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Tactile and auditory human-cattle interactions: Effects on behavioural reactions towards humans and during isolation

Master's thesis

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submitted by
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1. Introduction

The human-animal relationship is defined as the relatedness or distance between the animal and the human and expresses itself in their behaviour towards each other (Waiblinger, 2017; Waiblinger et al. 2006). In this study we focused on the animal's perspective, i.e. its relationship towards humans, the animal-human relationship (AHR). The AHR develops from the sum of interactions between humans and an animal, which are perceived as positive, negative or neutral by the animal (Waiblinger et al., 2006). The overall perception of humans, which is shaped by the animal's emotions during past interactions with humans, shapes the AHR (Waiblinger, 2017). The quality of the AHR also influences future interactions and the animal's perception of them (Waiblinger, 2017). If the AHR is negative, the consequences are stress reactions and a higher risk of injuries for the animals as well as more difficult handling and impaired health, welfare and productivity of the animals . In cattle, aversive handling during milking can lead to decreased milk yield (Rushen et al., 1999), and cows that are fearful of humans tend to have a lower productivity than cows that are less fearful (Breuer et al., 2000).

On the other hand, a positive AHR has an anti-stress potential and can improve animal welfare, e.g. by reducing stress reactions during necessary aversive management procedures like veterinary treatment (Waiblinger, 2004). It allows for the possibility of humans providing social support for animals in a stressful situation (Waiblinger, 2017). Coulon et al. (2015) showed that human caregivers were able to provide social support for lambs during isolation. The animals were stroked regularly before the test and reacted more calmly during isolation from conspecifics if they were stroked. Stroked veal calves interacted more with humans in a novel environment and defecated less than control animals (Lensink et al., 2000).

A positive AHR can lead to states of relaxation and pleasant emotions during interactions with humans which elicits physiological reactions like release of oxytocin and lower heart rate and blood pressure (Waiblinger, 2017; Waiblinger, 2019). In the long term a positive AHR is beneficial for social bonding and health (Waiblinger, 2017; Waiblinger, 2019). Gentle interactions decrease avoidance reactions towards humans and increase productivity in cattle (Breuer et al., 2000; Lensink et al., 2000; Lürzel et al., 2015a; Schmied et al., 2008a). Furthermore, it facilitates animal handling that is safer and easier for the animal and humans (Waiblinger, 2017).

A commonly used method of improving the AHR is stroking (e.g. Coulon et al., 2015; Lensink et al., 2000). Stroking, often combined with talking in a gentle voice, has been shown to reduce fear of humans in calves and cows (e.g. Lensink et al., 2000; Lürzel et al., 2015a; Windschnurer et al., 2009a). Bertenshaw and Rowlinson (2008) found that heifers approached the human voluntarily to be brushed. During the treatment the heifers offered the chin or poll and performed neck stretching. These behaviours might indicate that cattle perceive being brushed by a human as positive experience . Neck stretching is interpreted as a sign of pleasure during social licking and during stroking or brushing by humans (Bertenshaw & Rowlinson, 2008; Lürzel et al., 2015b; Schmied et al., 2008b).

Other studies also suggest that stroking is perceived as positive by cattle. In veal calves, stroking and letting calves suck the fingers of a person led to more approach behaviour towards and more interaction with a human in a novel environment (Lensink et. al, 2000). Dairy calves showed voluntary approach towards a human offering gentle interactions, which included stroking and talking in a gentle voice (Lürzel et al., 2015b). The behaviour of calves during gentle treatment includes a high amount of neck stretching and play behaviour (Lürzel et al., 2015a, Lürzel et al., 2015b). Play behaviour was shown to be an indicator for positive emotions in animals, mainly in juvenile farm animals like piglets, calves and lambs (Boissy et al., 2007). But play behaviour is not necessarily a sign of positive emotions in all species or situations, as shown for example in early-weaned kittens or riding school horses and might serve to reduce some forms of stress the animals are suffering from (Ahloy-Dallaire et al., 2018). Still, play is suppressed by many situations that have negative effects on the animal and therefore can be an indicator for welfare (Ahloy-Dallaire et al., 2018).

Stroking of cattle is most effective when applied on the withers and especially the ventral neck (Schmied et al., 2008b), body parts that are also licked by conspecifics during allogrooming (head and neck: Sato et al., 1991; withers and ventral neck: Schmied et al., 2005). Allogrooming in animals, including cattle, is an affiliative behaviour important for social bonding (Boissy et al., 2007; Val-Laillet et al., 2009). It is considered as rewarding and discussed as an indicator for positive emotions in animals (Boissy et al., 2007). The behavioural and physiological responses of cattle (neck stretching and ear hanging; decrease of heart rate) are comparable between situations when those regions are stroked by a human or licked by a herd mate (Schmied et al., 2008b). Stroking could thus have a similar effect on the AHR as social licking has on intraspecific bonds (Schmied et al., 2008a; Windschnurer et al., 2009a). In addition to stroking cattle at a specific body part like the ventral neck, ‘reactive stroking’ is another possible method. With ‘reactive stroking’ the handler strokes the head/neck region of the animal with reacting to momentary preferences indicated by the animal (Lange et al., 2020). Lange et al. (2020) showed that there were only minor differences between stroking the ventral neck and ‘reactive stroking’ of whole head/neck region.

The effect of stroking without talking on the AHR in cattle was already investigated (e.g. Schmied et al., 2008a), and stroking is often applied in combination with talking in a gentle voice (Lensink et al., 2000; Lürzel et al., 2015a; Waiblinger et al., 2004). The effect of talking in a gentle voice has not been investigated separately from the effect of stroking. The structure of an acoustic signal can affect the behavioural response of the receiving animal (McConnell, 1991). In animal training, handlers mostly use short and repeated notes to stimulate activity and long and continuous notes to decrease activity (McConnell, 1991). The fact that people are using the same structure of acoustic signal for similar tasks in animal training might indicate that the animals respond better to certain acoustic structure in a certain context (McConnell, 1991). Four short notes with rising frequency were more effective in training dog pups to come to the trainer than one long note with descending frequency (McConnell, 1990). In another study, horses reacted differently to a positive human acoustic signal, laughing, compared to a negative one, growling (Smith et al., 2018). When confronted with the negative signal the horses showed longer periods of freezing and vigilance. This finding provides evidence that horses can distinguish between human vocal cues carrying different emotional content (Smith et al., 2018). These studies indicate that the vocal structure and emotional content might affect animal behaviour. On the other hand it seems that soothing vocal cues do not facilitate learning a frightening task in horses, compared with harsh human vocalization (Heleski et al., 2015). The study found no statistical differences in failure at the task, time to success, time until task is performed calmly or heart rate. Alternatively, the results could be due to the fact that horses, highly familiar with negative reinforcement, paid more attention to the halter pressure than the vocal cues.

Although acoustic communication in cattle is not fully understood, there is some evidence they use distinct calls that contain information like age, sex, dominance and reproductive status (Watts & Stookey, 2000). Additionally, cows use low-pitched vocalizations in proximity of their calves during the first weeks of life and high-pitched vocalizations when they are separated from their calves (Padilla de la Torre et al., 2015). This shows that cattle differentiate between some acoustic signals for communication with conspecifics and use signals with distinct acoustic structures for different purposes. Kiley-Worthington and de la Plain (1996) described several different vocalizations used by cattle, e.g. in situations of conflict and threat or when greeting conspecifics. Cattle may also respond differently to human vocalizations of different acoustic structure. In a study by Breuer et al. (2000), restlessness in cows during milking was higher when stockperson used "harsh and loud vocalizations", and lower when the person used "soft and quiet vocalizations". Pajor et al. (2003) performed a Y-maze test where they found no significant preference for a human talking in a gentle voice over a human being present without talking or shouting. The cows chose talking in a gentle voice significantly more often than a human shouting but not more often than a human being present. But even though most of the cows chose the human being present during the first three trials, seven out of eight cows chose a human talking in a gentle voice in trial four to six; in trial 7 half of the animals chose talking in a gentle voice, in trial 8 six out of eight. Although there was no significant difference, it does not seem like the cows do not differentiate between a human talking in a gentle voice and a human being present without vocalising. Investigating this matter with a larger sample size over a longer time with more trials might show different results.

Although stroking is probably more rewarding, talking in a gentle voice could be a useful tool if it has a positive effect on the AHR, as it has some advantages over stroking. For example, talking can be used over a distance, avoiding close physical contact with a possibly fearful animal, and it can be applied to more than one animal at the same time. Still, we do expect that stroking is more rewarding than talking in a gentle voice, because stroking is adapted from allogrooming in cattle and there are indications that it has similar effects (Schmied et al., 2008a; Windschnurer et al., 2009a). For talking in a gentle voice there are no such – at least investigated – parallels in vocal behaviour of adult cattle during socio-positive interactions. Because it has not been investigated at all in cattle, we investigated the effect of talking in a gentle voice separately and in combination with the effect of stroking in our study.

In cattle, the combination of stroking and talking in a gentle voice has not been compared to stroking alone yet. In a study on shelter dogs a stroking style combined with an emphasized soothing voice was effective in reducing the cortisol response to a venepuncture, while another technique of stroking and talking did not have the same effect (Hennessy et al., 1998). In this case both – stroking and the way of talking – differed between the two treatments, so that it is not clear what aspect or if both aspects were responsible for the effect. Still, it might be a possible influence. However, human contact in early development of kittens was more effective for socialization when it included talking (Bernstein, 2005). This supports the assumption, that talking in a gentle voice is not indifferent to animals and that it might even magnify the effect of stroking alone. There is no evidence so far if this might be true in cattle. Still the combination is used frequently and successfully in many studies (Lensink et al., 2000; Lürzel et al., 2015a; Waiblinger et al., 2004).

The aim of our study was to test if different types of gentle interactions – stroking and talking in a gentle voice alone and combined – improve the AHR in heifers to a different degree. We investigated avoidance distance and the capacity of the human to provide social support to the heifers in an isolation test. When the AHR is positive, there is no avoidance, because the animal is not fearful of humans. The human is only able to provide social support when the AHR is good and the animal perceives human proximity as comforting during the isolation test. We hypothesized that both stroking and talking improve the AHR compared to a control treatment but to a different degree. Gentle interactions including stroking are expected to improve the AHR to a higher degree than talking in a gentle voice. The combination of both is hypothesized to have the biggest effect on AHR compared to stroking or talking alone.

We predicted that avoidance distances after the treatment are lower in heifers that experienced a treatment including stroking than in heifers that only experienced vocal interactions. Heifers that experienced vocal interactions were predicted to have lower avoidance distances than control animals. Regarding the isolation test with temporary human presence, we predicted a difference in the heifer´s behaviour due to social support provided by the human in the temporary human presence phase of the isolation test. Heifers that received the combined treatment were predicted to show the least stress reactions in the human presence phase (e.g. alert postures, locomotion, elimination, vocalisations) due to the improved capacity of the human to provide social support. The stroking treatment was expected to be less effective than the combination of stroking and talking but more effective than only talking in a gentle voice. Animals that experienced only vocal interactions were predicted to show fewer stress reactions in the human presence phase than control animals.

2. Methods

2.1 Animals, housing and management

The study was conducted in May and June 2019 on the young stock farm Rehgras (Furth an der Triesting, Austria), part of the VetFarm, the teaching and research farms of the University of Veterinary Medicine, Vienna. Sixty Austrian Simmental heifers (7 - 25 months of age) were used for the study. Before the study started the animals were grouped in two herds with 30 animals each according to age. Herd 1 contained the older animals with 15 - 25 months of age, herd 2 the younger animals aged 7 - 15 months. Twenty-nine heifers were born and raised at the VetFarm, 31 at the dairy farm of the leaseholder of Rehgras. The animals were raised similarly in both facilities. At the leaseholder's farm they were born in a group calving pen with visual contact to the herd. Calf and cow were usually separated 1 h after birth (up to 6 h after birth for night calving). Colostrum was fed in a bottle as soon as possible. Caretakers assisted the calf drinking during approximately the first 2 d (bottle-feeding) until the animal was able to drink independently from a teat bucket. They were fed with colostrum the first 2 d twice a day until satiation. After that they had access to a teat bucket twice a day, containing warmed milk from their dam. From day 7 on, they were fed with 10 L milk replacer per day (Sprayfro Royal Kälbermilch, 150 g/L in summer, > 160 g/L in winter). They were provided with water and a self-made calf starter that also contained roughage (straw) from day 5-6 on ad libitum. The animals were fed by hand or hay fork. The calves were housed individually in hutches and adjoining runs littered with straw for the first 14 d of life. They had visual contact to the other calves. The hutches measured 1.3 x 1.7 m², the adjoining runs 1.37 x 1.47 m². After 2 weeks, the calves were transferred into group housing until the 10 - 12th week of life. The calves were kept in two deep-litter pens next to each other with an elevated non-littered area in front of the feeding racks. The compartments for the groups of eight calves measured 4.92 x 5.22 m² each. The calves were fed milk replacer by an automatic feeder ("Urban Kälbermama Paula", Urban GmbH & Co.KG, Wüsting, Deutschland). The amount was controlled by the computer with individual sensor collars of the animals. Only one animal could feed on milk replacer at the same time. Weaning took place at an age of 10 - 14 weeks. Individual parameters were considered, such as growth and the intake of solid feed. After that, the animals were brought to another barn for a month, where they started to feed on hey, straw, silage and concentrate. With approximately 4 months of age, the heifers were transferred to the Rehgras farm. The animals had visual contact to humans in individual and group housing. The calves in the hutches were also visited by unfamiliar people including children quite regularly and could be touched. The animals had approximately 0,5 h contact to humans per day. At the VetFarm our heifers were born in a single calving pen with visual contact to the herd. Calf and cow were usually separated 1 h after birth (up to 6 h after birth for night calving). Colostrum was fed in a bottle as soon as possible. Caretakers assisted the calf drinking during approximately the first 2 d (bottle-feeding) until the animal was able to drink independently from a teat bucket. The calves were fed three times a day with 2 L colostrum during the first 5 d. After that they were fed with 3 L of pasteurised milk twice per day. The VetFarm provided water, hay and calf starter ("Kälberstart Vital") ad libitum since day 1. The animals were fed by hand or hay fork. The calves were housed individually in hutches and adjoining runs littered with straw for the first 14 d of life. They had visual contact to the other calves. The calf hutches measured 1.2 x 0.9 m^2, the adjoining runs 1.5 x 1.1 m^2. After 2 weeks, the calves were transferred into group housing until the 14^{th} week of life in groups of eight. The group-housing pen consisted in a 4.8 x 4.9 m^2 deep-litter area and a heightened feeding area with 8 individual feeding stalls where calves could be restrained, measuring 0.45 x 1.3 m^2. The calves were fed simultaneously with teat buckets in the feeding stalls. Weaning took place at an age of 12 weeks. After weaning they were fed with hay, silage and concentrate. Some calves had additional contact to humans apart from management routines depending on participation in studies. During housing in hutches, calves had contact to humans passing by.

At the farm Rehgras the heifers raised at the leaseholder's farm and at the VetFarm were kept together in groups of 12 – 16 animals; at the age of four months in a pen consisting of a roofed deep litter area and a concrete outdoor run. They were fed on hey, silage, straw and concentrate. The heifers we conducted our study on (7 – 25 months) were kept in groups on pasture during summer. In winter they were kept in pens including indoor (cubicle housing) and concrete outdoor areas. The animals only had human contact during feeding and cleaning their pens.

2.2 Experimental design and treatment

The project was discussed and approved by the institutional ethics and animal welfare committee in accordance with GSP guidelines and national legislation.

In our study we used five different treatments. During all of them the heifers were restraint in headlock feeding racks.

- The human is stroking the heifer at the ventral neck and talking in a gentle voice (ST).
- The human is talking in a gentle voice (T) to the heifer.
- The human is stroking the heifer at the ventral neck (S).
- The human is standing next to the heifer in close human presence (P).
- Control treatment: There is no human present (C).

The position of the human was the same in all treatments except C – directly next to the left shoulder of the heifer. The treatments were performed for 5 min/d. While approaching the heifers slowly, the experimenter sayed "Hallo, Mädchen" to the animals in all treatments except for C. In treatments ST and T, the experimenter continued talking in a gentle voice for the full duration of the treatment. In treatments S and P there was no further talking. The heifers were allocated to the five treatments, balanced for their avoidance distance (measured in a pre-test before AD test 1). One of the 31 heifers raised on the leaseholder farm was excluded from the study after the avoidance distance test because she showed an avoidance distance of 0 m from the beginning, so there was no scope for improvement (6 animals per treatment and herd). Thus, sample size was 6 animals per treatment and herd, except for treatment C in herd 2 with only 5 animals. For herd 1 and 2 the treatment was performed by one of two female experimenters each. The 'experimenter' was the one performing the treatment and was aware of the treatment allocation. The 'handler' helped with handling the heifers and sorting them for the treatment but was blinded to treatment allocation. Both experimenters were 'experimenter' to one herd and 'handler' in the other. Handling included bringing the herds in the stable for the experiment, sorting them in the stable for the treatment and help restraining them if necessary. The handling was the same for the animals of all treatments including treatment C. Experimenter A had long, blond hair, a height of 176 cm and was the experimenter to herd 1 and the handler to herd 2. Experimenter B had short, blond hair, a height of 172 cm and was the experimenter of herd 2 as well as the handler to herd 1. The experimenter and the handler were dressed in green overalls and black boots. The experimenter and the handler learnt to move the herds from pasture to stable before the start of the study. They walked behind the herd forming an imaginary line between them. To make a heifer and the herd move further the experimenter/handler stepped towards her to create pressure but stopped and withdrew immediately when the heifer showed the desired reaction.

Our study design included a three-week period of treatment with five treatment days per week. Before, directly after and two weeks after the treatment an avoidance distance test was performed. An isolation test was performed for each animal once after the treatment and before the last avoidance distance test. The isolation tests were conducted in total on six days; each day 10 animals (1 from each treatment per herd) were tested (Fig.1).

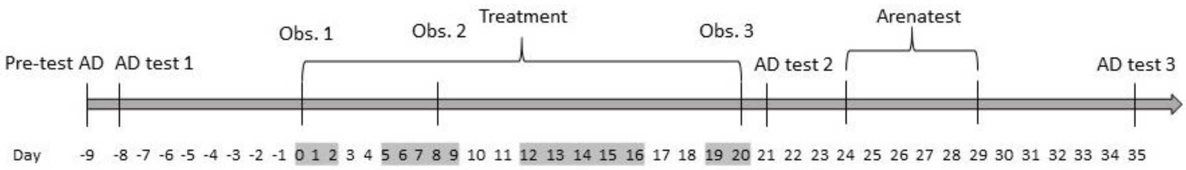

Figure 1: Time schedule of the experiment; treatment days are highlighted in grey; observations of the treatment by camera are labelled (Obs. 1, 2, 3). The pre-test was conducted one day before to assess avoidance distances for a balanced treatment allocation of the heifers.

2.3 Experimental procedures

The heifers were brought from the pasture to the barn before every treatment or test by both experimenters. The barn was subdivided in several compartments inside (A and B Deep litter, as well as the Cubicles C and D) and outside (A, B, C and D Outside, E). The outside compartments included headlock feeding racks and had concrete floor. The barn aisle was covered by a roof. To enter or leave the barn with a herd of heifers, the entrance of A Outside was used. For the sorting almost all compartments were used (except B Deep litter); the treatment of herd 1 was performed in C Outside and the treatment of herd 2 in D Outside and E (Fig. 2). Herd 1 received treatment in the morning, herd 2 in the afternoon.

For restraining and sorting the animals before the treatment compartments B and C were used for herd 1 and the compartments C, D and E for herd 2 (Fig. 2). Animals that were not treated in the current treatment session were kept in compartments where they could not observe the treatment (herd 1: A Outside and Deep litter, C Cubicles, D Cubicles, D/E; herd 2: A Outside and deep litter, C Cubicles, D Cubicles; Fig. 2). Twelve animals of herd 2 were too small to be restrained in feeding racks of compartments D and C and were not restrained for the sorting of groups. All heifers could be restrained in the headlock feeding racks designed for calves in compartment E, but there were not enough racks to restrain all twelve animals at once. For the treatment the number of feeding racks in compartment E was sufficient. Before the start of the treatment herd 2 was habituated to the feeding racks because they were not used to them at all. We habituated them to open feeding racks and the compartments on two days for 1.5 – 2 h each (total 3.5 h). After that we habituated herd 2 to sorting and standing in closed feeding racks on 3 days for 1 – 3 h each (in total 6.5 h). Herd 1 was habituated to the compartments and the sorting on 2 days for 1.5 – 2.5 h each (total 4 h). Before the animals were brought into the barn, concentrate was provided on the feeding alley in small amounts to encourage the animals to restrain themselves in the feeding racks without human interference. Individual animals belonging to the same treatment were released by the experimenter or handler and moved together into a compartment from which they could not observe the treatment. The heifers treated first stayed restrained. The order of the treatments was pseudo-randomized and changed every day in a rotating manner. The sorting of the animals was done by the experimenter and the handler. The handler was blinded to the identity of the treatment allocation.

During the treatment all heifers receiving it were restrained in the feeding rack with a minimal distance of one empty feeding place between two animals. After the first treatment was finished, the animals were released and brought into one of the compartments described above. The animals for the next group were moved out of a compartment and restrained in the headlock feeding racks in the treatment area. Before the heifers entered the treatment area, concentrate was provided again in small amounts to make sure that the animals restrained themselves in the feeding rack. This was done to avoid a possible effect on the AHR by the association of feeding with humans. With some animals, it was necessary that food was provided directly in front of them by a human in order to encourage them to enter the feeding rack. Treatment order within one group was from left to right (perspective from inside the compartment); the sequence of animals was not necessarily the same on every day, as the animals chose their feeding place freely. The experimenter treated the animals alone. After all heifers of one treatment were treated, the experimenter released them and brought them back into a compartment from which

they could not observe the treatment of the other groups. After the treatment of one herd was finished, the animals were returned together to their pasture.
The treatment was filmed with a handheld camera (Sony FDR-AX53) on the1st, 8th and 15th day (Fig. 1) by a third person.

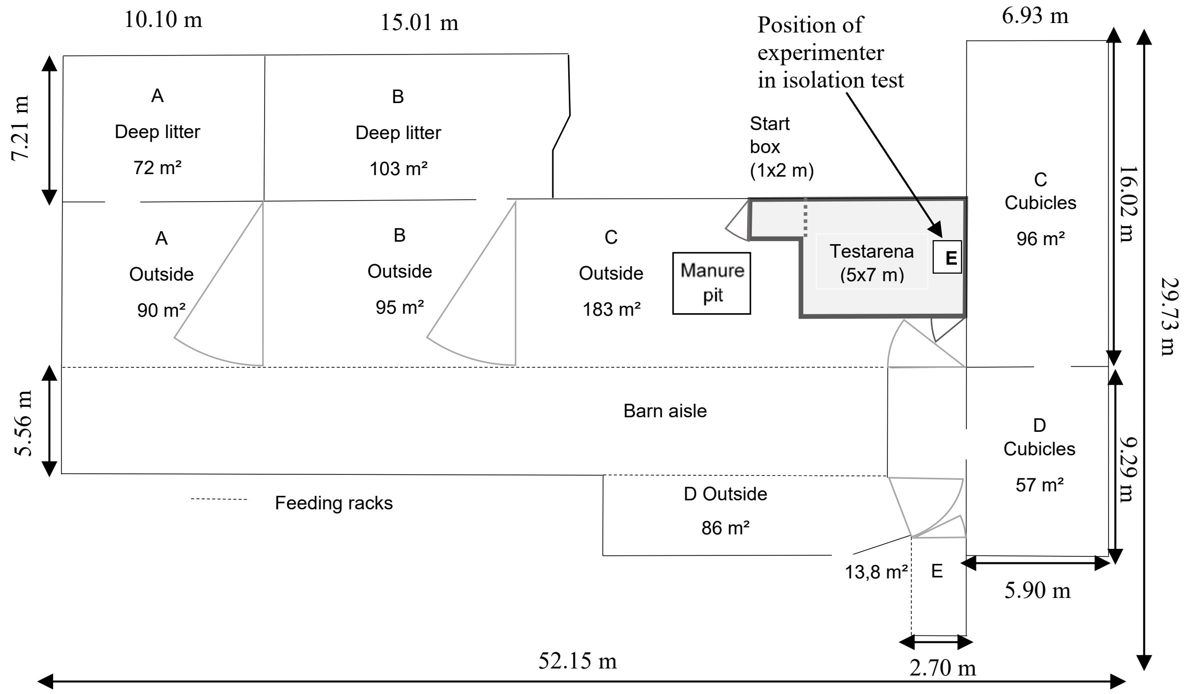

Figure 2: Schematic plan of the pens at the farm Rehgras including measurements and testing arena (adapted from © Regien van Hasselt). Entrance of the animals in the barn happened always over compartment A Outside. A and B Deep litter as well as the Cubicles were inside compartments. The others were all outside compartments with feeding racks and concrete floor. The barn aisle was covered by a roof. Testarena: location for the isolation test; d1: entrance door for heifer through start box; d2: entrance door for human experimenter; E: position of experimenter during the isolation test. The test arena was built in compartment C after the treatment period.

2.4 Avoidance distance test

Before and after the treatment, the heifer's avoidance distances were recorded as an indicator of the quality of the animal-human relationship. The avoidance distance test is a reliable and valid method to assess the AHR (Waiblinger et al., 2003; Windschnurer et al., 2009b). A third avoidance distance test was performed two weeks after the treatment to see if there were any longer-lasting effects. Each of the three avoidance tests was performed twice, once by the experimenter and once by the handler. The avoidance distances except for the third test were recorded in compartment C. The third test was done in compartments A and B because compartment C was still blocked by the test arena (see section 2.4 and Fig. 2). The avoidance distance test was performed in the barn on free moving animals and assessed similar as in Waiblinger et al. (2002). The test was started when the experimenter or handler stood in front (or slightly sideways, deviating up to approximately 30°) of the animal with a distance of approximately 3 m and the animal payed attention to her. The experimenter or handler approached the animal with a speed of 1 step/s and extended one arm in front with an angle of approximately 45°, with the back of the hand forward. If the heifer showed avoidance behaviour by taking a step away or withdrawing the head, the distance between the hand of the experimenter/handler and the muzzle of the heifer was estimated in steps of 10 cm at the moment when the avoidance behaviour started (Lürzel et al., 2015a; Waiblinger et al., 2002). If the heifer did not show avoidance, the experimenter/handler touched her nose and continued to stroke the cheek for a maximum of 5 s if the heifer did not stop the interaction earlier (similar as in Windschnurer et al., 2009b). An avoidance distance of 0 cm and the duration of the interaction were recorded in those cases. It was recorded whether the animals showed avoidance behaviour at the moment of touching (0A) or when the experimenter touched the animal but could not stroke it (0B); if the animal allowed stroking at the cheek, the time was recorded in seconds (0S1-0S5). When stroking for 5 s was possible, the test was ended after these 5 s. Herd 1 was tested in the morning, herd 2 in the afternoon.

2.5 Isolation test

An isolation test in a novel environment with temporary presence of a person was performed after the treatment to test if the human can provide social support in a challenging situation. The arena used for the test was constructed in compartment C after the treatment. The arena covered an enclosed area 5 m x 7 m and a startbox, which was a corridor measuring 1 m x 2 m with a sliding door to control the time the animals entered the arena. The walls were made of 1 m x 2 m plywood boards, which prevented visual contact to the other animals. Auditive contact was still possible. On the opposite side of the startbox was another door as an entrance for the experimenter (Fig. 2). The day before the beginning of the isolation tests, both herds were habituated to the arena from the outside. They were not habituated to the arena itself, because it was supposed to be a novel environment, but to the outside to facilitate moving them alone into the arena. The animals stayed approximately 1 h in compartment C and D and were moved along the arena several times (Fig. 2). For the isolation test the herds were brought in from pasture for six consecutive days (herd 1 in the morning, herd 2 in the afternoon). Each day ten animals were tested, five of each herd. Per herd one heifer of each treatment was tested per day. The order was pseudo-randomized. While the herd stayed in compartment A, the 5 animals were sorted by the experimenter and the handler and restrained in the feeding rack (as described in section 2.2) in compartment D (and E for herd 2) (Fig. 2). They stayed there until all five animals were tested and returned as a group to their herd before they were brought back to pasture. The animals were provided with feed (hay) and water during the whole time (except actual testing time for each animal). The handler moved the animals individually into the test arena. After the animal had entered the arena, the experimenter closed the door. The total test duration was 15 min. After 5 min the experimenter entered the test arena through the entrance door at the opposite site of the startbox and walked slowly to the middle of the shorter arena wall (5 m) (Fig.2, Fig. 3), and stayed there for 5 min. The experimenter talked in a gentle voice to animals of all treatments during the whole period of 5 min. If the animal approached, the experimenter tried to touch the nose first. If the animal allowed touching, the experimenter tried to touch the cheek and stroke the animal there. If the heifer allowed touching and stroking the experimenter tried to stroke the ventral neck. If the heifer withdrew from the touch, the experimenter stopped. She tried again only if the heifer approached again. If the animal withdrew for a third time, there were no further attempts to touch and stroke her. After 5 min, the experimenter left, and the heifer stayed alone for the remaining 5 min of the test. The behaviour of the heifer was recorded during the isolation test on video (Sony FDR-AX53) from 6.8 m above the arena. The handler moved the heifer back to compartment D to be restrained in the feeding rack (Fig. 2).

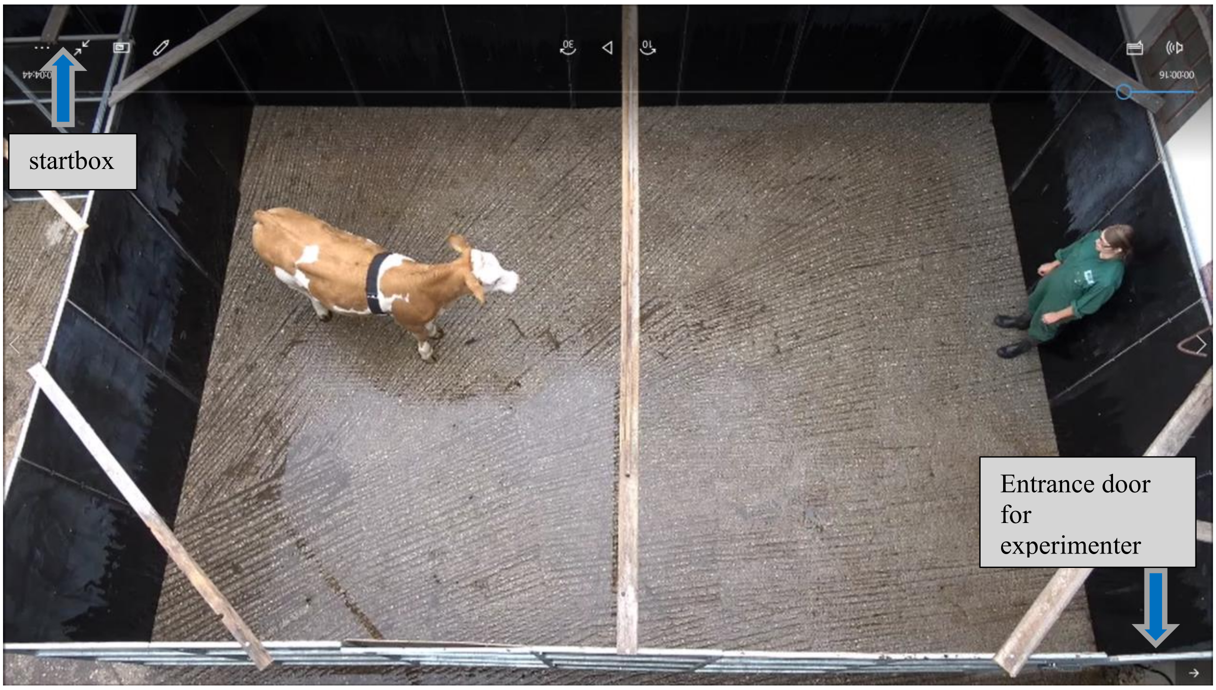

Figure 3: Screenshot of the testing arena, involving the experimenter at her position during the human phase, the heifer, the location of the entrance door for the experimenter and the location of the startbox.

2.6 Behavioural observations

The videos of the treatment and the isolation test were analysed with the event-logging software BORIS (v. 7.7.3; Friard and Gamba, 2016). The videos were coded by the two experimenters, because the observers were blinded for group allocation in the isolation test videos. The isolation test videos were analysed first, because in the treatment videos the type of treatment applied was visible and therefore the observers could not be blinded. One observer coded the isolation test videos in which the other was the test person/handler and vice versa. In order to record the location of the heifer in the isolation test videos the arena was divided into nine squares of similar size. *Vocalization* was recorded directly in the isolation test – counted by the handler from outside the arena – and recorded by video coding in the treatment. In the treatment videos the observers were blinded for the day the treatment was recorded (1^{st}, 8^{th}, 15^{th}). The behaviours coded are listed in two separate ethograms (Tab. 1, 2).

Inter-observer reliability was tested using BORIS (Cohen's kappa, interval time: 2 s). For this purpose, ten 1-min clips were cut from the original videos each – ten for the treatment videos and ten for the isolation test. Half of the clips were from herd 1, half from herd 2. The observers chose the clips randomly but made sure that all behaviours to be observed were included. The overall inter-observer reliability for the isolation test was kappa = 0.72. The inter-observer reliability for the individual behaviours ranged from kappa = 0.69 to kappa = 1.00. The overall inter-observer reliability for the treatment videos was kappa = 0.67. The inter-observer reliability for the individual behaviours ranged from kappa = 0.71 to kappa = 1.00.

2.7 Data preparation and statistical analysis

For statistical analysis we used the software package R version 3.6.2 (R Core Team, 2019). We did full/null model comparisons to reduce type I error and keep the probability of false positives at the nominal level of 0.05 (Forstmeier & Schielzeth, 2011). The statistical unit was the individual animal. The animal ID was included as random effect in all models, except *exploring human* in the isolation test, because this behaviour could occur only during the second phase and thus there was only one data point per animal in the data set. For all statistical models used p values ≤ 0.05 were considered significant.
Due to the not normally distributed residuals, the avoidance distance was analysed with a generalized linear mixed model (GLMM) based on the gamma distribution using the function glmer from the package "lme4" (Bates et al., 2015). As the avoidance distance data contained zeros, which are not contained in the gamma distribution, the dependent variable was transformed by adding 0.0000001 to every value. The original data included the avoidance distances towards the experimenter and the handler. Because none of our hypotheses target effects of the familiarity of the person, we tested if familiarity makes a difference before further analysing the data. In the full model, treatment, test number, herd, familiarity and all possible interactions of these four factors as well as origin were included as fixed effects. The origin is the farm where the heifers were born and brought up until the age of four months – at the leaseholder farm or the VetFarm (2.1 Animals, housing and management). In the null model, the interactions of familiarity with the other fixed effects were dropped. The full/null model comparison did not reveal a difference between the models, indicating that familiarity did not have an impact on the interaction of interest (test number * treatment).

Table 1: Ethogram of behaviours recorded in the isolation test, adapted from Lürzel et. al (2015b, 2020). The behaviours were recorded as durations, except for *elimination*, *moving away from human*, *avoiding human* and *exploring wall's height*, which were recorded as frequencies.

Behaviour	**Definition**
Square X	Square is counted if one front extremity enters it.
Exploring	The heifer's head is close (approximately ≤ 10 cm) to and directed toward the floor or the arena wall. (If the heifer holds her muzzle close to the wall during neck stretching while being stroked, it is not recorded as exploring.)
Exploring human	The heifer moves her muzzle towards the person into a perimeter of approximately 10 cm, muzzle pointing towards the person. The behaviour ends when the heifer's muzzle does not point towards the person anymore or leaves the perimeter of 10 cm.
Being touched	One of the test person's hands is in physical contact with the heifer.
Neck stretching	Positioning the neck and head actively in an outstretched line. Neck stretching is not recorded if the position is attained in the context of feeding, rubbing, licking or during exploration (= if an elongated neck is necessary to explore the object/person). If neck stretching is shown already before rubbing, licking or rubbing or licking of the person, it will be recorded as long as the neck is in an outstretched line. Neck stretching is only recorded during interactions with the person.
Self-grooming	The heifer touches her body with her tongue, a foot or the muzzle while standing.
Tail flicking	The heifer moves her tail to one or both sides, partly with an upward motion. The behaviour ends when the tail root is not moved for 2 seconds.
Elimination	The heifer urinates or defecates.
Alert	The heifer holds her head up (above the withers) with both ears directed to the front while not walking.
Avoiding human	The heifer moves out of reach of the person in reaction to a movement of the human.
Moving away from human	The heifer moves out of reach of the person, without a preceding movement of the person.
Exploring wall´s height	The heifer moves her head upward and close to the arena wall and shifts her weight to the hind legs. The forelegs may be lifted from the ground.
Rumination	The heifer moves her jaw regularly with approximately one movement per second. The behaviour begins when a sequence of at least 5 movements occurs. It ends when there are no jaw movements for 10 seconds.
Vocalization[1]	The heifer emits a sound.

[1] ***Vocalization*** was recorded directly.

Table 2: Ethogram of behaviours recorded during the treatment, adapted from Lürzel et al. (2020) and (Lange et al., in prep to be submitted). The behaviours were recorded as durations, except for *elimination*, *start of rumination*, *stop of rumination, pull back* and *vocalization*, which were recorded as frequencies.

Behaviour	**Definition**
Elimination	The heifer urinates or defecates.
Tail flicking	The heifer moves her tail to one or both sides, partly with an upward motion. The behaviour ends when there is no active movement of the tail for 2 seconds.
Rumination	The heifer moves her jaw regularly with approximately one movement per second. The behaviour begins when a sequence of at least 5 movements occurs. It ends when there are no jaw movements for 10 seconds.
Start of Rumination	The heifer moves her jaw regularly with approximately one movement per second. The behaviour starts with the first jaw movement shown. When the heifer already ruminates when the video starts, *start of rumination* is not recorded.
Stop of Rumination	The heifer stops moving her jaw regularly with approximately one movement per second. *Stop of rumination* is recorded when the jaw movements stop but only if there are no further jaw movements in the next 10 seconds.
Neck stretching	Positioning the neck and head actively in an outstretched line. *Neck stretching* is not recorded if the position is attained in the context of feeding, exploration rubbing or licking. If *neck stretching* is shown already before rubbing or licking, it will be recorded as long as the neck is in an outstretched line.
Movement	The heifer moves one or more legs or is shifting her weight while restrained in the feeding rack. A movement of the trunk or the dorsal line to the front, to the back or to the side is visible. If the trunk or dorsal line are not visible, but the distance between the shoulders of the animal and the feeding rack changes noticeably, the behaviour is also coded as *movement*. The behaviour stops when no other movement as described above is shown for 2 s.
Look away from experimenter	The heifer directs her head away from the experimenter (also recorded for treatment C – away from usual position of experimenter), deviating more than 30° from a neutral head position (head in line with spine*). Look away from the experimenter* is not recorded if the heifer is feeding or licking the floor.
Look at experimenter	The heifer directs her head towards the experimenter (also recorded for treatment C – towards usual position of experimenter), deviating more than 30° from a neutral head position (head in line with spine*).*

	Look at the experimenter is not recorded if the heifer is feeding or licking the floor.
Pull back	The heifer moves quickly backwards in the feeding rack until her head cannot move back any further.
Stand back	The heifer stands as far back as possible in feeding rack until the head cannot move back any further.
Vocalization	The heifer emits a sound.
Rubbing	The heifer rubs a part of her body on feeding rack.
Head down	The heifer holds her head lower than 30° from the horizontal head-neckline for at least 2 s. *Head down* is not recorded when the heifer is licking the floor, feeding, rubbing or stretching her neck.

Therefore, the GLMM to test our hypothesis was calculated only with the data collected by the experimenter. The full model included treatment, test number, herd and all possible interactions between them as well as origin as fixed factors. The interaction between test number and treatment was the effect of interest, as we wanted to investigate specifically if heifers of different treatments differ in their avoidance distance after receiving the treatment (test 2) and if that expected difference lasts beyond the treatment period (test 3). The main effects of treatment and test number were not relevant for our hypothesis, and a main effect of treatment was not to be expected, as the allocation to the treatments was balanced for the avoidance distance measured in the first test. Neither did we expect a change in avoidance distance in the control treatment, which would have been a prerequisite for a main effect of test number; however, after viewing the data, we found indications of a general calming effect of the experimental procedures. In order to quantify that effect, we reduced the model by removing also all two-way interactions, obtaining a result for the main effect of test number, which is, however, subject to the effects of multiple testing, as it was not included in the full-null model comparison. Multiple testing is leading to a higher false-positive rate and the results should be treated cautiously. Herd was included in the interaction because the heifers of the two herds could react differently in the avoidance distance tests and to the treatments, as one herd was younger than the other and the two herds were treated and tested by different persons. In the null model, the interaction between test number and treatment and thus also the three-way interaction of test number, treatment and herd were excluded.

The durations of *exploration*, *exploring human*, *tail flicking*, *self-grooming* and *alert* were analysed with GLMMs based on the beta distribution using the package "glmmTB" (Brooks et al., 2017). To that purpose, proportions were calculated by dividing durations by the total time the behaviours could be observed. Additionally, the proportions were transformed (y * (n- 1) + 0.5) / n; y = proportions of durations, n = number of observations) in order to make them suitable for the models (avoiding zeros and ones). The full GLMMs included treatment, phase (isolation I/human presence/isolation II), herd and all possible interactions between them as well as origin as a fixed effect. Herd was included as fixed factor in the interaction because the heifers of the two herds, also when in the same treatment, could react differently in the different phases of the isolation test. Because we were interested in the interaction of treatment and phase, it was excluded in the null model, leading to the exclusion of the higher-level interaction. We did not test the main effects of phase and treatment because we were not primarily interested in the general social support a human can provide, and neither for general differences between heifers of different treatments in their reactivity over the different phases of the test. We were specifically interested in the effect of the treatment on the human's capability to provide social support, i.e. in how the heifers of different treatments reacted during the phase with human presence. If the full/null model comparison revealed a significant difference between the models, but the three-way interaction was not significant, a reduced model without the three-way interaction was calculated. The main effects of treatment and phase were not relevant for our hypothesis, but after viewing the data, we found indications for a general social support a human can provide. In order to quantify that effect, we reduced the model by removing also all two-way interactions, obtaining a result for the main effect of phase, which is, however, subject to the effects of multiple testing, as it was not included in the full-null model comparison.

Exploring human was the only behaviour recorded as a duration not analysed with a GLMM but with a beta regression model (package "betareg", Cribari-Neto & Zeileis, 2010). *Exploring human* could only occur in the human presence phase, therefore only data from this phase were analysed and phase was not considered as a fixed factor; all other factors were the same as in the GLMMs. Treatment was excluded in the null model, leading to the exclusion of the higher-level interaction (treatment * herd). Here, we were interested in the main effect of treatment because it allows inferences about possible differences in behaviour between the treatments in the human phase.

Distance to human square was calculated by using the distances from the square centres to the position of the person. The distances were calculated from the x and y coordinates of the nine squares we used in video coding for describing the location of the heifers (distance = sqrt ($x^2 + y^2$); distance: distance from square centre to position of experimenter, x: distance of the x-coordinate to position of the experimenter, y: distance of the y-coordinate to position of experimenter). For the distances weighted means were calculated, that included the recorded durations. The distances were weighted according to the time the animals spent in each distance (square). The calculated weighted means were used as dependent variable for analysis with a linear mixed model using the function lmer from the package "lme4" (Bates et al., 2015). The full model included treatment, phase, herd and all possible interactions between them, as well as origin as fixed effects. The interaction of treatment and phase was excluded in the null model like in the GLMMs above except *explore human*, leading to the exclusion of the higher-level interaction.

The frequencies of *elimination*, *avoiding human* and *vocalization* could not be analysed statistically because the data contained too many zeros. Only the frequency of *changes of squares* could be analysed using a GLMM based on the Poisson distribution with the function glmer from the package "lme4" (Bates et al., 2015). The full model included treatment, phase, herd and all possible interactions between them as well as origin as fixed effects. the interaction of treatment and phase excluded in the null model, leading to the exclusion of the higher-level interactions, for the same reasons as in the models of the behaviours recorded as durations.

Descriptive analysis was performed using the R packages "ggplot2" (Wickham, 2016) and "cowplot" (Wilke, 2019). The graphical depiction included both data sets of the avoidance distance and all behaviours in the isolation test, including the frequencies that could not be analysed statistically. Tukey boxplots were used to show the data graphically. The upper and lower limits of the box depict Tukey's hinges, which approximate the 25th and 75th percentiles. The whiskers depict the most extreme values that are still within Tukey's fences. The upper fence is 1.5 times the box length added to the upper hinge, and the lower fence is 1.5 times the box length subtracted from the lower hinge. Data outside of the fences are defined as outliers and depicted as points.

3. Results

3.1 Behaviour during the treatment

The behavioural data recorded during the treatment was only analysed descriptively, thus, numeric differences are described in the following. We did not analyse the data statistically because we focused on our hypothesis that were aiming at the differences in the AHR between the treatments after the treatment in terms of avoidance distances and behaviour towards the human during the isolation test. Still, the data of the behaviour during the treatment is relevant in order to evaluate if the treatment was perceived as positive by the heifers and therefore could improve the AHR of the heifers. *Neck stretching* was performed only by two to five animals in each treatment (T: 5, S: 4, ST: 4, P: 2) and did not occur in the control treatment (Fig. 4b). The behaviour occurred 15 times in total in 14 different heifers. Half of these heifers had an avoidance distance of < 0.5 m in test 1, while the other half had avoidance distances between 0.9 to 2.9 m in test 1. The longest duration of neck stretching occurred in treatment S in a heifer that could be touched but not stroked in avoidance distance test 1 (Fig. 4b). Most of the *neck stretching* occurred on day 8 and 15 (d1: 3 times; d8: 6 times, d15: 6 times). The duration of *movement* (Fig. 4a) increased from day 1 to day 8 and decreased from day 8 to day 15 in all treatments. The duration of *looking at experimenter* (Fig. 4c) was the highest in T on day 1 and 8. In P, C and T the duration decreased from day 1 to 15, in treatments S and ST the duration increased from day 1 to day 8 and decreased from day 8 to day 15. *Looking away from experimenter* (Fig. 4d) mostly occurred in treatment C (looking away from theoretical position of the experimenter) and rarely and without an obvious pattern in the others. The duration of *looking at experimenter* was the highest in treatment T on day 1 and 8 and decreased from day 1 to 15 (C, P, T) or from day 8 to 15 (S, ST). The durations of *head down* (Fig. 5a) and *tail flicking* (Fig. 5b) decreased from day 1 to 15 of the treatment in most treatments. For *head down* in P and *tail flicking* in ST, the durations decreased from day 1 to 8 but increased again slightly from day 8 to 15. The shortest durations of *head down* occurred in C. *Standing back* (Fig. 5c) mostly occurred in the treatments experiencing tactile contact on day 1 of the treatment, decreasing to a very low occurrence already on day 8. On days 8 and 15, *standing back* occurred at very low levels in all treatments. Similarly, *pulling back* (Fig. 5d) occurred mostly in S and ST on day 1.

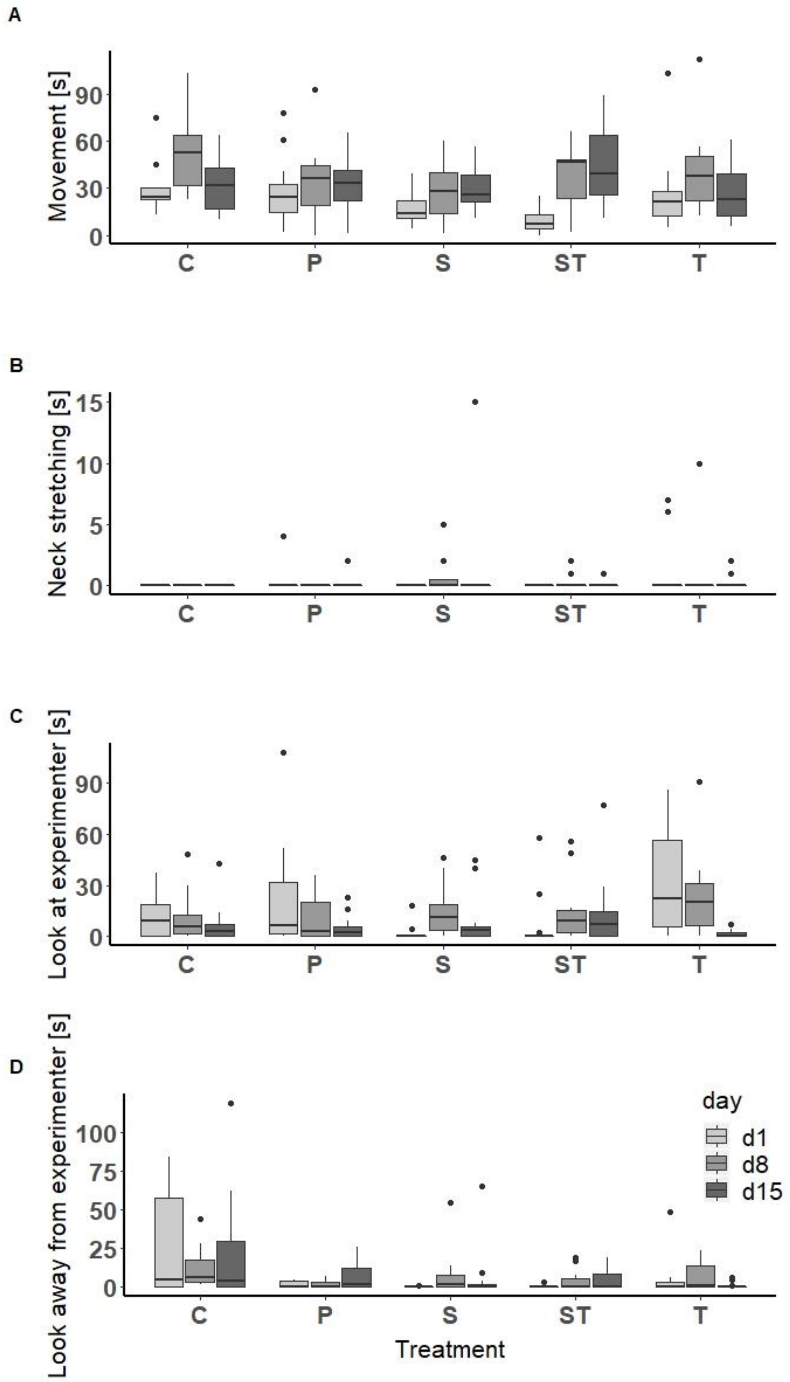

Figure 4: Durations [s] of *movement* (A), *neck stretching* (B) and *looking at the experimenter* (C) and *looking away from the experimenter* (D) in heifers (n = 59) belonging to five different treatments: C – control (n = 11), P – human presence (n = 12), S – stroking (n =12), ST – stroking and talking in a gentle voice (n = 12; day 8 n = 11), T – talking in a gentle voice (n = 12). The behaviours were recorded during the treatment.

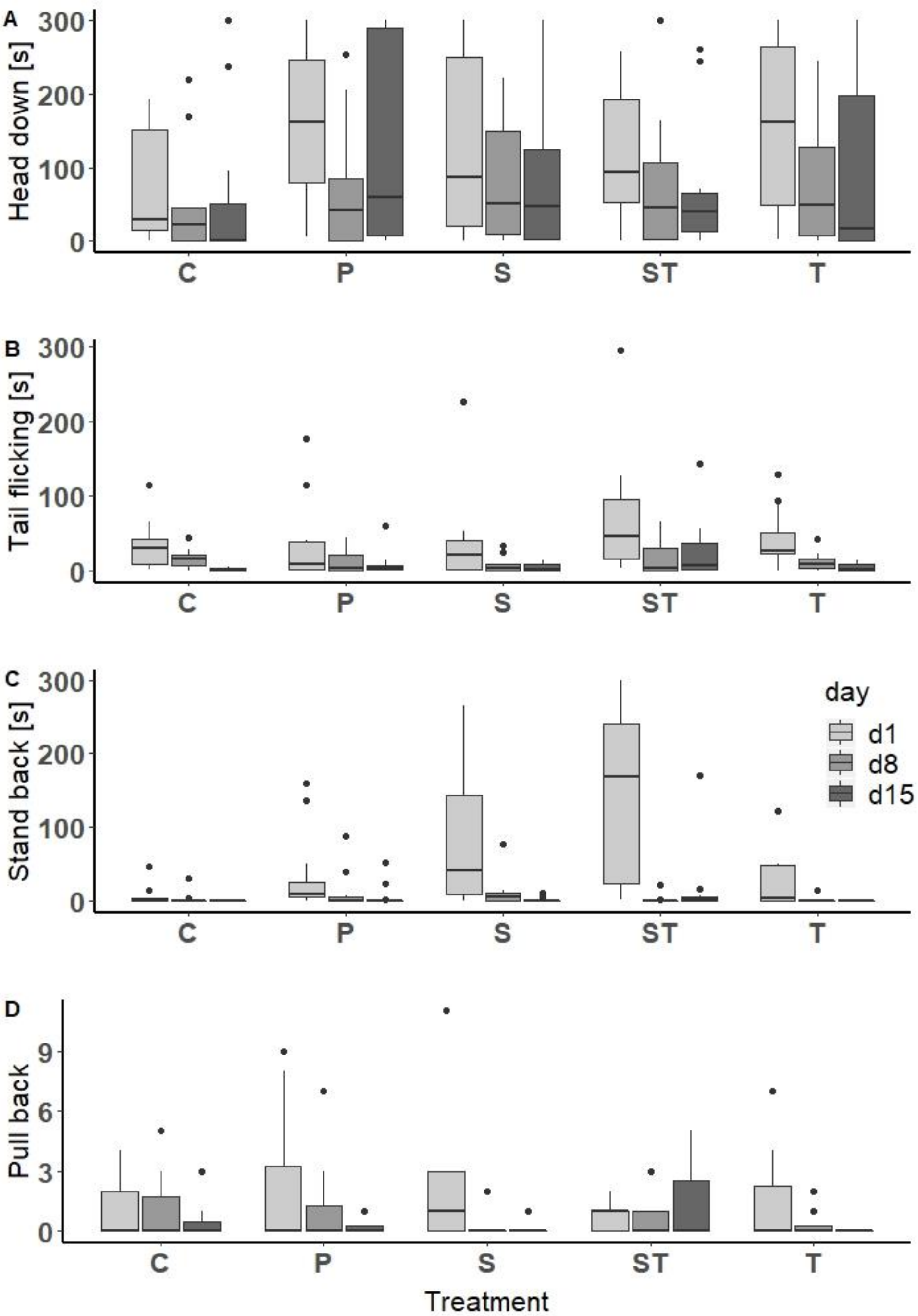

Figure 5: Durations [s] of *head down* (A), *tail flicking* (B) and *standing back* (C) and frequency of *pulling back* (D) in heifers (n = 59) belonging to five different treatments : C – control (n = 11), P – human presence (n = 12), S – stroking (n = 12), ST – stroking and talking in a gentle voice (n = 12; day 8 n = 11), T – talking in a gentle voice (n = 12). The behaviours were recorded during the treatment.

3.2 Avoidance distance test

The full/null model comparison showed that the interaction of familiarity with treatment, test number and herd did not make a significant difference (GLMM: $\chi^2 = 11.22$, df = 29, p = 1.00); thus, only the data of interest – the avoidance distances assessed by the experimenter – were analysed in detail. Across treatments, the avoidance distances decreased from test 1 to test 2 and increased again from test 2 to test 3 but stayed below the level of test 1 (Fig. 6). In test 2 the avoidance distances were lowest in the ST treatment. The T treatment had slightly higher avoidance distances than ST but lower ones than the S treatment. The avoidance distances of the C and P treatment were higher than those of the other treatments. However, the comparison between full and null model revealed no significant difference ($\chi^2 = 3.22$, df = 16, p = 1.00; Annex 2, Tab. A1). The reduced model revealed a significant effect of test number ($\chi^2 = 11.95$, df = 2, p = 0.003, Tab. A2) and origin ($\chi^2 = 9.536$, df = 1, p = 0.002; Tab. A2).

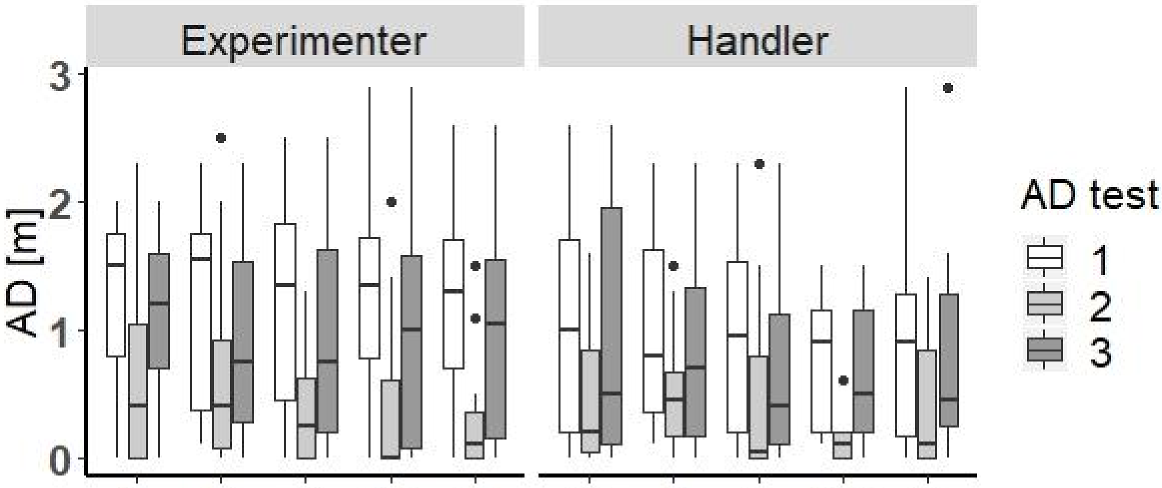

Figure 6: Avoidance distance (AD) [m] of heifers (n = 58) belonging to five different treatments: C – control, P – human presence, S – stroking, ST – stroking and talking in a gentle voice, T – talking in a gentle voice. The heifers were tested in three AD tests: 1 – before treatment, 2 – directly after treatment, 3 – two weeks after treatment, by the person that had treated them (experimenter) and a person that only helped with handling and sorting (handler). Statistics: Generalized Linear Mixed Models: comparison between models with and without four-way interaction including familiarity, p = 1.00; statistics for the avoidance distance towards the experimenter: interaction test number * treatment * herd: p = 1.00; main effect of test number, p = 0.003.

3.3 Behaviour in the isolation test

The full/null model comparisons for *alert*, *self-grooming, tail flicking* and *changes of squares* showed significant differences between models (GLMM; Tab. 3, Fig. 8). For *exploration,* the full/null model comparison showed a trend (Tab. 3, Fig. 9a). For *distance to human square* and *exploring human,* the full/null model comparison did not reveal a significant difference (*distance to human square*: Tab. 3, Fig. 9b; *exploring human*: Tab. 3, Fig. 7b).

The interaction between phase, treatment and herd was significant for *alert*, *self-grooming*, *tail flicking* and *changes of squares* (GLMM; Tab. 3, Fig. 8; Annex 3, Tab. A3, A6, A7, A9).

Table 3: P-values of the full/null model comparison, the three-way interaction phase * treatment * herd and the main effect of phase. Significant results appear in bold.

behaviour	full/null model			phase*treatment*herd			phase		
	χ^2	df	p	χ^2	df	p	χ^2	df	p
alert	28.81	16	**0.03**	22.35	8	**0.004**	-	-	-
self-grooming	27.67	16	**0.03**	25.97	8	**0.001**	-	-	-
tail flicking	37.00	16	**0.002**	27.64	8	**0.001**	-	-	-
changes of squares	83.59	16	**< 0.001**	36.97	8	**< 0.001**	-	-	-
exploration	25.32	16	0.06	12.58	8	0.13[2]	34.96	2	**< 0.001**[3]
distance to human square	16.71	16	0.40	-	-	-	-	-	-
exploring human	3.59	8	0.89	-	-	-	-	-	-

Alert (Fig. 8a) increased from phase 1 to phase 3 in most treatments. Self-grooming occurred mostly in treatment C in both herds (Fig. 8b). The frequency of *changes of squares* (Fig. 8d) decreased from phase 1 to phase 2 in all treatments. From phase 2 to phase 3 it increased again, so that the lowest frequency of *changes of squares* occurred in phase 2 in all treatments (Fig. 8d).

For *exploration* the full/null model comparison showed a trend, but the three-way interaction was not significant in the full model (GLMM; Tab. 3, Fig. 9a; Tab. A4). The reduced model for *exploration* without the three-way interaction showed no significant effect of the interaction of interest (Tab. 3; Tab. A5). The reduced model without the two-way interactions showed a

[2] Phase*treatment: $\chi^2 = 12.74$, df = 8, p = 0.12

[3] Treatment: $\chi^2 = 2.53$, df = 4, p = 0.64

significant main effect of phase (Tab. 3, Fig. 9a; Tab. A5), but no significant main effect of treatment (Tab. 3; Tab. A5). Heifers of most treatments in both herds showed a decrease from phase 1 to phase 2 and an increase from phase 2 to phase 3, with the median remaining below the one of phase 1 (Fig. 9a).

For *distance to the human square and exploring human* the full/null model comparison was not significant (Tab. 3). The *distance to human square* decreased from phase 1 to phase 2 and from phase 2 to phase 3 for all treatments (Fig. 9b).

Due to a high proportion of zeros (> 0.75) the data of *vocalization, elimination* and *avoiding human* was not analysed statistically. All differences described here are thus merely numeric differences. *Vocalization* seemed to occur mostly in treatment C. The median was highest in treatment C and phase 3. No animal of any treatment vocalized in phase 2 (Fig. 9c).

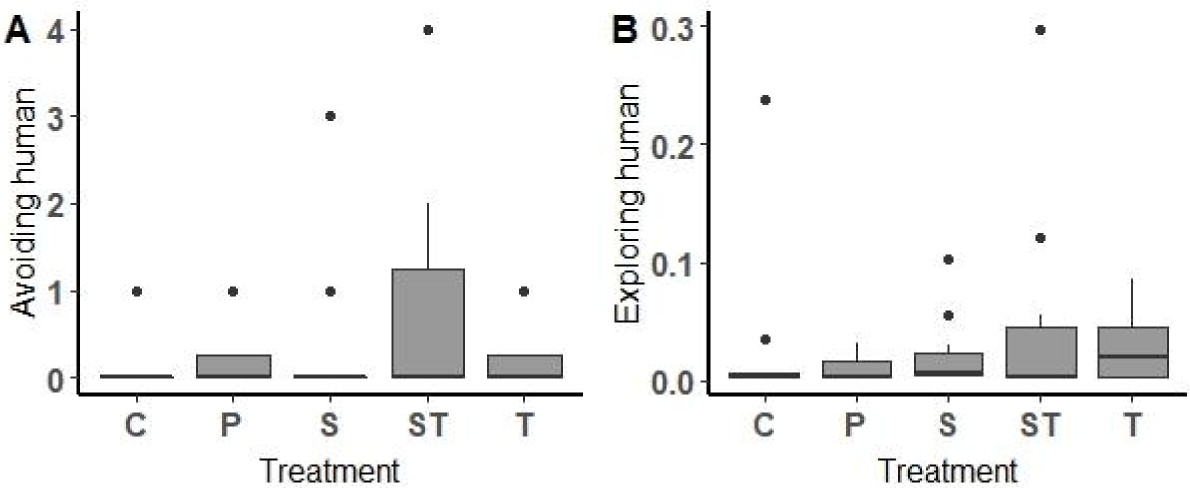

Figure 7: Frequency of *avoiding human* (A) and proportion of *exploring human* (B) in heifers (n = 58) belonging to five different treatments : C – control (n = 11), P – human presence (n = 12), S – stroking (n = 11), ST – stroking and talking in a gentle voice (n = 12) and T – talking in a gentle voice (n = 12). The behaviours were coded in phase 2 of an isolation test containing three phases: 1 – before experimenter enters, 2 – while the experimenter is there, 3 – after the experimenter left. Statistics: *Exploring human*: Generalized Linear Model based on the beta distribution: full/null model comparison p = 0.89. A*voiding human* was not analysed statistically.

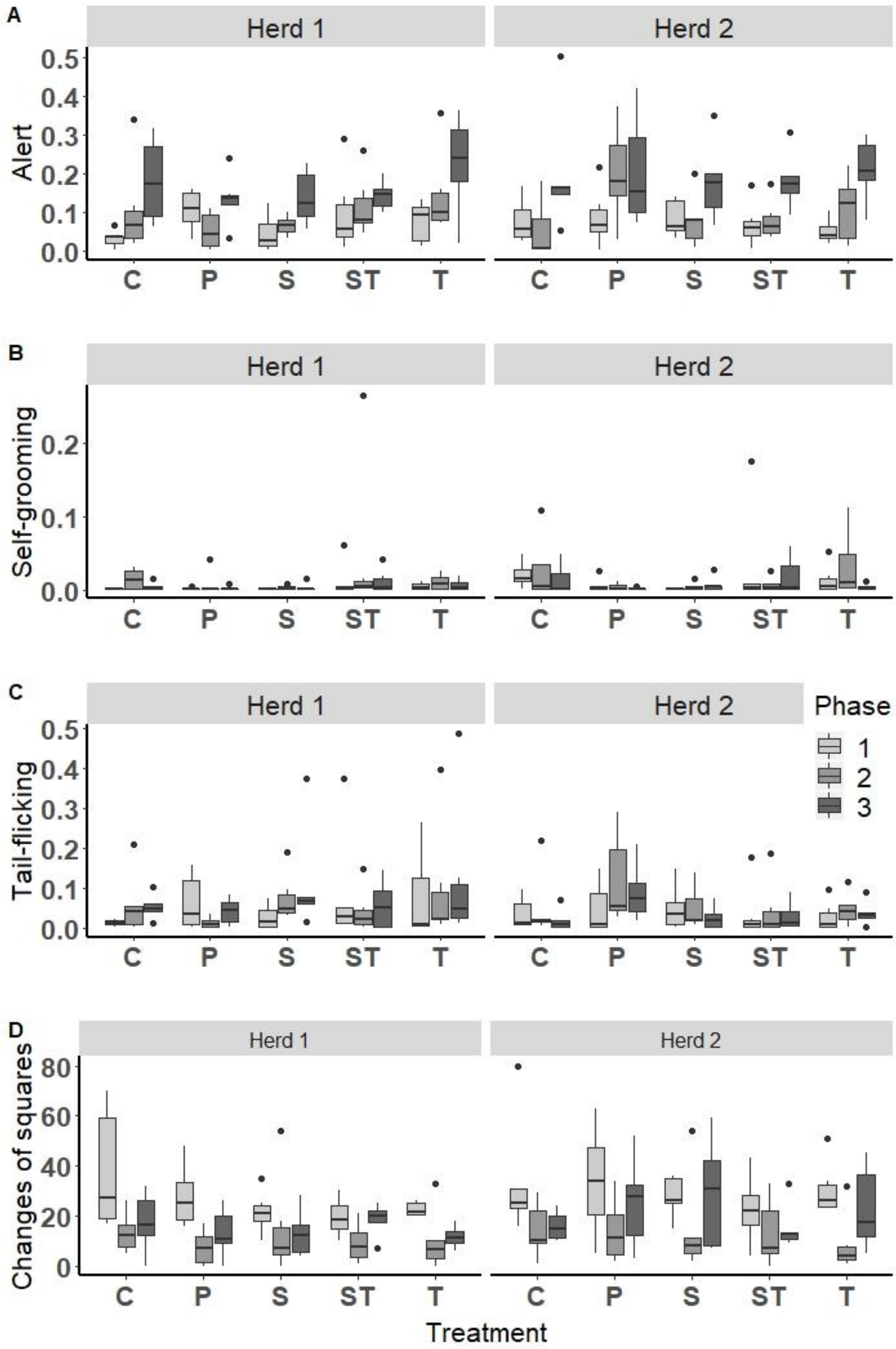

Figure 8: Proportions of *alert* (A), *self-grooming* (B) and *tail flicking* (C) and frequency of *changes of squares* (D) in heifers (n = 58) belonging to five different treatments: C – control (herd 1: n = 6; herd 2: n = 5), P – human presence (herd 1: n = 6; herd 2: n = 6), S – stroking (herd 1: n = 6; herd 2: n = 5), ST – stroking and talking in a gentle voice (herd 1: n = 6; herd 2: n = 6), T – talking in a gentle voice (herd 1: n = 6; herd 2: n = 6). The behaviours were recorded in an isolation test comprising three phases: 1 – before the experimenter entered, 2 – while experimenter was present, 3 – after the experimenter left. Statistics: Generalized Linear Mixed Models: interaction test number * treatment * herd: *alert* p = 0.004; *self-grooming* p = 0.001; *tail flicking* p = 0.001; *changes of squares* p < 0.001.

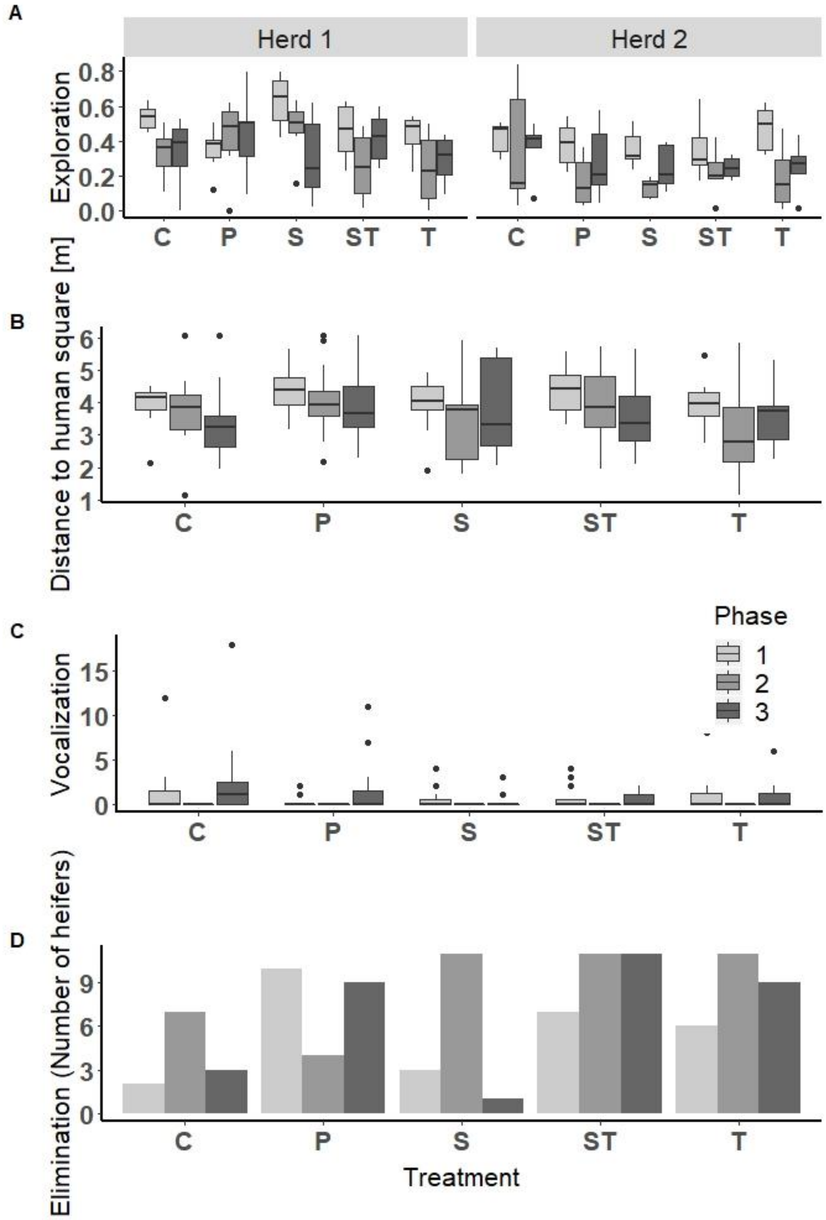

Figure 9: Duration of *exploration* (A) and the weighted duration of *distance to the human square* (B), *vocalization* (C) and *elimination* (D) in heifers (n = 58) belonging to five different treatments: C – control (herd 1: n = 6; herd 2: n = 5), P – human presence (herd 1: n = 6; herd 2: n = 6), S – stroking (herd 1: n = 6; herd 2: n = 5), ST – stroking and talking in a gentle voice (herd 1: n = 6; herd 2: n = 6) and T – talking in a gentle voice (herd 1: n = 6; herd 2: n = 5). The behaviours were coded in an isolation test containing three phases: 1 – before experimenter enters, 2 – while the experimenter is there, 3 – after the experimenter left. Statistics: *Exploration*: Generalized Linear Mixed Model: full/null model comparison p = 0.06, interaction phase * treatment * herd p = 0.13, interaction phase * treatment p = 0.12, main effect of phase p < 0.001; *distance to human square*: Linear Mixed Model: full/null model comparison p = 0.40. *Vocalization* and *elimination* were not analysed statistically.

4. Discussion

4.1 Behaviour during the treatment

We did not perform any statistical analysis on the behavioural data of the treatment period, because they are not directly relevant for our hypotheses regarding the improvement of the AHR. Our focus was on the effectiveness of different treatment types in reducing the heifers' avoidance distance and allowing the human to provide social support in an isolation test. Still, the behavioural data of the treatment period gives an indication about the heifers´ perception of the treatment. This perception is important for the interpretation of the data of the isolation test and the avoidance distance test, which is relevant for our hypotheses. The AHR is the deciding factor for the effect in the isolation test, but the perception of the treatment affects the AHR and therefore the amount of support the human can provide during the isolation test. The perception of the different treatments is crucial for interpreting differences in the behaviour during isolation or in avoidance distances between the treatments. which is the core of our study.

Neck stretching occurred only rarely during the treatment and the duration of the behaviour did not increase from day 1 to 15, but it was more frequent on days 8 and 15 compared to day 1. The behaviour is interpreted as a sign of positive perception during allogrooming or being stroked by a human (Schmied et al., 2008b; Schmied et al., 2005). We thus expected an increased occurrence of *neck stretching* over the course of the treatment period for the treatments S and ST due to an improvement of the heifers´ perception of the treatment. A positive perception of an interaction with the human leads also to an improvement of the AHR through the treatment. This finding indicates that neither the S treatment nor the ST treatment, which were predicted to be the most effective, were perceived as positive by most heifers. Still, *neck stretching* did occur for the longest duration by a heifer of treatment S. On the other hand, this heifer could be touched (but not stroked) before the treatment. Our findings do show some animals in treatments S an ST on day 8 and 15, partly with low and partly with high avoidance distances in test 1, that did show neck stretching. This might indicate that for some animals the treatment was perceived as positive over the treatment period. The fact, that it also occurred in the P and T treatment was unexpected, because neck stretching is associated with the context of stroking in interactions with humans (Schmied et al., 2008b). An explanation for this finding would be solicitation for the human to stroke them. In other studies that investigated the effect of gentle interactions on the AHR, *neck stretching* occurred for longer durations and in more animals as in our study (Lange et al., 2020; Lürzel et al., 2016; Schmied et al., 2008b).

Standing back and *pulling back*, which are probably indicators of a motivation to avoid the situation, were shown mostly by heifers of the S and ST treatment and suggest that especially human touch was perceived as negative on day 1. The high occurrence of *standing back* and *pulling back* on day 1 in contrast to a very low occurrence on the other days probably indicates that the treatment reduced the fear of humans to some extent, improving the perception of the treatment. The heifers learned that they have nothing negative or painful to expect from the treatment. Since most of our heifers were very shy (avoidance distances up to 2.9 m in test 1), it is possible that the increased *movement* later in the treatment period indicates also a moderate reduction of fear reactions from the human resulting from the treatment. Both, a high amount of fear and the absence of fear might be a cause for an inhibition of movement. According to Bremner (1997), very shy and very tame animals moved less during a treatment involving physical human contact in the milking parlour. Animals experiencing a high amount of fear might freeze, which is a possible reaction to an acute stressor and leads to an inhibition of movement (Løvlie, 2017). Animals with a moderate fear of humans might therefore move more, which would explain the increase in movement later in our treatment period.

The decrease of the duration of the behaviours *head down* and *tail flicking* from day 1 to 15 indicates that all treatments might have been perceived as negative at first, but less negative on day 8 and 15. *Head down* is interpreted as a sign of stress (Hemsworth et al., 2011) or submissive behaviour (Wagner et al., 2012), *tail flicking* as a sign of restlessness (Sylvester et al., 2004). In the control treatment, the occurrence of *head down* was lower than in the other treatments, but also decreased over the course of the treatment period. This might be due to habituation to restraint.

The decrease of the duration of *looking at the experimenter* in all treatments might indicate a habituation effect. However, the pattern was the same for treatment C, where no human was present. The duration of the behaviour was highest in T on day 1 and 8, which might suggest a higher interest in a human with a gentle voice. Still, the durations were lower in the ST treatment, but this might be due to the heifer focusing more on the stroking and human touch.

The animals of treatment C showed more of *looking away from experimenter* (looking away from the position of the experimenter) than heifers of all the other treatments. Animals that received a treatment seemed likely to not look away from the human, which can be interpreted as a sign of interest in the human.

Possibly, the treatment phase was not long enough for the heifers for their perception to change from negative to positive; alternatively, the treatment might have been not pleasant enough. Comparing with the literature, it seems unlikely that the total treatment duration of 75 min per animal was not long enough, as it is in line with other studies reporting an effective treatment (51 min in loose-housed dairy cows (Windschnurer et al., 2009a), 75 min in tethered dairy cows (Schmied et al., 2008a), 42 min in loose-housed heifers (Lürzel et al. 2016)). However, the dairy cows were used to human contact, e.g. around milking (Windschnurer et al., 2009a; Schmied et al., 2008a), in contrast to our heifers. It is possible that the duration of the treatment was still not sufficient for our heifers to improve the AHR effectively due to the limited human contact and resulting poor quality of the AHR compared to other studies.

Lürzel et al. (2018) did not find a difference in *neck stretching* behaviour during gentle treatment of dairy cows in the milking parlour between treatment and control group but they did find a significant difference in avoidance distance between the groups. Like our heifers, the dairy cows were not used to stroking. The duration of the treatment was 60 min in total, comparable to the studies mentioned above and our own study. It seems possible that even when the treatment is not pleasant enough for the animals to show *neck stretching*, it might still reduce fear of humans and improve the AHR. Human presence alone can still habituate animals to the human and improve the AHR (Waiblinger, 2017). This is also in line with our findings for treatment P – decrease of stress-related behaviours over the treatment period and decreased avoidance distances after the treatment.

The behavioural analysis of the treatment indicates that the treatment might not have been pleasant enough – indicated by the low occurrence of neck stretching and high level of fear and avoidance reactions to humans – to the animals to improve the AHR to a level where the human is perceived as positive. Still, the treatment seems to have habituated the heifers to the human, improving the AHR by reduction of fear.

4.2 Avoidance distance

The findings of the avoidance distance test were not in line with our predictions that heifers that experienced a treatment including gentle interactions have lower avoidance distances than control animals, and that heifers that experienced tactile gentle interactions have lower avoidance distances than animals that experienced only vocal gentle interactions. There was no significant effect of the interaction of test number and treatment. Still, in test 2 the heifers that received treatment including gentle interactions had numerically lower avoidance distances than the control heifers (treatments C and P), with the ST treatment having a median of zero. This numeric difference is in line of our hypothesis and several previous studies that showed (e.g. Lürzel et al., 2016; Schmied et al., 2008a) that gentle interactions are more effective in decreasing avoidance distances than human presence and the handling necessary for conducting our experiment.

After viewing the data, we found that the avoidance distance was reduced in test 2 in all treatments, pointing towards a main effect of test number. We reduced the model to quantify this decrease, finding a significant effect of test number; the result must, however, be interpreted with caution, because it was not included in the full-null model comparison and therefore may be influenced by multiple testing. The result indicates a reduced fear of humans due to the experimental procedures. Habituation to the test procedure might be an explanation (Waiblinger et al., 2006), but it seems unlikely that the effect of test number in test 2 should be due to habituation to the test procedure, because we did a pre-test to avoid the decrease in avoidance distances from the first to the second test seen in control animals in other studies (Lürzel et al., 2015b; Windschnurer et al., 2009a). During the treatment period, all animals were often exposed to the experimenter's presence and the experimenters had frequent contact with the animals while handling them and sorting them into their treatments. It seems more likely that the habituation of all animals to the experimenters altered the AHR, the animal's perception of the experimenters becoming less negative. We cannot conclude that the perception became more positive because the behavioural data from the treatment period does not support it – for example a very low occurrence of neck stretching. Even though only the data set including the tests with the experimenter was tested statistically, the data of the test with the handler showed the same pattern of decreased avoidance distances in test 2. This suggests that calm handling without stroking can improve the AHR to a certain degree.

Since there was no statistical effect of the interaction between test number and treatment, there was no evidence for a medium-term effect of the treatment. Because we did not find a significant short-term effect of the treatment in the avoidance distances, the absence of a medium-term effect is not surprising. Even if the treatment had some minimal effect on the improvement of the AHR, which might be conceivable on the basis of the numeric differences, it might still not be sufficient for a medium-term effect. A medium-term effect of a treatment including gentle interactions was reported by (Lürzel et al., 2016; Schmied et al., 2008a). They still found significantly lower avoidance distances five to eight weeks after the stroking treatment.

The fact that the animals were restrained could have affected the perception of the treatment by the animal and therefore be one reason of the limited effectiveness that our treatment had on the AHR. Performing the treatment on unrestrained animals (as in Bertenshaw and Rowlinson, 2008; Lürzel et al., 2016) might be a better approach to improve the pleasantness of the treatment, because the animals have a higher degree of control of the situation (as discussed in Lürzel et al., 2016; Windschnurer et al., 2009a). This might be especially important for animals that are fearful of humans, like the heifers in our study (AD up to 2.9 m in test 1). For these animals, close human contact is a negative stimulus and possibly stressful (Waiblinger et al., 2006; Lürzel et al., 2019). Lange et al. (2019) found no difference in the behaviour during the treatment between heifers that were restrained or unrestrained during a treatment including stroking and talking in a gentle voice in a similar study. In contrast to the animals in our study, the heifers were already familiar with being stroked from previous studies. Lürzel et al. (2019) investigated the difference between restrained and unrestrained dairy cows that received the same treatment including gentle interaction. They selected animals for the study that had an avoidance distance of at least 0.3 m. There were some indications that the unrestrained treatment was more effective in decreasing the avoidance distance, but both treatments were successful after 90 min of stroking in total. The AHR of our heifers was more similar to the dairy cows of Lürzel et al. (2019) than to the heifers of Lange et al. (2019). It is possible that the AHR in our study was of even lower quality in some animals, suggested by the high avoidance distances in test 1, probably caused by the reduced possibilities for human contact in our heifers compared to dairy cows. Therefore the restraint, possibly perceived as a lack of control over the situation, might be more relevant for shy animals like our heifers with regard to the positive perception of the treatment. Controllability is a major influencing factor for the perception of a potential stressful stimulus (Koolhaas et al., 2011). It is possible our treatment would have been more effective if the animals had been unrestrained.

Additionally, the younger herd of heifers were introduced to the feeding rack and restraint just before the study was conducted. Even though we habituated them to be restrained in the feeding rack, it might have been insufficient for some animals. Still, restraint does not necessarily reduce the effectiveness of a treatment including gentle interactions in heifers or cows (Lange et al., 2020b; Schmied et al., 2008a). However, Schmied et al. (2008a) performed their study on tethered dairy cows, which were accustomed to restraint in contrast to the heifers in our study. It seems possible that habituation to restraint plays a role in the animals' perception of the treatment, most likely due to a lack of control by the animals (Windschnurer et al., 2009a; Lürzel et al., 2018).

Additionally it might be possible that the two experimenters were very much focused on applying the treatment correctly and not paying enough attention to the animal in the sense of aiming to provide a pleasant experience to the animal. As a consequence, the animal's perception of the treatment might have been not as positive as expected.

4.3 Behaviour in the isolation test

The results of the isolation test were partly in line with our predictions that the treatment affects the stress reactions shown in the isolation test during human presence to a different degree. There was a significant interaction between phase, treatment and herd on *changes of squares*. The fact that the number of *changes of squares* in phase 2 was the highest in treatment C in herd 1 and in treatments C and P in herd 2 was in line with our prediction. Contrary to our prediction, the lowest number of *changes of squares* in herd 2 and phase 2 was in treatment T, not in the ST treatment as expected. The lower frequency of *changes of squares* in the treatments including gentle interactions compared to the control might indicate that the animals were less stressed in phase 2 due to an improved AHR. The fact that *vocalizations* never occurred in the phase with human presence, but in the other phases in every treatment, supports the findings for *changes of squares*. The presence of a human might be still preferable for some animals than being completely isolated even though they do not search proximity or have a particularly good AHR. Because of the low occurrence of *vocalizations*, no statistical analysis was possible and therefore we cannot draw any conclusions from this finding. Still, *vocalizations* appeared to a higher extent in unpleasant situations like isolation or during emotional states like fear or pain (Watts and Stookey, 2000; 1999). In isolation, they are seen as a distress reaction or an attempt to communicate with conspecifics (Watts and Stookey, 2000). In our study, the animals were only visually isolated and able to hear herd mates or other cattle vocalize. Therefore, some calls were responding calls to vocalizing herd mates. These are also due to isolation but not necessarily self-motivated. Our results are in line with findings of other studies. Farm animals tend to vocalize less in social isolation when a human is there (Rushen et al., 1998 cited in Watts and Stookey, 2000; Rault et al., 2011). Therefore, it seems likely that the human was able to provide some social support in the isolation test. Another explanation for a lower frequency of *changes of squares* in human presence could be that the heifers moved less because they were watching the human. In this case, the lower frequency of *changes of squares* in phase 2 would not indicate that the animals were less stressed but that they were vigilant towards or curious of the human. The median for *exploration* in treatment T was with 20% of total observation time also much lower in phase 2 than in phase 1 (48%) and phase 3 (30%). The same pattern – lowest occurrence of *exploration* in phase 2 – was visible for most treatments, except P and S of herd 1, although the differences were not significant. The lower occurrence of *exploration* in phase 2 could also contribute to the lower occurrence of *changes of squares* possibly due to focusing on the human. Additionally, there was a significant increase for *alert* in most treatments from phase 1 to phase 3, with a steeper increase from phase 2 to 3. We expected a decrease for *alert* from phase 1 to 2 due to the experimenter giving social support to the heifer, and an increase from phase 2 to 3, due to the experimenter leaving the arena. Therefore, our findings do support our prediction partly. The experimenter leaving the heifer during the isolation test seemed to increase stress reactions, which is in line with other studies (Waiblinger et al., 2006; Boivin et al., 2000). Alert behaviour or vigilance is seen as a sign of fearfulness in cattle (Welp et al., 2004).

Also, *distance to human square* did indicate that that the heifers did not spent much time in human proximity. There was no significant interaction between phase and treatment for *distance to human square,* but the value decreased from phase 1 to 3 in most treatments. Regarding only the relative changes, interest in the human and proximity seeking by the heifers could explain the smaller distances in phase 2. The decrease of *distance to human square* from phase 2 to 3 fits to the increase of *alert* from phase 2 to phase 3. After seeking proximity in phase 2 the heifers might have tried to follow or search for the experimenter after she left. However, the absolute distances were quite large (median 3.8 m in phase 2; median 3.5 m in phase 3). Most heifers seemed to avoid proximity to the human and were even closer to the human square after the human left, except for treatment T. The AHR was not improved to a point where close human presence and gentle interactions are perceived as positive by the heifers, as we expected at least in the ST treatment. Other studies found that stroked animals had a significantly lower latency to approach the experimenter (Lensink et al., 2000; Schmied et al., 2008a). Long latencies to approach, as observed in the control animals, were interpreted as a sign of fear (Lensink et al., 2000). We did not measure approach behaviour, so there we cannot compare these results directly to our study. The measures latency to approach and time spent in same area as the human, which are often used in similar studies (Waiblinger et al., 2006; Lensink et al., 2000; Lürzel et al., 2015b), would probably have been a more precise tool to assess the AHR during the isolation test. *Distance to human square* lacks accuracy, because it is calculated by using the mean distances – weighted by the durations spent there – of the entire phases. Still, it seems noticeable that our heifers spent most of the time in the arena away from the human. Lürzel et al. (2015b) found that the calves in an isolation test with temporary human presence spent almost the complete time in proximity of the human, but also stated that all calves had a good AHR from the beginning. The stroked calves of Lensink et al. (2000) spent approximately 25% of the test time interacting with the familiar person in an isolation test. However, it is also possible that the heifers in our study were not interested in staying in proximity to the human, because they did not perceive the human as positive but as neutral.

Furthermore, there was a significant effect of phase for the behaviour *exploration*. During human presence, the heifer's attention was expected to be on the experimenter rather than the arena. As expected, the duration decreased from phase 1 to phase 2 in most treatments. After the human left, the duration of *exploration* and the frequency of *changes of squares* increased again but were smaller than in phase 1. That is in line with our expectation that the animals habituate to the arena with time. Müller and Schrader (2018) also found a habituation effect with decreased duration of exploration, number of entered squares and release of saliva cortisol across repetitions of a social separation test in dairy cows.

For *tail flicking* and *self-grooming*, the three-way interaction was significant. For both behaviours, there was no pattern visible in our data, except for the highest occurrence of *self-grooming* in treatment C in both herds. *Self-grooming* might be a sign of displacement activity to reduce arousal elicited in novel or stressful situations (Boissy et al., 2007), and tail flicking is interpreted as a sign of restlessness and pain (Sylvester et al., 2004).

It has been shown before that gentle interactions can improve the AHR and give the human the ability to provide social support for cattle in stressful situations (Lensink et al. 2000; Waiblinger et al. 2004). In our study the improvement of the AHR was not as strong as expected. Therefore, talking in a gentle voice might have been preferred by the heifers, because it avoids close physical contact. The high occurrence of *standing back* and *pulling back* on day 1 of the treatment period in treatments including stroking suggests that most animals experienced more fear in the beginning of the treatment compared to the heifers that did not experience stroking. These fear reactions are possibly due to a poor AHR of most heifers in the beginning of the experiment. Also, the locomotion of heifers that experienced talking in a gentle voice was lowest in the isolation test, indicating a calming effect. Therefore, talking in a gentle voice might be a good method to start building a good AHR with animals that are fearful of humans. Our findings are not conclusive, but they might suggest that the heifers were not indifferent to talking in a gentle voice.

There are no studies published so far that support the effectiveness of talking in a gentle voice alone. Pajor et al. (2003) found no significant preference of cows for a human talking in a gentle voice over human presence without talking in a Y-maze test, but the data pointed in the direction that the animals might perceive talking in a gentle voice as positive (see 1. Introduction). Regarding on-farm studies, calm vocalizations of the stockperson were associated with a lower level of restlessness in cows during milking (Breuer et al., 2000; Waiblinger et al., 2002) Neither study presents strong evidence for a positive effect of talking in a gentle voice on the AHR, but they are an indication that further research on this topic might be worthwhile.

4.4 General discussion

Taken together, our data did not support a positive perception of the human after the treatment by the heifers. For example, occurrence of *neck stretching* during the treatment was low, the heifers remained at high distances to the human in the isolation test and showed alert postures in human presence. Still, a decrease of behaviours indicating fear of humans like *tail flicking*, submissive *head down* postures and avoidance reactions to physical contact over the treatment period as well as reduced avoidance distances after the treatment suggests some improvement of the initially very poor AHR – in terms of reducing fear or habituation to the human. In the phase with human presence of the isolation test, the heifers of all treatments moved around the least compared with the other phases, and they did not vocalize, which indicates that the human was able to provide some social support, even though the AHR was not improved to a point where close human presence is perceived as positive by the heifer. Because the stroking (and talking) treatment was not perceived as positive as expected, the differences between the treatments were too small or not as we expected them. Therefore, we could not confirm the hypothesis that different treatments alter the AHR to a different degree. Still, the numeric differences between test 1 and 2 in avoidance distance of the treatments including gentle interactions were higher compared to those of the controls, with ST showing the strongest reduction in avoidance distance after the treatment, which would be in line with our hypothesis that a combination of stroking and talking is the most effective in improving the AHR. On the other hand, close human presence, especially including stroking, led to negative reactions of the heifers at the beginning of the treatment period. Due to the poor AHR of the heifers, stroking was perceived as negative in the beginning of the treatment. Later on, the perception seemed to change to less negative, because the strong avoidance reactions during the treatment and the avoidance distances were reduced. Also, the locomotion of the heifers during the isolation test was lowest in the human presence phase in the T treatment. Therefore, the potential of talking in a gentle voice might be interesting for further research. It might be an important aspect when aiming to establish a good relationship with fearful animals.

4.5 Further Considerations

The experiment was conducted by two experimenters. Each experimenter was blinded to the treatment allocation of the heifers that she had not treated herself. The behavioural data of one herd was analysed by the experimenter who did not treat the herd and vice versa. Therefore, it can be excluded that experimenter bias affected the results. One exception from this is the avoidance distance test of the herd the experimenter treated herself. In that case, she was not blinded to treatment allocation.

We found differences in the behaviour of the heifers during the isolation test between the two herds. The three-way interaction including herd was significant for the behaviours *alert*, *self-grooming*, *tail flicking* and *changes of squares*. Herd 1 showed more *exploration* in all treatments compared to herd 2. Qualitative observations during the experimental procedures, e.g. introducing them to the outside of the arena or positioning the camera from the barn above them, suggested that herd 2, the herd consisting of the younger animals, showed more signs of fear compared to herd 1. Additionally, it is possible that there were differences between the herds due to the different experimenters. It was not possible to test for this effect because each experimenter tested one herd and not half of both herds. Therefore, we could not separate the effect of the experimenter from the effect of the herd.

Furthermore, potential effects of the treatment might have been overlooked due to a reduction of statistical power caused by the high number of treatments and by keeping the animals in two herds that had to be considered in the statistical analysis. Other studies usually compare fewer treatments: Windschnurer et al. (2009a) compared only two treatments with each other, Schmied et al. (2008a) and Lürzel et al. (2016) compared four different treatments.

5. Conclusion

The AHR of the heifers that experienced gentle (tactile) interactions was not improved as strongly as expected. Our data do not support a positive perception of the human and close human presence by the heifers after the treatment but give indications that the human still was able to provide some social support. Due to the reduced effectiveness of the treatments in general, we could not confirm the hypothesis that different treatments alter the AHR to a different degree. Still, there were indications of a greater influence of gentle interactions on the AHR in heifers compared to the control or mere human presence. Therefore, the potential of talking in a gentle voice might be interesting for further research. It might be an important aspect when aiming to establish a good relationship with fearful animals.

6. Summary

A positive AHR improves animal welfare and allows for the possibility of humans to provide social support for animals in a stressful situation. Gentle interactions – stroking and talking in a gentle voice – are a commonly used method of improving the AHR in cattle. The aim of our study was to test if different types of gentle interactions improve the AHR in heifers to a different degree. To that purpose, we allocated sixty Austrian Simmental heifers to five treatments (ST – stroking and talking in a gentle voice, S – stroking, T – talking in a gentle voice, P – human presence, C – control). Behavioural data were recorded during the treatment period, an isolation test with temporary human presence was performed after the treatment and avoidance distance tests were performed before and after the treatment. In the behaviour during treatment, *neck stretching* occurred rarely, but stress related behaviours decreased over the treatment period in all heifers. There were no significant differences between the treatments in the avoidance distance data, but larger numeric decreases in treatments that experienced gentle interactions. In the isolation test the frequency of *changes of squares* decreased in human presence, while the duration of *alert* increased after the human left. The results indicated that none of the treatments did improve the AHR to a level where the human is perceived as positive, but to a level of habituation to and reduced fear of the human. Due to the indications for a greater influence of gentle interactions compared to mere human presence on the AHR, it might be interesting for further research to study the potential of talking in a gentle voice for animals with a poor AHR.

7. Zusammenfassung

Eine positive Tier-Mensch Beziehung (TMB) verbessert das Wohlbefinden der Tiere und erlaubt Menschen die Möglichkeit Tieren in Situationen, die mit Stress verbunden sind, soziale Unterstützung zu bieten. Sanfte Interaktionen – Streicheln und ruhiges Sprechen – sind eine häufig genutzte Methode, um die TMB zu verbessern. Das Ziel dieser Studie war es herauszufinden, ob verschiedene Arten der sanften Interaktionen die TMB von Kalbinnen zu einem unterschiedlichen Grad verbessern. Zu diesem Zweck haben wir 60 Fleckvieh Kalbinnen in 5 Behandlungsgruppen aufgeteilt (ST – Streicheln und ruhiges Sprechen, S – Streicheln, T – ruhiges Sprechen, P – Präsenz des Menschen, C – Kontrolle). Verhaltensdaten wurden während der Behandlung aufgenommen, ein Isolationstest mit zeitweiser Anwesenheit eines Menschen wurde nach der Behandlung durchgeführt sowie Ausweichdistanztests vor und nach der Behandlungsperiode. Während der Behandlung trat bei allen Tieren nur wenig Hals strecken auf, aber Verhaltensweisen, die mit Stress zusammenhängen, nahmen über die Behandlungsperiode bei allen Kalbinnen ab. Es gab keine signifikanten Unterschiede zwischen den Behandlungen in den Daten zu den Ausweichdistanztests, aber größerer numerische Abnahmen in Tieren, die sanfte Interaktionen erfahren hatten. Im Isolationstest nahm die Häufigkeit der Wechsel zwischen den definierten Bereichen in der Arena in Anwesenheit des Menschen signifikant ab, während die Dauer von wachsamen Verhalten signifikant stieg, nachdem der Mensch die Arena verlassen hatte.

Die Ergebnisse deuten darauf hin, dass keine Behandlung die TMB soweit verbessert hat, dass der Mensch als positiv wahrgenommen wurde, aber zumindest soweit, dass eine Habituation an den Menschen und eine verringerte Furcht vor dem Menschen erreicht wurde. Aufgrund der Anhaltspunkte für einen größeren Effekt sanfter Interaktionen auf die TMB verglichen mit der Anwesenheit eines Menschen, könnte es interessant sein, das Potential von ruhigem Sprechen, vor allem bei Tieren mit einer schlechten TMB, in Zukunft genauer zu untersuchen.

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Annex 1: Behaviour during treatment

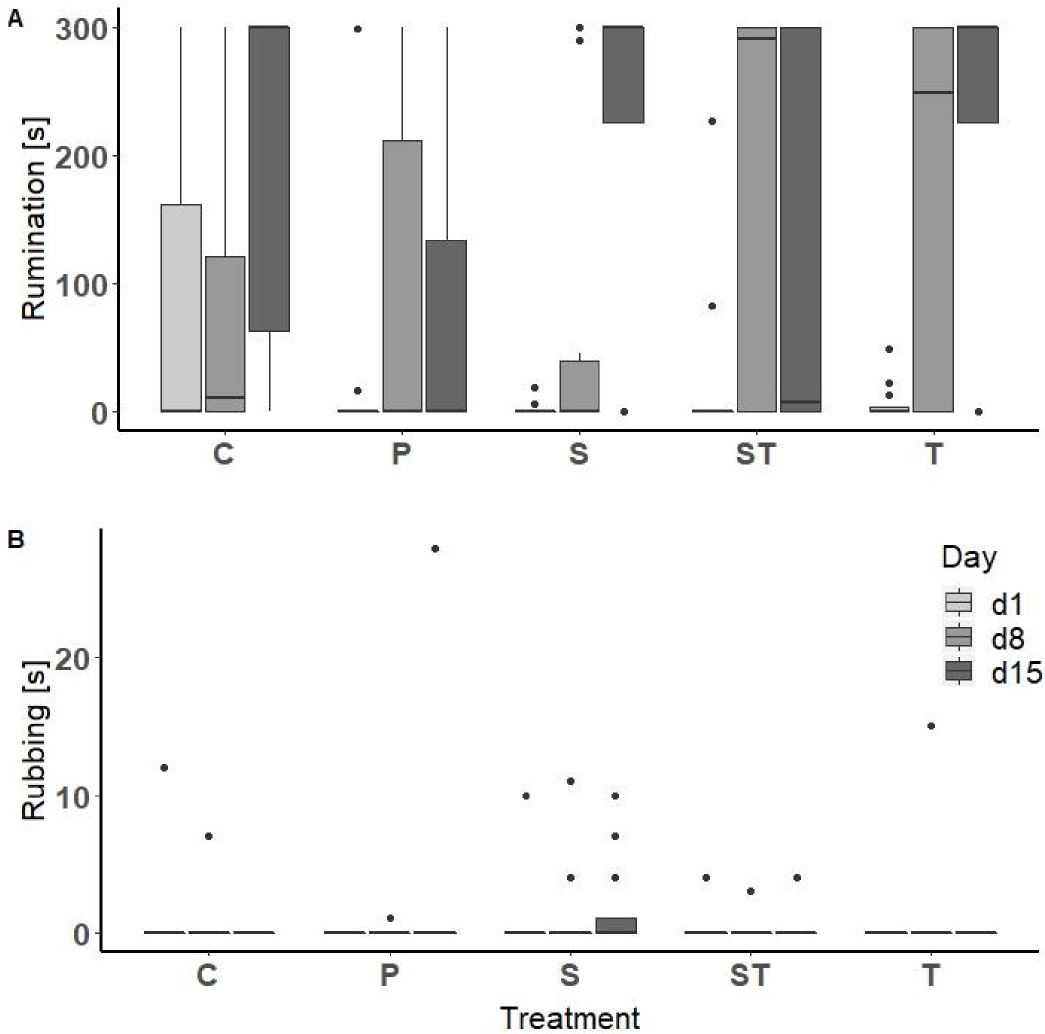

Figure A1: Durations [s] of *rumination* (A) and *rubbing* (D) in heifers belonging to five different treatments : C – control (n = 11), P – human presence (n = 12), S – stroking (n = 12), ST – stroking and talking in a gentle voice (n = 12; day 8 n = 11), T – talking in a gentle voice (n = 12). The behaviours were recorded during the treatment on days 1, 8 & 15.

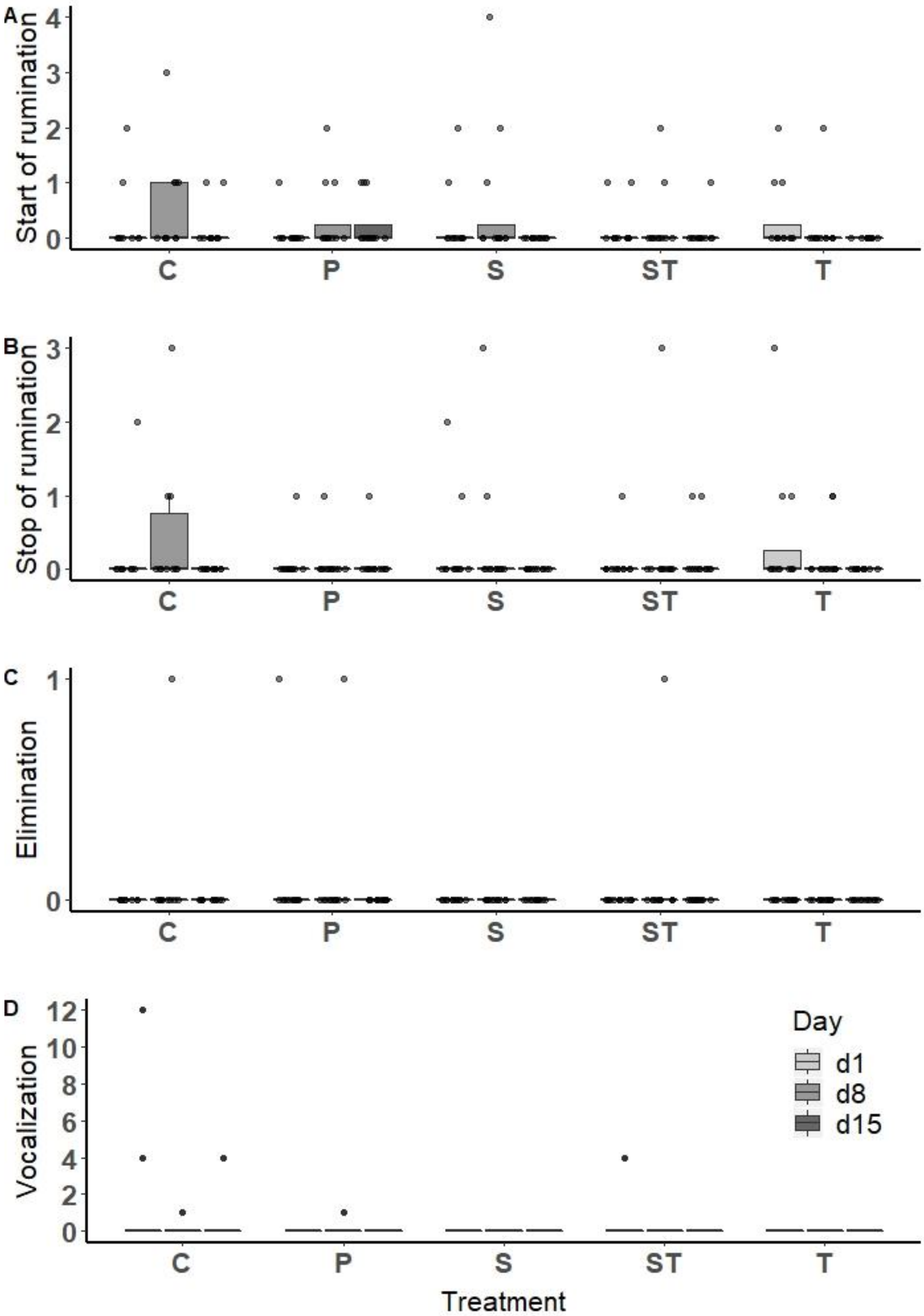

Figure A2: Frequencies of *start of rumination* (A), *stop of rumination* (B), *elimination* (C) and *vocalisation* (D) in heifers belonging to five different treatments: C – control (n = 11), P – human presence (n = 12), S – stroking (n = 12), ST – stroking and talking in a gentle voice (n = 12; day 8 n = 11), T – talking in a gentle voice (n = 12). The behaviours were recorded during the treatment on days 1, 8 & 15.

Annex 2: Statistical models for results of the avoidance distance tests

Table A1: Full model for the avoidance distances of the heifers towards the experimenter (n = 58): comparison of the five treatments (C – control, P – human presence, S – stroking, ST – stroking and talking in a gentle voice and T – talking in a gentle voice) over the three tests (1 – before treatment, 2 – directly after treatment, 3 – two weeks after treatment). Statistically significant results of variables tested in the full/null model comparison appear in bold. Statistics: General Linear Mixed Model.

Full model					
	Coefficient	SE	χ^2	df	p
(Intercept)	0.76	0.634	-[a]	-	-
Test number[b]			-[a]	-	-
Test 2	-0.731	0.837			
Test 3	-0.084	0.836			
Treatment[c]			-[a]	-	-
P	0.038	0.836			
S	-0.182	0.836			
ST	-0.092	0.836			
T	-0.062	0.836			
Herd[d]			-[a]	-	-
Herd 2	-0.612	0.906			
Origin[e]			10.855	1	0.001
Origin 2	-0.929	0.285			
Test number x treatment			-	-	-
Test 2 x P	0.384	1.183			
Test 3 x P	-0.052	1.182			
Test 2 x S	-0.912	1.183			
Test 3 x S	-0.207	1.182			
Test 2 x ST	-0.32	1.183			
Test 3 x ST	-0.037	1.182			
Test 2 x T	-0.912	1.185			
Test 3 x T	-0.055	1.182			
Test number x herd			-	-	-
Test 2 x herd 2	0.089	1.24			
Test 3 x herd 2	-0.006	1.239			
Treatment x herd			-	-	-
P x herd 2	-0.133	1.211			
S x herd 2	0.162	1.211			
ST x herd 2	0.058	1.217			
T x herd 2	0.489	1.223			
Test number x treatment x herd			1.783	8	0.987
Test 2 x P x herd 2	-0.722	1.714			
Test 3 x P x herd 2	-0.149	1.712			
Test 2 x S x herd 2	1.032	1.713			
Test 3 x S x herd 2	0.04	1.712			
Test 2 x ST x herd 2	-0.535	1.715			
Test 3 x ST x herd 2	-0.348	1.713			
Test 2 x T x herd 2	0.394	1.714			
Test 3 x T x herd 2	-0.294	1.713			

[a] not shown because of having a very limited interpretation
[b] dummy coded (‘Test 1’ as reference category)
[c] dummy coded (´C´ as reference category)
[d] dummy coded (´Herd 1´as reference category)
[e] dummy coded (´Origin 1´ as reference category)

Table A2: Reduced model for the avoidance distances of the heifers towards the experimenter (n = 58): comparison of the five treatments (C – control, P – human presence, S – stroking, ST – stroking and talking in a gentle voice and T – talking in a gentle voice) over the three tests (1 – before treatment, 2 – directly after treatment, 3 – two weeks after treatment). Statistically significant results of variables tested in the full/null model comparison appear in bold. Statistics: General Linear Mixed Model.

Reduced model after removal of non-significant three-way and two-way interactions					
	Coefficient	SE	χ^2	df	p
(Intercept)	0.796	0.37	-[a]	-	-
Test number[b]			11.952	2	0.003
Test 2	-0.942	0.27			
Test 3	-0.233	0.268			
Treatment[c]			1.251	4	0.87
P	-0.027	0.352			
S	-0.243	0.353			
ST	-0.32	0.352			
T	-0.099	0.358			
Herd[d]			3.107	1	0.078
Herd 2	-0.471	0.269			
Origin[e]			9.536	1	0.002
Origin 2	-0.835	0.271			

[a] not shown because of having a very limited interpretation
[b] dummy coded (‘Test 1’ as reference category)
[c] dummy coded (´C´ as reference category)
[d] dummy coded (´Herd 1´as reference category)
[e] dummy coded (´Origin 1´ as reference category)

Annex 3: Statistical models for results of the behaviours in the isolation test

Table A3: Full model for *alert* during the isolation test of the heifers (n = 58): comparison of the five treatments (C – control, P – human presence, S – stroking, ST – stroking and talking in a gentle voice and T – talking in a gentle voice) over the three different phases (1 – before experimenter enters, 2 – while experimenter is there, 3 – after experimenter left). Statistically significant results of variables tested in the full/null model comparison appear in bold. Statistics: General linear mixed model.

Full model *alert*					
	Coefficient	SE	χ^2	df	p
(Intercept)	-3.253	0.389	-[a]	-	-
Phase[b]			-[a]	-	-
Phase 2	1.013	0.419			
Phase 3	1.711	0.397			
Treatment[c]			-[a]	-	-
P	1.145	0.472			
S	0.165	0.518			
ST	0.84	0.488			
T	0.739	0.486			
Herd[d]			-[a]	-	-
Herd 2	0.754	0.512			
Origin[e]			0.027	1	0.87
Origin 1	-0.025	0.151			
Phase x treatment			-	-	-
Phase 2 x P	-1.846	0.573			
Phase 3 x P	-1.471	0.517			
Phase 2 x S	-0.428	0.594			
Phase 3 x S	-0.453	0.559			
Phase 2 x ST	-0.654	0.555			
Phase 3 x ST	-1.038	0.528			
Phase 2 x T	-0.296	0.543			
Phase 3 x T	-0.523	0.514			
Phase x herd			-	-	-
Phase 2 x herd 2	-1.75	0.634			
Phase 3 x herd 2	-0.672	0.545			
Treatment x herd			-	-	-
P x herd 2	-1.078	0.659			
S x herd 2	-0.018	0.707			
ST x herd 2	-1.021	0.685			
T x herd 2	-1.054	0.688			
Phase x treatment x herd			22.346	8	**0.004**
Phase 2 x P x herd 2	3.525	0.814			
Phase 3 x P x herd 2	1.425	0.716			
Phase 2 x S x herd 2	1.017	0.864			
Phase 3 x S x herd 2	0.216	0.765			
Phase 2 x ST x herd 2	1.688	0.831			
Phase 3 x ST x herd 2	1.175	0.741			
Phase 2 x T x herd 2	1.593	0.826			
Phase 3 x T x herd 2	0.976	0.73			

[a] not shown because of having a very limited interpretation
[b] dummy coded ('Phase 1' as reference category)
[c] dummy coded (´C´ as reference category)
[d] dummy coded (´Herd 1´as reference category)
[e] dummy coded (´Origin 1´ as reference category)

Table A4: Full model for *exploration* during the isolation test of the heifers (n = 58): comparison of the five treatments (C – control, P – human presence, S – stroking, ST – stroking and talking in a gentle voice and T – talking in a gentle voice) over the three different phases (1 – before experimenter enters, 2 – while experimenter is there, 3 – after experimenter left). Statistically significant results of variables tested in the full/null model comparison appear in bold. Statistics: General linear mixed model.

Full model *exploration*					
	Coefficient	SE	χ²	df	p
(Intercept)	0.134	0.343	-[a]	-	-
Phase[b]			-[a]	-	-
Phase 2	-0.839	0.394			
Phase 3	-1.114	0.396			
Treatment[b]			-[a]	-	-
P	-0.836	0.471			
S	0.383	0.458			
ST	-0.32	0.457			
T	-0.397	0.458			
Herd[c]			-[a]	-	-
Herd 2	-0.459	0.49			
Origin[d]			0	1	0.99
Origin 1	-0.002	0.168			
Phase x treatment			-	-	-
Phase 2 x P	0.848	0.568			
Phase 3 x P	1.547	0.561			
Phase 2 x S	0.17	0.552			
Phase 3 x S	-0.387	0.559			
Phase 2 x ST	-0.237	0.566			
Phase 3 x ST	0.967	0.554			
Phase 2 x T	-0.371	0.574			
Phase 3 x T	0.532	0.56			
Phase x herd			-	-	-
Phase 2 x herd 2	0.443	0.578			
Phase 3 x herd 2	0.805	0.587			
Treatment x herd			-	-	-
P x herd 2	0.693	0.675			
S x herd 2	-0.605	0.682			
ST x herd 2	0.047	0.668			
T x herd 2	0.609	0.665			
Phase x treatment x herd			12.578	8	0.127
Phase 2 x P x herd 2	-1.641	0.829			
Phase 3 x P x herd 2	-1.778	0.813			
Phase 2 x S x herd 2	-0.88	0.838			
Phase 3 x S x herd 2	0.187	0.833			
Phase 2 x ST x herd 2	-0.102	0.82			
Phase 3 x ST x herd 2	-1.099	0.812			
Phase 2 x T x herd 2	-0.826	0.832			
Phase 3 x T x herd 2	-1.303	0.816			

[a] not shown because of having a very limited interpretation
[b] dummy coded (‘Phase 1’ as reference category)
[c] dummy coded (´C´ as reference category)
[d] dummy coded (´Herd 1´as reference category)
[e] dummy coded (´Origin 1´ as reference category)

Table A5: Reduced models for *exploration* during the isolation test of the heifers (n = 58): comparison of the five treatments (C – control, P – human presence, S – stroking, ST – stroking and talking in a gentle voice and T – talking in a gentle voice) over the three different phases (1 – before experimenter enters, 2 – while experimenter is there, 3 – after experimenter left). Statistically significant results of variables tested in the full/null model comparison appear in bold. Statistics: General linear mixed models.

Reduced model of *exploration* after removal of non-significant three-way interaction					
	Coefficient	SE	χ^2	df	p
(Intercept)	-0.086	0.316	-[a]	-	-
Phase[b]			-[a]	-	-
Phase 2	-0.522	0.325			
Phase 3	-0.742	0.326			
Treatment[c]			-[a]	-	-
P	-0.282	0.407			
S	0.488	0.407			
ST	-0.132	0.406			
T	-0.07	0.405			
Herd[d]			-[a]	-	-
Herd 2	0.042	0.382			
Origin[e]			0.006	1	0.938
Origin 2	-0.013	0.165			
Phase x treatment			12.74	8	0.121
Phase 2 x P	0.063	0.427			
Phase 3 x P	0.7	0.422			
Phase 2 x S	-0.199	0.426			
Phase 3 x S	-0.349	0.437			
Phase 2 x ST	-0.289	0.429			
Phase 3 x ST	0.472	0.421			
Phase 2 x T	-0.75	0.432			
Phase 3 x T	-0.063	0.424			
Phase x herd			0.913	2	0.633
Phase 2 x herd 2	-0.243	0.273			
Phase 3 x herd 2	-0.03	0.27			
Treatment x herd			3.656	4	0.454
P x herd 2	-0.439	0.474			
S x herd 2	-0,846	0.49			
ST x herd 2	-0,367	0.479			
T x herd 2	-0,089	0.479			

Reduced model of *exploration* after removal of non-significant two-way interactions					
	Coefficient	SE	χ^2	df	p
(Intercept)	0.115	0.232	-[a]	-	-
Phase[b]			34.959	2	< 0.001
Phase 2	-0.862	0.141			
Phase 3	-0.591	0.14			
Treatment[c]			2.529	4	0.64
P	-0.219	0.245			
S	-0.057	0.248			
ST	-0.22	0.245			
T	-0.345	0.244			
Herd[d]			4.566	1	0.033
Herd 2	-0.363	0.167			
Origin[e]			0.005	1	0.944
Origin 2	-0.012	0.168			

[a] not shown because of having a very limited interpretation
[b] dummy coded (‘Phase 1’ as reference category)
[c] dummy coded (´C´ as reference category)
[d] dummy coded (´Herd 1´as reference category)
[e] dummy coded (´Origin 1´ as reference category)

Table A6: Full model for *self-grooming* during the isolation test of the heifers (n = 58): comparison of the five treatments (C – control, P – human presence, S – stroking, ST – stroking and talking in a gentle voice and T – talking in a gentle voice) over the three different phases (1 – before experimenter enters, 2 – while experimenter is there, 3 – after experimenter left). Statistically significant results of variables tested in the full/null model comparison appear in bold. Statistics: General linear mixed model.

Full model *self-grooming*					
	Coefficient	SE	χ^2	df	p
(Intercept)	-5.376	0.83	-[a]	-	-
Phase[b]			-[a]	-	-
Phase 2	1.059	0.745			
Phase 3	0.37	0.791			
Treatment[c]			-[a]	-	-
P	0.122	1.077			
S	0.071	1.077			
ST	0.53	1.016			
T	0.4	1.058			
Herd[d]			-[a]	-	-
Herd 2	1.24	1.052			
Origin[e]			0.009	1	0.924
Origin 2	-0.022	0,399			
Phase x treatment			-	-	-
Phase 2 x P	-0.717	1.094			
Phase 3 x P	-0.323	1.127			
Phase 2 x S	-0.853	1.09			
Phase 3 x S	-0.167	1.127			
Phase 2 x ST	0.078	0.884			
Phase 3 x ST	-1.708	1.103			
Phase 2 x T	-0.615	1.035			
Phase 3 x T	-0.172	1.082			
Phase x herd			-	-	-
Phase 2 x herd 2	-0.844	0.93			
Phase 3 x herd 2	-0.731	0.992			
Treatment x herd			-	-	-
P x herd 2	-0.952	1.471			
S x herd 2	-1.262	1.524			
ST x herd 2	-0.431	1.364			
T x herd 2	-0.72	1.414			
Phase x treatment x herd			25.966	8	**0.001**
Phase 2 x P x herd 2	0.445	1.463			
Phase 3 x P x herd 2	0.415	1.502			
Phase 2 x S x herd 2	0.985	1.501			
Phase 3 x S x herd 2	1.12	1.534			
Phase 2 x ST x herd 2	-1.372	1.214			
Phase 3 x ST x herd 2	1.54	1.351			
Phase 2 x T x herd 2	1.11	1.284			
Phase 3 x T x herd 2	-0.453	1.431			

[a] not shown because of having a very limited interpretation
[b] dummy coded (‘Phase 1’ as reference category)
[c] dummy coded (´C´ as reference category)
[d] dummy coded (´Herd 1´as reference category)
[e] dummy coded (´Origin 1´ as reference category)

Table A7: Full model for *tail flicking* during the isolation test of the heifers (n = 58): comparison of the five treatments (C – control, P – human presence, S – stroking, ST – stroking and talking in a gentle voice and T – talking in a gentle voice) over the three different phases (1 – before experimenter enters, 2 – while experimenter is there, 3 – after experimenter left). Statistically significant results of variables tested in the full/null model comparison appear in bold. Statistics: General linear mixed model.

Full model *tail flicking*					
	Coefficient	SE	χ^2	df	p
(Intercept)	-4.265	0.515	-[a]	-	-
Phase[b]			-[a]	-	-
Phase 2	0.982	0.411			
Phase 3	1.072	0.394			
Treatment[c]			-[a]	-	-
P	1.049	0.634			
S	0.412	0.654			
ST	1.253	0.627			
T	0.938	0.63			
Herd[d]			-[a]	-	-
Herd 2	0.633	0.697			
Origin[e]			1.169	1	0.28
Origin 2	0.29	0.266			
Phase x treatment			-	-	-
Phase 2 x P	-2.263	0.601			
Phase 3 x P	-1.306	0.525			
Phase 2 x S	-0.152	0.56			
Phase 3 x S	0.266	0.521			
Phase 2 x ST	-1.601	0.529			
Phase 3 x ST	-1.608	0.522			
Phase 2 x T	-0.601	0.502			
Phase 3 x T	-0.407	0.485			
Phase x herd			-	-	-
Phase 2 x herd 2	-0.594	0.569			
Phase 3 x herd 2	-1.469	0.593			
Treatment x herd			-	-	-
P x herd 2	-0.848	0.904			
S x herd 2	-0.024	0.938			
ST x herd 2	-1.418	0.912			
T x herd 2	-1.144	0.921			
Phase x treatment x herd			27.64	8	**0.001**
Phase 2 x P x herd 2	3.009	0.778			
Phase 3 x P x herd 2	2.474	0.76			
Phase 2 x S x herd 2	-0.126	0.777			
Phase 3 x S x herd 2	-0.585	0.816			
Phase 2 x ST x herd 2	1.363	0.756			
Phase 3 x ST x herd 2	1.894	0.788			
Phase 2 x T x herd 2	0.728	0.759			
Phase 3 x T x herd 2	1.217	0.768			

[a] not shown because of having a very limited interpretation
[b] dummy coded (‘Phase 1’ as reference category)
[c] dummy coded (´C´ as reference category)
[d] dummy coded (´Herd 1´as reference category)
[e] dummy coded (´Origin 1´ as reference category)

Table A8: Full model for *distance to human* square during the isolation test of the heifers (n = 58): comparison of the five treatments (C – control, P – human presence, S – stroking, ST – stroking and talking in a gentle voice and T – talking in a gentle voice) over the three different phases (1 – before experimenter enters, 2 – while experimenter is there, 3 – after experimenter left). Statistically significant results of variables tested in the full/null model comparison appear in bold. Statistics: Linear mixed model.

Full model *distance to human square*					
	Coefficient	SE	χ^2	df	p
(Intercept)	4.129	0.427	-[a]	-	-
Phase[b]			-[a]	-	-
Phase 2	0.06	0.458			
Phase 3	-0.288	0.458			
Treatment[c]			-[a]	-	-
P	0.225	0.568			
S	-0.223	0.568			
ST	0.357	0.569			
T	0.136	0.568			
Herd[d]			-[a]	-	-
Herd 2	-0.34	0.604			
Origin[e]			0.265	1	0.606
Origin 2	-0.111	0.215			
Phase x treatment			-	-	-
Phase 2 x P	-0.022	0.647			
Phase 3 x P	-0.076	0.647			
Phase 2 x S	-0.361	0.647			
Phase 3 x S	0.358	0.647			
Phase 2 x ST	-0.882	0.647			
Phase 3 x ST	-1.061	0.647			
Phase 2 x T	-1.679	0.647			
Phase 3 x T	-0.569	0.647			
Phase x herd			-	-	-
Phase 2 x herd 2	-0.539	0.679			
Phase 3 x herd 2	-0.598	0.679			
Treatment x herd			-	-	-
P x herd 2	0.481	0.824			
S x herd 2	0.538	0.843			
ST x herd 2	0.233	0.826			
T x herd 2	-0.144	0.824			
Phase x treatment x herd			9.919	8	0.271
Phase 2 x P x herd 2	-0.134	0.938			
Phase 3 x P x herd 2	0.304	0.938			
Phase 2 x S x herd 2	0.315	0.96			
Phase 3 x S x herd 2	0.37	0.96			
Phase 2 x ST x herd 2	1.27	0.938			
Phase 3 x ST x herd 2	1.578	0.938			
Phase 2 x T x herd 2	2.152	0.938			
Phase 3 x T x herd 2	1.523	0.938			

[a] not shown because of having a very limited interpretation
[b] dummy coded (‘Phase 1’ as reference category)
[c] dummy coded (´C´ as reference category)
[d] dummy coded (´Herd 1´as reference category)
[e] dummy coded (´Origin 1´ as reference category)

Table A9: Full model for *changes of squares* during the isolation test of the heifers (n = 58): comparison of the five treatments (C – control, P – human presence, S – stroking, ST – stroking and talking in a gentle voice and T – talking in a gentle voice) over the three different phases (1 – before experimenter enters, 2 – while experimenter is there, 3 – after experimenter left). Statistically significant results of variables tested in the full/null model comparison appear in bold. Statistics: General linear mixed model.

Full model *changes of squares*					
	Coefficient	SE	χ^2	df	p
(Intercept)	3.467	0.444	-[a]	-	-
Phase[b]			-[a]	-	-
Phase 2	-1.056	0.257			
Phase 3	-0.771	0.232			
Treatment[c]			-[a]	-	-
P	-0.285	0.575			
S	-0.512	0.58			
ST	-0.577	0.584			
T	-0.455	0.579			
Herd[d]			-[a]	-	-
Herd 2	-0.026	0.612			
Origin[e]			0.268	1	0.605
Origin 2	0.074	0.28			
Phase x treatment			-	-	-
Phase 2 x P	-0.272	0.42			
Phase 3 x P	0.028	0.355			
Phase 2 x S	0.695	0.372			
Phase 3 x S	0.255	0.366			
Phase 2 x ST	0.282	0.412			
Phase 3 x ST	0.718	0.348			
Phase 2 x T	0.228	0.399			
Phase 3 x T	0.1	0.371			
Phase x herd			-	-	-
Phase 2 x herd 2	0.153	0.377			
Phase 3 x herd 2	-0.012	0.352			
Treatment x herd			-	-	-
P x herd 2	0.136	0.833			
S x herd 2	0.218	0.857			
ST x herd 2	0.127	0.846			
T x herd 2	0.315	0.835			
Phase x treatment x herd			36.974	8	**< 0.001**
Phase 2 x P x herd 2	0.287	0.564			
Phase 3 x P x herd 2	0.453	0.49			
Phase 2 x S x herd 2	-0.331	0.54			
Phase 3 x S x herd 2	0.598	0.508			
Phase 2 x ST x herd 2	0.064	0.569			
Phase 3 x ST x herd 2	-0.348	0.513			
Phase 2 x T x herd 2	-0.603	0.577			
Phase 3 x T x herd 2	0.393	0.507			

[a] not shown because of having a very limited interpretation
[b] dummy coded (‘Phase 1’ as reference category)
[c] dummy coded (´C´ as reference category)
[d] dummy coded (´Herd 1´as reference category)
[e] dummy coded (´Origin 1´ as reference category)

Table A10: Full model for *exploring human* during the isolation test of the heifers (n = 58): comparison of the five treatments (C – control, P – human presence, S – stroking, ST – stroking and talking in a gentle voice and T – talking in a gentle voice) in phase 2 (while experimenter is there). Statistically significant results of variables tested in the full/null model comparison appear in bold. Statistics: Beta regression model.

Full model *exploring human*					
	Coefficient	SE	χ^2	df	p
(Intercept)	-4.252	0.637	-[a]	-	-
Treatment[c]			-[a]	-	-
P	-0.041	0.763			
S	0.149	0.754			
ST	0.676	0.732			
T	0.592	0.73			
Herd[d]			-	-	-
Herd 2	0.645	0.794			
Origin[e]			2.962	1	0.085
Origin 2	0.524	0.365			
Treatment x herd			-	-	-
P x herd 2	-0.153	1.081			
S x herd 2	-0.024	1.084			
ST x herd 2	-0.872	1.061			
T x herd 2	-0.683	1.052			

[a] not shown because of having a very limited interpretation
[b] dummy coded (‘Phase 1’ as reference category)
[c] dummy coded (´C´ as reference category)
[d] dummy coded (´Herd 1´as reference category)
[e] dummy coded (´Origin 1´ as reference category)