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Effects of gentle interactions on the relationship with humans and on stress-related parameters in group-housed calves

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Abstract

Although the relationship between farm animals and humans has strong implications for animal welfare and productivity, there have been few experimental studies on the influence of gentle interactions in group-housed calves. In the present study, Austrian Simmental calves were housed in groups of four under standard management conditions. Fourteen calves experienced 40 min of additional gentle interactions in the form of stroking and gentle talking during the first four weeks of life, whereas the remaining eleven calves did not. The animals' fear of humans was measured by avoidance distance tests on 33 and 76 days of age and by an arena test that comprised three phases — isolation, presence of a human, isolation — at 34 days of age. The very low avoidance distances did not differ significantly between the groups. In the arena test, there was less behaviour indicative of stress in the presence of the experimenter compared with the isolation phases. Heart-rate measurements showed a corresponding pattern. Control calves showed more tail-flicking than stroked calves and had higher concentrations of salivary cortisol before and after the test. There were no other significant differences between the groups. The minor number of behavioural differences may result from the control animals' good relationship with humans, ie there is a ceiling effect. If the general contact between stockpeople and calves is gentle and negative experiences are minimised, it is possible to achieve a good calf-human relationship without additional efforts.

Keywords: *animal welfare, avoidance distance, calves, cortisol, heart rate, human-animal relationship*

Introduction

Research on the relationship between cattle and stockpeople has gained importance in recent years, as it impacts upon economics as well as animal welfare. Not only is productivity higher on farms with a good relationship between animals and humans (Hemsworth *et al* 2000, 2002; Waiblinger *et al* 2002), there are also lower occurrences of disease (for mastitis: Ivemeyer *et al* 2011; for lameness: Chesterton *et al* 1989) and a lower risk of injury to the animals and to stockpeople because of reduced fear reactions (Rushen *et al* 1999; Waiblinger *et al* 2004). Some of the economic aspects are also relevant to animal welfare, which is itself becoming an economic factor due to rising consumer demand for ethically produced food (Bayvel *et al* 2012; Buller 2013) and provides possibilities for label production.

In improving animal welfare, it is important to go beyond merely reducing aversive experiences and work on creating situations in which animals can experience rewards and pleasures (Boissy *et al* 2007; Balcombe 2009; Green & Mellor 2011). Establishing a good relationship between animals and humans not only decreases the animals' fear of humans but also increases their quality of life by inducing positive emotions in situations where animals have contact with humans. The animals' relationship with humans is defined as their perception of humans in terms of the relative strength of positive and negative emotions elicited during interactions (Waiblinger *et al* 2006). Under specific circumstances, animals may perceive humans as conspecifics and form bonds with them (Estep & Hetts 1992; Raussi *et al* 2003) and social bonds are considered a source of positive emotions, for instance by facilitation of positive tactile contact between individuals (Balcombe 2009). Stroking, as a gentle form of tactile stimulation, not only reduces animals' fear of humans (in calves: Lensink *et al* 2000; Probst *et al* 2012; in cows: Schmied *et al* 2008a) but also has the potential to decrease heart rate (Schmied *et al* 2008b), which may be indicative of further physiological anti-stress effects (Waiblinger 2010).

There is a substantial body of literature pertaining to the relationship between calves (*Bos primigenius taurus*) and humans, both in beef suckler herds (eg Probst *et al* 2012) and in individually housed veal calves (eg Lensink *et al* 2000).

However, calves in suckler herds and conventionally raised dairy calves differ strongly in their social environment and in the amount of contact they have with humans, and the relationship with humans may be especially important for (female) dairy calves, as they will have regular close contact with humans later in life. As single housing of calves over eight weeks of age has been generally forbidden since 2006 by European legislation (Council Directive 2008/119/EC), nowadays dairy calves are usually kept in groups after a short period of individual housing. To our knowledge, only four studies have investigated the effect of gentle handling or stroking by a trained handler on calves in pair-wise or group housing: Schütz *et al* (2012) and Stewart *et al* (2013) compared responses to humans and reactions to painful routine husbandry procedures in group-housed calves that had previously experienced positive or mildly negative interactions with humans (lacking a non-handled control group), whereas Raussi *et al* (2003) and Lensink *et al* (2001) focused on the differences in the relationship with humans and ease of handling between individually housed and pair-housed calves. It seems that the relationship with humans is poorer in calves that are kept with conspecifics than singly housed, indicated for instance by more withdrawal responses (Lensink *et al* 2001; Duve *et al* 2012). Measures to improve cattle's relationship with humans are particularly important in group housing (Raussi 2003); the shortage of studies on the relationship with humans and especially on the effect of gentle contact in group-housed calves thus represents a considerable gap in the field of cattle welfare research. Further, only calves of typical dairy breeds were studied, with dual-purpose breeds not investigated. It is known that the type of breed influences temperament and fear reactions toward humans (Murphey *et al* 1980; Leruste *et al* 2012). Finally, the effects of regular additional gentle interactions in group-housed calves are yet to be studied in comparison with a routine rearing management that avoids aversive interactions but does not provide regular gentle contact.

We investigated the effect of gentle tactile and vocal interactions during early life on group-housed calves of a dual-purpose breed (Austrian Simmental cattle). We predicted that calves that experience such interactions would show less avoidance behaviour towards a human and, in a challenging situation, less stress-indicative and more contact-seeking behaviour in the presence of a human as well as a lower heart rate and lower increase in salivary cortisol than calves that had only experienced limited human contact in the context of routine management procedures.

Materials and methods

Animals, housing and management

The experiment was carried out at the Kremesberg Teaching and Research Estate of the University of Veterinary Medicine, Vienna. The Teaching and Research Estate maintains a dairy herd of approximately 90 cows, mainly comprising Austrian Simmental but also including some Brown Swiss and Holstein-Friesian cows. Twenty-five Austrian Simmental calves born between June and December 2012 were separated from their mothers within an hour of birth (except for night calvings). They were weighed (mean bodyweight [± SD]: 45.2 [± 5.6] kg) and disinfectant was applied to the umbilical stump. They were ear-tagged within the first three days of life. They received up to 2.5 L of colostrum from a bottle within the first 3 h of life and the same amount within the next 3 h and within the following 6 h, resulting in up to 7.5 L within the first 12 h of life. They were fed 1.5–3 L of milk from their dams via teat buckets three times daily (0800, 1300, 1800h) during the first six days of life, totalling 4.5–9 L per day, depending on how much the calves wanted to drink. Afterwards, the calves were fed 3–4 L of whole milk twice daily (0800, 1800h) in the second week of life and between 3.5 and 5 L of milk twice daily (0800, 1800h) according to age and body size until weaning. Water and hay were available *ad libitum* from the first day onwards; calf starter was provided *ad libitum* from around the third week of life (in group housing).

In the first two weeks of life, the calves were housed individually in 2 m^2 calf hutches with adjoining 2 m^2 runs, both littered with straw, which permitted visual and limited tactile contact with other calves as well as visual contact to humans. After 14 days, calves were moved to the calf barn, where they lived in groups of four in pens measuring 13.3–16.0 m^2 until weaning (housing was balanced for different pen sizes). Each pen consisted of a deep littered area of 10.5–12.6 m^2 and an elevated feeding area separated into four feeding boxes with group-locking levers. During feeding, the calves had visual contact with each other through railings. In front of the feeding boxes there was a wooden board with bucket holders, which limited visual contact to the stockpeople during milk feeding. On the wall opposite the feeding boxes, a separate, 2.5-m deep microclimate zone was created with a wooden cover 1.5 m above ground and a transparent strip curtain.

The youngest animal in any group was never more than 14 days younger than the oldest. If a group could not be composed exclusively of Austrian Simmental calves of the appropriate age, it was completed with Brown Swiss calves. These were treated in the same way as the experimental animals, although they were not tested. Females were usually disbudded under anaesthesia and analgesia (sedation: xylazine; local anaesthesia: procaine hydrochloride; analgesia: ketoprofen) at an age of 6 to 7 weeks, depending on bud growth. There were some exceptions: two stroked calves were disbudded in weeks 4 and 5 of life and one in week 9 and one control calf was disbudded in week 13. As disbudding was conducted under anaesthesia and analgesia and the animals were not conspicuous in the data analysis in any way, data from these animals are included in the analysis. The percentage of calves that had received injections in the context of veterinary treatment (before the first tests: stroked 46%, control 22%; until the last test: stroked 54%, control 44%) was higher in the stroked calves than in the controls, but not significantly so (Fisher's exact test; $P = 0.4$ and $P > 0.9$, respectively).

The calves in single housing were usually fed by one of two male milkers. Two stockmen were primarily responsible for the calves

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in group housing. The milkers and the main stockmen were replaced by one of three further stockmen for several weekends.

All procedures applied during the course of this study were discussed and approved by the institutional ethics committee in accordance with guidelines for Good Scientific Practice and with national legislation.

Experimental design and treatment

Calves were assigned to one of two experimental groups — the control group (six males, five females) and the gentle interaction group (seven males, seven females) — ensuring that the birth dates of the calves in the two groups were balanced. Both groups were cared for by the usual stockpeople encompassing the farm's routine procedures and in line with recommended standard procedures for rearing dairy calves. Feeding, spreading straw, rehousing to the calf barn and, where necessary, medical treatment and help entering the feeding box involved visual and sometimes physical contact between calves and humans. All calves were weighed, fed colostrum from a bottle and taught to drink from a teat bucket during the first days of life. Stockpeople were instructed not to talk to or stroke the calves during these procedures and not to let them suck their fingers. If physical contact was necessary, the stockpeople were to proceed in a calm and gentle manner, as usual on the farm, but to keep the interaction as short as possible and to avoid stroking except if it was necessary, for instance, to keep an animal calm during a veterinary procedure. The gentle contact treatment group, in contrast to the control group, was provided with gentle tactile stimulation and vocal interactions, ie stroking and talking, for 2 min per day, five days per week during the first four weeks of life. The animals were stroked on the ventral neck, as this has been shown to be most effective in reducing cows' fear of humans (Schmied *et al* 2008a). All animals in one pen belonged to either the control or the gentle contact group, as it was not possible to stroke some calves in a pen without having contact with their pen-mates. There were four pens with controls and four pens with stroked calves.

Gentle interactions were provided by one of three female experimenters (experimenter A: 1.65 m, dark blonde hair; experimenter B: 1.79 m, red hair; experimenter C: 1.68 m, blonde hair) and occasionally by one of two female stockpeople (stockperson 1: 1.65 m, light blonde hair; stockperson 2: 1.60 m, light brown hair). Each calf in the stroked group had contact with two or three handlers. The handler entered the pen and remained standing in close proximity to the entrance for 10 s before starting to talk in a gentle voice to the calf and approaching it. Depending on the calf's behaviour, she crouched or stood close to the calf, talking to it and stroking it on the ventral part of the neck (about 60 strokes per min in each direction) for 2 min. The handler then slowly stood and left the pen or, in group housing, stroked another calf. Every time a calf avoided the handler, she waited for 10 s and approached again. Calves in the control group were visually separated from the treatment calves during the experimental stroking treatment. However, they were able to watch the handlers during preparation for the treatment and so had ample opportunity to familiarise themselves with the experimenters without any physical contact.

Reaction of calves to stroking and talking gently

The behaviour of the stroked calves directly after the experimenter entered the pen and during the treatment was scored to assess whether the calves perceived the treatment as positive. Scores were assigned as follows — after entering: 1 = approach; 2 = no reaction or both approach and avoidance; and 3 = avoidance; during treatment: 1 = accepts stroking; 2 = accepts stroking more than half the time (estimated); 3 = accepts stroking about half the time; 4 = accepts stroking less than half the time; and 5 = stroking not possible (modified after Lensink *et al* 2000). If the calves showed play behaviour (jumping, running, play-fighting), neck-stretching (positioning neck and head in an outstretched line up, down, or forward; Schmied *et al* 2008b), or oral behaviour (licking and sucking on the handler's clothes and body) including butting (upward head-thrust, naturally directed against the dam's udder, in the present context against the experimenter), this was also recorded.

Behavioural testing

All tests were conducted by an experimenter with whom the calves were familiar from the treatment or, in the case of controls, from visual contact.

Avoidance distance test

Avoidance distance (AD) was measured as described by Waiblinger *et al* (2002) on days 33 (± 1) and 76 (± 1) of age. The test was started only if the animal was standing and looking at the experimenter (A or B, balanced across groups). The experimenter approached the animal from the front or the side at a speed of 1 step s^{-1}. She extended one arm in front at an angle of approximately 45°, with the back of the hand forwards. The distance between the calf's muzzle and the experimenter's hand was estimated (steps of 10 cm) at the moment when the calf avoided the experimenter by taking a step or withdrawing its head. If the calf did not avoid the experimenter, she touched the calf's nose with the back of her hand and an avoidance distance of 0 cm was assigned. The avoidance distance test (ADT) was conducted three times per calf to ensure robust data. Between repeated tests, the experimenter left the pen for at least 30 s. During tests, a voice tracer played a recording of one sound s^{-1} to allow accurate measurement of time.

Arena test

The arena test (adapted from Boivin *et al* 2000) was performed on day 34 (± 1) of age. Before the test, the experimenters (A and B or C) collected a saliva sample to measure the activity of the hypothalamic pituitary adrenal (HPA) axis (see *Collection and analysis of saliva samples*). The experimenters then fitted the calf with heart-rate recording equipment (see *Recording and analysis of heart rate measures*).

The test began with the experimenters loading the animal into a cart on wheels and transporting it to a room adjacent to the barn. The cart was placed next to the entrance of the

Table 1 Description of behavioural patterns in the arena test.

Behaviour	Description
Entrance in new area	The calf places both forefeet in a new area
Elimination	The calf urinates or defaecates
Escape attempt	The calf moves its head upward and close to the arena wall and shifts its weight to the hind legs. The forelegs may be lifted from the ground
Tail flicking	The calf moves its tail to the side, partly with an upward motion
Exploring	The calf's head is close to and directed toward the floor or the arena wall
Self-grooming	The calf touches its body with a hind foot or the muzzle
Locomotion	The calf takes at least two steps within 1 s
In same area as human	The calf stands with at least both forefeet in the area that contains the experimenter
In direct contact with human	The calf keeps its head close enough to the experimenter that no distance between them is visible

test arena, which measured 4.0 × 2.3 m (length × width). The walls were made of 1.4-m high wooden panels and the floor completely covered with black rubber stall mats, the edges of which were used to divide the arena into three similarly sized areas (each 2.3 × 1.3 m) for video analysis. The door was opened and if the animal did not enter the arena within 3 min the experimenters pushed it inside. The calf was isolated in the arena for 3 min (phase I). Experimenter A then entered the arena and crossed it to remain standing by the wall opposite the door (phase II). If the calf was not in the same area as the experimenter, she gently called to it every 30 s. If the calf approached the experimenter to within arms' reach, she stroked it for 30 s. After 3 min, the experimenter left the arena and the calf was isolated for a further 3 min (phase III) before the door was opened and the animal allowed to enter the cart. If it did not enter on its own, it was gently pushed into the cart. When the calf had been transported back to its home pen, a second saliva sample was collected before it was released.

The calf's behaviour in the arena was videotaped using a camera mounted close to the ceiling. The following behavioural patterns were analysed from the recordings by a trained observer using the software The Observer® XT 9.0 (Noldus Information Technology, Wageningen, The Netherlands): the frequencies of elimination, entrances into a new area, escape attempts and tail-flicking; the durations of exploration, self-grooming and locomotion; and the time spent in the same area as the human, the time spent in direct contact with the human and the latency until contact was established. For a description of behaviours, see Table 1. As the surveillance system did not record sound, the frequency of vocalisations was recorded manually. Due to a shortage of staff, it was not possible to have an observer 'blind' with regard to the experimental condition, evaluate the behaviour of treatment and control animals; considering the results, an observer bias can be excluded.

Collection and analysis of saliva samples

Before the arena test, basal saliva samples were taken in the feeding box 30 min after milk feeding using Salivettes® (Sarstedt AG, Nümbrecht, Germany). The samples were taken within 3 min of approaching the calf to avoid a handling effect. At the end of the arena test, reaction samples were taken in the cart before releasing the animal back into its home pen. To collect saliva, a cotton roll was inserted into the calf's mouth using dressing forceps, allowing the calf to chew on it for 1 min. Saliva was stored at –20°C until analysis. Cortisol concentrations were measured by enzyme immunoassay as previously described (Palme & Möstl 1997; Wagner *et al* 2013).

Recording and analysis of heart-rate measures

During the arena test, heart rate was recorded using commercial, non-invasive heart-rate monitors (Polar Electro Oy, Kempele, Finland). As the heart rate of each animal had been measured before (2 × 4 h; data still under analysis), a period of habituation to the equipment was not necessary. Heart-rate measuring equipment was attached after milk feeding, so that the recording started at least 20 min before the onset of the test, and left on the animal until at least 30 min after the test to provide a baseline value as well as a measure of the animal's recovery. Transmitters (T61-coded) were fastened to the animals using the chest belts provided, after wetting the coat and applying ample quantities of electrode gel for better electrode-skin contact. They were protected by stretch belts containing the receiver/data logger (S810i) in a sewn-on pocket. Animals were equipped with the heart-rate monitors in the feeding boxes to decrease contact between animals and experimenter.

To make data comparable to recordings during the test, 3-min time windows were analysed, one within the 20 min before the test and two immediately following it. Baseline and recovery heart-rate values were included in the analysis only if the animal was standing or walking slowly (ie not running/playing or lying), as determined from video recordings.

Statistical analysis

Data were not always obtained for all 25 animals due to technical failures, diarrhoea or unsuitable behaviour of the animals (for heart-rate measurements: lying down, running). The final sample sizes are given in *Results*. Data were analysed and presented graphically using PASW Statistics 20 software (SPSS Inc, Chicago, Illinois, USA). When data are depicted as box plots, the bold line corresponds to the median, the lower and upper line of the box to the first and third quartile, and the whiskers to the lowest and highest values that are still within a range of 1.5 × interquartile range. Outliers (all values between 1.5 × interquartile range and 3 × interquartile range) are marked with a circle, extreme values (outside of a range of 3 × interquartile range) with an asterisk. Differences, main effects and interactions with $P \leq 0.05$ are referred to as significant; with $0.5 \leq P \leq 0.1$ they

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are referred to as trends. AD was measured three times per animal at each age. The three values were averaged and mean ADs were analysed with repeated measures ANOVA. The results of the arena test (behaviour, cortisol concentrations, average heart-rate per phase) were analysed with repeated measures ANOVA, except for the behavioural pattern 'direct contact with the human' and the latency to establishing contact. These parameters were analysed using ANOVA. Non-significant ($P > 0.05$) treatment × sex interactions were removed from the models. In cases where Mauchly's Test of Sphericity indicated a violation of the assumption of sphericity ($P < 0.05$), F-values were Greenhouse-Geisser corrected (this concerned the measures: elimination, area close to the experimenter, exploration). If Levene's Test for Homogeneity of Variance was significant, data were log- (tail-flicking) or square root-transformed (escape attempt). If residuals were not normally distributed, data were log-transformed (vocalisations, latency to contact). If residuals were not normally distributed and the models' assumptions were not met after transformation, results were validated using non-parametric statistics (AD, elimination, grooming). *Post hoc* comparisons of heart rate between the different phases of the arena test were conducted using paired sample t-tests and the Bonferroni-Holm method for adjustment of α to correct for multiple testing. Means (± SEM), medians, 1st and 3rd quartiles and graphs of untransformed data are presented. To interpret the heart-rate data with regard to physical activity, Pearson's correlation coefficient was calculated for heart rate and locomotion during each phase of the arena test.

Results

Reaction of calves to stroking and talking gently

The calves approached the experimenter when she entered the pen in 30% of all occasions and avoided her in 3%. Calves accepted the stroking treatment in 76% of all occasions during the whole time and in a further 20% for more than half the time. There was only one occasion in which a calf accepted stroking for less than half of the time. Play behaviour occurred in 21%, oral behaviour in 43% and neck stretching in 70% of all occasions.

Avoidance distance test

There was no significant main effect on avoidance distance of treatment, age, or sex (Figure 1; repeated measures ANOVA: $n_{stroked} = 13$, $n_{control} = 9$; treatment $F_{1,19} = 0.45$; $P = 0.51$; age $F_{1,19} = 0.03$; $P = 0.87$; sex $F_{1,19} = 0.33$; $P = 0.57$), nor any significant interactions between these factors. In general, ADs were very low: more than 50% of the animals could be touched three times at 33 days of age and more than 70% on day 76.

Arena test

Behavioural measures

Repeated measures ANOVA ($n_{stroked} = 12$, $n_{control} = 9$, except for vocalisations, contact and latency to contact: $n_{stroked} = 12$, $n_{control} = 10$; Table 2) revealed a significant main effect of phase on the frequency of entrances into new areas ($F_{2,36} = 24.48$; $P < 0.001$), escape attempts ($F_{2,36} = 19.95$; $P < 0.001$), tail-flicking ($F_{2,36} = 5.65$; $P = 0.007$) and vocalisations ($F_{2,38} = 13.26$; $P < 0.001$), on the time spent in the same area as the experimenter ($F_{2,36} = 54.77$; $P < 0.001$), as well as on the duration of locomotion ($F_{2,36} = 63.99$; $P < 0.001$) and exploration ($F_{1.5,26.4} = 20.63$; $P < 0.001$). For all of these behaviours except time spent in the same area as the experimenter, occurrence was lower during phase II (experimenter present) than during phases I and III (experimenter absent). Calves spent more time during phase II in the area where the experimenter stood than they did during phases I and III. The main effect of phase was non-significant for the frequency of elimination and the duration of grooming.

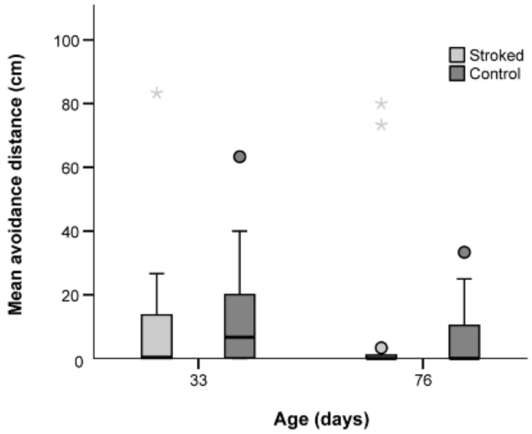
There was no significant effect of treatment or sex on any behaviour patterns except tail-flicking, for which there was a significant main effect of treatment: control animals showed the behaviour more often than stroked animals during all phases ($F_{1,18} = 4.41$; $P = 0.05$). Furthermore, there was a trend towards a main effect of sex on exploration ($F_{1,18} = 3.99$; $P = 0.06$), with higher levels in females.

No significant interactions between phase, treatment and/or sex were seen except for the number of entrances into a new area. There was a significant phase × sex interaction ($F_{2,36} = 4.07$; $P = 0.026$), with females entering new areas more often during phase 1 than males.

Salivary cortisol concentrations

There was a significant main effect of treatment ($n_{stroked} = 12$, $n_{control} = 11$; repeated measures ANOVA $F_{1,20} = 12.83$; $P = 0.002$) on log-transformed salivary cortisol concentrations. Stroked calves had lower cortisol values before and after the arena test (Figure 2). Cortisol levels tended to be higher after the test than before ($F_{1,20} = 3.05$; $P = 0.096$). There was neither a significant effect of sex nor any significant interaction.

Figure 1



Mean avoidance distances. Each calf was tested three times at each age, with the three measurements averaged to obtain the mean avoidance distance. Stroked: calves that had experienced gentle interactions with humans; control: calves with routine barn handling. Statistics: Mann-Whitney U test, one-tailed; day 33: $n_{stroked} = 14$, $n_{control} = 11$; day 76: $n_{stroked} = 13$, $n_{control} = 9$; ns at both ages.

Table 2 Behaviour during the arena test.

Behaviour	Treatment	Phase I (without human)		Phase 2 (with human)		Phase I (without human)	
		Median	1st, 3rd	Median	1st, 3rd	Median	1st, 3rd
Elimination	Control	0.0	0.0, 0.5	0.0	0.0, 0.5	0.0	0.0, 1.5
	Stroked	0.0	0.0, 1.0	0.0	0.0, 0.0	0.0	0.0, 0.8
Entrance into new area***	Control	12.0	7.5, 16.5	1.5	1.0, 5.0	10.0	6.0, 18.0
	Stroked	15.0	11.0, 21.0	2.5	1.3, 4.0	14.0	10.3, 20.0
Escape attempt***	Control	2.5	0.5, 3.5	0.0	0.0, 0.0	1.0	0.5, 2.0
	Stroked	0.0	0.0, 3.0	0.0	0.0, 0.0	1.0	0.0, 4.3
Tail-flicking**,*	Control	21.5	10.5, 33.5,	13.0	3.0, 42.0	29.0	9.0, 40.5
	Stroked	5.0	1.0, 8.5	0.0	0.0, 11.0	4.5	0.8, 41.0
Vocalisation***	Control	1.0	0.0, 3.8	0.0	0.0, 0.0	2.0	0.0, 5.0
	Stroked	0.0	0.0, 4.0	0.0	0.0, 0.0	0.0	0.0, 5.0
Same area as experimenter***	Control	8.5	0.0, 20.9	169.1	121.6, 170.2	54.6	12.3, 71.1
	Stroked	29.2	17.3, 39.4	131.1	94.8, 167.3	45.1	10.4, 57.5
Exploration***,T	Control	58.9	48.8, 64.4	28.5	7.2, 53.4	47.4	39.0, 55.1
	Stroked	71.8	51.1, 86.9	31.2	2.5, 42.1	70.2	51.4, 92.8
Locomotion***	Control	93.9	71.1, 113.4	24.6	10.6, 33.8	50.0	42.9, 73.3
	Stroked	87.3	73.5, 125.9	25.0	11.9, 36.6	75.9	64.8, 107.6
Grooming	Control	0.0	0.0, 1.1	0.0	0.0, 0.8	0.0	0.0, 1.1
	Stroked	0.0	0.0, 1.5	0.0	0.0, 1.7	0.0	0.0, 1.2
Contact	Control	–	–	65.3	41.4, 68.9	–	–
	Stroked	–	–	75.4	59.9, 146.3	–	–
Latency to contact	Control	–	–	2.0	0.7, 12.2	–	–
	Stroked	–	–	1.6	0.2, 31.4	–	–

*** Main effect of phase, $P \leq 0.001$; ** Main effect of phase, $P \leq 0.01$; * Main effect of treatment, $P \leq 0.05$; T Main effect of sex, $P \leq 0.1$. Number of occurrences per phase (170 s) is given for behaviours recorded as events (elimination, entrance into new area, escape attempt, tail-flicking, vocalisation). For behaviours recorded as states (time spent in the same area as the experimenter, exploration, locomotion, grooming, contact) and for latency to contact, the duration is given (s), with a possible maximum of 170 s. 1st, first quartile; 3rd, third quartile. $n_{stroked} = 12$, $n_{control} = 9$, except for vocalisations, contact and latency to contact, where $n_{stroked} = 12$, $n_{control} = 10$.

Heart rate

There was a significant effect of phase on average heart rate ($n_{stroked} = 5$, $n_{control} = 7$; repeated measures ANOVA $F_{2.51,22.62} = 21.8$; $P < 0.001$; Figure 3). Heart rate increased from before the test to phase I (isolation, $t = 6.96$; $P < 0.001$), decreased to phase II (presence of the experimenter, $t = 6.96$; $P < 0.001$) and increased again to phase III (absence of the experimenter, $t = -3.50$; $P = 0.004$). After the test, there was no significant decrease in heart rate until recovery 1 ($t = 1.20$; $P = 0.25$), but a further decrease from recovery 1 to recovery 2 ($t = 6.48$; $P < 0.001$). There was no significant effect of either treatment or sex (treatment $F_{1,9} = 3.8$; $P = 0.56$; sex, $F_{1,9} = 4.2$; $P = 0.53$) and no interaction. We found no significant correlation between duration of locomotion and heart rate for any of the test phases (Pearson's correlation coefficient: phase I, $r = 0.08$; $P = 0.79$; phase II, $r = -0.20$; $P = 0.48$; phase III, $r = 0.35$; $P = 0.20$).

Discussion

Group-housed, dual-purpose calves that experienced additional gentle tactile and vocal interactions were compared with calves that experienced only routine management. We investigated the animals' relationship with humans and parameters

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indicative of stress. We found significantly higher cortisol levels in controls than in stroked calves before and after the arena test and more tail-flicking in controls in the arena test but a lack of significant differences in other behaviours.

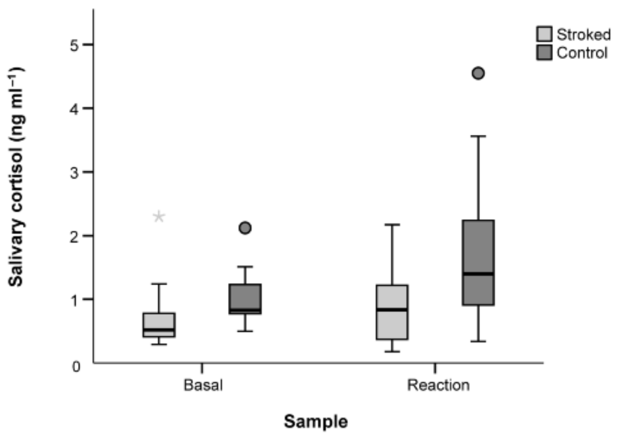
The minor number of significant differences in behavioural measures could theoretically mean that the treatment was not very effective — it was either not rewarding for the calves or the period of gentle interactions was too short to significantly reduce fear of humans. However, the stroked calves not only rarely avoided but also often actively approached the experimenter when she entered the pen for the treatment. Furthermore, levels of neck stretching were noticeably high during the treatment. Neck stretching is shown during social licking in cows and interpreted as indicative of relaxation (Reinhardt *et al* 1986; Bertenshaw & Rowlinson 2008; Schmied *et al* 2010). Play behaviour usually occurs rarely (Jensen & Kyhn 2000; Krachun *et al* 2010) but was shown in 21% of all occasions. It is considered an indicator of positive emotions and good welfare (Boissy *et al* 2007; Napolitano *et al* 2009; Held & Špinka 2011). Taken together, the behaviour during treatment indicates that it was indeed perceived as positive, in accordance with results in dairy heifers (Bertenshaw & Rowlinson 2008).

The animals experienced a total of 40 min of gentle interactions. The duration is comparable to that chosen for a study in dairy cows, where a total of 51 min of stroking led to a short-term decrease in AD one day after the end of each treatment phase (Windschnurer *et al* 2009). In addition, a total of 50 min of brushing of beef calves reduced avoidance reactions in the short term (Boivin *et al* 1998).

The small number of significant differences between the two groups may result from a ceiling effect. ADs were very low in both groups and it appears that the presence of the experimenter in the arena test was reassuring to calves regardless of the treatment experienced, as calves of both groups showed fewer behavioural signs of stress and lower heart rates when the experimenter was present. These findings indicate a good relationship with humans in the controls; consequently, it would have been very difficult to improve the relationship with humans by gentle interactions in the stroked group, resulting in a significant difference between groups. There are several possible factors that may have contributed to the low levels of fear observed in control calves. First, the unavoidable early close contact with humans during the first feedings may lay the foundation for a good relationship (Jago *et al* 1999; Krohn *et al* 2003). Further, a stockperson filled the milk buckets while being visible to the calves, reinforcing the connection of food with the presence of a human and thus possibly enhancing the effect of early contact with humans in the absence of negative interactions. A range of other farm-specific factors, such as farm size, space allowance and attributes of the stockperson may also play a role (Hemsworth *et al* 2000; Waiblinger *et al* 2002; Leruste *et al* 2012). Not only does the Teaching and Research Estate have a high ratio of stockpeople to calves, most of the time veterinary medicine students are also present, although as they were instructed not to interact with the calves we can assume that their presence had relatively limited effects.

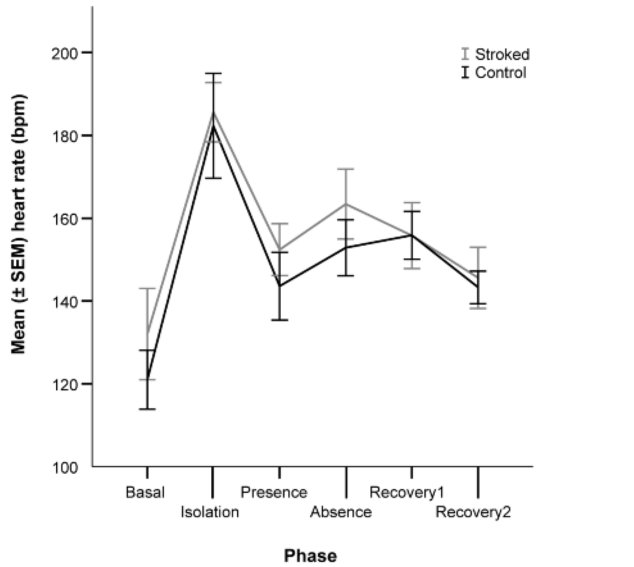
Another possible reason for the minor level of effects observed is that interactions during test procedures that were considered neutral to slightly aversive might have been perceived by the calves as stimulating and thus positive. In

Figure 2



Salivary cortisol concentrations before and after the arena test. Stroked: calves that had experienced gentle interactions with humans; control: calves with limited human contact. Statistics: repeated measures ANOVA; $n_{stroked}$ = 12, $n_{control}$ = 11; main effect of treatment: *P* = 0.002.

Figure 3



Mean (± SEM) heart rate before, during and after the arena test. Stroked: calves that had experienced gentle interactions with humans; control: calves with limited human contact. Basal, calf is standing or calmly walking in the home pen before the arena test; isolation, calf is isolated in the test arena; presence, the experimenter is present in the arena; absence, the experimenter has left the arena and the calf is again isolated; recovery1 and recovery2, calf is standing or calmly walking in the home pen after the arena test. Statistics: repeated measures ANOVA; $n_{stroked}$ = 5, $n_{control}$ = 7; main effect of phase *P* < 0.001.

addition to the procedures described in *Materials and methods*, eight faecal samples were taken from each calf over the course of the experiment. These were not analysed due to technical difficulties. Furthermore, heart rate was measured three additional times to calculate heart-rate variability (data still under analysis). It was often not possible to avoid contact between the experimenters and, particularly, the pen mates of the test animal. Calves generally reacted in a positive way towards the experimenters, with exploratory and play behaviour, which have both been described as self-rewarding and indicative of positive emotions (Boissy *et al* 2007). In addition, the effect of mere habituation should not be underestimated (Schütz *et al* 2012).

It is also possible that the calves are genetically predisposed towards a low level of fear of humans as a result of selection for breeding (Jago *et al* 1999). There are genetic factors underlying temperament, as shown by breed differences between (Murphey *et al* 1980, 1981) and even within dairy and beef breeds (Blanco *et al* 2009; Orbán *et al* 2011). Dual-purpose breeds seem less fearful of humans than dairy breeds, as indicated by lower avoidance distances (Mazurek *et al* 2011; Leruste *et al* 2012). It is possible that the Austrian Simmental is rather calm and predisposed towards a good relationship with humans, provided that there are no explicitly negative experiences with humans and that there are opportunities for habituation.

Although there were no differences in other behavioural measures, tail-flicking occurred more often in controls than in stroked calves during each phase in the arena test. As this behaviour is often seen in the context of painful procedures and in combination with other stress-indicating behaviour in cattle (Herskin *et al* 2003; Sylvester *et al* 2004) and in other species (Herskin *et al* 2009; von Borstel *et al* 2009), it can be interpreted as indicative of a negative affective state. Controls may have perceived the test situation in a more negative way than stroked calves.

Stroked calves also had significantly lower concentrations of cortisol in their saliva than control calves, both before and after the arena test. As animals were confined to the feeding boxes before the first sample and the duration of locomotion during the test did not differ between the groups, the difference does not merely reflect the metabolic demand of physical activity (Koolhaas *et al* 2011). It is also unlikely that the difference between the groups was caused by acute stress induced by the test, as cortisol concentrations were rather low, the numerical difference between groups was small and there was already a difference before the start of the test. An alternative explanation might be that tactile stimulation has an organisational effect on the activity of the HPA axis (Schulz *et al* 2009). A possible mechanism has been studied thoroughly in rats (*Rattus norvegicus*) (reviewed by Liu *et al* 1997): higher levels of maternal licking and grooming lead to enhanced glucocorticoid feedback sensitivity and, thus, decreased ACTH and cortisol concentrations in response to stress. An effect of stroking on HPA activity of calves via a similar mechanism is conceivable.

Animal welfare implications

A good relationship with humans may be achieved without specific efforts. If the general contact between stockpeople and calves is gentle and negative experiences are minimised, if calves are exposed to such contact on a regular basis and/or if calves belong to a breed with a rather calm temperament, the calves' relationship with humans can be good without the need for additional gentle interactions. In practice, stockpeople should provide additional gentle interactions whenever feasible, as this probably elicits positive emotions and thereby improves the calves' quality of life. Although the present study revealed minimal differences between groups, it is not possible to refute our hypothesis due to the ceiling effect. Under circumstances whereby the relationship between animals and humans has a bigger scope for improvement, additional gentle interactions might lead to a better relationship with humans in group-housed calves. This point warrants further exploration.

Conclusion

We did not find that gentle interactions, including stroking and talking to calves, led to a better relationship with humans compared with control animals. This negative finding may be due to the good relationship with humans in animals of the control group. The ceiling effect precludes an evaluation of the effectiveness of the use of gentle interactions, although the calves' immediate reactions indicate that they perceived the gentle interactions positively. Stroked calves had lower concentrations of salivary cortisol and showed less tail-flicking in the arena test; these parameters may be more sensitive to an effect of gentle interactions than the other parameters investigated.

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