaviour "Social interaction negative – receiver", where one or more rats are interacting with the enrichment.
Exploring – with focus on something inside the cage (EXI)	Standing/sitting on all four or two legs, with or without support towards cage wall/furnishing, with the head toward something inside the cage.
Wood chips (WC)	Chewing on or digging through wood chips, with the nose or front paws.
Pipe (P)	Touches the pipe with one or more paws or with the nose, gnawing or licking the pipe.
Resting in pipe (RP)	Chewing on or manipulating the wood stick.
Wood stick (WS)	Chewing on or manipulating the wood stick.
Wood wool (WW)	Head is under the wood wool, manipulating or chewing on the wood wool.

3.3. Data analysis

The data was processed in Excel 2016 and four line charts and two pie charts were created. The data is shown as frequency of number of interactions on average per rat. This was done to be able to compare between cages and weeks since one cage only had two rats and the other cages had three rats. Week one had six observations per cage, whereas week 2 and 3 had three observations per enrichment and cage each. The behaviour "Climbing" (Table 1) was not included in the data since this behaviour was not shown in any of the rats.

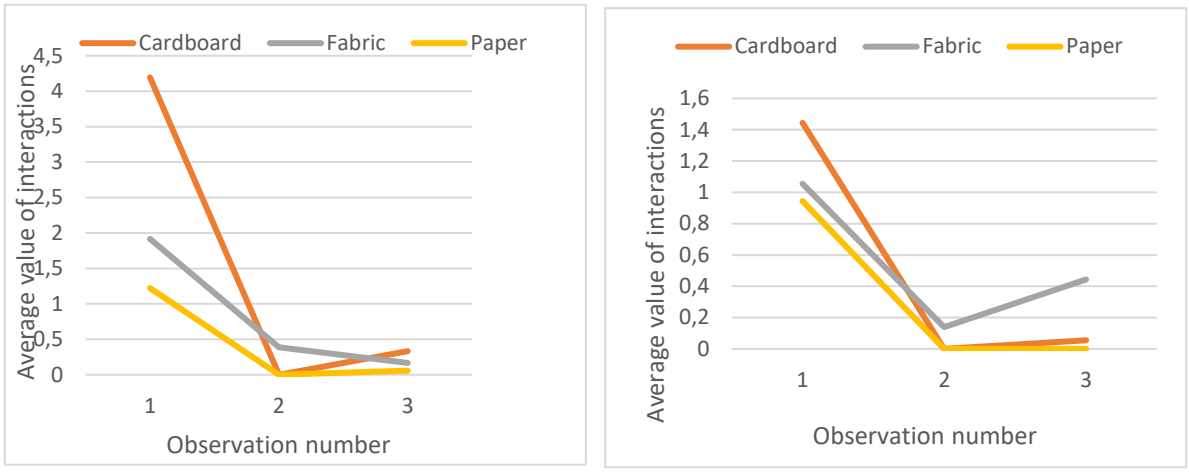
When analysing the activity levels of the rats with and without enrichment the behaviours "Resting" and "Resting in pipe" (Table 1) were compiled into one group; "Resting", and all other behaviours were compiled into another group; "Active".

When analysing the unwanted behaviours the behaviours "Social interaction positive – Instigator", "social interaction positive – Receiver", "Social interaction in connection to enrichment, positive – Instigator" and "Social interaction in connection to enrichment, positive – Receiver" (Table 2) were all compiled into one group called "Positive interactions". The behaviours "Social interaction negative – Instigator", "social interaction negative – Receiver", "Social interaction in connection to enrichment, negative – Instigator" and "Social interaction in connection to enrichment, negative – Receiver" (Table 2) were all compiled into another group called "Negative interactions". These new groups were created to show the results more clearly in the diagrams.

4. Results

4.1. Interaction with different types of enrichment

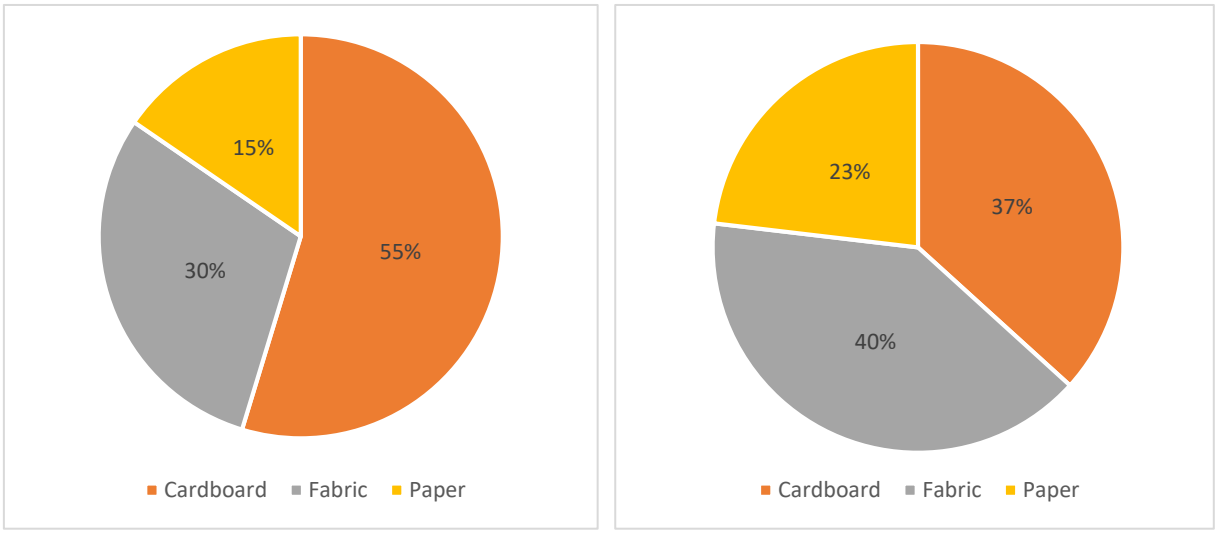
The rats showed more interactions with all the temporary enrichments during the first observation in both week 2 (Fig. 1a) and 3 (Fig. 1b). All the different enrichments had a higher frequency of interactions during the first observation of week 2 (Fig. 1a) compared to the same enrichment and observation number in week 3 (Fig. 1b). The frequency of interactions with cardboard was the highest during observation one in both weeks when temporary enrichment was present (Fig. 1a and Fig. 1b). The fabric hade the highest level of interactions in observation two in both week 2 (Fig. 1a) and week 3 (Fig. 1b) compared to the other enrichments. The cardboard accounted for the largest decrease of interactions in the first observation in week 2 compared to week 3 (Fig. 1a and Fig. 1b). The second observation in both weeks had the lowest number of interactions for cardboard and paper compared to observation one and three, where cardboard increased slightly more than paper in observation three (Fig. 1a and Fig. 1b). The fabric showed a different pattern, during week 2 the interactions decreased in observation three compared to observation two (Fig. 1a). In week 3 the interaction with fabric increased during observation three compared to observation two (Fig. 1b).



a) *b)*

Figure 1. Average frequency of interactions with cardboard, fabric and paper during week 2 (a) and week 3 (b).

When merging all three observations for each temporary enrichment, the cardboard had the highest percentage of interactions during week 2 (Fig. 2a). In week 3 fabric had the highest percentage of interactions (Fig. 2b). Both fabric and paper had an increased percentage of interactions in week 3 compared to week 2 whereas cardboard had a decreased number of interactions in week 3 compared to week 2 (Fig. 2a and 2b).



a) *b)*

Figure 2. The sum of all interactions during the three observations for cardboard, fabric and paper during week 2 (a) and week 3 (b).

4.2. Difference of activity levels with and without enrichment

The number of registrations with active behaviours were higher in all weeks compared to resting behaviours (Fig. 3). When temporary enrichments were introduced during week two, the number of registrations for active behaviours increased whilst the number of registrations of resting behaviours decreased (Fig. 3). The number of registrations for active was highest during week 2 and lowest during week 3 (Fig. 3). Number of registrations for resting were lowest during week 2 with a slight increase in week 3, and highest during week 1 (Fig. 3).

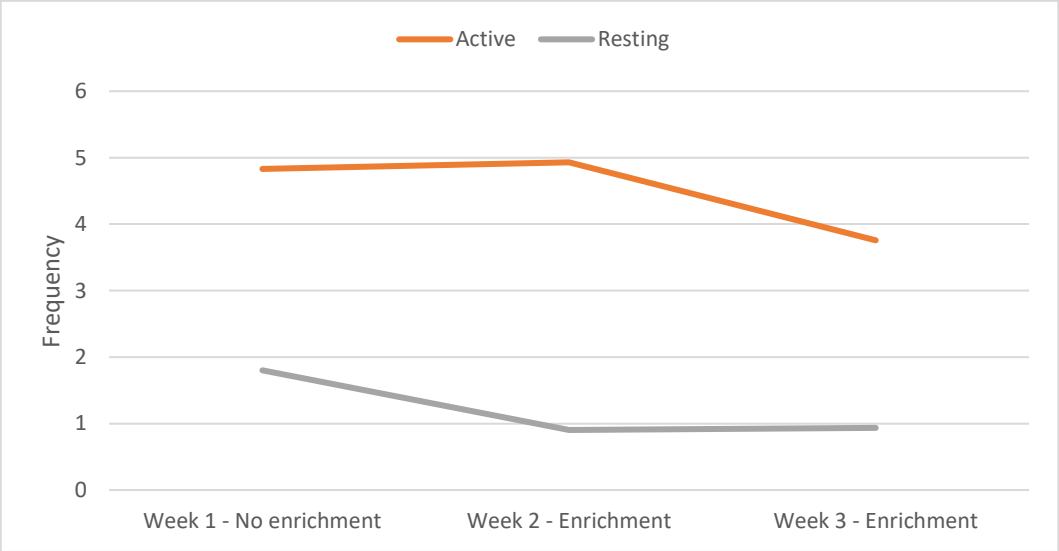


Figure 3. The sum of average frequency for resting behaviours and active behaviours during all three weeks

4.3. Unwanted behaviours with or without enrichment

During week 1 when no temporary enrichment was present, the number of negative interactions were higher than positive interactions (Fig. 4). During week 2 and 3, when temporary enrichment was present, the number of positive interactions increased and the number of negative interactions decreased. During week 3 the number of positive interactions decreased slightly compared to week 2, whilst the number of negative interactions continued to decrease.

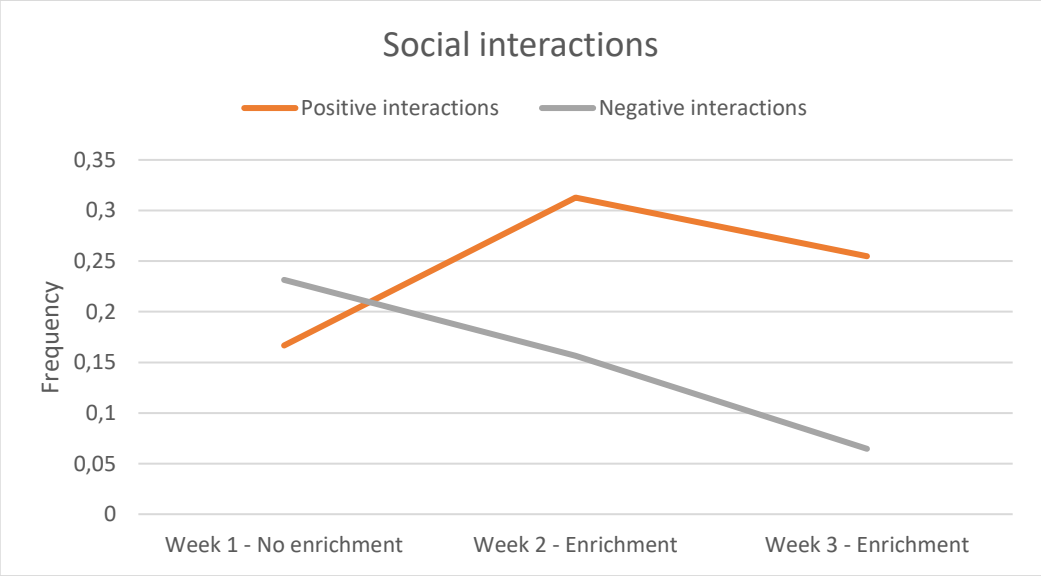


Figure 4. Average value per observation for positive and negative social interactions.

5. Discussion

5.1. The aim of the study

5.1.1. The interaction with the enrichment

The results showed that the rats interacted with the temporary enrichment in various frequency. They were most active with the temporary enrichment during the first observation in both week 2 and 3, this could be explained by the novelty of the object. The second observation was during the afternoon and since the rats are nocturnal, the afternoon is probably when they are sleeping. The third observation was during the next morning where the rats were a bit more awake and more active, but the novelty of the object had probably gone down. During week 2, the rats interacted with the cardboard most, but during week 3 it was the fabric that was most interacted with. When the enrichment was removed from the cage the cardboard was found ripped in very small pieces, whilst the fabric and paper had hardly been ripped apart at all. The cardboard could have been ripped apart more rapidly during week 3 and therefore lost its value faster. The fabric was mostly used by the rats to sleep on, which could explain why they interacted with it the most compared to the other enrichments during observation number two when they could have been resting. Since only the frequency and not the duration of the interaction was studied, they could also have been interacting with the cardboard during longer periods during week 3, which would not have shown in the data. The novelty of enrichment should be taken into consideration when deciding what type of enrichment should be given and how often.

5.1.2. The activity levels of the rats

During week 3 the activity levels were lower than week 1, although the resting behaviours were not lower week 3 compared to week 1. This could be explained by the registrations method since this study registered all behaviours as frequency and not as duration. The rats could have been active for a longer time during week 2 and 3 but it did not show in the number of registrations. Therefore the activity behaviours could have been performed less frequent but in longer durations, this is

more likely since the frequency of the resting behaviours did not increase during week two and three.

5.1.3. The frequency of unwanted behaviours

Even though the positive interactions were a bit lower week 3 compared to week 2, the negative interactions were at the highest during week 1, where no temporary enrichment was present. The results in this study support the earlier findings that enrichment can lower the amount of aggressive behaviour towards other rats (Abu-Ismail, 2011; Johnson *et al*., 2004).

The unwanted behaviours that were mostly shown in the rats was the negative interactions between the rats, i.e. fighting. The positive social interactions were mostly "play fighting". The negative interactions that were seen in week 2 and 3 were mostly rats that pushed another rat away from the temporary enrichment or the food, rarely was there any allogrooming towards another rat's lower part of the body.

According to Burke *et al*. (2021), lack of play-fighting behaviours can be seen as a sign of depression in rats. Since the rats in the present study were showing more play-fighting behaviours in the presence of temporary enrichments, conclusions could be drawn that temporary enrichment could result in happier rats.

5.2. Suggestions for improving enrichment

Since this study showed that the rats had different levels of interactions with different types of temporary enrichment it is probably important to vary what type of enrichment is given to the animals. Enrichment made with different types of materials that the animals can manipulate in different ways would be preferred to include as many natural behaviours as possible. According to Hutchinson *et al*. (2005) enrichment which provides the rats the opportunity to change the enrichment themselves could also help with bringing out a new interactive response. Different enrichment materials could be larger pieces of sturdy paper, for example cardboard which could be shredded to smaller pieces. Another example could be rubber that can be moved and thrown by the rats but harder to bite pieces off or fabric that can be stripped and used as bedding. The enrichment could also be given in different ways, for example being tied to the cage so the rats have to work to be able to carry it with them. The enrichment should be given according to a schedule to minimise the risk of repeating the same type too often and the rats losing interest due to the lack of novelty.

5.3. Method, literature and possible errors

This study had some possible errors. The study was carried out during three weeks in the light period of the day cycle. Since rats are normally nocturnal, this could have affected their behaviour and the outcome of the study. Many animal experiments are conducted during the light period of the day, and therefore it could be argued that it is positive to conduct these type of behaviour studies in the light phase as well. On the other hand, it has been shown that rats can show different types of behaviours and be more prone to stressors during the light period (Hawkins & Golledge, 2018; Aslani *et al*., 2014) and maybe they could also be less motivated to use enrichment during the light period.

Since the rats were filmed and a focal animal was used it was relatively easy to distinguish between which animal did different types of behaviour. It would have been interesting to be able to film the animals when no one was in the room to rule out the possible affect the observer had on the animals.

The rats in this study hade the possibility to hide from the light in the plastic tube or under wood wool, which could help them feel safer from different stressors, and thus lead to behaviours that are more normal. Because of the enrichment in the cage, it was sometimes hard to see if the rats were sleeping or just laying still, since the eyes were not always visible.

In the literature, it was a bit hard to distinguish relevant articles since there are no overall rule that decides what enrichment is. In some cases, enrichment was defined as having wood chips instead of wiring as flooring (Bradshaw & Poling, 1991), where in other cases enrichment was considered having access to toys or different types of nesting materials (Belz *et al*., 2003). de Azevedo *et al*. (2007) did a GAP analysis about enrichment and concluded that there need to be more studies made on the effect of enrichment on animals and the effect on experimental design.

The rats in this study were older male Lewis rats, an outbred strain that is not as commonly used as the strains Sprague Dawley and Wistar (Johnson, 2012). Although it may be more relevant to use a more commonly used strain, it is also positive to perform these types of studies on many different strains to be able to get an overall baseline on how enrichment affects different types of strains and sexes.

5.4. Ethical perspective

Animals in research is often a debated topic. According to the Swedish definition of what constitutes as a research animal, 5 801 463 animals were used in research during 2018, the majority of these were fish and octopuses and 17 945 were rats (less than 1%) (Jordbruksverket, 2019). By using animals in research, we take on a great responsibility of treating the animals in the best way we can based on the conditions that exists.

By constantly striving for replacing animal experiments, reducing the number of animals used and refining the way we keep and handle the animals (3R) (Swedish 3Rs Center, 2021), we can improve the life of many animals. Particularly the refinement aspect is important since this aspect affects the welfare of the animal in all aspects of the experiment. In particular, to ensure that the animals can perform as wide range of natural behaviours as possible, we can increase the welfare of the animals.

It is sometimes believed that a standardisation of housing is needed to be able to reproduce results in animal experiments and that different types of enrichment prevents this. This thought is now being argued with statements that a standardisation of the animals' environment can produce less validity regarding reproducibility between laboratories compared to animals held in an enriched environment (Van de Weerd *et al*., 2002; Wolfer *et al*., 2004; Würbel & Garner, 2007; Richter *et al*., 2009;; According to Richter *et al*. (2009) a standardised environment could make the researchers not notice and/or take in regard the differences in the environment that could vary (different sounds in the labs, different people that handle the animal, different waters etc.). Richter *et al*. (2009) concludes that animals in non-standardised environments are more alike since you can notice similarities between the animals even though they are being kept in different environments and therefore there is less of a risk for a false positive and/or false negative. By introducing enrichment you also introduce the refinement aspect of the 3Rs, but one should find out if the enrichment potentially could affect the study (Baumans, 2005; Bayne, 2005; van de Weerd *et al*., 2002). Van de Weerd *et al*. (2002) and Zaias *et al*. (2008) emphasize that some variations could exist depending on what type of parameters you study. For example, a growth difference between rats in enriched cages and non-enriched cages existed (Zaias *et al*., 2008). Where enrichment does not affect variability or where it decreases the varieties of the animals, there is no reason not to use enrichment since it affects the welfare in such a positive way (Van de Weerd *et al*., 2002).

By introducing enrichment to the animals' environment and by that getting a decrease in the variations, it could result in less animals needed in animal experiments. We will as well, most likely, have less stressed and less depressed animals, which makes the results of future studies more reliable and easier to replicate. In this way, we can also introduce the reduce aspect of the 3Rs, as fewer animals and studies need to be used to get significant results.

5.5. Further research

In further research, it would be interesting to see if animals that have had access to temporary enrichment from a young age would have behaved differently from animals who never had temporarily enrichment or from the rats in this study. This

is important since we can not always decide how early in life the animals gets the enrichment, it could all depend on the breeders enrichment program. It would also be interesting to see how temporary enrichment would affect their performance in experimental studies or overall handling of the rat, maybe in regard to different levels of stress hormones. Since not all researchers are comfortable with handling animals they could be more opposed to enrichment if the rats get more active when handled. The opposite could also happen, if the rats are less stressed since they have been able to perform their natural behaviours, maybe they could become even easier to handle. Further research should also focus on the individual variations of each rat to see if it is mostly one rat using the different types of enrichment in different ways, this to exclude the possibility of the preference of one individual to one type of enrichment affecting the whole group. Although, this could be easy to avoid by having a rotating schedule for the enrichment.

Previous studies has shown that chronic mild stress could increase the level of grooming (Jolles *et al*., 1979) in both frequency and duration with more short stops for grooming (Kalueff &Tuohimaa**,** 2005). Because of that more studies should be done in whether enrichment could affect grooming levels and how that would affect the rats stress levels. Unfortunately this part could not be fitted within this limited time in the present study.

By getting a larger number of animals with different variations to collect data from we could increase the knowledge of how the animals are affected by different situations as a group and maybe have a general idea on how enrichment effects rats in general. This would be important to be able to get more stable base levels and in turn more reliable data from the research, as stated previously.

6. Conclusion

This study showed that the rats interacted the least with paper and the most with cardboard and fabric, however the frequency of interactions with fabric and cardboard differed throughout the weeks. A conclusion could therefore be drawn that it is important to vary between different types of enrichment since the rats could use them for different purposes. It is also important to take into consideration that the novelty of the object is important when giving enrichment to rats. These rats also displayed a higher level of activity when they were offered the temporary enrichment, which could be a sign of higher welfare. The study also showed that the rats displayed a higher level of positive interactions with their cage mates when given the enrichment, and a decreasing level of negative interactions was shown. The literature show that by introducing enrichment to animals they will most likely be less stressed and depressed and it will also increase the likelihood of having more reliable data in research.

7. Popular science summary

Animal testing is a hot topic and it is often discussed whether or not it should exist. Something that a lot of people can find common grounds in is that when we are performing animal testing, the animals should have the best optimal care and they should be able to live as what they are: rats.

So what is important to a rat? Rats in the wild spend a lot of time on gathering different resources, this could be food, different materials to put in their nests or things to gnaw on. They also spend a lot of time interacting with other rats. In a laboratory setting it can be difficult to accommodate these different behaviours but a lot of studies have started to show the importance of giving the animals the opportunity to perform species-specific behaviours. In this study, the aim was to find out how the rats interacted with different types of materials, and how it affected them. Cardboard, paper and fabric were given to the rats and the behaviours they showed were then evaluated. It showed that the rats interacted with the cardboard and fabric the most. The rats were also more active when they were offered the materials, which is important to note since inactive rats can be a sign of depression. When the rats did not have the extra materials, they showed more negative behaviours towards other rats. When the materials were offered they showed more play-behaviours. Aggression in rats can be a sign of stress or anxiety, so it is important to see more behaviours that are playful in the animals.

Previous studies have shown that calm animals that can perform a lot of species-specific behaviour is important in research since it will give a more reliable result.

By giving the rats more materials so they can actually behave as rats, we will most likely have calmer and less depressed rats.

References

Abou-Ismail, U.A. 2011. The effects of cage enrichment on agonistic behaviour and dominance in male laboratory rats (Rattus norvegicus). *Research in Veterinary Science*, 90 (2), 346–351. https://doi.org/10.1016/j.rvsc.2010.06.010

Abou-Ismail, U.A., Burman, O.H.P., Nicol, C.J. & Mendl, M.T. 2007. Can sleep behaviour be used as an indicator of stress in group-housed rats (Rattus norvegicus)? *Animal Welfare*, 2007 (Volume 16, Issue 2), 185–188

Aslani, S., Harb, M.R., Costa, P.S., Almeida, O.F.X., Sousa, N. & Palha, J.A. 2014. Day and night: diurnal phase influences the response to chronic mild stress. *Frontiers in Behavioral Neuroscience*, 8. https://doi.org/10.3389/fnbeh.2014.00082

de Azevedo, C.S., Cipreste, C.F. & Young, R.J. 2007. Environmental enrichment: A GAP analysis. *Applied Animal Behaviour Science*, 102 (3–4), 329–343. https://doi.org/10.1016/j.applanim.2006.05.034

Barnett, S.A. 2007. *The rat: a study in behavior*. New Brunswick, N.J: AldineTransaction.

Baumans, V. 2005. Environmental Enrichment for Laboratory Rodents and Rabbits: Requirements of Rodents, Rabbits, and Research. *ILAR Journal*, 46 (2), 162–170. https://doi.org/10.1093/ilar.46.2.162

Bayne, K. 2005. Potential for Unintended Consequences of Environmental Enrihment for Laboratory Animals and Research Results. *ILAR Journal*, 46 (2), 129–139. https://doi.org/10.1093/ilar.46.2.129

Belz, E.E., Kennell, J.S., Czambel, R.K., Rubin, R.T. & Rhodes, M.E. 2003. Environmental enrichment lowers stress-responsive hormones in singly housed male and female rats. *Pharmacology Biochemistry and Behavior*, 76 (3–4), 481–486. https://doi.org/10.1016/j.pbb.2003.09.005

Bracke, M.B.M. & Hopster, H. 2006. Assessing the Importance of Natural Behavior for Animal Welfare. *Journal of Agricultural and Environmental Ethics*, 19 (1), 77–89. https://doi.org/10.1007/s10806-005-4493-7

Bradshaw, A.L. & Poling, A. 1991. Choice by rats for enriched versus standard home cages: plastic pipes, wood platforms, wood chips, and paper towels as enrichment items. *Journal of the Experimental Analysis of Behavior*, 55 (2), 245–250. https://doi.org/10.1901/jeab.1991.55-245

Brydges, N.M., Leach, M., Nicol, K., Wright, R. & Bateson, M. 2011. Environmental enrichment induces optimistic cognitive bias in rats. *Animal Behaviour*, 81 (1), 169–175. https://doi.org/10.1016/j.anbehav.2010.09.030

Burke, C.J., Modlinska, K., Mauro, M.H., Aleksandrova, L.R., Pellis, S.M., Phillips, A.G. & Euston, D.R. 2021. A naturalistic method to test depression: Anticipation of play. *Behavioural Brain Research*, 398, 112975. https://doi.org/10.1016/j.bbr.2020.112975

Burn, C.C. 2008. What is it like to be a rat? Rat sensory perception and its implications for experimental design and rat welfare. *Applied Animal Behaviour Science*, 112 (1–2), 1–32. https://doi.org/10.1016/j.applanim.2008.02.007

Foster, S., King, C., Patty, B. & Miller, S. 2011. Tree-climbing capabilities of Norway and ship rats. *New Zealand Journal of Zoology*, 38 (4), 285–296. https://doi.org/10.1080/03014223.2011.599400

Hawkins, P. & Golledge, H.D.R. 2018. The 9 to 5 Rodent − Time for Change? Scientific and animal welfare implications of circadian and light effects on laboratory mice and rats. *Journal of Neuroscience Methods*, 300, 20–25. https://doi.org/10.1016/j.jneumeth.2017.05.014

Himmler, S.M., Modlinska, K., Stryjek, R., Himmler, B.T., Pisula, W. & Pellis, S.M. 2014. Domestication and diversification: A comparative analysis of the play fighting of the Brown Norway, Sprague-Dawley, and Wistar laboratory strains of (Rattus norvegicus). *Journal of Comparative Psychology*, 128 (3), 318–327. https://doi.org/10.1037/a0036104

Hosey, G.R., Melfi, V. & Pankhurst, S. 2013. *Zoo animals: behaviour, management and welfare*. 2nd edition. Oxford: Oxford University Press.

Hutchinson, E., Avery, A. & VandeWoude, S. 2005. Environmental Enrichment for Laboratory Rodents. *ILAR Journal*, 46 (2), 148–161. https://doi.org/10.1093/ilar.46.2.148

Johnson, M. 2012. Laboratory Mice and Rats. *Materials and Methods*, 2. https://doi.org/10.13070/mm.en.2.113

Johnson, S., Patterson-Kane, E. & Niel, L. 2004. Foraging enrichment for laboratory rats. *Universities Federation for Animal Welfare*, 13 (3), 305-312(9)

Jolles, J., Rompa-Barendregt, J. & Gispen, W.H. 1979. Novelty and grooming behavior in the rat. *Behavioral and Neural Biology*, 25 (4), 563–572. https://doi.org/10.1016/S0163-1047(79)90362-5

Jordbruksverket. 2019. Användning av försöksdjur i Sverige under 2018. (Dnr: 5.2.17-17593/2019).

Kalueff, A.V. & Tuohimaa, P. 2005. The grooming analysis algorithm discriminates between different levels of anxiety in rats: potential utility for neurobehavioural stress research. *Journal of Neuroscience Methods*, 143 (2), 169–177. https://doi.org/10.1016/j.jneumeth.2004.10.001

Kompagne, H., Bárdos, G., Szénási, G., Gacsályi, I., Hársing, L.G. & Lévay, G. 2008. Chronic mild stress generates clear depressive but ambiguous anxiety-like

behaviour in rats. *Behavioural Brain Research*, 193 (2), 311–314. https://doi.org/10.1016/j.bbr.2008.06.008

Krinke, G.J. 2000. *The Laboratory Rat*. Elsevier. https://doi.org/10.1016/B978-0-12-426400-7.X5037-7

Modlinska, K. & Pisula, W. 2020. The Norway rat, from an obnoxious pest to a laboratory pet. *eLife*, 9, e50651. https://doi.org/10.7554/eLife.50651

Modlinska, K., Stryjek, R. & Pisula, W. 2015. Food neophobia in wild and laboratory rats (multi-strain comparison). *Behavioural Processes*, 113, 41–50. https://doi.org/10.1016/j.beproc.2014.12.005

Pellis, S.M. & Pellis, V.C. 1987. Play-fighting differs from serious fighting in both target of attack and tactics of fighting in the laboratory rat Ratus norvegicus. *Aggressive behavior*, 13 (4), 227–242. https://doi.org/10.1002/1098-2337(1987)13:4<227::AID-AB2480130406>3.0.CO;2-C

Pellis, S.M. & Pellis, V.C. 2007. Rough-and-Tumble Play and the Development of the Social Brain. *Current Directions in Psychological Science*, 16 (2), 95–98. https://doi.org/10.1111/j.1467-8721.2007.00483.x

Richter, C.P. 1959. Rats, man, and the welfare state. *American Psychologist*, 14 (1), 18–28. https://doi.org/10.1037/h0043834

Richter, S.H., Garner, J.P. & Würbel, H. 2009. Environmental standardization: cure or cause of poor reproducibility in animal experiments? *Nature Methods*, 6 (4), 257–261. https://doi.org/10.1038/nmeth.1312

Sheperdson, D.J., Mellen, J.D. & Hutchins, M. 1998. *Second Nature: Environmental Enrichment fpr Captive Animals*. Washington DC: Smithsonian Institution Press.

Stryjek, R., Modlińska, K. & Pisula, W. 2012. Species Specific Behavioural Patterns (Digging and Swimming) and Reaction to Novel Objects in Wild Type, Wistar, Sprague-Dawley and Brown Norway Rats. (McGregor, A. P., red.) *PLoS ONE*, 7 (7), e40642. https://doi.org/10.1371/journal.pone.0040642

The swedish 3Rs center. https://jordbruksverket.se/languages/english/the-swedish-3rs-center/these-are-the-3rs. Used 2021-03-21.

Van de Weerd, H.A., Aarsen, E.L., Mulder, A., Kruitwagen, C.L.J.J., Hendriksen, C.F.M. & Baumans, V. 2002. Effects of Environmental Enrichment for Mice: Variation in Experimental Results. *Journal of Applied Animal Welfare Science*, 5 (2), 87–109. https://doi.org/10.1207/S15327604JAWS0502_01

Westlund, K. 2014. Training is enrichment—And beyond. *Applied Animal Behaviour Science*, 152, 1–6. https://doi.org/10.1016/j.applanim.2013.12.009

Wolfer, D.P., Litvin, O., Morf, S., Nitsch, R.M., Lipp, H.-P. & Würbel, H. 2004. Cage enrichment and mouse behaviour. *Nature*, 432 (7019), 821–822. https://doi.org/10.1038/432821a

Würbel, H. & Garner, J.P. 2007. Refinement of rodent research through environmental enrichment and systematic randomization. *NC3Rs*, (9), 1–9

Young, R.J. (red.). 2003. *Environmental Enrichment for Captive Animals*. Oxford, UK: Blackwell Science Ltd. https://doi.org/10.1002/9780470751046

Zaias, J., Queeney, T.J., Kelley, J.B., Zakharova, E.S. & Izenwasser, S. 2008. Social and Physical Environmental Enrichment Differentially Affect Growth and Activity of Preadolescent and Adolescent Male Rats. *American Association for Laboratory Animal Science*, 47 (2), 30-34(5)

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