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Gentle human-animal interactions and positive emotions in cattle

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Declaration

I hereby confirm that I followed the rules of good scientific practice to the best of my knowledge.

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Abbreviations

ADT	avoidance distance test
AHR	animal-human relationship
HAI	human-animal interaction
HAR	human-animal relationship
HF	normalized powers of high frequency
HR	heart rate
HRV	heart rate variability
IBI	inter-beat interval
LF	normalized powers of low frequency
SDNN	standard deviation of the inter-beat intervals
RMSSD	square root of the mean squared differences of successive inter-beat intervals

1. Introduction

1.1. Affective states in animals

Affective states of animals – or, how animals *feel* – are commonly considered one of the three main constituents of animal welfare, alongside intact physical functioning and the opportunity to lead natural lives (Fraser, 2008; Fraser et al., 1997). Understanding, describing and measuring the affective experiences of animals has been an integral challenge in animal welfare research (Paul and Mendl, 2018). To address this topic scientifically, it is a prerequisite to establish an understanding of what is an emotion – to have a shared concept that facilitates collaborative thinking, collective discussion and scientific investigations of subjective experiences in animals. Whole articles have been written solely on the purpose of finding definitions for affective states in animals (e.g. Kremer, 2020; Paul, 2018) and different frameworks for the study of animal emotions have been proposed (e.g. Bliss-Moreau, 2017; Mendl, 2010). David Fraser describes the problem in his work investigating the role of values for the animal welfare debate: "[A] key concern [is] centred on words like 'pleasure', 'pain', 'suffering', and 'happiness'. There is no simple English word to capture this class of concepts. They are sometimes called 'feelings', but that term seems too insubstantial for states like pain and suffering. They are sometimes called 'emotions', but emotions do not include states like hunger and thirst. Perhaps the most accurate, if rather technical, term is 'affective states', a term that refers to emotions and other feelings that are experienced as pleasant or unpleasant rather than hedonically neutral." (Fraser, 2008). The absence of consistent definitions of terms used in research investigating subjective experiences has been observed and tackled in recent publications (Kremer et al., 2020; Paul and Mendl, 2018). Following these recent discussions, we will refer to “affective state” as an umbrella term for emotions, mood or other subjective experiences of animals, and specify it more clearly if needed. Paul and Mendl (2018) propose that “emotion is a multicomponent (subjective, physiological, behavioural and cognitive) response to a stimulus or event that is typically of importance to the individual, it is always valenced (pleasant or unpleasant) and can vary in activation/arousal and duration/persistence”. This is in line with the framework we chose to use for the present project: the widely recognized two-dimensional framework for studying animal emotions that Mendl (2010) adapted from human emotion literature (e.g. Burgdorf and Panksepp, 2006; Russell and Barrett, 1999). It proposes that each affective state can be described along two dimensions (Fig. 1): *arousal*, which refers to the level of activation associated with an emotion, and *valence*, indicating whether an emotion is a positive or negative experience.

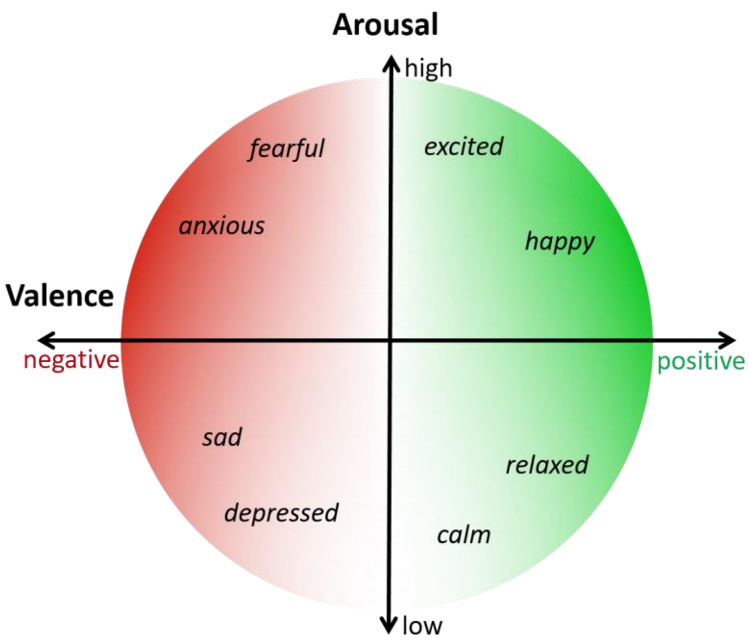

Figure 1: Two-dimensional framework of affective states. The words in italics describe examples of discrete emotions. Modified after Mendl et al. (2010).

While it is possible to integrate discrete emotions like sadness, calmness, happiness into this framework, it also allows the assessment of affective states without categorizing them into such discrete emotions. To some degree, it thus allows to work around the question whether, or to what degree, animals are aware of their affective states and *how* exactly they are experiencing a certain affective state.

1.1.1. Positive affective states and animal welfare

Nowadays, positive experiences are considered a hallmark of good animal welfare (Boissy et al., 2007; Lawrence et al., 2019; Rault et al., 2020a; Yeates and Main, 2008), but this has not always been the case (Fraser et al., 1997). Especially industrialization and the concomitant establishment of modern intensive farming systems raised public concerns and intensified the debate over the quality of lives of animals (Fraser, 2008; Fraser et al., 2013; Hemsworth et al., 2015). Probably among the first to raise large-scale public awareness on positive affective states in animals was Ruth Harrison in her book "Animal Machines": "How far have we the right to take our domination of the animal world? Have we the right to rob them of all pleasure in life simply to make more money more quickly out of their carcasses?" (Harrison, 1964). Her book and the subsequent public interest in the topic was one of the factors leading the UK government to commission an investigation of the welfare of intensively farmed animals. In the resulting document, also known as the Brambell report, the ability of animals "to experience emotions such as rage, fear, apprehension, frustration and pleasure […]" (Brambell, 1965) are acknowledged, explicitly encompassing positive affective states. Nevertheless, for the following decades, the main focus of animal welfare science remained on investigating and alleviating negative influences on animal welfare first – reflecting the fact that "modern" intensive farming systems such as the use of cages severely impaired even basic needs of animals (Waiblinger, 2012), and prevention of suffering was generally prioritized over the promotion of pleasures for animals (Fraser and Duncan, 1998). Still, the general idea that animals are able to "feel" or experience emotions and should have the opportunity for positive experiences was not forgotten and remained lively debated (e.g. Fraser and Duncan, 1998; Tschanz, 1997, Désiré et al., 2002).

In the beginning of the 21st century, the concept of "positive animal welfare" was increasingly investigated and discussed (Boissy et al., 2007; Yeates and Main, 2008), and terms like "positive emotions", "quality of life" and even "happiness" became more common in animal welfare research (Lawrence et al., 2019; Rault et al., 2020a). Research into indicators of positive welfare intensified (e.g. Knierim and Winckler, 2009; Napolitano et al., 2009). The idea that animal management should "promote their feelings of contentment and happiness" (Broom, 2007) and could thus promote a high quality of life for animals gained further popularity (Boissy et al., 2007; Green and Mellor, 2011; Mellor, 2012). The quality of life of an animal reflects the net balance between negative and positive experiences, and in order to get closer to the goal of a good quality of life, our ability to understand, measure and manage affective states of animals needs to be improved further (Mellor, 2016).

Positive affective states may also improve animal welfare by enhancing the animals' health (Boissy et al., 2007), e.g., improved immunity was found in cats that showed behaviour that was classified as positive (Gourkow et al., 2014), and several studies have shown that gentle human-animal interaction provide health benefits (see also section 1.2.1).

1.1.2. Affective experiences and controllability

The idea that reduced controllability or predictability influences the animal's experience of a certain stimulus and increases the negative effects of stressors in animals is hardly a new one (Veissier and Boissy, 2007; Weiss, 1971; Wiepkema, 1987). It therefore seems reasonable to assume that the modulation of the perceived controllability of a situation can influence the affective state elicited in the animal, and providing animals a sense of control over situations may even lead to positive affective experiences (Boissy et al., 2007). Recently, in the debate of animal welfare and positive affective experiences, concepts of controllability, perceived control or animal agency are increasingly being addressed. "Sense of agency" was embedded as one of the core facets of positive welfare research in the "Vienna Framework" (Rault et al., 2020a). Agency has been defined as an "inner-motivated behavioural engagement with the environment" with four different levels, each connected to certain affective or cognitive implications: passive/reactive agency (behaviour in direct reaction to external stimuli), action-driven agency (actively behaving to achieve current outcomes), competence-building agency (actively behaving to build skills and acquire information) and aspirational agency (actively behaving in pursuit of planned and reflected goals), and at least action-driven and competence building agency are proposed to directly promote the experience of pleasurable emotions (Špinka, 2019). Unfortunately, the standard living conditions of captive animals are often leading to restricted agency and are thus compromising their welfare (Špinka, 2019), possibly even more severely so in farmed animals that are often living in even less enriched environments than companion or zoo animals. Exercising agency, on the other hand, is thought to allow animals to experience mental security and positive affective engagement (Mellor et al., 2020). Investigating opportunities for enhancing farm animals' sense of agency could therefore directly contribute to their well-being, and thus even their productivity (Špinka, 2019). One area where animals can express agency is in interaction with human beings (Mellor et al., 2020).

1.2. The relationship of animals and humans

1.2.1. Importance of a positive animal-human relationship for animal welfare

How animals perceive humans and how they experience interactions with them can strongly affect their welfare (Boivin et al., 2003; Waiblinger, 2019). The perception of the human by the animals is influenced directly by the behaviour of the human, such as the way they move, speak or interact with animals (Waiblinger, 2017). The relationship of an animal towards humans (AHR) is determined by the relative strength of the animals' positive and negative emotions elicited during interactions with humans. These emotions are determined by the experience of HAI in the past, and in turn influence interactions in the future (Waiblinger et al., 2006). A positive AHR is characterized by the animal voluntarily approaching and seeking proximity to humans, and showing signs of pleasure, relaxation or other rewarding experiences during HAI (Rault et al., 2020b). Recent reviews are supporting the relevance of the relations between animals and humans for animal welfare: a positive AHR is thought to benefit the animal directly by promoting positive emotions during interactions with humans (Waiblinger, 2019), lower the risk of injuries and increase stress resilience (Rault et al., 2020b). Good human-animal relationship was included as a welfare criterion in the Welfare Quality project in 2009 (Winckler et al., 2007), and in 2012, the World Organisation for Animal Health included the creation of positive human-animal relationships as one of the ten 'General Principles for the Welfare of Animals in Livestock Production Systems' (Fraser et al., 2013). Studies have shown that a positive AHR has positive effects on the physiology, health and productivity of cattle. The observed anti-stress effects and health benefits have been linked to increased oxytocin levels after positive social interactions (Uvnäs-Moberg, 1998), which have also been observed after positive interactions between humans and animals (Uvnäs-Moberg et al., 2014). A positive AHR increases weight gain in calves (Lürzel et al., 2015), affects udder health (Ivemeyer et al., 2018, 2011), improves fertility (Hemsworth et al., 2000), can lower the heart rate during veterinary treatments (Schmied et al., 2010), and gentle tactile contact with young beef cattle seemed to lead to lower cortisol increases during slaughter 9 months later (Probst et al., 2012). Previous gentle handling reduced fear responses during veterinary treatments, thereby decreasing stress for animals and increasing safety for the handlers (Waiblinger et al., 2004).

1.2.2. Characteristics of human-animal interactions

Human–animal interactions can be perceived through different sensory channels: visual, olfactory, tactile and auditory (Waiblinger et al., 2006). How a human behaves has direct effects on the perception of HAI by the animals: different qualities of movement, forms of tactile interactions and how the voice is used all contribute to the evaluation of an experience as positive, neutral or negative (Waiblinger, 2017). Thus, identifying the characteristics of gentle interactions that enhance their positive perception will contribute to increasing the wellbeing of animals (Hemsworth, 2003).

Tactile interactions such as gentle touch or stroking are traditionally being used in the work with animals. Studies have shown that close positive social interactions between humans and animals can increase levels of oxytocin, which in turn increase wellbeing and reduce stress (Uvnäs-Moberg et al., 2014). In the social affiliative relationships of cattle, tactile contact plays an important role, especially in the form of social licking (Reinhardt et al., 1986; Sambraus, 1969). The function of this mutual grooming behaviour is hereby not only restricted to the purpose of cleaning, it is also used to reduce aggression (Sambraus, 1969) and serves to form and maintain social bonds as well as stabilize social relationships (Sato et al., 1991). Lastly, being licked also seems to have a calming effect, as indicated by decreased heart rates in cows receiving licking (Laister et al., 2011). Especially when being licked at the ventral neck and withers, cows reacted with stretching their necks and letting their ears hang, indicating a positive experience of the social licking of especially these body parts (Schmied et al., 2005).

Multiple studies found that cattle enjoy tactile stimulation also in interaction with humans (Bertenshaw and Rowlinson, 2008; Schmied et al., 2008b; Schulze Westerath et al., 2014), while other studies indicated that cattle did not perceive it positively (Boivin et al., 1998; Pajor et al., 2003, 2000). This might be caused by different characteristics of the interactions, such as overwhelming novelty due to a lack of habituation (Boivin et al., 1998; Pajor et al., 2000), a lack of momentary motivation for social interactions or stroking of body regions that are not preferred by the animals. Several studies have shown that the body region stroked seems to have an influence on the perception of the treatment and identified the ventral neck as the region eliciting the most prominent physiological and behavioural changes (Schmied et al., 2008a, 2008b). In response, subsequent studies focused on stroking that area in a standardized way (Lürzel et al., 2016, 2015; I. Windschnurer et al., 2009). However, during intraspecific social licking, one licking bout may include several body areas, and not one generally preferred body part could be identified (Laister et al., 2011). This raises the question whether including several body parts and reacting to the signals of the receiving animal might help to mimic the social interactions of cattle more precisely, and thus lead to more positive reactions. This might also allow the animal to engage more actively and feel more in control over the interaction and thus further improve their affective experience(Boissy et al., 2007; Windschnurer et al., 2009).

Another important means of communication in cattle herds are auditory interactions in the form of vocalizations (Kiley, 1972; Padilla de la Torre et al., 2015). As Watts and Stookey put it, “vocalization may be viewed as a subjective commentary, by an individual, on its own internal state” (Watts and Stookey, 2000) and thus provides meaningful information about the producer to the conspecific receiver. Cows use low-frequency calls when in close proximity to their calves during the first three or four weeks post-partum (Padilla de la Torre et al., 2015). But cattle also respond to human vocalizations: they can learn to react to certain calls (Albright et al., 1966) or even individual names (Murphey and Moura Duarte, 1983) and seem to prefer handlers talking gently over shouting humans (Pajor et al., 2003). Correspondingly, low-pitch speaking and drawn-out vowels have been considered positive and friendly HAI in the context of milking (Ivemeyer et al., 2011; Waiblinger et al., 2002), and studies on gentle interaction have often included stroking in combination with talking in a gentle voice (Rushen et al., 1999; Schütz et al., 2012). Still, not much is known about the distinct effects of vocal stimulation during HAI (Waiblinger, 2017). Different sensory channels open up different possibilities for interactions (Waiblinger, 2017), and as auditory interactions can reach several animals at the same time and across some distance, it is proposed to be practical especially in larger herds (Waiblinger, 2019).

It is considered good scientific practice to standardize experimental parameters, as in keeping them identical across study subjects (Pascual-Leone et al., 2016), in order to increase comparability, reproducibility and internal validity, and decrease variation in data (Richter et al., 2009). Correspondingly, in research settings, HAI commonly include highly standardized features, such as predetermined behaviours of the human, in order to provide experimental control (Rault et al., 2020b). The use of playback recordings has been suggested to broadcast sound repeatably and in a controlled fashion, and thus simplify experimental designs (Watts and Stookey, 2000). Correspondingly, stroking procedures in scientific settings have been standardized to include only one body region and a very similar style for all animals and during the whole procedure. However, the use of highly standardized stimuli might also carry some disadvantages: especially in the field of studying HAI, highly standardized interactions counteract the flexibility needed to maintain a certain naturalness of mutual interactions and not let them become too mechanical and automatic. Additionally, the field of HAI also aims to maintain a certain practical applicability, so that research findings can be transferred into non-research settings, e.g. on farms. In that context, standardizing interactions to very high degrees may lead to a loss of practical applicability (i.e. in using too narrowly defined stimuli, highly elaborated stroking procedures or using audio recordings requiring expensive technical equipment). Additionally, recent studies have proposed that higher standardization might actually produce results that are only true under very specific circumstances and therefore decrease external validity (Richter et al., 2009; Voelkl et al., 2021). In order to reach a sensible balance between high standardization for achieving low variability in the data and maintaining external validity and applicability, it is useful to compare the effects of stimuli that are standardized to a higher or lower degree on the resulting variation in data. To our knowledge, no studies have investigated whether the use of a higher degree of standardization in a stimulus in HAI indeed leads to lower variability in the data.

1.2.3. Effects of perceived control over the situation during human-animal interactions

In a similar way that they can influence the affective experience of any situation, different levels of perceived control over a situation or agency can also impact the qualitative experience of HAI. Different aspects of interactions with humans can enhance or impede the animals' capability to exercise agency (Mellor et al., 2020). As interactions typically involve active participation from both agents, situations involving active social engagement and reciprocal behaviours should be preferred over more passive situations that decrease agency (Rault et al., 2020b). Different levels of control over the situation, such as being restrained, or having influence on duration and manner of stroking might improve the experience of HAI compared to when these factors are being determined solely by the human (Waiblinger, 2019). However, our understanding of the importance of a sense of agency during HAI and its effect on the HAR remains incomplete and warrants further research (Rault et al., 2020b).

1.2.4. Improving the animal-human relationship

In an effort to enhance animal welfare, it is important to develop and test strategies of how a negative AHR can be improved. Aforementioned HAI such as gentle talking and stroking have been successfully used in studies to improve the relationship of animals to humans, indicated by reduced avoidance behaviour of cows (Lürzel et al., 2018; Schmied et al., 2008a;

Windschnurer et al., 2009) and calves (Lürzel et al., 2015; Probst et al., 2012). However, as such interactions require a relatively close contact to the animal, it can be difficult to facilitate such procedures with animals that are fearful of humans, because they try to avoid close proximity to humans. Close presence (Hemsworth et al., 1987) and touch are perceived as threatening by cattle with a poor AHR (Waiblinger et al., 2006). A human trying to establish contact, albeit gentle contact, may already elicit a stress response in fearful animals and feed into the loop of negative experiences. If the spatial proximity necessary for gentle tactile interactions cannot be reached without causing stress or fear, the animal has no opportunity to experience the rewards of receiving gentle touch. One measure to facilitate tactile interactions with fearful animals is using restraint to restrict their ability to avoid the interactions. It would permit the animals to experience from the first day on that the interactions are not causing them harm, and the higher exposure rate might accelerate the process of habituation. Restraint, however, might decrease their feeling of agency or control over the situation, and in combination with forced human contact lead to inescapable sensory impositions which might lead to negative affective states (Rault et al., 2019) and thus compromise the rewarding experience of the gentle HAI. Forced contact without the possibility to avoid it might also elicit aversive reactions in early stages. Research on the effect of restraint or control over the situation during HAIs is scarce. In one study, calves experienced either 'forced' or 'voluntary' brushing treatments (Boivin et al., 1998), but neither group approached the stockman more than a control group not experiencing HAIs. However, the rewarding nature of the interactions might have been undermined by aversive effects of social isolation on the unweaned calves during the treatment. In another study, foals that experienced forced stroking for 14 days after weaning while being restrained by means of a halter showed more approach behaviour than foals experiencing stroking while being unrestrained (Ligout et al., 2008). However, the amount of time the foals actually received gentle tactile contact was significantly greater in the group that experienced the forced stroking than the unrestrained group (2 h vs. 30 min), possibly hampering the efficiency of the unconstrained stroking treatment.

1.3. Indicators of affective states in cattle

Affective states are by definition subjective, and thus, hard to be assessed, especially in the absence of verbal communication. Animals cannot tell us verbally how they feel – but they have other means of expressing their emotions (Dawkins, 2015), and it is up to us to improve our understanding of their ways of communication. The notion that animals can experience positive affective states and their importance for animal welfare intensified the interest in the development of reliable and valid approaches to assess positive emotions (Lawrence et al., 2019). Typically, studies of affective experiences investigate a combination of behavioural and physiological parameters (Mendl et al., 2010).

1.3.1. Behaviour

A classical approach in animal studies is behavioural observation. It is non-invasive and can be used in routine situations, experiments or behavioural tests. Several behaviours have been associated with the expression of positive affective states in cattle, such as stretching the neck, which has been observed during intraspecific grooming behaviour like social licking (Laister et al., 2011; Sato et al., 1991; Schmied et al., 2005), but also during interactions with humans (Bertenshaw and Rowlinson, 2008; Schmied et al., 2008b; Waiblinger et al., 2004). In the context of HAI also voluntary spatial proximity or contact to humans can indicate a positive

HAR (Rault et al., 2020b; Tschanz, 1997), as well as approach behaviour (Waiblinger et al., 2006). Despite the idea that animals express emotions on their faces similarly to humans dating back almost 150 years (Darwin, 1872), research on animal affective states has only recently started to seriously focus on facial expressions of animals (for review see Descovich et al., 2017), leading to welfare-relevant findings like the "pain face" (in cattle: Gleerup et al., 2015). In cattle, especially ear positions and movements have been investigated in the context of different affective states (Battini et al., 2019; de Oliveira and Keeling, 2018; Mandel et al., 2019; Proctor and Carder, 2014), but findings have been contradictive (Mattiello et al., 2019), possibly also due to differing definitions and categorization of ear positions. The subtle nature of facial expressions makes their assessment quite challenging: with changes and movements often being small and volatile, direct observation is difficult, and video analysis is time-intensive. Still, advancing our knowledge of micro-expressions might be the key to increase our understanding especially of low-arousal states, as they seem to be associated with such subtle minute movements (Camerlink, 2020). This may particularly be true for prey species, that avoid openly displaying emotions (Guesgen and Bench, 2017).

1.3.2. Behavioural tests as indicators for the animal human relationship

Observing the reactions of animals to humans in specially designed test situations can inform us about specific aspects of the AHR, e.g. to assess different qualities of the animal-human relationship we can test the avoidance and approach reactions of animals to humans (Waiblinger et al., 2006). The avoidance distance test allows conclusions on the relationship of cattle towards humans (Waiblinger et al., 2006). As described previously and validated by Waiblinger et al. (2002, 2003) and Windschnurer et al. (2009), the test person approaches the animal in a standardized way and estimates the distance between the hand and the muzzle at the moment the animal shows a sign of avoidance. If the animal does not withdraw, it is recorded whether the animal accepts touching or stroking. Direct approach by a human can elicit fear in many animals (Waiblinger et al., 2006), thus, in contrast to an approach test, the avoidance distance test allows discrimination of animals that are fearful of humans from animals that are simply disinterested (Waiblinger et al., 2006). To investigate the approach behaviour of the animal towards the human, a different test can be used: the approach test is initiated by an experimenter taking position at 3 m distance (within the field of vision of the animal), and remaining passively for 3 min without encouraging contact. If the animal establishes contact, after 10 s the experimenter starts stroking. Approach behaviour is influenced by several, possibly conflicting emotions, such as fear, curiosity or positive expectations (Waiblinger et al., 2003), and can thus provide useful information on positive or negative AHR (Waiblinger et al., 2006).

1.3.3. Cardiac parameters

1.3.3.1. Heart rate

Heart rate (HR), the number of heart beats per minute, has been used for a long time to assess internal processes in cattle, often in terms of assessing stress (e.g. Lefcourt, 1999; Mohr, 2002; von Borell, 2007). Furthermore, HR has been investigated in terms of social interactions with conspecifics (Laister et al., 2011; Sato and Tarumizu, 1993) or with humans (Rushen et al., 1999; Schmied et al., 2008b). HR can be assessed non-invasively with wireless, portable heart rate monitors such as the POLAR® system (Hopster and Blokhuis, 1994). It reflects the net interactions between vagal (HR-reducing) and sympathetic (HR-increasing) influences (von Borell et al., 2007). However, it does not allow separation of the activity of the two branches of the autonomous nervous system: a rise in HR can be caused by an increase in sympathetic activity, but it may also result from a decrease in vagal regulation or from simultaneous changes in both (von Borell et al., 2007). While HR is often interpreted as an indicator of arousal (Briefer et al., 2015; Lambert and Carder, 2019; Travain et al., 2016; Zebunke et al., 2013), in order to obtain information on valence, we can investigate parameters of heart rate variability (HRV) (Boissy et al., 2007).

1.3.3.2. Heart rate variability

"A healthy heart is not a metronome" – it does not always beat regularly (Shaffer et al., 2014). The variation in the intervals between the single beats (inter-beat interval, IBI), the HRV, can reveal more detailed information about the activity of the autonomic nervous system and thus may aid investigation of emotional states of animals (von Borell et al., 2007). Various HRV parameters give information on the balance between the two branches of the autonomic system: the sympathetic ("fight or flight") and the parasympathetic ("rest and digest") system (Ede et al., 2019). HRV is often assessed using time domain and frequency domain analyses. The time domain reflects statistical variation in heart beat intervals (von Borell et al., 2007). A very common parameter is the standard deviation of the IBIs (SDNN), which is thought to reflect both sympathetic and parasympathetic influences (Shaffer et al., 2014; von Borell et al., 2007). A more informative parameter is the root mean square of successive differences between successive IBIs (RMSSD), which represents short-term variability and is mainly influenced by the vagal tone (Shaffer et al., 2014; von Borell et al., 2007). The ratio of these two time domain parameters, RMSSD divided by SDNN, is often investigated to obtain information on general sympathovagal balance (Langbein et al., 2004). In the frequency domain, power spectral analysis is used to separate the component rhythms of HRV that operate within different frequency ranges, providing information on both frequency and amplitude of the specific rhythms within the HRV waveform, and quantifying the various oscillations over any given period in the HRV recording (Shaffer et al., 2014). The high frequency (HF) band is widely accepted to reflect vagal activity (Task Force of ESP and NASPE, 1996; von Borell et al., 2007), and is also associated with positive affective states (McCraty et al., 1995; von Borell et al., 2007). Because HF is influenced by the respiration rate, species-specific respiration rates must be considered when locating the HF band (Kovács et al., 2014; von Borell et al., 2007). For mature cattle, von Borell (von Borell et al., 2007) proposes a HF range of 0.2 – 0.58 Hz (corresponding to a respiratory rate of 12 – 35 breaths/min), and for calves a range of 0.5 – 0.83 Hz (corresponding to a respiratory rate of 30 – 50 breaths/min). The low frequency (LF) band ranges from 0.05 – 0.2 Hz (von Borell et al., 2007). The physiological meaning of this parameter is more controversial (von Borell et al., 2007): While it originally has been suggested to reflect sympathetic activity (Task Force of ESP and NASPE, 1996), this is debated (Billman, 2013), especially in cattle (Hagen et al., 2005; Kovács et al., 2014; Mohr et al., 2002). It is now assumed that the LF band reflects both vagal and sympathetic influences, as well as other physiological mechanisms like baroreflex and thermoregulation (Billman, 2013; von Borell et al., 2007). Correspondingly, this debate includes discussions about the meaning of the parameter LF/HF, which previously was assumed to reflect sympathovagal balance, and great care should be taken when interpreting these parameters (Billman, 2013).

1.4. Aims of the project and hypotheses

Enriching the lives of farmed animals with positive experiences, pleasurable interactions and alleviating their fear can enhance their well-being and contribute to good welfare in a rather simple and direct way. The aim of this thesis was to reach a more comprehensive understanding of the effects of different characteristics of gentle HAI on positive emotions in cattle, and how we can best use gentle HAI to improve the AHR of cattle that are fearful of humans. To this end, we first examined different characteristics of HAI with heifers that had a positive relationship with humans. In three experiments we investigated different forms of tactile and auditory stimuli and different levels of control over the situation, as well as varying degrees of standardization. In a subsequent experiment, we analyzed the effects of restraint during applying gentle HAI on improving the AHR of cows that are fearful of humans.

Four experiments were conducted:

Experiment A (Comparison of playback and 'live' talking)

In experiment A, we compared the reactions of habituated heifers' to stroking while an experimenter was talking in a gentle voice or while a recording of an experimenter talking in a gentle voice was played.

We hypothesized that:

1) Both forms of auditory stimulation in combination with stroking will lead to a positive, low-arousal state in the heifers
2) Some of the effects will last until shortly after the stroking
3) Talking directly to the animals will elicit stronger positive reactions than the playing of a record of talking in a gentle voice
4) The higher degree of standardization in the recorded auditory stimulus will lead to decreased variation in the resulting data

→ Publication of experiment A: Talking to cows – reactions to different auditory stimuli during gentle human-animal interactions

Experiment B (Comparison of stroking at the ventral neck to 'reactive' stroking)

In experiment B, we investigated the effects of two different forms of gentle tactile interactions on the behaviour and cardiac parameters of habituated dairy heifers. We compared the reactions to stroking exclusively the ventral neck with the reactions to stroking in a reactive way by focussing on parts for which the animal indicates a preference, including the whole head/neck region.

We hypothesized that:

1) Both forms of tactile stimulation will lead to a positive, low-arousal state in the heifers
2) Some of the effects will last until shortly after the stroking
3) Stroking in a reactive way will elicit stronger positive reactions than stroking exclusively the ventral neck
4) The higher degree of standardization when stroking only the ventral neck will lead to decreased variation in the resulting data

→ Publication of experiment B: Effects of Different Stroking Styles on Behaviour and Cardiac Parameters in Heifers

Experiment C (Effects of restraint during stroking)

In experiment C, we investigated whether restraint has a negative impact on the perception of gentle human-animal interactions by habituated dairy heifers. We compared the reactions to stroking while they were either restrained in a headlock or free to move around in an arena.

We hypothesized that:

1) Independently of restraint, stroking and gentle talking will lead to a positive, low-arousal state in the heifers
2) Some of the effects will last until shortly after the stroking
3) Stroking will elicit stronger positive reactions when the animals are free to move than when they are restrained in the headlock

→ Publication of experiment C: Effects of restraint during gentle human-animal interactions

Experiment D (Effects of restraint during habituation to stroking)

In experiment D, we tested whether the animal-human relationship of cows that are fearful of humans is improved more effectively by gentle interactions during restraint or when the gentle interactions are offered while the animals are free to move.

We hypothesized that:

1) The improvement of the animal-human relationship is influenced by the level of control the animal has over the situation
2) The animal-human relationship will improve more strongly (though at a later point in time) when the animals are free to move
3) The improvement of the animal-human relationship will last longer when the animals are free to move

→ Publication of experiment D: Gentle interactions with restrained and free-moving cows: effects on the improvement of the animal-human relationship

2. Publications

2.1. Experiment A: Talking to cows – reactions to different auditory stimuli during gentle human-animal interactions

Lange, A., Bauer, L., Futschik, A., Waiblinger, S., Lürzel, S. (2020) Talking to cows – reactions to different auditory stimuli during gentle human-animal interactions. Frontiers in Psychology 11, 1–14.
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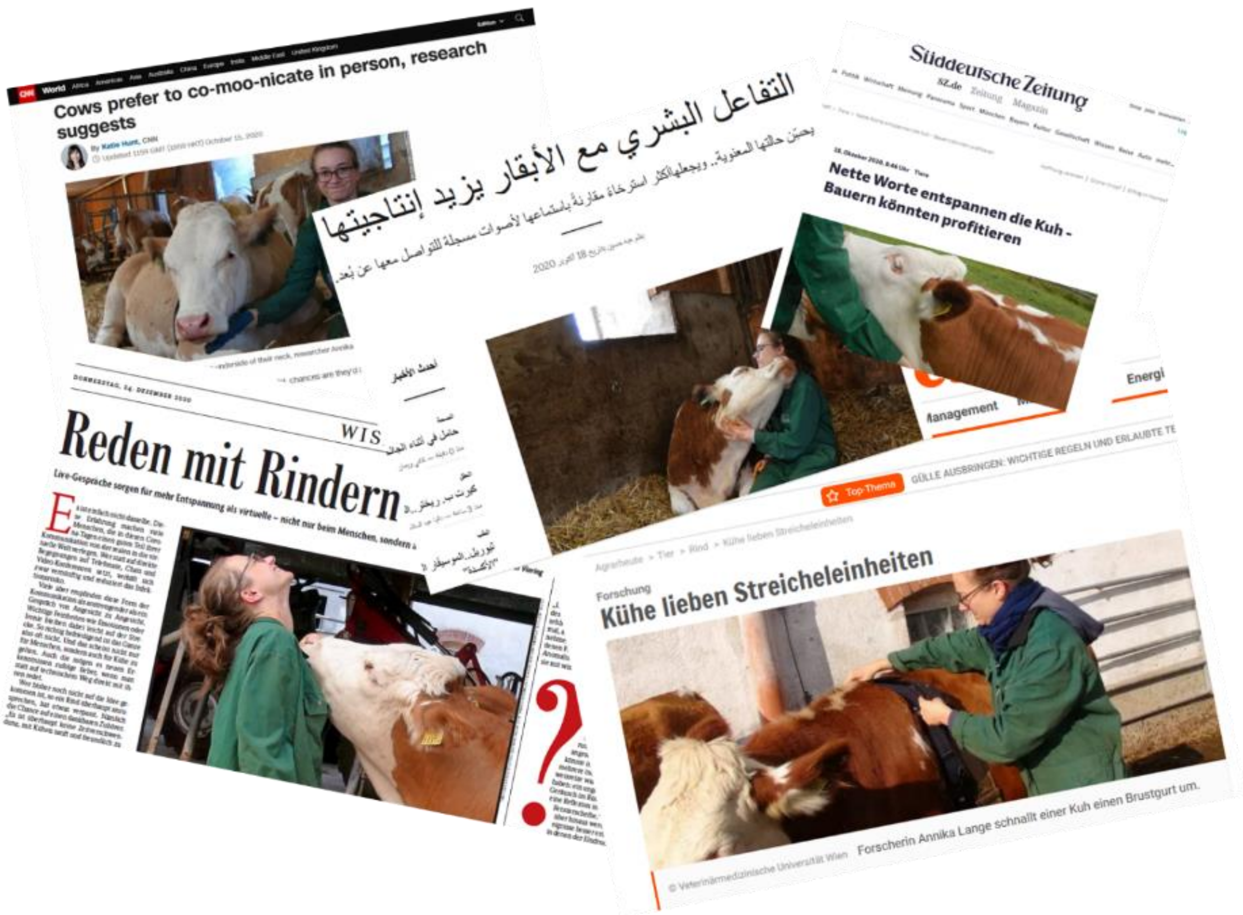

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Talking to Cows: Reactions to Different Auditory Stimuli During Gentle Human-Animal Interactions

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The quality of the animal-human relationship and, consequently, the welfare of animals can be improved by gentle interactions such as stroking and talking. The perception of different stimuli during these interactions likely plays a key role in their emotional experience, but studies are scarce. During experiments, the standardization of verbal stimuli could be increased by using a recording. However, the use of a playback might influence the perception differently than "live" talking, which is closer to on-farm practice. Thus, we compared heifers' ($n = 28$) reactions to stroking while an experimenter was talking soothingly ("live") or while a recording of the experimenter talking soothingly was played ("playback"). Each animal was tested three times per condition and each trial comprised three phases: pre-stimulus, stimulus (stroking and talking) and post-stimulus. In both conditions, similar phrases with positive content were spoken calmly, using long low-pitched vowels. All tests were video recorded and analyzed for behaviors associated with different affective states. Effects on the heifers' cardiac parameters were assessed using analysis of heart rate variability. Independently of the auditory stimuli, longer durations of neck stretching occurred during stroking, supporting our hypothesis of a positive perception of stroking. Observation of ear positions revealed longer durations of the "back up" position and less ear flicking and changes of ear positions during stroking. The predicted decrease in HR during stroking was not confirmed; instead we found a slightly increased mean HR during stroking with a subsequent decrease in HR, which was stronger after stroking with live talking. In combination with differences in HRV parameters, our findings suggest that live talking might have been more pleasurable to the animals and had a stronger relaxing effect than "playback." The results regarding the effects of the degree of standardization of the stimulus on the variability of the data were inconclusive. We thus conclude that the use of recorded auditory stimuli to promote positive affective states during human-animal interactions in experimental settings is possible, but not necessarily preferable.

Keywords: cattle, animal welfare, human-animal communication, auditory perception, gentle talking, affective states, positive emotions, expressive behavior

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INTRODUCTION

The welfare of animals is strongly influenced by the animals' perception and evaluation of their environment and the affective reactions induced by it (Veissier and Boissy, 2007). Humans constitute a substantial part of their environment, especially in farm animals. The way animals perceive humans and the quality of their interactions has a strong impact on their welfare (Boivin et al., 2003; Waiblinger, 2019). How an interaction is perceived by an animal can be influenced by the behavior shown by the human: characteristics of movements, tactile interactions and the use of voice all contribute to whether an interaction is experienced positively, neutrally or negatively (Waiblinger, 2017). While the perception of tactile stimulation has been investigated in cattle (Schmied et al., 2008; Lange et al., 2020), less is known about the effects of vocal stimulation (Waiblinger, 2017). Despite possible benefits of applying auditory stimuli in farm environments (Waiblinger, 2019), research on the effects of gentle vocal interactions on farm animals is scarce.

Cattle have highly developed auditory abilities: their hearing ranges from 23 Hz to 37 kHz (Heffner, 1998). Vocalizations are an integral part of their intraspecific communication (Kiley, 1972; Watts and Stookey, 2000; Green et al., 2019); for instance, in an affiliative context, cows direct low-frequency calls toward their calves (Padilla de la Torre et al., 2016). But cattle are also responsive to human vocalizations: calves can learn to be called by individual names (Murphey and Moura Duarte, 1983) and cows learn to follow specific calls to go to the milking parlor (Albright et al., 1966). They also seem to be sensitive to characteristics of voice reflecting the human's affective state: heifers showed a clear preference for handlers talking gently compared to handlers shouting at them (Pajor et al., 2003); however, visual signals might have influenced their choice in this experiment.

Low-pitched vocal interactions with drawn-out vowels are considered part of positive, friendly milker behavior (Waiblinger et al., 2002; Ivemeyer et al., 2011). Both in practice (e.g., Waiblinger et al., 2003; Hanna et al., 2006) and in research (e.g., Rushen et al., 1999; Schütz et al., 2012), gentle interactions with cattle often include gentle tactile stimulation in combination with talking in a gentle, soothing voice. However, it is difficult to standardize talking in the context of scientific experiments without introducing artificiality by repeatedly using the same phrases. Using playback of recordings facilitates the repeated presentation of auditory stimuli and might be useful for simplification of experimental designs (Watts and Stookey, 2000). There is evidence that calves recognize recorded samples of their mother's calls (Barfield et al., 1994), and the playback of recorded calls of calves stimulated milk production in cows (Pollock and Hurnik, 1978; McCowan et al., 2002) and lowered their heart rate (Zipp et al., 2014). The playback of a recording of gentle talking over a loudspeaker could increase standardization while retaining a natural speech melody. However, there are no studies that investigated if the use of speakers is equally effective as talking directly to cattle, as the animals might perceive the vocal stimulus differently. Recorded speech differs in frequency composition, harmonics and resonance from speech generated directly by a human (Howard and Angus, 2006), and losses in lower and higher frequencies are visible in sonographic recordings of recorded compared to live spoken voice commands (Fukuzawa et al., 2005). Another difference might be the loss of multimodal information when the auditory stimulus is produced artificially and presented via the single channel of a playback, excluding other multimodal components (Watts and Stookey, 2000). Furthermore, if one single recording is used for multiple experimenters to achieve increased standardization, the resulting mismatch between the broadcasted voice and the individual experimenter might disturb the animal, since studies show that domestic animal species such as horses can form cross-modal representations about familiar human individuals (Proops and McComb, 2012). In addition, talking in a gentle voice might also change the handler's affective state and body language, as vocalization, breathing and posture are closely related to the quality of sound produced (Partan, 2013), and that way might influence the animals' perception of the interactions and the resulting affective state.

To investigate the effects of human-animal interactions on the affective states of animals, different behavioral and physiological parameters can be measured (Mendl et al., 2010). The valence of animals' affective experience can be evaluated by observing their behavior (Dawkins, 2015; Kremer et al., 2020), including their facial expressions (for a review see Descovich et al., 2017). During social licking (Sato et al., 1991; Laister et al., 2011) and stroking by humans (Schmied et al., 2008) cattle often show neck stretching, a behavior interpreted as indicative of a positive experience. Additionally, recent studies suggest that ear positions and movements can be helpful in the assessment of affective states in cattle (e.g., Lambert and Carder, 2019; Lange et al., 2020). Other indicators for affective states are cardiac parameters, e.g., the HR of heifers accelerated when exposed to recordings of human shouting (Waynert et al., 1999). Heart rate (HR) is regulated by sympathetic and parasympathetic activity. Heart rate variability (HRV) parameters reveal more detailed information about sympathovagal balance and thus allow investigation of internal states of animals (von Borell et al., 2007).

We compared heifers' reactions to stroking while an experimenter was talking soothingly ("live") or while a recording of an experimenter talking soothingly was played ("playback"). Even though earlier studies suggest that stroking in combination with auditory stimuli can elicit a positive, low-arousal state in cattle, this has not been shown for a stroking treatment with a playback auditory stimulus. We thus hypothesized that both forms of auditory stimulation in combination with stroking would lead to a positive, low-arousal state in the heifers; thus, we predicted a decrease of HR, an increase of HRV and an increase of behaviors indicating low arousal and positive valence. We expected some of these effects to last until shortly after stroking. Further, we hypothesized that live talking would elicit a more positive emotional state than talking played by a speaker. Finally, we hypothesized that the higher degree of standardization in the "playback" stimulus leads to lower variability in the data.

MATERIALS AND METHODS

Animals, Housing and Management

The experiment was discussed and approved by the institutional ethics committee in accordance with the Good Scientific Practice guidelines and national legislation (project number ETK-02/04/2017).

The study was performed with 28 heifers (27 Austrian Simmental, one Austrian Simmental × Brown Swiss) on the young stock farm of the University of Veterinary Medicine, Vienna (Rehgras, Furth an der Triesting, Austria) between May and November 2017. As we aimed to investigate positive emotions during human–animal interactions, a generally positive perception of close human contact was a prerequisite. Based on their positive animal-human relationship, we pre-selected 32 heifers. Twenty-eight of these animals were later used for the tests. The heifers' age ranged from 7 to 24 months. According to their age, two groups of 16 animals were formed. Housing, feed and general treatment was the same for both groups, which were kept mainly on pasture. Only during poor weather conditions and for testing the animals were brought into deep-litter pens with adjoining outdoor runs, where they were fed hay and concentrate. Water and mineral blocks were provided ad libitum.

The animals were carefully habituated to the camera (Sony HDR-CX730,Weybridge, UK) and HRV equipment (Polar Electro Oy, Kempele, Finland) as well as the experimenters (both female, green overalls; A: brown hair, 1.63 m; B: brown-reddish hair, 1.70 m), the loudspeakers (Denon Envaya mini™ DSB-100, Kawasaki, Japan; fixed to the strokers' chest, but not playing sound) and the stroking procedure, until it was possible to equip the free-moving heifers with the HRV girths and stroke the animals for 3 min without them walking away or showing any visible signs of unease. Animals were considered fully habituated when a full 9-min trial (see Section Experimental Procedure, no vocal stimulation) could be performed on them while they were lying without inducing any avoidance reactions. For further details of the selection and habituation process, see Lange et al. (2020).

Experimental Design

We applied a crossover design, i.e., each animal acted as its own control and was thus subjected to both treatments. To ensure robustness of the data, each animal experienced each treatment three times in an alternating pattern, i.e., in a total of six trials (trial numbers 1–6). Each trial consisted of three phases of 180 s (3 min) each: (1) pre-stimulus (PRE), where the experimenter stood next to the animal so that baseline values could be recorded; (2) stimulus (STIM), with the experimenter stroking the ventral neck while talking in a gentle voice ("live") or while a recording of the experimenter talking in a gentle voice was played ("playback"), and (3) post-stimulus (POST), where the experimenter was standing next to the animal again so that possible carry-over effects could be observed. Approximately half of the animals started with the "live" auditory stimulus, the other half with the "playback" stimulus. The experimenters aimed to balance the order of the treatments over each testing day, but complete balancing was not always possible.

Experimental Procedure

General Procedure

All trials were carried out in a deep-litter barn of 182 m^2 (min. 11 m^2/animal), which was familiar to all animals. Each animal was prepared and equipped for HRV measurement (POLAR® horse trainer transmitters and S810i monitors, Polar Elektro Oy, Kempele, Finland) by thoroughly wetting the coat and applying ultrasound gel at electrode sites, before using elastic girths to fix the electrodes and transmitters to the chest. The transmitters were protected by a second girth with a sewn-on pocket to contain the monitor. All trials were conducted on lying animals during resting phases to minimize the influence of physical activity on cardiac parameters. Before starting a trial, the handler (i.e., stroker) started a POLAR® monitor and placed it in the pocket of the girth. When an animal had been lying for at least 5 min, the camera operator assumed a position ∼2 m from the heifer with the camera approximately at the height of the heifer's eyes, filming the head/neck region from the heifer's left side with special focus on the left eye and ear. The stroker assumed a standing position next to the animal's left shoulder and started the trial. She wore rubber gloves with a rough surface and, when the STIM phase started, applied a constant, previously practiced pressure while stroking at a frequency of 40–60 strokes/min (Schmied et al., 2008). The loudspeaker was hanging around the strokers' neck and fixed to the stroker's chest. A trial was completed after 9 min or aborted earlier at the occurrence of an event likely to influence the animal's emotional or physiological state, e.g., standing up, falling asleep or social interactions (Lange et al., 2020). If a trial was stopped, the experimenters waited for at least 1 h before testing the animal again.

Auditory Stimuli

During the stimulus phase, all animals experienced tactile stimulation on the ventral neck as described in Lange et al. (2020). Additionally, they were exposed to different auditory stimuli. In the "live" condition, the stroker talked directly to the animals in a gentle voice as in previous studies (Lürzel et al., 2015b, 2016), using phrases with positive content (in German) that were spoken calmly, with long low-pitched vowels and a decrease in pitch toward the end of the words or phrases. For the "playback" condition, a sample of Experimenter A talking in a gentle voice in the same way as in the "live" condition was recorded in WAV format via a digital voice recorder (Linear PCM Recorder LS-3, Olympus, Japan). It was integrated into an audio file (see **Supplementary Data 1**) that was played via an MP3 player (SanDisk Clip Sport MP3 Player, SanDisk Corporation, Milpitas, USA) connected to the loudspeaker fixed to the strokers' chest. The volume of the loudspeaker was adapted (using the Smartphone Android App SoundMeter) to the volume of the experimenter talking before each sequence of trials, as the experimenter adjusted the volume of her voice to the surroundings (e.g., wind, farm work). We determined an average volume of 35–47 dB per day, while staying under a maximum level of 70 dB. To assess the acoustic qualities of our recording we used the free acoustic analysis software Praat (Boersma and Weenink, 2020). The mean pitch was

190.7 Hz (± 43.4 Hz standard deviation), which is lower than the mean pitch of a sample that was described as a soothing voice cue (236.2 Hz) in contrast to a harsh voice cue (322.1 Hz) (Heleski et al., 2015).

While the experimenter was stroking the animals continuously during the 3 min of the STIM phase, the vocal stimulus was only present in the first and last minute. In both conditions, spoken signals in the audio file announced the start and end of these 1-min periods as well as of the phases. Between the phases, there were 10-s breaks to allow the stroker to assume or leave the stroking position. Possible effects of the loudspeaker itself were thus present in both conditions and the auditory stimulus of the playback was as similar as possible to the "live" condition with respect to duration of speech. Two persons conducted the experiments; one stroked the animals, the other filmed the treatment. In two thirds of the trials the stroking treatment was performed by Experimenter A and in one third by Experimenter B, in a semi-randomized order.

Behavioral Observations

All trials were video recorded and the behavior was analyzed with the coding software Solomon Coder (version: beta 17.03.22, András Péter, Budapest, Hungary), using focal animal sampling and continuous recording (Martin and Bateson, 2007). The observer was blinded to the test condition as the head of the stroking person was covered on the screen during coding, so that possible lip movements were not visible. The observer recorded ear and head positions and movements as well as other behavior according to an ethogram (**Table 1**; for photographs of ear positions, see **Supplementary Figures 1**, **2**). To determine the intra-observer reliability, ten 2-min video sequences were chosen from videos not used for further analyses and coded twice. Cohen's kappa for ear postures was 0.61, for eye aperture 0.63 and for the head postures 0.71. Cohen's kappa for rumination and lying position was 1 and for miscellaneous behaviors 0.64.

Heart Rate Measurements

Inter-beat intervals were error-corrected and processed according to Hagen et al. (2005) using the Polar Precision Performance Software, version 4.03.050 (Polar Electro Oy, Kempele, Finland), and HR and HRV parameters were calculated using Kubios, version 2.0 (Biosignal Analysis and Medical Imaging Group, Department of Applied Physics, University of Eastern Finland, Kuopio, Finland). To account for the respiratory rate, frequency bands were set to 0.04–0.2 Hz for the low frequency band and 0.2–0.58 Hz for the high frequency band (von Borell et al., 2007). The following parameters were analyzed statistically: mean heart rate (*HR*); time domain: standard deviation of the inter-beat intervals (*SDNN*) and square root of the mean squared differences of successive inter-beat intervals (*RMSSD*), and the ratio of *RMSSD* and *SDNN* (*RMSSD*/*SDNN*); frequency domain (using fast Fourier transform): normalized powers of high (*HF*) and low frequency (*LF*), and the ratio of *LF* and *HF* (*LF*/*HF*).

Statistical Analysis

Behavioral Data

We used the software package R, version 3.5.2 (R Core Team, 2019). The durations of behaviors that occurred often enough to be suitable for analysis were transformed to proportions by dividing them by the total time during which they could be observed. To account for the fact that the ear positions are mutually exclusive and their proportions always amount to one, we tried to fit a compositional model but the large amount of zeros led to convergence problems. Therefore, we selected the three ear positions that were observed often enough for statistical analysis. They were analyzed using generalized linear mixed models (GLMMs) (Baayen, 2008) with a beta error structure and logit link function (McCullagh and Nelder, 1989; Bolker, 2008) using the package "glmmTMB," version 0.2.3 (Brooks et al., 2017). Because values of the responses being exactly 0 or 1 can lead to infinite point probabilities in beta distributions, the response variables were transformed according to $(y \times (n - 1) + 0.5)/n$, where y is the original response and n the number of observations (Smithson and Verkuilen, 2006), resulting in regular small shifts of the values away from 0 and 1 (e.g., for $n = 534$, 0 becomes 0.00094, 1 becomes 0.99906).

Ear hanging and the other downward ear positions did not occur often enough to be evaluated statistically on their own [median duration in s (min–max): *hanging* 0 (0–155)]. Thus, we calculated the variable *low ear* by summing up the durations of downward ear positions (*hanging* + *back down* + *center down* + *forward down*; summed up to *low*). The result of *low ear* was still dominated by zeros, causing difficulties with the beta error distribution; therefore, it was dichotomized (occurrence: yes/no) and analyzed using a GLMM with a binomial structure and logit link function. The behavior *changes of ear position* was calculated by summarizing the frequency of different ear positions and subtracting 1 (for the initial ear position), and analyzed using a GLMM based on the negative-binomial distribution with a log link function. A minimum of three observations per condition per animal were included in statistical analyses. If additional tests were performed due to technical problems in HR(V) data collection, up to four tests per condition could be included (9 cases), which resulted in a sample size for models of 534 measures in total made for 28 individuals in a total of 178 trials with 3 phases each. For all full models, fixed effects were treatment (factor with two levels: live, playback), phase (factor with three levels: PRE, STIM, POST) and their interaction, and individual as well as trial ID (trial number nested in individual) as random effects. Trial ID was included as a random effect to account for the fact that each trial consisted of three phases and thus contributed three data points, where it seemed plausible to assume that there was random variation between the trials. We included random slopes within individual for trial number (to account for possible changes caused by treatment repetition), treatment and phase to allow their effects to vary between individuals (Barr et al., 2013). To address the issue of cryptic multiple testing (Forstmeier and Schielzeth, 2011), we compared each full model with a respective null model that lacked the variables of interest (phase and the interaction of phase and treatment) but was otherwise identical. We used a likelihood ratio test (R

TABLE 1 | Ethogram (Lange et al., 2020).

Category	Behavior[a]	Definition
Inactive ear posture[b]	Ear hanging	The ear loosely hangs downwards (referring to the ground). There is no visible muscle tension, leading often to a slightly bouncing movement when the position is assumed.
Active ear postures[b,c]	Back up	The ear is held behind and above the latero-lateral axis.
	Back center	The ear is held behind and at the same height as the latero-lateral axis.
	Back down	The ear is held behind and below the latero-lateral axis.
	Center up	The ear is held perpendicular to the head and above the latero-lateral axis.
	Center	The ear is held perpendicular to the head along the latero-lateral axis.
	Center down	The ear is held perpendicular to the head and below the latero-lateral axis.
	Forward up	The ear is held in front of and above the latero-lateral axis.
	Forward center	The ear is held in front of and at the same height as the latero-lateral axis.
	Forward down	The ear is held in front of and below the latero-lateral axis.
	Ear flicking	The ear is quickly (within max. 0.5 s) moved back and forth at least once. The behavior is coded until one of the other ear postures is clearly visible again. The residual movement after the active movement is still part of ear flicking.
Head/neck postures	Held without touching	The head is actively held up and does not touch the stroker.
	Held with touching	The head is actively held up and touches the stroker.
	Rest head without touching	The heifer does not actively carry the head's weight. The heifer's head is in contact with the ground, barn equipment, another animal or with the heifer's leg(s). The heifer's head is not in contact with the stroker.
	Rest head with touching	The heifer does not actively carry the head's weight. The heifer's head is lying on the ground, barn equipment, another animal or the heifer's leg(s) while being in contact with the stroker, or it is lying on the stroker's leg.
	Head shaking/tossing	Successive quick movements of the head. The movements can be rotational or up and down.
	Neck stretching	Positioning neck and head actively in an outstretched line, either up, down, or forward.
Eyes[d]	Open	The iris is at least partly visible.
	Closed	The iris is not visible at all for longer than 0.5 s.
	Not visible	Neither eye is visible.
Miscellaneous	Rubbing the stroker	The heifer touches the stroker and moves the touching body part while in contact with the experimenter. The behavior ends when the contact between the heifer and the person is interrupted for at least 3 s.
	Rubbing	The heifer moves the head/neck region while in contact with the ground or barn equipment. The behavior ends when the contact between the heifer's head/neck region and the ground/equipment has ended.
	Nose close	The heifer moves her muzzle toward the stroker within a range of 5 cm. The behavior ends when the heifer's nose does not point toward the stroker anymore, leaves the range of 5 cm or if another behavior of the "miscellaneous" category starts.
	Licking the stroker	The heifer's tongue touches the stroker at least once. The behavior ends when the heifer's tongue does not touch the stroker again within 3 s.
	Ruminating	The heifer's jaw moves regularly sideways with a frequency of about one movement per second. This movement is recorded as rumination if it occurs in a series of at least five movements (which may start before and end after the observation). Rumination ends when the jaw movement is paused for more than 10 s.
Calculated measures	Contact	The time in which the heifer's head and neck area was in contact with the stroker. Sum of durations of "rest head with touching", "held with touching", "nose close", "rubbing experimenter" and "licking experimenter", not including contact established by stroking.
	Resting head	Sum of durations of "rest head with touching" and "rest head without touching".
	Ear low	The sum of the durations of the ear hanging or held below the latero-lateral axis ("hanging" + "back down" + "center down" + "forward down").
	Changes of ear positions	Sum of the frequencies of different ear positions per trial minus 1.

[a] All behaviors were coded as durations, except changes of ear positions (count data).
[b] The left ear was recorded; if it was not visible, the right ear was recorded.
[c] The latero-lateral axis refers to an imaginary line between the bases of the ears. "Behind" means the ear is pointing toward the back of the head, "in front" refers to the rostral end of the head, "above" describes the ear pointing dorsally and "below" pointing ventrally. If the observed ear was moved by the experimenter, the position before the movement was recorded until the next unambiguous ear posture was assumed.
[d] The left eye was recorded; if it was not visible, the right eye was recorded.

function "anova") for these comparisons. The significance of the individual independent variables was determined by dropping them one at a time and using a likelihood ratio test to compare the resulting models to the full model (Barr et al., 2013). Values of $p \leq 0.05$ are referred to as significant, and $0.05 < p \leq 0.1$ as a trend (Stoehr, 1999). If the full–null model comparison was, or tended to be, significant and the interaction was non-significant, the interactions were removed from the models and reduced models were fitted to investigate the main effect of phase. Main effects of treatment were not tested, as they were not of interest.

As stated above, the mismatch between the broadcasted voice and the individual experimenter stroking the animal, as was the case when experimenter B was stroking during the playback of the voice of experimenter A, could drive the results regarding the interaction between condition and phase. If this were the case, one would expect the pattern of this interaction to depend on whether the mismatch was present or not. To address this question, we fitted one model in addition to each full model. This model included the three-way interaction between phase, treatment and presence of the mismatch and all terms comprised therein (and a random slope of presence of the mismatch), but was otherwise identical to the respective full model. Subsequently, we compared this model to a reduced model lacking the three-way-interaction but otherwise being identical, again using a likelihood ratio test (R function "anova"). If this comparison reveals significance, it indicates that the effects of condition and phase were indeed driven by the mismatch. In the case of the model for the behavior *neck stretching*, the reduced model did not converge, but we inspected the coefficients of the full model to reveal possible effects of the mismatched experimenter/voice combination on the duration of *neck stretching*. We found no evidence for significant effects of the mismatch between the broadcasted voice and the individual experimenter stroking the animal for any of the behaviors (*neck stretching* $z = 0.534$, $p = 0.593$ (full model coefficients); *contact* $\chi^2 = 0.223$, df = 2, $p = 0.895$; *eye closed* $\chi^2 = 0.025$, df = 2, $p = 0.988$; *head resting* $\chi^2 = 0.451$, df = 2, $p = 0.798$; *ear flicking* $\chi^2 = 0.916$, df = 2, $p = 0.632$; *changes of ear position* $\chi^2 = 0.522$, df = 2, $p = 0.770$; *back up* $\chi^2 = 0.077$, df = 2, $p = 0.962$; *back center* $\chi^2 = 2.746$, df = $p = 0.253$; *forward up* $\chi^2 = 0.937$, df = 2, $p = 0.626$; *ear low* $\chi^2 = 0.684$, df = 2, $p = 0.710$). Hence, we report results of the models not including the mismatch.

Since the "playback" stimulus had a higher degree of standardization than the "live" stimulus, it seemed plausible that the variation in a given behavior would be smaller in the "playback" treatment than in the "live" treatment. We explicitly estimated this potential effect by modeling the precision parameter of the response as a function of treatment in each model (Lange et al., 2020). With a higher degree of standardization in "playback" stroking, we expected smaller variation in behaviors, and thus, larger estimated precision parameters. For the models where we found overdispersion (*neck stretching*, *changes of ear positions, contact, head resting* and *forward up*), we corrected standard errors and *p*-values based on Wald's z-approximation (Field, 2005); therefore no degrees of freedom are reported and χ^2s were replaced by z-values (Gelman and Hill, 2006). We determined 95% confidence limits using the function "simulate.glmmTMB" of the "glmmTMB" package. We assessed the model stability by comparing the estimates of models based on the full dataset with estimates of models fitted to subsets where the levels of each random effect were dropped one at a time (Nieuwenhuis et al., 2012). This revealed a fairly good stability of the models.

For graphical depiction, we used the R packages "ggplot2" (Wickham, 2016) and "cowplot" (Wilke, 2019). Data were depicted as boxplots for each treatment and phase, using the mean values of behaviors per animal (averaged across the three trials per treatment). The bold line corresponds to the median; the lower and upper lines of the box to the first and third quartile, respectively; and the whiskers correspond to the lowest and highest values that were still within 1.5 × interquartile range from the margins of the box. Outliers (all values outside of 1.5 × interquartile range) are depicted as circles.

Cardiac Data

Due to technical problems during HRV recording (i.e., >5% of errors per minute), we obtained a sample size of 26 animals, which resulted in 176 total measures as sample size for models. Because of an insufficient number of recordings with experimenter B, only recordings of tests where experimenter A stroked the animals were used for HRV analysis. Cardiac variables were analyzed using linear mixed models (LMMs) with the package "lme4" (Bates et al., 2015), including treatment, phase and their interaction, age (d), time of day, *HR* (unless it was the response variable) and duration of rumination (s) as fixed effects. Heart rate was included as a fixed effect because it is often strongly correlated to HRV indicators (Zaza and Lombardi, 2001; Monfredi et al., 2014; Sacha, 2014; McCraty and Shaffer, 2015). While HR is often regarded as an indicator of arousal (Zebunke et al., 2013; Briefer et al., 2015; Travain et al., 2016; Lambert and Carder, 2019), HRV might also provide information on valence (Boissy et al., 2007). By correcting for *HR* in the models, the results represent the influence of the other independent variables (mainly the interaction of treatment and phase) on HRV parameters independently of their influence on *HR*, allowing conclusions in addition to those that can be drawn from *HR*. To account for the cyclical nature of circadian rhythms that influence HRV (Hagen et al., 2005; Kovács et al., 2016), we modeled time of day turning time into radians: first we transformed time to decimal numbers by summarizing hours, minutes divided by 60 and seconds divided by 3,600. The result was multiplied with 2 × π and divided by 24, and the resulting variable was included together with its sine and cosine into the model (Stolwijk et al., 1999). The individual and trial number nested in individual were considered as random effects. We included random intercepts and random slopes within individual for trial number (to account for possible effects of treatment repetition), treatment and phase to allow their effects to vary between individuals. Where possible, we also included estimates of the correlations between the random intercept and slopes into the model (Barr et al., 2013). However, for the response variables *SDNN* and *LF*, the models including the correlations did not converge and we dropped the correlation estimates from

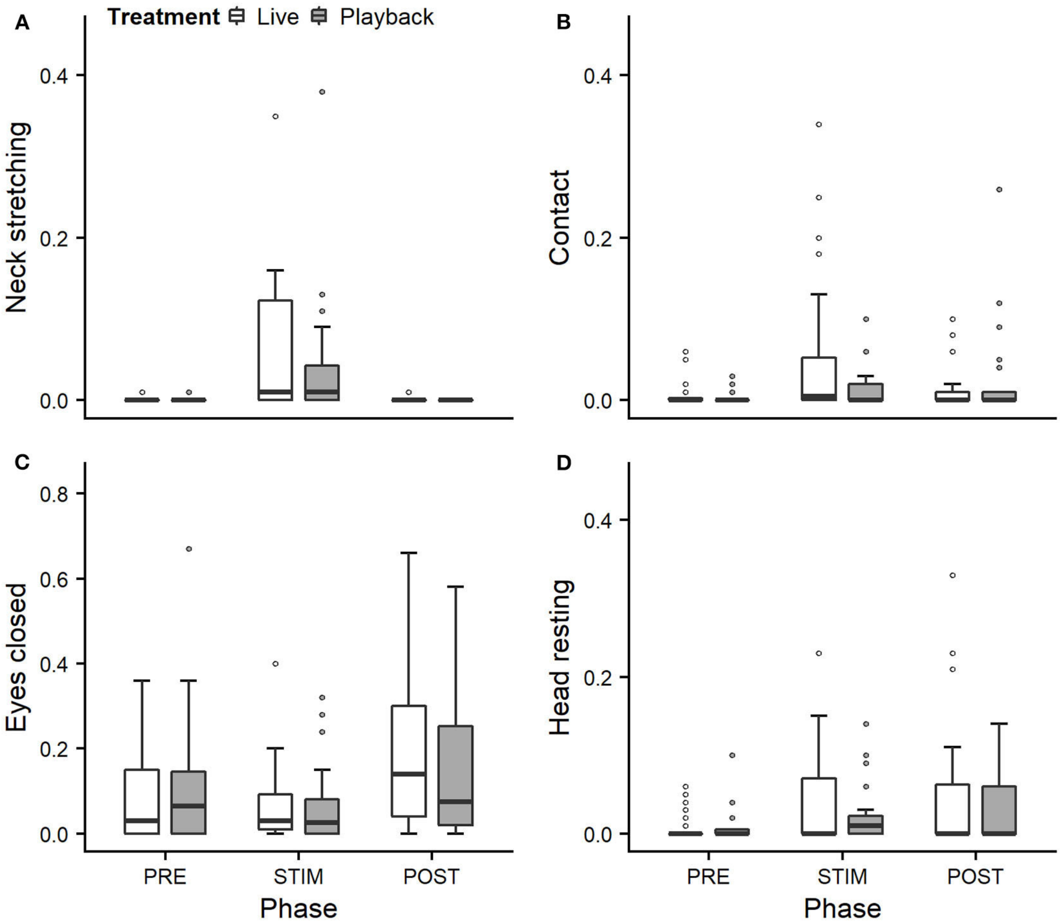

FIGURE 1 | Mean durations (as a proportion of the total time observed) of *neck stretching* **(A)**, *contact* **(B)**, *eyes closed* **(C)**, and *head resting* **(D)** of heifers ($n = 28$) during the experimental trials. Means were calculated across the three trials per treatment and are depicted according to the treatment used (white = "live," dark gray = "playback") and phase (PRE = pre-stimulus, STIM = stimulus, POST = post-stimulus). Statistics for GLMMs: significant main effect of phase for *neck stretching* **(A)**, $p < 0.05$. Note that the y-axis scale varies to allow for sufficient resolution for rare behaviors.

the model. We then proceeded in the same way as described above: we fitted a null model that lacked the variables of interest (phase and the interaction of phase and treatment), and if the full-null model comparison revealed significant differences and the interaction was non-significant, it was removed from the model and reduced models were fitted to test for the significance of the main effect of phase.

RESULTS

Behavior During Gentle Interactions

We statistically analyzed *neck stretching* (median duration in s; min–max: 0; 0–112), *contact* (0; 0–175), *eye closed* (0; 0–180) and *head resting* (0; 0–180) (**Figure 1**); the ear positions *back up* (124; 0–180), *back center* (8; 0–180), *forward up* (0; 0–164), *ear low* (0; 0–169); and the ear movements *ear flicking* (1; 0–76) and *changes of ear position* (9; 0–63) (**Figure 2**).

Full and null models differed significantly for the response variables *neck stretching* (**Figure 1**; GLMM: $\chi^2 = 10.811$, df = 4, $p = 0.029$), *ear flicking* (**Figure 2**; $\chi^2 = 32.426$, df = 4, $p < 0.001$) and *changes of ear position* (**Figure 2**; $\chi^2 = 35.907$, df = 4, $p < 0.001$) as well as for all the tested ear positions except for *forward up* (**Figure 2**; *back up*: $\chi^2 = 31.371$, df = 4, $p < 0.001$; *back center*: $\chi^2 = 13.613$, df = 4, $p = 0.009$; *ear low*: $\chi^2 = 19.758$, df = 4, $p = 0.001$). The full–null model comparisons revealed a statistical tendency toward a difference for *forward up* (**Figure 2**; $\chi^2 = 9.332$, df = 4, $p = 0.053$) and no significant difference for *contact* (**Figure 1**; $\chi^2 = 2.067$, df = 4, $p = 0.723$), *head resting* (**Figure 1**; $\chi^2 = 2.024$, df = 4, $p = 0.731$) and *eyes closed* (**Figure 1**; $\chi^2 = 6.113$, df = 4, $p = 0.191$).

As the interaction of phase and treatment was not significant for any of the behaviors we analyzed, effects of the phase were not influenced by the type of auditory stimulus used in the treatment. However, independently of which treatment was used, the phase had a significant effect on several of the behaviors. The reduced models revealed a significant main effect of phase for *neck stretching* ($z = 2.594$, $p = 0.009$), *ear flicking* ($\chi^2 = 32.520$, df = 2, $p < 0.001$) and *changes of ear position* ($\chi^2 = 31.526$, df = 2, $p < 0.001$): while the durations of *neck stretching* increased during STIM (**Figure 1**), the durations of

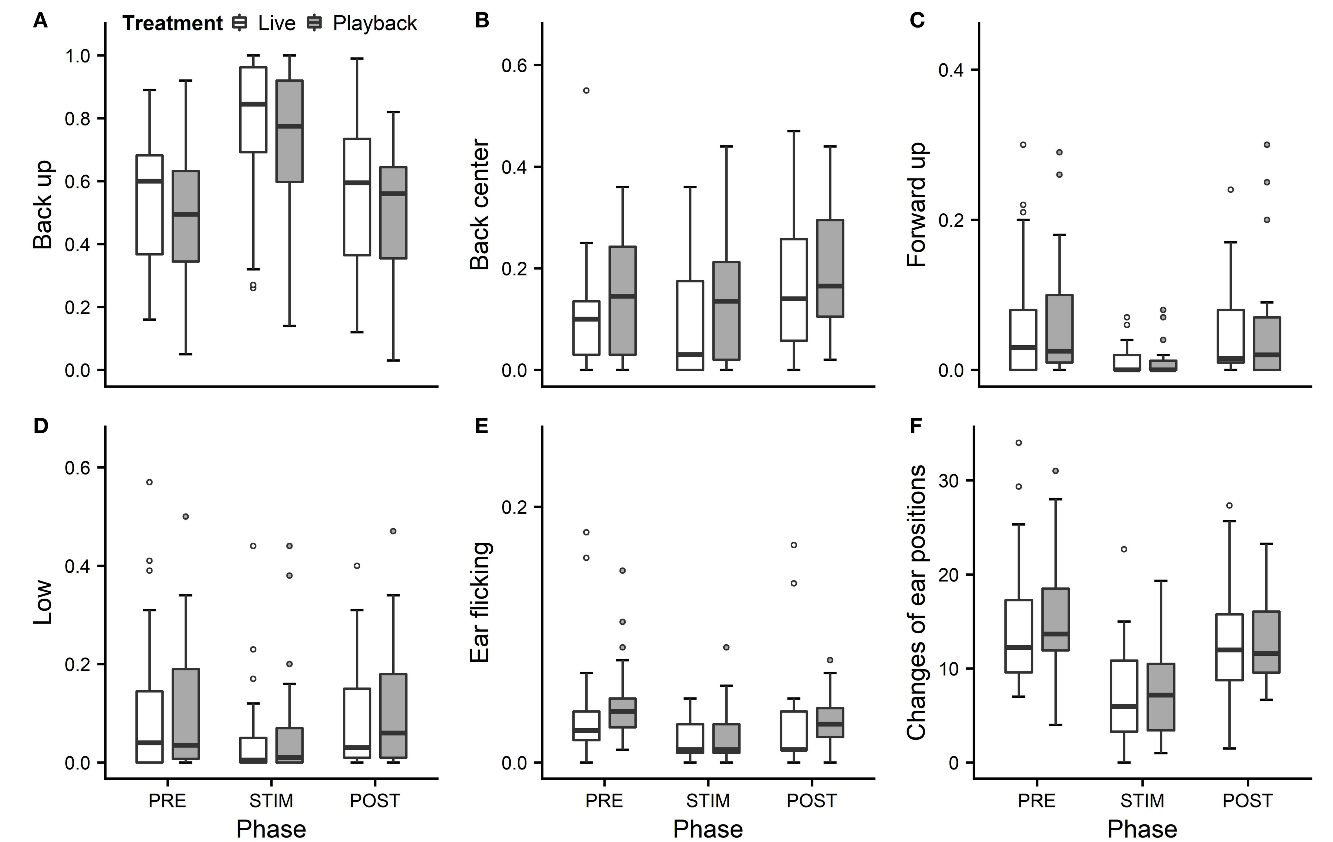

FIGURE 2 | Mean durations of ear positions **(A–D)** and ear flicking **(E)** as a proportion of the total time observed and **(F)** mean number of changes of ear positions of heifers (n = 28) during the experimental trials. Means were calculated across the three trials per treatment and are depicted according to the treatment used (white = "live," dark gray = "playback") and phase (PRE = pre-stimulus, STIM = stimulus, POST = post-stimulus). Statistics for GLMMs: significant main effect of phase for *back up* **(A)**, *back center* **(B)**, *ear low* **(D)**, *ear flicking* **(E)**, and *changes of ear positions* **(F)**, $p < 0.05$; and trend for *forward up* **(C)**, $p < 0.1$. Note that the y-axis scale varies to allow for sufficient resolution for rare behaviors.

ear flicking and the numbers of *changes of ear position* decreased (**Figure 2**). Phase also had a significant effect on the ear positions *back up* (χ^2 = 30.705, df = 2, $p < 0.001$), *back center* (χ^2 = 13.500, df = 2, $p = 0.001$), *forward up* ($z = -0.216$, $p = 0.027$), and *ear low* (χ^2 = 19.094, df = 2, $p < 0.001$): during STIM, the durations of *back up* increased significantly, whereas the durations of the other tested ear positions decreased (**Figure 2**).

The variability was significantly smaller in the "playback" treatment for *neck stretching* (χ^2 = 16.177, df = 1, $p < 0.001$) and *contact* (χ^2 = 4.321, df = 1, $p < 0.001$), but higher for the ear position *back center* (χ^2 = 10.273, df = 1, $p < 0.001$). It did not differ significantly for the other behaviors. For statistical details, including model coefficients, standard errors and confidence intervals, see Supplementary Material (**Supplementary Table 1**).

The number of tests aborted because of heifers standing up during STR without any obvious reason (e.g., being chased up) was higher in the "playback" condition (n = 13) than the "live" condition (n = 6) but did not differ significantly (χ^2 = 2.3, df = 1, $p = 0.127$).

Cardiac Data

Full and null models differed significantly for the response variables *HR* (LMM: *HR*: χ^2 = 26.688, df = 4, $p < 0.001$), *SDNN* (χ^2 = 13.185, df = 4, $p = 0.010$), *RMSSD/SDNN* (χ^2 = 13.091, df = 4, $p = 0.011$) and *HF* (χ^2 = 12.272, df = 4, $p = 0.015$). The full–null model comparison revealed no significant difference for *RMSSD* (χ^2 = 2.933, df = 4, $p = 0.569$), *LF* (χ^2 = 0.645, df = 4, $p = 0.958$) and *LF/HF* (χ^2 = 2.784, df = 4, $p = 0.595$).

The interaction of phase and treatment was significant for all cardiac parameters with a significant full-null model comparison (**Supplementary Table 2**, *HR*: χ^2 = 9.917, df = 2, $p = 0.007$; *SDNN*: χ^2 = 8.738, df = 2, $p = 0.013$; *HF*: χ^2 = 7.657, df = 2, $p = 0.022$; *RMSSD/SDNN*: χ^2 = 8.378, df = 2, $p = 0.015$). Whereas *HR* increased slightly during stroking in both conditions, it decreased more strongly in the "live" condition after the treatment (**Figure 3**). There was a distinct increase in SDNN during STIM in the "live" condition, followed by a decrease in POST, whereas the strongest increase in the "playback" condition took place in POST. *RMSSD/SDNN* mirrored this pattern: in "live" it decreased during STIM, increasing again in POST, and in "playback" it decreased during POST. *HF* increased by nearly

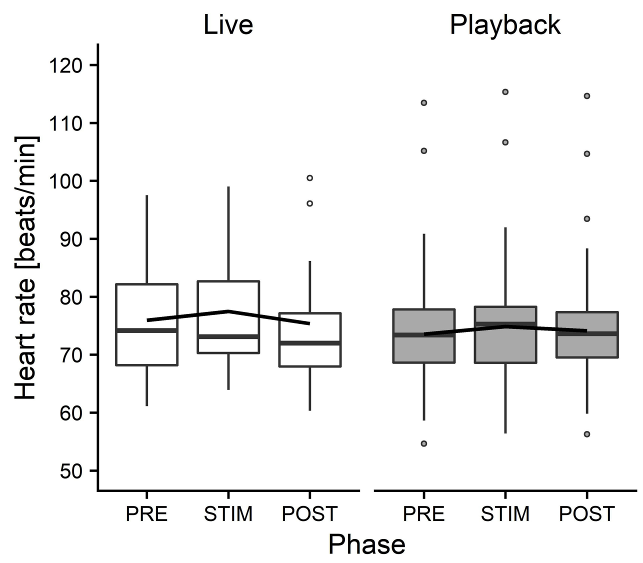

FIGURE 3 | Means of heart rate of heifers ($n = 26$), calculated across the three trials per treatment and depicted according to treatment (white = "live," gray = "playback") and phase (PRE = pre-stimulus, STIM = stimulus, POST = post-stimulus). The black line indicates the estimated means of the models. Statistics for LMM: significant interaction of condition and phase, $p < 0.05$.

30% during POST of the "live" condition whereas it decreased during POST in "playback" (**Figure 4**). The models revealed a significant negative effect of *HR* on all the HRV parameters except *LF* and *LF/HF*, where it had a significant positive effect (see **Supplementary Table 2**).

DISCUSSION

We compared the reactions of heifers to stroking while applying two different auditory stimuli: the stroker talking directly to the animals in a gentle voice or a recording of the stroker's talking. We found behavioral and physiological indications of a positive perception of the interactions for both auditory stimuli. While the behavioral reactions to gentle interactions did not differ statistically, some of the cardiac parameters indicated differences between the auditory stimuli, also shortly after the presentation of the stimulus had ended.

Perception of Each Treatment

Both treatments led to changes in behavior during the STIM phase that indicate a positive perception: During stroking, the heifers showed significantly longer durations of *neck stretching*, a behavior shown during intraspecific social grooming (Sambraus, 1969; Reinhardt et al., 1986; Schmied et al., 2005), which is often actively solicited, and stroking by humans (Waiblinger et al., 2004; Schmied et al., 2008; Lürzel et al., 2015a). It is interpreted a sign of enjoyment, and it can thus be assumed that the situation is perceived as positive.

In a previous, similar experiment (Lange et al., 2020), we observed decreases of *ear flicking* and *changes of ear position* during stroking with no auditory stimuli. The present study confirms this pattern. The animals showed less *ear flicking* during STIM than PRE, a behavior mostly associated with negative affective states, such as pain after dehorning (Heinrich et al., 2010; Neave et al., 2013) or reactions to insect attacks (Mooring et al., 2007).

During STIM, the animals also changed the positions of their ears less often than in PRE. Frequencies of *changes of ear positions* were lower in sheep feeding (Reefmann et al., 2009a) or voluntarily being groomed by a human (Reefmann et al., 2009b) than during separation from the herd. In contrast, dairy cows showed an increased frequency of *changes of ear positions* during stroking compared to before or after (Proctor and Carder, 2014), which might however have been caused by small differences in experimental design, such as the stroker approaching at the beginning of the stroking phase. In contrast, the decrease in *changes of ear positions* and *ear flicking* during stroking in the current as well as in our previous study (Lange et al., 2020) indicates an association of a reduction of these behaviors with a positive, low-arousal state also in cattle.

However, for some of the behaviors we expected to indicate affective states, the treatment did not lead to significant differences: previously observed effects of stroking (Lange et al., 2020) on the duration of the animal *resting* its *head* and the time spent in *contact* with the experimenter were not confirmed in this study. These findings might be connected with the auditory stimulus, which might keep the animal comparatively more attentive to a certain degree and thus limit the intensity of the relaxation.

In an attempt to reflect the continuous nature of ear positions, we recorded nine different positions along the vertical and the horizontal axis: *back up, back center, back down, center up, center, center down, forward up, forward center* and *forward down*, plus *ear hanging*. During stroking, durations of the *back up* position increased significantly, while durations of *forward up* and *ear low* decreased, mostly in line with our previous experiment (Lange et al., 2020). The tendency toward decreased durations of *forward up* might indicate lowered vigilance (Boissy and Dumont, 2002), which is associated with less fear (Welp et al., 2004), and could corroborate the hypothesis that stroking induces positive low-arousal states.

We predicted to find longer durations of *ear low* during stroking, because *low ear* positions, including *ear hanging*, were associated with low-arousal, positive affective states in dairy cows in previous studies (Schmied et al., 2008; Proctor and Carder, 2014). However, we observed predominantly *back up* positions and surprisingly rare occurrences of *ear low*. One possible reason might have been the strokers' position kneeling next to the lying animal and resulting in the auditory signal being located above and behind the heifers' ears in both conditions. Since the ear position pattern was very similar to the one found in our previous study without vocal stimulation (Lange et al., 2020), however, the effect of the auditory stimulus seems not to have had a strong influence on ear positions, possibly

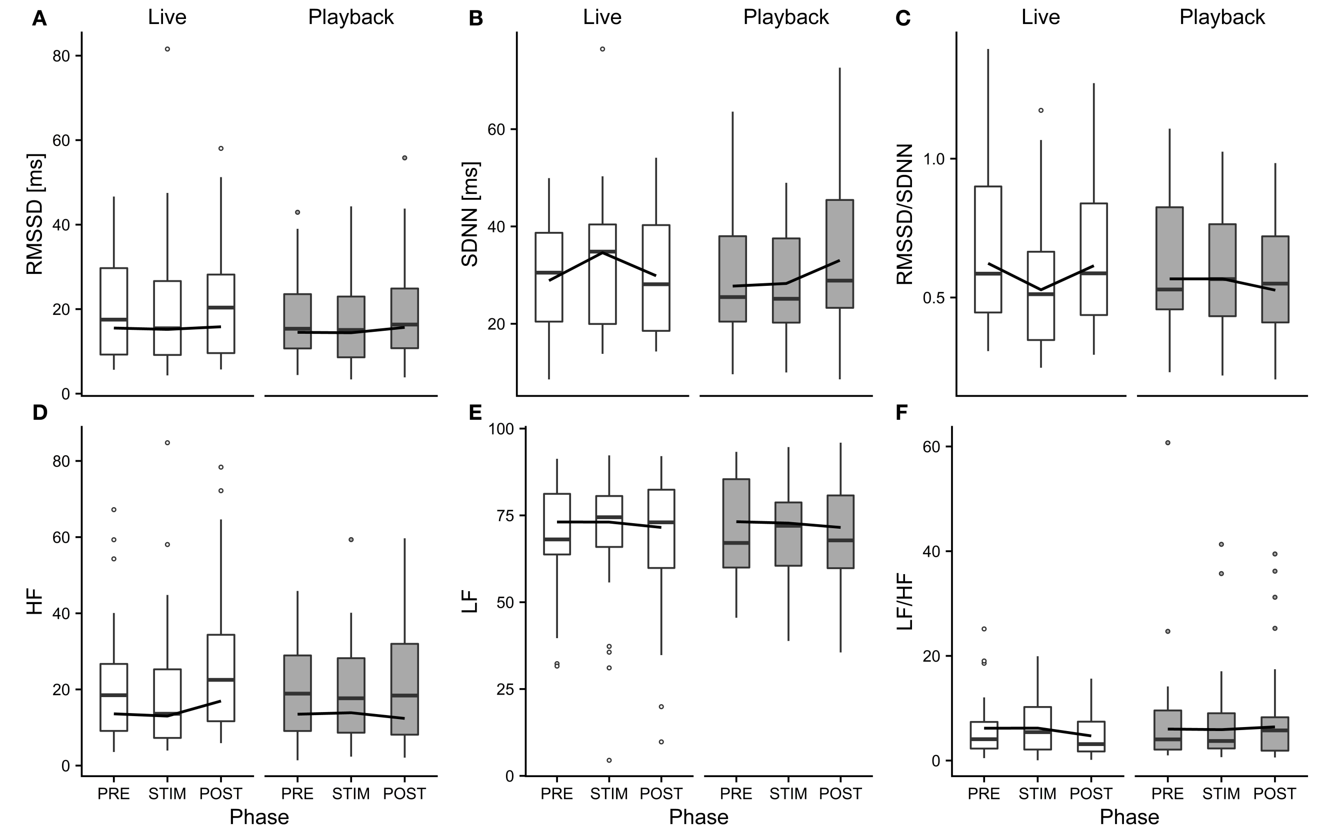

FIGURE 4 | Means of heart rate variability parameters *RMSSD* **(A)**, *SDNN* **(B)**, *RMSSD/SDNN* **(C)**, *HF* **(D)**, *LF* **(E)**, and LF/*HF* **(F)** of heifers ($n = 26$), calculated across the three trials per treatment and depicted according to treatment (white = "live," gray = "playback") and phase (PRE = pre-stimulus, STIM = stimulus, POST = post-stimulus). The black line indicates the estimated means of the models. Statistics for LMMs: significant interaction of condition and phase for *SDNN* **(B)**, *RMSSD/SDNN* **(C)**, *HF* **(D)**, $p < 0.05$.

because cattle have a relatively low sound-localization acuity compared with other mammals (Heffner and Heffner, 1992); the stroker's position relative to the animal's head may nevertheless be relevant.

Furthermore, the effects that we saw in STIM were not observed in POST, contrary to our hypothesis of longer-lasting effects of the treatment on behavior. However, some of the observed behaviors (such as *neck stretching* and the different ear positions) are more immediate reactions to positive stimuli and do not allow to observe longer-lasting changes in affective states.

Comparison of the Treatments

As there were no significant differences in the behavioral reactions to the two different auditory stimuli, stroking and talking in a gentle voice *per se* seem to have a stronger effect on the behavior than the source of the auditory stimulus. As this experiment did not include a treatment where the animals were stroked without any auditory stimulation, we cannot infer any information on whether gentle talking in general enhances or diminishes the positive effects of stroking, but the results are very similar to our previous study, where the animals were stroked without acoustic stimulation. Stroking can elicit quite strong effects on physiology and behavior in different species (rats: Holst et al., 2005; cows: Schmied et al., 2010; cats: Gourkow et al., 2014; lambs: Coulon et al., 2015; horses: Lansade et al., 2018), which might exceed possible consequences of small differences in auditory stimuli. Regarding the absence of significant differences in behavior, it seems plausible that the heifers did not discern the two auditory stimuli, at least not to an extent where it would have affected their behavior. Furthermore, the mismatch of experimenter and playback voice did not have a significant effect on any of the behaviors. Indeed, there is a substantial amount of literature in different species indicating that they do not necessarily distinguish playback from live auditory stimuli: playback is used successfully in studies investigating bird behavior (Douglas and Mennill, 2010), dogs react to dog-directed human speech played back from a loudspeaker (Ben-Aderet et al., 2017; Benjamin and Slocombe, 2018), and dairy cows increase their production when exposed to a playback of calf vocalizations (Pollock and Hurnik, 1978; McCowan et al., 2002; no effect if calves are reared with their mothers: Zipp et al., 2013). Other characteristics of speech might thus have a stronger impact on the animals' behavior than the characteristics induced by the type of source.

On the other hand, the analysis of cardiac parameters points toward a different perception of the two auditory stimuli. In both conditions, *HR* increased from PRE to STIM and decreased from STIM to POST, but this decrease was significantly more pronounced in the "live" condition, indicating a stronger relaxation effect of live talking after the presentation of the stimulus. The slight increase of *HR* during STIM in both conditions seems to contradict our expectation that our treatment would induce a low-arousal state. However, it is in line with previous findings reporting an increased *HR* of lying animals that were licked by conspecifics (Laister et al., 2011) or receiving a stroking treatment (Lange et al., 2020) and might be caused by physical reactions to stroking (e.g., *neck stretching*) more than by a meaningful change in arousal or affective state (Lange et al., 2020).

Independently of the changes in *HR*, there were some significant effects of the conditions on HRV parameters: *HF* increased in POST in the "live" condition, but decreased in POST in the "playback" condition. It is widely accepted that *HF* increases with increasing activity of the parasympathetic branch of the autonomic nervous system (Task Force of ESP and NASPE, 1996; von Borell et al., 2007). The increased values suggest a higher parasympathetic activity after stroking in the "live," but not the "playback" condition. An increased *HF* may be associated with positive emotions (McCraty et al., 1995; von Borell et al., 2007) and was found in horses regularly receiving a relaxing massage (Kowalik et al., 2017). This increase in *HF* was not accompanied by an increase in *RMSSD*, although both represent vagal activity and are often correlated (Task Force of ESP and NASPE, 1996; Hagen et al., 2005; von Borell et al., 2007; Shaffer et al., 2014). However, changes in *RMSSD* were not consistently observed in other studies investigating different affective states in animals (Reefmann et al., 2012; Travain et al., 2016). *RMSSD* might therefore be a suboptimal indicator of animal affective states (Gygax et al., 2013; Tamioso et al., 2018). A different pattern emerged for *SDNN*: values increased from PRE to STIM in the "live" condition, and decreased again in POST, whereas in the "playback" condition, *SDNN* reached its highest values in POST. *SDNN* reflects influences of both parasympathetic and sympathetic activity (von Borell et al., 2007; Shaffer et al., 2014). Together with the decrease of *RMSSD/SDNN* during live talking, these findings might indicate that the "live" condition led to higher sympathetic activity during stroking and talking, possibly indicating positive arousal in response to being stroked (Tamioso et al., 2018). The increase of *RMSSD/SDNN* in "live" in POST is in line with increased values observed in sheep being brushed by a familiar human (Tamioso et al., 2018), and, in combination with the observed increase of *HF* in POST in "live," indicates a shift toward vagal dominance after live talking. These patterns were not observed in the "playback" condition; contrarily, *SDNN* increased in POST, while *RMSSD/SDNN* and *HF* decreased slightly, possibly indicating a relative shift towards sympathetic regulation after stroking with "playback" stimulation.

In combination, the HRV results suggest that live talking may have been more pleasurable to the animals than "playback" and led to increased parasympathetic activity in the POST phase. They thus support the interpretation of a more pronounced relaxation effect indicated by the stronger decrease of *HR* in POST in "live" than in "playback." The difference between the two auditory stimuli might be caused by losses of lower and higher frequencies of recorded sound, which have been found to cause a decline in dog's responses to commands, especially in the absence of certain non-verbal cues (Fukuzawa et al., 2005). As we could not measure the actual sound pressure reaching the animals' ears directly, we can neither exclude the possibility that there might have been other systematic differences between the acoustic signals produced by two sources, such as consistent differences in volume, which might have contributed to eliciting higher or lower arousal. Another difference between the situations might have been produced by a subconscious change of the stroker's body language or attention toward the animal during live talking. However, stroker behavior was standardized as far as possible – in both conditions, the stroker was calmly sitting next to the heifer's shoulder, focused on stroking the animal. Great care was taken to match the "playback" condition not only in body posture and calm breathing, but also in mental focus and intention of interacting gently with the animal, trying to minimize possible differences in non-verbal communication.

We hypothesized that the higher degree of standardization in the "playback" stimulus would lead to decreased variability in the data. However, the variability of the responses as indicated by the precision parameters revealed a conflicting pattern, indicating that the relationship between the degree of standardization of the treatment and the variability in the observed behavior is more complex than expected or has different effects on different parameters. The higher degree of standardization in "playback" stimuli did not lead to a generally reduced variability and therefore should not be the main criterion for preference of playback stimuli for gentle human-animal interactions in experimental settings.

CONCLUSION

Our experiment leads to the conclusion that gentle stroking in combination with gentle vocal stimulation can induce positive affective states in habituated heifers, both when the experimenter is talking directly to the animal and when the vocal stimulus is played back from a recording. However, changes in cardiac parameters point toward a more positive experience and longer-lasting relaxation effects of live talking. Taking into account the inconclusive results regarding the effects of a higher degree of standardization on the variability of the data, we conclude that the use of recorded auditory stimuli to promote positive affective states in human-animal interactions in experimental settings is possible, but not necessarily preferable.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The animal study was reviewed and approved by Ethics and Animal Welfare Committee of Vetmeduni Vienna (Ethik und Tierschutzkommission), project number ETK-02/04/2017; Veterinärplatz 1, 1210 Vienna.

AUTHOR CONTRIBUTIONS

SL and SW: conceptualization. SL, SW, LB, AL, and AF: methodology. AL: formal analysis, visualization, and writing—original draft preparation. AL and LB: investigation. SL, SW, and AL: writing—review and editing. SW: supervision. SL: project administration and funding acquisition. All authors have read and agreed to the published version of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpsyg.2020.579346/full#supplementary-material

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2.2. Experiment B: Effects of different stroking styles on behaviour and cardiac parameters in heifers

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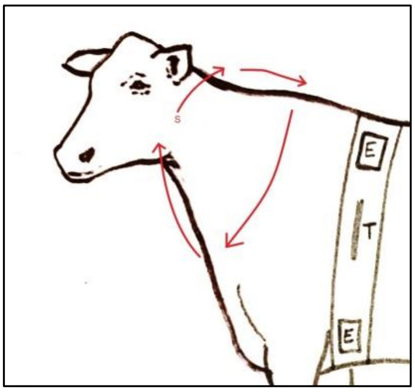

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Article

Effects of Different Stroking Styles on Behaviour and Cardiac Parameters in Heifers

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Simple Summary: Positive emotions can improve the welfare of animals. Humans can induce positive emotions in cattle via gentle interactions, such as stroking. While previous studies showed that stroking at the lower side of the neck elicited the most positive reactions in cows, cattle groom each other on different body regions and probably react to each other's signals. We compared the reactions of dairy heifers to two different stroking styles: stroking exclusively on the lower neck or stroking the whole head/neck region and reactively following the signals of the animal. For both styles, we observed longer durations of behaviours indicating positive emotions and relaxation during stroking, suggesting that the animals enjoyed the treatment. The different stroking styles led to differences in the positions of the heifers' ears: during "reactive" stroking, the animals held their ears longer in low positions, whereas during stroking of the lower neck, the ears spent longer pointing backwards-upwards. However, we did not observe significant differences in other behaviours, indicating that the manner of stroking of the head/neck region seemed to be not very important for the positive perception of stroking. We conclude that both ways of stroking can elicit positive emotions in cattle and increase the animals' well-being.

Abstract: Gentle animal–human interactions, such as stroking, can promote positive emotions and thus welfare in cattle. While previous studies showed that stroking at the ventral neck elicited the most positive reactions in cows, intra-specific allogrooming in cattle includes different body regions and is probably guided partly by the receiver. Thus, we compared heifers' (n = 28) reactions to stroking with the experimenter either reactively responding to perceived momentary preferences of the heifers or exclusively stroking the ventral neck. Independently of the stroking style, longer durations of neck stretching and contact occurred during stroking, supporting our hypothesis of a positive perception of stroking. We did not confirm the predicted decrease in heart rate and increase in heart rate variability, but instead found a slightly increased mean heart rate during stroking. The different stroking styles elicited differences in the heifers' ear positions: "reactive" stroking led to longer durations of low ear positions during stroking, while during "ventral neck" stroking, the duration of back up increased. However, no other behaviours differed significantly between different stroking styles, indicating that the exact manner of stroking applied in our treatments seemed to be less important in the promotion of positive affective states in cattle through gentle human–animal interactions.

Keywords: human–animal interactions; affective states; positive emotion; expressive behaviour; cattle welfare; stroking; ear positions

1. Introduction

The promotion of positive emotional states in animals has recently gained more attention in animal welfare science [1–3]. The emotional states and welfare of farm animals are strongly influenced by the relationships with the humans they interact with [4,5]. The animal–human relationship is determined by the relative strength of positive and negative emotions of the animal during its interactions with humans [6]. Gentle interactions between humans and animals can induce positive emotions [7–9], but are not always effective in doing so, depending on the exact manner of interaction or individual differences [10–12] Thus, identifying the characteristics of gentle interactions that enhance their positive perception will contribute to increasing the animals' wellbeing.

Human–animal interactions can occur through different sensory channels: visual, olfactory, tactile and auditory [6]. Tactile interactions are an important part of the social life of cattle, as evidenced by social licking [13] and its frequent solicitation [14,15]. There is evidence that tactile stimulation is perceived as positive by cattle also if delivered by humans [9,16,17], but the perception is influenced by the body region stroked [9,18].

In several previous studies [19,20] cattle were stroked on the ventral neck, which is the area that led to the most positive reactions compared with stroking of the withers or chest [9]; stroking of the ventral neck improved the animals' relationship with humans most effectively [18]. Focusing on one defined area allows for higher standardisation of stroking. However, intra-specific allogrooming in cattle includes different body regions [15,21], with animals often moving from one region to another [14], probably at least in part following the receiver's signals [21,22]. Therefore, reacting in a flexible way to the animal's behavioural indications of preference, and consequently stroking different body areas, would mimic the social behaviour of cattle more closely and might lead to more positive reactions. While studies in horses have shown that grooming styles reacting to the animals' signals lead to more positive reactions [23], there is no study investigating reactive stroking treatments in cattle yet.

The effects of human–animal interactions on animal affective states can be assessed using behavioural and physiological indicators [24]. One behaviour that is often observed in cattle during social licking [15,21] and during stroking or brushing by humans is neck stretching [16,17,19], which is therefore interpreted as indicating a positive perception. Recently, facial expressions have been investigated as potential indicators of affective states in animals (reviewed by Descovich et al. [25]). In cattle, ear positions and movements in particular have come into the focus of scientific research on affective states [26–29]. Social interactions and positive affective states can also affect cardiac parameters. Tactile stimulation in the form of social licking [15] or stroking on the ventral neck [9] has been shown to decrease the heart rate (HR) of cattle. Calculating heart rate variability (HRV) parameters allows for the investigation of changes in sympathetic or vagal activation [30].

We investigated the effects of two different forms of gentle tactile interactions on the behaviour and cardiac parameters of dairy heifers (n = 28) habituated to gentle interactions with humans. We compared the heifers' reactions to exclusively stroking the ventral neck with the reactions to stroking in a reactive way, i.e., with the experimenter including the whole head/neck region and focussing on parts for which the animal indicated a preference.

We hypothesised that both the "ventral neck" and the "reactive" treatment would elicit a positive, low-arousal state in habituated heifers and thus predicted a decrease of HR, an increase of HRV, and an increase of behavioural indicators of low arousal and positive valence. We expected some of these effects to last long enough to be still observed shortly after stroking. Furthermore, we hypothesised that stroking in a reactive way would be perceived as more positive than exclusively stroking the ventral neck. Lastly, we hypothesised that the higher degree of standardisation in the "ventral neck" treatment would lead to a lower variability in the data.

2. Materials and Methods

2.1. Animals, Housing and Management

The experiment was conducted between May and November 2017 on 28 heifers (27 Austrian Simmental, one Austrian Simmental ×Brown Swiss) kept at the young stock farm (Rehgras, Furth an der Triesting, Austria) of the University of Veterinary Medicine, Vienna. Out of approximately 90 heifers housed there, 32 heifers between 7 and 24 months of age were selected based on their positive animal–human relationship (see Section 2.2). According to their age, they were divided into two stable groups of 16 animals that were housed, fed and treated in the same way; they were kept mainly on pasture and brought into deep litter pens with adjoining outdoor runs only during adverse weather conditions and for testing. There, they were fed hay and a small quantity of concentrate. The animals had ad libitum access to water and mineral blocks.

The experiment was discussed and approved by the institutional ethics committee in accordance with the Good Scientific Practice guidelines and national legislation (project number ETK-02/04/2017).

2.2. Selection and Habituation

As we aimed to investigate positive emotions during human–animal interactions, a generally positive perception of close human contact was a prerequisite. The experimenters selected heifers that were actively seeking human contact when approached on pasture and accepted short periods of stroking. Most heifers were already habituated to the experimenters, procedures and equipment from a previous study. The remaining animals were carefully habituated to the camera (SONY HDR-CX730, Weybridge, UK) and HRV equipment (POLAR® Electro Oy, Kempele, Finland), as well as the experimenters (both female, green overalls; stroker: brown hair, 1.63 m; cameraperson: blonde hair, 1.60 m) and the stroking procedure. We used a stepwise habituation approach that developed from letting the heifers explore the experimenters over approaching them while talking in a gentle voice to slowly touching and finally stroking them. We aimed to stop the interaction before the animals showed any sign of avoidance. If needed, concentrate was provided as a food reward until it was possible to equip the free-moving heifers with the HRV girths and stroke the animals for 3 min without any visible signs of fear or walking away. Animals were considered fully habituated when a full 9-min trial (see Section 2.3, stroking of the ventral neck) could be performed without inducing any avoidance reactions. Tests were performed on 28 of the 32 pre-selected heifers.

2.3. Experimental Design

We applied a crossover design, i.e., each animal acted as its own control and was thus subjected to both treatments (see Section 2.4.2). To ensure robustness of the data, each animal experienced each treatment three times in an alternating pattern, i.e., in total six trials (trial numbers 1–6). Each trial consisted of three phases of 180 s (3 min) each: (1) pre-stroking (PRE), where the experimenter stood next to the animal so that baseline values could be recorded; (2) stroking (STR), with the experimenter either stroking in a reactive way ("reactive") by responding to perceived momentary preferences of the heifer or exclusively stroking the ventral neck ("ventral neck"); and (3) post-stroking (POST), where the experimenter was again standing next to the animal so that possible carry-over effects could be observed. Approximately half of the animals started with "ventral neck" stroking and the other half with "reactive" stroking. The experimenters aimed to balance the order of the treatments over each testing day, but due to trial repetitions, complete balancing was not always possible.

2.4. Experimental Procedure

2.4.1. General Procedure

All trials were carried out in a deep litter barn of 182 m^2 (min. 11 m^2/animal), which was familiar to all animals. Each animal was prepared and equipped for HRV measurement (POLAR®, S810i, Polar Elektro Oy, Kempele, Finland), as described previously [19]. All trials were conducted on lying animals during resting phases to minimise the influence of physical activity on cardiac parameters. Before starting a trial, the handler (i.e., stroker) started a POLAR® monitor and placed it in the pocket of the girth. When an animal had been lying for at least 5 min, the camera operator took up position approximately 2 m from the heifer with the camera approximately at the height of the heifer's eyes, filming the head/neck region from the heifer's left side, with special focus on the left eye and ear. The stroker assumed a standing position next to the animal's left shoulder and started the trial. The stroker wore rubber gloves with a rough surface and applied a constant, previously practiced pressure while stroking at a frequency of 40–60 strokes/min [9].

A trial was stopped after its completion of 9 min or aborted earlier at the occurrence of an event likely to influence the animal's emotional or physiological state (including obvious distractions, the animal standing up or falling asleep (i.e., sleeping position and eyes closed for >10 s) before or during stroking, self-grooming for more than 10 s, or being chased away or showing other social interactions with a herd member). If a trial was stopped, the experimenters waited for at least 1 h before testing the animal again.

2.4.2. Stroking Styles

In the "ventral neck" treatment, the heifer was stroked only on the ventral neck, as in previous studies [9,19]. In the "reactive" treatment, the stroker included the whole head/neck region, always starting behind the left jaw and following a predetermined route until the heifer showed a behaviour indicating a momentary preference, such as presenting a body part, (partly) closing the eyes, stretching the neck or leaning towards the stroker's hand. The stroker remained at the indicated area of the head/neck region for as long as the heifer showed the indicative behaviour. In both treatments, the animals were stroked continuously for 3 min with one hand.

2.5. Behavioural Observations

All trials were video recorded and the behaviour was analysed with the coding software Interact®, version 16.1.3.0. (Mangold International GmbH, Arnstorf, Germany), using focal animal sampling and continuous recording [31]. While it was not possible to conceal the treatment, the observers were blinded to the research questions and hypotheses, as well as to the experimental design; the videos were cut to contain one phase each so that the observers were also blinded towards the sequence of the phases. The observers recorded ear and head positions and movements, as well as other behaviour according to an ethogram (Table 1, for photographs of ear positions, see Supplementary Material, Figure S1). Two trained observers conducted the behavioural observations, where one observer analysed the ear positions (intra-observer reliability (IOR): Cohen's κ = 0.78) and the other observer analysed the other behaviours (IOR: Cohen's κ = 0.89–1.00).

Table 1. Ethogram adapted from References [9,32].

Behaviour [(1)]		Definition
Inactive ear posture [(2)]	Ear hanging	The ear loosely hangs downwards (referring to the ground). There is no visible muscle tension, leading often to a slightly bouncing movement when the position is assumed.
Active ear postures [(2)] [(3)]	Back up	The ear is held behind and above the latero-lateral axis.
	Back centre	The ear is held behind at the same height as the latero-lateral axis.
	Back down	The ear is held behind and below the latero-lateral axis.
	Centre up	The ear is held perpendicular to the head and above the latero-lateral axis.
	Centre	The ear is held perpendicular to the head along the latero-lateral axis.
	Centre down	The ear is held perpendicular to the head and below the latero-lateral axis.
	Forward up	The ear is held in front of and above the latero-lateral axis.
	Forward centre	The ear is held in front of and at the same height as the latero-lateral axis.
	Forward down	The ear is held in front of and below the latero-lateral axis.
	Ear flicking	The ear is quickly (within max. 0.5 s) moved back and forth at least once. The behaviour is coded until one of the other ear postures is clearly visible again. The residual movement after the active movement is still part of ear flicking.
Head/neck postures	Held without touching	The head is actively held up and does not touch the stroker.
	Held with touching	The head is actively held up and touches the stroker.
	Rest head without touching	The heifer does not actively carry the head’s weight. The heifer’s head is in contact with the ground, barn equipment, another animal or with the heifer’s leg(s). The heifer’s head is not in contact with the stroker.
	Rest head with touching	The heifer does not actively carry the head’s weight. The heifer’s head is lying on the ground, barn equipment, another animal or the heifer’s leg(s) while being in contact with the stroker, or it is lying on the stroker’s leg.
	Head shaking/tossing	Successive quick movements of the head. The movements can be rotational or up and down.
	Neck stretching	Positioning neck and head actively in an outstretched line, either up, down, or forward.
Eyes [(4)]	Open	The iris is at least partly visible.
	Closed	The iris is not visible at all for longer than 0.5 s.
	Not visible	Neither eye is visible.
Miscellaneous	Rubbing the stroker	The heifer touches the stroker and moves the touching body part while in contact with the experimenter. The behaviour ends when the contact between the heifer and the person is interrupted for at least 3 s.
	Rubbing	The heifer moves the head/neck region while in contact with the ground or barn equipment. The behaviour ends when the contact between the heifer’s head/neck region and the ground/equipment has ended.
	Nose close	The heifer moves her muzzle towards the stroker within a range of 5 cm. The behaviour ends when the heifer’s nose does not point towards the stroker anymore, leaves the range of 5 cm or if another behaviour of the “miscellaneous” category starts.
	Licking the stroker	The heifer’s tongue touches the stroker at least once. The behaviour ends when the heifer’s tongue does not touch the stroker again within 3 s.
	Ruminating	The heifer’s jaw moves regularly sideways with a frequency of about one movement per second. This movement is recorded as rumination if it occurs in a series of at least five movements (which may start before and end after the observation). Rumination ends when the jaw movement is paused for more than 10 s.
Calculated measures	Contact	The time in which the heifer’s head and neck area was in contact with the stroker. Sum of durations of “rest head with touching”, “held with touching”, “nose close”, “rubbing experimenter” and “licking experimenter”, not including contact established by stroking.
	Resting head	Sum of durations of “rest head with touching” and “rest head without touching”.
	Ear low	The sum of the durations of the ear hanging or held below the latero-lateral axis (“hanging” + “back down” + “centre down” + “forward down”).
	Changes of ear positions	Sum of the frequencies of different ear positions per trial minus 1.

[(1)] All behaviours were coded as durations, except changes of ear positions (count data). [(2)] The left ear was recorded; if it was not visible, the right ear was recorded. [(3)] The latero-lateral axis refers to an imaginary line between the bases of the ears. “Behind” means the ear is pointing towards the back of the head, “in front” refers to the rostral end of the head, “above” describes the ear pointing towards the dorsal and “below” towards the ventral part of the head. If the observed ear was moved by the experimenter, the position before the movement was recorded until the next unambiguous ear posture was assumed. [(4)] The left eye was recorded; if it was not visible, the right eye was recorded.

2.6. Heart Rate Measurements

Data were error-corrected and processed according to Hagen et al. [33] using the Polar Precision Performance Software, version 4.03.050 (Polar Electro Oy, Kempele, Finland), and Kubios, version 2.0 (Biosignal Analysis and Medical Imaging Group, Department of Applied Physics, University of Eastern Finland, Kuopio, Finland). To account for the respiratory rate, frequency bands were set to 0.04–0.2 Hz for the low frequency band and 0.2–0.58 Hz for the high frequency band [30]. The following parameters were statistically analysed: mean heart rate (HR); time domain: standard deviation of the inter-beat intervals (SDNN) and square root of the mean squared differences of successive inter-beat intervals (RMSSD); frequency domain (using a fast Fourier transform): normalised powers of high (HF) and low frequency (LF), LF/HF power ratio (LF/HF).

2.7. Statistical Analysis

2.7.1. Behavioural Data

For the statistical analysis of behavioural data, we used the software package R, version 3.5.2 [34]. The durations of behaviours that occurred often enough to be suitable for analysis were transformed to proportions by dividing them by the total time in which they could be observed. To account for the fact that the ear positions are mutually exclusive and their proportions always amount to one, we tried to fit compositional models but the large amount of zeros led to convergence problems. Therefore, we selected the four ear positions that were observed often enough for statistical analysis (median duration in s (min–max): back up, 122 (0–180); back centre, 8 (0–180); centre, 1 (0–170); forward up, 0 (0–148)). They were analysed using generalised linear mixed models (GLMMs) [35] with a beta error structure and logit link function [36,37] using the package "glmmTMB", version 0.2.3 [38]. To rule out that values of the responses were exactly zero or one, the response variables were transformed according to $(y \times (n - 1) + 0.5)/n)$, where y is the original response and n the number of observations [39]. The hanging ear position and the other down positions did not occur often enough to be evaluated statistically on their own (median duration in s (min–max): hanging 0 (0–2)). Thus, we calculated the variable low by summing up the durations of down positions (hanging + back down + centre down + forward down; summed up to low 0 (0–160)). The result was still dominated by zeros, causing difficulties with the beta error distribution; therefore, it was dichotomised (occurrence: yes/no) and analysed using a GLMM with a binomial structure. The sample sizes for models were 516 total measures made for 28 individuals in a total of 172 trials with 3 phases each. For all full models, we included treatment, phase and their interaction as fixed effects, and individual as well as trial ID (trial number nested in individual) as random effects. Trial ID was included as a random effect to account for the fact that each trial consisted of three phases and thus contributed three data points, where it seemed plausible to assume that there was random variation between the trials. We included random slopes within individual for trial number (to account for possible habituation effects with treatment repetition), treatment and phase to allow their effects to vary between individuals [40]. Due to convergence problems with the model for ear flicking, full statistical analysis of this parameter was not possible and the results were inspected graphically.

Since the "ventral neck" stroking style involved a higher degree of standardisation than the "reactive" stroking style, it seemed plausible that the variation in a given observed behaviour would be smaller in the "ventral neck" treatment than in the "reactive" treatment. We explicitly estimated this potential effect by modelling the precision parameter of the response as a function of treatment in each model. Beta distributions can be characterised by a mean and dispersion parameter describing the variation in the distribution around its mean. However, since the variation in the response is actually inversely related to the dispersion parameter, we henceforth label it a precision parameter since a larger precision parameter means a greater concentration of the variable around its mean. Therefore, with a higher degree of standardisation in "ventral neck" stroking, we expected smaller variation in behaviours, and thus, larger estimated precision parameters.

To avoid cryptic multiple testing [41], we compared each full model with a respective null model that lacked the variables of interest (phase and the interaction of phase and treatment) but was otherwise identical. We used a likelihood ratio test (R function "anova") for these comparisons. The significance of the individual effects was determined by dropping them one at a time and using a likelihood ratio test to compare the resulting models to the full model [40]. Values of $p \leq 0.05$ are referred to as significant, and $0.05 < p \leq 0.1$ as a trend [42]. If the full–null model comparison was significant, non-significant interactions were removed from the models and reduced models were fitted. The significant main effects of treatment are not discussed, as they were not of interest and thus not part of the full–null model comparisons. We determined 95% confidence limits using the function "simulate.glmmTMB" of the "glmmTMB" package.

We assessed the model stability by comparing the estimates of models based on the full dataset with estimates of models fitted to subsets where the levels of the random effects were dropped one at a time [43]. This revealed a fairly good stability of the models, with the exception of the model for low ear positions: in this binomial model, the exclusion of levels of random effects led to more extreme estimates. However, as the direction of these extreme estimates remained the same as in the model for the full dataset, this did not change our interpretation.

Over-dispersion was not an issue in most of the models (range of dispersion parameters: 0.69–1.16; high values indicate high dispersion). In the case of the two models that were overdispersed (contact and forward up), we report standard errors and *p*-values corrected for overdispersion (based on Wald's z-approximation, therefore no degrees of freedom are indicated and χ^2s were replaced by z-values) [44].

The variables rumination, changes of ear positions and resting head were not analysed using statistical tests, but inspected graphically, as no directed hypotheses could be formulated based on the available literature, however the behaviours might still be affected by emotional state.

For graphical depiction, we used the R packages "ggplot2" [45] and "cowplot" [46]. Data were depicted as Tukey-style boxplots for each treatment and phase, using the mean values of behaviours per animal (averaged across the three trials per treatment). The bold line corresponds to the median; the lower and upper lines of the box to the first and third quartile, respectively; and the whiskers correspond to the lowest and highest values that were still within 1.5 × interquartile range from the margins of the box. Outliers (all values outside of 1.5 × interquartile range) are depicted as circles.

2.7.2. Cardiac Data

Due to technical failure during HRV recording, we obtained a sample size of 27 animals. Cardiac variables were analysed using linear mixed models (LMMs), including treatment; phase and their interaction; age (days); time of day and its quadratic, third and fourth polynomials; HR (unless it was the response variable) and duration of rumination (s) as fixed effects. Trial number nested in animal nested in housing group was considered as a random effect. Heart rate was included as a fixed effect in order to avoid double presentation of the same findings as it is associated with the HRV characteristics [47,48]. By including HR in the models, the results represent the influence of the other independent factors on HRV parameters independently from their influence on HR. The model assumptions were checked via visual inspection of the residuals, and HR, RMSSD, SDNN, HF and LF/HF ratio were log-transformed to meet the assumption of a normal distribution. Likelihood-ratio tests were used to compare models, including the time of day with the models without time and its polynomials; if the difference was not significant, the time of day was excluded from the model. For further model selection, the Akaike information criterion (AIC) was used. Fixed effects (except for the variables of interest treatment, phase and their interaction) were removed from the model if their removal did not result in an increase of the AIC of the resulting model. A false discovery rate control (FDRC) was performed using the Benjamini-Hochberg correction for multiple testing [49] with $n = 6$ and d = 0.05 for significant differences; d = 0.1 was used for trends. The results of the analysis of covariates are not reported as doing so is beyond the scope of this paper.

3. Results

3.1. Behaviour during Gentle Interactions

Following the animals' preferences in the "reactive" stroking style led to varying durations of stroking of the different areas of the head/neck region (Figure 1); the dorsal neck was stroked most for the longest duratio[n] … [m]uzzle, forehead, ear, poll and back were …

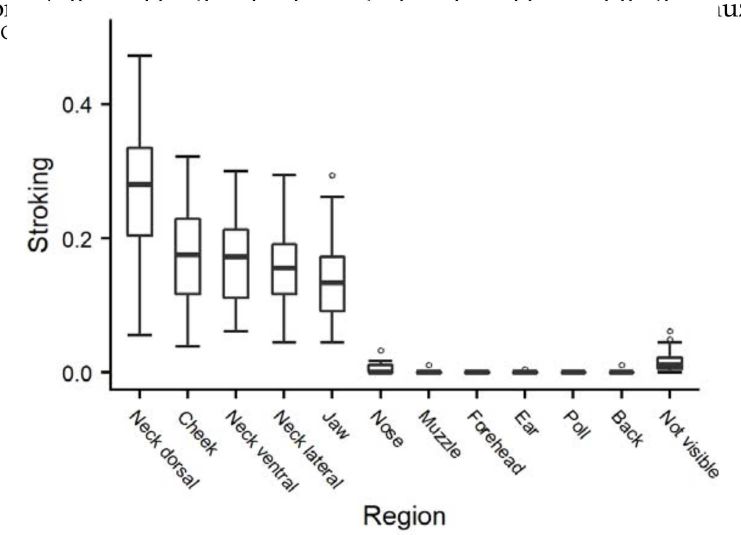

Figure 1. Durations (as a proportion of the total time observed) of stroking for each area of the head/neck region of heifers (n = 28) in the "reactive" treatment, averaged across the three trials per animal.

As behaviours indicating positive affective states, we statistically analysed the behaviours neck stretching, contact, eye closed and ear flicking (Figure 2; median duration in seconds (min–max): neck stretching 0 (0–180), contact 0 (0–159), eye closed 12 (0–180); Figure 3f: ear flicking 2 (0–68)).

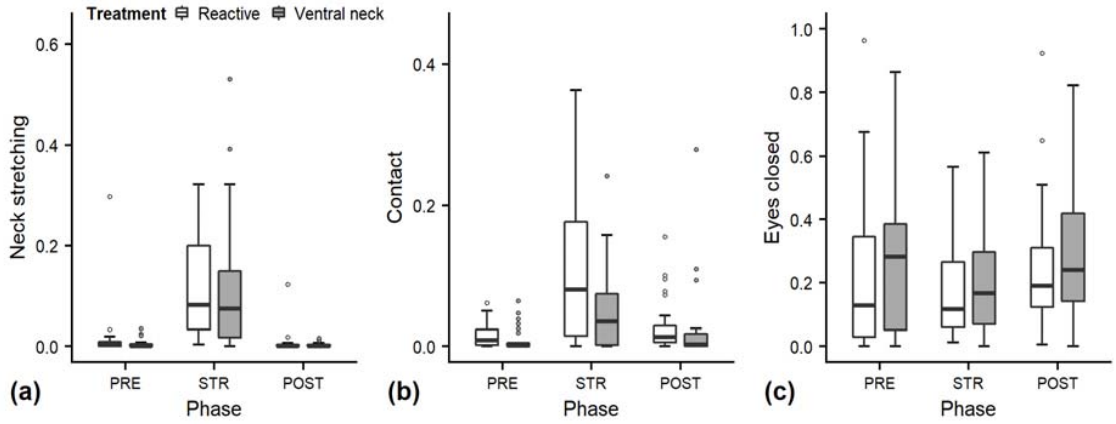

Figure 2. Mean durations (as a proportion of the total time observed) of neck stretching (**a**), contact (**b**) and eyes closed (**c**) of heifers (n = 28) during the experimental trials. Means were calculated across the three trials per treatment and are depicted according to the treatment used (white = "reactive", dark grey = "ventral neck") and phase (PRE = pre-stroking, STR = stroking, POST = post-stroking). Statistics for GLMMs: significant main effect of phase for neck stretching (**a**) and contact (**b**), $p < 0.001$. Note that the y-axis scale varies to allow for sufficient resolution for rare behaviours.

Full and null models differed significantly for the response variables neck stretching and contact (Figure 2; GLMM: neck stretching: χ^2 = 28.838, df = 4, $p < 0.001$; contact: χ^2 = 16.336, df = 4, p = 0.003), as well as for all the tested ear positions (Figure 3; back up: χ^2 = 55.738, df = 4, $p < 0.001$; back centre: χ^2 = 27.717, df = 4, $p < 0.001$; ear low: χ^2 = 29.458, df = 4, $p < 0.001$; centre: χ^2 = 15.010, df = 4, p = 0.005; forward up: χ^2 = 10.294, df = 4, p = 0.04). The full-null model comparison revealed no significant difference for eyes closed (χ^2 = 4.532, df = 4, p = 0.3).

$\chi^2 = 27.177$, df = 4, $p < 0.001$; ear low: $\chi^2 = 29.458$, df = 4, $p < 0.001$; centre: $\chi^2 = 15.010$, df = 4, $p = 0.005$; forward up: $\chi^2 = 10.294$, df = 4, $p = 0.04$). The full–null model comparison revealed no significant difference for eyes closed ($\chi^2 = 4.532$, df = 4, $p = 0.3$).

Effects of the phase on ear positions depended on the treatment for back up ($\chi^2 = 30.100$, df = 2, $p < 0.001$), back centre ($\chi^2 = 23.835$, df = 2, $p < 0.001$) and ear low ($\chi^2 = 24.324$, df = 2, $p < 0.001$). During "ventral neck" stroking, the durations of back up increased (Figure 3a), durations of back centre (Figure 3b) decreased and durations of ear low did not change substantially, while during "reactive" stroking, no clear changes occurred for back up, and durations of both back centre and ear low increased (Figure 3c).

The interaction of treatment and phase did not have a significant effect on any of the other behaviours. The reduced models for neck stretching and contact revealed a significant main effect of phase (neck stretching: $\chi^2 = 27.527$, df = 2, $p < 0.001$; contact: $z = 2.996$, $p = 0.003$), with increases during STR independent of stroking style (Figure 2a,b). Phase also had a significant effect on the ear positions centre and forward up (centre: $\chi^2 = 12.350$, df = 2, $p = 0.002$; forward up: $z = -2.852$, $p = 0.004$); during STR, durations of both ear positions decreased independently of stroking style (Figure 3d,e).

The variability was significantly smaller in the "ventral neck" treatment for contact ($\chi^2 = 4.851$, df = 1, $p < 0.001$) and the ear position centre ($\chi^2 = 11.192$, df = 1, $p < 0.001$), but higher for neck stretching ($\chi^2 = 5.258$, df = 1, $p = 0.022$). For statistical details, including model coefficients, standard errors and confidence intervals, see Supplementary Material (Table S1).

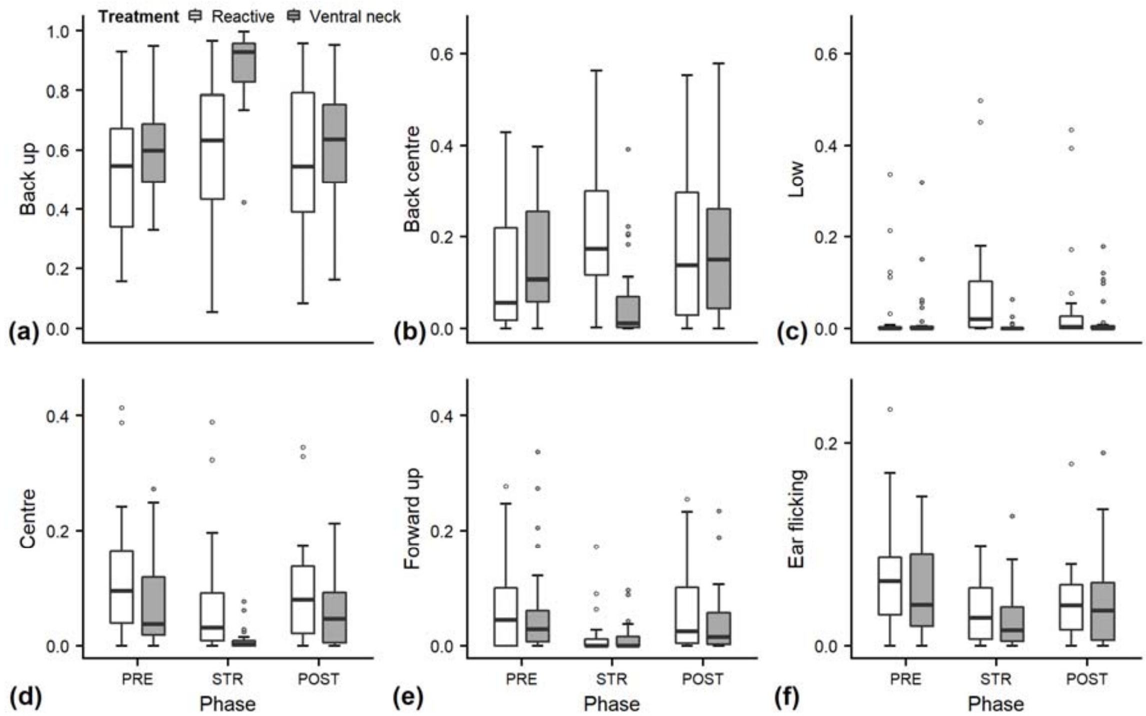

Figure 3. Durations of ear positions of heifers ($n = 28$) (as a proportion of the total time observed) during the experimental trials. Means were calculated across the three trials per treatment and are depicted according to the treatment used (white = "reactive", dark grey = "ventral neck") and phase (PRE = pre-stroking, STR = stroking, POST = post-stroking). Statistics for GLMMs: significant effect of treatment × phase for back up (**a**), back centre (**b**) and ear low (**c**), $p < 0.001$; significant main effect of phase: centre (**d**) and forward up (**e**), $p < 0.05$. Ear flicking (**f**) was not evaluated statistically. Note that the y-axis scale varies to allow for sufficient resolution for rare ear positions.

As there were problems with model convergence, ear flicking was only evaluated at the descriptive level, along with head resting, rumination and changes of ear positions (Figure 4). Ear flicking (Figure 3f) and changes of ear positions (Figure 4a) had numerically lower values in STR than PRE and POST. There was no conclusive pattern for the duration of rumination (Figure 4c). The duration of resting head increased numerically over the three phases in the "reactive" treatment but not in the "ventral neck" treatment (Figure 4b). The number of trials stopped because heifers stood

There was no conclusive pattern for the duration of rumination (Figure 4c). The duration of resting head increased numerically over the three phases in the "reactive" treatment but not in the "ventral neck" treatment (Figure 4b). The number of trials stopped because heifers stood up during the stroking phase with the approaching person was higher in the "ventral neck" treatment (*n* = 7) than the "reactive" treatment (*n* = 4).

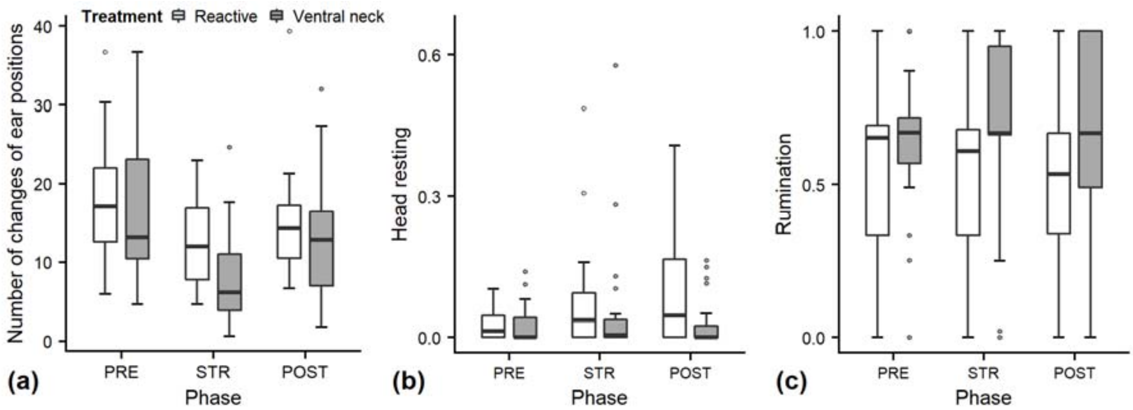

Figure 4. Means of changes of ear position (**a**), head resting (**b**) and rumination (**c**) of heifers (*n* = 28). (**a**): number of changes of ear positions; (**b**,**c**): durations of behaviour as a proportion of the total time observed. Means were calculated across the three trials per treatment and are depicted according to the treatment used (white = "reactive", dark grey = "ventral neck") and phase (PRE = pre-stroking, STR = stroking, POST = post-stroking). Note that the y-axis scale varies to allow for sufficient resolution for rare behaviours.

3.2. Cardiac Data

Phase affected the HR of the animals independently of treatment (Table 2, LMM; $\chi^2 = 47.0$, df = 2, $p < 0.05$); the HR increased slightly (Table 3, estimated increase of 2.04 bpm in "reactive" compared with 1.60 bpm in "ventral neck") in STR compared with PRE, but not in POST compared with PRE, according to the contrasts. There was no other significant effect of phase, treatment or the interaction of treatment and phase on any response variable, but a trend towards a main effect of phase on LF/HF with a decrease in STR (Table 2, $\chi^2 = 7.0$, df = 2, $p < 0.1$).

Table 2. Results of statistical analysis of heart rate (HR) and heart rate variability (HRV) parameters of heifers (*n* = 28). Results that remained significant after false discovery rate control (FDRC; *p* < 0.05) appear in bold, trends (*p* < 0.1) in italics; statistics: LMMs. SDNN: standard deviation of the inter-beat intervals, RMSSD: square root of the mean squared differences of successive inter-beat intervals, LF: normalised power of low frequency, HF: normalised power of high frequency.

Parameter	Treatment			Phase			Treatment × Phase		
	χ^2	df	*p*	χ^2	df	*p*	χ^2	df	*p*
HR (bpm)	1.14	1	0.29	**46.99**	**2**	**0.00**	1.67	2	0.43
SDNN (ms)	0.15	1	0.70	1.57	2	0.46	1.88	2	0.39
RMSSD (ms)	0.16	1	0.69	0.78	2	0.68	0.88	2	0.64
LF (ms²)	0.18	1	0.68	4.64	2	0.10	0.67	2	0.72
HF (ms²)	0.08	1	0.79	5.58	2	0.06	0.54	2	0.76
LF/HF	0.13	1	0.71	*6.99*	*2*	*0.03*	0.95	2	0.62

Table 3. Estimated marginal means, standard errors (SE) and lower and upper confidence limits (CL_L, CL_U) of HR and HRV parameters of heifers (*n* = 27) for each treatment and phase. PRE = pre-stroking, STR = stroking, POST = post-stroking.

		PRE				STR				POST			
Parameter	**Treatment**	**Mean**	**SE**	**CL_L**	**CL_U**	**Mean**	**SE**	**CL_L**	**CL_U**	**Mean**	**SE**	**CL_L**	**CL_U**
HR (bpm) (1)	Reactive	81.1	2.3	56.8	115.8	83.3	2.3	58.3	119.0	81.6	2.3	57.1	116.5
	Ventral neck	83.0	2.3	58.3	118.1	84.7	2.3	59.5	120.5	83.0	2.3	58.3	118.0
SDNN (ms) (1)	Reactive	27.3	2.0	10.8	68.6	25.6	1.9	10.2	64.3	26.6	1.9	10.6	66.9
	Ventral neck	26.6	1.9	10.9	64.9	25.5	1.8	10.4	62.3	24.2	1.7	9.9	59.0
	Reactive	14.7	1.3	4.6	46.3	14.1	1.3	4.5	44.3	14.2	1.3	4.5	44.9

Table 3. Estimated marginal means, standard errors (SE) and lower and upper confidence limits (CL_L, CL_U) of HR and HRV parameters of heifers (*n* = 27) for each treatment and phase. PRE = pre-stroking, STR = stroking, POST = post-stroking.

		PRE				STR				POST			
Parameter	**Treatment**	**Mean**	**SE**	**CL_L**	**CL_U**	**Mean**	**SE**	**CL_L**	**CL_U**	**Mean**	**SE**	**CL_L**	**CL_U**
HR (bpm) [(1)]	Reactive	81.1	2.3	56.8	115.8	83.3	2.3	58.3	119.0	81.6	2.3	57.1	116.5
	Ventral neck	83.0	2.3	58.3	118.1	84.7	2.3	59.5	120.5	83.0	2.3	58.3	118.0
SDNN (ms) [(1)]	Reactive	27.3	2.0	10.8	68.6	25.6	1.9	10.2	64.3	26.6	1.9	10.6	66.9
	Ventral neck	26.6	1.9	10.9	64.9	25.5	1.8	10.4	62.3	24.2	1.7	9.9	59.0
RMSSD (ms) [(1)]	Reactive	14.7	1.3	4.6	46.3	14.1	1.3	4.5	44.3	14.2	1.3	4.5	44.9
	Ventral neck	14.2	1.3	4.7	43.6	13.1	1.2	4.3	40.1	14.0	1.2	4.6	42.8
HF (ms^2) [(1)]	Reactive	12.2	1.4	2.7	54.6	15.2	1.8	3.4	67.5	12.2	1.4	2.7	54.5
	Ventral neck	11.7	1.3	2.8	48.6	14.0	1.6	3.4	58.3	12.6	1.4	3.0	52.1
LF (ms^2)	Reactive	71.8	2.5	40.3	103.4	68.9	2.5	37.5	100.3	74.5	2.5	43.1	106.0
	Ventral neck	73.0	2.3	43.2	102.9	68.9	2.4	39.0	98.8	72.8	2.3	43.0	102.7
LF/HF [(1)]	Reactive	5.6	0.8	0.9	36.2	4.3	0.6	0.7	27.3	6.0	0.9	0.9	37.9
	Ventral neck	6.0	0.8	1.0	35.2	4.8	0.7	0.8	27.9	5.6	0.8	1.0	32.6

[(1)] Back-transformed from log-scale using the R package "emmeans" [50].

4. Discussion

In line with our hypothesis, different stroking styles ("reactive" vs. "ventral neck") elicited differences in the heifers' ear positions. However, no other behaviours differed significantly in reaction to stroking with different stroking styles. Independently of the stroking style, the heifers reacted with longer durations of neck stretching and contact and decreased durations of the ear positions centre and forward up during STR compared with PRE, supporting our hypothesis of a positive perception of stroking. We did not confirm the predicted changes in HR and HRV, but instead found a slightly increased mean HR during stroking, and no changes in HRV parameters.

4.1. General Effects of Gentle Tactile Interactions on Behaviour and Cardiac Parameters

We found a significant effect of phase on behaviours indicating a positive affective state during STR for both stroking styles. For instance, the duration of neck stretching increased from PRE to STR. Neck stretching is shown by cattle during intraspecific social grooming [13,21,22] after they actively solicited it, and during stroking by humans [9,19,51] after they voluntarily approached them. It can thus be assumed that the situation is perceived as positive and neck stretching can be interpreted as a sign of enjoyment. The animals also established physical contact with the stroker for longer durations in STR than in PRE. This concurs with other studies where calves leaned against the brush during brushing by a human [17] and heifers approached and proactively offered body parts to a human during positive tactile contact [16]. Following the concept that animals seek out situations of positive valence [2,52,53], seeking proximity to humans indicates that our stroking treatment was perceived as positive.

We expected to induce a low-arousal state during STR. Surprisingly, the mean HR of the animals was significantly higher during STR than PRE; however, the increase was low with less than 2 bpm on average. Although this finding contradicts our hypothesis of a decrease of arousal through stroking, it is in line with the slightly accelerated HR found in animals licked by conspecifics while lying [15]. Since the animals were lying for a minimum of five minutes before we started a trial, we can assume that they were already in a low-arousal state. This is reflected in the low values of baseline HR (raw data, mean ± SD: "reactive" 75 ± 9 bpm, "ventral neck" 77 ± 8 bpm) that were found in PRE and fall below the reported HR of standing cattle that reacted with HR decreases to allogrooming [15,54]. Such low baseline values might have caused a physiological floor effect, where a further decrease of HR is quite impossible, even if stroking is perceived as calming. Additionally, compared to resting in PRE, any physical reaction to the stroking treatment (such as neck stretching, seeking contact to the stroker or presenting body parts) would lead to an increase of HR and might therefore mask the calming effect of stroking. In conclusion, our hypothesis that both stroking styles would elicit a positive, low-arousal state can only be confirmed with regard to valence, but not to arousal. Although there was an effect of phase on HR, there was none on HRV parameters that would have surpassed the effect of the phase on HR. Thus, the stroking of lying heifers did not seem to exert an additional psychophysiological effect on the autonomous nervous system, likely due to the already existing low-arousal, relaxed state and the dominance of vagal regulation during rest [30].

To meaningfully compare our results regarding ear positions with previous findings, differences in the definitions of ear positions need to be considered. Often, specific discrete ear postures are defined [26,32] and their frequency or duration is recorded, which means that ear positions divergent from the predefined postures might not be recorded or analysed. It is not reported which degree of divergence from the definition is allowed for an ear position to still be included in that definition. To cover the continuous spectrum of possible ear positions, we described them according to their position along the vertical and the horizontal axis. This resulted in nine different ear positions: back up, back centre, back down, centre up, centre, centre down, forward up, forward centre and forward down, which were then analysed for their duration, plus ear flicking and ear hanging. This different way of defining ear positions, in our opinion, better reflects the continuous nature of ear positions, but leads to a reduced comparability of our findings with previous studies.

Looking at the proportions of the individual ear positions, we found a decrease of centre and forward up during STR. Erect ears and ears directed forwards have been associated with heightened attention or high-arousal states in dairy cows [28,29]. A decrease of these positions might indicate reduced vigilance or a decrease in arousal during the stroking phase. The graphs show a similar pattern for changes of ear positions and ear flicking, which have numerically lower values during STR. Frequent changes of ear positions were found in reaction to a presumably negative, high-arousal situation in sheep [55] and in dairy cows [27], but also during a positive, presumably low-arousal stroking situation [32]. Ear flicking is a behaviour that is mostly associated with negative affective states [56,57] or reactions to insect attacks [58]. Changes of ear positions and ear flicking should therefore be investigated further as indicators of emotional state.

The effects of the treatment on behaviour and cardiac activity that we saw in STR were not observed in POST, indicating that the positive effects of stroking in lying heifers did not last long enough for carry-over effects to be observed. Some of the observed behaviours (such as neck stretching) are more immediate reactions to positive stimuli and do not allow observation of longer-lasting changes in affective states.

4.2. Effects of Stroking Style

Responding to the animals' signals in the "reactive" stroking style resulted in the longest duration of stroking on the areas of dorsal neck, cheek, ventral neck, lateral neck and jaw (order according to descending duration). This distribution between the neck and head during "reactive" stroking is quite similar to the one found during allogrooming ([21]: neck 65%, head 25% of total duration), which may indicate that the stroker correctly identified the animals' preferences.

Nevertheless, we found only limited support for our hypothesis that "reactive" stroking would elicit a more positive emotional state than stroking the ventral neck only. The two different tactile stimuli did not lead to significant differences in behaviours or cardiac parameters, except for ear positions. Animals stroked in a "reactive" style showed an increase in low ear positions and in back centre during STR, while animals stroked at the ventral neck showed a significant and strong increase in back up with a concurrent decrease in back centre.

The significant increase of low ear positions during STR in the "reactive" treatment partly confirmed our prediction of lower ear positions during the low-arousal state elicited during STR. However, there was no increase of ear low during "ventral neck" stroking. While we found a similar HR in "reactive" stroking as in "ventral neck" stroking, ear low only increased during "reactive" stroking, possibly indicating that low ear positions are reflecting not only arousal, but must be influenced by other factors as well, such as affective valence or attention. However, low ear positions generally occurred for small proportions of time and far less often than expected. In previous studies, dairy cows showed hanging ears for about 5%–65% of the time [9,32]. Reasons for the short durations of low ear positions, especially ear hanging, in our experiment might be specific to our study population: unlike other studies, which were conducted on adult cattle, we worked with young stock, who might show shorter durations of low ear positions due to a higher reactivity [59]. There are no studies yet investigating the relation of age and low ear positions in cattle.

By far the most common position in our study was the back up position. One factor that possibly influenced the position of the heifers' ears was the location of the stroker. In our study, the stroker was kneeling beside the animal's shoulder, possibly causing the heifer to turn her ears backwards and upwards while directing attention to the human. However, the stroker's position was the same for both stroking styles, but back up increased significantly during the stroking phase only in the "ventral neck" treatment. When interpreting this position, the aforementioned differences in definitions across literature must be taken into account. Backwards ear positions have been found to be associated with both negative and positive affective states. They are part of the facial expressions shown by cattle in pain [60]. However, in a study with a similar design [32], more "back" positions occurred during a low-arousal, positive situation similar to our stroking phase, which might correspond to our result regarding the back up position; however, neither study differentiated the height of the ear in the "back" ear position and it is unclear what position was recorded if the ear was held both backwards and upwards.

One study that defined a position similar to our back up position found higher frequencies during positive states, such as using the brush or feeding compared to queuing to be milked, and suggested it might indicate a higher-arousal positive state than ears back down [26]. The significant increase of back up and the concurrent decrease of back centre that we observed during stroking in the "ventral neck" treatment might thus indicate a higher-arousal state during "ventral neck" stroking than in "reactive" stroking. However, this was not supported by the HR values, which did not differ between the stroking styles. In general, this absence of differences in HR between treatments indicates that the different distributions of ear positions occurring with different stroking styles were probably influenced by factors other than arousal. Therefore, ear positions could be helpful indicators of subtle differences in the valence of affective states of cattle in the future. However, especially regarding the lack of significant differences of HRV or behavioural parameters between the two treatments in our study and the ambiguous findings of previous studies, more research is necessary before making clear interpretations towards the meaning of different ear positions with regard to valence.

One possible alternative explanation for the lack of other differences between the two stroking treatments could be that during "reactive" stroking, the ventral neck was also stroked. An individual might thus have also been stroked mainly at the ventral neck in the "reactive" treatment if it indicated such a preference, which could have led to the absence of obvious differences between the two treatments. However, over all trials of the "reactive" treatment, stroking was performed for the longest durations in the region of the dorsal neck, followed by the cheek, ventral neck, lateral neck, jaw and nose, indicating that there was a meaningful difference between the stroking treatments. Another explanation for the lack of differences lies in a potential masking effect: stroking on the neck might specifically elicit neck stretching as a direct reaction in the case of a positive perception, while stroking another region may induce other behavioural signs of positive perception as well. It is possible that animals perceived the interaction as more pleasant during "reactive" stroking than during "ventral neck" stroking, but because the neck was stroked for shorter durations of the overall treatment time, less neck stretching was induced and similar durations of neck stretching occurred with both stroking styles.

Regarding the behaviours evaluated at the descriptive level, the duration of resting head increased numerically over the three phases in the "reactive" treatment but not in the "ventral neck" treatment, which is comparable with the results of ear low. This behaviour should be recorded and evaluated statistically in future studies.

The conflicting results regarding the variability indicate that the relationship between the degree of standardisation of the treatment and the variability in the observed behaviour is more complex than expected or the standardisation has different effects on different parameters. The higher degree of standardisation in the "ventral neck" treatment did not necessarily lead to a reduction in variability and therefore should not be the sole criterion for the selection of stroking style for gentle human–animal interactions in experimental settings.

5. Conclusions

Although we found some differences in ear positions depending on the stroking style, the exact manner of stroking did not have a strong influence on the perception by the animal and thus seems to be less important. Our study supports previous studies indicating that gentle tactile interactions with cattle, provided that the animals have a good relationship with humans, can induce positive emotional states and thus improve their welfare.

Supplementary Materials: The following are available online at http://www.mdpi.com/2076-2615/10/3/426/s1. Figure S1: Example photographs of ear positions, Figure S2: Example photographs of ear positions with lines indicating (a) the vertical axis (yellow, through the poll and the caudo-ventral edge of the mandible angle) and (b) the horizontal axis (red, between the bases of the ears), Table S1: Full and reduced models for the different behaviours of the heifers (n = 28): comparison between the different stroking styles over the three phases.

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2.3. Experiment C: Effects of restraint during gentle human-animal interactions

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Effects of restraint on heifers during gentle human-animal interactions

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ABSTRACT

Gentle human-animal interactions can induce positive emotions in cattle and enhance their welfare. We investigated whether a change in the animals' perceived control over the situation influences their perception of the interactions. We compared the reactions of habituated heifers ($n = 28$) to stroking and talking in a gentle voice while they were restrained in a feeding rack as routinely practiced on farms ('lock') or free to move in an arena ('free'), which allowed for a higher level of control over the situation and thus, probably a higher sense of agency. All heifers had a positive relationship to humans, i.e. freely accepted human touch, and were habituated to gentle human-animal interactions. Each animal was tested three times per condition and each trial comprised three phases: pre-stroking, stroking and post-stroking. Video recordings of the trials were analysed for behaviours associated with different affective states. We also assessed heart rate and heart rate variability (HRV). In line with our hypotheses, stroking and gentle talking led to longer durations of neck stretching, indicating a positive affective state in both conditions, with stronger effects in the 'free' condition. Longer durations of lower ear positions occurred during stroking primarily in 'lock'; however, the ear positions differed already in the pre-stroking phase, suggesting that restraint itself affected the ear positions independently of the human-animal interactions. Decreased heart rates during stroking in 'free' suggest a calming effect of the gentle interactions when the animals were free to move, and HRV parameters imply a greater relaxation effect shortly after 'free' interactions. We thus conclude that heifers with a good animal-human relationship enjoy gentle interactions with humans also when they are restrained, but they seem to perceive them even more positively when allowed to move freely, possibly due to a higher degree of agency. Furthermore, the results of this study confirm ear postures as promising indicators of the affective states of cattle, but underline that external factors such as restraint can substantially influence ear positions and need to be considered in the interpretation of the results.

1. Introduction

Positive experiences are considered a hallmark of good animal welfare (Boissy et al., 2007; Lawrence et al., 2019; Yeates and Main, 2008). Human behaviour plays an important role, as it can evoke different affective states in animals and promote the experience of positive emotions (Mellor et al., 2020; Rault et al., 2020a). Furthermore, a good human-animal relationship has benefits for animal health and production and for work safety (e.g. Hemsworth et al., 2011; Waiblinger, 2019). Gentle human-animal interactions, such as brushing and stroking, can induce positive emotions in cattle (e.g.(Bertenshaw et al., 2008; Schulze Westerath et al., 2014) and thus improve their relationship with humans and their well-being (Lange et al., 2020a; Schmied et al., 2008b).

Whether human-animal interactions are experienced as positive or negative depends on different internal and external factors (Waiblinger et al., 2006). One factor influencing the affective quality of interactions is the perceived control over the situation, or agency (Lange et al., 2020c; Mellor et al., 2020; Špinka, 2019). Decreased controllability can lead to the evaluation of a stimulus as a stressor (Koolhaas et al., 2011). Stress is often associated with negative affective states (Mendl et al., 2010) and should be avoided during human-animal interactions. An

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animals' capability to freely exercise agency may be enhanced or decreased by different aspects of situations (Rault et al., 2020b) and human-animal interactions (Mellor et al., 2020). Agency is thought to contribute to good welfare (Špinka, 2019) and thus the effects of gentle interactions with humans likely are more beneficial when the animal is able to actively choose or avoid them. This has been suggested previously based on individual cows' reactions to gentle interactions (Le Neindre et al., 1993; Lürzel et al., 2016; Windschnurer et al., 2009) and confirmed for dairy cows that were fearful of humans (Lange et al., 2020c).

One factor often compromising the agency of farmed cattle is restraint in headlocks. Depending on the previous experience of an animal, this may be perceived as stressful, aversive or, if associated, e.g., with feeding, even as positive (Grandin and Shivley, 2015), affecting the animal's behaviour and physiology (Grandin, 1997). In terms of human-animal interactions, restraint may be helpful for interacting with fearful cattle (Lange et al., 2020c). Providing gentle interactions during restraint could not only improve the human-animal relationship (Schmied et al., 2008a; Windschnurer et al., 2009), but also mitigate the stress often linked with procedures requiring movement restriction (Waiblinger et al., 2004). However, this only holds true if gentle human-animal interactions are still perceived positively when animals are restrained and have a lower degree of agency. One experiment that distinguished between 'forced' and 'voluntary' brushing treatments applied to calves found that neither group approached a human more often than calves that had not experienced any brushing by humans (Boivin et al., 1998). However, the authors questioned whether the brushing treatment was actually rewarding for the animals, possibly due to social isolation during the treatment. Therefore, the effects of control over the situation on animals that enjoy gentle human-animal contact remain to be investigated.

Affective experiences can be described along two dimensional scales: arousal (intensity) and valence (positivity/negativity), and they can be assessed using behavioural and physiological parameters (Mendl et al., 2010). For example, during social licking (Laister et al., 2011; Sato et al., 1991; Schmied et al., 2005) or stroking by humans (Lange et al., 2020a; Schmied et al., 2008b), cattle often show neck stretching. But also small changes in facial expressions seem to convey emotional meaning; especially ear positions and movements have recently been investigated in cattle (e.g. de Oliveira and Keeling, 2018; Lambert, 2019), though sometimes with contradicting results (Battini et al., 2019; Lange et al., 2020b). Positive interactions such as social licking (Laister et al., 2011) or being stroked by a human (Schmied et al., 2008b) can decrease the heart rate (HR). Computing heart rate variability (HRV) parameters reveals further information on the balance between sympathetic and vagal activity (von Borell et al., 2007).

We investigated whether restraint has a negative impact on the perception of gentle human-animal interactions. We compared the reactions of habituated heifers (n = 28) to stroking while they were either restrained in a headlock or free to move around in an arena. We hypothesized that, independently of restraint, stroking and gentle talking induces a positive, low-arousal state in the heifers, indicated by a decrease of HR, increase of HRV, and an increase of behavioural indicators of low arousal and positive valence, with some of these effects persisting until shortly after stroking. Furthermore, we expected that the gentle interactions are perceived more positively when the animals are free to move than when they are restrained in the headlock. In addition, we aimed to identify variation in the ear positions of heifers, expecting lower ear positions during the stroking phase.

2. Material and methods

2.1. Animals, housing and management

The study was conducted with 28 Austrian Simmental heifers on the young stock farm of the University of Veterinary Medicine, Vienna (Rehgras, Furth an der Triesting, Austria) between March and May 2018. The heifers' age ranged from 10 to 26 months. The animals were grouped according to age and fertility status in three stable groups (A, B, C) in three different sections of the barn. Section A was a deep litter pen, sections B and C were cubicle systems with rubber mats. All sections offered permanent access to adjoining outdoor runs with feeding racks in which the animals were used to being fed and restrained. The total space allowance per animal was approximately 21 m^2 (A), 13 m^2 (B) and 10 m^2 (C). Mechanical brushes for autogrooming were present in all sections. Management and feeding were the same for all groups, only the group in section B was pastured for the last two test days due to barn management reasons. On these days, they were moved to their familiar outdoor runs and left for acclimatization for at least 2 h before the testing started.

The study was discussed and approved by the Ethics and Animal Welfare Committee of the University of Veterinary Medicine, Vienna in accordance with the Good Scientific Practice guidelines and national legislation (project number ETK-02/04/2017).

2.2. Selection and habituation

As we aimed to investigate positive emotions during human–animal interactions, a generally positive perception of close human contact and stroking was a prerequisite. Twenty-one of the heifers were already habituated to gentle tactile and vocal interactions with humans, equipment and general procedures. For the remaining animals, we selected heifers that were actively seeking human contact when approached and accepted short periods of stroking when standing free in the barn. We used a stepwise habituation approach, first letting the heifers explore the experimenters, then approaching them while talking in a gentle voice and in the end touching and stroking them. We aimed to stop each interaction before the animals showed any sign of avoidance. If needed, concentrate was provided as a food reward until it was possible to equip the free-moving heifers with the HRV girths. We carefully habituated them to the camera (SONY HDR-CX730, Weybridge, UK) and HRV equipment (POLAR® Electro Oy, Kempele, Finland), as well as to the experimenters (both female, green overalls, brown hair; stroker: 1.63 m; cameraperson: 1.78 m) and the test procedure. Habituation was complete when it was possible to stroke the animal for at least 3 min without them showing any signs of avoidance, both while standing unrestrained and while being restrained in the feeding rack of their home pen. For further details of the selection and habituation process, see Lange et al. (2020a), (2020b). Before the experiment started, all heifers had worn the HRV girth for at least 1 h and had been allowed to explore the test arena (for details see 2.4) for at least 1 h.

2.3. Experimental design

We applied a within-subject design, i.e., each animal acted as its own control and was thus subjected to both conditions. To ensure robustness of the data, each animal experienced each condition three times i.e., in total six trials (trial numbers 1–6). The conditions were 'free', in which the animal was free to move around, and 'lock', in which it was restrained in a headlock. Half of the animals started with the 'lock' condition, the other half with the 'free' condition; subsequently the conditions were interchanged in an alternating pattern. The experimenters aimed to balance the order of the conditions over each testing day, but complete balancing was not always possible. One trial consisted of three phases of 3 min each: (1) pre-stroking (PRE), in which the experimenter calmly stood next to the heifer at a distance of approximately one meter so that baseline values could be recorded; (2) stroking (STROKE), with the experimenter stroking and talking gently to the animal; and (3) post-stroking (POST), in which the experimenter was again standing calmly next to the animal so that possible carry-over effects could be observed.

2.4. Experimental procedure

The animals were prepared and equipped for HRV measurement as described previously (Lange et al., 2020a). The cameraperson activated the monitor and placed it in the pocket of the girth of the next animal to be tested and gently moved it to the pen. Before each trial there was a 3-min acclimatization phase for the animals to calm down after entering the test pen. Groups A and B were tested in test pen 1 (22 m^2), and group C in test pen 2 (23 m^2). The pens consisted of metal fences that allowed visual, auditory and olfactory, but no physical contact to other animals; one side consisted of a feeding rack.

After entering the test pen and approaching the feeding rack, the animals received a small amount of concentrate to maintain their motivation to access the headlocks voluntarily. To ensure standardization, the procedure was executed in exactly the same way in the 'free' condition, with the only difference that the self-locking mechanism of the headlock was disabled. After the 3-min acclimatization phase, the stroker entered the test pen and took position in the centre of the test pen ('free') or next to the animal on its left side ('lock'). After the 3-min PRE phase, a 10-s break followed, during which the stroker approached the animal and established contact. The stroker wore rubber gloves with a rough surface (LUX paver's gloves, OBI Bau-und Heimwerkermärkte, Vienna, Austria) and when the STROKE phase began, applied a constant pressure while stroking at a frequency of 40–60 strokes/min, thereby imitating the speed of intraspecific social licking (Schmied et al., 2005). While talking gently to the animal (Lange et al., 2020a), the stroker started stroking at the ventral neck and included head, dorsal and lateral neck regions and withers, reacting to the animals' signals such as moving a body part towards the stroker or stretching the neck (Lange et al., 2020b). In the 'free' condition, during PRE and POST, the stroker stood in the centre of the test pen, not interacting with the test heifer in any way. Only if the stroker's safety was potentially threatened (intense play behaviour, pushing or rubbing), the stroker followed a behavioural cascade to protect herself, progressing from a loud, sharp exclamation over clapping the hands, stamping the foot to giving the animal a light slap. If the animal moved out of reach of the stroker during STROKE, the stroker waited until the animal stood still and approached it again. If the animal moved away more than three times in 1 min, or more than five times in one stroking phase, the test was stopped and excluded from analysis to make sure the animal was not tested if it had no motivation to be stroked. If a trial was stopped, the experimenters waited for at least 1 h before repeating the test, with a maximum of three trials per day. After a maximum of five unsuccessful trials, an animal would have been excluded from the study, but this never occurred.

2.5. Behavioural observations

All trials were video recorded. Due to technical problems in cardiac data collection, six tests needed to be repeated. The original recordings were excluded from the behavioural analysis and replaced by the recordings from the tests that were used for HRV analysis. The focus of the camera was on the head of the heifer, especially the left eye and ear; if they were not visible, the right eye and ear were filmed. The behaviour was analysed with the coding software BORIS (Friard and Gamba, 2016; version 7.4.6) using focal animal sampling and continuous recording (Bateson and Martin, 2007). While it was not possible to conceal the condition, a person blinded to the research questions and hypotheses analysed the videos. The videos were cut to contain one phase each so that the observer was also blinded towards the sequence of the phases. The observer recorded ear and head positions and movements as well as other behaviours according to an ethogram (Table 1; for photographs of ear positions, see Supplementary Material, Fig. S1). To assure a high quality of the behavioural observation, 12 video sequences of 3 min each were chosen from videos not used for further analyses and coded twice by the observer and another experienced person (S.L.) to calculate intra- as well as inter-observer reliability. Cohen's kappa for ear postures was

Table 1
Ethogram adapted from Lange et al. (2020b). Example photographs of the ear positions are included in the Supplementary Materials (Fig. S1 and S2).

Behaviour[1]		Definition
Inactive ear posture[2]	*Ear hanging*	The ear loosely hangs downwards (referring to the ground). There is no visible muscle tension, leading often to a slightly bouncing movement when the position is assumed.
Active ear postures[2,3]	*Back up*	The ear is held behind and above the latero-lateral axis.
	Back centre	The ear is held behind at the same height as the latero-lateral axis.
	Back down	The ear is held behind and below the latero-lateral axis.
	Centre up	The ear is held perpendicular to the head and above the latero-lateral axis.
	Centre	The ear is held perpendicular to the head along the latero-lateral axis.
	Centre down	The ear is held perpendicular to the head and below the latero-lateral axis.
	Forward up	The ear is held in front of and above the latero-lateral axis.
	Forward centre	The ear is held in front of and at the same height as the latero-lateral axis.
	Forward down	The ear is held in front of and below the latero-lateral axis.
	Ear flicking	The ear is quickly (within max. 0.5 s) moved back and forth at least once. The behaviour is coded until one of the other ear postures is clearly visible again. The residual movement after the active movement is still part of ear flicking.
Head/neck postures	*Head play*	Up-and-down head movements, often while the animal is oriented towards the person; often with the poll directed forwards/chin pulled to the chest; can look like rubbing without establishing contact, but can also include physical contact; part of the movement often slightly rotational, not straight.
	Head shaking	Successive quick rotational movements of the head.
	Neck stretching	Positioning neck and head actively in an outstretched line, either up, down, or forward.
Eyes[4]	*Open*	The iris is at least partly visible.
	Closed	The iris is not visible at all for longer than 0.5 s
	Not visible	Neither eye is visible.
Miscellaneous	*Rubbing*	The heifer moves the head/neck region while in contact with the ground or barn equipment. The behaviour ends when the contact between the heifer's head/neck region and the ground/equipment has ended.
	Rubbing the stroker	The heifer touches the stroker and moves the touching body part while in contact with the stroker. The behaviour ends when the contact is interrupted for at least 3 s
	Exploring	The heifer moves its muzzle towards an object into a perimeter of 10 cm, muzzle pointing towards the object which can be touched or licked. The behaviour ends when the animal's muzzle does not point towards the object anymore or leaves the perimeter of 10 cm.
	Exploring the stroker	The heifer moves its muzzle towards the person into a perimeter of 10 cm, muzzle pointing towards the person. The behaviour ends when the animal's muzzle does not point towards the person anymore or leaves the perimeter of 10 cm.
	Ruminating	The heifer's jaw moves regularly sideways with a frequency of about one movement per second. This movement is recorded as rumination if it occurs in a series of at least five movements (which may start before and

(*continued on next page*)

Table 1 (*continued*)

Behaviour[1]		Definition
		end after the observation). Rumination ends when the jaw movement is paused for more than 10 s
	Movement[5]	The heifer moves at least one leg or shifts her weight so that a movement of the back sideways, backwards or forwards is visible; or, if the back cannot be seen, when the distance between feeding rack and shoulder changes visibly. The behaviour ends when no movement is visible for more than 2 s
	Locomotion[6]	The heifer moves her legs (at least two steps within 4 s). The behaviour ends if interrupted for > 2 s
	Kick	The heifer lifts one of her legs quickly at least to the height of the carpal/tarsal joint.
	Threat	The heifer presents her forehead: The head is lowered, the heifer's nose drawn towards to her chest. The eyes are widened. The position is not counted as threat if it occurs in the context of play behaviour. Another form of threat is head-tossing: The animal throws its head sideways/backwards towards the threatened subject (human, conspecific).
	Tongue rolling	The heifer moves her tongue outside of the mouth in a repetitive way, with the mouth open and without the tongue touching an object including the heifer's body. As an exception, the mouth region may be touched by the tongue within a bout of tongue rolling.
	Self-grooming	The heifer's tongue or mouth touches her own body (excl. muzzle) or the typical up-and-down or forward-backward head movement is shown (if tongue/mouth is not visible). It is also scored if the heifer touches her body with her claws for more than 2 s. The behaviour ends when the contact between tongue or nose and body is interrupted and the grooming movement ends.
	Reprimand	The person speaks with a loud voice or makes a sudden movement (such as stamping the foot, smacking the hand against experimenter's thigh or slapping the animal with the hand, if other measures were unsuccessful).
	Stroking	The person actively touches the animal with hand or finger movements such as petting, scratching, stroking. The behaviour ends if interrupted for > 2 s
Calculated measures	*Changes of ear positions*	Sum of the frequencies of different ear positions per trial minus 1.
	Contact[6]	Sum of "Rubbing the stroker" and "Exploring the stroker".

[1] All behaviours were coded as durations, except *changes of ear positions, kicking, threat* and *reprimand*, which were coded as frequency.

[2] The left ear was recorded; if it was not visible, the right ear was recorded.

[3] The latero-lateral axis refers to an imaginary line between the bases of the ears. "Behind" means the ear is pointing towards the back of the head, "in front" refers to the rostral end of the head, "above" describes the ear pointing towards the dorsal and "below" towards the ventral part of the head. If the observed ear was moved by the experimenter, the position before the movement was recorded until the next unambiguous ear posture was assumed.

[4] The left eye was recorded; if it was not visible, the right eye was recorded.

[5] The behaviour *movement* could only occur in the 'lock' condition, and was thus not recorded in 'free'.

[6] The behaviours *locomotion* and *contact* could only occur in the 'free' condition, and were thus not recorded in 'lock'.

0.82 (intra: 0.83), for eye aperture 0.97 (0.98), for the head postures 0.69 (0.79) and for the remaining behaviours 0.92 (0.95).

2.6. Heart rate measurements

Data were error-corrected and processed according to Hagen et al. (2005) using the Polar Precision Performance Software, version 4.03.050 (Polar Electro Oy, Kempele, Finland), and HRV parameters were calculated using Kubios, version 2.1 (Biosignal Analysis and Medical Imaging Group, Department of Applied Physics, University of Eastern Finland, Kuopio, Finland). To account for the respiratory rate, the high frequency band was set 0.2–0.58 Hz (von Borell et al., 2007). The following parameters were analysed statistically: mean heart rate (*HR*); time domain: standard deviation of the inter-beat intervals (*SDNN*) and square root of the mean squared differences of successive inter-beat intervals (*RMSSD*), and the ratio of *RMSSD* and *SDNN* (*RMSSD/SDNN*); frequency domain (using a fast Fourier transform): normalized powers of high frequency (*HF*). After excluding all recordings with >5.0% artefacts per minute, the remaining recordings were cut into 1-minute segments, resulting in a maximum of nine 1-minute segments per recording (3 min per phase). If less than two 1-minute segments per phase and condition were obtained, the observation was excluded from the analysis. To account for the different number of 1-minute segments and their dependency, the data obtained from the remaining two or three 1-minute segments within one phase were averaged.

2.7. Statistical analysis

For the statistical analysis, we used the software package R, version 3.6.3 (R Core Team, 2019). A detailed description of the statistical analyses is given in the Supplement (Supplementary Methods).

2.7.1. Behavioural data

The durations of the behaviours were transformed to proportions by dividing them by the total time during which they could be observed (subtracting the time that a behaviour was not visible from the total duration of the phase). They were analysed using Generalized Linear Mixed Models (GLMMs) (Baayen, 2008) with a beta error structure and logit link function (Bolker, 2008; McCullagh and Nelder, 1989). The behaviour *contact* was calculated by summarizing the behaviours *rubbing the stroker* and *exploring the stroker*. The behaviour *changes of ear position* was calculated by summarizing the frequency of different ear positions and subtracting 1 (for the initial ear position), and analysed using a GLMM with a negative-binomial error distribution and a log link function.

Three observations per condition per animal were included in statistical analyses. This resulted in a sample size for models of 504 measures made for 28 individuals in a total of 168 trials with 3 phases each. For the full models, fixed effects were condition (factor with two levels 'free' and 'lock'), phase (factor with three levels: PRE, STROKE, POST) and their interaction as well as the test number; random intercepts effects were individual as well as trial_ID (trial number nested in individual). We included random slopes within individual for trial number (to account for possible changes caused by condition repetition), condition and phase to allow their effects to vary between individuals. To avoid cryptic multiple testing (Forstmeier and Schielzeth, 2011), we compared each full model with a respective null model that lacked the fixed effects of interest (phase, and the interaction of phase and condition) but was otherwise identical, using a likelihood ratio test (R function "anova"). The significance of the individual independent variables was determined by dropping them one at a time from the fixed effects and using a likelihood ratio test to compare the resulting models to the full model (Barr et al., 2013). Values of $p \leq 0.05$ are referred to as significant, and $0.05 < p \leq 0.1$ as a trend towards significance (Stoehr, 1999). If the full–null model comparison was, or tended to be, significant and the interaction was non-significant, the interactions were removed from the models and reduced models were fitted to investigate the main effect of phase. Main effects of condition were not tested, as they were not relevant for the testing of our hypotheses. As some of the behaviours could only occur in one condition (*locomotion* and *contact* in 'free', *movement* in 'lock'), models for these behaviours were fitted for subsets only containing the relevant condition and included only phase as a fixed effect.

To account for the fact that the nine ear positions and *ear hanging* are mutually exclusive and interdependent and that their proportions always amount to one, we analysed them using a compositional model (Pawlowsky-Glahn and Buccianti, 2011) following an additive log-ratio-transformation (ALR; Bolker, 2008). We fitted a linear mixed model with Gaussian error structure and identity link (LMM; Baayen, 2008), with the ALR-transformed proportions as the dependent variable. The model roughly followed the structure of the models for the other behaviours: the full model comprised the fixed effects of condition, phase, and their interaction as well as the test number. Individual, ear position, and trial_ID were included as a random intercept effects. Furthermore, to account for the non-independence of the mutually exclusive ear positions we included an additional random intercepts effect for the combination individual, test and phase (phase_ID). Finally, we included a random intercepts effect for the ear position (see below). Trial_ID was included as a random effect to account for the fact that each trial consisted of three phases and thus contributed three data points (each consisting of nine ALR-transformed proportions), and it seemed plausible to assume that there was random variation between the trials. We included random slopes for condition and phase and their interaction and test number within individual and also of phase within trial_ID to allow their effects to vary between individuals and trials (Schielzeth and Forstmeier, 2009; Barr et al., 2013). We included the same random slopes of condition and phase and their interaction and also test number within ear position. The random slopes effect of the condition*phase interaction within ear position is the key term representing the research question, as it models the possibility that preferred ear positions vary depending on condition and phase. Thus, if the proportion of time the ears are held in different positions varies depending on the particular constellation of condition and phase, this random slopes effect will contribute substantially to explaining the response. As an overall test of the random slopes of condition and phase and their interaction within ear position, we compared the full model with a null model that lacked these random slopes but was otherwise identical to the full model. Since the determination of the degrees of freedom of tests of random effects are not straightforward (Bolker et al., 2009) we used a permutation test (Adams and Anthony, 1996; Manly, 1997). Since this test addresses the effect of the random slopes of condition and phase and their interaction within ear position, we fitted an additional reduced model lacking specifically the random slopes of the interaction. We compared this reduced model with the full model using the same permutation test to obtain a p-value specifically for the interaction. The sample analysed for this model comprised a total of 4536 ALR-transformed measures of proportion of time made for 28 individuals in a total of 168 trials (random effect of trial_ID), each consisting of three phases leading to a total 504 levels of the factor phase_ID. After fitting the model we checked whether the assumptions of normally distributed and homogeneous residuals were fulfilled by visual inspection of a QQ plot (Field, 2005) of residuals and residuals plotted against fitted values (Quinn and Keough, 2002). These indicated no severe deviations from these assumptions.

For graphical depiction, we used the R packages "ggplot2″ (Wickham, 2016), "cowplot" (Wilke, 2019), and "graphics". Data were depicted as Tukey-style boxplots for each condition and phase, using the mean values of behaviours per animal (averaged across the three trials per condition). The bold line corresponds to the median; the lower and upper lines of the box to the first and third quartile, respectively; and the whiskers correspond to the lowest and highest values that were still within 1.5 × interquartile range from the margins of the box. Outliers (all values outside of 1.5 × interquartile range) are depicted as circles.

2.7.2. Cardiac data

Due to technical problems during HRV recording (i.e., >5% of errors per minute), one heifer with less than two 1-minute segments per phase and condition was excluded from the analysis, resulting in a sample size of 27 heifers with 118 tests. We cut 1001 valid 1-minute segments: 553 during 'lock' and 448 during 'free'. The minutes within one phase were averaged, because for some phases there were only two of the three 1-minute segments available.

Cardiac variables were analysed using linear mixed models (LMMs). As fixed effects we included condition, phase and their interaction, as well as age (d), time of day, *HR* (unless it was the response variable) and duration of rumination (s), which were entered as quantitative predictor variables. *Movement* and *locomotion* could not be included into the model because those variables could occur only in one condition each. Heart rate was included as a fixed effect on the one hand to account for differences in physical activity between the two conditions, on the other hand because it is often strongly correlated with HRV indicators (McCraty and Shaffer, 2015; Monfredi et al., 2014; Sacha, 2014; Zaza and Lombardi, 2001). While HR is often regarded as an indicator of arousal (Briefer et al., 2015; Lambert and Carder, 2019; Travain et al., 2016; Zebunke et al., 2013), HRV might also provide information on valence (Boissy et al., 2007). By correcting for *HR* in the models, the results represent the influence of the other independent variables (mainly the interaction of condition and phase) on HRV parameters independently of *HR* (Billman, 2013). The individual and trial_ID (trial number nested in individual) were considered as random intercepts effects. We included random slopes for trial number (to account for possible effects of condition repetition), condition and phase within individual to allow their effects to vary between individuals. The model assumptions were checked via plotting residuals against fitted values and visual inspection of the residuals, and *RMSSD, SDNN, RMSSD/SDNN* and *HF* were log-transformed to meet the assumption of a normally distributed residuals. We then proceeded in the same way as described above: we fitted a null model that lacked the variables of interest (phase and the interaction of phase and condition), and if the full-null model comparison was significant and the interaction was non-significant, it was removed from the model and reduced models were fitted to test for the significance of the main effect of phase.

3. Results

3.1. Behaviour during Gentle Interactions

While in the 'lock' condition stroking lasted for the entire STROKE phase (180 s), heifers in the 'free' condition were free to walk away and avoid stroking. However, they showed a very high acceptance of *stroking* (median duration in s: 179; min–max: 88–180; first-third quartile: 167–180). Due to the methodological differences between the conditions, some of the behaviours could only occur in one condition (*locomotion* and *contact* in 'free', *movement* in 'lock'). Of the behaviours that occurred in both conditions (Fig. 1), we statistically analysed the behaviours *neck stretching* (median duration in s; min–max: 0; 0–59), *ear flicking* (0; 0–24), and *changes of ear position* (mean number; 27; 2– 59). The behaviours that could only occur in one condition (Fig. 2) were analysed on subsets of data only containing the relevant condition; in 'free', these were *locomotion* (median duration in s; min–max: 23; 0–63) and *contact* (13; 0–120), and in 'lock', it was *movement* (23; 0–78).

The full models (including phase and the interaction of phase and condition) differed significantly from the null models (not including these terms) for the response variables *neck stretching* (Fig. 1; GLMM: $\chi^2 = 63.457$, df = 4, $p < 0.001$), *ear flicking* (Fig. 1; $\chi^2 = 17.651$, df = 4, $p = 0.001$) and *changes of ear position* (Fig. 1; $\chi^2 = 18.579$, df = 4, $p = 0.001$). In the PRE and POST phases, *neck stretching* occurred for

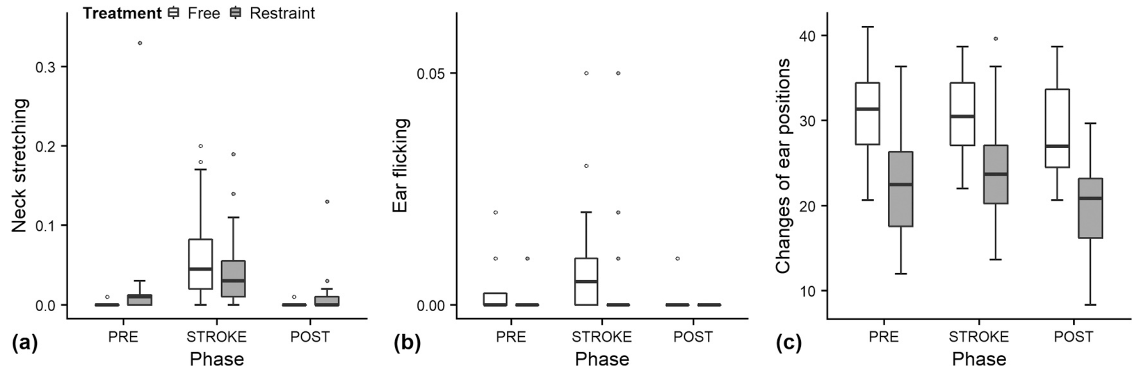

Fig. 1. Durations (as a proportion of the total time observed) of *neck stretching* (**a**) and *ear flicking* (**b**) and mean number of *changes of ear positions* (**c**) of heifers (n = 28) during the experimental trials, averaged across the three trials per condition and are depicted according to the condition (white = 'lock', dark grey = 'free') and phase (PRE = pre-stroking, STROKE = stroking, POST = post-stroking). Statistics for GLMMs: significant effect of the interaction of condition and phase for *neck stretching* (**a**) and *ear flicking* (**b**) $p < 0.05$, and trend towards significance for *changes of ear positions* (**c**), $p < 0.1$. Note that the y-axis scale varies to allow a sufficient resolution for rare behaviours.

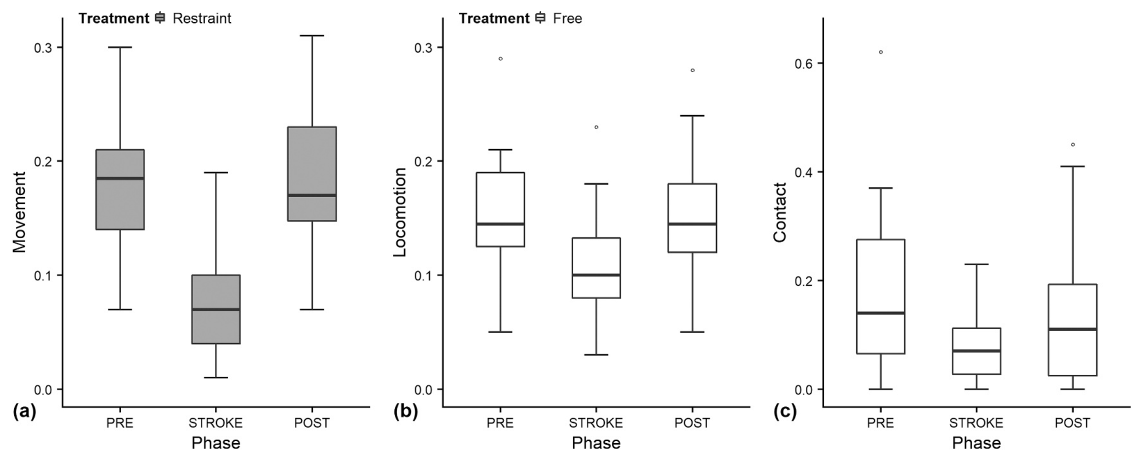

Fig. 2. (a) – (**c**): Durations (as a proportion of the total time observed) of *movement* (**a**), *locomotion* (**b**) and *contact* (**c**) of heifers (n = 28) during the experimental trials, averaged across the three trials in a subset of the data comprising only the condition the behaviours could be observed in, and are depicted according to the condition (white = 'lock', dark grey = 'free') and phase (PRE = pre-stroking, STROKE = stroking, POST = post-stroking). Statistics for GLMMs: significant main effect of phase for *movement* (**a**) and *locomotion* (**b**) $p < 0.001$. Note that the y-axis scale varies to allow a sufficient resolution for rare behaviours.

slightly longer durations in 'lock' than in 'free', but stroking led to longer durations of neck stretching in 'free' than in 'lock' (interaction phase*-condition: $\chi^2 = 8.121$, df = 2, $p = 0.017$). Durations of e*ar flicking* were generally relatively short, especially in the 'lock' condition; in 'free', *ear flicking* increased significantly during STROKE (interaction phase*-condition: $\chi^2 = 6.217$, df = 4, $p = 0.045$). The number of *changes of ear position* was generally lower in 'lock' than in 'free', but they tended to increase slightly during STROKE in the 'lock' condition, whereas there was a minimal decrease in the 'free' condition (interaction phase*-conditon: $\chi^2 = 5.246$, df = 4, $p = 0.073$).

Within the 'lock' condition, durations of *movement* decreased during STROKE (Fig. 2; $\chi^2 = 42.685$, df = 2, $p < 0.001$). Similarly, within the 'free' condition, durations of *locomotion* decreased (Fig. 2; $\chi^2 = 17.542$, df = 2, $p < 0.001$), but there was no obvious change in durations of *contact* across phases (Fig. 2; $\chi^2 = -1459.809$, df = 2, $p = 1$). For further details on results of statistical analysis of behaviours, see Supplementary Table S1.

The analysis of the ear positions revealed a clear effect of the random slopes of condition, phase and/or their interaction on the preferred ear positions (permutation test of full-null model comparison: $\chi^2 = 509.725$, $p = 0.001$), and the random slopes of the interaction between condition and phase within ear position were also significant (permutation test: $\chi^2 = 17.621$, $p = 0.001$). More specifically, the proportion of time the ears were held in different positions was similar in all combinations of condition and phase except in the stroking phase of 'lock'. For instance, positions *back down*, *centre down*, and *hanging* were the rarest in all combinations of condition and phase, but in the stroking phase of 'lock', *centre down* was the fifth most common one. Similarly, *back up* was the most common in all combinations of condition and phase, but still relatively less common in the stroking phase of 'lock' (Fig. 3). For further details on results of statistical analysis of behaviours, see Supplementary Tables S2 and S3.

3.2. Cardiac Data

Full and null models differed significantly for the response variables *HR* (LMM: *HR*: $\chi^2 = 13.177$, df = 4, $p < 0.010$), *RMSSD* ($\chi^2 = 12.684$, df = 4, $p = 0.013$), *SDNN* ($\chi^2 = 19.018$, df = 4, $p = 0.001$ and *RMSSD/SDNN* ($\chi^2 = 13.098$, df = 4, $p = 0.011$). The full–null model comparison revealed no significant difference for *HF* ($\chi^2 = 0.000$, df = 4, $p > 0.9999$), so it was not analysed further.

The interaction of phase and condition was significant only for *RMSSD/SDNN* ($\chi^2 = 10.179$, df = 2, $p = 0.006$), showing an increase during STROKE and a decrease in POST in the 'lock' condition and a less pronounced, opposite pattern in 'free'. There was a statistical tendency towards an effect of the interaction of phase and condition on *HR* ($\chi^2 = 5.483$, df = 2, $p = 0.064$): *HR* stayed almost equal during all phases of the 'lock' condition but decreased slightly during stroking in 'free'

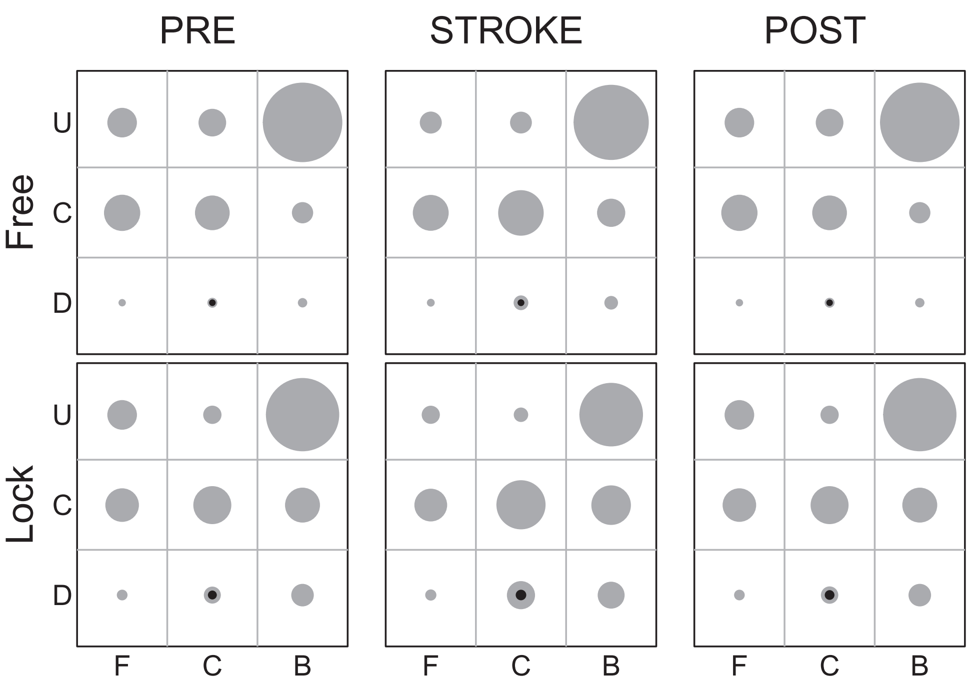

Fig. 3. Overview of mean durations of ear positions of heifers (n = 28) as a proportion of the total time observed. Means were calculated across the three trials per condition and are depicted according to the condition ('lock', 'free') and phase (PRE = pre-stroking, STROKE = stroking, POST = post-stroking). The area of the circles represents the mean relative duration of the ear position across phases and conditions, the black circle represents the duration of *ear hanging*. The letters account for the position on the horizontal and the vertical axis: B = *back*, C = *centre*, F = *forward*, D = *down*, U = *up*. Statistics for LME: significant effect of condition and phase and the interaction between condition and phase within ear position, $p < 0.001$. See Supplementary Fig. S3 for a more detailed depiction of the results.

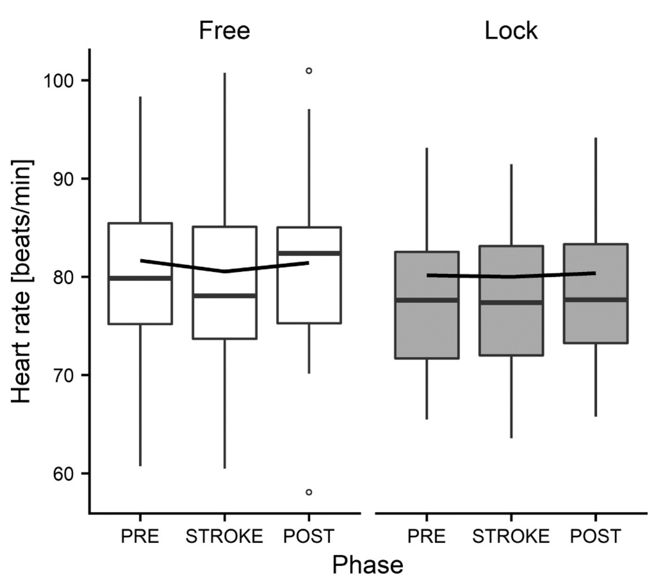

Fig. 4. Heart rate of heifers ($n = 27$), averaged across the three trials per condition and depicted according to the condition (white = 'lock', dark grey = 'free') and phase (PRE = pre-stroking, STROKE = stroking, POST = post-stroking). The black line indicates the estimated means of the models. Statistics for LMM: tendency for interaction of condition and phase, $p = 0.06$.

(Fig. 4). Independently of the condition, phase had a significant effect on the parameters *RMSSD* and *SDNN* (Fig. 5; *RMSSD*: $\chi^2 = 10.505$, df = 2, $p = 0.005$; *SDNN*: $\chi^2 = 14.932$, df = 2, $p = 0.001$). *RMSSD* decreased from PRE to STROKE (although in 'lock' the decrease was only minimal) and then increased in both conditions to POST; *SDNN* decreased during stroking, but increased again in POST in both conditions (Fig. 5). The models showed a significant negative association of *HR* with all of the HRV parameters (see Supplementary Materials, Table S4).

4. Discussion

To investigate whether restraint in a headlock has a negative impact on the perception of human-animal interactions, we compared the reactions of habituated heifers to gentle interactions with humans while they were restrained in the headlock or free to move. In line with our hypotheses, stroking and gentle talking led to longer durations of lower ear positions and increased durations of behaviours indicating positive affective states in both conditions, with stronger effects in the 'free' condition. The changes of the cardiac parameters over the phases suggest positive effects of the gentle interactions, especially when the animals were free to move.

4.1. Behavioural reactions

Stroking and talking gently to the heifers led to longer durations of *neck stretching* while they were free to move than when they were restrained. This behaviour is often shown during social grooming (Reinhardt et al., 1986; Schmied et al., 2005) and stroking by humans (Lürzel et al., 2015; Waiblinger et al., 2004). As heifers seek out these interactions and actively solicit them (Bertenshaw and Rowlinson, 2008; Sambraus, 1969), it can be assumed that they are positively valenced and *neck stretching* indicates that the animals enjoy them. While able to move around freely, the heifers stretched their necks almost exclusively during stroking, but in the 'lock' condition, the animals showed *neck stretching* also during the PRE and POST phase. It is possible that in the context of restraint in the headlock, the heifers stretched their necks not only as a reaction to a pleasurable tactile stimulus, but also to increase their range of sensory perception or in an attempt to compensate for their restricted mobility. In both conditions, however, longer durations of *neck stretching* were observed during stroking, with the duration in 'free' surpassing that of 'lock' despite the higher baseline duration in 'lock'. These results suggest that the heifers enjoyed the gentle interactions in both conditions, but more so when they were free to move. This is in line with a previous study investigating effects of restraint during gentle interactions on the improvement of the animal-human relationship (Lange et al., 2020c), where improvements were strongest when the animals were free to move during the interactions. Our results might be due to the animals experiencing a higher level of agency during the gentle interactions when they are able to move around freely, which might enhance their experience of positive affective engagement (Mellor et al., 2020). In addition, they did not only have control over being

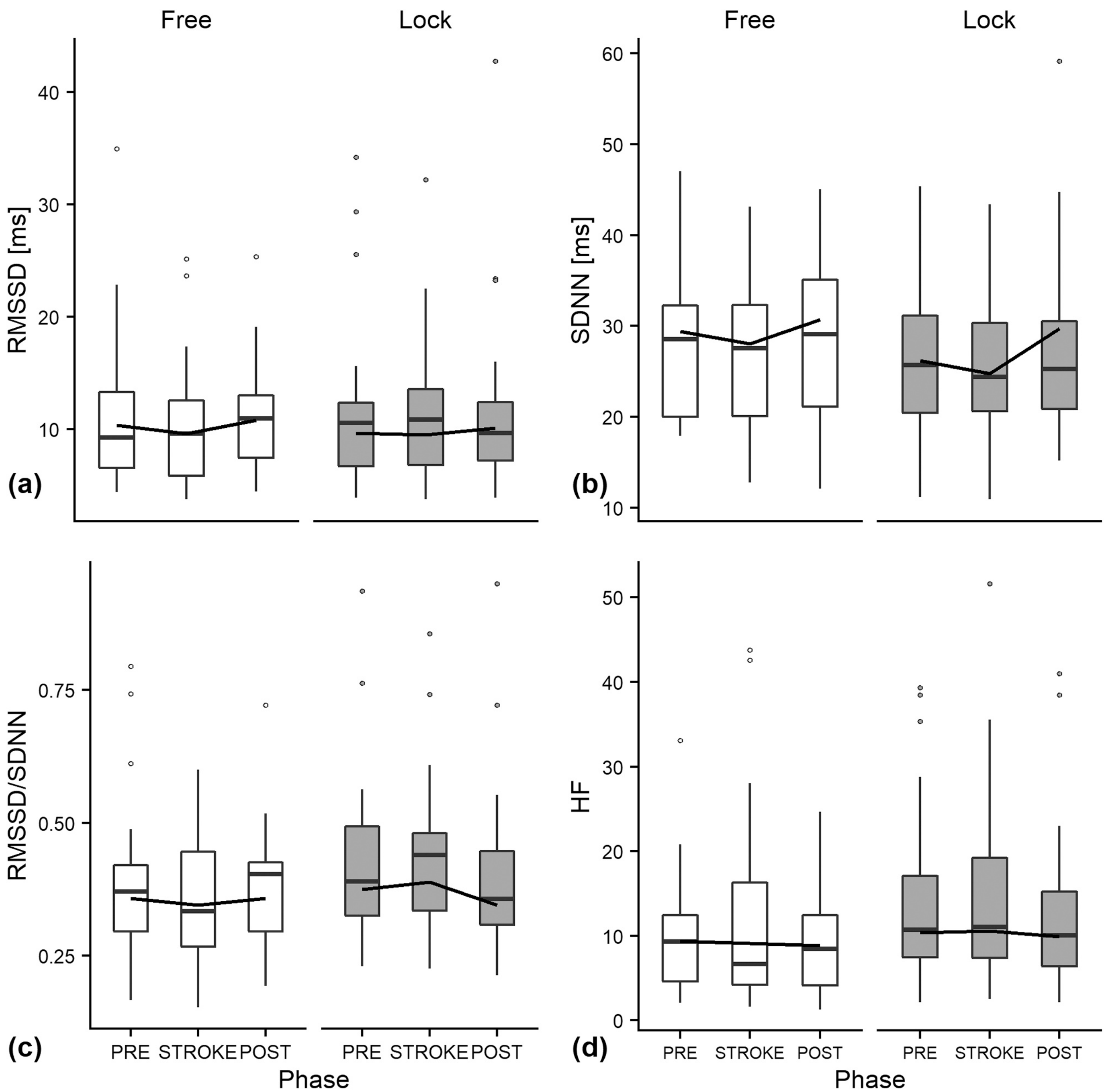

Fig. 5. HRV parameters of heifers ($n = 27$), averaged across the three trials per condition and depicted according to the condition (white = 'lock', dark grey = 'free') and phase (PRE = pre- stroking, STROKE = stroking, POST = post- stroking). The black line indicates the estimated means of the models. Statistics for LMMs: significant interaction of condition and phase for *RMSSD/SDNN* (**c**), significant effect of phase for *RMSSD* (**a**), *SDNN* (**b**), $p < 0.05$. Note that the y-axis scale varies to allow a sufficient resolution of the different cardiac parameters.

stroked by staying or leaving, but could also more actively engage in the interactions, affecting the exact way of interactions as they had more freedom to position themselves and present preferred body parts for stroking. However, it cannot be completely ruled out that other differences between the conditions influenced the results, e.g. the stroking could have been more pleasurable in 'free', as it might have been easier for the stroker to reach the preferred body parts of the animals. Although the heifers could have avoided the interactions in 'free', durations of stroking were only minimally shorter than those in 'lock', suggesting that the animals enjoyed the treatment. The significant decrease of *movement* and *locomotion* during the stroking phase further supports our hypothesis that the animals experienced a low-arousal state and indicates a calming effect of the human-animal interaction in both conditions. Locomotion is, however, partly related negatively to the duration of stroking in 'free', as moving away would have interrupted it, thus not necessarily an indicator for a calming effect of stroking.

Regarding ear positions and movements, not all results were as expected. In previous studies we used a similar design to investigate reactions of lying heifers to gentle interactions (Lange et al., 2020a,b) and we expected patterns in the behaviour to be similar in our present study. However, some behaviours were affected differently by the gentle interactions: *ear flicking* increased significantly during STROKE in the 'free' condition; in 'lock' it occurred very rarely. This behaviour is usually reported in the context of pain after disbudding (Heinrich et al., 2010; Neave et al., 2013) or defence against insects (Mooring et al., 2007). As pain can be ruled out as a cause in our test situation, we suspected that the lower frequency of *ear flicking* in 'lock' might have been due to an overall lower presence of insects in the headlock area. Even though all tests took place in the same arena, the headlock was located under the roof, which provided protection from sun. However, studies showed that shade does not significantly decrease insect-avoiding behaviours (Kendall et al., 2007; Palacio et al., 2015), which renders this explanation less plausible. Another possibility is that the arena attracted more insects than the feeding area due to traces of manure or urine that might have been present even despite regular cleaning. Calmly standing animals may attract insects more than animals walking around, which may explain the higher frequency of ear flicking during stroking.

Interestingly, a similar pattern occurred also for the frequency of *changes of ear positions*, which was generally lower in the 'lock' condition. Possibly, more varied sensory input in 'free' could have caused increased attention and consequently an increased number of *changes of ear positions*. The low numbers in 'lock' could reflect reduced attention due to a limited exposure to environmental stimuli during restraint, which was then significantly increased during the interaction with the stroker. Another possibly influential aspect is the more variable position of the stroker relative to the animal in the 'free' condition, where the animals were free to choose their orientation in relation to the stroker, which might have prompted them to change the positions of their ears more often. While our previous studies indicate that the number of *ear position changes* decreases during positive, low-arousal states in lying heifers (Lange et al., 2020a, 2020b), another study found that they increased during stroking in cows that were lying or standing (Proctor and Carder, 2014). This might be caused by the differing body postures (although most cows were lying in Proctor and Carder's, 2014 study as well), but also by the stroker approaching the cow at the beginning of the stroking phase, unlike in our studies, in which the experimenter already stood next to the animal in the PRE phase. The meaning of the parameter *changes of ear position* for affective states in cattle might depend on the context and has therefore yet to be investigated further (Mattiello et al., 2019).

In addition to the ear movements, we recorded nine different ear positions along the vertical and the horizontal axis – *back up, back centre, back down, centre up, centre, centre down, forward up, forward centre* and *forward down* – plus *ear hanging.* Lower ear positions are associated with states of low arousal and positive valence (Proctor and Carder, 2014; Schmied et al., 2008b), and indeed, gentle interactions led to longer durations of low ear positions, especially while the animals were restrained in the headlock. While this supports our hypothesis that stroking and talking in a gentle voice elicited a low-arousal, positive state in the heifers, it is surprising that this effect was more obvious in the 'lock' than in the 'free' condition. However, already in the PRE phase, longer durations of low ear positions were observed during restraint in the headlock than in free-moving animals, suggesting that restraint by itself affected the ear positions of the heifers. Again, the presumed higher variety of environmental influences and sensory stimulation in 'free' might have led to increased attention, which is associated with higher ear positions (Battini et al., 2019; Mandel et al., 2019). In combination, the analysis of ear movements and ear positions in this study underlines that, while they seem to be promising indicators for affective states of cattle, situational context and influences of the environment need to be considered for their interpretation. For instance, in lambs the qualitative behaviour assessment approach was used to confirm the interpretation of previous findings on ear positions (Serrapica et al., 2017).

4.2. Effects on cardiac parameters

When the heifers were free to move, their *HR* decreased slightly during the gentle interactions, which is in line with previous studies showing a lower *HR* in horses during grooming by a human at a preferred body area (Feh and de Mazierès, 1993) and in cattle during allogrooming (Sato and Tarumizu, 1993). Contrarily, we had observed increased *HR* values during stroking in our previous studies investigating gentle interactions with lying heifers (Lange et al., 2020a, 2020b): most likely, the gentle interactions had elicited an increase in arousal during the stroking phase in the lying animals, in comparison to the very relaxed resting states of the animals during the PRE and POST phases. Body posture seems to play an important role in the direction and strength of the effect - the present findings are in line with more pronounced decreases in *HR* observed in dairy cows receiving allogrooming when they were standing than lying (Laister et al., 2011). Interestingly, during restraint in the headlock, no decrease in *HR* could be seen. Especially in combination with the parameters *locomotion* and *movement*, which imply a decrease of physical activity in either condition during STROKE, these results suggest that stroking and gentle talking had a stronger calming effect in heifers when they are free to move than when they are restrained. However, we were not able to control for physical activity, as the possibility for activity differed considerably between the two conditions: restraint only allowed for small movements such as weight shifting or moving the legs, whereas *locomotion* in 'free' implied a higher degree of physical activity. Thus, the difference in physical activity between the phases might be more strongly pronounced in 'free' than in 'lock'. This means that the decrease in *HR* in 'free' should not be interpreted only in terms of the affective state but is also affected by physical activity, the decrease of which however indicates a calming effect in itself.

Independently of the changes in *HR*, which we corrected for by including it in the statistical models, the treatments had some significant effects on HRV parameters: in 'free', *RMSSD/SDNN* was not strongly affected by the gentle interactions, but in the 'lock' condition, *RMSSD/SDNN* increased during STROKE and decreased in POST. This parameter reflects sympathovagal balance, and decreased values could be caused by an increase in sympathetic activity, a decrease of vagal tone or a combination of both. The increase of *RMSSD/SDNN* during the gentle interactions is in line with increased values in sheep during brushing (Tamioso et al., 2018) and pigs during feeding (Zebunke et al., 2011). The decrease during POST in the 'lock' condition could reflect increased arousal caused by rising frustration at being restrained in the feeding rack, as some animals became restless towards the end of the trials (qualitative observation).

According to the model, *RMSSD* increased in POST in both conditions. Because *RMSSD* represents vagal activity (Hagen et al., 2005; Task Force of ESP and NASPE, 1996), its increase suggests vagal activation after the gentle interaction, and thus, relaxation. *SDNN* increased in both conditions after the gentle human-animal interactions. It represents overall variability and is influenced by both parasympathetic and sympathetic nervous system activity (Shaffer et al., 2014; von Borell et al., 2007). These results suggest that the treatments had positive effects in both conditions, especially shortly after the gentle interactions. In combination with the pattern in *RMSSD/SDNN* however, it seems that the lasting relaxation effect was weaker when the animals were restrained in the headlock than when they were free to move.

5. Conclusion

Our study leads to the conclusion that stroking and gentle talking can induce positive affective states in heifers with a good animal-human relationship both when they are restrained in a headlock or free to move around in an arena. Increasing durations of behaviours indicating positive affective states and cardiac parameters suggest that gentle interactions are perceived more positively when the animals are free to move. Furthermore, the results of this study confirm ear postures as promising indicators of the affective states of cattle, but underline that influences of the environment and situational context need to be considered for their interpretation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.applanim.2021.105445.

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2.4. Experiment D: Gentle interactions with restrained and free-moving cows: effects on the improvement of the animal-human relationship

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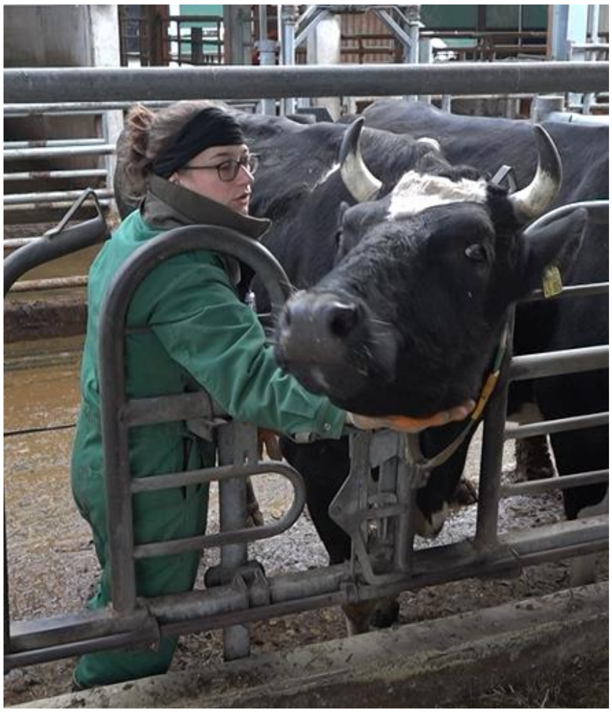

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RESEARCH ARTICLE

Gentle interactions with restrained and free-moving cows: Effects on the improvement of the animal-human relationship

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Abstract

The animal-human relationship is essential for farm animal welfare and production. Generally, gentle tactile and vocal interactions improve the animal-human relationship in cattle. However, cows that are fearful of humans avoid their close presence and touch; thus, the animal-human relationship first has to be improved to a point where the animals accept stroking before their perception of the interactions and consequently the animal-human relationship can become positive. We tested whether the animal-human relationship of cows fearful of humans is improved more effectively by gentle interactions during restraint, allowing physical contact from the beginning, or if the gentle interactions are offered while the animals are free to move, giving them more control over the situation and thus probably a higher level of agency and a more positive perception of the interactions. Thirty-six dairy cows (median avoidance distance 1.6 m) were assigned to three treatments (each n = 12): gentle vocal and tactile interactions during restraint in the feeding rack (LOCK); gentle vocal and, if possible, tactile interactions while free in the barn (FREE); routine management without additional interactions (CON). Treatments were applied for 3 min per cow on 10 d per fortnight for 6 weeks (i.e., three periods). Avoidance and approach behaviour towards humans was tested before the start of the treatment period, and then at 2-week intervals. The recorded variables were reduced to one score by Principal Component Analysis. The resulting relationship score (higher values implying a better relationship with humans) increased in all groups; the increase was stronger in FREE than in CON, with the increase in LOCK being not significantly different from the other treatment groups. Thus, we recommend that gentle interactions with cows should take place while they are unrestrained, if possible.

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1 Introduction

Nowadays, there is largely a consensus in the field of animal welfare science that farmed animals should have a good quality of life, not only a life mostly free of aversive experiences [1,2]. This means that the balance of positive and negative emotions should be tipped towards the positive side [1], which requires not only the avoidance of negative emotional states as far as possible, but also the presence of opportunities in the animals' lives to experience positive emotional states. A good animal-human relationship not only reduces negative emotional states and associated stress, but also provides a possibility for the animals to experience positive emotions, e.g. pleasure during gentle tactile interactions with humans, in addition to being associated positively with good health and production as well as work safety (for reviews, see [3–5]).

An effective method to improve the relationship between cattle and humans is gentle tactile stimulation [6,7], which is thought to mimic social licking, an affiliative behaviour shown by cows [8]. Gentle tactile contact, often combined with talking in a gentle voice, reduces the avoidance distance of cows [9,10] and calves [11,12] and thus improves the animals' relationship to humans, including a reduction of fear of humans [5,13]. Although gentle tactile interactions are generally effective in improving the animal-human relationship in cattle and seem to be perceived as positive by most of the experimental animals at the end of a treatment period [14–16], a neutral to good quality of the animal's relationship to humans is necessary as a prerequisite. If the animal-human relationship is poor, animals perceive humans, their close presence and touch as threatening stimuli [5] and animals free to choose will not accept close contact. In order to take advantage of the benefits of a good animal-human relationship mentioned above, it is necessary to improve the perception of the human from negative to neutral and possibly already positive, so that gentle tactile interactions can take place. This happens in the beginning mainly by habituation, first to the human's presence, decreasing the distance to the animals over time, and probably also to physical contact with the human, so that the animals do not fear the person anymore, resulting in a more neutral perception of humans. At some point, the animals are usually able to enjoy the interactions, and once this point is reached, the improvement of the animal-human relationship can proceed due to positive reinforcement.

Two major approaches to habituation are possible: either the animals are restrained and touched without the possibility to avoid the contact or the animals are free to move. The first approach has the advantage that the animals can learn already from the first day that this contact does not cause them harm. The higher exposure might accelerate the habituation process; then, the cows are able to enjoy the interactions at an earlier time, allowing positive reinforcement and a positive perception of the human. On the other hand, the restraint approach carries the risk that the animals might perceive the treatment initially as aversive due to their fear of humans and lack of possibilities to avoid close contact with them, which might also affect the later perception of the treatment, although we expect it to change quickly to neutral and then to positive. The second approach has the advantage of allowing the animal control over the situation, which should reduce or eliminate the potential stress caused by the approach or presence of a human [17,18] and allow a more positive perception of the interactions. Moreover, recent literature suggests that animal agency in itself can contribute to good welfare [19]. Accordingly, it has been proposed that an experimental stroking treatment might be more effective if the animal plays an active role in the situation [9,16,20]. However, animals with fear of humans cannot be approached closely enough to touch them from the very beginning; first, the relationship to humans has to be improved to a degree that they accept close presence and then touch. The habituation process might thus take longer; the transition from the acceptance of the close presence of a human to a positive perception might occur relatively quickly, though, once the animal can be touched and experiences the positive tactile stimulus. In addition, it might be the case that the effect of the treatment will be more stable over time in the free-moving animals, as the possible occurrence of negative emotions such as fear is minimized.

We tested the hypothesis that the improvement of the animal-human relationship, assessed via avoidance and approach behaviour towards humans, is influenced by the level of control the animal has over the situation. We predicted that the animal-human relationship is improved more quickly in cows that were approached and stroked while restrained (LOCK) than in cows that were able to move freely (FREE) due to a faster process of habituation. However, we expected the animal-human relationship in FREE to improve more strongly (though at a later point in time) due to a more positive perception of the interactions. For the same reason, we expected the positive effect on the animal-human relationship to last longer in FREE than in LOCK after the end of the treatment.

2 Methods

2.1 Animals, housing and management

The experiment was conducted from January to March 2019 on 36 animals from two herds housed separately on the research farm of the Thünen Institute of Organic Farming, Trenthorst, Germany. Herd 1 consisted of 40 black and white German Holstein cattle (14 with horns, 26 genetically polled). Herd 2 consisted of 44 black and white German Holstein cattle and one German Red Pied (all horned). If necessary for other experiments, the farm rears calves together with their mothers; during the experimental period, there were 5 to 7 calves in both herds. The whole-day contact of dams with their calves during the first three months of lactation and the resulting unlimited suckling is the main reason for the relatively low average milk yield of 6,430 kg/305 days.

The two herds were kept in loose housing, with a roofed lying area consisting of two rows of cubicles (1.24 m x 3 m including headspace) separated by a rubber-floored alley, a partly roofed feeding area and an unroofed alley between the two areas (total space allowance: 785 m^2 per herd; 17–19 m^2/animal). The feeding area consisted of two sections accessible via transponder-controlled selection gates only for the respective yield group; for details, see [21]. The cows were milked twice daily (at 05:15h and 15:45h) in a 2x4 tandem milking parlour (GEA Farm Technologies, Bönen, Germany) located between the two compartments; for details, see [10]. A fresh mixed ration for ad libitum consumption was provided twice per day after morning and evening milking. After each milking time, the cows were restrained in the feeding rack approximately until 08:30 h and 18:00 h, respectively, to prevent them from lying down immediately after milking and thus reduce the risk of intra-mammary infections.

From each herd, 18 German Holstein cows with an avoidance distance (see test description, section 2.5) of at least 0.3 m were each assigned to one of three treatments randomly, but balanced for category of lactation (1, first lactation; 2, second or third lactation; 3, fourth lactation or higher), lactation day, horn status and avoidance distance. No cows with calf at foot were involved in the experiment. The mean lactation number of the experimental cows was 3 (min. 1 –max. 9), and at the beginning of the treatment period (day 1 in Fig 1), the experimental cows were on average in milk since 125 (10–244) days. The cows remained in their respective herd during the entire study period. The study was registered and approved by the responsible authority, the Ministry of Energy, Agriculture, the Environment, Nature and Digitalization in Schleswig-Holstein (file number V244-1713/2019).

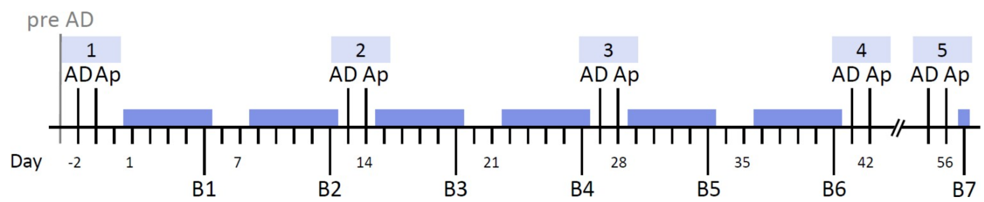

Fig 1. Experimental design. pre AD, pre-experimental avoidance distance test; AD, avoidance distance test; Ap, approach test; B1–7, behavioural observations. Treatment days are marked with a blue bar. Test numbers are given above AD and Ap indications, highlighted in light blue. For B7, the animals were treated as during the treatment period.

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2.2 Experimental design

All treatments and tests were conducted in the barn. The handler stroked the cows in the first treatment and talked to them in a gentle voice while they were restrained in the feeding rack (LOCK, n = 12). The cows in the second treatment experienced the presence of the handler, talking in a gentle voice and, if possible, stroking while unrestrained and able to move freely in the barn (FREE, n = 12). The third treatment group comprised control cows, which were not stroked or talked to at all (CON, n = 12).

The treatments were applied on 10 days out of each 14-day interval over the course of 6 weeks (Fig 1). For testing the animal-human relationship, avoidance distance tests and approach tests [5] were conducted before the start of the treatment period (test 1) and after every 14-day interval (tests 2–4), as well as 2 weeks after the end of the treatment period (test 5). The cows' behaviour during the treatment was recorded on video once a week and additionally 2 weeks after the end of the treatment period to assess the reactions after 2 weeks without treatment.

2.3 Experimental treatment

Two handlers (both female, brown hair, green overall; height person A: 1.80 m, person B: 1.63 m) applied the treatments, one handler always treating the same herd. Before the start of the experiment, both handlers moved through both herds, talking in a gentle voice to the animals, so that they were not completely unfamiliar to the animals when they conducted the behavioural tests. This procedure was repeated on the afternoon before every testing day.

The handlers fed a small amount of concentrate (170 g) to all experimental animals while they were restrained in the feeding rack after the morning milking during the first 5 days of treatment to facilitate the establishment of a positive relationship. Also the CON animals received the concentrate to make sure that any differences between CON and LOCK or FREE were due to the treatment and not to the provision of feed. The CON animals did not experience any additional experimental treatment and were only subjected to the approach and avoidance tests.

The treatment of LOCK animals took place after the morning milking, when all animals were restrained in the feeding rack. On treatment days, all cows remained in the feeding rack during the duration of the treatment of the LOCK group, except for animals standing next to LOCK animals, which were released to provide space for the handler (these could also be FREE, CON or other LOCK animals). The treatment started only when the animal had finished feeding or had fed for at least 30 min. The handler addressed the animal verbally before establishing physical contact at the back or shoulder. She approached from the right side, as the cows were more used to physical contact starting at the right side from regular ratings of body condition score and injuries. The handler stroked the cow for 3 min while talking to her in a gentle voice. She reacted to the animal's expressive behaviour (e.g. neck stretching, presentation of specific body parts) and stroked the parts of the head/neck region the cow seemed to prefer [22]. Stroking speed was 40–60 strokes/min as in previous studies [10,15], and the handlers had standardized the applied pressure between themselves. They wore rubber gloves with a rough surface (LUX paver's gloves, OBI Bau-und Heimwerkermärkte, Vienna, Austria) during the treatments. If an animal showed defensive behaviour (e.g. head tossing, moving away from the handler), the handler continued stroking at the withers or shoulder until she could stroke the head/neck region again. Once all the LOCK animals in one feeding group were treated, the handler opened the feeding rack and animals of all treatments were free to leave the feeding area. The stroking treatment was started alternatingly in the early-lactation and in the late-lactation feeding group of each herd.

The treatment of FREE animals took place in the morning (approximately between 09:00h and 11:30h) when the animals were free to move in the barn. The handler approached FREE animals in a non-threatening way (no excessive body tension, slow movements, avoiding eye contact), paying attention to the body language of the animal and aiming to stop the approach before the animal showed an overt avoidance reaction. When the animals could be approached without the handler eliciting avoidance behaviour, or even sought contact, the handler slowly started to touch and stroke them, similar to the LOCK animals. The body region that was touched first depended on the behaviour of the animal: if the cow approached the handler, she touched the head first; if the cow did not show approach behaviour, the handler touched the back or shoulder first, as in the LOCK animals. The treatment was conducted for 3 min per day; the time counted from the first approach of the handler and was paused if the treatment was stopped due to the animal moving away or showing threatening behaviour towards the handler, to be started again with the next approach.

2.4 Observations of behaviour during treatment

During every fifth treatment, the animals' behaviour was recorded by a technician using an HD Camcorder (SONY HDR-CX730, Weybridge, UK) for later analysis with the software BORIS [version 7.8.2; 23] according to an ethogram (S1 Table). While it was not possible to blind the observers with regard to the treatment, the order of observations was randomized so that the observers did not know whether they observed a treatment of the beginning or end of the experimental phase.

2.5 Avoidance distance tests

Avoidance distance tests in the barn [9,24] were conducted by both persons on both herds, so that either herd was tested twice on any given testing day–first by the handler, a familiar person that was not blinded towards treatment allocation, and after that by the handler of the other herd, a less familiar (in the following: "unfamiliar") person that was blinded. All cows were tested in the morning, the tests starting after the morning milking and finishing at noon. If a cow could not be tested in the morning, the test was done in the afternoon or, on one occasion, on the next day before the handlers conducted the approach tests, but not directly before the approach test. If an animal stood in the alley in a suitable position (e.g. its way of retreat should not be blocked), the test person started from a distance of 3 m and approached the animal from the front at a speed of 1 step/s. One arm was extended in front of her at an angle of about 45˚, with the back of the hand pointing forwards. The distance between the animal's muzzle and the test person's hand was estimated in steps of 10 cm at the moment when the cow avoided the test person by taking a step or withdrawing the head. If the cow did not avoid her, she touched the cow's nose with the back of her hand. If the cow was touched, an avoidance distance of 0 cm was assigned, and the handler tried to stroke the cow's cheek for 5 s. The touch score was recorded as 'avoidance at touching', 'possible to touch (but not stroke)' and 'stroking possible + duration in s'. If an experimental cow reacted to the test of a neighbouring animal, she was not tested directly afterwards but at a later point in time.

Prior to the start of the experimental phase, there was an additional avoidance distance test, conducted by the future handler of each herd, that was not evaluated. The avoidance distance can decrease from the first to the second test, probably due to habituation to the testing procedure [9], and by ensuring that every animal was tested at least once before the data collection started, we aimed to diminish this effect.

2.6 Approach test

The approach test in the barn was conducted by the handler of the respective herd (the familiar person) in the morning of the day after the avoidance distance test. The test was started when a cow was standing in an alley in a suitable position, i.e. standing in a way that she could see the test person and that her way was not blocked, e.g. by other animals or the cubicles. The test person went to a position at 3 m distance from the cow's head and remained there passively without encouraging contact for the test duration of 3 min, looking at her but not directly into her eyes. If the cow approached until establishing contact, the test person waited for 10 s after she established contact and started to stroke her until the test was terminated because the 3 min had passed or the animal walked away. If the cow moved away for more than 3 m (without having approached to contact) or started feeding, using the brush or interacting with another animal before the 3 min had passed, the test was terminated ahead of time and repeated some time later, as well as when another animal interrupted the test. This procedure was adopted in order to reduce the influence of competing motivations. There were no more than three test attempts per animal per testing day. If three attempts were unsuccessful, the maximal latency (180 s) was assigned.

2.7 Statistical analysis

The effect size could not be reliably estimated for sample size calculations, as there were no comparable data in the literature. We nevertheless carried out a power calculation for an unpaired t-test on avoidance distances with the software G*Power, version 3.1.9.2 [25], assuming a difference of one standard deviation between two of the groups and requiring a power of 80% and a significance level of $\alpha = 0.05$, leading to a necessary sample size of n = 12 for these groups.

Data were analysed and presented graphically using the statistics environment R, version 3.5.2 [26]. Differences, main effects and interactions with $P \leq 0.05$ are referred to as significant, with $P \leq 0.1$ as a tendency. Statistics were calculated using the individual animal as statistical unit. Observations of animals that were obviously lame on the day of testing (three observations in tests 4 and 5, two animals) were removed from the data set, and there were no data for one animal's third approach test and another animal's first avoidance distance test with the unfamiliar person, resulting in a total sample size of 175 observations (59 CON, 56 LOCK, 60 FREE).

The seven variables derived from the behavioural tests–avoidance distance towards the familiar and unfamiliar person, touch score in the avoidance distance tests with the familiar and unfamiliar person, latencies to approach into a perimeter of 1 m around the familiar person and to contact, duration of contact with the familiar person–were reduced to one score using principal component analysis (PCA; function *prcomp*). The first resulting principal component (Table 1) was well interpretable, with all variables indicating acceptance or seeking of

Table 1. Eigenvalue of the first principal component (PC1, denoted "relationship") derived by the PCA on the outcomes of the behavioural tests, variance explained, and loadings of the behavioural variables.

	PC1
Eigenvalue	**1.97**
Variance explained	**0.56**
Loadings	
AD familiar	-0.34
Touch score familiar	0.39
AD unfamiliar	-0.35
Touch score unfamiliar	0.39
Latency to 1m	-0.41
Latency to contact	-0.40
Duration of contact	0.37

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physical contact with the test person loading positively and all variables indicating a motivation to keep a distance to the person or a lack of motivation to approach loading negatively. Thus we denote the values of the first component as "relationship score", with higher values indicating a stronger motivation for or acceptance of physical contact with the person and lower values indicating a stronger avoidance of the person.

We used the relationship score as our response in a linear mixed model (LMM) with the package *lme4* [27], with treatment and test number and their interaction as fixed factors and the animal nested in the herd as random factor to take into account the repeated measures. The distributions of residuals and homogeneity of variance were checked visually, and to fulfil model assumptions, the relationship score was log-transformed after adding a value of 2.3 to obtain positive values.

To investigate the interaction between treatment and test number in more detail, we calculated the change in the relationship score from test 1 to test 4 and from test 1 to test 5, as the most obvious effects of the treatment were expected for tests 4 and 5. We evaluated both resulting variables with LMMs including the same random effects as the main model and treatment as the only fixed effect, and corrected the results for multiple testing using false discovery rate control (FDRC) [28]. Subsequently, we calculated pairwise comparisons with Tukey correction using the package *emmeans* [29]. For detailed model descriptions, see S2 Table. The behaviour during the treatment is presented descriptively.

3 Results

3.1 Behaviour during the treatment

The behaviour during the treatment was not analysed using statistical tests, but evaluated on the descriptive level. Over the course of the experimental period, the FREE cows accepted increasingly longer durations of stroking (Fig 2A). In observation 1 the median stroking duration was 3 s (first–third quartile: 0–86 s), and the largest increase was between observation 3 (median 52 s, 4–124 s) and observation 4 (144 s, 105–162 s). After observation 4, the duration of stroking stayed relatively stable until it reached its maximum in observation 7, two weeks after the treatment period ended (154 s, 129–174 s). In the LOCK treatment, the median duration of stroking was around 180 s throughout the whole experimental phase, reflecting that LOCK animals could not avoid stroking.

The duration of neck stretching (Fig 2B) was very low in both groups, the median staying at 0 s throughout the experimental phase. In the LOCK treatment group, the third quartile

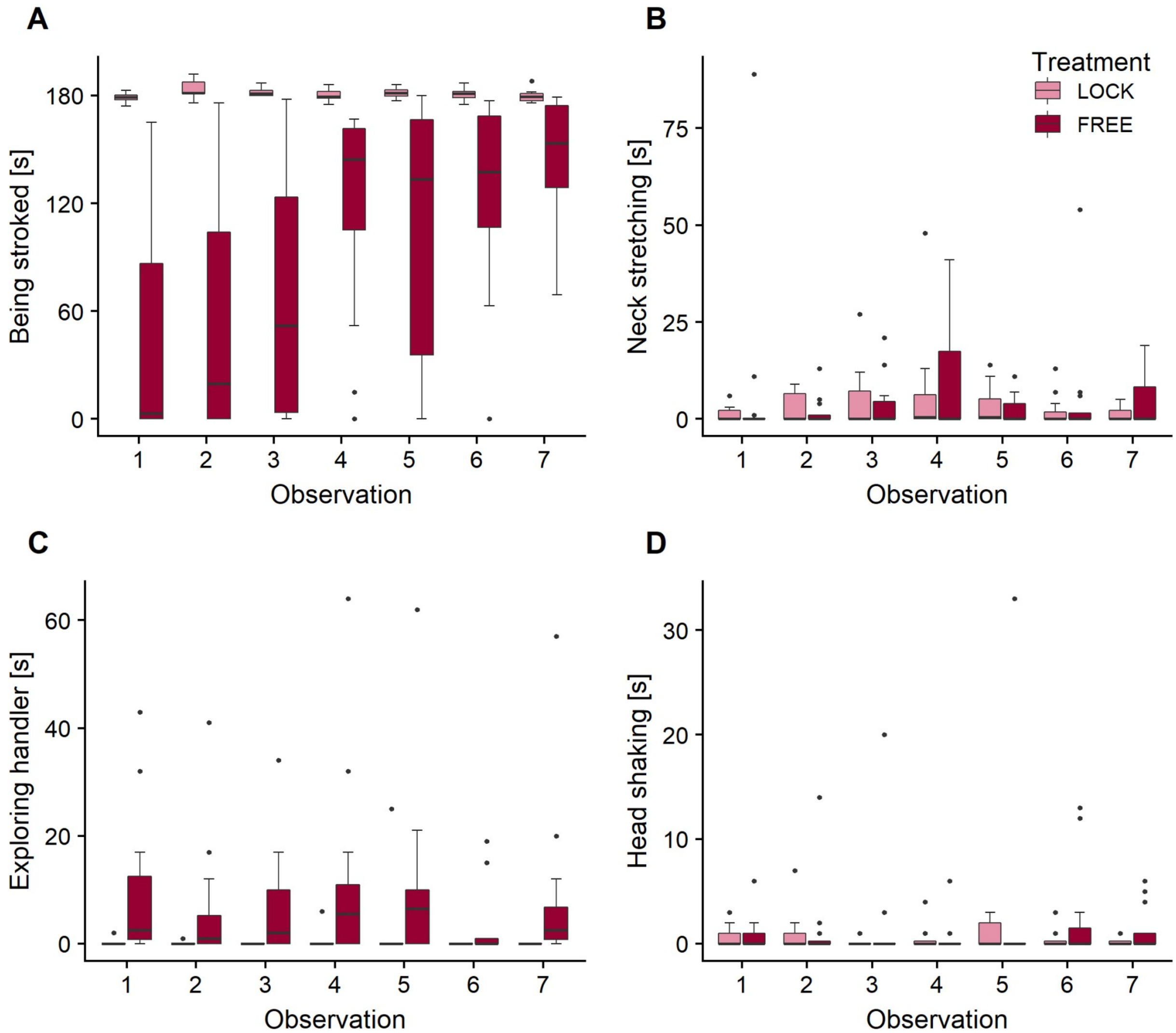

Fig 2. Behaviours shown by cows in the LOCK and FREE groups during gentle interactions. A) Being stroked, B) neck stretching, C) exploring the handler, D) head shaking. The FREE group (n = 12) experienced gentle interactions with a person while free in the barn, the LOCK group (n = 12) while restrained in the feeding rack. The treatment period comprised 6 weeks, with a total of 30 treatment days; behavioural observation took place during each fifth treatment. Observation 7 was not part of the regular treatment but served as a test situation in order to assess the animals' reactions after 2 weeks without gentle interactions.

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increased from observation 1 (2 s) to observation 2 (6 s) and stabilized at that level before decreasing again in observation 6 (2 s). In the FREE group, the third quartile increased more slowly, peaked at observation 4 (18 s) and decreased thereafter, only increasing again slightly in observation 7, two weeks after the end of the treatment period (8 s).

Some behaviours, especially contact-seeking behaviours such as exploration (Fig 2C), rubbing or licking of the handler, occurred numerically more often in FREE than in LOCK (overall median & Q3: FREE 2 s, 10 s; LOCK 0 s, 0 s). Behaviours possibly indicating a negative perception occurred rarely (head shaking, Fig 2D; overall frequencies for FREE, as there was no occurrence in LOCK: walking away 8, threatening 7). Graphs of behaviours not depicted in Fig 2 are included in the supporting information (S1 Fig).

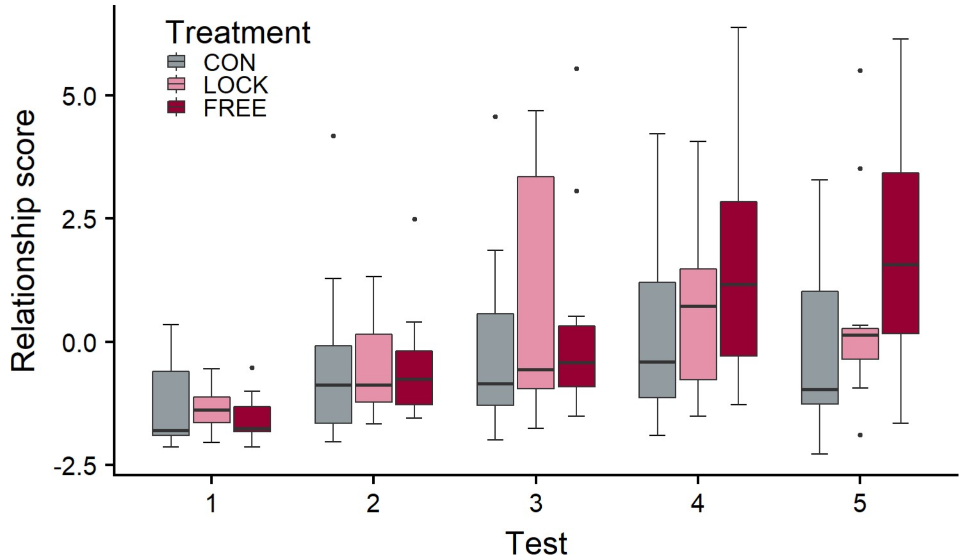

Fig 3. The relationship score in the three treatment groups over the course of the study. The FREE group experienced gentle interactions with a person while free in the barn, the LOCK group while restrained in the feeding rack; the CON group did not experience gentle interactions. The treatment period comprised 6 weeks with a total of 30 treatment days between tests 1 and 4; during the 2 weeks between tests 4 and 5, no treatment took place. LMM: test number p = 0.008, test number × treatment p = 0.014; n = 12 per treatment group and test number, except for test 1 n_{CON} = 11, test 3 n_{LOCK} = 10, test 4 n_{LOCK} = 11, test 5 n_{LOCK} = 11.

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3.2 Avoidance distance and approach tests

The results of the individual behavioural variables are depicted in S2 Fig. The relationship score increased over the course of the experiment in all groups (Fig 3; main effect of test number: χ^2 = 7.0, df = 1, p = 0.008), and there was a significant interaction of test number and treatment (χ^2 = 8.5, df = 2, p = 0.014), with the relationship score being highest in FREE, lowest in CON and intermediate in LOCK at the end of the experimental period.

The change in the relationship score from test 1 to test 4 (Fig 4) was not significantly different between groups (χ^2 = 14.1, df = 2, p = 0.19), but the comparison of the change between tests 1 and 5 revealed a trend towards a difference between groups (χ^2 = 27.6, df = 2, $p < 0.1$ after FDRC). The increase in the relationship score from test 1 to test 5 was significantly higher in FREE than in CON (df = 31, t = -2.55, p = 0.041), whereas CON and LOCK (df = 31, t = -0.52, p = 0.86) and LOCK and FREE (df = 31, t = -2.02, p = 0.12) did not differ significantly.

4 Discussion

The main finding was a general reduction of avoidance distance over the course of the treatment period, which was strongest in FREE, followed by LOCK. It was paralleled by an increase in approach behaviour, which again was most pronounced in FREE animals, followed by LOCK, but not statistically significant.

4.1 Behaviour during the treatment

The descriptive evaluation of the behavioural observations revealed that FREE animals accepted increasingly longer durations of stroking over the course of the experiment, which indicates a successful process of habituation and, perhaps, positive reinforcement due to a positive perception of the interactions. The biggest increase was between observation 3 (after 15 treatments) to observation 4 (after 20 treatments), indicating that more than 15 treatments (45

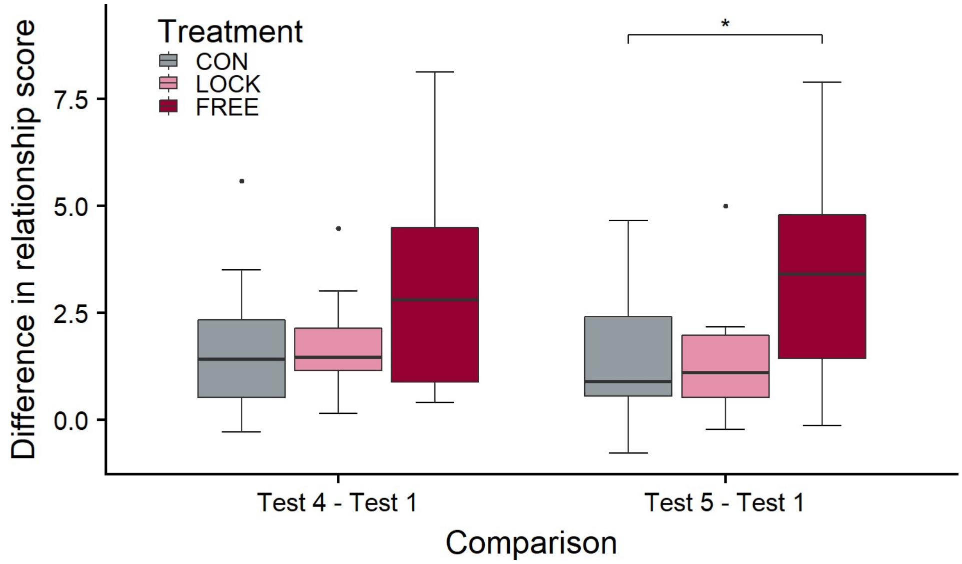

Fig 4. Change in the relationship score from test 1 to test 4 and from test 1 to test 5 in the three treatment groups. The FREE group experienced gentle interactions with a person while free in the barn, the LOCK group while restrained in the feeding rack; the CON group did not experience gentle interactions. Test 4 took place after the treatment period of 6 weeks with a total of 30 treatment days, test 5 2 weeks later. No treatment took place between tests 4 and 5. LMM: treatment $p < 0.1$ for test 5, ns for test 4; *: $p < 0.05$ for pairwise comparison; $n_{CON} = 11$, $n_{LOCK} = 11$, $n_{FREE} = 12$.

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min) were necessary to improve the animal-human relationship to a degree where most cows could be stroked for more than half of the treatment time. The treatment was not equally accepted by all FREE animals: even in observation 6, after 87 min of treatment, one cow did not accept stroking. The longer durations of stroking in observation 7, two weeks after the end of the treatment period, might indicate a rebound effect: the cows' increased acceptance of stroking after they lacked the opportunity to engage in positive human-animal interactions for two weeks might indicate an increased motivation for gentle tactile stimulation. It is noteworthy that in this last observation, all FREE cows accepted stroking for at least 1 min, indicating that there were longer-term effects of the treatment, improving the animal-human relationship further even after the end of the treatment period, at least in individual animals.

During stroking in both treatments, some cows showed neck stretching, although with relatively low durations. As this behaviour is shown during actively solicited social grooming [8,30,31] and stroking by humans [14], it is interpreted as a sign of enjoyment [14,15,32] and thus indicates a positive perception of the stroking treatment. As could be expected, considering the lower acceptance of stroking in FREE animals during the first three observations, the LOCK group showed more neck stretching during the first three observations. In observation 4, when the acceptance of stroking was higher, more neck stretching occurred in the FREE group as well. This pattern might indicate that once the animals can be touched, the transition to a positive perception of the interaction occurs relatively quickly.

With regard to the other behaviours, it has to be considered that most of them could be more easily expressed by the FREE animals, as the possibilities of movement of the LOCK animals were restricted. However, the numerically higher occurrence of some behaviours, such as exploration of the handler or walking away, might indicate that the cows indeed used their behavioural freedom to control actively the intensity of the interaction with the handler or to avoid the interaction.

4.2 Avoidance distance and approach tests

In line with our hypothesis, the relationship score increased in the three treatment groups to different degrees. We predicted that this increase should be visible earlier in LOCK than in FREE animals due to the increased opportunity to habituate to close human presence and physical contact in this context, also allowing positive reinforcement at an earlier time point during the study. However, the increase from test 1 to test 2 was similar in all three groups. In test 4, the median relationship score was lowest in the CON and highest in the FREE group, with the score of the LOCK animals being more similar to that of FREE than of CON animals, but the increase from baseline (test 1) to test 4 was not significantly different between groups. In contrast, the relationship score of the FREE animals increased more strongly from test 1 to test 5 than in CON animals, with that of LOCK animals being intermediate and not significantly different from the other groups' scores. This result is in line with our expectation that the effects of the treatment on avoidance and approach behaviour would be more sustained and thus more pronounced in FREE than in LOCK animals two weeks after the end of the treatment. Although it was unexpected that the relationship score increased even after the end of the treatment period, the finding confirms an earlier study in tied dairy cows, where the avoidance reaction towards the handler was lower 4 weeks after the end of the treatment (3 weeks of stroking the ventral neck) compared with the test directly after the treatment [6].

It seems thus that the increased controllability of the situation as perceived by cows that are able to move freely during gentle interactions with humans has a beneficial influence on the improvement of their relationship with humans, as already hypothesized by Le Neindre et al. [20], and that this effect outweighs the benefits of restraint regarding a faster habituation of the animals. It is possible that the first treatments were perceived as aversive by the LOCK animals because all experimental animals had a suboptimal relationship with humans, as indicated by their moderate to high avoidance distances. In this case, close physical contact with humans might be perceived negatively by the animals until habituation sets in, and this negative affective state will be exacerbated by the lack of possibilities to avoid the treatment. In contrast, even the first treatments of the FREE animals probably had a positive component, as the animals could satisfy their curiosity, and the negative component was most likely smaller than in the LOCK group, as the animals were able to control to which extent they accepted the contact with the person. Correspondingly, they had a higher level of agency, which again evokes positive emotions [19]. In addition, the interactions were potentially more mutual in this situation, as it was easier for the animals to explore and lick the person as well as to present specific body parts they preferred to have stroked. In the LOCK animals, a true, mutual interaction was much more difficult, as the restraint not only prevented the animals from avoiding the treatment but also hampered active participation, possibly reducing the positive perception of the interaction.

The relationship score of the CON group increased over the study period as well, although to a lower degree. This might seem surprising because they did not experience gentle interactions with the handler, but there are several mechanisms that can explain the result. The increase from test 1 to test 2 might have been influenced by the feeding of concentrate by the handlers on the first five days [33–35]. In general, CON animals probably lost some of their fear towards humans by the frequent presence of the handlers in the barn [36–38]. Through a process of habituation, they might have started to learn that the person poses no threat to them (the person imposing neither negative nor positive interactions), which led to an improvement of their relationship with humans. Close human presence did not reduce avoidance reactions in tied dairy cattle in contrast to stroking [6]; however, as the cows were tied in that study, they had no control over the distance to the human during the 'presence' treatment, in contrast to our CON cows, which were able to keep a distance to the person. A third mechanism might be social transmission [39]: the CON cows witnessed the interactions with the LOCK and FREE cows and their reactions and consequently adapted their behaviour accordingly. While social transmission has not been studied thoroughly in cattle, there are some studies indicating that cows are able to adapt their behaviour according to the behaviour of conspecifics [40,41]. Regarding the animal-human relationship, gentle interactions between handlers and tethered cows led to a decreased distance to handlers not only in the treated cows but also in neighbouring cows that could observe the treatment, and the distance the observing cows kept was correlated with the distance the treated cows kept [18].

Another point that needs to be addressed is the actual duration of gentle tactile contact in the LOCK and the FREE treatments. Our experiment was not designed to investigate primarily the effect of the level of control over the situation as perceived by the animal. To this purpose, we would have needed to record the duration of vocal and tactile contact with the FREE animals and also the distance kept by the animals and then to treat matched LOCK animals in the same way, as yoked controls [42]. Instead, we opted for an approach that would be more relevant for practice, answering the question whether gentle interactions with or without restraint were more effective at improving the animal-human relationship under the condition that the farmer invests the same amount of time in interacting with the animals. The FREE animals were thus stroked for a shorter time, in total, than the LOCK animals, but still showed a stronger improvement of their relationship with humans. Thus, we can conclude that the duration of the tactile interaction is not the main factor influencing the effectiveness of gentle human-cow interactions. Other characteristics have to play a role, and one of the characteristics that differ clearly between the situations is animal agency or perceived controllability of the situation.

5 Conclusion

Interacting gently with free-moving dairy cows in the barn improved the animal-human relationship to a higher degree than interactions during restraint in the feeding rack. This might be due to a stronger sense of control over the situation, and thus agency, and the ability to avoid or intensify the contact with the person according to the animal's motivation, potentially leading to a more pleasurable experience. Thus, we recommend that gentle interactions with dairy cows should take place while they are unrestrained, if possible.

Supporting information

S1 Fig. Behaviours shown by cows in the LOCK and FREE groups during gentle interactions.
(DOCX)

S2 Fig. Results of the avoidance distance and approach tests.
(DOCX)

S1 Table. Ethogram for behaviours coded during gentle interactions.
(DOCX)

S2 Table. Model descriptions.
(DOCX)

S1 File. Raw data.
(XLSX)

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3. General discussion

The current thesis aimed to achieve a more comprehensive understanding of how human-animal interactions can be used to elicit positive affective states in cattle. In three experiments, we investigated the effects of different modalities of HAI in regards to changes in behaviour and physiology associated with positive emotions in heifers with a positive relationship to humans. Then, we examined effects of gentle HAI in the context of improving the AHR of cows that are fearful of humans. After a brief summary of the project, this section aims to integrate the key results of this project's experiments by analysing overarching commonalities and contradictions across the individual experiments as well as with previous findings.

To explore the effects of different auditory stimuli during gentle HAI, we compared heifers' reactions to stroking while an experimenter was talking soothingly or while a recording of an experimenter talking soothingly was played. Both forms of auditory stimulation in combination with stroking led to a positive, low-arousal state in the heifers. Changes in cardiac parameters suggested a more positive experience and longer-lasting relaxation effects of live talking.

Taking a closer look at tactile stimulation, we compared reactions to stroking with the experimenter either reactively responding to perceived momentary preferences of the heifers and stroking the whole head/neck region or exclusively stroking the ventral neck. Although we found some differences in ear positions, no other parameters differed depending on the stroking style, suggesting that the exact manner of stroking did not have a strong influence on the perception by the animal.

To learn more about the role of perceived control over the situation for the experience of HAI, we compared the heifers' reactions to stroking while they were restrained in the feeding rack or free to move around. We found that heifers enjoy gentle interactions with humans also when they are restrained, but they seem to perceive them even more positively when allowed to move freely, possibly due to a higher degree of agency.

To investigate the role of perceived control over the situation during habituation of cows that are fearful of humans, we tested whether the animal-human relationship is improved more effectively by gentle interactions during restraint, allowing physical contact from the beginning, or when the gentle interactions are offered while the animals are free to move. We found that interacting gently with free-moving dairy cows in the barn improved the animal-human relationship to a higher degree than interactions during restraint in the feeding rack. This might be connected to a stronger sense of control over the situation, further corroborating the hypothesis that higher degrees of agency contribute to the positive experience of gentle HAI.

The experiments of this thesis confirm our hypothesis that gentle HAI in the form of stroking and gentle talking can elicit positive affective states and improve the AHR of cattle, and thereby contribute to animal welfare.

3.1. General effects of gentle interactions on parameters associated with affective states

One focus of this project was the measurement of affective states, especially regarding the positive spectrum. We analysed several behavioural parameters that are associated with the expression of positive emotions in cattle, as well as cardiac parameters that are associated with affective states. Considering the results of the individual experiments, there seem to be a few overarching trends and consistent effects, as well as changes in parameters that seem to contradict each other. To get a clearer picture and try to discern which parameters could be valid and reliable indicators of positive affective states, it is interesting to explore how the parameters changed over the different experiments of this project. Because each of the articles discusses in more detail how the observed changes in the parameters compare to findings in previous literature, the following section will focus on investigating similarities and differences across the experiments within this project, especially regarding the first three experiments. Considering the very similar protocols of experiments A, B and C, a comparison of their results seems reasonable. However, some limitations come up when comparing results of different, albeit analogous experiments: while the study population was very similar over these three experiments, it was not identical. Although subjects were drawn from the same pool, not every animal participated in every experiment, as some animals left the farm and needed to be replaced. Furthermore, the data collection for experiment C was started only when experiment A and B were already finished, causing slight differences in mean age of the animals' age and external conditions such as season and whether. This necessitates a very careful interpretation of effects that were observed across the different experiments.

3.1.1. Behaviour

In the quest to identify indicators suitable to measure positive affective states, it is prerequisite to validate that the animal is in a positive affective state during the observation (Keeling et al., 2021). By allowing our animals to stop the treatment by walking away (in all but the restraint conditions), we can assume that they enjoyed the treatment or at least did not have any aversive experiences. The voluntary nature of our interactions allows the conclusion that the treatment was pleasurable for the animals, and indicates that the associated parameters do indeed reflect positive valence.

In all experiments, the gentle interactions consistently led to longer durations of *neck stretching* during the stroking segments than the non-stroking segments. Cattle show this behaviour during intraspecific social grooming (Reinhardt et al., 1986; Sambraus, 1969; Schmied et al., 2005), but also during stroking by humans (Lürzel et al., 2015; Schmied et al., 2008b; Waiblinger et al., 2004). Both interactions are actively solicited and sought out by cattle (Bertenshaw and Rowlinson, 2008; Sambraus, 1969), indicating a positive appreciation of the activities. *Neck stretching* has also been associated with higher concentrations of salivary oxytocin in cattle after a stroking treatment (Lürzel et al., 2020), a hormone that is linked to positive social interactions. Our results confirm *neck stretching* as a suitable indicator of positive affect in the context of gentle tactile stimulation including HAI.

According to the concept that animals approach positively valenced situations (Boissy et al., 2007; Désiré et al., 2002; Fraser and Duncan, 1998; Tschanz, 1997), seeking proximity or even establishing physical contact to humans further suggests that the animals perceived the HAI as positive. Correspondingly, longer durations of *contact* were observed during the stroking phase than before or after stroking in experiments A and B, but not in the free condition of experiment C. *Contact* was defined as the sum of durations of behaviours such as exploring, licking or rubbing the experimenter as well as resting the head on the experimenter's body, but it did not include contact that was established directly through stroking. One factor impacting the differences of durations of *contact* between the experiments are the differences in mobility: in the restraint condition in experiment C, the ability of the animals to initiate contact with the stroker was extremely restricted, making a meaningful analysis of the behaviour impossible; therefore, we only analysed *contact* in the free condition of experiment C. In experiments A and B, the heifers were lying down while the experimenter was sitting by their side, while in experiment C, the animals were in a standing position with the experimenter standing next to them. Only when the animals were standing and free to move in experiment C, they established more *contact* before and after stroking, trying to initiate stroking or play, than during the stroking phase, when their motivation to be stroked was satisfied. Generally, when evaluating *contact* behaviour the positioning of the experimenter to the animals and correspondingly the possibility for physical contact needs to be taken into consideration as it might affect the comparability of results.

3.1.2. Ear positions

Additionally, smaller and less obvious changes in behaviour, like facial expressions, convey emotional meaning. Especially when researching subtle changes in and differentiating between different affective states, looking at such micro-expressions can be helpful (Guesgen and Bench, 2017). Particularly ear movements and positions are regarded promising indicators of affective states in cattle and are recently increasingly being investigated as possible indicators for positive emotions (e.g. de Oliveira and Keeling, 2018; Lambert and Carder, 2019).

However, sampling methods and definitions of different ear positions vary, making it hard to meaningfully compare results of different studies (Battini et al., 2019; Keeling et al., 2021). When specific discrete ear positions are being defined, postures that digress from these predetermined definitions might not be recorded or analysed, and it is often not clear which degree of divergence is still allowed (Lange et al., 2020b). To more comprehensively cover the continuum of possible ear positions and allow for a more granular sampling, we established a system to describe ear positions according to their position along the vertical and the horizontal axis. This resulted in nine different ear positions: *back up, back centre, back down, centre up, centre, centre down, forward up, forward centre* and *forward down*, plus the movements *ear flicking* and *ear hanging*. While this way of sampling ear positions may better reflect the continuous nature of ear positions, it may also compromise the comparability of our findings with other studies, necessitating careful consideration of differences in the definitions of ear positions when comparing our results with previous findings. For example, other studies defined positions like “ears upwards” or “ears backwards” (Mandel et al., 2019; Proctor and Carder, 2014), but it is unclear what position was recorded if the ear was in an ambiguous and/or intermediate position. The position we defined as *back up* may not have been regarded as a “high” ear position but labelled as “backwards”, or not have been recorded at all.

Figure 2. Example photographs of ear positions. The ear postures are described relative to the vertical axis, an imaginary line through the poll and the caudo-ventral edge of the mandible angle, and the horizontal axis, an imaginary line between the bases of the ears. "Back" means the ear is pointing towards the back of the head, "forward" refers to the rostral end of the head, "up" describes the ear pointing dorsally and "down" pointing ventrally.

Because low ear positions and ear hanging were found to be associated with low-arousal positive states (Proctor and Carder, 2014; Schmied et al., 2008b), we expected increased durations of these ear postures during and after stroking. However, these expectations were not, or only partly, confirmed. We observed only very short durations of ear hanging, not allowing statistical analysis. In general, low ear positions occurred relatively rarely and did not consistently increase during the stroking phases: they decreased during the stroking phase in experiment A in both conditions, and only increased during reactive stroking and in the restraint condition of experiment C. One possibly influencing factor may be the body position of the heifers. While the patterns of ear positions were quite similar in the experiments that were performed on lying animals, a different pattern emerged in the third experiment, that was performed on standing animals in the feeding rack or free in the arena. Especially while being restrained, longer durations of low ear positions could be observed. However, these comparatively long durations of low ear positions occurred over all phases and were present even before stroking, indicating that restraint itself has an influence on the ear positions of heifers (Lange et al., 2021). The generally predominant ear position in all of our experiments was *back up*. This ear position has also been observed to increase in positive contexts like feeding and using the brush (de Oliveira and Keeling, 2018) and the authors suggest it may indicate higher arousal states than *back down*. The results of our *HR* measurements however did not confirm such a clear correlation between arousal and the position of the ear on the vertical axis, e.g., in experiment B, reactive stroking led to longer durations of low ear positions, but the *HR* of the heifers was not influenced differently by the stroking styles. The unexpectedly short durations of low ear positions and hanging ears that we observed in our experiments might be specific to our study population: while most other studies were performed on adult cattle, we worked with heifers of six to 24 months of age. Additionally, we used only one breed, Simmental, for these experiments. To my knowledge, there are no studies yet investigating the influence of age and breed on ear positions in cattle.

In conclusion, the analysis of ear postures in the current thesis indicates that they may be promising indicators for affective states of cattle, but their interpretation is not uncomplicated. *Back up* occurred most commonly across the different experiments, suggesting an association with low-arousal positive affective states; however, more research is needed before drawing strong conclusions about the meaning of different ear positions in regards to affective states of cattle. Because small differences in positions, such as *back up* or *back down,* seem to be associated with different contexts and might convey quite different affective states, great care should be taken when defining distinct ear postures, as well as when observing, recording and analysing them. Furthermore, situational context, such as restraint, body position of the animal, position of possible interaction partners, and environmental influences such as sounds or other distractions always need to be taken into account for the interpretation of ear positions.

From a practical perspective, the observation and analysis of ear postures can be quite time-consuming, especially if an appropriately high resolution is desired. In our experiments, some videos needed to be sampled frame-by-frame to correctly categorize transition poses or more complex cases. The development of automatic detection might alleviate such processes in the future (see also section 3.4).

3.1.3. Cardiac parameters

We expected the gentle HAI to induce a low-arousal state and therefore predicted *HR* to be lower during and after stroking. However, in experiments A and B, the *HR* of the animals was significantly higher during stroking than before the stroking started. Although this finding contradicts our hypothesis, it is in line with an accelerated *HR* found in animals that were licked by conspecifics while lying (Laister et al., 2011) and we suspect that – compared to the very relaxed resting states of the recumbent animals before and after stroking – the gentle interactions had an activating effect and elicited a slight increase in arousal (Lange et al., 2020a, 2020b). However, in experiment C, which was performed with standing animals, the *HR* of the heifers decreased slightly during stroking when the animals were free to move. This is again in line with the observations of Laister et al. (2011), who found decreases in *HR* in cows that were standing while receiving social licking. However, the different conditions of our experiments (lying down, restraint and free moving) differed considerably in their allowance for physical activity, limiting the comparability across the different experiments of this project. In summary, we come to the conclusion that differences not only in physical activity but also in body posture should always be considered when using cardiac parameter to assess affective states of animals.

To obtain more detailed information about the activity of the different branches of the autonomic nervous system we analysed different HRV parameters. However, our findings were not consistent across the different experiments of our project, and none of the parameters consistently reacted to the HAI in a reliable way. Furthermore, the recording of HRV proved challenging: frequent signal losses and a high occurrence of artefacts resulted in a great number of failed recordings, even though great care was taken during the preparation of the contact sites and the placement of the electrodes. Movements of the heifers appeared to increase the risk of signal disruptions, indicated by even poorer quality of the recordings when the animals were free to move around. Next to a considerable delay in the projects timeline, these technical difficulties resulted in a substantial loss of data which may have contributed to the inconclusive nature of our findings. Development of a more robust, less error-prone method of recording cardiac activity would be beneficial to obtain larger and more reliable data sets, enhancing our understanding of the meaning of different HRV parameters in relation to the affective states of animals.

3.2. Characteristics of gentle interactions leading to a positive perception of HAI

Identifying characteristics of gentle interactions that positively influence the animals' perception of the contact is essential for understanding how human-animal interactions can best promote positive emotions in animals. This project investigated how humans can modify their behaviour or the environment in which the interaction takes place, and how different modalities of interactions, such as vocal and tactile stimuli, as well as differences in control over the situation, affect the animals' experience of HAI.

In a comparison of two methods of vocal stimulation we used either direct voice or a playback version of the same voice during stroking. To our knowledge this is the first study comparing reactions of cattle to verbal stimulation being delivered directly or played back from a speaker. Durations of behaviours that indicate positive affective states increased during the stimulation compared to before or after, suggesting that the animals enjoyed the interactions independently of which type of vocal stimulation was used. Changes in cardiac parameters indicated that live talking was perceived more positively and led to longer lasting relaxation than talking played by a speaker. These results are in line with studies finding that dogs respond better to direct verbal cues than to tape-recorded commands (Fukuzawa et al., 2005). Additionally, the higher degree of standardization of the recorded stimuli did not generally reduce variability in data. In combination, these results suggest that while the use of recorded auditory stimuli during gentle HAI in order to promote positive affective states in cattle is possible, it is not per se preferable.

In our study investigating different styles of tactile interaction, stroking the whole head/neck region in a reactive way led to longer durations of low ear positions during stroking, which have been found to be associated with low-arousal positive states in cattle. However, no other behaviours, nor cardiac parameters, differed significantly between the two stroking styles. In combination with the incomplete state of knowledge regarding the meaning of different ear positions, this discourages drawing strong conclusions as to which stroking style was more beneficial to the heifers. The exact manner of stroking seems to be less important for the experience of gentle stroking by humans. The higher degree of standardization of stroking only the ventral neck did not lead to a substantial reduction of variability in the resulting data, but it also did not notably reduce the positive experience of the heifers.

In a subsequent study, we investigated the role of control over the situation for the experience of gentle HAI by stroking them either while they were restrained in the feeding rack or free to move around. Longer durations of neck stretching suggested a positive affective state in both conditions. However, the effects were even stronger when the animals were able to move freely, indicating that the free-moving heifers had an even more positive experience of the HAI, possibly caused by higher levels of agency.

Taking a broader look at the results of the different experiments that were part of this study, it appears that the characteristics of HAI that allowed for more flexibility and naturalness (live talking, reactive stroking, unrestrained animals) tended to be superior to the more rigid approaches. Possibly, when interactions reach a certain degree of standardization, they may become artificial and be perceived as less positive, as true mutual interactions allow for a certain fluidity and reactiveness. Furthermore, higher degrees of standardization did not generally lead to reduced variation in resulting data, refuting the most important argument for the use of highly standardized protocols. This takes the same line as recent articles arguing that increased standardization decreases external validity of the findings (Richter et al., 2009; Voelkl et al., 2021). In combination, it seems that high degrees of standardization should not be the sole criterion for the design and selection of protocols for gentle human–animal interactions in experimental settings.

Despite these differences in efficacy between characteristics of gentle HAI, it should not be disregarded that in all of the experiments the animals showed signs of positive affective states in all conditions. This implies that the exact way in which interactions with animals are carried out is less important than the question whether they are performed at all. This is very positive from a practical point of view: chances are better that easy, fast and cheap measures will be applied in practice than complicated or elaborate protocols.

3.3. Using gentle interactions to improve the AHR – effects of control over the situation

However, the use of gentle HAI with cattle is not limited to promoting positive experiences; gentle HAI have also been shown to decrease the avoidance distance of cows (Lürzel et al., 2018; Schmied et al., 2008a; Windschnurer et al., 2009), reducing fear and improving their AHR, and thus, animal welfare. Especially in animals with a poor AHR, e.g. when they are fearful of humans, close human contact and handling can cause stress and negative reactions from both humans and animals, elevating the risk for trauma and injury, and thus severely compromise animal welfare (Waiblinger et al., 2006). Investigating how we can effectively use gentle HAI to improve the AHR therefore provides the opportunity to make a significant contribution to improving animal welfare.

Thus, we aimed to apply our findings about which characteristics of gentle HAI are best suited to promote positive experiences in heifers with a good relationship to humans to the subject of improving the AHR of cows fearful of humans. Particularly the question of restraint is highly relevant in this context, as animals with fear of humans naturally try to avoid interactions with humans and therefore cannot benefit from gentle tactile HAI without being restrained, at least before having reached at least a neutral perception of humans. To investigate the effects of restraint during gentle HAI on cows that are, at the start of the experiment, fearful of humans, we tested whether the animal-human relationship is improved more effectively by gentle interactions during restraint in the feeding rack, allowing physical contact and thus gentle tactile stimulation from the beginning, or when the gentle interactions are offered while the animals are able to move around freely and avoid or intensify the interaction, allowing for greater agency. We found that gentle interactions with free-moving dairy cows improved the animal-human relationship to a greater extent than with cows that where restrained. We conclude that gentle interactions show greatest benefits with cattle when they are free to move and recommend that HAI should be performed with unrestrained animals, if possible. Yet, the AHR improved for all animals over the course of the experiment. This concurs with the results from experiment C, indicating that gentle HAI are also perceived positively whilst animals are being restrained. Interestingly, the AHR improved also for the animals in the control group that received no special treatment, albeit to a lesser extent than in the treatment groups. The positive effects that could be observed in the control group are also in line with previous studies observing that gentle interactions with tethered cows did not only reduce the fear of the animals receiving the treatments, but also for the neighbouring cows that observed the treatments

(Munksgaard et al., 2001). Analogous to our findings regarding efficacy of different characteristics of gentle HAI, this aspect is interesting from a perspective of applicability: the prospect that the AHR of fearful cows could be improved substantially without even interacting with the individual fearful cows, but by merely being present and interacting positively with other cows in the barn, could be a strong incentive for caretakers to not just finish their daily work as quickly as possible, but allow some time for a few nice words or gentle stroking.

3.4. Contribution and Implications

The intention of this project was to investigate how humans can best promote positive affective states in cattle in order to improve their welfare. This thesis helped gain important insights into how HAI can be used to provide positive experiences for the animals.

Our findings on the effects of different modalities of verbal and tactile stimulation study can be a useful resource for the growing field of positive animal welfare science, informing researchers interested in eliciting positive affective states in cattle in experimental settings. Moreover, since our methods are very applicable, they can easily be adopted in the daily routine of farms, veterinary clinics or in the context of farm animal-assisted interventions with cattle, and can relatively easily be taught to stockpersons, students, and caretakers. Using such simple forms of enjoyable interactions allows to weave in more positive experiences for the animals, directly contributing to their quality of life. As some of our findings have attracted interest from media and were featured internationally on CNN, in the Daily Mail, American and German radio stations and even all the way to the Akhbar Elyom newspaper in Egypt, they helped to raise awareness of animal sentience and affective wellbeing all over the world. The articles have been shared and picked up by social media, reaching a large audience that may not normally be concerned about animal welfare issues, much less animal sentience. In addition, this project contributed to the knowledge about behavioural and physiological parameters in relation to positive affective states in cattle. A comprehensive new system of describing ear positions according to their position along the vertical and the horizontal axis allows to better cover the continuum of possible ear positions in a more detailed fashion, and hopefully contributes to improve comparability of results across different studies. It may also aid the development of automatic recording of ear positions, e.g. by using digital ear tags with acceleration sensors, as they are already in use on farms. As first pilot trials yielded promising results, this could be a way to realize continuous, exact and cheap analysis of ear positions and warrants a promising opportunity for further research. However, while new technologies such as precision livestock farming and automatic monitoring of animals hold great potential to improve the lives of both farmers and animals, concerns are being raised that the relationships of farmers with their animals may deteriorate (Winckler, 2017). Concerns include the loss of skills or personal care of the animal-caretakers (Cornou, 2009; Werkheiser, 2018), or the risk of less frequent and shorter interactions between farmer and animal or opportunities for direct observation, e.g. when daily interactions during milking or feeding are falling away (Hostiou et al., 2017). This project illuminates one way of mitigating this proposed estrangement of farmers and livestock by exploring how humans can create opportunities for positive experiences using gentle HAI. Over all the experiments of this project, we aimed to upkeep a practical perspective and employed methods relevant for practice that can easily be applied on farm. It can be assumed that the importance of the relationships between animals and their caretakers will receive further attention in the societal debate surrounding animal welfare and will increasingly be picked up by guidelines and legislation. When measurements of human-animal relationships will be formally monitored, as it is already being practiced for certain welfare labels for milk or meat, farmers need recommendations on how to improve in that area. This project enhances our capability to establish efficient and easily manageable procedures for improving the AHR particularly in animals that are fearful of humans, which will help to actively apply HAI to improve cattle welfare. Ideally, our findings will not only be applied in scientific contexts, but will be adopted in conventional farms to provide meaningful improvements to the day-to-day lives of cattle.

4. General conclusion

The present thesis shows that gentle human-animal interactions can elicit positive affective states in cattle and improve the animal-human relationship, and therefore enhance their well-being. We found that the exact manner of gentle human-animal interaction did not strongly impact the affective experience for cattle with a positive animal-human relationship, but more flexible, natural approaches seem to lead to more positive reactions. Generally, styles of interaction that allowed for a certain reactivity and naturalness tended to be superior over more inflexible approaches. Thus, cattle should be stroked in a reactive manner, following the animals' reactions and signals. While the use of recorded auditory stimuli is possible, it is not necessarily preferable over direct verbal stimulation, as the higher standardization of the stimulus does not lead to less variability in the resulting data. Higher degrees of control over the situation seem to elicit more positive effects. Therefore, we recommend that interactions with cattle should be conducted when they are unrestrained, if possible. Within this thesis, no evidence could be found that stimuli with a higher degree of standardization lead to lower variation in data, suggesting that high degrees of standardization should not be the sole criterion when designing HAI protocols for experimental settings. Finally, this project confirms neck stretching as a valid parameter for positive affective states in cattle during gentle HAI and presents a new, comprehensive system of evaluating ear positions, both on the level of observation, as on the level of statistical analysis, which might benefit the development of automatic collection of ear position data.

5. Summary

Alongside intact biological functioning and the opportunity to express natural behaviour, affective experiences are considered a key aspect of animal welfare. Positive emotions have been found to improve animals' health, thereby benefitting both mental and physical aspects of animal welfare. The affective states and wellbeing of farm animals strongly depend on the relationships with the humans they are interacting with. Those relationships are determined by the animals' perception of humans and the relative intensity of positive or negative emotions the animals experience during interactions with humans. Different characteristics of human-animal interactions may affect the quality of their emotional experience, but research on the effects of different stimuli during these interactions is scarce.

The overarching aim of this project was to reach a more comprehensive understanding of the effects of different characteristics of gentle human-animal interactions on positive emotions in cattle and the improvement of the animal-human relationship of cattle that are fearful of humans. First, we investigated the effects of different modalities of human-animal interactions on heifers that had a positive relationship with humans: in three experiments, we compared different forms of tactile and auditory stimulation and different levels of control over the situation, as well as varying degrees of standardization. Then, we examined effects of restraint during gentle interactions in the context of improving the animal-human relationship of cows that are fearful of humans.

In experiment A, we compared the reactions of heifers to stroking while an experimenter was talking in a gentle voice or while a recording of the experimenter talking in a gentle voice was played. While both forms of auditory stimulation combined with stroking elicited a positive,

low-arousal state, the direct verbal stimulus seemed to have a stronger relaxation effect. The use of the playback stimulus did not lead to a consistently reduced variability in data.

Experiment B compared the reactions to stroking with the experimenter either reactively responding to perceived momentary preferences of the heifers and stroking the whole head/neck region or exclusively stroking the ventral neck. Both stroking styles elicited positive reactions. Although we observed differences in ear positions, the different stroking styles did not lead to significant differences in any other parameters, indicating that the exact manner of stroking does not strongly impact the animals' perception of gentle human-animal interactions.

In experiment C, we compared heifers' reactions to stroking during restraint in the feeding rack or when they were free to move in an arena. Although the heifers showed signs of positive, low-arousal affective states during both forms of gentle interactions, behavioural and cardiac parameters suggest the positive effects to be stronger when the animals are free to move.

Experiment D explored the effects of restraint on the habituation of cows that are fearful of humans to gentle human-animal interactions. We found that interacting gently with free-moving dairy cows in the barn improved the animal-human relationship to a higher degree than interactions during restraint in the feeding rack, possibly due to a higher sense of agency.

In summary, this project confirms that gentle human-animal interactions can elicit positive affective states in cattle, improve the animal-human relationship, and thereby contribute to the wellbeing of cattle. We found that cattle with positive animal-human relationships respond positively to gentle human-animal interactions, regardless of the exact form of interactions. However, more flexible, natural forms seem to lead to more positive reactions. Therefore, gentle human-animal interactions should implement reactive styles of stroking, direct verbal stimulation and should be performed with unrestrained animals, if possible. Increased degrees of standardization did not consistently reduce variability in resulting data. Neck stretching was confirmed as a valid parameter for positive affective of gentle tactile interaction, and a new system to describe and analyse ear positions was developed.

6. Zusammenfassung

Neben der Möglichkeit, sich natürlich zu verhalten, und einer intakten biologischen Funktion werden auch mentale Zustände als wichtige Aspekte des Wohlbefindens von Tieren angesehen. Positive Emotionen können die Gesundheit von Tiere verbessern und dienen damit sowohl psychischen als auch physischen Aspekten des Tierschutzes. Affektive Zustände und Wohlbefinden von Nutztieren hängen stark von den Beziehungen zu den Menschen ab, mit denen sie in Kontakt sind. Diese Beziehungen werden durch die Wahrnehmung des Menschen und die relative Intensität positiver oder negativer Emotionen bestimmt, die die Tiere während der Interaktion mit dem Menschen erleben. Verschiedene Merkmale von Mensch-Tier-Interaktionen können die Qualität der emotionalen Erfahrung der Tiere beeinflussen, aber es gibt nur wenige wissenschaftliche Erkenntnisse über die Auswirkungen verschiedener Stimuli während dieser Interaktionen.

Das übergeordnete Ziel dieses Projekts war es, ein umfassenderes Verständnis dafür zu erlangen, welche Auswirkungen verschiedene Merkmale freundlicher Mensch-Tier-Interaktionen haben auf positive Emotionen bei Rindern und auf die Verbesserung der Tier-Mensch-Beziehung bei Rindern, die Angst vor Menschen haben. Zunächst untersuchten wir die Auswirkungen verschiedener Modalitäten von Mensch-Tier-Interaktionen auf Kalbinnen mit positiver Mensch-Tier-Beziehung: In drei Experimenten verglichen wir verschiedene Formen taktiler und auditiver Stimulation und unterschiedliche Level der Kontrolle über die Situation sowie die Effekte von unterschiedlich intensiver Standardisierung der Stimuli. Anschließend untersuchten wir die Auswirkungen von Fixierung während sanfter Mensch-Tier-Interaktionen im Zusammenhang mit der Verbesserung der Tier-Mensch-Beziehung von Kühen, die Angst vor Menschen haben.

In Versuch A verglichen wir die Reaktionen von Kalbinnen auf Streicheln, während die Experimentatorin mit freundlicher Stimme sprach oder während eine Aufnahme der freundlichen Stimme abgespielt wurde. Während beide Formen der auditiven Stimulation in Kombination mit Streicheln einen positiven Zustand mit geringer Erregung hervorriefen, deuteten Veränderungen der Herzparameter auf einen stärkeren Entspannungseffekt des direkten verbalen Stimulus hin. Die Verwendung der Playback-Aufnahme führte nicht zu einer durchgehend geringeren Variabilität der Daten.

In Versuch B wurden die Reaktionen auf das Streicheln verglichen, während der Versuchsleiter entweder reaktiv auf die wahrgenommenen momentanen Präferenzen der Kalbinnen reagierte und die gesamte Kopf-/Halsregion streichelte oder ausschließlich den ventralen Hals streichelte. Beide Streichelarten lösten positive Reaktionen aus. Obwohl wir Unterschiede bei den Ohrpositionen beobachteten, führten die verschiedenen Arten des Streichelns zu keinen signifikanten Änderungen bei anderen Parametern, was darauf hindeutet, dass die genaue Art des Streichelns keinen sehr großen Einfluss auf die Wahrnehmung von freundlichen Mensch-Tier-Interaktionen hat.

In Versuch C verglichen wir die Reaktionen der Kalbinnen auf Streicheln, wenn sie in einem Fressgitter eingesperrt waren oder wenn sie sich frei bewegen konnten. Obwohl die Kalbinnen bei beiden Formen der freundlichen Interaktion Anzeichen positiver Emotionen zeigten, deuteten Verhaltens- und Herzparameter darauf hin, dass die positiven Effekte stärker waren, wenn die Tiere sich frei bewegen konnten.

Versuch D untersuchte die Auswirkungen einer Fixierung im Fressgitter auf die Gewöhnung von Kühen, die Angst vor Menschen haben, an freundliche Mensch-Tier-Interaktionen. Es zeigte sich, dass freundliche Interaktionen mit freilaufenden Milchkühen im Stall die Tier-Mensch-Beziehung in höherem Maße verbesserte als Interaktionen während einer Fixierung im Fressgitter, was möglicherweise auf ein Gefühl höherer Kontrollierbarkeit zurückzuführen ist.

Zusammenfassend bestätigt dieses Projekt, dass freundliche Mensch-Tier-Interaktionen positive affektive Zustände bei Rindern hervorrufen, ihre Tier-Mensch-Beziehung verbessern und damit zu ihrem Wohlbefinden beitragen können. Wir fanden heraus, dass Rindern mit einer positiven Tier-Mensch-Beziehung positiv auf freundliche Mensch-Tier-Interaktionen reagieren, unabhängig von der genauen Form der Interaktion. Flexiblere, natürlichere Formen scheinen aber zu positiveren Reaktionen zu führen. Deshalb sollten bei freundlichen Mensch-Tier-Interaktionen reaktive Formen des Streichelns und direkte verbale Stimulation eingesetzt werden, und sie sollten mit nicht fixierten Tieren durchgeführt werden, wenn möglich. Ein höherer Grad an Standardisierung führte nicht zu einer durchgängigen Verringerung der Variabilität der Ergebnisse. Halsstrecken konnte als valider Indikator für die positive Wirkung freundlicher taktiler Interaktionen bestätigt werden, und ein neues System zur Beschreibung und Analyse von Ohrpositionen wurde entwickelt.

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