THE EFFECTS OF SWITCHING LIGHT-DARK REGIME ON THE BEHAVIOR OF WISTAR RATS

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1. Summary

Most living beings have a circadian rhythm that spans about 24 hours. Rodents are nocturnal animals, which means they are active during nighttime when there is no or little light present. Therefore, when evaluating behavioral aspects of nocturnal animals, this should preferably be done during the dark-phase. The goal of this research is to determine how this light-dark switch has an effect on the behavior of laboratory Wistar Unilever rats, and to determine the time needed for acclimatization. Overall we can state that transport itself had a clear effect on animals. Both reversal and control groups display a significant increase of resting behavior in week 1 comparing to week -1. Autogrooming shows an increase in the first week after transport in both the control groups, and there does seem to be a higher amount of autogrooming overall in both control groups. The amount of eating behavior clearly decreases during the first week in both reversal groups however this is not the case in the control groups. Playing and social behavior are both very variable during the entire experiment. In the male groups there is a decrease of playing and social behavior during the first week after transport however in the female groups this is not the case. When looking at active behavior we see that both the control and reversal groups display a decrease in week 1 in comparison to week -1. Here again the control groups also seem to be affected by the transport itself. We can conclude that it may be preferred to keep nocturnal animals in a reversed day-night cycle so that they won't be disturbed during their resting period, however this requires more research.

2. Introduction

Most living beings have a circadian rhythm that spans about 24 hours. In this circadian rhythm we distinguish an active phase and a resting phase of which the timing is species-dependent. Rodents are nocturnal animals, which means they are active during nighttime when there is no or little light present. Therefore, when evaluating behavioral aspects of nocturnal animals, this should preferably be done during the dark-phase.
Many researchers choose to switch the light-dark phases in behavioral researches in rodents so that during daytime, when the researchers are awake and present, the animals are in the dark-phase and therefore in their activity-phase. This means that, after transportation of the animals from their breeder to the research lab, the animals have a 'jet-lag' of 12 hours. The goal of this research is to determine how this light-dark switch has an effect on the behavior of laboratory rats, and to determine the time needed for acclimatization. Also we will determine if there is a significant difference in the response on the light-dark shift in males and females.

2.1. The circadian rhythm

Most living beings like plants, animals, and even bacteria have a circadian rhythm. This is a 24 hour day-night rhythm of activity and rest, the timing of which are dependent on the species (for example nocturnal or diurnal species). The circadian rhythm provides a way to 'anticipate external cues and to adapt molecular and behavioral processes to specific day-time with the advantage of temporally separating incompatible metabolic processes'[1].
The core of the circadian rhythm is the circadian clock, an "internal clock" in a living being which has a period of approximately 24 hours. It is influenced by different Zeitgebers. A Zeitgeber (German for "time giver" or "synchronizer") is an exogenous cue which synchronizes the internal clock with the earth's day-night cycle. Light is the most prominent Zeitgeber of the circadian rhythm in mammals. There are other factors like food intake and social interaction but the effect of these are minimal in comparison to the effect of light.[2, 3]
In mammals the circadian clock is present in the suprachiasmatic nucleus (SCN); a portion of the brain the size of a grain of rice containing over 20.000 neurons. It is present in the anterior part of the hypothalamus.[1] The SCN can be divided in a ventrolateral and dorsomedial part. The ventrolateral SCN responds to light influences. [4]
The SCN works as a pacemaker which in turn synchronizes peripheral clocks as seen in fig. 1. Thus the SCN influences multiple organs like the liver, heart, lungs and kidney[5]. This way, the body can activate multiple organs necessary during the active phase and limit unnecessary processes during the resting phase.[6]

Light enters the eye and is absorbed by specialized photoreceptors in the retina. These send a signal via the retinohypothalamic tract (RHT) to the circadian pacemaker in the SCN which is located in the anterior hypothalamus. In response to this signal, the SCN then sends signals to the pineal gland and other hypothalamic nuclei.[7]
The SCN regulates the 24-hour cycle of sleep and wakefulness, but also maintains other physiological processes. For one, the SCN stimulates the release of CRH and vasopressin (AVP) by the hypothalamus. CRH and AVP stimulate the release of ACTH by the pituitary, which in turn leads to a release of glucocorticoids by the adrenal gland (*fig 1*).[3, 6] AVP also functions as an antidiuretic hormone and causes vasoconstriction.
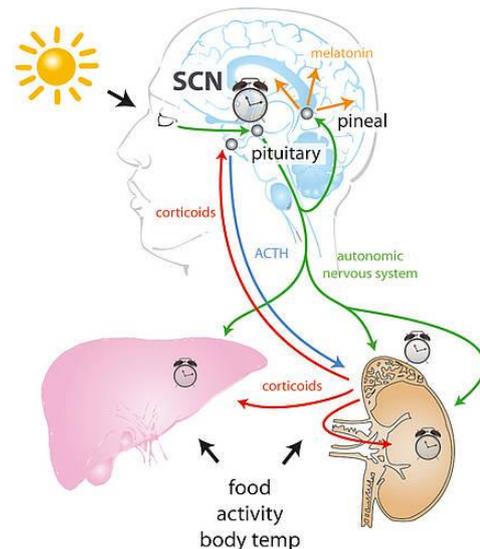
Fig. 1: The circadian rhythm.
Light reaches the photoreceptors in the retina which send a signal to the SCN. The SCN then regulates indirectly the release of ACTH by the hypothalamus and is inhibits (when influenced by light) the release of melatonin. This way the SCN synchronizes the peripheral circadian clocks.

Furthermore the SCN inhibits the production of melatonin by the pineal gland. This inhibition is also directly caused by light.[3]
Glucocorticoids peak during the time of activity (daytime in diurnal and nighttime in nocturnal animals) and melatonin peaks always during nighttime[8].

2.2. Melatonin

Melatonin has two important functions. Firstly it inhibits the LH production by the pituitary gland and therefore has an influence on the estrous cycle.[9]
Secondly it has an important function in the circadian rhythm although this is not fully understood. In general it seems to have an effect on the physiology and behavior that are appropriate to a nocturnal state. However it is not necessarily associated with sleep, since both nocturnal and diurnal species have high concentrations of melatonin during nighttime (*fig. 2*).[8, 10-12]

2.3. Glucocorticoïds

Glucocorticoids are released during the active phase of the animal. This is daytime in diurnal and nighttime in nocturnal animals. Glucocorticoids have many different functions in homeostasis and stress. Most of the functions are to maximize one's ability to deal with a stressor (with epinephrine and norepinephrine). For example, glucocorticoids lead to an increased cardiovascular tone, concentration of energy to muscles (for fight or flight), inhibition of reproductive physiology and appetite, increases in cerebral perfusion rates and sharpened cognition.[1]

Furthermore glucocorticoids have an immunesuppressive effect. They also have a stimulating effect on the peripheral clocks in the liver, heart and finally they possibly also have an effect on the circadian clock in the hypothalamus.[6]


Fig 2: Regulation of cortisolrelease.
Stress induces the release of CRH which leads to a release of ACTH. This in turn leads to a release of cortisol. Cortisol binds to a glucocorticoid-receptor, which has an inhibitory effect on CRH and ACTH. This way cortisol has a negative feedback on its own release.{{158 Sriram,K. 2012}}

The metabolic function of glucocorticoids is to increase the glucose concentration by stimulating gluconeogenesis by the liver, mobilization of amino acids, inhibition of glucose uptake by muscles and adipose tissues and they stimulate fat breakdown.[6]
The most important glucocorticoid is cortisol.

Glucocorticoids are released both pulsatile (short peaks) during stress and circadian rhythmic by the adrenal gland.[6] Under the influence of stress or in response to a signal from de SCN, the hypothalamus releases corticotrophin-releasing hormone (CRH) and arginin vasopressin (AVP). These induce the release of adrenocorticotropic hormone (ACTH) by the pituary gland. In turn ACTH stimulates the synthesis of corticosteroids by the adrenal gland (*fig. 2*).[13]
The circadian peak occurs early in the morning (diurnal) or early in the night (nocturnal) at the start of the active phase.[6]

2.4. Diurnal and nocturnal animals

Animals can be divided into nocturnal (active during nighttime), diurnal (active during daytime) and crepuscular (active during twilight). [12, 14] Humans for example are diurnal and therefore active during daytime, while most rodents are nocturnal.[11] Most hormones are therefore reversed so the organs are active when necessary (during the activity phase). The concentration of melatonin however is high during nighttime in both diurnal and nocturnal animals (*fig. 3*).
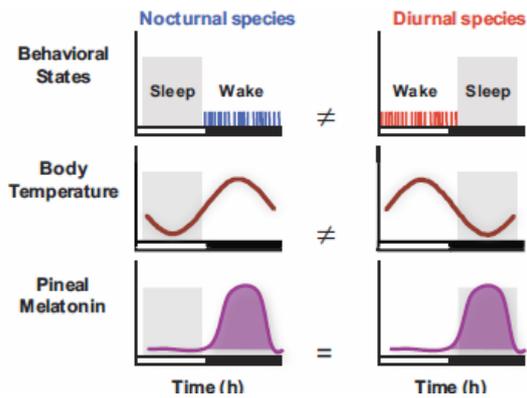
Fig. 3: The differences between nocturnal and diurnal species in body temperature and melatonin. *Day- and nighttime are indicated by the black (night) and white (day) bars on the x-axis. While the sleep period and the body temperatures are oppositely phased, the melatonin synthesis takes place during nighttime in both categories.[11]*

The difference in mechanism between diurnal and nocturnal animals is still not completely understood. What we do know is that during daytime (light-phase), the SCN of both diurnal and nocturnal animals have a higher fire rate. Apparently, in nocturnal animals this signal inhibits awakening and promotes sleeping[15]. There is no clear difference within the SCN of nocturnal or diurnal species however this does not exclude a possibility of a switch between the patterns of coupling between the clock and its outputs that resides within the SCN[16].

2.5. Phase shift

The circadian rhythm has the possibility to adjust to the light-dark phase. The circadian time is 0 at the beginning of the light phase and it is 12 at the beginning of the dark phase[10]. When the retina absorbs light early in the morning this sets the circadian clock to an earlier time; it produces a phase advance. Light exposure in the evening sets the circadian clock to a later time; it produces a phase delay (*fig. 4*).[10, 11, 13, 14]
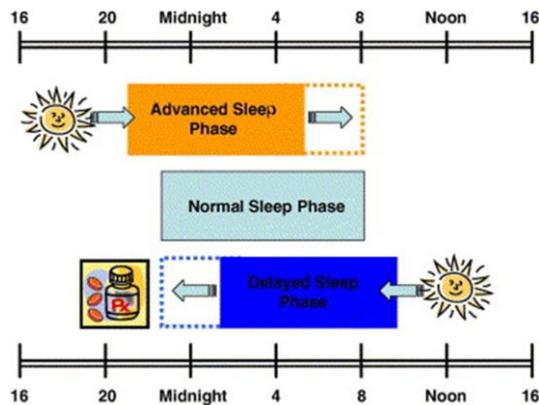
Fig. 4: Phase shift mechanism. *Light exposure in the morning leads to an advanced sleep phase i.e. early morning awakening and early night resting. Light exposure in the evening leads to a delayed sleep phase i.e. late awakening and late resting in comparison to the normal range.[13]*

When a shift in the external cycle of light-dark rhythm takes place, neurons of the SCN resynchronize with different pace. The circadian system however requires several days to adjust to this shift. During his period of readjusting, a temporal disruption of daily rhythm is observed. This is called jet lag disorder (JLD) or jet lag.[2]

2.6. Jet lag disorder

Jet lag disorder is a sleep disorder caused by the inability of the circadian rhythm to fully adapt to a different light-dark rhythm.[13] Thus the pathophysiologic mechanism of jet lag disorder is a mismatch between the endogenous circadian rhythms and exogenous signals.[10]
As stated before, the SCN has a dorsomedial part (DMSCN) and a ventrolateral part (VLSCN). The latter responds to light, while the first is unresponsive to light. These two are in synchrony with each other. When there is a shift in the night-day regime, the VLSCN

also shifts rapidly. The DMSCN however shifts slowly. This leads to dissociation and an altered sleep pattern (fig 5).[4]
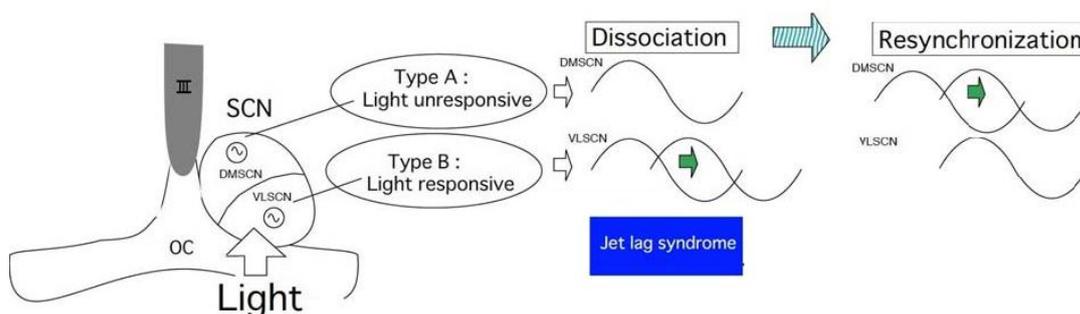
Fig 5: The mechanism of jet lag.
A change in the light-dark phase leads to a rapid shift in the VLSCN, however the DMSCN shift is less rapid. This leads to a dissociation which has to resynchronize.[4]

In humans, jet lag is associated with the rapid crossing of time zones during inter-continental flights. The circadian clock has no time to adjust to the different time zone. It has been stated that it takes about a day per time zone for the circadian clock to adjust.[17]

Symptoms regarding jet lag are[10, 17]:
1. Insomnia (mostly during eastward travel) or excessive sleepiness during daytime (mostly during westward travel)[13]
2. Depression
3. Decreased physical performance
4. Cognitive impairment, decreased alertness
5. Gastrointestinal disturbances, general malaise

Although mostly discussed in humans because of intercontinental travelling, jet lag syndrome also occurs in animals. Nagano et al. described the effect of an abrupt shift in the light-dark cycle on Wistar rats.[4] They found that after a 10 hour delaying shift the rats had an extended resting period.

2.7. Why do researchers switch the light-dark phases?

As mentioned before, many researchers chose to change the light-dark rhythm so that nocturnal animals can be evaluated during their active phase. Results of behavioral testing can be affected when carried out in the light phase. For example, rats in the forced-swim test during the light phase were more stressed than those in the dark phase.[18] Roedel et al. found that light has a significant impact on the behavior of DBA mice. Locomotor activity and general exploration were significant lower during the resting phase compared with the active phase. Also the DBA mice showed a more pronounced inhibition of food intake and unfamiliar food exploration during the resting phase. Furthermore, the animals preformed a higher number of wrong choices, showed a higher anxiety-related behavior, and took more time on the modified hole board during resting phase.[19]This is confirmed by Bertogio and Carobrez, who also stated that general exploration reduces during the resting phase.[18]
Kelliher et al. revealed that rats put in a forced swim test react different depending on the light- or dark phase. Animals that were tested in the dark phase (active phase) displayed a significant decrease in time spent in escape-oriented activity. They also observed that these rats displayed signs of reduced stress, and that they were less agitated or aggravated when being handled on removal from the swim apparatus. From this Kelliher concluded that the animals were less stressed when tested during the nocturnal phase.[20]

Because of these influences on experimental results, many researchers choose to change the light-dark rhythm in behavioral experiments.

2.8. Rat behavior

In order to establish if our animals are affected by the light-dark shift, we will evaluate a number of behavioral patterns (appendix 1). First we will discuss the meaning of some behavioral patterns.

2.8.1. Grooming
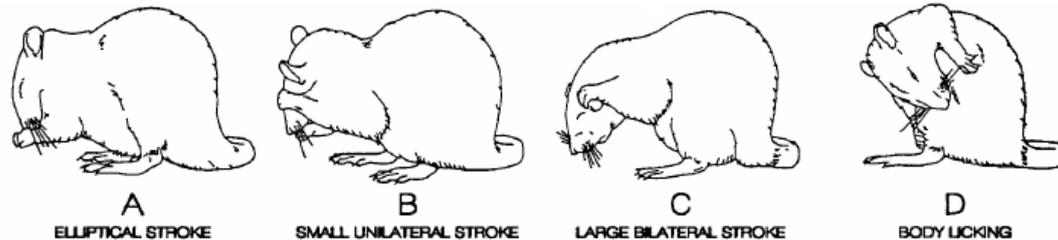
Fig 6: grooming behavior in rats.
Firstly the animal conducts 5-9 rapid elliptical strokes over the nose and mystacial vibrissae (A). This is followed by (B) small asymmetrical strokes of increasing amplitude around the face. Thirdly (C) the animal conducts large bilateral strokes, followed by (D) a posturnal turn followed by body licking of the flank. After this the genitals and tail are groomed.

Grooming behavior is divided in self grooming and allogrooming (grooming of a companion), the latter we will discuss in social behavior. Self grooming (autogrooming) is seen after many different activities, for example after social contact, sexual behavior, exploratory behavior. It also usually precedes sleeping. Autogrooming usually acts as a response to arousal and it seems to reflect the process of dearousal.[21, 22]
Grooming behavior includes licking, scratching and nibbling the fur. It has many functions including the removal of parasites, thermoregulation (mostly in desert animals) and chemocommunication. It may also have an antibacterial effect because of the antibacterial properties of rat saliva.[21, 22]
Grooming plays an important role in behavioral adaptation to stress (stress-coping and de-arousal). The exact function of autogrooming in stressfull situations is still not fully understood as it can increase in both high and low amounts of stress. In stressfull situations (for example a novel environment) rodents display a higher amount of short, unfinished grooming bursts. Non-stressfull grooming is mostly displayed as a transition from rest to activity (or reversed) and, unless the animal is disturbed, includes the entire grooming bout.[23]
Rats spend almost half of their time during waking hours at grooming and have a certain pattern in which they express grooming (fig 6). The grooming bout starts with paw-licking or face-washing followed by grooming of the fur around the head, neck and eventually the body. This is then followed by licking of the genitals and finally the tail.[24]
We will determine the amount of self-grooming in our different experimental groups.

2.8.2. Social behavior

Social behavior includes social grooming (allogrooming), social exploration and all other social interactions with the exception of social playing/fighting which we will determine separately.
Tactile social contact (like social grooming) is part of the communicatory tool kit of many species. Social grooming involves gently nibbling and licking the fur of a cage companion. This seems to induce a positive affective state, as the recipient adapts a relaxed body tone and droopy eyelids. Therefore tactile social contact seems to manipulate the affective state of the recipient.[25]
As in self-grooming the amount of social interaction will decrease when experiencing chronic stress. [26]

2.8.3. Playing
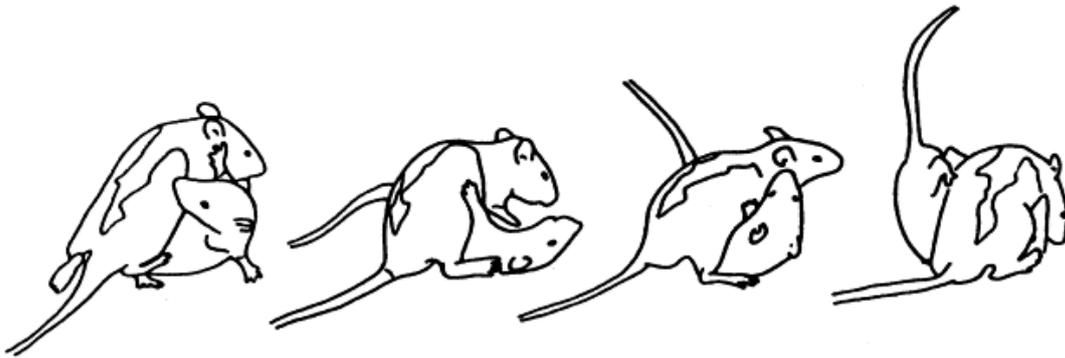
Fig 7: Pinning in rats*. [27]*

Social play behavior has a high reward value just like feeding, drinking and sexual behavior. Therefore it may be inferred that play behavior is very important as well. In rats it has been found that play deprivation leads to abnormal social, sexual or aggressive behavior in the adult animal. This leads to the conclusion that play behavior has an important role in the development of normal social behavior in the adult. Male rats display more play behavior than female rats, because female rats are more likely to withdraw from a play initiation.[28, 29]

It seems that the goal of playing in rats is to touch the nape of its playing companion. Social play includes pinning (fig 7), bouncing, punching, lateral display, charging, chasing and boxing or wrestling.[28, 29]

In this experiment we will evaluate the amount of social playing. As in social behavior and self-grooming, also the amount of social play will decrease during chronic stress. [26]

2.8.3. Resting/activity

As mentioned before, rodents are nocturnal animals whose activity phase lies in the dark phase (at night). Rats sleep about 80% of a 12 hour light period, but also about 30% of a 12 hour dark period.[24] The most active periods are the beginning and end of the dark period (fig 8).[29, 30]

In our study, "active" consist of all behavior except playing, eating or social behavior.

As this is a study of changing the light-dark switch, the amount of resting behavior (including sleeping, laying down or sitting without being alert) will give us an indication of the 'jet lag' the rats will experience.
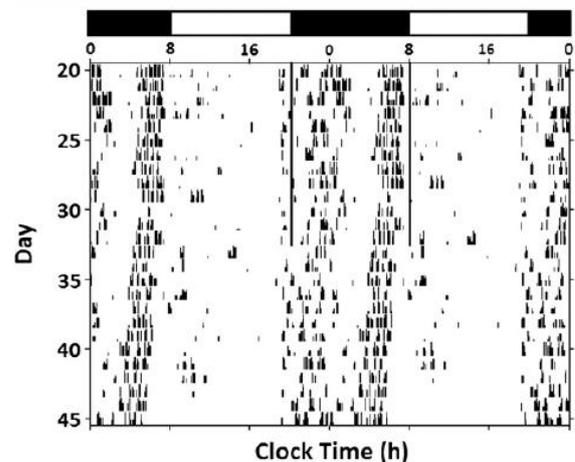
Fig 8*: Wheel running activity in rats.*
The black bar represents the dark phase and the white bar the light phase. The highest activity (black stripes) rates lie at the beginning and end of the dark phase. {{153 Oncil, Bayer, R. 2010}}

3. Materials and Methods

3.1 Animals and husbandry

Wistar Unilever rats (HsdCpb:WU) (n = 96) of which 48 males and 48 females were used. Breeding and weaning was executed in Harlan Laboratories B.V. Horst. After weaning all animals were housed in the surgical unit of Harlan Laboratories B.V. Horst, where baseline measurements were collected.

The animals were transported according to Harlan protocols. After transport the animals were housed in the animal unit of the Department of Anatomy and Neurosciences, VU University Medical Centre in Amsterdam. Here the post-transport measurements of the experiment were collected.

From weaning on the rats were housed in standard UNO type IV cages (556x334x195mm). All cages were marked with a number. Three animals were kept per cage and the group composition remained unchanged during the entire experiment. The cages were filled with bedding (Abedd LTE E-001) and cage cleaning occurred once a week. A plastic shelter was added per cage as enrichment.

During the experiment the animals were housed in controlled animal rooms with relative ambient temperature of 19.3-22.5ºC and a relative humidity of 58.5-67.1%. Light regime was maintained at 12 hours light on and 12 hours red light with a switch at 6.00 am and 18.00 pm.

Acified water and standard rodent food pellets (Harlan Teklad irradiated 18% protein rodent diet 2918) were provided ad libitum.

3.2. Experimental design

In this experiment we tried to mimic a regular transport of laboratory animals according to Harlan protocols.
The animals were divided into two batches, one male batch (n = 48) and one female batch (n = 48). Data was collected from these animals for 5 weeks, one week before transport and the weeks following transport. Male animals were moved immediately after weaning, female animals after a few days. With exception of that, both batches had the same experimental design.

The week before transport all animals were monitored under similar conditions to record baseline values. After transport the animals were randomly divided into two groups, a control group (n = 24) and an experimental group (n = 24). The control group remained under the same conditions as prior to transport; light phase from 6 am to 6 pm and dark phase from 6 pm to 6 am. The experimental group was held under opposite conditions; 6 am to 6 pm dark phase and 6 pm to 6 am light phase (Tabel 1).

Stage experiment	Group	Switch to dim light	Recording times
Baseline	All groups	6.00 pm	5.30 pm-6.30 pm 8.00 pm-9.00 pm
Post transport	Control Group	6.00 pm	5.30 pm-6.30 pm 8.00 pm-9.00 pm
Post transport	Experimental group	6.00 am	5.30 am-6.30 am 8.00 am-9.00 am

Tabel 1: Design of the experiment.

Recordings were made one hour at the time of the light switch (half an hour before – half and hour after) and again one hour after two hours of red light. Two cages were recorded by one camera, and four cameras were recording simultaneously. The recordings thus consist of 8 cages of animals (fig. 9).

Fig 9: Image recorded by the cameras.

Behavior was only evaluated from the recordings one hour after 2 hours of lights off, thus 8:00 pm – 9:00 pm in baseline and control groups and 8:00 am – 9:00 am in the experimental groups. This was decided because the light-dark switch itself had a large impact on the behavior of the rats (the transition from light to dark creates such a big impulse that the animals almost immediately become active all at once [29, 30]). In this hour the animals were followed twice for 5 minutes, the first starting at 05:00 after the beginning of the recording, and the second starting at 50:00 after the beginning of the recording.

One cage holding 3 animals was evaluated blind at an interval of 10 seconds per behavioral observation, thus 29 observations were made in 5 minutes.

5:30 – 6:30	8:00 – 9:00	
No observations were made	8:05 – 8:10 5 minutes of observation	8:50 – 8:55 5 minutes of observation

3.3 Behavioral parameters

In this experiment 7 different behavioral parameters will be evaluated; autogrooming, eating/drinking, resting, social exploration/social grooming, playing/fighting, all other behaviors (active) and not visible (see appendix 1). These were scored by one person whit the use of Noldus' The Observer 5.0.

3.4 Statistics

Since the amount of "not visible" data differed per day (during to quality differences and preferred cage places) the remaining behavioral parameters were calculated in percentages. This also solved the problem we had with some missing files due to camera problems.
Only the "active" data was normally distributed, for the other behaviors we ranked the percentages.

All data was analyzed by SPSS 16.0. We used a repeated measures ANOVA to analyze the overall daily and weekly data, and a dependent t-test to analyze the days and weeks individually. Furthermore a independent t-test was used to analyze the significance between the four groups.

We analyzed the development of the behavioral parameters during the experiment with the means per week (week -1 – week 4), and the development of the behavioral parameters per week with daily means (week -1; day -8 – day -4, week 1; day 1 – day 3, week 2; day 6, day 8 – day 10, week 3; day 13 – day 17, week 4; day 20 – day 23). This we have applied to the different genders and treatments (control groups and reversal groups of the male and female batches). Furthermore we compared the weeks following transport with the mean of the week prior to transport (= week -1, which we took as a "baseline").
We also compared the control and reversal groups per gender. In every behavioral parameter we also compared the reversal and control group during week -1 to determine the reliability of the baseline.
Finally a comparison was made between the genders for both treatments.

4. Results

The means and standard deviations of both the weekly and daily results are included in Appendix 2, 3 and 4.

4.1 Resting

Weekly development:
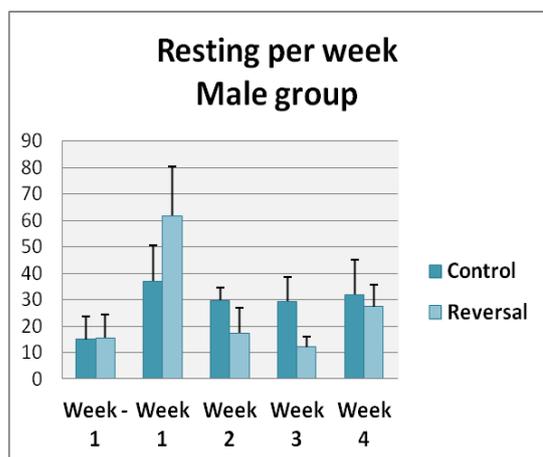
Graph 1a: Average amount of resting per 8 cages per week. Male control and reversal groups.
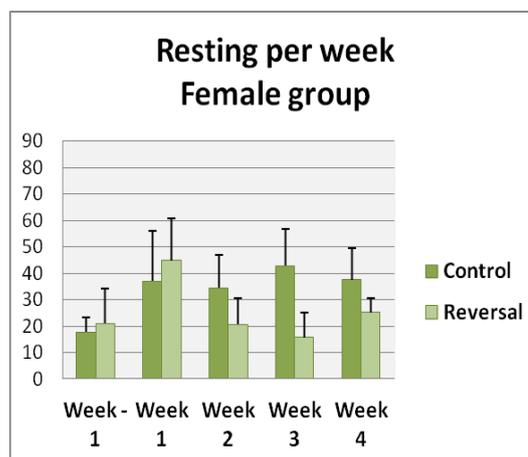
Graph 1b: Average amount of resting per 8 cages per week. Female control and reversal groups.
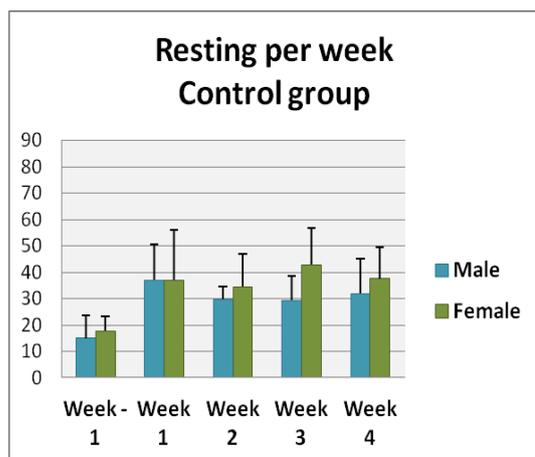
Graph 1c: Average amount of resting per 8 cages per week. Male and female control groups.
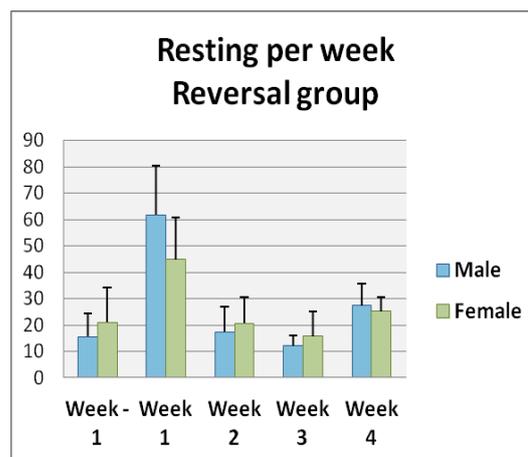
Graph 1d: Average amount of resting per 8 cages per week. Male and female reversal groups.

Male group (*see graph 1a*):
Control: In comparison to week -1 all weeks post-transport (wk1>wk-1; *P*=.005, wk2>wk-1; *P*=.006, wk3>wk-1; *P*=.009, wk4>wk-1; *P*=.014) display a significantly increase in resting behavior. In the male control group the decrease in resting behavior per week is not significantly in comparison with the previous week. The total decrease of resting (week 1 - week 4) is not significant.
Reversal: In comparison to week -1 the male reversal group displays a significant difference in resting behavior during week 1 (wk1>wk-1; *P*=.000) and week 4 (wk4>wk-1; *P*=.005). The peak of increased resting behavior in week 1 is significant from all other weeks post transport (wk1>wk2; *P*=.001, wk1>wk3; *P*=.000, wk1>wk4;*P*=.005). Also in week 4 the amount of resting is higher than in week 2 and 3 (wk4>wk2; *P*=.010, wk4>wk3; *P*=.000).
Comparing treatments: During the week prior to transport there were no significant differences between the reversal and control group in resting behavior. Resting behavior is higher in the male reversal group during week 1 although not significant (*P*=.061). In week 2 and 3 however, the control group displays significantly more resting behavior than

the reversal groups (ctr2>rvrs2; *P*=.009, ctr3>rvrs3; *P*=.000). In week 4 there is no significant difference between the control group and the reversal group.

Female group (*see graph 1b*):
Control: The amount of resting behavior is significantly different from week -1 in all the weeks following transport (wk-1<wk1; *P*=,001, wk-1<wk2; *P*=.002, wk-1<wk3; *P*=.000, wk-1<wk4; *P*=.002). In the weeks following transport there is a peak in resting behavior in week 3, however this is only significant higher than week 2 (wk3>wk2; *P*=.044) and does not differ significantly from any of the other weeks. The total amount of resting (week 1 – week 4) does not decrease significantly.
Reversal: In comparison to week -1 the female reversal group also displays a significant increase during week 1 (wk-1<wk1; *P*=.011). After this peak the amount of resting behavior strongly decreases in the following 3 weeks and it is no longer significantly different from week -1. The female reversal group also displays a high peak of resting behavior in week 1 that is significantly higher in comparison to any of the other weeks (wk1>wk2; *P*=.003, wk1>wk3; *P*=.000, wk1>wk4; *P*=.020). After this peak the amount of resting behavior is about on the same level as week -1. Between week 2, 3 and 4 no significant differences are displayed.
Comparing treatments:
During the week prior to transport there were no significant differences between the reversal and control group in resting behavior. In the female group there is no significant difference between the control and reversal group during the first week after transport (*P*=.311). In the following weeks however there is significantly more resting behavior in the control group (ctr2>rvrs2; *P*=.032, ctr3>rvrs3; *P*=.000, ctr4>rvrs4; *P*=.019).

Comparing genders *(graph 1c/d)*:
Control group: only in week 3 do the male and female groups differ significantly (male>female; *P*=.039).
Reversal group: There is no significant difference between the two genders in any of the weeks.

Daily development (*See graph 2a/b*):
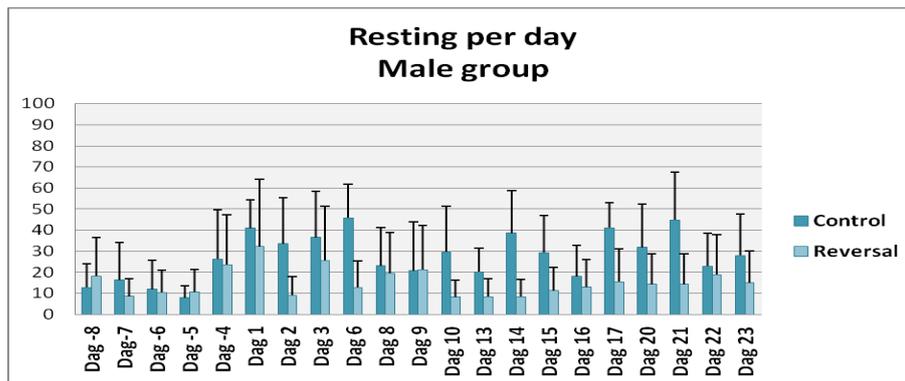
Graph 2a: Average amount of resting behavior per day. Male control and reversal groups.
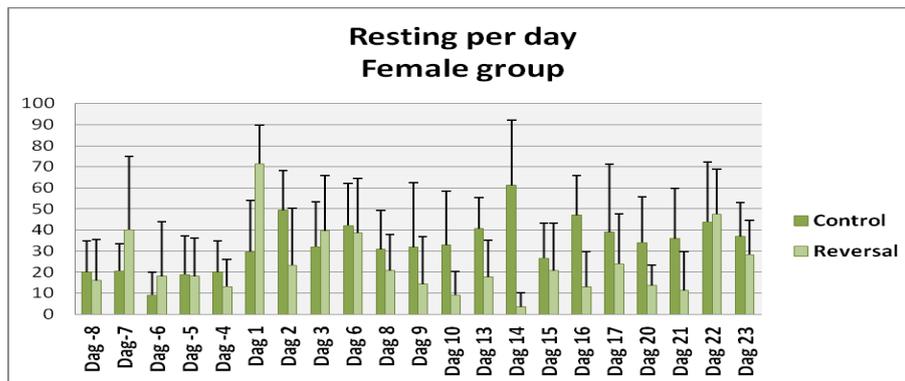
Graph 2b: Average amount of resting behavior per day. Female control and reversal groups.

Week -1:
Male group: There are no significant differences in week -1 in the male group.
*Female group:* Both the female control and reversal groups display a significant difference between day -7 and day -6 (ctrl -7>-6; *P*=.018, rvrsl -7>-6; *P*=.021).
Comparing treatments: There are no significant differences in week 1 between the two treatments.
Comparing genders: When comparing the two genders a significant higher amount of resting is displayed in the reversal group by the female group on day -7 (male<female; *P*=.022).

Week 1:
Male group: In the male groups there is an increase in resting behavior during week 1. This increase lasts all three days and there are no significant changes.
Female group: In the female groups however there are significant changes: in the control group day 2 has a significantly higher amount of resting behavior than day 1 or 3 (2>1; *P*=.024 and 2>3; *P*=.032). In the reversal group the increased resting behavior peaks on day 1, which has a significantly higher amount of resting behavior than day 2 or 3 (1>2; *P*=.006 and 1>3; *P*=.011).
Comparing treatments: When comparing the two treatments the male reversal group displays a significant higher amount of resting behavior on day 2 (Mctr2<Mrvrs2; *P*=.013). The female reversal group has a higher resting amount on day 1 (Fctr1<Frvrs1; *P*=.010) and day 2 (Fctr2<Frvrs2; *P*=.024).
Comparing genders: When comparing the two genders we see a significant difference in the reversal group on day 2 (male>female; *P*=.004).

Week 2:
Male group: During week 2 the male control group displays a increased resting on day 6 which is significant higher than day 8 and 9 (6>8; *P*=.017, 6>9;*P*=.043).
Female group: In the female control group no significant differences were found however in the reversal group displayed on day 6 a significant higher amount of resting behavior than on day 10 (6>10; *P*=.012).

Comparing treatments: When comparing the treatments there is a significant lower amount of resting behavior in the female reversal group on day 10 (Fctr10>Frvrs10; *P*=.023). The male reversal group displays a significant lower amount of resting than the control group on day 6 (Mctr6<Mrvrs6; *P*=.004).
Comparing genders: On day 13 and 16 there is a significant difference between the male and female control groups (day 13 male<female; *P*=.007, day 16 male<female; *P*=.006).

Week 3:
Male group: In the male control group there was a significant difference between day 13 - day 17 (13<17; *P*=.006), day 15 – day 16 (15>16; *P*=.001) and day 16 – day 17 (16<17; *P*=.007).
Female group: In the female control group we only found a significant difference between day 14 and day 17 (14>17; *P*=.049). In the reversal groups only the female group displayed a significant change in resting behavior between day 14 and day 15 (14<15; *P*=.033).
Comparing treatments: On day 13 (Mctr13>Mrvrs13; *P*=.025), 14 (Mctr14>Mrvrs14; *P*=.011), day 15 (Mctr15>Mrvrs15; *P*=.017) and day 17 (Mctr17>Mrvrs17; *P*=.004) the male reversal group displays less resting behavior than the control group. In the female group this significant difference occurs on day 13 (Fctr13>Frvrs13; *P*=.009), day 14 (Fctr14>Frvrs14; *P*=.000) and day 16 (Fctr16>Frvrs16; *P*=.002).
Comparing genders: Between the two genders we see a significant difference in the reversal group on day 14 (male>female; *P*=.002).

Week 4:
Male group: In week 4 the male control group displayed a significant change between day 21 and day 22 (21>22; *P*=.026). In the male reversal group a significant change was found between day 20 and day 21 (20>21; *P*=.013).
Female group: The female control group displayed no significant changes during the week however the reversal group displays a significant higher amount of resting behavior on day 22 than on day 20 and 21 (22>20; *P*=.001, 22>21; *P*=.007).
Comparing treatments: The male control and reversal groups display no significant differences, while the female reversal group displays a lower amount of resting behavior than the female control group on day 21 (*P*=.016).
Comparing genders: On day 21 the male reversal group displays a significant higher amount of resting behavior than the female reversal group (male>female; *P*=.005) while on day 22 the female reversal group displays a higher amount of resting behavior (male<female; *P*=.019).

4.2 Autogrooming

<u>Weekly development:</u>
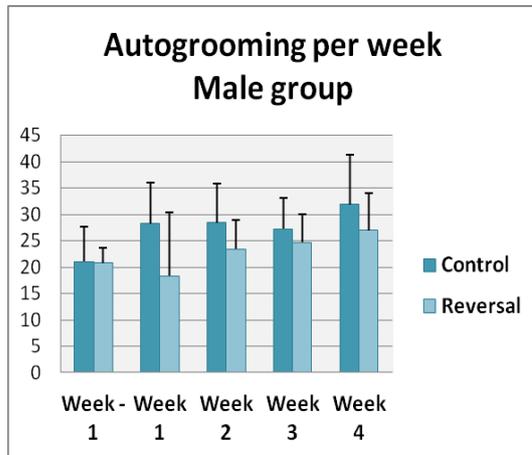
Graph 3a: Average amount of autogrooming per 8 cages per week. Male control and reversal groups.
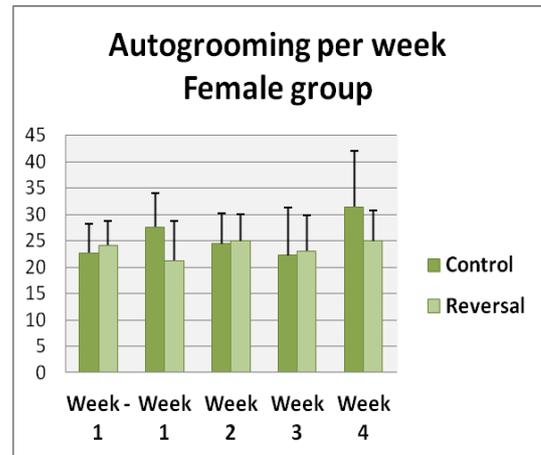
Graph 3b: Average amount of autogrooming per 8 cages per week. Female control and reversal groups.
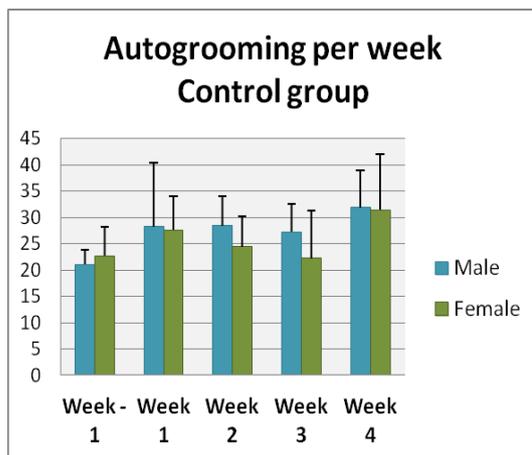
Graph 3c: Average amount of autogrooming per 8 cages per week. Male and female control groups.
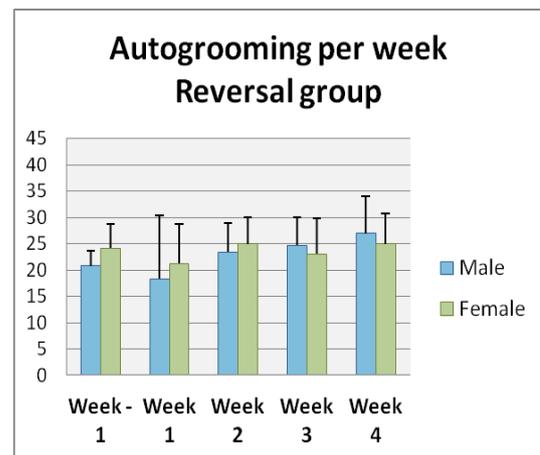
Graph 3d: Average amount of autogrooming per 8 cages per week. Male and female reversal groups.

Male group (*see graph 3a*):
Control: In comparison to the week prior to transport the male control animals display a significant increase in autogrooming during the first, second and fourth week (wk-1<wk1; *P*=.044, wk-1<wk2; *P*=.009, wk-1<wk4; *P*=.015). There are no significant differences between any of the weeks following transport.
Reversal: In comparison to week -1 the male group displays no significant differences and there are no significant differences between any of the weeks following transport.
*Comparing treatments*: The male group displays no significant differences between the treatments.

Female group (*see graph 3b*):
Control: In the female control group there is an increase in autogrooming in week 1, however this is not significant in comparison to week -1. In the weeks following transport the female control group displays no further significant differences.
Reversal: In comparison to the week prior to transport the female reversal group displays no significant differences in the weeks post-transport. In the weeks following transport the female reversal group displays no further significant differences.
Comparing treatments: The female group displays no significant differences between the treatments.

Comparing genders (*graph 3c/d*):

Control group: There are no significant differences between the male and female control groups.
Reversal group: There are no significant differences between the male and female reversal groups.

Daily development (*see graph 4*):
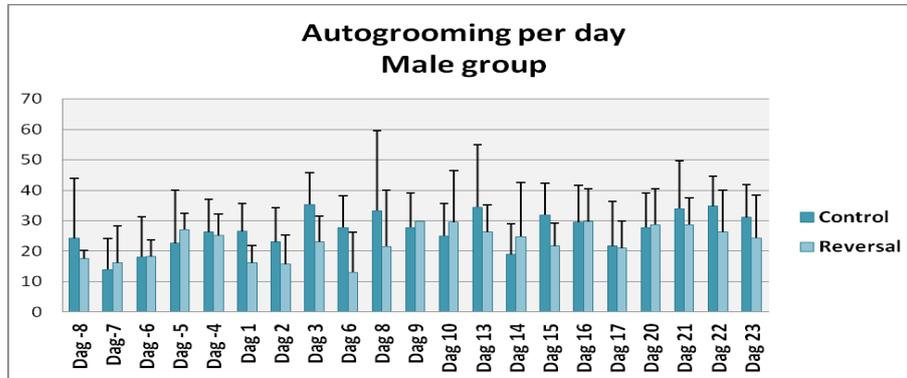
Graph 4a: Average amount of autogrooming per day per group. Male control and reversal groups.
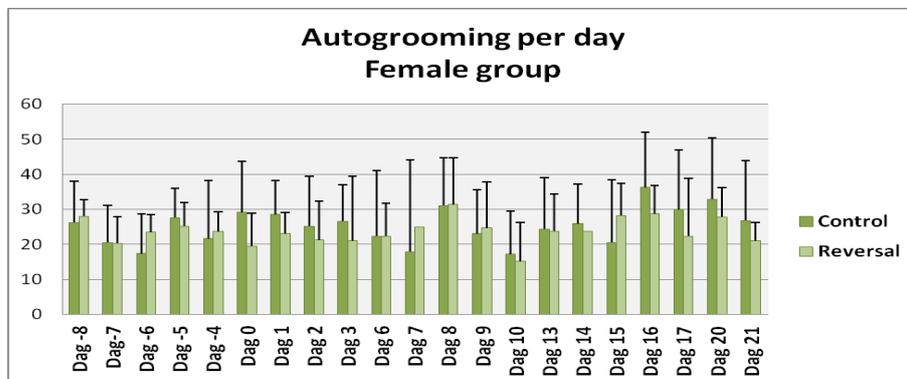
Graph 4b: Average amount of autogrooming per day per group. Male control and reversal groups.

Week -1:
Male group: Throughout week -1 the male control group displays a significant difference where day -4 displays a higher amount of autogrooming than day -7 (-4>-7; *P*=.025). The male reversal group displays this same significant difference (-4>-7; *P*=.029).
Female group: There are no significant differences in the female group.
Comparing treatments: There are no significant differences between the two treatments during week -1.
Comparing genders: There is a significant difference between the male and female reversal group on day -8 (male<female; *P*=.013). There are no further significant differences between the genders.

Week 1:
Male group: Throughout week 1 there is a significant difference between day 2 and day 3 (2<3; *P*=.034) in the male control group.
Female group: There is no significant difference in or between the female groups .
Comparing treatments: There are no significant differences between the two treatments during week 1.
Comparing genders: There are no significant differences between the genders.

Week 2:
Male group: During week 2 the male reversal group displayed a decrease of autogrooming on day 6 which is significant lower than all the other days of week 2 (6<8; *P*=.042, 6<9; *P*=.002, 6<10; *P*=.001).
Female group: There is no significant difference in or between the female groups.
Comparing treatments: On day 6 the male reversal group displays a significantly lower amount of autogrooming than the control group (ctr6>rvrs6; *P*=.001).
Comparing genders: There are no significant differences between the genders.

Week 3:
Male group: The male control group displays a significant difference between day 13 and 14 (13>14; P=.040) and day 14 and 15 (14<15; P=.045).
Female group: 'The female reversal group also displays a significant difference between day 13 and 14 (13>14; P=.029), day 14 and 16 (14<16; P=.043) and day 14 and 17 (14<17; P=.038).
Comparing treatments: There is a significant difference between the male control and reversal groups on day 15 (ctrl15>rvrsl15; P=.032).
Comparing genders: There are no significant differences between the genders.

Week 4:
Throughout week 4 none of the groups or treatments displayed any significant changes.

4.3 Eating

Weekly development:
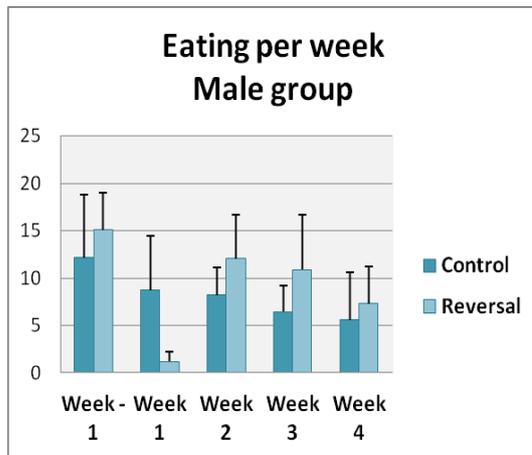
Graph 5a: Average amount of eating per 8 cages per week. Male control and reversal groups.
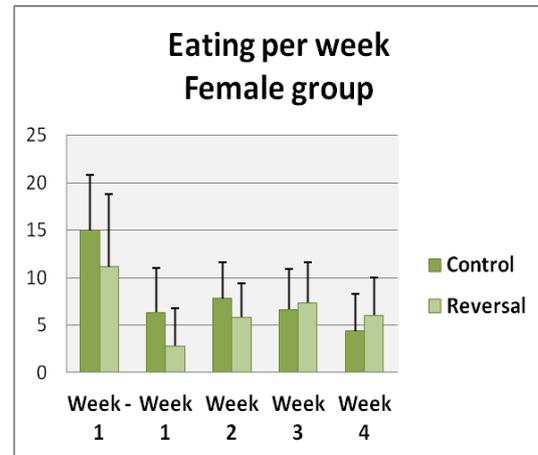
Graph 5b: Average amount of eating per 8 cages per week. Female control and reversal groups.
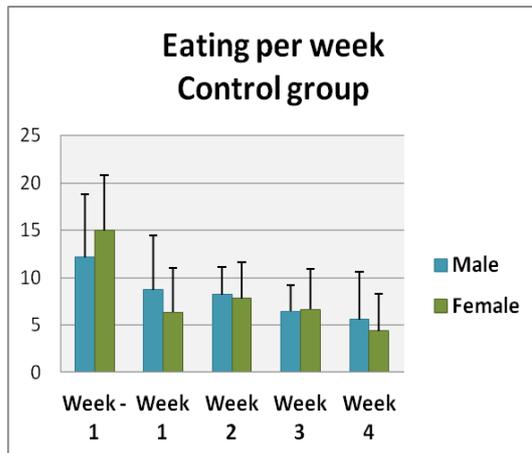
Graph 5c: Average amount of eating per 8 cages per week. Male and female control groups.
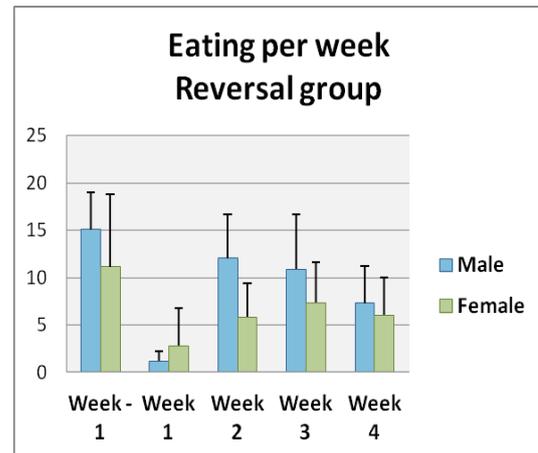
Graph 5d: Average amount of eating per 8 cages per week. Male and female reversal groups.

Male group (*see graph 5a*):
In the male group there is a clear decreasing line of eating behavior in both control and reversal groups.
Control: In the male control group there is a clear decreasing line of eating behavior, although it is only significantly different from week -1 in week 3 (wk-1>wk3; *P*=.009). During the weeks following transport the male control group does not display a significant decrease of total eating behavior (week 1 - week 4) nor a significant decrease of any week comparing with its preceding week. There is however a significant decrease when comparing week 4 with week 2 (wk2>wk4; *P*=.032).
Reversal: In comparison to week -1, eating behavior significantly decreases in week 1 (wk-1>wk1; *P*=.000). In week 2 and 3 the amount of eating behavior is not significantly lower compared to week -1, but in week 4 it is again significantly decreased (wk-1>wk4; *P*=.006). The male reversal group also seems to display a descending trend in eating behavior however it is more variable during the weeks following transport. In the weeks following transport the male group displays a decrease in eating behavior in week 1. This decrease is significantly lower in comparison to all the other weeks (wk1<wk2; *P*=.000, wk1<wk3; *P*=.000, wk1<wk4; *P*=.002).
Comparing treatments: The male control and reversal groups are not significantly different in week -1. In week 1 however the male reversal group displays a significantly lower amount of eating behavior than the control group (ctr1>rvrs1; *P*=.002). The male reversal group displays a *higher* eating behavior during week 2, 3 and 4 but this is not significant.

Female group (*see graph 5b*):
In the weeks following transport the female group also has a decrease of eating behavior in week 1 however it is only significantly different from week 3
Control: In comparison to week -1 the female control group displays a significant difference during all four weeks following transport (wk-1>wk1; *P*=.003, wk-1>wk2; *P*=.014, wk-1>wk3; *P*=.008, wk-1>wk4; *P*=.003). Furthermore the amount of eating behavior decreases significantly in week 4 in comparison to week 3 (wk3>wk4; *P*=.011).
Reversal: The female reversal group does not display a significant decrease during week 1 in comparison to week -1 but gets very close to a significant difference (*P*=.055). The weeks post-transport in female reversal group never differ significantly from week -1. Week 3 has significantly more eating behavior than week 1 (wk3>wk1; *P*=.043). There are no other significantly differences during the weeks following transport.
Comparing treatments: The female reversal and control groups are not significantly different during the experiment.

Comparing genders (*see graph 5c/d*):
Control group: There are no significant differences between the male and female control groups.
Reversal group: In week 2 the male group displays significant more eating behavior than the female group (male>female; *P*=.007).

Daily development (*see graph 6*):
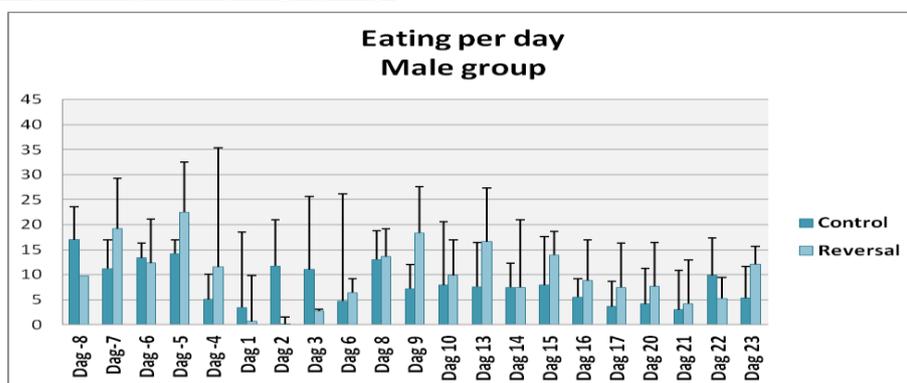
Graph 6a: amount of eating behavior per day per group. Male control and reversal groups.
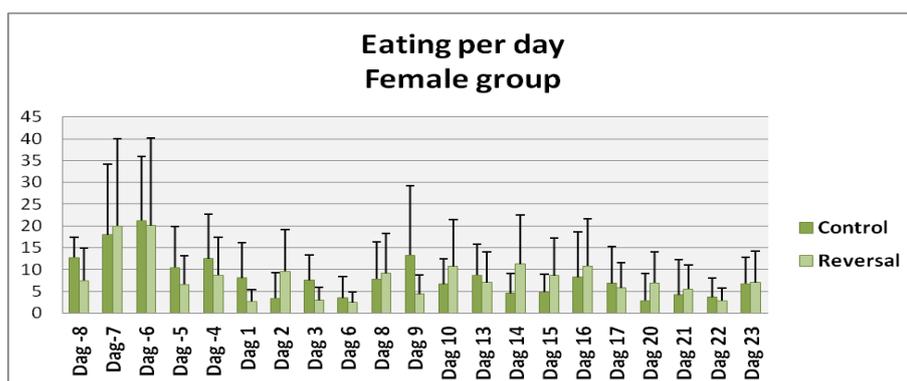
Graph 6b: amount of eating behavior per day per group. Female control and reversal groups.

Week -1:
Male group: Throughout week -1 the male reversal group displays a significant change: day -7 has a significant higher amount of eating behavior than day -8 (-8<-7; *P*=.022).
Female group: The female reversal group displays a significant difference between day -7 and -6 (-7<-6;*P*=.021).
Comparing treatments: When comparing the treatments however the female reversal group is significantly lower than the control group in day -8 (Fctr-8>Frvrs-8; *P*=.028).
Comparing genders: There are no differences between the genders in the control group. On day -7 the female reversal group displays more eating behavior than the male reversal group (male<female; *F*=.030).

Week 1:
Male group: Throughout week 1 the male groups do not display any significant changes.
Female group: Throughout week 1 the female groups do not display any significant changes.
Comparing treatments: The male reversal group has significantly less eating behavior than the control group on day 1 (Mctr1>Mrvrs1; *P*=.020), day 2 (Mctr2>Mrvrs2; *P*=.006) and day 3 (Mctr3>Mrvrs3; *P*=.038). The female reversal group only displays significantly less eating than the control group on day 3 (Fctr3>Frvrs3; *P*=.027).
Comparing genders: There are no differences between the genders in the control groups. In the reversal group there is a significant difference on day 2 (female>male; *P*=.022).

Week 2:
Male group: In week 2 the male reversal group displays a significant difference between day 6 and day 9 (6<9; *P*=.002).
Female group: The female reversal group displays a lower amount of eating behavior on day 6 which is significant to all other days in this week (6<8; *P*=.027, 6<9; *P*=.009, 6<10; *P*=.024).
Comparing treatments: On day 9 the male reversal group has significantly more eating behavior than the control (ctrl9>rvrsl9; *P*=.000). The female control and reversal display no significant differences.
Comparing genders: There are no differences between the genders in the control group. In the reversal group there is a significant difference on day 6 (male>female; *P*=.009). On day 9 the male reversal group displays more eating behavior than the female reversal group (male>female; *P*=.003).

Week 3:
Male group: In week 3 the male reversal group displays a significant difference between day 13 and 14 (13>14; *P*=.032).
Female group: Throughout week 3 the female groups do not display any significant changes.
Comparing treatments: There are no differences between the control and reversal groups.
Comparing genders: There are no differences between the genders.

Week 4:
Male group: In week 4 the male reversal group displays a significant difference between day 21 and 23 (21<23; *P*.031).
Female group: The female reversal group displays a significant lower amount of eating on day 22 in comparison to day 20 (22>20; *P*=.010). On day 20 however the female reversal group displays significantly more eating behavior than the control (Fctr20<Frvrs20; *P*=.015).
Comparing treatments: There are no differences between the control and reversal groups.
Comparing genders: There are no differences between the genders.

4.5 Playing/fighting

Weekly development:
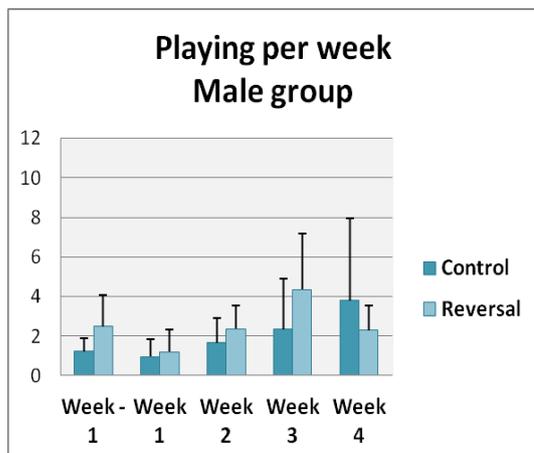
Graph 7a: Average amount of eating per
8 cages per week. Male control and reversal groups.
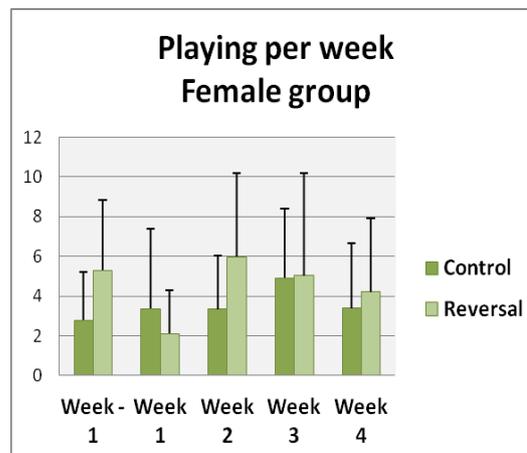
Graph 7b: Average amount of eating per 8
cages per week. Female control and reversal groups.
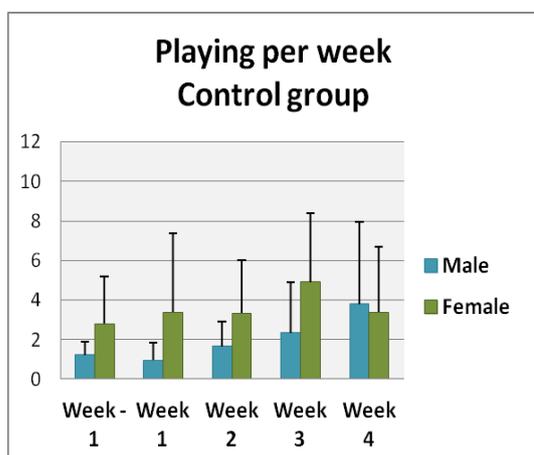
Graph 7c: Average amount of eating per
8 cages per week. Male and female control groups.
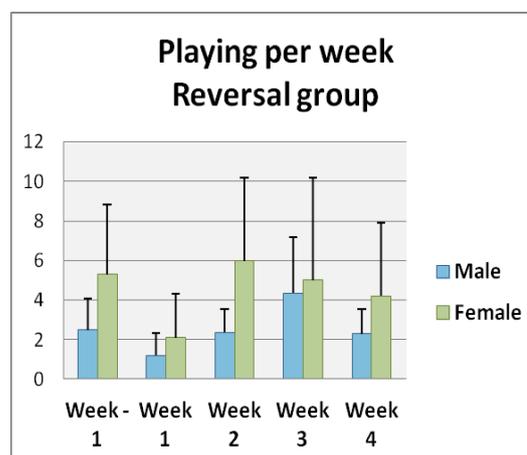
Graph 7d: Average amount of eating per
8 cages per week. Male and female reversal groups.

Male group (*see graph 7a*):
Control: In comparison to week -1 the male control group displays no significant differences in the week post-transport. There is however a significant difference between week 2 and week 4 (wk2>wk4; *P*=.038).
Reversal: In comparison to week -1 the male reversal group displays no significant differences in the weeks post-transport. The reversal group does display a decrease in playing during week 1 which is significant from week 3 and 4 (wk1<wk3; *P*=.011, wk1<wk4; *P*=.035).
Comparing treatments: During the entire experiment there were no significant differences between the control and reversal groups.

Female group (*see graph 7b*):
Control: The female control group displays no significant differences during the experiment.
Reversal: The female reversal group displays no significant differences during the experiment.
Comparing treatments: During the entire experiment there were no significant differences between the control and reversal groups.

Comparing genders (*see graph 7c/d*):

Control group: There were no significant differences between the male and female control groups during the experiment.
Reversal group: There were no significant differences between the male and female reversal groups during the experiment.

Daily development (*see graph 8*):
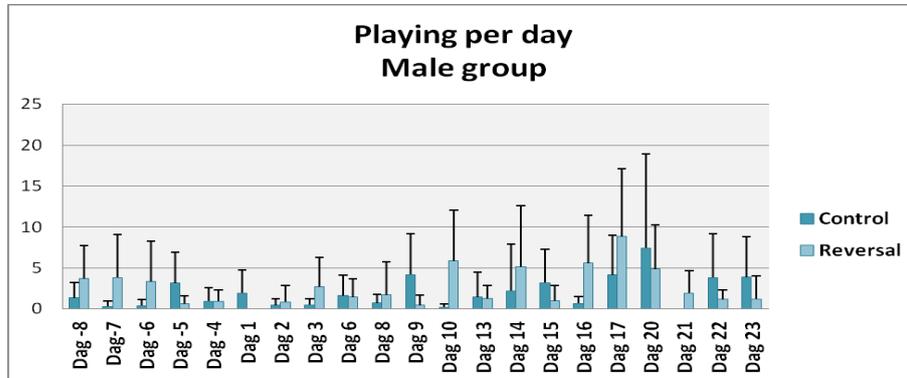
Graph 8a: amount of play-behavior per day per group. Male control and reversal groups.
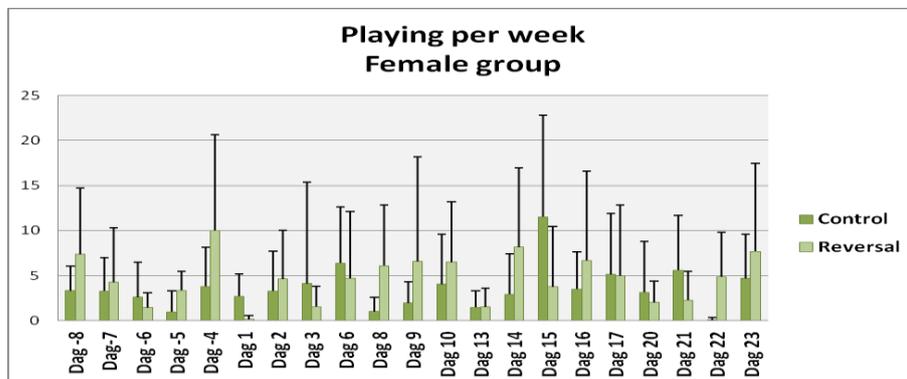
Graph 8b: amount of play-behavior per day per group. Female control and reversal groups.

Week -1:
Male group: In the male control group day -6 has a significant higher amount of playing than day -7 (-6>-7; *P*=.038). Furthermore there is a significant difference between day -6 and day -4 (-6>-4; *P*=.025).
Female group: There are no significant differences in the female group.
Comparing treatments: There are no differences between the control and reversal groups.
Comparing genders: There are no differences between the genders.

Week 1:
Male group: The male control group displayed no significant differences during the first week. In the male reversal group there was no playing behavior at all on day 1 and 2. The female control group displays a high peak of playing behavior on day 1 which is significant to a very low amount of play behavior on day 2 (1>2; *P*=.010). Neither of these days is significantly different from day 3.
Female group: The female reversal group displayed an increase of playing throughout week 1 however not significantly.
Comparing treatments: When comparing the treatments the male reversal group displays a significant lower amount of playing behavior on day 2 (MaleControl2>MaleReversal2; *P*=.013). The female reversal group displayed a significant lower amount of playing behavior on day 1 (FemaleControl1>FemaleReversal1; *P*=.013).
Comparing genders: There are no differences between the genders.

Week 2:
Male group: In the male control group there is a significant higher amount of play-behavior on day 8 than day 9 (8>9; *P*=.016).
Female group: There are no significant differences in the female groups.

Comparing treatments: There are no differences between the control and reversal groups.
*Comparing genders:* On day 6 the male control group displays more play-behavior than the female control group (MaleControl6>FemaleControl6; *P*=.027).

Week 3:
Male group: There are no significant differences in the male group.
Female group: In week 3 the female reversal group has a significant difference between day 14-16 and day 14-17 (14>16; *P*=.027, 14>17; *P*=.015).
Comparing treatments: When comparing the treatments however the male reversal group displays a significant higher amount of playing behavior on day 16 (MaleControl16<MaleReversal16; *P*=.009). The female reversal group displays a significant higher amount than the control group of playing on day 13 (Fctr13<Frvrs13; *P*=.032) and day 14 (Fctr14<Frvrs14; *P*=.000).
Comparing genders: On day 13 the male control group displays more play-behavior than the female control group (MaleControl13>FemaleControl13; *P*=.043). The male reversal group displays more play-behavior than the female reversal group on day 16 (MaleReversal16>FemaleReversal16; *P*=.04=.045).

Week 4:
Male group: The male reversal group displays a peak of play-behavior on day 22 which is significantly different from day 20 and day 21 (22>20; *P*=.041, 21>20;*P*=.030). The male reversal group displays a significant higher amount of playing behavior on day 22 in comparison to the control group (Mctr22<Mrvrs22; *P*=.031).
Female group: On day 21 the female reversal group displays a significant higher amount of play behavior (Fctr21<Frvrs21; *P*=.048).
Comparing treatments: There are no differences between the control and reversal groups.
Comparing genders: On day 22 the male reversal group displays more play-behavior than the female reversal group (MaleReversal22>FemaleReversal22; *P*=.015).

4.6 Social

Weekly development:
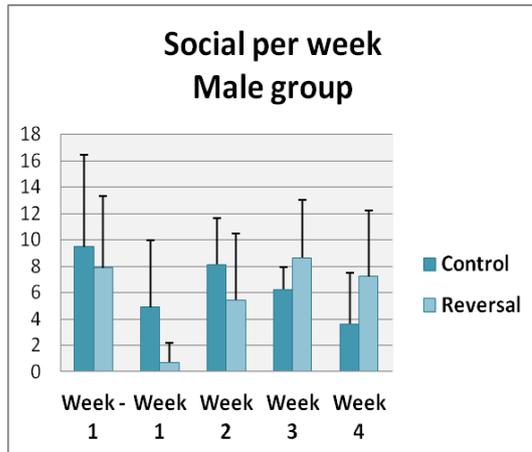
Graph 9a: Average amount of social per 8 cages per week. Male control and reversal groups. groups.
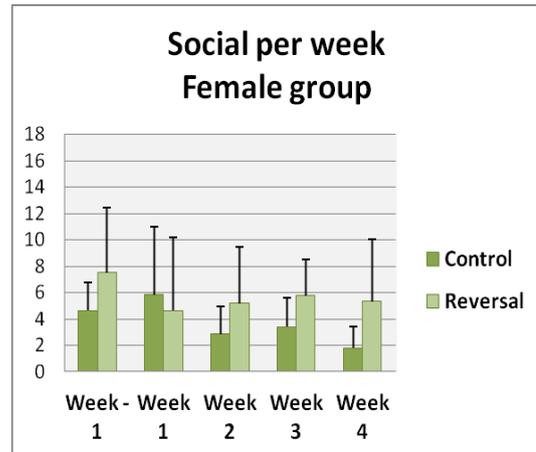
Graph 9b: Average amount of social per 8 cages per week. Female control and reversal
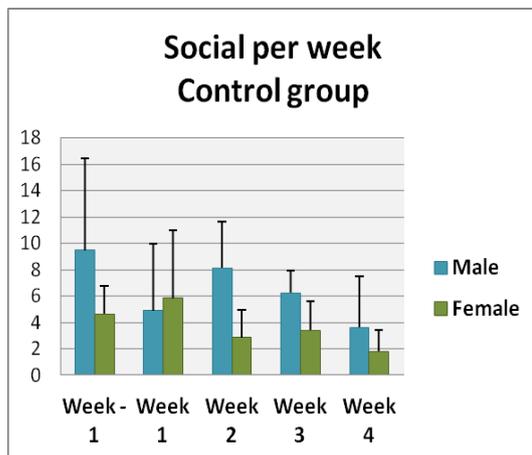
Graph 9c: Average amount of eating per 8 cages per week. Male and female control groups.
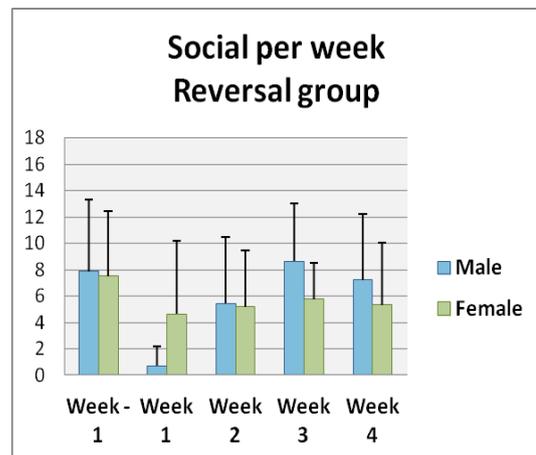
Graph 9d: Average amount of eating per 8 cages per week. Male and female reversal groups.

Male group (*see graph 9a*):
Control: Week 1 is significant lower than week -1 due to a very high standard deviation (wk-1>wk1; *P*=.036). There were no significant differences between the weeks following transport.
Reversal: Again the amount of social behavior is significant lower in week 1 in comparison to week 1 (wk1>wk-1; *P*=.006). Between the weeks following transport however the male reversal group displays a significant decrease in week 2, week 3 and week 4 in comparison to week 1 (wk1<wk2; *P*=.004, wk1<wk3; *P*=.001, wk1<wk4; *P*=.005).
Comparing treatments: There were no significant differences between the male control and reversal groups.

Female group (*see graph 9b*):
Control: In week 4 the female control group displays significantly less social behavior in comparison to week -1 (wk-1>wk4; *P*=.029). There are no further significant differences in the female control group.
Reversal: In week 4 the female reversal group displays significantly less social behavior in comparison to week -1 (wk-1>wk4; *P*=.044). There are no further significant differences in the female control group.
Comparing treatments: There were no significant differences between the female control and reversal groups.

Comparing genders (*see graph 9c/d*):

Control group: In week 3 the male control group displays significantly more social behavior than the female control group (male>female; *P*=.009).
Reversal group: There are no significant differences between the male and female reversal groups during the entire experiment.

Daily development (*see graph 10*):
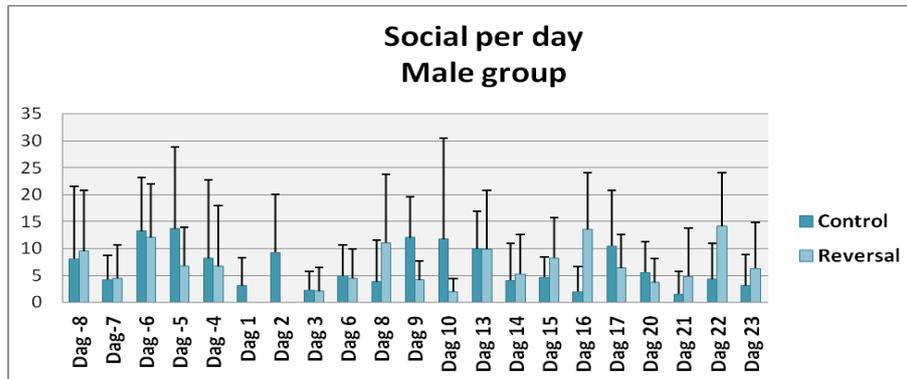
Graph 10a: amount of social behavior per day per group. Male control and reversal groups.
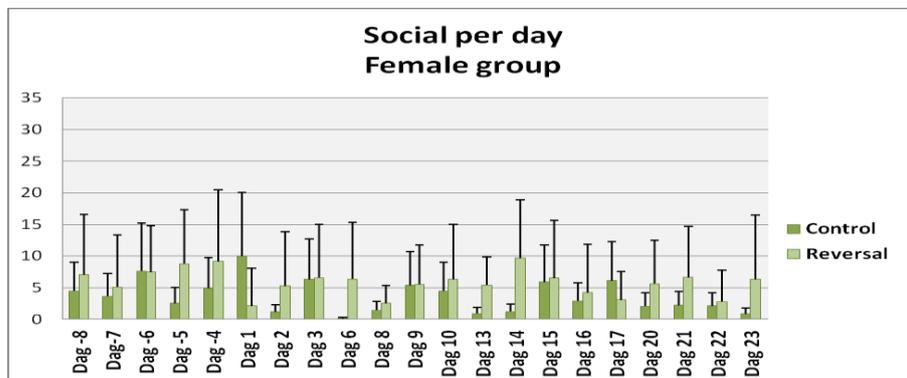
Graph 10b: amount of social behavior per day per group. Female control and reversal groups.

Week -1:
Male group: There are no significant differences in the male group during week -1.
Female group: During week -1 the female control group displays a decrease of social behavior on day -5 which is significant lower than day -8 (-5<-8; *P*=.025), day -7 (-5<-7; *P*=.038) and day -4 (-5<-4; *P*=.024).
Comparing treatments: On day -5 there is a significant difference between the female treatments (Control<reversal; *P*=.003).
Comparing genders: On day -7 there is a significant difference between the male and female control groups (Male<Female; *P*=.019). Between the reversal groups there is a significant difference on day -5 (Male<Female; *P*=.002).

Week 1:
Male group: In week 1 the male reversal group displays a difference between day 1 and day 3 (1<3; *P*=.014).
Female group: The female reversal group displays a peak on day 2 which is significant to both day 1 and day 3 (*P*=.000 and *P*=.024).
Comparing treatments: On day 1 the amount of social behavior is significantly lower in both the male reversal group (MaleReversal1<MaleControl1; *P*=.014) and female reversal group (FemaleReversal1<FemaleControl1; *P*=.024).
*Comparing genders:* On day 2 the male reversal group displays less social behavior than the female reversal group (Male<Female; .003).

Week 2:
Male group: During week 2 the male control group displays a significant difference between day 9 and day 10 (9>10; *P*=.049). The male reversal group also displays a significant difference between day 9 and day 10 (9<10; *P*=.029).

Female group: There are no significant differences in the female group.
Comparing treatments: Between the reversal and control groups there are however significant differences. The male reversal group displays a significant lower amount of social behavior on day 9 (MaleReversal9<MaleControl9; *P*=.010) and a significant higher amount of social behavior on day 10 (MaleReversal10>MaleControl10; *P*=.042). On day 8 the female reversal group displays more social behavior than the female control group (FemaleReversal8<FemaleControl8; *P*=.034).
Comparing genders: On day 8 and 9 the female reversal group displays more social behavior than the male reversal group (Male8<Female8; *P*=.024, Male9<Female9; *P*=.0,15).

Week 3:
Male group: In week 3 the male reversal group displays a significant difference between day 13 -17, day 15-16 and day 15-17 (13<17; *P*=.013, 15<16; *P*=.004, 15<17; *P*=.005).
Female group: The female reversal group displays a significant difference between day 13 and day 14 (13<14; *P*=.025).
Comparing treatments: Between the treatments there are significant differences. On day 16 the male reversal group displays significant more social behavior than the control group (*P*=.020).
Comparing genders: There are no significant differences between the male and female groups.

Week 4:
Male group: In week 4 the male control group display a significant decrease in social behavior on day 21 in comparison to day 20, 22 and 23 (*P*=.000, *P*=.013 and *P*=.013).
Female group: The female control group has a significant lower amount of social behavior on day 22 in comparison to day 21 and day 23 (*P*=.002 and *P*=.005). The female reversal group displays significant more social behavior on day 22 in comparison to day 20 (22>20; *P*=.035).
Comparing treatments: When comparing the treatments there is significant more social behavior on day 21 in the male reversal group (FemaleReversal21>FemaleControl21; *P*=.038). The female reversal group displays however significant more social behavior on day 22 (FemaleReversal20>FemaleControl20; *P*=.000).
Comparing genders: On day 20 and 22 the male control group displays more social behavior than the female control group (Male20>Female20; *P*=.046, Male22>Female22; *P*=.025). on day 21 the female control group displays more soial behavior than the male control group (Male21<Female21; *P*=.000).
On day 23 the male reversal group displays less social behavior than the female reversal group (male<female; .007).

4.7 Active

Weekly development:
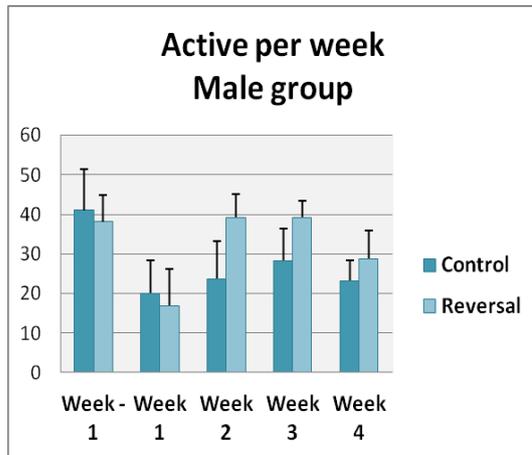
Graph 9a: Average amount of activity per
8 cages per week. Male control and reversal groups.
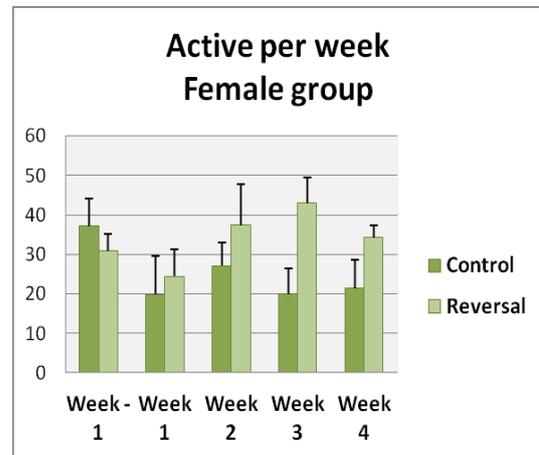
Graph 9b: Average amount of activity per 8
cages per week. Female control and reversal groups.
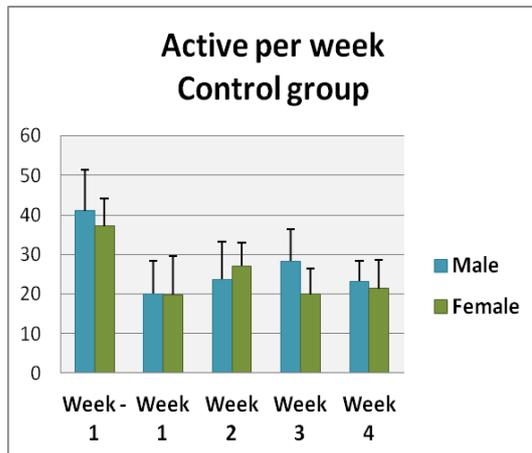
Graph 9c: Average amount of activity per
8 cages per week. Male and female control groups.
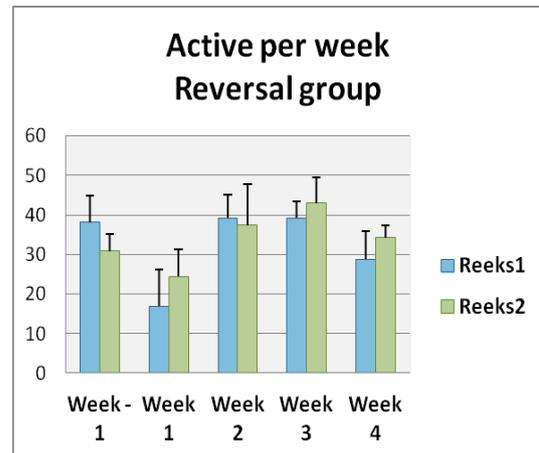
Graph 9d: Average amount of activity per
8 cages per week. Male and female reversal groups.

Male group (*see graph 11a*):
Control: The male control group displays a significantly lower active behavior in comparison to week -1 during week 1, 2, 3 and week 4 (wk-1>wk1; *P*=.002, wk-1>wk2; *P*=.007, wk-1>wk3; *P*=.011, wk-1>wk4; *P*=.001).
In the weeks following transport the male group displays a significant difference between week 1 and week 3 (wk1<wk3; *P*=.022) Furthermore the male control group displays decrease of active behavior in week 4 in comparison to week 3 (wk3>wk4; *P*=.005).
Reversal: In comparison to week -1 the male reversal group displays a significant decrease in "active" behavior in week 1 (wk-1>wk1; *P*=.000). In week 2 and 3 the amount of activity is almost the same as in week -1. In week 4 activity is again significantly lower than week -1 (wk-1>wk4; *P*=.019). As stated the male group displays a very low amount of active behavior during week 1. This is significantly lower than week 2 and 3 (*P*=.000 for both). In week 2 and 3 the activity is almost the same, however in week 4 the male group again displays a significant lower amount of "active" behavior than in week 2 (wk2>wk4; *P*=.006) or 3 (wk3>wk4; *P*=.004)
Comparing treatments: For "active" behavior the reversal and control of the male groups did not differ significantly during week -1. The reversal and the control groups differed significantly in the male group during week 2 (Ctr2<rvrs2; *P*=.001) and week 3 (ctr3<rvrs3; *P*=.003) were the reversal group had a significantly higher amount of "active" behavior.

Female group (*see graph 11b*):
Control: In comparison with week -1 "active" behavior decreased significantly during the first week after transport in the female control group (wk-1>wk1; *P*=.001). The female control group displays a significantly lower active behavior in comparison to week -1 during week 2, 3 and week 4 (wk-1>wk2; *P*=.003, wk-1>wk3; *P*=.000 wk-1>wk4; *P*=.011). Week 3 is significantly higher than week 2 (wk3>wk2; *P*=.003).
Reversal: The female group displays a decrease (although not significantly) during week 1 and increasing "active" behavior in the following weeks. In week 3 the amount of "active" behavior is significantly higher than in week -1 (wk-1<wk3; *P*=.001). The female reversal group also displays a significantly low amount of activity in week 1 in comparison to all the other weeks (wk1<wk2; *P*=.001, wk1<wk3; *P*=.000 and wk1<wk4; *P*=.004). In week 4 the female group displays a significant lower amount of "active" behavior than in week 3 (wk3>wk4; *P*=.000).
Comparing treatments: In the female group there is a significant difference between the control and reversal group during week -1. In the female reversal group there was a significant higher amount of "active" behavior during week 2 (ctr2<rvrs2; *P*=.022), week 3 (ctr3<rvrs3; *P*=.000) and week 4 (ctr4<rvrs4; *P*=.001).

Comparing genders (*see graph 11c/d*):
Control group: in week 3 the male and female group differ significantly (male>female; *P*=.031).
Reversal group: in week -1 male and female groups differ significantly (male>female; *P*=.008).

Daily development (*see graph 12*):
When looking at the development per day we see a high variety in amounts of active behavior.
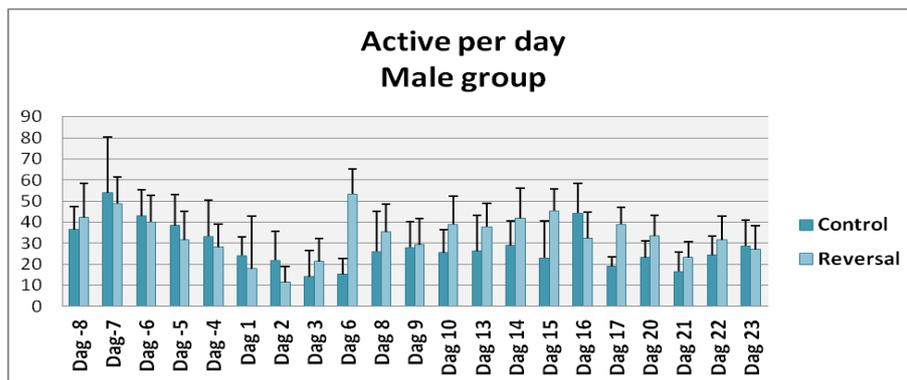
Graph 12a: amount of activity per day per group. Male control and reversal groups.
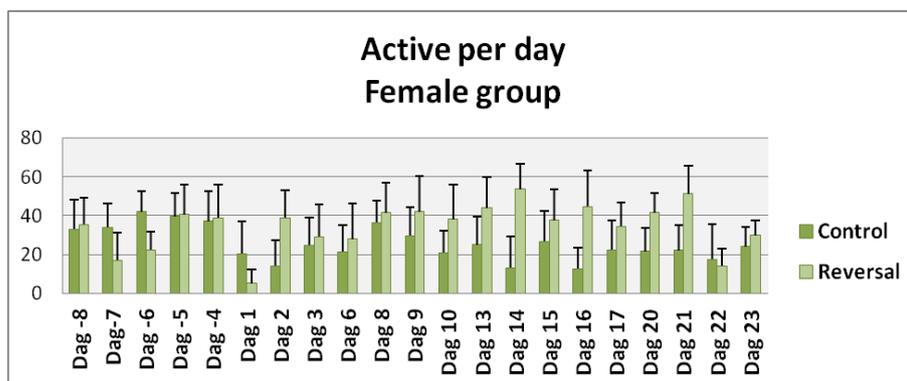
Graph 12b: amount of activity per day per group. Female control and reversal groups.

Week -1:
Male group: Throughout week -1 both male groups display a slight decrease however this is not significant when comparing the days with their preceding day. There is however a significant difference in the male reversal group between day -7 and the days -5 and -4 (-7>-5; *P*=.038 and -7>-4; *P*=.014).

Female group: The female groups display a much more variable trend during the week. The female reversal group displays a significant difference between day -7 – day -5, and day -6 - day -5 (-7<-5; *P*=.018 and -6<-5; *P*=.041).
Comparing treatments: When comparing the control and reversal groups there is a significant difference between the female groups on day -7 (Fctr-7>Frvrs-7; *P*=.024) and day -6 (Fctr-6>Frvrs-6; *P*=.001). The male groups do not display significant differences.
Comparing genders: There are no significant differences between the genders in the control group. In the reversal group the males display more activity on day -7 and -6 (male-7>female-7; *P*=.000, Male-6>female-6; *P*=.006).

Week 1:
Male group: There are no significant differences in the male group during week 1.
Female group: During week 1 the female reversal group displays a significant change between day 1 and the days 2 and 3 (1<2; *P*=.001 and 1<3; *P*=.003).
Comparing treatments: When comparing the control and reversal groups the female groups differ significantly on day 1 and 2 (Fctr1>Frvrs1; *P*=.033 and Fctr2<Frvrs2; *P*=.003). The male groups do not differ significantly during week 1.
Comparing genders: There are no significant differences between the genders in the control group. On day 2 the male and female groups of the reversal group differ significantly (female2>male2; .000).

Week 2:
Male group: The male control group displays a significant difference between day 6 and day 10 (6<10; *P*=.012). The male reversal group displays a significant difference between day 6 - day 8 and day 6 - day 9 (6>8; *P*=.024, 6>9; *P*=.014).
Female group: The female control group displays a significant difference between day 8 and the days 6 and 10 (8>6; *P*=.013 and 8>10; *P*=.034)
Comparing treatments: When comparing the control and reversal group the male reversal group displays a significant higher amount of active behavior on day 6 (Mctr6>Mrvrs6; *P*=.000) and day 10 (Mctr10>Mrvrs10; *P*=.047). The female reversal group displays also a significant higher amount of active behavior on day 10 (Fctr10<Frvrs10; *P*=.036).
Comparing genders: There are no significant differences between the genders in the control group. In the reversal group the males and females differ significantly on day 6 (male>female; *P*=.005).

Week 3:
Male group: Throughout week 3 the male control group displays a significant increase in the amount of active behavior on day 16 in comparison to the other days (16>13; *P*=.033, 16>14; *P*=.035, 16>15; *P*=.003, 16>17; *P*=.001*)*. Furthermore the male control group displays a significant difference between day 14 and 17 (14>17; *P*=.040). The male reversal group displays a significant difference between day 15 and day 16 (15>16; *P*=.045).
Female group: The female reversal group displays a significant drop on day 14 in comparison to day 15 and day 17 (14<15; *P*=.023, 14<17; *P*=.007).
Comparing treatments: In comparing the reversal group with the control group the male reversal group displays a significant higher amount of active behavior on day 15 (Mctr15<Mrvrs15; *P*=.007) and day 17 (Mctr17<Mrvrs17; *P*=.000). On day 16 the control group displays a higher amount of active behavior however this is not significant. The female reversal group has a significant higher amount of active behavior on day 13 (Fctr13<Frvrs13; *P*=.026), day 14 (Fctr14<Frvrs14; *P*=.000) and day 16 (Fctr16<Frvrs16; *P*=.001).
Comparing genders: On day 14 and day 16 the male and female control groups differ significantly (male14>female14; *P*.041, male16>female16; *P*=.000)

Week 4:
Male group: In the male reversal group day 20 – day 21 display significant different amounts of activity (20>21; *P*=.004).
Female group: On day 22 the female reversal group displays a significant lower amount of active behavior than all the other days of that week (22<20; *P*=001, 22<21; *P*=.001, 22<23; *P*=012). Furthermore day 20 and day 21 are significantly different from day 23 (20>23; *P*=.046, 21>23; *P*=.003).
Comparing treatments: When comparing the two treatments the female reversal group displays a significant higher amount of active behavior on day 20 (Fctr20<Frvrs20;

P=.003) and day 21 (Fctr21<Frvrs21; *P*=.001). The activity of the male reversal group is significant higher on day 20 (Mctr20<Mrvrs20; *P*=.034).
Comparing genders: There are no significant differences between the genders in the control group. On day 21 and 22 the reversal males and females differ significantly (male21>female21; *P*=.000, Male22<female22; *P*=.004).

5. Discussion

Our goal in this research was to determine the effects of transport and a 12-hour light-dark switch on the behavior of laboratory rats.

Overall many of the behavioral parameters display a line of increasing or decreasing amounts of behavior throughout the weeks following transport. The daily amounts are very variable with many peaks and low percentages. One reason for this variety might be that sometimes the animals were not clearly visible. Since we used percentages this could lead to an incorrect "higher" percentage of that behavioral parameter. However, since we have a total of 464 observations per day (58 per cage per day), the effects of one cage with animals that are not clearly visible should not have a significant influence on the average amount of the behavioral parameter per week. This could however explain the high variety in daily amounts of behavior.
Furthermore over the entire experiment there were no clear differences between the male and female groups. There are a few significant differences, but the overall amounts are on the same level and the development is similar in both genders.

Some behavioral parameters in the control groups at week 4 are still significantly different from week -1. This is the case with resting (male control and reversal and female control group), activity (male control and reversal and female control group), social (both female groups) and in eating (male reversal and female control group). These could be the long-term effects of transport itself, but are probably due to aging and a less need for food intake due to a lower grow rate. The peak in growth in rats is at 7-8 weeks, and in week -1 the animals in our research are 8 weeks old. Thus the amount of food intake is much higher in this week than in the weeks following.[28]

Overall we can state that transport itself has an effect on the animals as seen in both of the control groups. Both reversal and control groups display significant increases of resting behavior in week 1 comparing to week -1. This could be due to the stress of transport and a novel environment. Several researchers state that this can lead to a increased activity.[31-34] However, S. Suchecki et al. stated that the kind and duration of a stressor can lead to either reduced or increased resting, with increasing resting at a longer duration of the stressor. Acute stress leads to an increased rest, while chronic stress leads to insomnia.[35] The transport and novel environment can be seen as acute stress-full situations which leads to an increase in sleeping behavior during the first days after transport.
Also, the animals were kept in a dark environment during transport (darkness is a trigger for activity in rats) and could be sleep deprivated by the time they were brought in the new facility. This would also explain the increased resting in the first days.
After week 1 resting behavior is higher in the control (non-reversal) group in both genders. Furthermore, when looking at the resting behavior, it appears that even in the control group the values in week 4 are still significantly different from week -1. This is also the case in the male reversal group, however the other weeks (week 2 and 3) the amount of resting is not significantly different from week -1. Both reversal groups seem to recover faster from the transport and the 12 hour light-dark shift (week 2) than the control group from transport only (> week 4). This could be due to the fact that during the resting period of the animals (which is during the light-period) researchers and animal caretakers can disturb the animals, leading to a poor resting period with reduced REM-sleep which the animals compromise by sleeping more during the active period.[36, 37]

Autogrooming does not seem to display a specific increase or decrease during the weeks following transport. In fact, it seems to stay on the same level. As discussed in the introduction, situations that cause both a high or low amount of stress result in an increase in autogrooming. We do see an overall *increase* of autogrooming in both control groups in week 1, although this is only significant in the male group. An increase in autogrooming could be due to the novel environment after transport which in turn leads to stress. However this would lead to a higher frequency of shorter autogrooming bursts[23] and since we evaluated with an interval of 10 seconds for 10 minutes spread over 1 hour, it seems unlikely that the animals by chance display a short autogrooming burst exactly at every second of evaluation. It should therefore be explained due to the higher activity in the control groups and therefore more "comfort grooming" between arousal and de-

arousal.[23,21, 22] The fact that the reversal group does not show this increase is due to the higher percentage of sleeping behavior.

The amount of eating behavior clearly decreases during the first week in both reversal groups. This again could be due to an increased amount of resting in this week. The control groups however do not display the same decrease in week 1.
Furthermore the amount of eating behavior seems decrease throughout the weeks. As stated before this is probably due to a lower growth-rate of the animals, however it could also be due to stress as mentioned by van Ruiven [38].

Playing and social behavior are both very variable during the experiment, and this is clearly visible when looking at the daily development. Again in the male groups there is a decrease of playing and social behavior during the first week after transport however in the female groups this is not the case. The amount of playing and social behavior is very low in comparison to the other behavioral parameters; the maximum amount of playing per day is 14 percent, and the maximum amount of social behavior is just below 12. Since the overall amounts are much smaller and the variety much higher, there are almost no significant differences. There is only a significant decrease in week 1 in comparison to week -1 in the male group. Z.A. Klein et al. found a decrease in social behavior in adolescent male rats when confronted with acute stress (restraint-induced stress).[39] Our female groups however show no decrease of social behavior or play behavior in week 1.

When looking at active behavior we see that both the control and reversal groups display a decrease in week 1 in comparison to week -1. Here again the control groups also seem to be affected by the transport itself. Both the male and female reversal groups display a lower amount of activity, which could be due to the higher amount of resting behavior in the reversal groups during these weeks. This seems likely since in the following weeks, when the amount of resting is higher in the control group, the amount of activity is lower than in the reversal group.
The effect of stress on the activity of animals has already been researched before. Many situations have an increasing influence on the amount of activity including cage cleaning, transport (and a novel environment) and handling.[31-34] However these were all experiments with in-house transportation and these effects were only clear in the first 60-90 minutes. In-house transportation has a lesser impact on the animals since the transportation time is shorter and the contrast of the novel environment could be lower (the animals do not leave the building). However as stated before the transport and novel environment can be seen as acute stress-full situations which leads to an increase in sleeping behavior during the first days after transport. This increase of sleep behavior logically goes together with an decrease of activity.

Previous researches only determined the physiological changes that occurred in animals during and after transport. S. Capdevilla et al. concluded that laboratory animals need at least a three day adaptation period after a transport of 5 hours without a light-dark shift [40]. This period is also mentioned by L.A. Conour [41] who recommends at least a 3-5 day period. However both researchers mention that the acclimatization period is dependent on the specific research. This 3-5 day period seems to also be the case in our reversal groups, where the amount of resting behavior is almost the same as week -1 in week 2, 3 and 4. Furthermore most of the behavioral parameters are back to baseline from week 2 and on. When looking at the daily development most behavioral parameters are on day 6 significantly different from day 8 and on, so a acclimatization period of 6 days seems preferred.
However the results of our research also seem to indicate that the control group may need a longer adaptation period when taking only resting behavior in account, maybe well over 4 weeks since the amount of resting is significantly higher during the 4 weeks after transport. J.A. Obernier mentions that young and easily stressed animals may take a longer period to acclimatize, and mentions that circadian rhythm may take several weeks to months to normalize [42]. However as mentioned before this increased resting could also be due to the fact that during the resting period of the animals researchers and animal caretakers can disturb the animals, leading to a poor resting period. In order to determine whether if this is indeed the case more research is needed. Further research could include breeding animals in a reversed day-night cycle, or a test where the animals are only disturbed during their activity-phase in order to exclude the effect of human activity in the resting period.

6. Attachments

Appendix 1

Ethogram

Behavior		Description of behavior	
Active	A	Active behavior including sniffing of surroundings excluding cage companions, mobile exploration, root/digging, gnaw/nibbling, manipulating shelter, locomotion.	State
Resting	R	Resting behavior including lying and sitting. Looking around without exploration (sniffing) or alertness.	State
Eating/drinking	E	Intake of food or water.	State
Auto-grooming	W	Self-grooming; licking or nibbling the animal's own fur.	State
Social interaction			
Social exploration/ social grooming	S	Sniffing/exploring of a cage companion. Licking or nibbling the fur of a cage companion.	State
Playing/fighting	D	Play behavior with cage companion(s) including pinning, pouncing, and chasing. Also fighting behavior, this could not be evaluated separately due to the quality of the recordings.	State
Not visible	X	Animal is not visible.	State

	Treatment	Male											
		Active		Resting		Eating		Autogrooming		Social		Playing	
		Mean	Sd	Mean	Sd	Mean	Sd	Mean	Sd	Mean	Sd	Mean	Sd
Day -8	Control	36,47	10,89	12.85	11.21	16.97	15.01	24.20	20.45	8,13	13.48	1.35	1.87
	Reversal	42,22	16,25	17.34	18.25	9.75	10.04	17.45	5.75	9.54	11.19	3.68	4.04
Day -7	Control	54,01	26,45	16.30	17.71	11.19	9.19	13.97	10.17	4.22	4.44	0.29	0.63
	Reversal	48,85	12,72	7.54	8.50	19.18	8.76	16.13	9.53	4.47	6.26	3.80	5.27
Day -6	Control	42,94	12,58	11.93	13.66	13.38	14.63	18.08	10.33	13.25	9.90	0.39	0.74
	Reversal	40,14	12,59	14.02	10.42	12.32	10.01	18.15	8.33	12.01	10.03	3.32	4.90
Day -5	Control	38,40	14,79	7.86	5.67	14.23	21.44	22.62	12.07	13.70	15.16	3.16	3.77
	Reversal	31,47	13,50	11.51	10.56	22.50	23.75	27.10	13.17	6.78	7.18	0.61	0.94
Day -4	Control	33,36	17,03	26.25	23.36	5.06	5.75	26.24	14.81	8.14	14.62	0.93	1.63
	Reversal	28,22	10,94	27.47	23.64	11.56	9.18	25.13	18.36	6.67	11.24	0.93	1.38
Day 1	Control	24,10	8,92	40.97	13.55	3.43	4.84	26.43	15.68	3.12	5.21	1.29	2.80
	Reversal	17,82	25,02	65.33	32.13	0.66	1.32	16.16	17.04	0.	0	0	0
Day 2	Control	21,91	13,63	33.58	21.66	11.71	12.67	23.03	9.83	9.28	10.77	0.45	0.73
	Reversal	11,53	7,24	71.75	9.01	0.14	0.41	15.69	8.87	0	0	0.86	1.99
Day 3	Control	14,20	12,2	36.59	21.73	11.04	8.84	35.38	10.72	2.27	5.82	0.49	0.73
	Reversal	21,23	11,15	48.22	25.61	2.70	2.77	23.04	17.94	2.08	4.47	2.70	3.56
Day 6	Control	15,21	7,43	45.78	16.10	4.76	4.87	27.74	7.31	4.90	5.82	1.59	2.49
	Reversal	53,29	11,88	21.38	12.73	6.42	5.55	12.98	7.50	4.46	5.52	1.44	2.19
Day 8	Control	26,03	18,93	23.04	18.26	13.01	9.70	33.33	19.76	3.86	7.65	0.70	1.06
	Reversal	35,35	13,07	16.68	19.45	13.61	9.16	21.54	8.94	11.10	12.61	1.69	4.02
Day 9	Control	28,03	12,23	20.78	23.01	7.21	3.63	27.75	10.13	12.04	7.61	4.17	4.96
	Reversal	29,19	12,60	17.96	21.15	18.38	7.10	29.85	11.81	4.16	3.57	0.44	1.25
Day 10	Control	25,58	10,98	29.59	21.60	7.93	5.04	24.93	13.21	11.72	18.76	0.21	0.40
	Reversal	38,97	13,47	13.78	8.18	9.88	10.76	29.85	11.81	1.98	2.44	5.87	6.16
Day 13	Control	26,32	16,87	20.14	11.30	7.62	7.04	24.93	17.38	9.98	7.01	1.43	3.07
	Reversal	37,85	10,91	8.22	8.40	16.58	13.54	26.20	13.87	9.87	10.99	1.25	1.55
Day 14	Control	28,86	11,53	38.53	20.37	7.44	7.93	18.86	10.72	4.08	6.94	2.20	5.72
	Reversal	41,86	14,35	15.57	8.36	7.46	4.69	24.71	14.14	5.25	7.40	5.12	7.49
Day 15	Control	22,99	17,50	29.28	17.48	7.93	7.45	31.92	9.29	4.65	3.85	3.21	4.07
	Reversal	45,44	10,33	9.64	11.20	13.97	8.08	21.66	7.92	8.27	7.53	0.99	1.88
Day 16	Control	44,31	14,08	18.00	14.67	5.52	6.39	29.47	11.21	2.01	4.61	0.66	0.82
	Reversal	32,23	12,61	10.09	12.98	8.84	8.87	29.71	23.08	13.51	10.57	5.59	5.84
Day 17	Control	18,98	4,65	41.12	11.94	3.65	5.88	21.62	10.32	10.47	10.33	4.14	4.88
	Reversal	39,01	8,04	17.29	15.52	7.48	8.75	21.00	10.97	6.35	6.19	8.84	8.24
Day 20	Control	23,18	7,84	31.89	20.43	4.17	5.42	27.73	10.32	5.59	5.73	7.42	11.48
	Reversal	33,52	9,61	21.58	14.36	7.66	8.63	28.64	14.17	3.71	4.43	4.85	5.44
Day 21	Control	16,65	8,94	44.87	22.60	2.97	5.07	33.98	26.29	1.52	4.30	0	0
	Reversal	23,25	7,56	37.12	14.30	4.25	4.21	28.62	12.43	4.80	8.97	1.93	2.71
Day 22	Control	24,28	9,22	22.95	15.62	9.85	13.32	34.78	11.32	4.28	6.74	3.84	5.30
	Reversal	31,58	11,12	21.62	18.84	5.26	3.54	26.22	9.26	14.11	9.98	1.17	1.16
Day 23	Control	28,65	12,35	28.02	19.52	5.30	5.99	31.05	10.73	3.09	5.74	3.87	4.96
	Reversal	27,01	11,37	28.99	15.04	12.09	10.43	24.35	12.66	6.32	8.53	1.22	2.82

Appendix 2: Means in percentages (active) and ranked numbers (resting, eating, autogrooming, playing and social) and standard deviations of the male groups during the experiment per day.

Appendix 3

	Treatment	Female											
		Active		Resting		Eating		Autogrooming		Social		Playing	
		Mean	Sd	Mean	Sd	Mean	Sd	Mean	Sd	Mean	Sd	Mean	Sd
Day -8	Control	33,12	14,99	20.05	14.66	12.74	4.70	26.24	11.68	4.50	4.32	3.32	2.74
	Reversal	35,41	13,69	15.95	19.48	6.11	7.44	28.07	9.41	7.06	9.52	7.36	7.31
Day -7	Control	33,97	12,43	20.58	12.76	17.94	16.27	20.55	10.52	3.63	5.10	3.28	3.70
	Reversal	17,06	14,31	40.14	34.65	13.04	20.04	20.35	5.93	5.10	8.17	4.27	6.06
Day -6	Control	42,10	10,36	9.07	10.78	21.24	14.69	17.35	11.30	7.62	6.17	2.58	3.91
	Reversal	22,27	9,47	18.04	25.77	27.24	20.12	23.51	11.07	7.47	7.31	1.45	1.67
Day -5	Control	39,53	12,31	18.83	18.23	10.49	9.42	27.63	8.35	2.51	4.09	0.98	2.34
	Reversal	40,48	15,48	17.94	18.31	4.22	6.55	25.20	18.38	8.76	8.59	3.36	2.08
Day -4	Control	37,19	15,30	19.98	14.91	12.47	10.13	21.63	16.57	4.90	3.74	3.79	4.34
	Reversal	38,84	17,15	13.15	12.78	5.19	8.68	23.65	9.48	9.17	11.28	9.98	10.64
Day 1	Control	20,39	16,74	29.70	24.33	8.04	8.06	29.12	9.71	10.01	7.06	2.71	2.48
	Reversal	5,15	7,18	71.51	18.10	1.57	2.71	19.49	13.31	2.10	5.96	0.14	0.41
Day 2	Control	14,17	13,39	49.38	18.89	3.42	5.83	28.58	14.15	1.14	2.79	3.28	4.39
	Reversal	38,90	14,41	23.25	27.22	4.87	9.59	23.08	13.21	5.25	8.61	4.61	5.40
Day 3	Control	24,77	14,12	32.02	21.25	7.51	5.86	25.22	10.34	6.32	8.8	4.13	11.22
	Reversal	29,08	16,61	39.58	26.22	1.90	2.95	21.28	11.03	6.59	8.37	1.55	2.23
Day 6	Control	21,26	14,04	42.08	20.00	3.50	4.92	26.61	18.75	0.14	0.40	6.37	6.23
	Reversal	27,84	18,22	38.60	26.01	1.42	2.40	21.07	10.68	6.36	8.93	4.68	7.42
Day 8	Control	36,51	11,02	30.88	18.49	7.83	8.56	22.30	13.54	1.43	2.51	1.02	1.58
	Reversal	41,52	15,44	20.69	17.21	6.77	9.15	22.35	9.16	2.55	2.84	6.10	6.75
Day 9	Control	29,70	14,77	31.85	30.53	13.28	15.86	17.86	12.55	5.35	7.74	1.93	2.38
	Reversal	42,11	18,26	14.29	22.61	6.55	4.35	24.92	7.98	5.53	6.21	6.58	11.57
Day 10	Control	20,92	11,30	32.78	25.62	6.67	5.79	31.10	12.36	4.48	6.74	4.04	5.57
	Reversal	38,28	17,86	9.00	11.15	8.50	10.72	31.39	16.411	6.30	8.67	6.51	6.67
Day 13	Control	25,09	14,16	40.68	14.79	8.74	6.98	23.05	14.60	0.92	1.08	1.49	1.83
	Reversal	43,99	16,10	17.70	17.46	6.70	7.04	24.64	8.34	5.42	4.48	1.52	2.04
Day 14	Control	13,04	16,13	61.13	31.09	4.53	4.48	17.17	11.44	1.19	2.30	2.91	4.47
	Reversal	53,77	13,13	3.58	6.62	9.64	11.29	15.15	5.16	9.69	9.18	8.14	8.83
Day 15	Control	26,85	15,79	26.56	16.74	4.79	4.17	24.38	17.91	5.88	7.02	11.51	11.25
	Reversal	37,66	16,11	20.72	22.52	7.60	8.57	23.72	14.58	6.51	9.08	3.76	6.65
Day 16	Control	12,51	10,83	46.96	18.99	8.24	10.33	25.86	15.81	2.88	4.30	3.52	4.12
	Reversal	44,81	18,53	12.86	16.91	7.61	10.82	23.79	13.23	4.20	7.63	6.69	9.86
Day 17	Control	22,33	15,24	38.99	32.34	6.87	8.35	20.53	16.89	6.12	7.85	5.13	6.76
	Reversal	34,53	12,22	23.93	23.67	5.30	5.79	28.20	15.27	3.04	4.46	4.97	7.85
Day 20	Control	21,66	12,09	33.96	21.79	2.85	6.27	36.28	17.58	2.06	3.01	3.16	5.65
	Reversal	41,59	9,83	13.70	9.71	8.31	6.98	28.74	7.57	5.62	6.91	2.01	2.37
Day 21	Control	22,21	13,07	35.81	23.90	4.19	8.10	29.96	17.02	2.21	3.95	5.59	6.05
	Reversal	51,39	14,48	11.20	18.67	6.13	5.50	22.35	11.03	6.62	8.02	2.29	3.16
Day 22	Control	17,53	18,25	43.76	28.37	3.66	4.29	32.85	18.51	2.09	4.11	0.08	0.24
	Reversal	14,03	8,89	47.55	21.38	2.86	2.84	27.86	15.78	2.81	4.95	4.86	4.95
Day 23	Control	23,99	10,00	36.88	16.27	6.74	6.00	26.81	11.74	0.86	1.34	4.69	4.86
	Reversal	29,91	7,59	28.24	16.43	6.77	7.06	21.06	11.72	6.30	10.19	7.70	9.77

Appendix 3: Means in percentages (active) and ranked numbers (resting, eating, autogrooming, playing and social) and standard deviations of the female groups during the experiment per day.

Appendix 4

		Male											
	Treatment	Active		Auto-grooming		Resting		Eating		Playing		Social	
		Mean	Sd	Mean	Sd	Mean	Sd	Mean	Sd	Mean	Sd	Mean	Sd
Week -1	Control	41.03	10.37	21.02	6.68	15,03	8.48	12.17	6.60	1.23	0.99	9.49	3.89
	Reversal	38.18	6.76	20.79	2.81	15.58	8.75	15.06	3.91	2.47	1.59	7.89	4.97
Week 1	Control	20.07	8.36	28.28	7.75	37.05	13.71	8.73	5.71	0.95	0.87	4.89	1.72
	Reversal	16.86	9.30	18.30	12.11	61.77	18.73	1.17	1.02	1.19	1.16	0.69	4.37
Week 2	Control	23.71	9.54	28.44	7.35	29.80	4.73	8.23	2.89	1.67	1.21	8.13	3.53
	Reversal	39.20	5.91	23.46	5.51	17.45	9.48	12.07	4.57	2.36	1.19	5.43	5.02
Week 3	Control	28.29	8,18	27.27	5.77	29.42	9.05	6.43	2.78	2.33	2.56	6.24	5.09
	Reversal	39.28	4,11	24.66	5.30	12.16	3.97	10.86	5.76	4.36	2.81	8.65	1.49
Week 4	Control	23.19	5.24	31.88	9.45	31.93	13.13	5.57	5.00	3.78	4.15	3.62	6.93
	Reversal	28.84	7.02	26.96	7.11	27.33	8.35	7.32	3.93	2.29	1.24	7.24	5.39
		Female											
	Treatment	Active		Auto-grooming		Resting		Eating		Playing		Social	
		Mean	Sd	Mean	Sd	Mean	Sd	Mean	Sd	Mean	Sd	Mean	Sd
Week -1	Control	37.19	7.05	22.68	5.52	17.70	5.61	14.98	5.81	2.79	2.40	4.63	2.12
	Reversal	30.81	4.25	24.16	4.66	21.04	13.12	11.16	7.65	5.29	3.52	7.51	4.92
Week 1	Control	19.78	9.84	27.64	6.39	37.03	19.01	6.32	4.69	3.37	4.02	5.83	5.15
	Reversal	24.38	6.99	21.28	7.48	44.78	15.95	2.78	4.02	2.10	2.20	4.65	5.56
Week 2	Control	27.10	5.87	24.47	5.69	34.40	12.68	7.82	3.79	3.34	2.66	2.85	2.06
	Reversal	37.44	10.28	24.93	5.07	20.64	9.91	5.81	3.60	5.97	4.21	5.18	4.29
Week 3	Control	19.96	6.43	22.20	9.12	42.86	13.84	6.63	4.31	4.91	3.48	3.40	2.20
	Reversal	42.95	6.43	23.10	6.67	15.76	9.24	7.37	4.29	5.02	5.15	5.77	2.72
Week 4	Control	21.35	7.29	31.47	10.57	37.60	11.75	4.36	3.93	3.38	3.29	1.81	1.58
	Reversal	34.23	3.15	25.72	5.72	25.17	5.57	6.02	4.03	4.21	3.70	5.34	4.70

Appendix 4: Means in ranked numbers and standard deviations in of the different behavioral parameters during the experiment per week.

7. References

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