HABITUATION TO HUMAN PRESENCE AND TRANSPORT BOX TRAINING

An explorative study on the possibilities of habituation and positive reinforcement training in Dunkin Hartley guinea pigs

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Abstract

Dunkin Hartley guinea pigs are widely used as laboratory animals. At Utrecht University these guinea pigs participate during practical lessons in which Veterinary Medicine students learn how to handle and determine the sex of the animals. The guinea pigs are housed in a floor housing system (+/- 3m2 of floor space) that includes enrichment. While providing the animals with more space and opportunities to fulfill their behavioral needs, this type of housing can complicate catching the guinea pigs, for example when they need to be transported to the location of the practical lessons (the 'practical room'), or to perform regular health checks. Catching the guinea pigs can result in anti-predator reactions due to the prey animal nature of the guinea pigs. In bigger housing systems there will be more space for the guinea pigs to flee. In the case this leads to having to chase the animals in order to catch them, this could lead to stress. In this study we developed and tested a habituation and trainings protocol which could be used to avoid these stressful situations. The animals were habituated to human presence and movement and trained to walk into a transport box voluntarily with positive reinforcement training. Over the course of the habituation period we found a significant increase in behaviors in which the animals made contact with a human, also we found a significant decrease for hiding behavior. The mean fear level of the guinea pigs (which includes the length of the flight, flight initiation distance and flight probability) decreased over the habituation to movement sessions and the approach time of the animals decreased as well. The training was focused on teaching the animals to voluntarily enter a transport box, and undergo transportation. The training protocol was split into 17 different levels, building up from walking into the box into full transport (a 2 minute walk) to the practical room. The levels were created to habituate the animals to the box and give them a positive association with transport, which was accomplished by little steps like moving the box door, closing the door, lifting the box etc. Desired behaviors within each level were rewarded with ESVE drops, a food reward for which the guinea pigs were highly motivated. All guineapigs showed a significant progression in their training level over time and all animals reached the level in which they were transported towards the practical room, indicating that training had been successful.

Introduction

Background of the study

Guinea pigs used as laboratory animals

Since guinea pigs, *Cavia porcellus*, were domesticated in the Central Andes around 6000 years ago, the species has been used in a lot of different ways by humans. In South America, as well as in the Caribbean, guinea pig meat was used as a protein source in the human diet. The animals were also used in rituals [1–3]. Nowadays, the domesticated guinea pig is commonly used as a pet and as laboratory animal. The wild guinea pig *Cavia aperea* still lives in South America. They use open grassland areas which they use for foraging alternating this with vegetation dens areas in which they can hide for predators[4].

The introduction of guinea pigs in laboratories started around 200 years ago when they were used as a model for infectious diseases like diphtheria. The usage peak lays around the year 1960 when 2.5 million guinea pigs were used per year, mostly for research on tuberculosis. Even though other animals like rats and mice have replaced the guinea pigs for certain disease models, guinea pigs are still used as models in laboratory for a wide range of studies such as (infectious) pulmonary diseases, osteoarthrosis and genetic research[3,5]. In the Netherlands, 9108 guinea pigs were used in laboratory in the year 2019. 7710 guinea pigs were used for fundamental scientific research. The other guinea pigs were used for vaccine and medicine development, research about animal diseases and welfare, research for quality control and toxicological research[6].

A strain of guinea pigs that is widely used in laboratories is the albino Dunkin Hartley out-bred strain. The strain was bred nearly 100 years ago, and is still used today[3].

Animal welfare in laboratory animals

In ancient Greece, animals were used for (medical) experiments by physicians like Hippocrates. Over time, the vision on animals and animal welfare changed a lot. For example, Rene Descartes stated in the 16th century that animals did not have a mind nor the ability of feeling pain like humans do. Nowadays, this statement is proven incorrect which leads to the fact that people attach more importance to animal welfare in laboratories [7,8].

An early definition of animal welfare has been described in the "the five freedoms" which were defined in 1965 by the Brambell committee. The five points on this list are: "freedom from hunger and thirst", "freedom from discomfort", "freedom from pain, injury or disease", "freedom to express normal behavior" and "freedom from fear and distress" from which we could conclude that stress and fear in animals could result in lower welfare [7,8].

However, while the five freedoms can be useful for acute welfare assessments, they do not take into account variability in environmental (positive or negative) stimuli, and the abilities of animals to deal with them. Therewithal, the concept is mainly based on the exclusion of negative experiences while the importance of positive emotions and experiences for the animals is mostly ignored[9]. For a definition of animal welfare in the long term, the Faculty of Veterinary Medicine in Utrecht uses a different, far-reaching definition, namely: "An individual is in a

state of well-being when it is able to actively adapt to his life circumstances and thus achieve a state that it experiences as positive."[10,11]
The minimal sizes of laboratory animal enclosures are stated by law, and for guinea pigs they depend on the body weight of the animals. For all body weights a minimum enclosure height is stated on 23 centimeters. Animals up to 450 grams require a minimum enclosure size of 1800 square centimeter and animals over 450 grams require a minimum enclosure size of 2500 square centimeter. The floor areas for animals of 0-200 grams, 200-300 grams, 300-450 grams, 450-700 grams and 700 or more grams are respectively 200, 350, 500, 700 and 900 square centimeter per animal[12]. In laboratories, guinea pigs are mostly housed in a traditional plastic "shoebox" housing system, or GP-suite guinea pig racks which are bigger than the shoebox system and contain a hiding house. However, the "shoebox" housing system, and to a lesser extend the GP-suite, do not allow the guinea pigs to adapt properly to the environment[13,14]. Group housing with bedding, access to hay, hiding places and gnawing material is important, which could be known from behavioral studies about wild living guinea pigs *C. aperea*[4,15]. The traditional housing systems are relatively small and mainly based on a hygienic and cost efficient system. The lack of floor space makes it harder to offer the guinea pigs an enriched environment with branches for gnawing and hiding houses that are big enough for the whole group of animals to hide. Furthermore, not all the traditional systems allow the use of bedding in the cages due to the cage construction[4,13,14].
The faculty of Veterinary Medicine of Utrecht University (the Netherlands) uses Dunkin Hartley guinea pigs for educational purposes in the bachelor Veterinary medicine and in courses on laboratory animal science. Here, the animals are used in practical lessons where students are taught how to safely handle guinea pigs and how to determine the sex of the animal. These guinea pigs are housed in the 'Central laboratory animal research facility' in floor housing systems (with approximately 3m2 of floor space – see materials and methods for more details) with access to a hiding house and a tunnel.
When the guinea pigs have to be transported to the practical rooms, catching the animals in these large floor pens could be harder for the caretakers and teachers when compared to the traditional housing systems, due to the fact that there is more space for the animals to run away and escape. As a prey animal, being chased and caught by a caretaker or teacher could result in stress for the guinea pigs[16,17].

TRAINING ANIMALS TO COOPERATE TO INCREASE ANIMAL WELFARE

To improve animal welfare, looking for alternatives for the situations that induce stress in the guinea pigs, such as the moments in which they need to be caught for transport, could be an option. A training in which the animals learn to walk into a transport box voluntarily could make catching the animals unnecessary. To teach an animal to perform a certain behavior, the trainer could use a form of operant conditioning, namely reinforcement training[18]. In this training process a positive or negative stimulus is used that increases the behavior or response the trainer wants from the animal. Negative reinforcement training, in which an animal is exposed to a negative stimulus until it shows the wanted behavior, is known for its possibility to induce stress and discomfort in animals[19,20]. Therefore, with animal welfare improvement as one of the goals, positive reinforcement rather than negative reinforcement should be used.
In positive reinforcement training a positive reinforcer, for example a food reward, is used to reward desired behavior[21]. This method is used often in training with marine mammals[22],

laboratory animals[23,24] and zoo animals[21] to train animals for cooperation with certain (medical) procedures and as mental enrichment strategy. Research showed that horses which were trained with positive reinforcement to load for transport had a shortened loading time and a showed reduced amount of stress related behavior than horses in a (no training) control group[25]. Also, a study with dogs showed that dogs trained with negative reinforcement expressed more stress related behaviors compared to dogs which were trained with positive reinforcement. Thereby, dogs trained with positive reinforcement showed a better dog-owner relationship[19]. In an experiment with cats that were trained and habituated towards a transport box Pratsch et al. showed in 2018 that trained cats experienced less stress after transport to a veterinarian clinic than untrained cats[26].

To teach an animal to walk into a transport box, the act of voluntarily entering the transport box should be rewarded which could lead to a process called signal approached learning, a form of classical conditioning. The tendency to approach an auditive or visual signal could increase because the approach results in a positive reinforcer. This could occur when the guinea pigs associate the transport box (conditioned stimulus) with a food reward (unconditioned stimulus). The animals will then get attracted towards the transport box (a process called sign tracking or towards the food reward (a process called goal tracking) depending on the animals nature. Both processes could help with training the animal to enter a transport box voluntarily[18,27,28].

The success of animal training depends on several factors. First of all, it is important to choose a food reward for which the animal is highly motivated. Furthermore, the length of the training sessions and the attitude of the trainer can both influence the rapidity of the process. Training sessions must be kept short enough to keep the attention of the animals and the trainer should have the ability of self-reflection, empathize with the animals and have a consequent and calm character[29]. The process between the starting point (the animals is not trained) and the end goal of the training could be visualized as a road which is broken into small steps, described in training levels, which are followed to reach the end goal succesfully[26,29,30].

A successful training protocol could lead to a more predictable situation for the guinea pigs, because a cue or signal announces which behavior is wanted and results in a reward. Due to training, the animals are then able to anticipate to the situation and could influence their own state of wellbeing by performing the wanted behavior[31,32]. The relationship between the predictability of events and animal welfare has been demonstrated in a study with monkeys[33].

It should be kept in mind that guinea pigs which are not used to human interaction can experience stress when humans are around. It is known that rodents in stress situations show decreased cognitive performance, which includes learning and problem solving behavior, due to increased corticosterone levels in the blood plasma [34,35]. Therefore, with reduction of the amount of stress the guinea pigs experience during human-animal interactions, training success could increase. This could be done with the process of habituation.

THE PROCESS OF HABITUATION

Habituation could be described as a form of non-associative learning in which the amplitude of the behavioral response to a stimulus decreases with repeated exposure to this specific stimulus [36]. This process allows animals to adapt to stressors in their environment. Adaptation towards stressors that are not a high-risk for survival could positively affect the fitness of individuals due to less flight reactions. The animal is able now to spend more time foraging and doing other activities that could increase fitness[37].

The rate at which animals habituate to stressors can be influenced by several factors, and these factors need to be taken into account when aiming to successfully habituate animals to certain stressors. First of all, a stimulus that initiates low levels of stress results overall in a faster habituation process than a stimulus that initiates higher stress levels[38]. Also, the rate of habituation depends on the interval at which the stimulus is presented. A high exposure frequency towards the stimulus causes a fast short-term habituation. However, with high stimulus frequency's the process of spontaneous recovery, in which the fear-response towards the stimulus increases again, occurs more often[39]. A study with rats showed that long-term habituation to a noise was more successful, measured in a decrease in startle behavior, with an 16 second interval than with an 2 second interval. However, animals exposure to a 2 second interval showed stronger short-term habituation[40]. When the intervals are too long, habituation does not take place[41]. Another point of attention is that an aversive stimulus such as pain due to medical procedures could influence the habituation process negatively[41].
The counterpart of habituation is sensitization, a learning process in which the fear response towards a stimulus increases when the stimulus is repeated. This process could teach animals to adapt on high risk situations which could induce survival[41]. Unpleasant stimuli should be avoided to make the habituation process successful. A study with macaques showed increased sensitization when pain as unpleasant stimulus was used to dislodge the animals from agricultural land[42].
In laboratory guinea pigs, habituation to humans could be used to improve animal welfare by lowering the stress the animals experience in the presence, by movement and during touch of a human. Also, animals that repeatedly have positive contact and interactions with humans could couple a more positive emotion to the human presence, which could lead to an increase of welfare as the animals reach a more positive state of mind[43]. The decrease of anti-predator behavior and decreasing of the flight initiation distance (FID) could be used as indicators for habituation progress[44,45]. Reduction of food intake could indicate stress due the phenomenon stress induced anorexia[46]. A study with guinea pigs[47] as well as with cats[26] show a decrease in interest for food when stress animals experienced more stress. The increase of explorative behavior of mice was also found to be an indicator of reduced stress [48,49].
In this study with Dunkin Hartley guinea pigs, a successful habituation process could lead to reduction of the amount of stress the animals experience in the presence of a human. Therefore, successful habituation plays a key role in animal training and could be integrated in the initial training steps in a trainings protocol[29,30].

AIM OF THE STUDY

The aim of this explorative study was testing the effect of habituation of the guinea pigs to human presence. The development of a habituation protocol was an important part of this experiment. Another goal of this study was investigating the possibility of training guinea pigs with positive reinforcement to walk voluntarily into the transport cage. Therefore, the development of a training protocol was a second important task. The end goal of training was the transport of the guinea pigs towards the practical room. The training program consists of training levels (habituation to the transport box, entering the box, closing the door, lifting the box, movement on a transport cart - further details see materials & methods) that work towards this end goal.

To test the hypothesis that habituation to human presence had effect, the behavior of the guinea pigs was scored. When habituation takes effect, the following behavioral changes could be expected: a decrease of anti-predator behavior such as hiding and fleeing, an increase of locomotive and explorative behavior, an increase of food uptake and an increase of contact making with the human observer in the cage. With an effective transport box training, a significant progression in training levels over time should be expected.

In case the habituation and training protocols are successful, this might help improving ease of handling and catching the guinea pigs in the future, thereby hopefully making these events less stressful for the animals and more predictable. This, in turn, might have a positive influence on the welfare of the animals.

Materials and methods

ANIMALS AND HUSBANDRY

The animals that participated in this experiment were six Dunkin Hartley guinea pigs (strain: HsdDhl:DH). Three female animals were housed in a 193x164x300 cm (lxwxh) ground cage and three males were housed in a 193x160x300 cm ground cage at the 'Central laboratory animal research facility' of Utrecht University in the department of 'big animals (grote dieren)'. The animals were delivered to the institution on 19 august 2020 with the age of 3-4 weeks old. The work protocol, describing animal husbandry, teaching activities involving the animals, etc. was assessed and approved by the Animal Welfare Body Utrecht (work protocol number 105146-1 – title: 'onderwijs cavia').

The guinea pigs had access to two shelters standing on the bottom of the cage. One was a big shelter with one opening. The other shelter was a red transparent tunnel. In each cage a pink Jolly ball and three wooden branches were available for further enrichment. Water (in a bowl and drinking bottle), autoclaved hay and guinea pig pellets from "Special diet services" with batch number 4540 were available at libitum. Autoclaved straw was used as bedding. Male and female guinea pigs were able to make physical contact (nose-nose touch) through the metal separation between the two cages. The food and water of the guinea pigs was refilled each day before 10 AM. On each Thursday morning the cage was cleaned. The housing of the guinea pigs was not changed prior to or during this experiment. **Figure 1** shows the guinea pig housing.

Figure 1. The cage of the males with on the left (behind the iron bars) the cage of the females.

On 22 January the guinea pigs were marked with Kerbl top marker in blue and pink. One animal in each group was left unmarked and was coded as "white" during the scoring procedure.

VIDEO RECORDING

After the first week of observation, the total habituation and training sessions were all recorded on video. From 26 January until 12 February systema security camera system (Bascom®) was used for the males as well as the females. The cages were filmed with an helicopter view during this time. The camera used for the males was replaced on 16 February with a Parasonic HC-V180 handcamera standing on a tripod on the big shelter in the male cage. A helicopter view was not achievable with this camera setup. The different viewpoints per camera per cage are shown in **figure 2**.

Figure 2. Left to right: 1. Female camera, 2. Male camera 26 January-12 February, 3. Male camera after 12 February

USE OF FOOD REWARDS

During the habituation sessions five different food rewards were used:

- ESVE drops (flavors: “yoghurt”, “bosbes”, “melk-honing”, “sinaasappel” and “wortel”)
- Nature flakes from Vitakraft (contains: dried carrot flakes, crushed peas, crushed beans)
- Fresh cucumber, washed then peeled and cut in pieces smaller than 1 by 0,5 centimeter.

Availability of the different food rewards during the habituation sessions and the notations about animal reactions are described in **appendix 1.**
During the training sessions only ESVE drops were used, because all the animals were motivated for this reward while the animals had an unequal personal preference for the other food rewards (personal observations during the habituation sessions).

TIME SCHEDULE

Totally, there were 28 habituation/training sessions which were divided over 8 weeks. The habituation process consisted of different phases. The time schedule of the habituation and training sessions is visualized in **figure 3** and the dates at which each session took place are noted in **appendix 1**.

In the first week the initial observation and habituation to human presence started with four sessions with the duration of 1-1.5 hour per cage. No cameras were available this week, because this study was based on the study of J. van Eupen[50]. Because of the results in this previous study (in another group of guinea pigs) we did not expect a lot of change in behavior in the first week, therefore it was expected that the lack of this data would not be a problematic for the statistical analysis.
In session 2, the use of food rewards was introduced. Further habituation sessions then continued with the presence of food rewards (ESVE drops and dried vegetables), so the animals were able to make a positive association with the presence of the observer[29]. Habituation sessions were shortened to 45 minutes after the first week, because it was observed that the animals lost interest in the observer after 45 minutes and retreated to eat or sleep.

On Tuesday, Wednesday and Friday the further habituation sessions started at 10.30 AM. On Thursday the habituation sessions started at 2.30 PM because cage cleaning was planned on Thursday mornings. Training sessions (see ‘training protocol’ for further details) took only place on Tuesdays, Wednesdays and Fridays and started around 10.30 AM.
During training, animals are exposed to the movements of a human, therefore a phase in which guinea pigs were habituated to human movement was added (see ‘habituation protocol’ for more details). The “habituation to human movement” phase started when all guinea pigs in a group accepted the ESVE drops from the observers hands for two consecutive days. **Figure 3** shows when each group (males or females) entered the specific habituation phases.
Males and females showed a difference in habituation rate (females progresses slower than males), so the habituation schedule was adjusted to the progress each group (males or females) made. This is why males and females were not in the same habituation phase on the same date (see **figure 3**).
Because males progresses quicker than females, we added an explorative phase for males at the end of the habituation period, in which habituation to human touch was introduced. The habituation to human touch started for an individual animal when it showed a “no flight” in a habituation to movement session for at least 50% of the movements. However, because habituation to human touch was not required for transport box training, this phase was skipped

for the females, and once they finished the habituated to human movement phase, transport box training commenced for all animals on session 18 (see **figure 3**, and see 'training protocol' for further details).

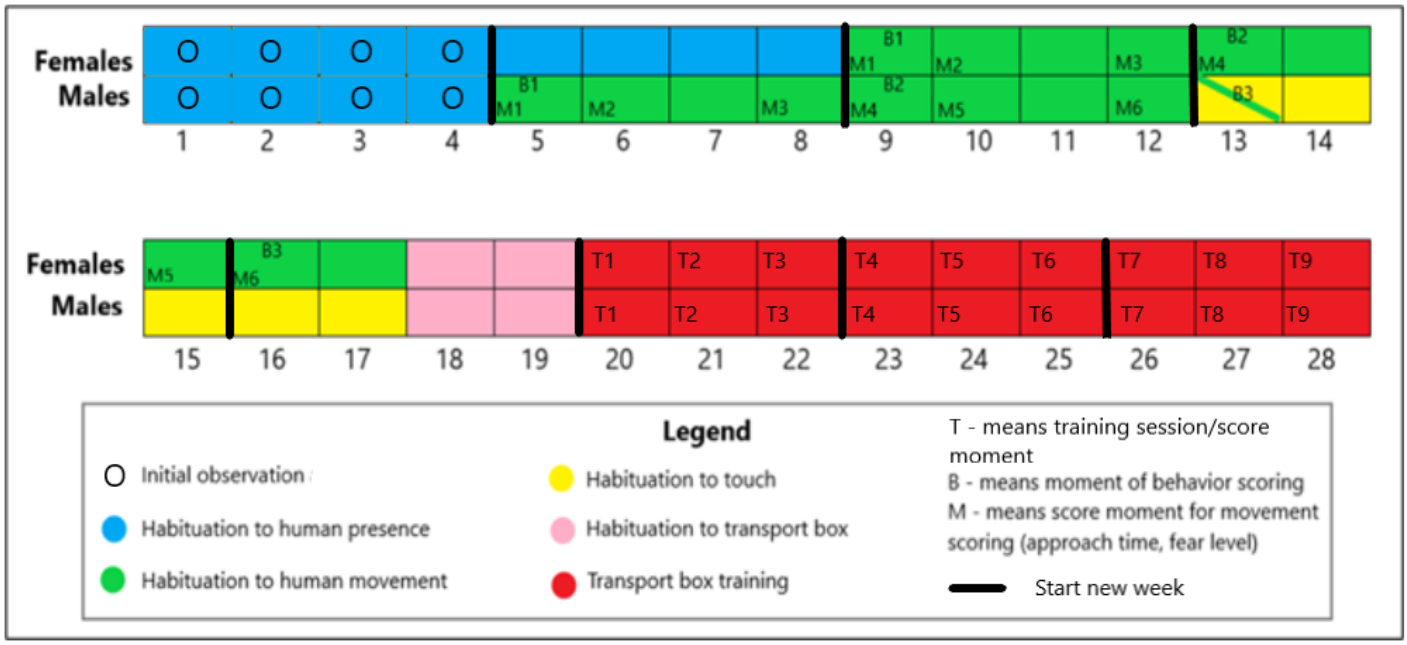

Figure 3. The schedule for the male and female guinea pigs with the different habituation phases and the transport box training over the 28 sessions. Session 13 for males was comprised of habituation to human touch for one of the animals ('male blue'), while the two other individuals ('male pink' and 'male white') were still at the habituation to human movement phase. B1, B2 and B3 are the sessions from which the 15 minutes of behavior were scored. M1-M6 are the sessions on which the scoring specific for the "habituation to human movement" phase was done.

HABITUATION PROTOCOL

HABITUATION TO HUMAN PRESENCE

The observer entered the cage with a serene attitude and tried to make no loud noises. The observer was sitting still on the cage floor without moving on purpose (besides some movements when the observer shifted sitting posture). The two different sitting postures used by the observer are visualized in **figure 4**. The guinea pigs were not touched by the observer and the observer avoided direct eye contact with the animals.

Figure 4. The two different sitting postures from the observer during the habituation sessions.

During this phase the animals were also habituated towards handfeeding which could be useful for offering food rewards during the training[29]. Food rewards were placed on the a terracotta plate, that was used as feeding plateau, and on the clothes of the observer. The feeding plateau was placed no further than 10 centimeters from the observer. The observer placed her hand with a food reward in between the thumb and forefinger next to the food rewards on the clothes or the feeding plateau so the guinea pigs could take the reward.
When all three guinea pigs in a group accepted the food rewards from the observers hand, the feeding plateau was no longer used.
During the other habituation phases the hand feeding was further expanded from a still hand towards a hand that moved carefully towards the animal to offer the reward.

HABITUATION TO HUMAN MOVEMENTS

The observer made slow movements (half a circle) with the upper body. The movements were not directed towards the guinea pigs and hands/arms did not approach the guinea pigs on purpose. After a movement the observer was sitting still again. Movements were only made when there was at least one guinea pig was in zone A or zone B (zones see figure 7) for at least 10 seconds.
When the guinea pigs had fled after a movement, the observer tried to sit still until at least one animal entered zone A or B again. The observer did not move when an animal was still making the approach, but waited until the animal was showing other behavior or was making contact with the observer by sniffling or putting paws on the observer. When the guinea pigs approached again, they were offered a food reward from the observer.

HABITUATION TO HUMAN TOUCH

This was an explorative habituation phase in which no data was collected for statistical research.

When a guinea pig was around the observer, the observer gave the guinea pig a food reward with one hand and tried to touch the guinea pigs side softly with the other hand. The guinea pigs were able to flee all the time and the observer did not touch a guinea pig when it was not able to flee due to the position of the animal in the cage, like when the animal was trapped in a corner or when another guinea pig blocked its way.
The main goal of this study was habituating the guinea pigs to human presence and learning them to walk into the transport box voluntarily. For the transport box training, it was not necessary that the animals were habituated to touch. Therefore, the habituation to touch phase was stopped so transport box training could start on time.

HABITUATION SCORING

BEHAVIOR SCORING

The behavior of the guinea pigs during the habituation phases was scored for three weeks, one day a week. Due to the difference in habituation schedules, the scoring was not performed on the same session/date but it was taken into account that the habituation phase was equal. **Figure 3** shows which sessions for the males and the females were scored for behavior coded as B1, B2 and B3.
The scoring started from the moment that the observer was settled in de cage, from that moment exactly 15 minutes were scored. The software used for scoring was Solomon coder (version 19.08.02)[51]. The videos were uploaded in the Solomon coder in AVI format.
First an ethogram **(appendix 2)** was build based on literature research and field observations in week one. This ethogram was used to define which behaviors were scored and if the behaviors were scored for frequency only, or for both frequency and total duration (s) of the behavior in the 15 minutes that were scored (frequency and/or duration see **appendix 2**).
The following behaviors were scored:

A. Hiding
   There was hypothesized that the frequency and duration of hiding, as an anti-predator behavior decreases over time when habituation is successful[15,47].
   - Hiding tunnel
   - Hiding behind the shelter (zone c minus the big shelter, visualized in figure 7)
   - Hiding under the big shelter
B. Animal-human interactions
   With successful habituation towards humans, an increase in animal-human interactions/explorative behavior towards the human over time is expected.
   - Sniffling observer
   - Gnawing observer
   - Taking food reward from hands
   - Taking food from clothes
   - Approach
   - Behind the observers back
   - Paws on observer
C. Flight behavior

A decrease in flight behavior over time is expected when habituation towards humans occurred[15,47].

- Fleeing
- Freezing
- Startle

D. Ingestion behavior

Based on literature there is hypothesized that reduction of stress levels of the animals in human presence due to habituation could result in increase of ingestion behavior over time[46].

- Eating hay or straw
- Eating pallets
- Drinking
- Eating from feeding plateau
- Caecotrophy

E. Exploration towards environment

The habituation process is directed to the presence of a human while the animals are still in the home cage, so no environmental changes are made. Therefore, there will be tested for the possibility of change in exploration to the environment, but no specific hypothesis about in- or decrease are made.

- Gnawing object
- Sniffing object
- Pushing object
- Digging

F. Active locomotive behavior

There is hypothesized that with success of habituation the active locomotion behavior increases[52].

- Walking
- Jumping
- Frisky hops

G. Passive locomotive behavior

Passive locomotive behavior was tested for change over time.

- Rest/lay down

H. Grooming

Grooming behavior was tested for change over time.

I. Social behavior

Research with guinea pigs showed that a social buffering effect occurred in guinea pigs in stressful situation but no differences in social behaviors were found[53]*. In other species change in social behavior under stressful conditions had been observed*[54]*. Therefore, in this study social behavior was tested for change over time.*

- Socio-positive behavior (in total [15])
  - Nose-nose
  - Sniffing other guinea pig
  - Allogrooming
  - Following
- Socio-negative behavior (in total [15])
  - Biting

- Chasing
- Stand-threat
- Head up
- Fighting
- Attack lunge
- Fleeing from other guinea pig
- Heath-thrust

- Social contact between the sexes
  - Unsuccessful contact between the sexes
  - Nose-nose contact between the sexes
  - Rumba-rumble

J. Other behaviors
The other behaviors were tested for change over time in no specific direction.
- Scratching
- Shaking
- Stretching
- Yawning

SCORING HABITUATION TO HUMAN MOVEMENT

Six habituation to human movement sessions, coded M1-M6 visualized in **figure 3**, were scored for each group of guinea pigs. In each session, the reactions towards the first six movements of the observer per guinea pig were scored. On score moment M3 for the males, this amount of movements was not reached because the males were not around the observer a lot (and the guinea pigs reaction to movement was only tested once they were within zone A or zone B, see **figure 5** for specifications). Therefore, this day only three movements for male blue, and five movements for male pink and male white were scored.
For the habituation to human movement, two specific parameters were scored. First there was scored if the guinea pigs were fleeing from the observers movement and to which zone. The cage was divided into three zones, shown in **figure 5**. Zone A is the green zone which is half a circle at a distance of 15 centimeter from the observer. Zone C is the red zone which is defined as the zone "hiding big" and "hiding behind shelter" from the behavior scoring combined. Zone B is the space in between zone A and zone C.
When a guinea pig was partly in one zone and partly in another, the zone that contained the biggest percentage of the guinea pigs body was counted. However, zone A is relatively small, therefore the guinea pig was scored as "being in zone A" when the head of the animal was in zone A.
There are 7 possibilities that could be scored:

- No flight A = the guinea pig does not flee when the guinea pig is inside zone A
- No flight B = the guinea pig does not flee when the guinea pig is inside zone B
- Flight inside A = the guinea pig is in zone a and flees, but it stays in zone A
- Flight inside B = the guinea pig is in zone a and flees, but it stays in zone B
- A to B = the guinea pig flees from zone A towards zone B
- A to C = the guinea pig flees from zone A towards zone C
- B to C = the guinea pig flees from zone B towards zone C

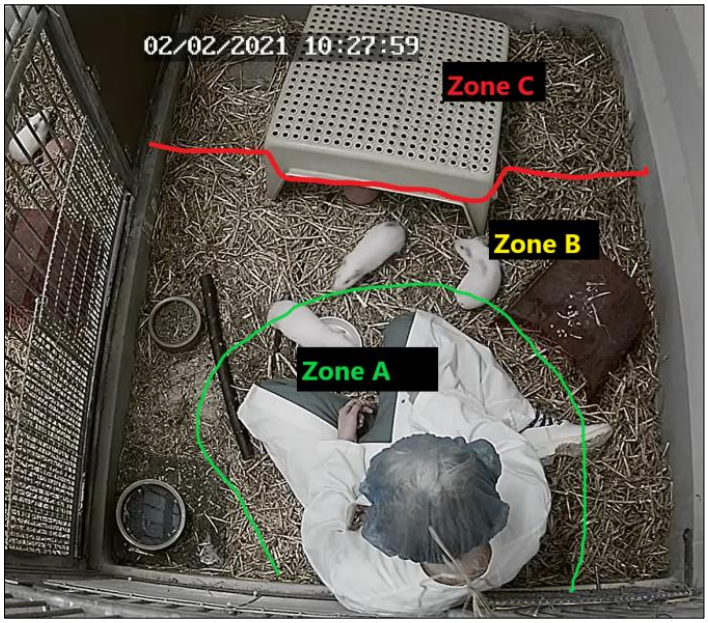

Figure 5. The three different zones (A, B and C) in which the cage was divided for the scoring towards were the guinea pigs fled after a movement from the observer.

The flight zone data was processed into "fear levels" which could be used to indicate the flight motivation/amount of fear which the guinea pigs experienced after a movement of the observer. A higher fear level could indicate higher stress levels for the guinea pig. The calculation used for the fear levels could be explained with help of **table 1** in which the possibilities for "start place", "fleeing yes/no" and "fleeing zones" are coupled to a numerical value. **Table 2** shows the fear level per flight option. *Example calculation "fear level" from "flight inside A": Start place A = score 1, fleeing yes = score 2, fleeing zones is inside the zone = score 1. Fear level = 1+2+1=4.*

Table 1. The possibilities for start place (zone A or zone B), fleeing (yes or no) and flight length (inside the zone, one zone, two zone's) couplet to a numerical value.

Start place	Fleeing yes/no	Flight length
A = 1	No = 1	Inside zone = 1
B = 2	Yes = 2	One zone = 2
		Two zones = 3

Table 2. The seven "flight options" and the coupled fear level that could be calculated with help of table 1.

Flight reaction	Fear level
No flight in A	2
No flight in B	3
Flight inside A	4
Flight inside B	5
Flight A to B	5
Flight B to C	6
Flight A to C	6

The second parameter that was scored is the “approach time” in seconds, which is defined as the time takes the guinea pig to approach the observer again after a flight. Approach time was measured using a stopwatch. The time starts the moment of the movement and is stopped when the guinea pig turns its nose towards the observer again, or when it approaches again after hiding (which means that the head of the guinea pig crosses the line that separates zone B from zone C.)

When the guinea pig did not flee or only startled, the approach time was defined as 0. The approach time was stopped and noted as 60 seconds when a guinea pig does not approach in less than a minute. This is done because after this time the guinea pigs would switch to different behaviors, such as eating, or social interactions (based on personal observations) and were therefore assumed to not be in active flight anymore.

TRAINING PROTOCOL

Training started for both the male and the female group in session 18. The training phase started with two days habituation towards the transport box for 45 minutes each day in presence of the observer. A box measuring 40,5 by 25 by 29 centimeters (lxwxh) was used, see **figure 6**. During the two habituation days, the box was placed inside the guinea pig enclosure without the door, so the guinea pigs could explore the inside as well as the outside of the transport box. The observer, from now called “trainer”, had placed pieces of ESVE drops inside the transport box before the box was placed in the guinea pig cage.

Figure 6. The transport box that was used for the training.

After these two habituation days towards the transport box, the training was started using levels and criteria for each guinea pig as an individual. The duration of the sessions lay between 15 and 40 minutes. At level 11 the trainer started to move the transport cart and with every

following level the length of the road increased. Therefore, with increase of the level, the duration of the trainings sessions increased as well.

Materials

2 transport boxes, transport cart, green cloth for covering the transport boxes when the guinea pigs left the guineapig hallway, ESVE drops broken in pieces

Description of the levels

At the beginning of each training session pieces of ESVE drops were placed inside the transport box. The trainer placed extra drops inside the cage during the training so there were always pieces of drops precent inside the cage.

LEVEL 1. The transport box is placed inside the cage and the trainer besides it. The level is achieved when the guinea pig enters the transport box and eats a food reward.

LEVEL 2. Repeat level 1, but now the trainer stands in the cage. The level is achieved when the guinea pig enters the transport box and eats a food reward.

LEVEL 3. When a guinea pig is inside the transport box the trainer makes a soft sound with the fingers on the entrance of the box. The level is achieved when the guinea pig eats a food reward during or after the sound.

LEVEL 4. When a guinea pig is inside the transport box, the trainer moves the door of the transport box. The level is achieved when the guinea pig eats a food reward during or after the movement of the door.

LEVEL 5. When a guinea pig is inside the transport box, the trainer closes the door of the transport box for around 10 seconds and opens it again. The level is achieved when the guinea pig eats a food reward with the door closed or after the door is opened again.

LEVEL 6. When a guinea pig is inside the transport box, the trainer closes the transport box and makes a sound with the handle at the top of the box. The level is achieved when the guinea pig eats a food reward during or after the sound making.

LEVEL 7. When a guinea pig is inside the transport box, the trainer closes the transport box and lift it 15 centimeters from the ground. The level is achieved when the guinea pig eats a food reward after the box is placed on the ground again.

LEVEL 8. When a guinea pig is inside the transport box, the trainer closes the transport box and lift it 50 centimeters from the ground. The level is achieved when the guinea pig eats a food reward after the box is placed on the ground again.

LEVEL 9. When a guinea pig is inside the transport box, the trainer closes the transport box and takes it a couple steps outside the guinea pig cage. After this, the transport box is placed inside the guinea pig cage again. The level is achieved when the guinea pig eats a food reward when the box is in the air or after the box is placed on the ground again. *This level was skipped after 2-03-2021 because then the transport cart was available and the transport box was placed directly onto the transport cart after the trainer stepped outside the cage.*

LEVEL 10. When a guinea pig is inside the transport box, the trainer closes the transport box and takes the box outside the guinea pig cage and places it on the transport cart. After this, the transport box is placed inside the guinea pig cage again. The level is achieved when the guinea pig eats a food reward when the box stands on the transport box.

LEVEL 11. Repeat level 10, but now the transport cart is moved towards the door of the guinea pig hallway and back (route A in **figure 7**). After this, the box is placed inside the guinea pig cage again. The level is achieved when the guinea pig eats a food reward when the box stands on the transport cart after the ride.

LEVEL 12. Repeat level 11, but now the transport cart is moved towards the hallway "grote dieren" and back (route A + B in **figure 7**). After this, the transport box is placed inside the guinea pig cage again. The level is achieved when the guinea pig eats a food reward when the box stands on the transport cart after the ride.

LEVEL 13. Repeat level 12, but now the transport cart is moved towards the reception (route A + B + C in **figure 7**). At the reception the cart is stopped and the trainer will try to give the guinea pig a food reward. After this, the cart will be moved back towards the guinea pig enclosure, where again a food reward will be offered. The level is achieved when the guinea pig eats a food reward when the box stands on the transport cart after the total ride.

LEVEL 14. Repeat level 13, but now the transport cart is moved towards the practical room (a two minute walk, route A + B + C + D in **figure 7**). In the practical room the cart is stopped and the trainer will try to give the guinea pig a food reward. After this, the cart will be moved back towards the guinea pig enclosure where again a food reward will be offered. The level is achieved when the guinea pig eats a food reward when the box stands on the transport cart after the total ride.

LEVEL 15. Repeat level 14, but this time the transport box will be opened from above and the trainer will offer the guinea pig a food reward. The level is achieved when the guinea pig accepts the food reward from the trainers hands inside the opened transport box.

LEVEL 16. Repeat level 15, but this time the transport box will be opened and the trainer will take out the guinea pig. The trainer will hold the guinea pig for around 30 seconds against her body. After that the guinea pig will be gently put back into the transport box and a food reward will be offered from the trainers hands. The level is achieved when the guinea pig accepts the food reward from the trainers hand when it is back in the transport box.

LEVEL 17. Repeat level 16. The level is achieved when the guinea pig accepts a food reward during the handling procedure.

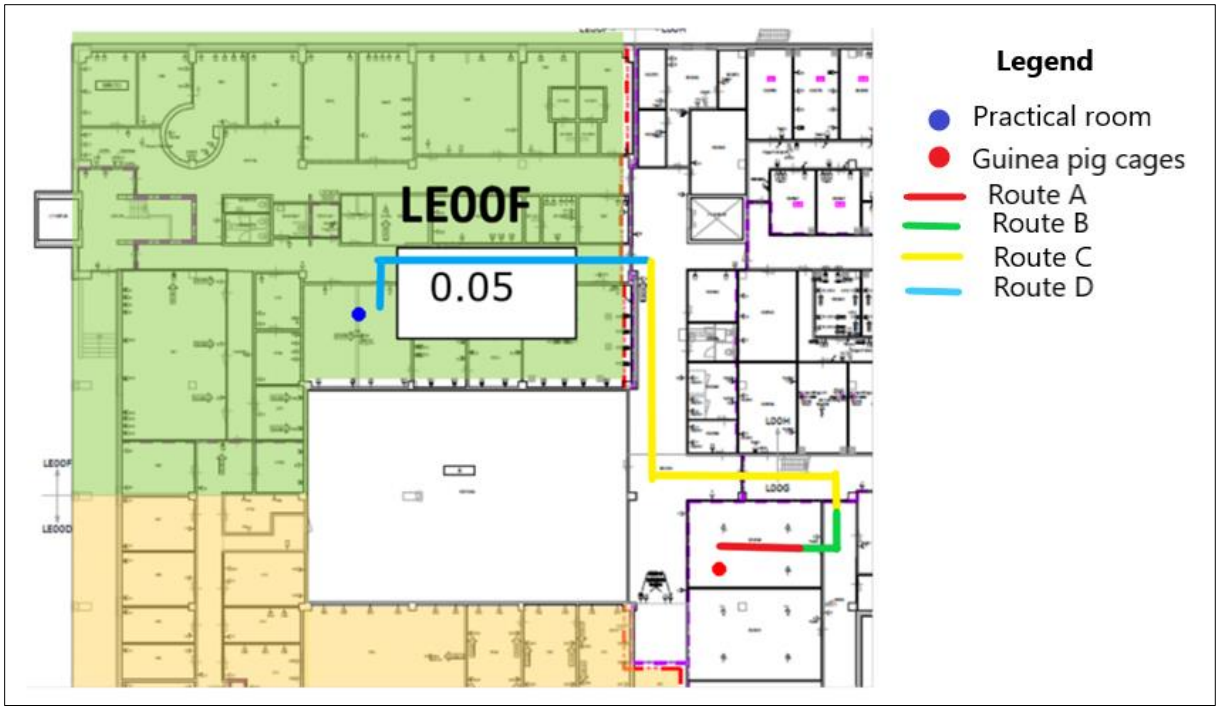

Figure 7. The floor map of the first floor of the GDL building to visualize the routes that were taken during the transport box training. The blue dot marks the practical room, the red dot marks the guinea pig enclosure. The colored lines show the routes that were walked during the training levels.

POINTS OF ATTENTION

- For each individual guinea pig a new training session started with the next level after the highest achieved level in the session before. In some cases, more than one guinea pig entered the transport box. If this was the case, the trainer performed the level of the guinea pig with the lowest level of those present in the transport box.
- The female guinea pigs did show anti-predator behavior (hiding, fleeing) to the trainer when she was standing in the cage. Therefore the trainer performed level three and four sitting (females only). These levels were scored as "end level" two, because the trainer was sitting. The trainer habituated the females to a standing person on short moments during and after the training, therefore after three days sitting was unnecessary.
- Eating a food reward means both eating a reward that was placed inside the transport box, or eating a reward from the hands from the trainer. However, some levels were only achieved when the guinea pig took the food reward from the hands of the trainer. When this was the case, this is specially described in the levels (level 15, 16, 17). Refusal or acceptation of the food reward could be used as a stress parameter, by the existence of stress induces anorexia. Therefore the acceptance of the food reward is used as an indication for completing a level[47,55–57].

TRANSPORT BOX SCORING

The levels were scored for each training session (9 sessions total). The "end level" was the highest level a guinea pig completed in a trainings session. For level one to nine, the videos were watched back because the trainer was inside the guinea pig cage and there was no room

and time for notations on paper. From level 10, the trainer made notations in a notebook that laid on the transport cart.

STATISTICAL ANALYSIS

For behavior during the habituation phases, we aimed to investigate changes in behavior across the different sessions (B1, B2 and B3 in **figure 3**) and changes in approach time and fear levels were investigated over 6 habituation to movement sessions (M1-M6 in **figure 3**). Training progress was evaluated by investigating changes in training levels over the 9 trainings session. The statistical analysis was performed over the data of the total group (with N = 6.)

IBM SPSS statistics version 26 for windows was used for the statistical analysis and for visualizing the data into figures. In this study, a p-value under 0.05 was assumed as significant and a p-value between 0.05 and 0.100 was assumed as a trend. Habituation or training session-number was always the independent variable, the outcome variables - all behavioral frequencies/durations, "approach time", "fear level" and training levels - were the dependent variables.

On the frequency data of the behaviors, a Friedman's ANOVA was performed to find out if there was any behavioral change over time. When the Friedman ANOVA indicated a significant difference, a Wilcoxon post hoc test was used to identify statistical differences between specific sessions. Bonferroni correction (p values x 3) was applied to correct the post hoc tests for multiple testing.

The data for total behavior durations in a session was tested for normality with the Shapiro-Wilk test. The duration of "hiding tunnel", "hiding total", "sniffling observer", "eating straw/hay", "sniffling an object", "socio-positive behavior total", "paws on" and "fleeing" showed a normal distribution. After a log transformation only the duration of "hiding big" showed a normal distribution.

On (log transformed) parametric data, an one way repeated measure ANOVA was performed to find changes in durations of behavior over the three habituation sessions. When a one way repeated measure ANOVA indicated a significant difference, this data was also post hoc tested to identify in between which sessions the difference occurred. Bonferroni correction was applied on the post hoc tests to correct for multiple testing which creates a new significance level of < 0.017 (0.05/3) for the post hoc tests. Before performance of an one way repeated measure ANOVA the sphericity was tested with a Mauchly test. When the assumption of sphericity was violated the degrees of freedom were corrected.

On non-parametric data for the durations a Friedman's ANOVA was performed. When data showed significant difference, the same post hoc test procedure was followed as described by the Friedman's ANOVA's for the frequency date.

On the fear levels in "habituation to movement" and the training levels in the transport box training a Friedman's ANOVA was performed to find out if there was a change in reached level over the "habituation to movement"- or trainings sessions. The approach time in "habituation to movement" was tested for normality which was not proven, therefore also a Friedman's ANOVA was performed.

Results

BEHAVIOR OVER THREE HABITUATION SESSIONS

Excluded behaviors and descriptive statistics

Some behaviors hardly ever occurred and were therefore not described in the results (freezing n = 3, eating pallets n = 0, drinking n = 4, coprophagy n = 1, digging n = 0, frisky hops n = 3, grooming n = 2, duration socio-negative, stretching n = 0, yawning n = 0.)

No statistical analysis was performed on "taking food from clothes" and "eating from feeding plateau" because these feeding methods were stopped after 2 weeks of habituation by the observer.

The means descriptive statistics for all the scored behaviors per score moment are shown in **table 3**.

Table 3. The mean values and standard deviations per behavior on the score moments B1, B2 and B3.

Behavior Freq.	B1	B2	B3	Behavior Duration (s)	B1	B2	B3
Hiding under big house	Mean: 4,50 Sd: 3,209	Mean: 5,17 Sd: 2,787	Mean: 4,17 Sd: 2,972	**Hiding under big house**	Mean: 84,06 Sd: 76.67	Mean: 58,80 Sd: 58.05	Mean: 53,63 Sd: 23,81
Hiding behind big house	Mean: 7,33 Sd: 3,077	Mean: 3,67 Sd: 2,066	Mean: 3,50 Sd: 1,517	**Hiding behind big house**	Mean: 217,00 Sd: 138,66	Mean: 192,30 Sd:138,66	Mean: 86,27 Sd: 97,47
Hiding tunnel	Mean: 4,64 Sd: 3,777	Mean: 1,83 Sd: 0,408	Mean: 2,83 Sd: 3,656	**Hiding tunnel**	Mean: 46,06 Sd: 45,54	Mean: 11,13 Sd: 9,48	Mean: 30,30 Sd: 21,45
Hiding total	Mean: 16,50 Sd: 4,324	Mean: 10,67 Sd: 1,506	Mean: 11,50 Sd: 3,834	**Hiding total**	Mean: 347,13 Sd: 130,86	Mean: 263,00 Sd: 95,07	Mean: 170,20 Sd: 95,09
Sniffling observer	Mean: 28,00 Sd: 10,752	Mean: 19,50 Sd: 11,274	Mean: 19,83 Sd: 9,131	**Sniffling observer**	Mean: 60,16 Sd: 15,44	Mean: 39,13 Sd: 25,46	Mean: 48,96 Sd:29,70
Gnawing observer	Mean: 2,17 Sd: 2,137	Mean: 2,67 Sd: 2,875	Mean: 0,00 Sd: 0,00	**Gnawing observer**	Mean: 9,20 Sd: 7,43	Mean: 9,30 Sd: 10,56	Mean: 0,00 Sd: 0,00
Taking food from hands	Mean: 8,67 Sd: 7,581	Mean: 14,17 Sd: 9,725	Mean: 17,83 Sd: 8,635	-	-	-	-
Paws on	Mean: 5,00 Sd: 4,141	Mean: 15,17 Sd: 7,026	Mean: 18,17 Sd: 5,382	**Paws on**	Mean: 63,96 Sd: 48,43	Mean: 166,76 Sd: 110,20	Mean: 201,53 Sd: 103,30
Approach	Mean: 14,17 Sd: 4,119	Mean: 10,50 Sd: 2,739	Mean: 12,17 Sd: 2,927	-	-	-	-
Behind observers back	Mean: 2,33 Sd: 5,067	Mean: 2,17 Sd: 2,567	Mean: 2,00 Sd: 1,414	**Behind observers back**	Mean: 56,73 Sd: 71,91	Mean: 13,93 Sd: 11,85	Mean: 24,13 Sd: 31,21
Fleeing	Mean: 12,33 Sd: 4,179	Mean: 13,17 Sd: 4,215	Mean: 9,50 Sd: 4,183	**Fleeing**	Mean: 16,90 Sd: 5,79	Mean: 16,93 Sd: 5,25	Mean: 11,96 Sd: 5,24
Freeze	Mean: 0,33 Sd: 0,516	Mean: 0,17 Sd: 0,408	Mean: 0,00 Sd: 0,00	**Freezing**	Mean: 0,767 Sd: 1,60	Mean: 0,133 Sd: 0,327	Mean: 0,00 Sd: 0,00
Startle	Mean: 1,17 Sd: 1,472	Mean: 1,00 Sd: 1,200	Mean: 3,67 Sd: 6,563	-	-	-	-
Eating hay or straw	Mean: 5,17 Sd: 3,601	Mean: 8,17 Sd: 2,401	Mean: 10,67 Sd: 4,885	**Eating hay or straw**	Mean: 37,97 Sd: 22,81	Mean: 116,67 Sd: 48,96	Mean: 90,83 Sd: 55,26
Eating pellets	Mean: 0,00 Sd: 0,00	Mean: 0,00 Sd: 0,00	Mean: 0,00 Sd: 0,00	**Eating pellets**	Mean: 0,00 Sd: 0,00	Mean: 0,00 Sd: 0,00	Mean: 0,00 Sd: 0,00
Drinking	Mean: 0,33 Sd: 0,516	Mean: 0,33 Sd: 0,816	Mean: 0,00 Sd: 0,00	**Drinking**	Mean: 8,00 Sd: 17,44	Mean: 1,167 Sd: 2,858	Mean: 0,00 Sd: 0,00
Caecotrophy	Mean: 0,00 Sd: 0,00	Mean: 0,00 Sd: 0,00	Mean: 0,17 Sd: 0,408	-	-	-	-

Gnawing object	Mean: 0,33 Sd: 0,516	Mean: 0,17 Sd: 0,408	Mean: 1,67 Sd: 3,141	**Gnawing object**	Mean: 0,933 Sd: 2,096	Mean: 0,167 Sd: 0,408	Mean: 4,93 Sd: 11,03
Sniffling object	Mean: 9,17 Sd: 4,750	Mean: 4,83 Sd: 4,750	Mean: 1,17 Sd: 0,983	**Sniffling object**	Mean: 19,33 Sd: 14,20	Mean: 9,80 Sd: 8,35	Mean: 2,80 Sd: 3,05
Pushing object	Mean: 0,00 Sd: 0,00	Mean: 0,17 Sd: 0,408	Mean: 0,67 Sd: 1,211	**Pushing object**	Mean: 0,00 Sd: 0,00	Mean: 0,167 Sd: 0,408	Mean: 0,800 Sd: 1,391
Digging	Mean: 0,00 Sd: 0,00	Mean: 0,00 Sd: 0,00	Mean: 0,00 Sd: 0,00	**Digging**	Mean: 0,00 Sd: 0,00	Mean: 0,00 Sd: 0,00	Mean: 0,00 Sd: 0,00
Walking	Mean: 39,33 Sd: 11,656	Mean: 37,33 Sd: 10,013	Mean: 46,83 Sd: 10,226	**Walking**	Mean: 121,20 Sd: 36,60	Mean: 110,73 Sd: 34,93	Mean: 149,97 Sd: 13,67
Lay/rest	Mean: 0,00 Sd: 0,00	Mean: 0,00 Sd: 0,00	Mean: 0,00 Sd: 0,00	**Lay/rest**	Mean: 0,00 Sd: 0,00	Mean: 0,00 Sd: 0,00	Mean: 0,00 Sd: 0,00
Jumping	Mean: 0,00 Sd: 0,00	Mean: 2,00 Sd: 1,673	Mean: 0,33 Sd: 0,516	-	-	-	-
Scratching	Mean: 0,17 Sd: 0,408	Mean: 0,33 Sd: 0,516	Mean: 0,67 Sd: 0,516	-	-	-	-
Shaking	Mean: 0,00 Sd: 0,00	Mean: 0,50 Sd: 0,837	Mean: 1,00 Sd: 0,894	-	-	-	-
Frisky hops	Mean: 0,00 Sd: 0,00	Mean: 0,33 Sd: 0,816	Mean: 0,00 Sd: 0,00	-	-	-	-
Stretching	Mean: 0,00 Sd: 0,00	Mean: 0,00 Sd: 0,00	Mean: 0,00 Sd: 0,00	-	-	-	-
Grooming	Mean: 0,00 Sd: 0,00	Mean: 0,17 Sd: 0,408	Mean: 0,00 Sd: 0,00	**Grooming**	Mean: 0,00 Sd: 0,00	Mean: 0,100 Sd: 0,245	Mean: 0,00 Sd: 0,00
Yawning	Mean: 0,00 Sd: 0,00	Mean: 0,00 Sd: 0,00	Mean: 0,00 Sd: 0,00	-	-	-	-
Socio-positive Total	Mean: 4,33 Sd: 2,503	Mean: 3,33 Sd: 1,366	Mean: 1,67 Sd: 1,506	**Socio-positive total**	Mean: 4,067 Sd: 2,643	Mean: 6,100 Sd: 2,779	Mean: 2,400 Sd: 2,773
Socio-negative Total	Mean: 1,00 Sd: 1,673	Mean: 1,00 Sd: 1,265	Mean: 1,00 Sd: 1,095	**Socio negative total**	Mean: 0,113 Sd: 0,326	Mean: 0,00 Sd: 0,00	Mean: 0,167 Sd: 0,408
Unsuccessful contact between sexes	Mean: 0,17 Sd: 0,408	Mean: 0,50 Sd: 1,225	Mean: 0,17 Sd: 0,408	**Unsuccessful contact between sexes**	Mean: 0,367 Sd: 0,898	Mean: 2,400 Sd: 5,879	Mean: 0,700 Sd: 1,715
Nose-nose between sexes	Mean: 0,67 Sd: 0,816	Mean: 1,67 Sd: 2,422	Mean: 0,17 Sd: 0,407	**Nose-nose between sexes**	Mean: 2,166 Sd: 2,587	Mean: 6,800 Sd: 9,680	Mean: 0,133 Sd: 0,326
Rumba-rumble	Mean: 0,17 Sd: 0,408	Mean: 2,00 Sd: 3,633	Mean: 0,17 Sd: 0,408	-	-	-	-

Hiding behavior

The frequency of "hiding behind the shelter" significantly changed over time, $\chi 2\ (2) = 8.455$, $p = 0.012$. The change is visualized in **figure 8.** B1: mean 7,33, Sd 3,077, B2: mean 3,67, Sd 2,066 and B3: mean 3,50, Sd 1,517 Post-hoc tests indicated a trend for decrease between B1 and B2 ($p = 0.095$ one-tailed), no significant decrease between B2 and B3 ($p = 0.500$ one-tailed), and a significant decrease between B1 and B3 ($p = 0.047$ one-tailed).

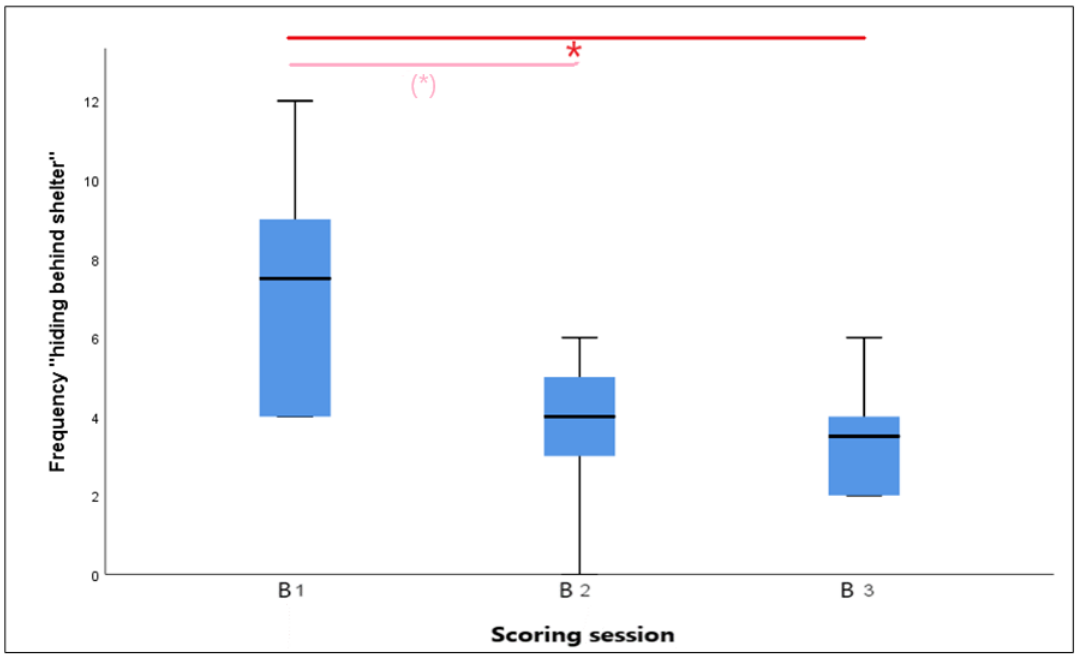

Figure 8. The changes of the frequency “hiding behind the shelter” over time. -* shows the significant decrease ($p<0.05$) between B1 and B3 and –(*) shows the trend for decrease ($p<0.10$) between B1 and B2 (time schedule see figure 3).

Significant change over time was also found for the duration “hiding behind the shelter” (χ 2 (2) = 7.000, p = 0.029) as visualized in **figure 9**. B1: mean 217,00, Sd 138,66, B2: mean 192,07, Sd 138,66 and B3: mean 86,26, Sd 94,37 The post-hoc tests indicated no significant decrease between B1 and B2 (p = 0.500 one-tailed), a trend for decrease between B2 and B3 (p = 0.093 one-tailed), and significant decrease between B1 and B3 (p = 0.047 one-tailed).

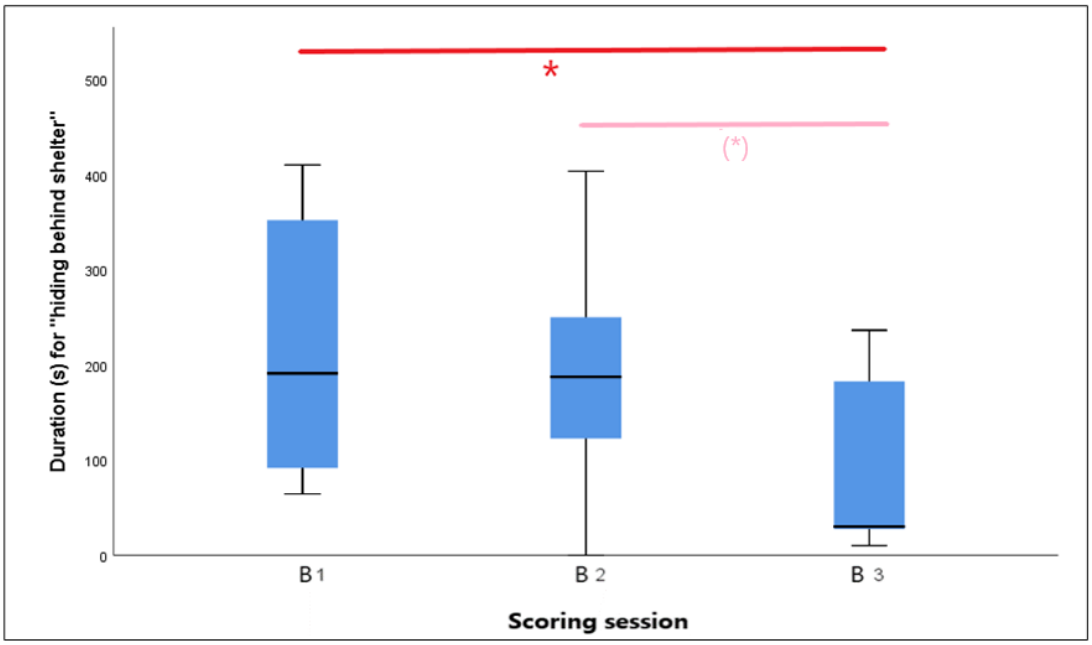

Figure 9. The changes in duration of “hiding behind shelter” over time. -* Shows the significant decrease ($p<0.05$) between B1 and B3 and –(*) shows the trend for decrease ($p<0.10$) between B2 and B3 (time schedule see figure 3).

When grouping all hiding behaviors together (irrespective of location) we found no significant change over time in the total number of hiding bouts (χ 2 (2) = 4.364, p = 0.123). The frequencies of "hiding under big shelter" (χ 2 (2) = 0.381, p = 0.880) and "hiding under tunnel" (χ 2 (2) = 1.091, p = 0.652) and the durations for "hiding under the tunnel" (F(2, 10) = 2.343, p = 0.146) and "hiding under big shelter" (F(2, 10) = 0.047, p = 0.954) did also not significantly change over time. However, the total time the guinea pigs spent hiding did significantly change over time (F (1.099, 5.495) = 11.284, p = 0.016)**.** B1: mean 347,113, Sd 53,42, B2: mean 263,00, Sd 38,81 and B3: mean 170,20, Sd 38,82. The post-hoc tests indicated a trend for decrease between B1 and B2 (p = 0.077 one-tailed), and significant decrease between B2 and B3 (p = 0.013 one-tailed) and B1 and B3 (p = 0.027 one-tailed). The data for the total time of hiding is visualized in **figure 10**.

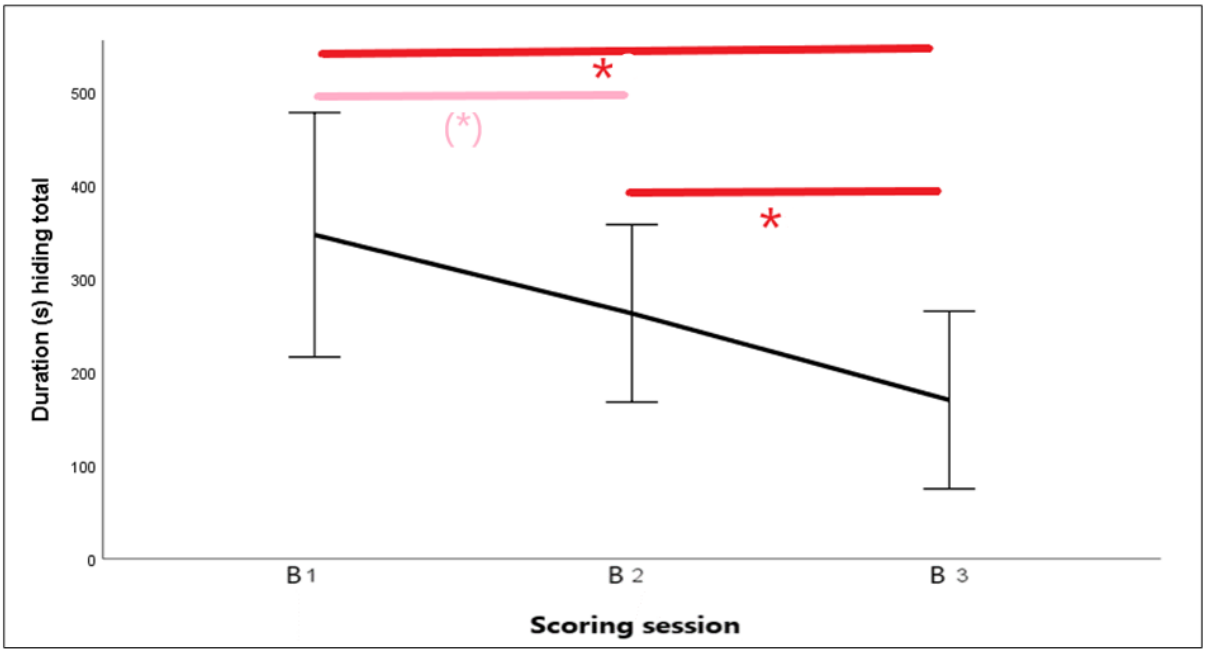

Figure 10. The changes of the duration in seconds of total hiding behavior in the guinea pigs over time with -* showing a significant decrease (p<0.05) between B1 and B3, and between B2 and B3. – (*) shows a trend for decrease (p<0.10) between B1 and B2 (time schedule see figure 3). The error bars indicate +/- 1 standard deviation.

Flight behavior

The frequencies of "fleeing" (χ 2 (2) = 2.455, p = 0.325) and "startle" (χ 2 (2) = 2.100, p = 0.409) and the duration of "fleeing" (F (2, 10) = 2.569, p = 0.126) did not show significant change over time.

Animal human interactions

Significant change over time was found for the frequency "gnawing on the observer" (χ 2 (2) = 8.400, p = 0.010). The post-hoc tests (B1: mean 2,17, Sd 2,14, B2: mean 2,67, Sd 2,88 and B3: mean 0,00, Sd 0,00) indicated no significant change between B1 and B2 (p = 1.000), a trend for change between B2 and B3 (p = 0.093) and no significant change between B1 and B3 (p = 0.375).

The duration of "gnawing on the observer" also significantly changed over time ($\chi 2$ (2) = 6.909, p = 0.031). The post-hoc tests over B1 (mean 9,20, Sd 7,43), B2 (mean 9,30, Sd 10,56) B3 (mean 0,00, Sd 0,00) showed no significant change between B1 and B2 (p = 1.000), a trend for change between B2 and B3 (p = 0.093), and no significant change between B1 and B3 (p = 0.375). The changes for the frequency and the duration of gnawing are made visible in **figure 11 and 12**.

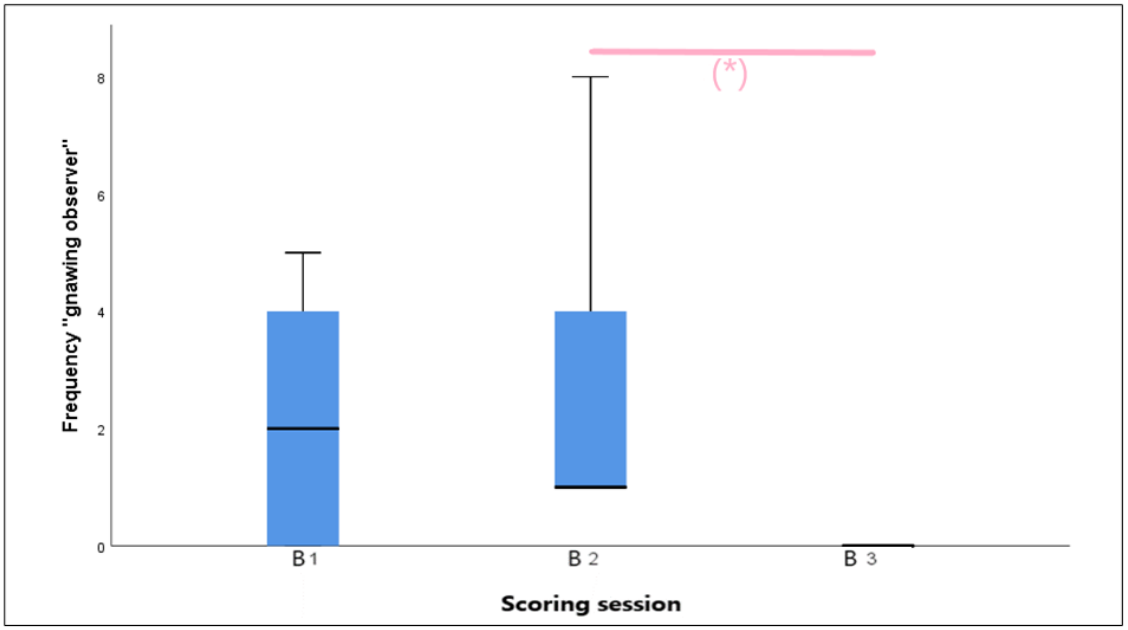

Figure 11. The change in the frequency of the guinea pigs gnawing on the observers clothes over time. –(*) indicates a trend for difference (p<0.10) between B2 and B3.

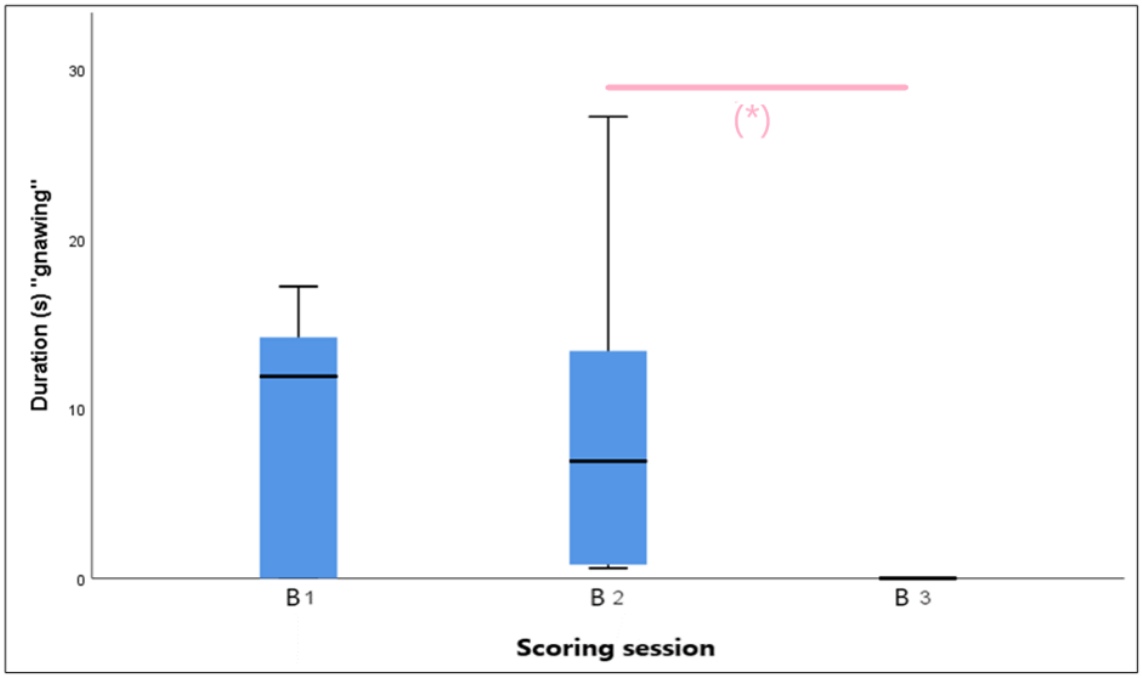

Figure 12. The change of the duration of gnawing on the observers clothes over time. –(*) indicates a trend for significant difference (p<0.10) between B2 and B3.

The frequency of “taking food from the observers hands” (χ 2 (2) = 7.913, p = 0.017) did significantly change over time as visualized in **figure 13**. No significant difference was found between B1 and B2 (p = 0.234 one-tailed), and between B2 and B3 (p = 0.469 one-tailed). A significant increase between B1 and B3 (p = 0.047 one-tailed) was found with the post-hoc tests (B1: mean 8,67, Sd 7,58, B2: mean 14,17, Sd 9,73 and B3: mean 17,83, Sd 8,64).

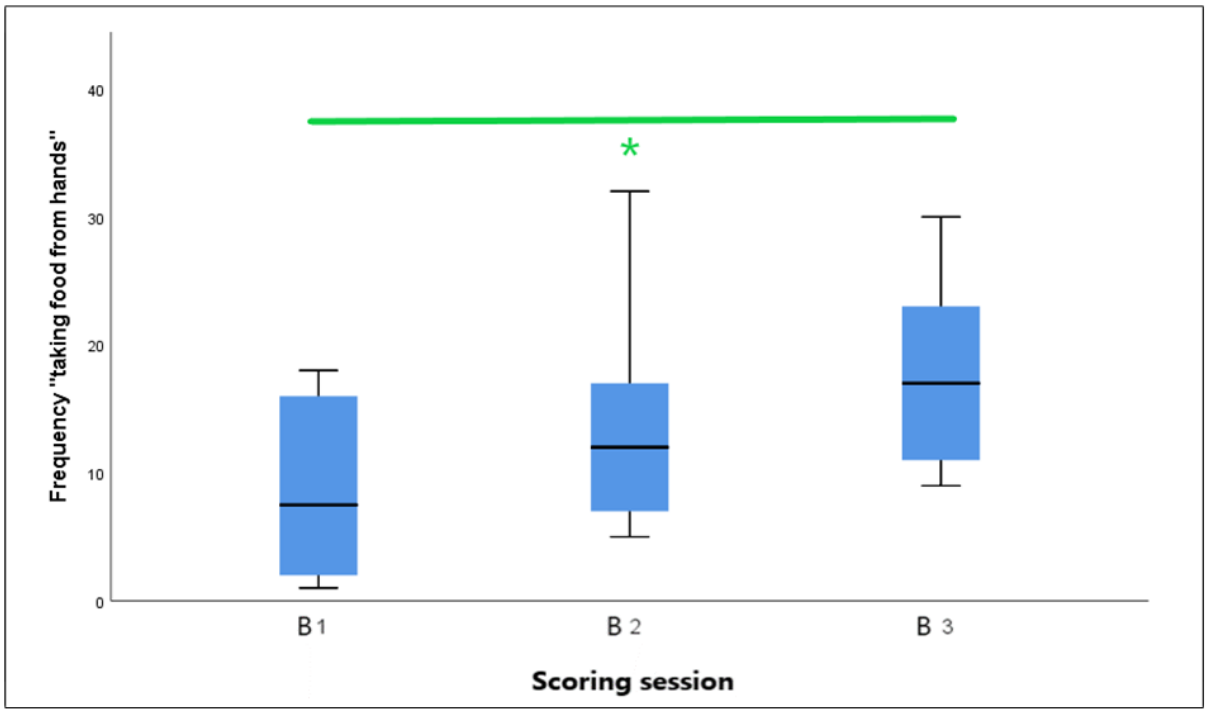

Figure 13. The change in the frequency of taking food from the observers hands over time. -* indicates the significant increase (p<0.05) between B1 and B3.

The guinea pigs did show significant change for the frequency “paws on the observer” over time (χ 2 (2) = 9.333, p = 0.006). The post-hoc tests (B1: mean 5,00, Sd 4,15, B2: mean 15,17, Sd 7,03 and B3: mean 18,17, Sd 5,38) showed significant increase between B1 and B2 (p = 0.047 one-tailed), no significant change between B2 and B3 (p = 0.500 one-tailed) and significant increase between B1 and B3 (p = 0.047 one-tailed) as shown in **figure 14.**

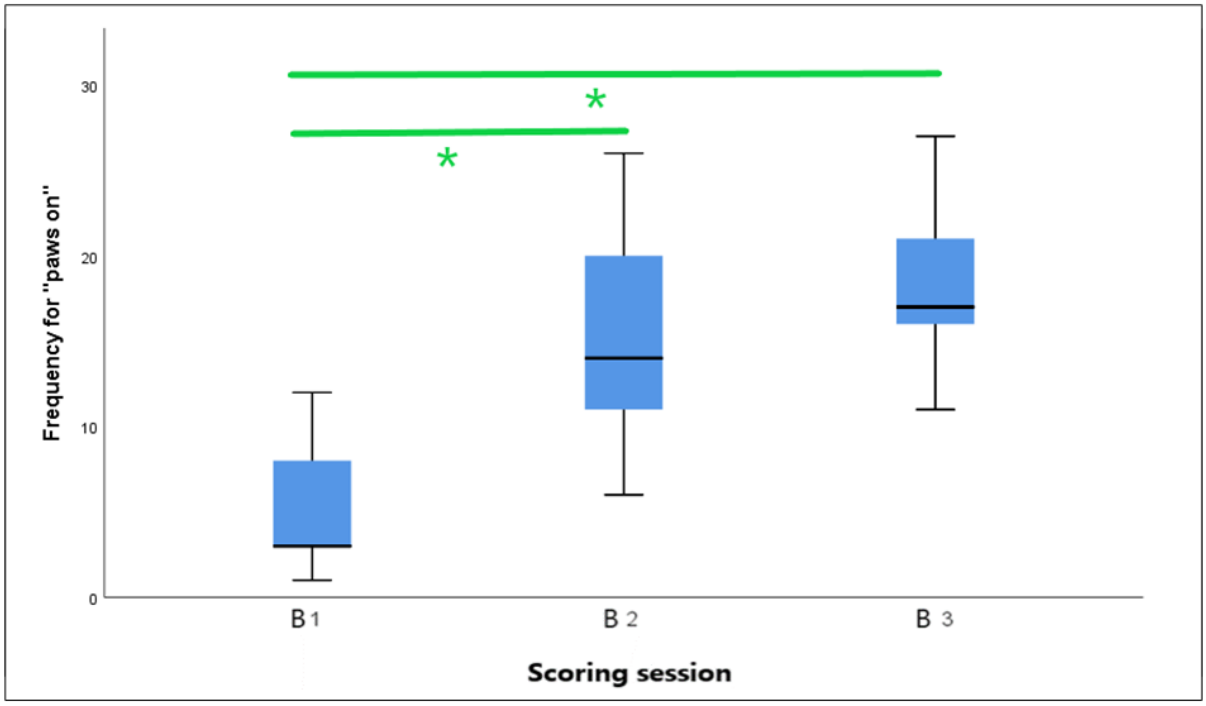

Figure 14. The change in the frequency of "paws on the observer" over time. -* shows the significant increases ($p<0.05$) between B1 and B2 and between B1 and B3.

The duration paws on the observer did significantly change over time ($F(2, 10) = 7.947$, $p = 0.009$) which is visualized in **figure 15**. The post hoc test (B1 (mean 63,97, Sd 19,78), B2 (mean 166,77, Sd 44,99), B3 (mean 201,53, Sd 42,17)) showed a significant increase between B1 and B2 ($p = 0.050$ one-tailed), no significant change between B2 and B3 (0.405 one-tailed) and a significant increase between B1 and B3 ($p = 0.036$ one-tailed)

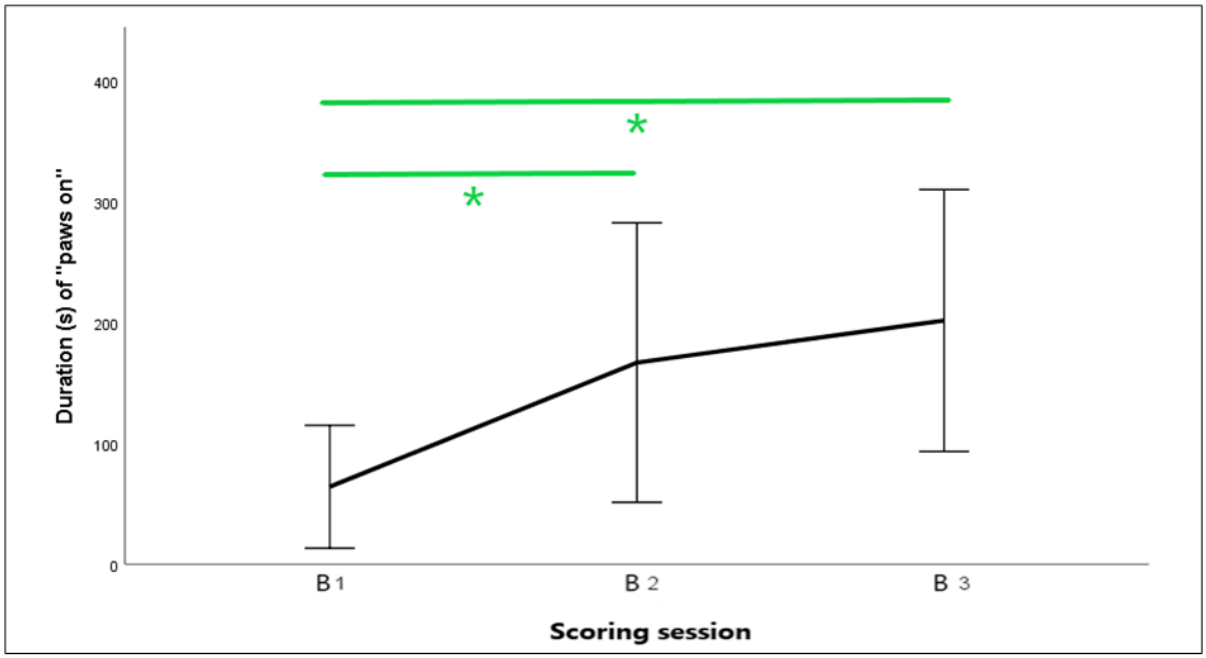

Figure 15. The change of the duration in seconds of the guinea pigs having their paws on the observer over the time. -* marks the significant increase ($p<0.05$) between B1 and B2, and between B1 and B3 (time schedule see figure 3). The error bars indicate +/- 1 standard deviation.

The frequencies of “approaching” (χ 2 (2) = 1.455, p = 0.519) “sniffling the observer” (χ 2 (2) = 4.000, p = 0.184) and “behind the back of the observer” (χ 2 (2) = 0.273, p = 0.873) did not significantly change over time. Also, the duration “sniffling the observer” (F (2, 10) = 2.622, p = 0.121) and the log transformed data for duration “behind the back of the observer” (F (2, 10) = 0.190, p = 0.830) did not show significant change over time.

Ingestion behavior

The guinea pigs did not show significant change for frequency “eating hay or straw” over time, χ 2 (2) = 3.909, p = 0.155. The duration of eating hay or straw did show a trend for change over time, F (2, 10) = 3.814, p = 0.059.

Exploration towards environment

The frequency “sniffling objects” significantly changed over time (χ 2 (2) = 9.652, p = 0.003) which is visualized in **figure 16**. The post-hoc tests (B1: mean 9.17, Sd 4,75, B2: mean 4,83, Sd 2,48 and B3: mean 1,17, Sd 0,98) showed no significant difference between B1 and B2 (p = 0.189) and between B2 and B3 (p = 0.189). A trend for difference was found between B1 and B3 (p = 0.093).

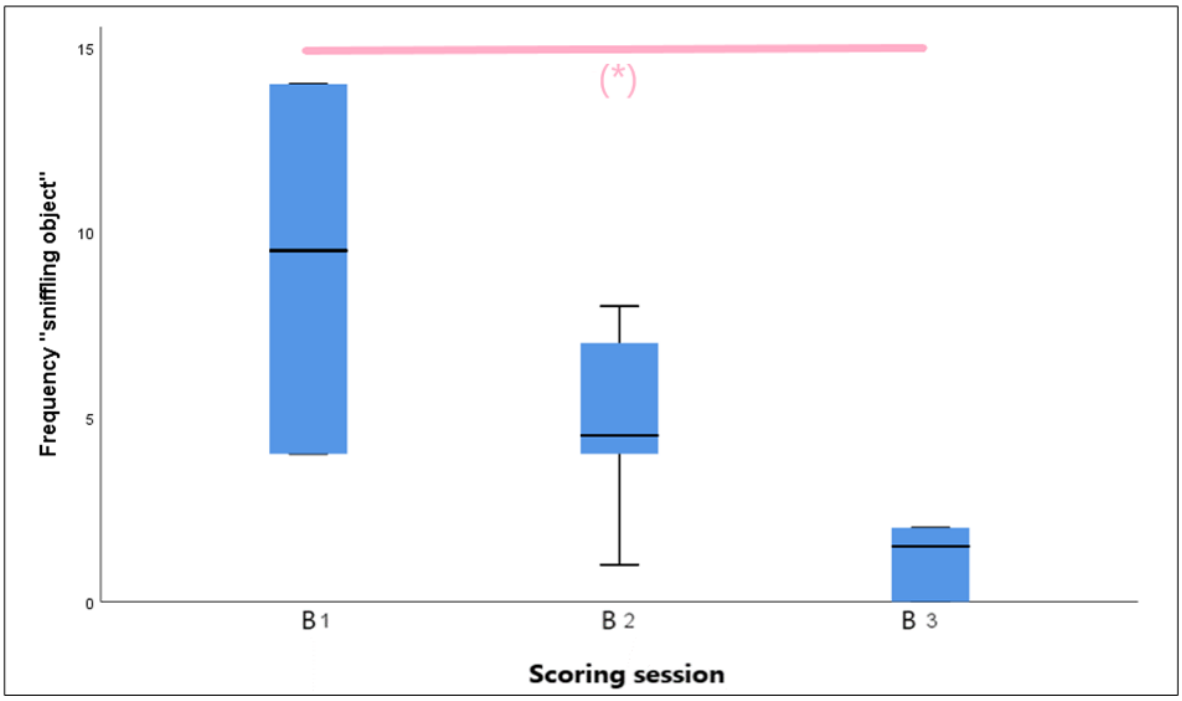

Figure 16. The change of the frequency for the behavior “sniffling objects” over time. –(*) shows the trend (p<0.10) for change between B1 and B3 (time schedule see figure 3).

The guinea pigs showed significant change over time, shown in **figure 17**, for the duration of “sniffling objects” (F (2, 10) = 4.389, p = 0.043). The post-hoc tests (B1: mean 19,33, Sd 5,80, B2: mean 9,80, Sd 3,41, B3: mean 2,80, Sd 1,25) found no significant evidence for change was found between B1 and B2 (p = 0.738) and between B2 and B3 (p = 0.400). A trend for change was found between B1 and B3 (p = 0.070).

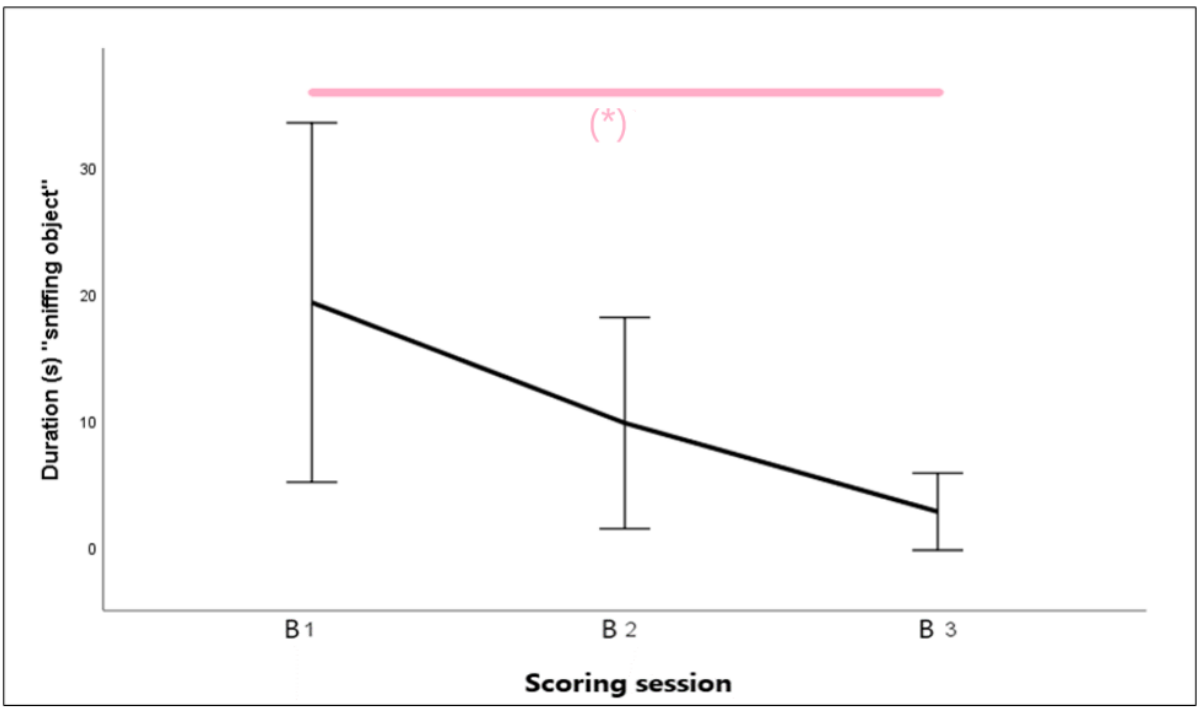

Figure 17. The duration in seconds of "sniffling object" visualized over the time. With –(*) the trend ($p<0.01$) for change between B1 and B3 is shown (time schedule see figure 3). The error bars indicate +/- 1 standard deviation.

The guinea pigs did not show significant change for the frequency of "gnawing on objects" (χ 2 (2) = 2.000, p = 0.519) and for the frequency "pushing objects" (χ 2 (2) = 2.000, p = 0.778) over time. Also the guinea pigs did not show significant change over time for the duration "gnawing on objects" (χ 2 (2) = 1.714, p = 0.519) "pushing objects" (χ 2 (2) = 2.000, p = 0.778).

Active locomotion behavior

A significant change over time, as visualized in **figure 18**, was found for the frequency for "jumping" (χ 2 (2) = 7.429, p = 0.019). No significant change was found between B1 and B2 (p = 0.188 one-tailed), B2 and B3 (p = 0.188 one-tailed) and B1 and B3 (p = 0.500 one-tailed) with the post-hoc tests (B1: mean 0,00, Sd 0,00, B2: mean 2,00, Sd 1,67, B3: mean 0,33, Sd 0,516).

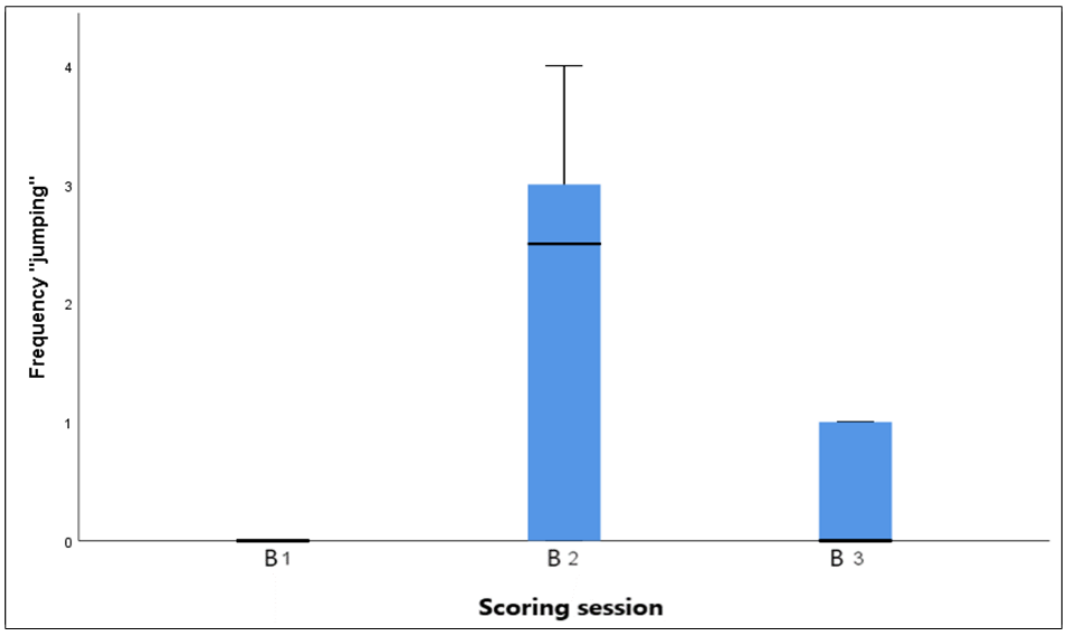

Figure 18. Changes of the frequency of "jumping" over score moments B1, B2 and B3 (time schedule see figure 3).

The frequency for "walking" did not show significant change over time (χ 2 (2) = 3.739, p = 0.172) as well as the duration of "walking" (χ 2 (2) = 4.333, p = 0.142)

Social behavior

The guinea pigs showed only a significant change over time for the frequency of "total socio-positive behavior" (χ 2 (2) = 8.455, p = 0.012), **figure 19**. With the post-hoc tests (B1: mean 4,33, Sd 2,50, B2: mean 3,33 Sd 1,37, B3: mean 1,67, Sd 1,51) a trend for change was found between B1 and B3 (p = 0.093) and no significant change was found between B1 and B2 (p = 1.000), and B2 and B3 (p = 0.189).

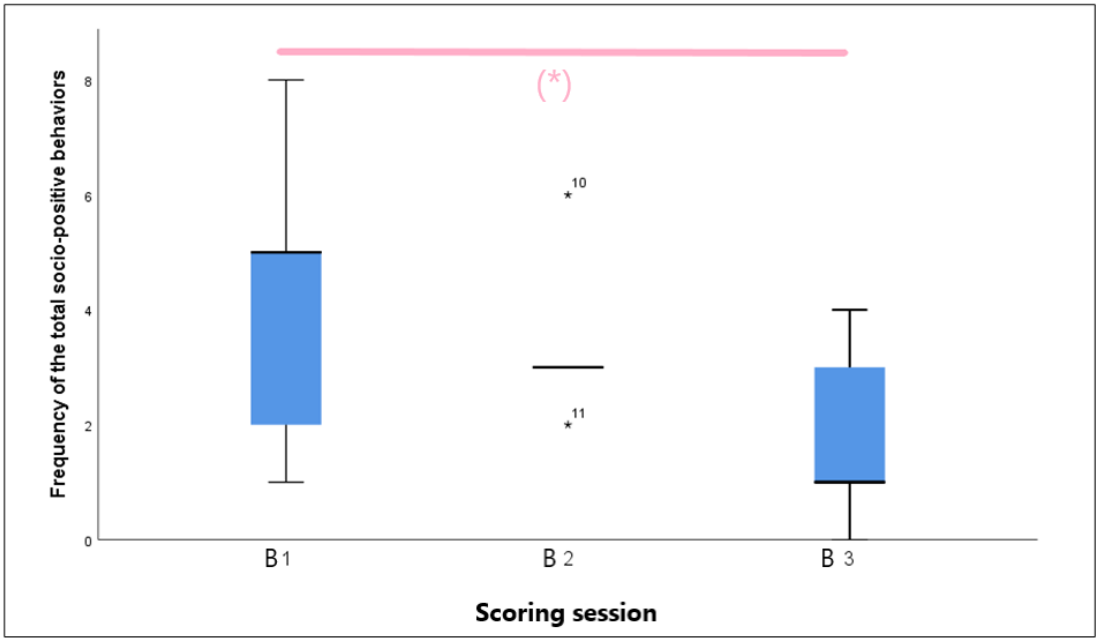

Figure 19. The change in total socio-positive behavior over time. –(*) shows a trend (p<0.01) for change between B1 and B3 (time schedule see figure 3).

The duration of socio-positive behavior showed a trend for change over time, F (2, 10) = 3.844, p = 0.058.

The guinea pigs did not show significant change in the frequency of "socio-negative behavior total" over time, χ 2 (2) = 0.111, p = 0.991. The frequency "unsuccessful contact between the sexes" (χ 2 (2) = 0.000, p = 1.000) as well as the duration (χ 2 (2) = 0.000, p = 1.000) did not show significant change. Also, no significant change over time was found for the frequency (χ 2 (2) = 1.882, p = 0.438) and duration (χ 2 (2) = 1.882, p = 0.438) for "nose-nose contact between the sexes" and for the frequency of "rumba-rumble" (χ 2 (2) = 3.000, p = 0.500).

Other behaviors

The frequency of "scratching" (χ 2 (2) = 2.800, p = 0.395) did not significantly change over time. The frequency of "shaking" did show a trend for change over time, χ 2 (2) = 5.375, p = 0.074.

HABITUATION TO HUMAN MOVEMENT

The guinea pigs showed a significant change in the group mean for fear levels over time, χ 2 (5) = 14.525, p = 0.005. A significantly change over time was also found for the group mean of the approach time, χ 2 (5) = 14.762, p = 0.005. The change in mean values of the fear levels and the mean values of the approach time are visualized in respectively **figure 19 and 20.**

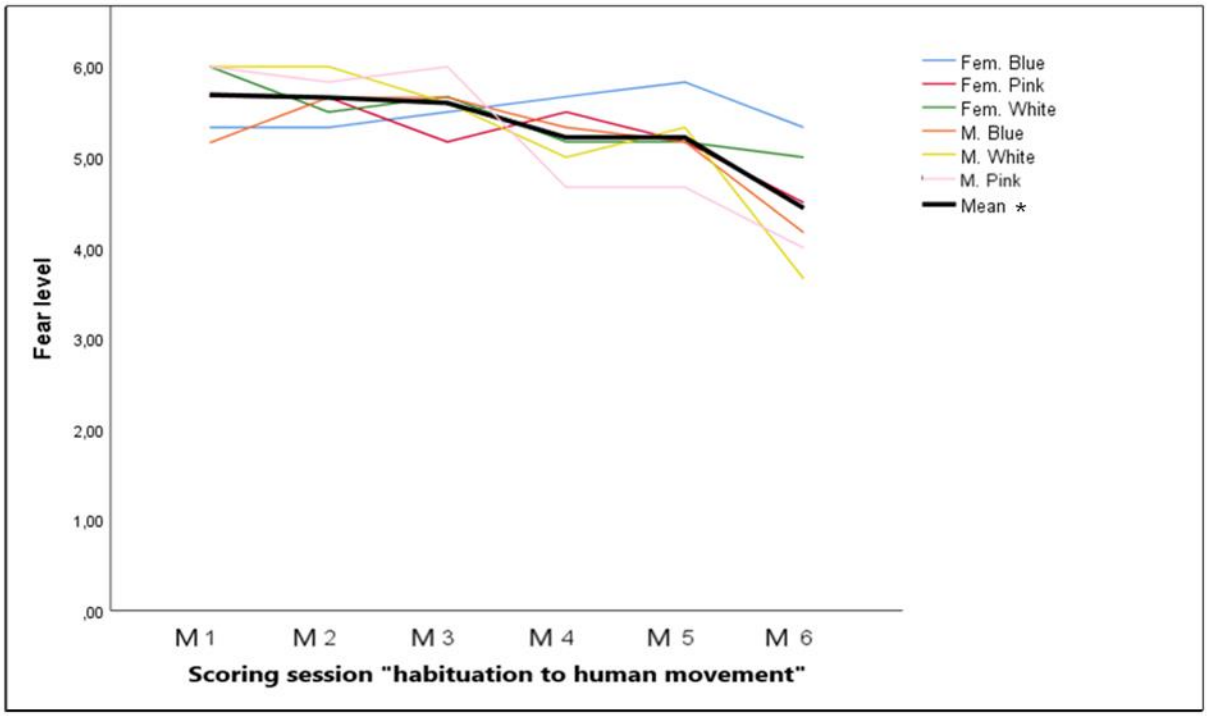

Figure 19. The development of the fear levels for the individual guinea pigs and the group mean over the six habituation sessions (M1-6, time schedule see figure 3) that were scored for habituation to human movement. The different colored lines represent the different individuals. The thick black line depicts the mean change in fear levels over time.

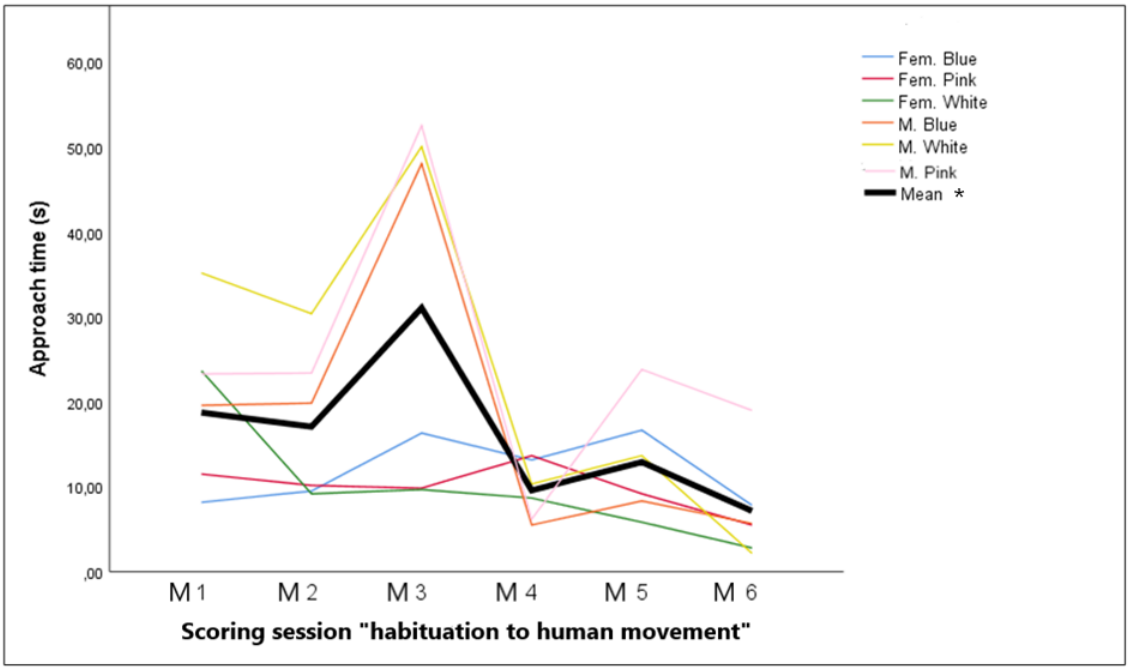

Figure 20. The mean values of the approach time in seconds for the individual guinea pigs and the group mean plotted against the six sessions (M1-6, time schedule see figure 3) that were scored for habituation towards human movement. The different colored lines represent the different individuals. The thick black line depicts the mean change in approach time over time.

TRANSPORT BOX TRAINING

The guinea pigs showed a significant change in their training level over time, $\chi 2\ (8) = 47.046$, $p = 0.000$. The development of the training expressed in training levels is shown in **figure 21.**

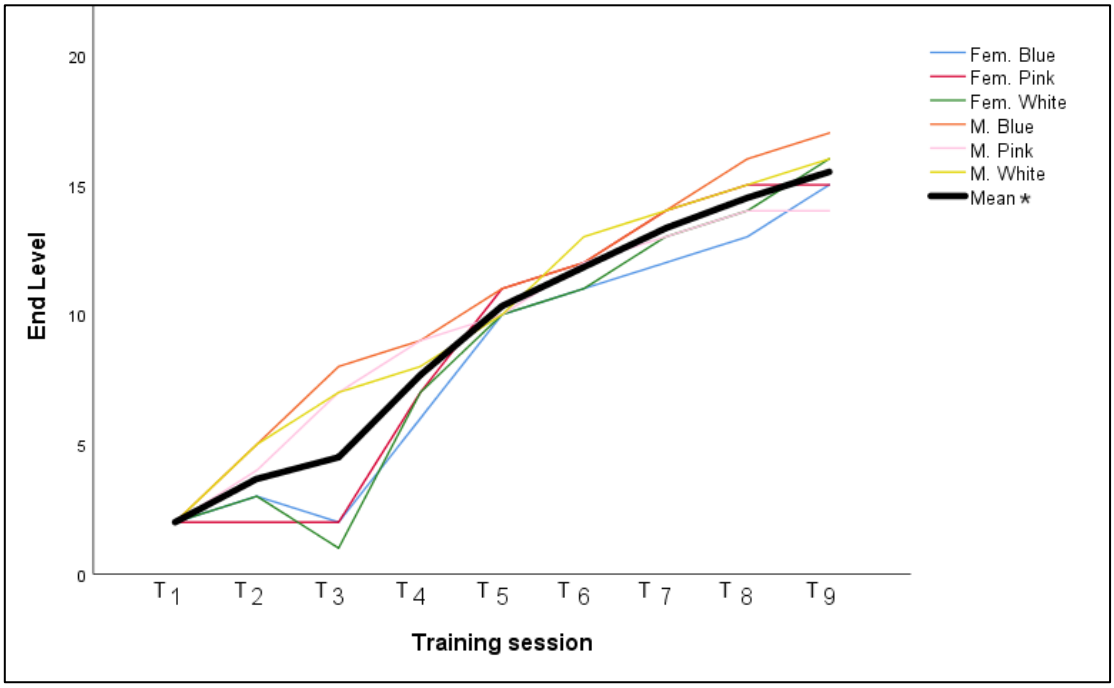

Figure 21. The group mean values of the end levels and the end level per guinea pig per session. (end level = highest achieved training level in a session) plotted against the nine training sessions. The different colored lines represent the different individuals. The thick black line depicts the mean end levels over time.

Discussion

Results

The goal of this explorative study was testing the effects of habituation towards humans and the possibility of training Dunkin Hartley guinea pigs to walk into a transport box voluntarily. Field observations and the success that was achieved with the transport box training indicate that the guinea pigs in all probability successfully habituated towards the observer/trainer. These findings could be supported with behavioral changes that occurred during the study.
First of all, a statistical decrease was seen for the frequency as well as for the duration of "hiding behind the shelter". When looking at hiding behavior irrespective of hiding location ("total hiding"), guinea pigs decreased the time spent in hiding over time. Wirth et al. found in 2020 that guinea pigs that were exposed to the presence of humans in animal-assisted therapy showed more frequent hiding behavior than animals in a control setting without humans[47]. This increase of the hiding frequency from guinea pigs in human presence compared to a setting without humans was also found in a similar study of Gut et al[58]. A decrease of duration in hiding behavior, as an anti-predator behavior, could be an indication for habituation progress, and similar changes in hiding behavior during habituation have been found in other rodents, for example rats[59].
No significant change was found for the frequency and duration for hiding under the big shelter and hiding under the tunnel. The tunnel was transparent and stood relatively close to the observer and the entrance of the big shelter was facing the observer. A possible explanation could be that the animals preferred the hiding place behind the shelter when they got anxious and did not see the tunnel as a proper hiding place. Behind the shelter, the sightline with the observer was broken. This hypothesis could be substantiated by the findings in a study with rats that preferred a shelter with physical as well as optical protection above a place that provided only physical shelter[60].

Besides the decrease in hiding behavior, an increase in some of the scored interactions between the animals and the observer was found. The guinea pigs showed an increase in taking food rewards from the observers hands, as well as an increase in frequency and duration of placing the paws on the observers body.
Making more body contact could be used as an indicator for habituation success, as rats habituated to humans by tickling (thought to mimic socio-positive play behavior in rats) made more contact with an human hand than non-habituated animals[61,62]. Also, rabbits that were habituated to handling by humans showed more cooperative behavior in procedures were body contact was needed[63].
The increase in acceptance of food from the observer could indicate that the stress level around the observer lowered over time, as stress could induce anorexia – a phenomenon that had been observed in different species including cats[26,57] and rats[46,55,64]. A reduced food intake of guinea pigs in a situation with humans compared to control situations was seen in two studies in which guinea pigs were used in animal-assisted therapy. In these studies the animals showed also more startle and freeze behavior when they were exposed to humans[47,58]. With this information, an increase in ingestion behavior of hay and straw was expected. However, the guinea pigs showed no significant change in the frequency of eating hay or straw and only a trend for change in de

duration of eating hay or straw. The ingestion of hay and straw could be influenced by the time the animals spend on contact making with the observer and eating food rewards from her hands. Therefore, it would be better to score the total frequency and duration of ingestion behavior (including the ingestion from the feeding plateau and the observers hands). This was not possible due to the camera angle in the female cage in which the head of the observer sometimes blocked the view when the guinea pigs were chewing on the food rewards, so the duration could not specifically be measured. More research about the total time of all ingestion behaviors together could be valuable.
Another animal-human interaction was gnawing behavior, which was seen as an expression of explorative behavior in a study with mice[65]. The decrease between measure moment B2 and B3 is remarkable. Despite the finding of Salomons et al. (2010)[52] in mice which show an increase in explorative behavior over time to a new environment after the initial anxiety (voiced in avoidance behavior) decreases, the decrease in gnawing could also be declared by habituation. When the animals habituate towards an object or person, their interest in it (and therefore their explorative behavior) could decrease over time which has been observed in a studies with mice[65] as well as rats and hamsters[66].

Besides the explorative behavior towards the observer, also the exploration of the home cage of the animals was scored. No significant changes were seen in the behaviors “pushing objects” and “gnawing objects” but the animals showed a significant decrease for both the frequency and the duration of “sniffling objects”. This decrease could be explained by the findings that the animals were spending more time with the observer (taking food rewards and placing the paws on the observer) and had less time for interactions with the environment.

The guinea pigs showed no significant change in the frequency and duration of walking. A significant change was found for jumping, but a post hoc test could not show between which sessions the difference occurred. With successful habituation, an increase of active locomotive behavior could be expected. This hypothesis was be supported by a study in mice which showed a reduction in locomotion behavior when exposed to a fear inducing situation (exposure to predator odor and ethanol)[49]. Also mice that were habituated to a new environment showed an increase in locomotion over time[52].
There should be kept in mind that in our study the behavioral measurements only started in the second week for the males and the third week for the females. Field observations indicated the animals used the shelters more frequently in the first week, and were less explorative towards the observer and the environment. Therefore, for drawing conclusions on locomotive behavior more research including data collection in the beginning of the habituation process is needed.

Other behaviors that did not show significant changes were the flight behaviors. However, it was expected that the flight responses of the animals decrease when animals experience less stress due to successful habituation. As prey animals, guinea pigs could show flight reactions towards a stressor in their environment[15] and Wirth et al. (2020) found that guinea pigs showed more startles during a setting in which a human was present compared to the control setting without a human[47].
There are some points of attention in the setting of this experiment that could have influenced the flight behavior of the guinea pigs. First of all, during the sessions in which the animals were habituated to movement, the observer did not made an equal amount of movements each session because the moments on which the observer moved depended on the presence of the animals

around the observer. The time that the animals were around the observer differed between the sessions and also the amount of animals that were around the observer at the moment of a movement differed. The difference in movement frequency of the observer could influence the flight and startle data.
In addition, the animals were in a room in which rabbits were also housed. The rabbit cages were positioned almost directly behind the sitting position of the observer. The rabbits sometimes made loud noises that induced fleeing in the guinea pigs. However, the camera recordings did not include sound, so it was not possible to differ between the cause of the flights. This could lead to noise in the dataset, making it more difficult to detect significant differences in flight behavior between the different time points. Also, during field observations it looked like that the speed at which the animals fled away decreased, which could influence the flight duration. It might be recommended to look deeper into the flight reactions and set new definitions on the behaviors that include speed which could help making a stricter distinction between fleeing and walking. With the knowledge of these limitations, no direct conclusions could be drawn from the lack of significant decrease in flight behavior and more research would be recommended.

In the scored social behaviors, only a significant decrease was seen in the frequency of socio-positive behavior. Two studies with guinea pigs showed a social buffering effect in stressful situations. When the guinea pigs were placed in a new environment together they showed lower cortisol levels than guinea pigs that were alone. However no change in socio-positive or socio-negative behavior was found when the behavior in the new environment was compared to that in the home cage[53,67]. A social interaction test with rats showed that paired rats showed less social behavior when they were introduced to an environment in which they experienced more stress[54], which contradicts the findings in the previous mentioned guinea pig studies[53,67]. No direct conclusions could be drawn from decrease that was found in frequency of socio-positive behavior in our study, although an explanation could be that the animals paid more attention to the observer over time due to habituation, and therefore had less time to interact with each other.

More research on this topic with a larger sample size (see limitations and recommendations) could help with investigating the effect of habituation on the other behaviors that did not show significant change or only a trend (shaking.)

The guinea pigs showed also evidence for successful habituation in the specific measurements that were done for “habituation to human movement” as the approach time as well as the fear level of the guinea pigs decreased significantly over time.
The fear level contained the appearance of a flight reaction (flight yes/no), the flight length (flight inside zone or towards another zone) and the flight initiation distance (start flight reaction in zone A or B). Studies in other animals indicate that the decrease in our fear levels could be caused by habituation. First of all, McGowan et al. (2014) found that the flight initiation distance of wild skinks increases when the animals were approached by a group of more than three humans, which is a stronger fear inducing trigger, compared to approach of a single human[44]. Also, a study in populations of hyrax, a wild rodent species living in Tanzania, showed that populations habituated towards humans showed a lower flight initiation distance than populations that had not been habituated to humans[68].
A study in wild living lamas showed that the appearance of a flight reaction due to traffic decreased after habituation to traffic[69], and the length of a flight reduced in wild kangaroos

when humans approached in an adapted way that seems less threatening[70]. The decrease in approach time also could show the positive effect of habituation, as it could be expected the more fearful an animal is, the more it avoids its trigger that induces fear[52].
There must be mentioned that the graph of the approach time showed a peak on M3, which is caused by the males that were hiding a lot that day and did not make a lot of contact with the observer, for which no direct explanation could be found.
The decrease in fear level and approach times could indicate that habituation to the human movements was successful, although they did not decreased to a moment in which no flights at al occurred. To reach lower fear levels and approach times, a longer habituation period could be necessary.

The guinea pigs showed a significant increase in training levels and all animals reached the level in which they were transported towards the practical room. The guinea pigs also did not show avoidance towards the transport box after transport, as all guinea pigs walked voluntarily into the transport box for at least once in each training session. This could be an indication of success, because animals could react with an avoidance response towards a fearful situation, which was found in studies in mice[71,72] and rats[73]. The acceptance of an food reward was also used to determine if animals achieved a training level, as food refusal or a reduction in food intake could be a stress indicator as explained before[46,47,55,58,64]. The increase in training levels and absence of avoidance response towards the transport box indicate that the training protocol was successful. The increase of the training levels could also say something about the success of earlier habituation phases, as stress and fear (which would be expected towards the presence of a human when habituation was not successful) could influence a learning process in a negative way[34,35].

LIMITATIONS AND RECOMMENDATIONS

This explorative study had some limitations that should be mentioned including some recommendations for further research on the topic.
First of all, the behavioral scoring was done by the same person that observed the guinea pigs in chronologic session order. To prevent bias, it would be better to randomize the scoring and use a different person for this task, who is blind for the level of habituation or training of animals in the video.
Another point of attention is the camera setup. The camera for the males and the one for the females did not give the same range of visibility of the cage, as the female camera showed a smaller representation of the cage in which some corners were excluded. Also the exchange of the male camera with the hand camera changed the viewpoint, which made the scoring of the hiding behavior less specific. No cameras were available for filming under the big hiding house and behind the hiding house. Therefore, when a guinea pig was out of view this was scored as hiding, but no nuances were made about other behaviors that they performed during hiding. However, during field observations the observer noticed that the guinea pigs were often eating or resting in a laying position when they were hidden. These behaviors in particular could help with measuring the habituation progress, as a reduction in food intake could be caused by stress[46] and laying down could indicate relaxation[58]. A camera placed on the same level as the guinea pigs (for scoring subtle behaviors like chewing) in combination with camera with

helicopter view over the whole cage and cameras under the hiding houses could help with scoring the behaviors more precisely.

Also, the sample size in this study was very small (n=6) which causes low power in the statistical analyses. Therefore, it could be harder to detect change. Due to the small sample size, the male and female group were not analyzed separately, while in the field observations differences in habituation rate and interaction with the human were observed between the sexes. These observations are in conformity with a study of Jolles et al. in which male rats showed more risk-taking behavior towards an environment with predator odor than females[74]. Also, in a population of yellow eyed pinguins was seen that females needed a longer recovery time at the beginning of an habituation experiment to human approach than the males, however after 5 days there was no longer a significant difference between males and females[75]. Male and female rats show differences in classical and operant conditioning learning processes, which includes active avoidance tests and lever-pressing tasks[76]. Thereby, study with pet rabbits showed that males made more social contact with their owner than females[77]. With this information it should be recommended that when a larger group of guinea pigs is available for research, the differences in habituation progress between the sexes get determined so a habituation protocol could be created that fits the needs of the different genders.
In this study, the partition between the male and female cage consisted of transparent metal mesh, so the animals were able to see the observer when she was with the other gender group. This should be no problem when all animals follow the same habituation schedule, which was not the case in our study. The males started a week earlier with the habituation towards human movement. Due to the transparent wall between the cages there is a possibility that the females were already passively habituated towards movement at the moment this phase started for them.

The set-up of this study was based on findings in the previous study from J. van Eupen [50], in which only a light effect of habituation to humans was found. Therefore, the habituation phase to human presence in this study was started a week before the cameras were available because no big changes in behavior were expected in these four days. However, field observations indicated in the first week already behavioral changes in anti-predator behavior and contact seeking with the observer. Due to the lack of imagery, this week is excluded from the statistical analysis, while it could have influenced the findings.
Another point of attention could be that the guinea pig group was used in practical's already before habituation started, which could be a negative experience with humans for the animals. A study with yellow-eyed pinguins showed that animals with a negative experience (blood sample taking) with humans before habituation started, did show less habituation progress than naïve animals[75]. When a new group of guinea pigs arrives from the breeder, a start with habituation before the animals interact with humans in a (for them) negative way could possibly result in a faster and more successful habituation process.
In this study, not all the cameras were able to record sounds. Therefore the vocalizations of the guinea pigs were not scored. However, vocalizations are an important component in guinea pig communication and vocal behavior starts at young age[15,78]. The recording of vocal behavior could help with the interpretation and scoring of social behavior and make the scoring more subtle.

After the training was completed the guinea pigs were tested for their reaction to a new human (which was not included in the schedule). Field observations indicated that especially the

females were reserved towards the new observer/trainer. They did not accept a food reward from the hands and were hiding relatively more than with their usual observer/trainer. During the practical's the animals will be approached and handled by different humans, therefore it should be recommended to habituate the animals to more humans. Research about the effect habituation to more than one observer could be useful for the development of an successful habituation protocol.
Also, the females showed a fear response in the beginning of the training when the trainer was standing. To increase training success and speed, habituation to a standing/walking person in the cage could be recommended.
To increase the welfare of the guinea pigs used in this study during the practical's, it could be recommended to habituate the animals further towards handling by humans. This could be done by repeating training level 17, but now the animals should be handled gently for 10 minutes long. The study of Rocha et al. in 2017 showed that guinea pigs that were handled this way for ten days showed a decrease in tonic immobility, which is an anti-predator response[45]. The use of food rewards during the handling and practical's could be recommended so the experiences could become more positive for the animals.
During this study, the observation has been made that the guinea pigs showed a reserved attitude towards new food rewards (dried vegetables). Neophobia in guinea pigs to sour, sweet and salty flavors was also seen in a study from Miller and Holzman in 1981[79]. The guinea pigs in our study were habituated to ESVE drops before the study started which made the training easier because they were highly motivated for this food reward. Therefore, it is strongly recommended to habituate new groups of guinea pigs to food rewards as was done with our animals.
Lastly, in a study with a new group of guinea pigs the statistical analyses could be strengthened with a behavior measurement of the guinea pigs without the presence of a human before habituation starts. The undisturbed guinea pig behavior could be compared with the behavior of different habituation sessions to determine if, for example hiding behavior increases initially due to fearfulness towards humans. Thereby it could be measured if the behaviors that we used to indicate stress or fear decrease to a "normal" level (the frequency or duration measured without the presence of a stressor) during the habituation process. This could help with determining the habituation success.

Conclusion

There could be concluded that the developed habituation protocol led to habituation success, as the guinea pigs made more body contact with the observer and the acceptance of food rewards increased over time. The decrease of hiding behavior could also support this statement. Also, the animals showed reduction of the fear level that was used to determine success in the habituation to human movements, as well as a reduction in approach time. The increase of the training levels indicates that it is possible to train guinea pigs with positive reinforcement to enter a transport box. For better insights in the habituation process, more research about flight behavior, social behavior, active locomotive behavior and explorative behavior towards the observer and the environment could be recommended. To increase the welfare of the guinea pigs that participated in this study, it might be recommended to continue the habituation to humans with more different observers and start with habituation to handling as well. This study

indicates that non habituated guinea pigs could experience stress due to the presence of humans or during handling. Early habituation to humans could be a possibility to increase the welfare of laboratory guinea pigs and could help with making the interactions between the animals and humans more positive for both the handlers and the guinea pigs. The training success in this study indicates that the training of guinea pigs in other facility's and research could be useful. More cooperation and a reduction of stress in animals during (medical procedures) could increase welfare and also improve the ease of work for caretakers and researchers.

Acknowledgement

Hereby, I would like to thank the people who supported me during this thesis. First of all, I would like to thank the caretakers of the animals Jeroen van Ark and Helma Avezaat for giving me a lot of information about the facility and the guinea pigs. I would also like to thank Kim Kalapnatsing for her support and tips during my learning process to work with the program SPSS statistics, Joost Hoogeveen for prereading my research proposal and Sybren Huitink and Samantha van Heuven for helping me with some Exel/computer tips and tricks. Last but not least I would really like to thank my supervisor Esther Langen, for learning me a lot about animal behavior research and being always so open to my questions. I really appreciated al the feedback which helped me to improve my work.

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Appendixes

Appendix 1 – Schedule and notations

Date	Program	Notations	Availability of food rewards
Week 1 19-01-2021 Session 1	**First - Females** (1.5 hour habituation session towards "human presence") **Second - Males** (1.5 hour habituation session towards "human presence") .	*Cameras were not available this week, therefore notations were made but scoring could not occur. Guinea pigs were marked on Friday 22 January, so the first three days no individual differences could be notated. No food rewards were used during this first session. Overall observation was that the guinea pigs, male as well as female, approached faster when the observer was crackling with straw in the hands.* **Females:** approached the observer but showed a flight reaction when the observer moved. **Males:** approached the observer and showed a flight reaction when the observer moved. The males recovered easier from a flight or freeze reaction. This was seen due to the fact that the males approached the observer again faster after a flight or freeze reaction and were hiding for a shorter time	*No*
20-01-2021 Session 2	**First - Males** (1.5 hour habituation session towards "human presence") **Second - Females** (1.5 hour habituation session towards "human presence")	*The observer started using food rewards (yogurt drops broken into small pieces) during this session and continued this in the following sessions.* **Males:** the guinea pigs ate yogurt drops broken in small pieces from the back of a mirror that was used as a feeding plateau laying 5-10 centimeters from the observers legs. Also, the guinea pigs were eating hay and straw within a range of 30 centimeters from the observer. When the observer moved, the guinea pigs fled behind the biggest hiding house. They approached again when the observer was crackling with straw. **Females:** same as for the male guinea pigs, but approaching after a flight reaction took more time for the females.	*ESVE drops*
21-01-2021 Session 3	**First - Males** (1.5 hour habituation session towards "human presence") **Second - Females** (1.5 hour habituation session towards "human presence")	*A different reaction was seen in between the sexes. The males showed far more explorative behavior than the females. The caretaker of the animals (Jeroen) confirmed this and told that the males were hiding less than the females, and also relaxed more when handled.* **The males:** ate yogurt drops from the feeding plateau that was placed 5-10 centimeters from the leg of the observer. They were hiding in hay and eating from it when the hay lay against the leg of the observer. The males started also exploring the observer by licking, sniffling and nibbling on the observers clothes and book. They walked around the observer and walked through the "tunnel" that existed between the back of the observer and the wall of the cage. Two of the three males took a yogurt drop from the observers hands. Guinea pigs were still fleeing when the observer moved.	*ESVE drops*

		The females: took the yogurt drops from the feeding plateau that was placed 5-10 centimeters from the observers leg. They came closer towards the observer than the previous days, but they did not explore the observer.	
22-01-2021 Session 4	**First - Males** (1.5 hour habituation session towards "human presence") **Second - Females** (1.5 hour habituation session towards "human presence")	*The guinea pigs were marked this day, so individual differences could be seen.* **The males**: took yogurt drops from the feeding plateau again. They were also gnawing on the observers clothes and exploring the observer by licking and sniffling. All the males took a yogurt drop from the observers hand. (There must be mentioned that the observer kept the hand still and waited till the animals approached. The observer did not make a movement with the hand with the yogurt drop towards the animals, cause this induced a flight reaction.) The blue male reacted the most open towards the observer, by spending the most time around the observer and exploring the observer and clothes a lot. **Females**: sniffed the hand of the observer in which the observer was crackling the straw. Yogurt drops were taken from the feeding plateau again. The pink female was the most open guinea pig towards the observer. She was from the females the one that came the closest towards the observer and was around the observer more time than the other guinea pigs.	*ESVE drops*
Week 2 26-01-2021 Session 5	**First - Males** (45 minutes habituation towards "Human movement") **Second - Females** (45 minutes habituation towards "Human presence")	*Towards the males as well as the females, new treads were presented on the feeding plateau. Dried carrot, pea flakes, bean flakes and fresh cucumber cut into little parts were present.* **The males:** approached almost immediately and put their front legs onto the knee of the observer to lake yogurt drops. With each consecutive movement of the observer the guinea pigs reacted with less stress. They took food rewards from the humans hands. The males were very explorative this day and were sniffing, licking and gnawing on the humans clothes many times. Males did not try the new treats. **Females:** They started to take food rewards from the humans hands and put their front legs on the humans leg in sitting position. The females were more explorative in case of the new treats, they licked cucumber and dried carrot but they did not eat it or take it into the mouth.	*ESVE drops* *Pea flakes* *Bean flakes* *Dried carrot* *Cucumber*
27-01-2021 Session 6	**First - Females** (45 minutes habituation towards "Human presence") **Second - Males** (45 minutes habituation towards "Human movement")	**Males:** did not try new treats. **Females:** ate dried carrot.	*ESVE drops* *Pea flakes* *Bean flakes* *Dried carrot*
28-01-2021 Session 7	**First - Males** (45 minutes habituation towards "Human movement") **Second - Females** (45 minutes habituation towards "Human presence")	*Males were done before cage cleaning. Females after. The females looked very stressed, were high reactive to impulses and hide a lot. Keep this in mind when statistics are performed.* **Females**: ate dried carrot, and pea flakes. They have maybe ate the bean flakes but that is not for sure. During the habituation session the white (not marked) female ate cucumber.	*ESVE drops* *Pea flakes* *Bean flakes* *Dried carrot* *Cucumber*

29-01-2021 Session 8	**First - Females** (45 minutes habituation towards "Human presence") **Second - Males** (45 minutes habituation towards "Human movement")	**Males** ate nothing but the yogurt drops. Males were not interested in observer after +-15 minutes. They were all hiding under the shelter, but when observer looked under the shelter she noticed that the males were sleeping. **Females** ate pea flakes, bean flakes, cucumber (was gone from the day before) and dried carrot. Females were hiding a lot. Only the pink marked female made a lot of contact and was near the observer for most of the time.	*ESVE drops* *Pea flakes* *Bean flakes* *Dried carrot*
Week 3 2-02-2021 Session 9	**First - Males** (45 minutes habituation towards "Human movement") Second - Females (45 minutes habituation towards "Human movement")	-	*ESVE drops* *Pea flakes* *Bean flakes* *Dried carrot*
3-2-2021 Session 10	**First - Females** (45 minutes habituation towards "Human movement") Second - Males (45 minutes habituation towards "Human movement")	-	*ESVE drops* *Pea flakes* *Bean flakes* *Dried carrot*
4-2-2021 Session 11	**First - Males** (45 minutes habituation towards "Human movement") **Second - Females** (45 minutes habituation towards "Human movement")	**Females:** Pink female did refuse the bean flakes and sometimes the dried carrots. She did this by taking the foods from the observers hands, but when she tasted what it was, she dropped it from her mouth. Yogurt drops and pea flakes were still accepted by this female.	*ESVE drops* *Pea flakes* *Bean flakes* *Dried carrot*
5-02-2021 Session 12	**First - Females** (45 minutes habituation towards "Human movement") **Second - Males** (45 minutes habituation towards "Human movement")	-	*ESVE drops* *Pea flakes* *Bean flakes* *Dried carrot*
Week 4 9-02-2021	*Cancelled due to snow problems with traveling*	-	-
10-02-2021 Session 13	**First - Females** (45 minutes habituation towards "human movement") **Second - Males** (45 minutes habituation: male bleu start "human touch", male white and male pink "human movement")	-	*ESVE drops* *Pea flakes* *Bean flakes* *Dried carrot*
11-02-2021 Session 14	**First - Males** (45 minutes towards "human touch" for all males) **Second - Females** (45 minutes habituation towards "human movement")	**The males:** tried the pea flakes and bean flakes, but when given from the observers hands the males refused these. Dried carrot was refused from the observers hands and from the feeding plateau as well. **Females:** Pink female ate cucumber but refused the bean flakes. Ate dried carrots, yogurt drops and pea flakes. White female ate cucumber (more than the pink female) but refused bean flakes. Ate dried carrots, yogurt drops and pea flakes. Blue female refused to try cucumber. Ate bean flakes, pea flakes, dried carrot and yogurt drops.	*ESVE drops* *Pea flakes* *Bean flakes* *Dried carrot* *Cucumber*
12-02-2021 Session 15	**First - Females** (45 minutes habituation towards "human movement")	**Females:** Blue female tried cucumber but did not eat a lot from it (more tasting/licking). Other females ate cucumber.	*ESVE drops* *Pea flakes* *Bean flakes* *Dried carrot*

	Second - Males (45 minutes habituation towards "human touch")		*Cucumber*
Week 5 16-02-2021 Session 16	**First - Females** (45 minutes habituation towards "human movement") **Second - Males** (45 minutes habituation towards "human touch")	-	*ESVE drops* *Pea flakes* *Bean flakes* *Dried carrot*
17-02-2021 Session 17	**First - Females** (45 minutes habituation towards "human movement") **Second - Males** (45 minutes habituation towards "human touch")	-	*ESVE drops* *Pea flakes* *Bean flakes* *Dried carrot*
18-02-2021 Session 18	Transport box habituation	Box was placed inside the cage, open with food rewards inside it. The guinea pigs were allowed to explore the box freely.	*ESVE drops* *Pea flakes* *Bean flakes* *Dried carrot*
19-02-2021 Session 19	Transport box habituation	" "	*ESVE drops* *Pea flakes* *Bean flakes* *Dried carrot*
Week 6 23-02-2021 Session 20 Training 1	Transport box training	-	*ESVE drops*
24-02-2021 Session 21 Training 2	Transport box training	-	*ESVE drops*
26-02-2021 Session 22 Training 3	Transport box training	-	*ESVE drops*
Week 7 2-3-2021 Session 23 Training 3	Transport box training	-	*ESVE drops*
3-3-2021 Session 24 Training 5	Transport box training	-	*ESVE drops*
5-3-2021 Session 25 Training 6	Transport box training	-	*ESVE drops*
Week 8 9-3-2021 Session 26 Training 7	Transport box training	-	*ESVE drops*
10-3-2021 Session 27 Training 8	Transport box training	-	*ESVE drops*
12-3-2021 Session 28 Training 9	Transport box training	-	*ESVE drops*
Week 9 17-03-2021	Tryout habituation and transport box training with another observer/trainer	Specially the females reacted different towards the "new observer". They were hiding more and did not made body contact or accept a food reward. The females did make body	*ESVE drops*

		contact and accepted food rewards from the “old observer” while the “new observer” was next to them in the male cage. The males were trainable by the “new observer” as they walked into the transport box and the box could be closed. They also made body contact and accepted food rewards while the “new observer” was sitting inside their cage.	

Appendix 2 - Ethogram table *Cavia porcellus*

1. Hiding	
*Not hiding	Guinea pig is visible and not hiding (Definition of hiding: see the next three definitions)
Hiding big shelter	Guinea pig is hidden under the big shelter (and not visible on camera)
Hiding transparent tunnel	Guinea pig is under the red transparent tunnel
Hiding behind shelter	Guinea pig is behind/next to the big shelter house (red zone in figure 5 minus the place under the big hiding house).
2. Social and explorative behavior towards observer	
Sniffling observer	The guinea pig directs its nose towards the observer or body parts of the observer and inhales short and fast (little nose movements) 2 centimeters or closer from the observer
Gnawing observer	The guinea pig rasps with its teeth on the clothes or hands of the observer
*Licking observer	The guinea pig goes with its tongue over the observers clothes, shoes or hands
Taking food from hands	The guinea pig takes a treat from the observer (food is between two fingers of the observer) and consumes it
Taking food from clothes	The guinea pig takes a treat that lays on the observers clothes (observer sits on the ground , legs flat, and puts a treat on the leg) and consumes it
*Touch with nose	The guinea pig touches the observers clothes or hands with its nose
Paws on the observer	The guinea pig touches the observers clothes or hands with its paws (put paws on the leg of the observer)
Behind observers back	The guinea pig is located in between the back of the observer and the wall or tries to get in this position by pushing the observer and the wall with its head (a small tunnel is formed in between the observers back and the wall)
Approach	The guinea pig walks with the face in the direction of the observer (the distance between the observer and the guinea pig is reduced)
3. Social behavior	
3.1 Social positive interactions	
Nose touching	The guinea pigs nose is touching the nose of another guinea pig
Sniffling guinea pig	The guinea pig directs its nose towards another guinea pigs body (other parts than the nose, because when there is nose-nose contact this will be defined as a nose touch) and inhales short and fast (little nose movements) 2 centimeters or closer from the other guinea pig
Allogrooming	Guinea pig is nibbling, licking or rubbing (with the face) a body part of another guinea pig
Following	The guinea pig starts to follow another guinea pig that is moving (the guinea pig does not chase the other guinea pig, it does not make the other guinea pig move)
3.2 Social negative interactions	
Biting	The guinea pig bites another guinea pig
Chasing	The guinea pig is chasing another guinea pig that moves away in reaction to the chase (makes the guinea pig move with social negative behavior)

Stand-threat	The guinea pig stands towards another guinea pig, the body is in a curved position (hindquarter and head is directed towards the other guinea pig do a curve in the body/spinal court forms) and the other guinea pig reacts in the same way
Head-up	The guinea pig throws the head up, the nose is directed upwards
Head-thrust	The guinea pig thrusts its head towards another guinea pig, body could go with this movement but the body/paws stays in place (when the guinea pig moves the body from its place see next, attack-lunge)
Attack-lunge	The guinea pig moves with a fast and short movement (jump or run) towards another guinea pig, teeth could be visible sometimes
Fighting	The guinea pig is reacting towards another guinea pig that reacts back, both with fast up following social negative behaviors like biting, head-thrusts etc. (normally the social negative interactions could be scored one by one, but when the animals do show these interactions fast towards each other for 4 seconds or more, this could be indicated as fighting)
Fleeing/retreat from another guinea pig	The guinea pig moves away from another guinea pig in the cage that shows social negative behavior towards the fleeing guinea pig
3.3 Contact between the sexes (trough cage wall)	
Nose to nose contact	The noses of a male and a female guinea pig touch trough the opening in the cage wall
Unsuccessful contact seeking	The guinea pig seeks contact (stands with its paws on the opening in the cage wall) but no guinea pig on the other side reacts
Rumba-rumble	The guinea pig is swinging with the hind quarter and makes an purring sound (mostly males towards females, sign of sexual interest)
4. Individual behavior	
4.1 Ingestion	
Eating straw/hay	The guinea pig consumes hay or straw (takes straw of hay in its mouth and then an chewing movement follows)
Eating pellets	The guinea pig consumes pallets (takes a pellet in its mouth and then an chewing movement follows)
Drinking water	The guinea pig consumes water from the water bowl or the bottle with drinking nipple
Eating treats from feeding plateau	The guinea pig consumes food (treats) from the feeding plateau
Caecotrophy	The guinea pig eats feces directly from the anus
4.2 Elimination	
*Defecating	Guinea pig leaves feces from the anus
*Urinating	Guinea pig leaves urine from the urinal tractus
4.3 Exploration environment	
Digging	The guinea pig is moving the bedding on the floor with both its front paws
Gnawing on objects	The guinea pig rasps with its teeth on the surface of an object
Pushing objects	The guinea pig makes objects move by using its nose/head to push the objects
Sniffing objects	The guinea pig directs its nose towards an object and inhales short and fast (little nose movements) 2 centimeters of closer from the object
4.4 Locomotive	
Walking	The guinea pig walks from one place towards another place
*Sitting still	Guinea pig stands still on a place for at least 3 seconds, the animal is not tense and light movements of the face are possible
Rest/lay down	The guinea pig lays dawn on one side or on the belly, eyes could be open or closed
Frisky hops	The guinea pig makes upward leaps (or series of these movements) in the air and could make turns with the head or forequarter while doing this (mostly seen in young animals or in sexually heated males)
Jumping	Four paws of the guinea pig leave the ground for a moment (this happens when the guinea pig leaves a higher place, like the observers leg, or need to get over an object, like a stick or feeding bowl)

4.5 Other behaviors	
Yawning	The guinea pig opens the mouth widely and inhales air
Stretching	The guinea pig elongates its body, mostly this is seen with a lowering hindquarter and a front quarter that is pushed up with the front paws.
Scratching	The nails of a paw scratch a body part of the guinea pig
Shaking	The guinea pig shakes with the whole body
Grooming	Guinea pig uses its paws to clean its own face, licking the own fur on other body parts than the face
4.6 Flight/freeze	
Fleeing from observer	The guinea pig moves/runs away from a stimulus caused by the observer
Freezing	The guinea pig stands completely still and tense for at least 3 seconds, all other behaviors stop (in reaction towards a stimulus)
Startle	A fast and short movement of the body, like the body shocks, but the guinea pig stays in place, that follows on a stimulus

Legenda Ethogram:

- A star before a behavior = this behavior is not scored during this experiment.
- Red = scored for duration and frequency.
- Blue = scored for frequency only.

Literature for ethogram building: [15,47,58,80–84]

Appendix 3 – Data behavior scoring

Date	Hiding Big	Hiding Tunnel	Hiding BS	Sniffing Observer	Gnawing observer	Food hands	Food clothes	Approach	Behind back	Under legs	Fleeing observer
Fem. Blue 26-1-2021	3	0	4	20	6	0	0	6	0	0	3
Fem. Pink 26-1-2021	6	2	3	28	5	0	6	10	0	0	4
Fem. White 26-1-2021	6	3	3	21	8	0	3	10	0	0	7
M. Blue 26-1-2021	2	8	7	43	5	3	1	10	6	0	7
M. Pink 26-1-2021	4	8	8	30	3	2	1	12	3	0	9
M. White 26-1-2021	1	8	9	12	1	1	1	10	2	0	10
Fem. Blue 2-2-2021	4	0	4	33	0	18	0	16	0	2	16
Fem. Pink 2-2-2021	10	3	4	30	0	16	0	20	3	2	17
Fem. White 2-2-2021	6	1	12	20	4	12	0	17	0	0	15
M. Blue 2-2-2021	8	1	3	38	8	11	0	13	2	0	13
M. Pink 2-2-2021	6	2	4	23	1	7	0	13	2	0	13
M. White 2-2-2021	1	2	5	18	1	5	0	6	0	0	6
Fem. Blue 10-2-2021	5	2	4	10	1	13	0	12	2	0	19
Fem. Pink 10-2-2021	8	2	0	22	1	32	0	10	5	0	15
Fem. White 10-2-2021	3	2	6	6	4	17	0	9	2	0	13
M. Blue 10-2-2021	10	3	2	33	0	11	1	17	3	3	12
M. Pink 10-2-2021	3	1	4	18	0	11	0	9	2	0	4
M. White 10-2-2021	2	4	6	27	0	9	0	11	4	0	6
Fem. Blue 16-2-2021	4	2	2	15	0	23	0	12	0	4	12
Fem. Pink16-2-2021	3	11	3	19	0	23	0	10	2	3	8
Fem. White	3	2	4	7	0	30	0	14	1	9	15
M. Blue 16-2-2021	3	3	4	18	1	15	0	14	2	7	6
M. Pink 16-2-2021	3	7	10	9	0	4	0	12	3	1	2
M. White 16-2-2021	9	5	9	15	0	15	0	19	0	1	2

Date	Freeze	Startle	Eat strawhay	Eat pellets	Drinking	Eat FP	Ceaco	Gnawing object	Sniffing object	Pushing object
Fem. Blue 26-1-2021	0	1	5	0	0	9	0	0	12	0
Fem. Pink 26-1-2021	0	0	10	0	0	2	0	0	21	1
Fem. White 26-1-2021	0	3	3	0	0	2	0	0	10	0
M. Blue 26-1-2021	1	0	9	0	0	2	0	1	12	0
M. Pink 26-1-2021	1	0	9	0	1	3	0	0	14	0
M. White 26-1-2021	0	1	7	0	1	0	0	1	4	0
Fem. Blue 2-2-2021	0	1	2	0	0	0	0	0	14	0
Fem. Pink 2-2-2021	0	1	3	0	0	0	0	0	4	0
Fem. White 2-2-2021	0	4	1	0	0	0	0	0	7	0
M. Blue 2-2-2021	0	3	7	0	0	0	0	0	8	0
M. Pink 2-2-2021	1	1	7	0	0	0	0	0	7	0
M. White 2-2-2021	0	1	7	0	0	0	0	1	4	0
Fem. Blue 10-2-2021	0	0	8	0	2	0	0	0	4	0
Fem. Pink 10-2-2021	0	0	13	0	0	0	0	0	1	1
Fem. White 10-2-2021	0	1	7	0	0	0	0	0	5	0
M. Blue 10-2-2021	0	17	15	0	0	0	0	8	1	0
M. Pink 10-2-2021	0	1	12	0	0	0	0	1	2	3
M. White 10-2-2021	0	2	14	0	0	0	0	0	2	1
Fem. Blue 16-2-2021	0	1	2	0	0	0	0	0	0	0
Fem. Pink16-2-2021	0	1	8	0	0	0	0	1	2	0
Fem. White	0	0	13	0	0	0	1	0	0	0
M. Blue 16-2-2021	0	10	12	0	0	0	0	0	0	0
M. Pink 16-2-2021	0	1	9	0	0	0	0	0	3	0
M. White 16-2-2021	0	4	8	0	0	0	0	0	1	0

Date	Digging	Walking	Lay/rest	Jumping	Scratching	Shaking	Frisky hops	Stretching	Grooming	Yawning
Fem. Blue 26-1-2021	0	28	0	0	1	0	0	0	1	0
Fem. Pink 26-1-2021	0	38	0	0	2	1	1	0	0	0
Fem. White 26-1-2021	0	28	0	0	1	0	0	0	0	0
M. Blue 26-1-2021	0	56	0	0	0	0	0	0	0	0
M. Pink 26-1-2021	0	50	0	0	1	0	0	0	0	0
M. White 26-1-2021	0	33	0	0	0	0	0	0	0	0
Fem. Blue 2-2-2021	0	31	0	0	0	0	0	0	0	0
Fem. Pink 2-2-2021	0	40	0	0	0	0	0	0	0	0
Fem. White 2-2-2021	0	26	0	0	0	0	0	0	0	0
M. Blue 2-2-2021	0	48	0	3	0	2	0	0	0	0
M. Pink 2-2-2021	0	50	0	3	0	1	2	0	0	0
M. White 2-2-2021	0	35	0	0	0	0	0	0	0	0
Fem. Blue 10-2-2021	0	29	0	4	1	0	0	0	0	0
Fem. Pink 10-2-2021	0	37	0	2	1	0	0	0	0	0
Fem. White 10-2-2021	0	25	0	0	0	0	0	0	1	0
M. Blue 10-2-2021	0	60	0	0	0	0	0	0	0	0
M. Pink 10-2-2021	0	36	0	0	1	2	0	0	0	0
M. White 10-2-2021	0	43	0	0	1	1	0	0	0	0
Fem. Blue 16-2-2021	0	44	0	1	0	0	0	0	0	0
Fem. Pink16-2-2021	0	39	0	1	1	1	0	0	0	0
Fem. White	0	59	0	0	1	2	0	0	0	0
M. Blue 16-2-2021	0	43	0	2	0	0	0	0	0	0
M. Pink 16-2-2021	0	26	0	0	0	0	0	0	0	0
M. White 16-2-2021	0	51	0	2	0	0	0	0	0	0

Date	Sniff guinea	Allogrooming	Following	TOTAL socio-positive	Biting	Chasing	Stand-threat	Head-up
Fem. Blue 26-1-2021	4	0	0	8	0	0	0	1
Fem. Pink 26-1-2021	2	0	0	5	0	0	0	3
Fem. White 26-1-2021	3	0	0	6	0	0	0	2
M. Blue 26-1-2021	2	0	1	5	0	0	0	2
M. Pink 26-1-2021	1	0	2	5	0	0	0	0
M. White 26-1-2021	1	0	1	8	0	0	0	2
Fem. Blue 2-2-2021	4	0	0	5	0	0	0	0
Fem. Pink 2-2-2021	0	0	0	2	0	0	0	0
Fem. White 2-2-2021	1	0	0	1	0	0	0	0
M. Blue 2-2-2021	2	0	0	3	0	0	0	1
M. Pink 2-2-2021	2	0	0	3	0	0	0	0
M. White 2-2-2021	1	0	0	3	0	0	0	0
Fem. Blue 10-2-2021	3	0	0	6	0	0	0	1
Fem. Pink 10-2-2021	1	0	0	2	0	0	0	0
Fem. White 10-2-2021	1	0	0	3	0	0	0	2
M. Blue 10-2-2021	2	0	1	3	0	0	0	0
M. Pink 10-2-2021	0	0	0	1	0	0	0	0
M. White 10-2-2021	1	0	0	1	0	0	0	0
Fem. Blue 16-2-2021	3	0	0	4	0	0	0	2
Fem. Pink16-2-2021	0	0	0	1	1	0	0	0
Fem. White	0	0	0	0	0	0	0	1
M. Blue 16-2-2021	0	0	0	1	0	0	0	0
M. Pink 16-2-2021	0	0	0	0	0	0	0	0
M. White 16-2-2021	0	0	0	1	0	0	0	0

Date	Fighting	Attack-lunge	Fleeing Guinea	Head-thrust	TOTAL socio-negative	Unsuc. contact	Rumba	S Nose-Nose	Paws ON
Fem. Blue 26-1-2021	0	0	0	0	1	0	0	0	4
Fem. Pink 26-1-2021	0	0	2	0	5	0	0	0	9
Fem. White 26-1-2021	0	0	0	0	2	0	0	0	5
M. Blue 26-1-2021	0	0	1	1	4	0	0	1	8
M. Pink 26-1-2021	0	0	0	0	0	0	1	2	3
M. White 26-1-2021	0	0	0	0	2	1	0	1	3
Fem. Blue 2-2-2021	0	0	0	0	0	0	0	0	3
Fem. Pink 2-2-2021	0	0	0	0	0	0	0	0	12
Fem. White 2-2-2021	0	0	0	0	0	0	0	0	1
M. Blue 2-2-2021	0	1	0	0	2	3	9	6	26
M. Pink 2-2-2021	0	0	0	0	0	0	3	3	13
M. White 2-2-2021	0	0	0	0	0	0	0	0	6
Fem. Blue 10-2-2021	0	0	0	0	1	0	0	1	15
Fem. Pink 10-2-2021	0	0	0	0	0	0	0	0	20
Fem. White 10-2-2021	0	0	0	1	3	0	0	0	11
M. Blue 10-2-2021	0	0	0	1	1	0	1	0	27
M. Pink 10-2-2021	0	0	0	0	0	1	0	0	11
M. White 10-2-2021	0	0	0	0	0	0	0	0	17
Fem. Blue 16-2-2021	0	0	1	0	3	0	0	0	16
Fem. Pink16-2-2021	0	0	0	0	1	0	0	0	17
Fem. White	0	0	0	0	1	0	0	1	21
M. Blue 16-2-2021	0	0	0	0	0	0	0	0	24
M. Pink 16-2-2021	0	0	0	0	0	0	0	0	2
M. White 16-2-2021	0	0	0	0	0	0	0	0	18

Date	DUR. Hiding Big	DUR. Hiding Tunnel	DUR. Hiding BS	DUR. Sniffling	DUR. Gnawing	DUR. Food hands	DUR. Food clothes	DUR. Behind back	DUR. Under legs
Fem. Blue 26-1-2021	132,8	0	224,4	49,6	42	0	0	0	0
Fem. Pink 26-1-2021	198,2	5,4	83,8	64,6	42	0	1,2	0	0
Fem. White 26-1-2021	267	19,2	135,6	105	58,2	0	0,6	0	0
M. Blue 26-1-2021	5,8	70	91,8	76	13,2	0,6	0,2	192,2	0
M. Pink 26-1-2021	111,4	68,8	184	69,6	14,2	0,4	0,2	42	0
M. White 26-1-2021	6,4	113,6	410,6	48,8	10,6	0,2	0,2	32,8	0
Fem. Blue 2-2-2021	70,4	0	198,6	75,4	0	3,6	0	0	9,8
Fem. Pink 2-2-2021	210,8	23,8	64,6	51,4	0	3,2	0	73,4	7,6
Fem. White 2-2-2021	99,6	0,2	352,4	39,8	17,2	2,4	0	0	0
M. Blue 2-2-2021	43	1,8	164,4	81,2	12,4	2,2	0	30,2	0
M. Pink 2-2-2021	49,6	24,6	250,4	40,8	1,4	1,4	0	26,4	0
M. White 2-2-2021	15	4,4	404	46,4	0,6	1	0	0	0
Fem. Blue 10-2-2021	55,6	8	122,8	13,8	13,4	2,6	0	8,4	0
Fem. Pink 10-2-2021	173,8	6,6	0	41,8	0,8	6,4	0	12	0
Fem. White 10-2-2021	21,8	21,4	210,8	11,4	27,2	3,4	0	6,6	0
M. Blue 10-2-2021	64,6	49,4	32,4	81,8	0	2,2	0,2	7,6	24
M. Pink 10-2-2021	41,4	38,6	183	80,8	0	2,2	0	46,2	0
M. White 10-2-2021	43,4	28,4	236,6	58,6	0	1,8	0	78	0
Fem. Blue 16-2-2021	95	2,8	10,2	21	0	4,6	0	0	122,8
Fem. Pink16-2-2021	51	54,8	27,6	39,4	0	4,6	0	5,2	48,8
Fem. White	26,4	7,8	27,8	12,2	0	6	0	7,8	131,8
M. Blue 16-2-2021	90,2	31,4	68	55,4	8	3	0	9,6	82,6
M. Pink 16-2-2021	103,4	70,6	282	25,4	0	0,8	0	24,6	16,8
M. White 16-2-2021	191	23,6	124,8	51,6	0	3	0	0	2,6

Date	DUR. Fleeing	DUR. Freeze	DUR. Eat straw/hay	DUR. Eat pellets	DUR. Drinking	DUR. Eat FP	DUR. Gnawing object	DUR. Sniffing object	DUR. Pushing object
Fem. Blue 26-1-2021	3,6	0	68,2	0	0	106,4	0	37,2	0
Fem. Pink 26-1-2021	3,8	0	105,6	0	0	14	0	62,6	0,8
Fem. White 26-1-2021	6,4	0	27,8	0	0	18	0	27,4	0
M. Blue 26-1-2021	9,8	4	77,2	0	0	11,6	5,2	10,2	0
M. Pink 26-1-2021	13,2	0,6	45,8	0	4,6	17,6	0	45,2	0
M. White 26-1-2021	13,4	0	37,6	0	43,4	0	0,4	4,8	0
Fem. Blue 2-2-2021	17,8	0	35,6	0	0	0	0	23	0
Fem. Pink 2-2-2021	24,2	0	18,2	0	0	0	0	19	0
Fem. White 2-2-2021	23	0	13,4	0	0	0	0	13,8	0
M. Blue 2-2-2021	21	0	47,6	0	0	0	0	23,6	0
M. Pink 2-2-2021	14,2	0,8	76,4	0	0	0	0	8,4	0
M. White 2-2-2021	9,6	0	176,2	0	0	0	1	3,6	0
Fem. Blue 10-2-2021	23,2	0	130,2	0	7	0	0	6,8	0
Fem. Pink 10-2-2021	13,6	0	110,2	0	0	0	0	1	1
Fem. White 10-2-2021	20	0	159,4	0	0	0	0	15,4	0
M. Blue 10-2-2021	13	0	168	0	0	0	27,4	2,8	0
M. Pink 10-2-2021	4,8	0	129,6	0	0	0	1,8	6,8	3,4
M. White 10-2-2021	9,8	0	99	0	0	0	0	1	1,4
Fem. Blue 16-2-2021	17	0	8,4	0	0	0	0	0	0
Fem. Pink16-2-2021	8,6	0	76,4	0	0	0	0,4	6,2	0
Fem. White	18,6	0	63,6	0	0	0	0	0	0
M. Blue 16-2-2021	7,4	0	142	0	0	0	0	0	0
M. Pink 16-2-2021	2,6	0	122,6	0	0	0	0	6	0
M. White 16-2-2021	1,2	0	93	0	0	0	0	1,6	0

Date	DUR. Digging	DUR. Walking	DUR. Lay/rest	DUR. Grooming	DUR. Nose-nose	DUR. Sniff guinea	DUR. Allogrooming	DUR. Following	TOTAL DUR socio-positive
Fem. Blue 26-1-2021	0	95,6	0	1,2	5	1,8	0	0	6,8
Fem. Pink 26-1-2021	0	123,2	0	0	2,6	1,2	0	0	3,8
Fem. White 26-1-2021	0	63,8	0	0	1,4	2	0	0	3,4
M. Blue 26-1-2021	0	163,4	0	0	1	2	0	1,4	4,4
M. Pink 26-1-2021	0	170,6	0	0	1,4	0,8	0	5,6	7,8
M. White 26-1-2021	0	107	0	0	3,8	0,6	0	1,6	6
Fem. Blue 2-2-2021	0	95,8	0	0	0,2	3,4	0	0	3,6
Fem. Pink 2-2-2021	0	107	0	0	2,2	0	0	0	2,2
Fem. White 2-2-2021	0	83,4	0	0	0	0,4	0	0	0,4
M. Blue 2-2-2021	0	145	0	0	0,8	4,4	0	0	5,2
M. Pink 2-2-2021	0	158,4	0	0	6,8	2,2	0	0	9
M. White 2-2-2021	0	97,6	0	0	7,4	0,6	0	0	8
Fem. Blue 10-2-2021	0	83,2	0	0	4,4	3,4	0	0	7,8
Fem. Pink 10-2-2021	0	111	0	0	0,6	0,8	0	0	1,4
Fem. White 10-2-2021	0	69,2	0	0,6	2,6	2,6	0	0	5,2
M. Blue 10-2-2021	0	161	0	0	0	3	0	2,2	5,2
M. Pink 10-2-2021	0	151,4	0	0	0,6	0	0	0	0,6
M. White 10-2-2021	0	158	0	0	0	1	0	0	1
Fem. Blue 16-2-2021	0	150,8	0	0	2,6	2,2	0	0	4,8
Fem. Pink16-2-2021	0	123,2	0	0	2,8	0	0	0	2,8
Fem. White	0	155,4	0	0	0	0	0	0	0
M. Blue 16-2-2021	0	133,6	0	0	1,6	0	0	0	1,6
M. Pink 16-2-2021	0	93,6	0	0	0	0	0	0	0
M. White 16-2-2021	0	149,4	0	0	2,2	0	0	0	2,2

Date	DUR. Chasing	DUR. Fighting	DUR. Fleeing Guinea	TOTAL DUR socio-negative	DUR. Unsuc. contact	DUR. Sex Nose-Nose	DUR. Paws ON
Fem. Blue 26-1-2021	0	0	0	0	0	0	31,6
Fem. Pink 26-1-2021	0	0	2,6	2,6	0	0	133,6
Fem. White 26-1-2021	0	0	0	0	0	0	35,4
M. Blue 26-1-2021	0	0	0,8	0,8	0	3,6	119,6
M. Pink 26-1-2021	0	0	0	0	0	3,2	65
M. White 26-1-2021	0	0	0	0	2,2	6,2	45,4
Fem. Blue 2-2-2021	0	0	0	0	0	0	30
Fem. Pink 2-2-2021	0	0	0	0	0	0	121,6
Fem. White 2-2-2021	0	0	0	0	0	0	2,2
M. Blue 2-2-2021	0	0	0	0	14,4	22,6	195,8
M. Pink 2-2-2021	0	0	0	0	0	15	113,6
M. White 2-2-2021	0	0	0	0	0	0	39
Fem. Blue 10-2-2021	0	0	0	0	0	3,2	141,6
Fem. Pink 10-2-2021	0	0	0	0	0	0	365,8
Fem. White 10-2-2021	0	0	0	0	0	0	144,8
M. Blue 10-2-2021	0	0	0	0	0	0	181,4
M. Pink 10-2-2021	0	0	0	0	4,2	0	93
M. White 10-2-2021	0	0	0	0	0	0	125,2
Fem. Blue 16-2-2021	0	0	1	1	0	0	156,4
Fem. Pink16-2-2021	0	0	0	0	0	0	358,4
Fem. White	0	0	0	0	0	0,8	294,8
M. Blue 16-2-2021	0	0	0	0	0	0	115,2
M. Pink 16-2-2021	0	0	0	0	0	0	14,4
M. White 16-2-2021	0	0	0	0	0	0	116,2

Appendix 3 – Data “fear levels” and “approach time” in habituation to movement

Date	Guinea	Gender	Session number	Movement numb.	No Fl. A	No Fl. B	Fl. inside A	Fl. A to B	Fl. A to C	Fl. inside B	Fl. B to C	Time until approach	"Fear level"
26-1-2021	M. Blue	M	1	1							1	12	6
26-1-2021	M. Pink	M	1	1							1	15,8	6
26-1-2021	M. White	M	1	1							1	38,8	6
26-1-2021	M. Blue	M	1	2					1			20,4	6
26-1-2021	M. Pink	M	1	2					1			10	6
26-1-2021	M. White	M	1	2							1	22,4	6
26-1-2021	M. Blue	M	1	3							1	17	6
26-1-2021	M. Pink	M	1	3					1			23,6	6
26-1-2021	M. White	M	1	3					1			60	6
26-1-2021	M. Blue	M	1	4				1				8,4	5
26-1-2021	M. Pink	M	1	4							1	6,8	6
26-1-2021	M. White	M	1	4							1	9,8	6
26-1-2021	M. Blue	M	1	5	1							0	2
26-1-2021	M. Pink	M	1	5							1	56	6
26-1-2021	M. White	M	1	5							1	60	6
26-1-2021	M. Blue	M	1	6					1			60	6
26-1-2021	M. Pink	M	1	6					1			9,6	6
26-1-2021	M. White	M	1	6							1	20,4	6

Date	Guinea	Gender	Session number	Movement numb.	No Fl. A	No Fl. B	Fl. inside A	Fl. A to B	Fl. A to C	Fl. inside B	Fl. B to C	Time until approach	"Fear level"
27-1-2021	M. Blue	M	2	1					1			21,2	6
27-1-2021	M. Pink	M	2	1					1			28	6
27-1-2021	M. White	M	2	1					1			24,6	6
27-1-2021	M. Blue	M	2	2					1			18,2	6
27-1-2021	M. Pink	M	2	2					1			10,2	6
27-1-2021	M. White	M	2	2					1			60	6
27-1-2021	M. Blue	M	2	3				1				5	5
27-1-2021	M. Pink	M	2	3					1			11,6	6
27-1-2021	M. White	M	2	3							1	19,4	6
27-1-2021	M. Blue	M	2	4							1	29,4	6
27-1-2021	M. Pink	M	2	4							1	60	6
27-1-2021	M. White	M	2	4							1	26,4	6
27-1-2021	M. Blue	M	2	5						1		25	5
27-1-2021	M. Pink	M	2	5						1		5	5
27-1-2021	M. White	M	2	5							1	19,8	6
27-1-2021	M. Blue	M	2	6					1			20,4	6
27-1-2021	M. Pink	M	2	6							1	25,6	6
27-1-2021	M. White	M	2	6							1	32,2	6

Date	Guinea	Gender	Session number	Movement numb.	No Fl. A	No Fl. B	Fl. inside A	Fl. A to B	Fl. A to C	Fl. inside B	Fl. B to C	Time until approach	"Fear level"
29-1-2021	M. Blue	M	3	1							1	60	6
29-1-2021	M. Pink	M	3	1							1	60	6
29-1-2021	M. White	M	3	1				1				59	5
29-1-2021	M. Blue	M	3	2				1				24,2	5
29-1-2021	M. Pink	M	3	2					1			56,8	6
29-1-2021	M. White	M	3	2							1	54,6	6
29-1-2021	M. Blue	M	3	3							1	60	6
29-1-2021	M. Pink	M	3	3							1	26	6
29-1-2021	M. White	M	3	3							1	53,6	6
29-1-2021	M. Pink	M	3	4							1	60	6
29-1-2021	M. White	M	3	4				1				23,2	5
29-1-2021	M. Pink	M	3	5					1			60	6
29-1-2021	M. White	M	3	5							1	60	6

Date	Guinea	Gender	Session number	Movement numb.	No Fl. A	No Fl. B	Fl. inside A	Fl. A to B	Fl. A to C	Fl. inside B	Fl. B to C	Time until approach	"Fear level"
2-2-2021	M. Blue	M	4	1				1				4	5
2-2-2021	M. Pink	M	4	1						1		3	5
2-2-2021	M. White	M	4	1				1				10	5
2-2-2021	M. Blue	M	4	2					1			4	6
2-2-2021	M. Pink	M	4	2						1		5	5
2-2-2021	M. White	M	4	2						1		29	5
2-2-2021	M. Blue	M	4	3					1			9	6
2-2-2021	M. Pink	M	4	3				1				6	5
2-2-2021	M. White	M	4	3				1				5	5
2-2-2021	M. Blue	M	4	4					1			9	6
2-2-2021	M. Pink	M	4	4				1				4	5
2-2-2021	M. White	M	4	4				1				8	5
2-2-2021	M. Blue	M	4	5		1						0	3
2-2-2021	M. Pink	M	4	5						1		19	5
2-2-2021	M. White	M	4	5						1		5	5
2-2-2021	M. Blue	M	4	6					1			7	6
2-2-2021	M. Pink	M	4	6		1						0	3
2-2-2021	M. White	M	4	6				1				5	5

Date	Guinea	Gender	Session number	Movement numb.	No Fl. A	No Fl. B	Fl. inside A	Fl. A to B	Fl. A to C	Fl. inside B	Fl. B to C	Time until approach	"Fear level"
3-2-2021	M. Blue	M	5	1					1			3	6
3-2-2021	M. Pink	M	5	1				1				55	5
3-2-2021	M. White	M	5	1					1			5	6
3-2-2021	M. Blue	M	5	2				1				3	5
3-2-2021	M. Pink	M	5	2		1						0	3
3-2-2021	M. White	M	5	2				1				3	5
3-2-2021	M. Blue	M	5	3				1				5	5
3-2-2021	M. Pink	M	5	3						1		17	5
3-2-2021	M. White	M	5	3				1				7	5
3-2-2021	M. Blue	M	5	4				1				4	5
3-2-2021	M. Pink	M	5	4				1				8	5
3-2-2021	M. White	M	5	4						1		4	5
3-2-2021	M. Blue	M	5	5					1			35	6
3-2-2021	M. Pink	M	5	5						1		60	5
3-2-2021	M. White	M	5	5							1	60	6
3-2-2021	M. Blue	M	5	6			1					0	4
3-2-2021	M. Pink	M	5	6						1		3	5
3-2-2021	M. White	M	5	6				1				3	5

Date	Guinea	Gender	Session number	Movement numb.	No Fl. A	No Fl. B	Fl. inside A	Fl. A to B	Fl. A to C	Fl. inside B	Fl. B to C	Time until approach	"Fear level"
5-2-2021	M. Blue	M	6	1						1		1	5
5-2-2021	M. Pink	M	6	1						1		41	5
5-2-2021	M. White	M	6	1							1	7	6
5-2-2021	M. Blue	M	6	2				1				2	5
5-2-2021	M. Pink	M	6	2						1		13	5
5-2-2021	M. White	M	6	2						1		6	5
5-2-2021	M. Blue	M	6	3	1							0	2
5-2-2021	M. Pink	M	6	3		1						0	3
5-2-2021	M. White	M	6	3	1							0	2
5-2-2021	M. Blue	M	6	4		1						0	3
5-2-2021	M. Pink	M	6	4		1						0	3
5-2-2021	M. White	M	6	4		1						0	3
5-2-2021	M. Blue	M	6	5				1				29	5
5-2-2021	M. Pink	M	6	5		1						0	3
5-2-2021	M. White	M	6	5		1						0	3
5-2-2021	M. Blue	M	6	6						1		2	5
5-2-2021	M. Pink	M	6	6						1		60	5
5-2-2021	M. White	M	6	6		1						0	3

Date	Guinea	Gender	Session number	Movement numb.	No Fl. A	No Fl. B	Fl. inside A	Fl. A to B	Fl. A to C	Fl. inside B	Fl. B to C	Time until approach	"Fear level"
2-2-2021	Fem. Blue	V	1	1							1	11	6
2-2-2021	Fem. Pink	V	1	1					1			13	6
2-2-2021	Fem. White	V	1	1					1			16	6
2-2-2021	Fem. Blue	V	1	2						1		6	5
2-2-2021	Fem. Pink	V	1	2					1			26	6
2-2-2021	Fem. White	V	1	2							1	11	6
2-2-2021	Fem. Blue	V	1	3				1				6	5
2-2-2021	Fem. Pink	V	1	3				1				7	5
2-2-2021	Fem. White	V	1	3					1			9	6
2-2-2021	Fem. Blue	V	1	4					1			17	6
2-2-2021	Fem. Pink	V	1	4							1	10	6
2-2-2021	Fem. White	V	1	4					1			42	6
2-2-2021	Fem. Blue	V	1	5				1				4	5
2-2-2021	Fem. Pink	V	1	5							1	8	6
2-2-2021	Fem. White	V	1	5					1			20	6
2-2-2021	Fem. Blue	V	1	6				1				5	5
2-2-2021	Fem. Pink	V	1	6				1				5	5
2-2-2021	Fem. White	V	1	6					1			44	6

Date	Guinea	Gender	Session number	Movement numb.	No Fl. A	No Fl. B	Fl. inside A	Fl. A to B	Fl. A to C	Fl. inside B	Fl. B to C	Time until approach	"Fear level"
3-2-2021	Fem. Blue	V	2	1					1			13	6
3-2-2021	Fem. Pink	V	2	1					1			5	6
3-2-2021	Fem. White	V	2	1					1			9	6
3-2-2021	Fem. Blue	V	2	2			1					0	4
3-2-2021	Fem. Pink	V	2	2				1				3	5
3-2-2021	Fem. White	V	2	2			1					0	4
3-2-2021	Fem. Blue	V	2	3							1	16	6
3-2-2021	Fem. Pink	V	2	3					1			16	6
3-2-2021	Fem. White	V	2	3					1			15	6
3-2-2021	Fem. Blue	V	2	4			1					3	4
3-2-2021	Fem. Pink	V	2	4					1			6	6
3-2-2021	Fem. White	V	2	4					1			3	6
3-2-2021	Fem. Blue	V	2	5					1			11	6
3-2-2021	Fem. Pink	V	2	5				1				13	5
3-2-2021	Fem. White	V	2	5					1			19	6
3-2-2021	Fem. Blue	V	2	6					1			14	6
3-2-2021	Fem. Pink	V	2	6					1			18	6
3-2-2021	Fem. White	V	2	6				1				9	5

Date	Guinea	Gender	Session number	Movement numb.	No Fl. A	No Fl. B	Fl. inside A	Fl. A to B	Fl. A to C	Fl. inside B	Fl. B to C	Time until approach	"Fear level"
5-2-2021	Fem. Blue	V	3	1				1				10	5
5-2-2021	Fem. Pink	V	3	1							1	19	6
5-2-2021	Fem. White	V	3	1					1			14	6
5-2-2021	Fem. Blue	V	3	2				1				16	5
5-2-2021	Fem. Pink	V	3	2					1			14	6
5-2-2021	Fem. White	V	3	2					1			11	6
5-2-2021	Fem. Blue	V	3	3					1			12	6
5-2-2021	Fem. Pink	V	3	3		1						0	3
5-2-2021	Fem. White	V	3	3					1			15	6
5-2-2021	Fem. Blue	V	3	4					1			13	6
5-2-2021	Fem. Pink	V	3	4							1	8	6
5-2-2021	Fem. White	V	3	4				1				6	5
5-2-2021	Fem. Blue	V	3	5				1				9	5
5-2-2021	Fem. Pink	V	3	5				1				11	5
5-2-2021	Fem. White	V	3	5							1	4	6
5-2-2021	Fem. Blue	V	3	6					1			38	6
5-2-2021	Fem. Pink	V	3	6						1		7	5
5-2-2021	Fem. White	V	3	6				1				8	5

Date	Guinea	Gender	Session number	Movement numb.	No Fl. A	No Fl. B	Fl. inside A	Fl. A to B	Fl. A to C	Fl. inside B	Fl. B to C	Time until approach	"Fear level"
10-12-2021	Fem. Blue	V	4	1					1			8	6
10-2-2021	Fem. Pink	V	4	1				1				5	5
10-2-2021	Fem. White	V	4	1							1	5	6
10-2-2021	Fem. Blue	V	4	2					1			14	6
10-2-2021	Fem. Pink	V	4	2					1			33	6
10-2-2021	Fem. White	V	4	2							1	9	6
10-2-2021	Fem. Blue	V	4	3				1				5	5
10-2-2021	Fem. Pink	V	4	3				1				8	5
10-2-2021	Fem. White	V	4	3					1			2	6
10-2-2021	Fem. Blue	V	4	4				1				6	5
10-2-2021	Fem. Pink	V	4	4				1				6	5
10-2-2021	Fem. White	V	4	4				1				18	5
10-2-2021	Fem. Blue	V	4	5					1			21	6
10-2-2021	Fem. Pink	V	4	5					1			15	6
10-2-2021	Fem. White	V	4	5	1							0	2
10-2-2021	Fem. Blue	V	4	6					1			25	6
10-2-2021	Fem. Pink	V	4	6					1			15	6
10-2-2021	Fem. White	V	4	6							1	18	6

Date	Guinea	Gender	Session number	Movement numb.	No Fl. A	No Fl. B	Fl. inside A	Fl. A to B	Fl. A to C	Fl. inside B	Fl. B to C	Time until approach	"Fear level"
12-2-2021	Fem. Blue	V	5	1							1	37	6
12-2-2021	Fem. Pink	V	5	1				1				7	5
12-2-2021	Fem. White	V	5	1				1				6	5
12-2-2021	Fem. Blue	V	5	2							1	5	6
12-2-2021	Fem. Pink	V	5	2				1				21	5
12-2-2021	Fem. White	V	5	2				1				3	5
12-2-2021	Fem. Blue	V	5	3				1				8	5
12-2-2021	Fem. Pink	V	5	3				1				7	5
12-2-2021	Fem. White	V	5	3					1			8	6
12-2-2021	Fem. Blue	V	5	4					1			11	6
12-2-2021	Fem. Pink	V	5	4					1			6	6
12-2-2021	Fem. White	V	5	4				1				3	5
12-2-2021	Fem. Blue	V	5	5					1			7	6
12-2-2021	Fem. Pink	V	5	5				1				9	5
12-2-2021	Fem. White	V	5	5				1				10	5
12-2-2021	Fem. Blue	V	5	6							1	32	6
12-2-2021	Fem. Pink	V	5	6				1				5	5
12-2-2021	Fem. White	V	5	6				1				5	5

Date	Guinea	Gender	Session number	Movement numb.	No Fl. A	No Fl. B	Fl. inside A	Fl. A to B	Fl. A to C	Fl. inside B	Fl. B to C	Time until approach	"Fear level"
16-2-2021	Fem. Blue	V	6	1					1			9	6
16-2-2021	Fem. Pink	V	6	1		1						0	3
16-2-2021	Fem. White	V	6	1					1			5	6
16-2-2021	Fem. Blue	V	6	2				1				23	5
16-2-2021	Fem. Pink	V	6	2				1				6	5
16-2-2021	Fem. White	V	6	2				1				3	5
16-2-2021	Fem. Blue	V	6	3					1			3	6
16-2-2021	Fem. Pink	V	6	3				1				5	5
16-2-2021	Fem. White	V	6	3				1				5	5
16-2-2021	Fem. Blue	V	6	4				1				3	5
16-2-2021	Fem. Pink	V	6	4				1				6	5
16-2-2021	Fem. White	V	6	4				1				2	5
16-2-2021	Fem. Blue	V	6	5				1				4	5
16-2-2021	Fem. Pink	V	6	5			1					2	4
16-2-2021	Fem. White	V	6	5			1					1	4
16-2-2021	Fem. Blue	V	6	6				1				5	5
16-2-2021	Fem. Pink	V	6	6				1				14	5
16-2-2021	Fem. White	V	6	6				1				1	5