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Effects of positive reinforcement training for heifers on responses to a subcutaneous injection

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ABSTRACT

Cattle are subjected to routine procedures that require restraint and close contact to humans, which are both potentially aversive to the animal. Positive reinforcement training techniques may affect how animals perceive and respond to these procedures. The objectives of the current study were to describe a positive reinforcement regimen used to train cattle to stand still for a sham injection, and to assess the effects of this training on the responses to an actual injection. Eight "agency" heifers were trained, over an average of 85 ± 4.6 sessions, with positive reinforcement (i.e., animals received a grain reinforcer for desired behaviors) to enter a headlock, and they were habituated with counterconditioning and desensitization to a sham injection (i.e., animals were gradually exposed to the sensation of the sham injection, paired with access to grain). The headlock remained open at all times to allow heifers to leave. Eight "habituation" heifers were exposed to the treatment area and headlock for an equal number of sessions and duration as agency heifers, and 7 "naïve" heifers were provided no exposure to the treatment area. Once agency heifers tolerated the sham injection, all animals received a 1-mL subcutaneous injection of 0.9% NaCl while in the head lock (habituation and naïve heifers were locked in but agency heifers were free to withdraw). Immediate responses to the injection, starting with tenting of the skin, were video recorded and summarized as a reactivity score, which included the number of steps, head tosses, and backing-up movements; we also recorded the latency to approach the treatment area and headlock for 3 d after the injection. Of the agency heifers, 5 remained standing for the actual injection, whereas 3 heifers moved out of the headlock for a brief period (1, 3, and 5 s, respectively). Habituation heifers had a higher reactivity score [17.5 (10.5–28); median (IQR)] than agency [6 (2–13.5)] and naïve heifers [6 (5–7)]. Averaged over the 3 d after injection, agency heifers showed lower latencies to come to the treatment area [8.7 (7.2–24.2) s] than did habituation [50.5 (28–60) s] and naïve [53.7 (18–60) s] heifers. Agency heifers voluntarily entered the headlock within 1.3 (1–1.5) s but, with one exception, none of the other heifers did so within the allowed 15 s. These results indicate that dairy heifers can be trained with positive reinforcement and counterconditioning to voluntarily accept a painful procedure, and that training can reduce avoidance behaviors during and after the procedure.

Key words: agency, stress, pain, human–animal relationship, management

INTRODUCTION

Dairy cattle are routinely subjected to husbandry procedures for health (e.g., vaccinations, hoof trimming) and management (e.g., rectal exams) reasons. Even though some routine procedures contribute to the longer-term welfare of the animals (e.g., vaccinations), they require restraint and close human contact that can be stressful for cattle (e.g., Lewis and Hurnik, 1998; Rushen et al., 1999), and the procedures themselves can be painful (Adcock and Tucker, 2018).

Efforts to make husbandry procedures less aversive show promise. For example, repeated handling of veal calves reduced fear responses to humans (Lensink et al., 2000). Further, dairy cows were less responsive to rectal palpation when in the presence of a person with whom they had prior positive experiences (Waiblinger et al., 2004). Operant conditioning, in particular positive reinforcement (where desired behaviors of the animals are followed and strengthened with provision of a reinforcer, typically food) and counterconditioning (where negative associations with an aversive event are replaced with positive associations), is often used with zoo and laboratory animals to mitigate negative responses to husbandry procedures. For example, studies on primates (Behringer et al., 2014) and grizzly bears (Joyce-Zuniga et al., 2016) have shown that these animals can be trained with such techniques for "vol-

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untary" venipuncture (i.e., without restraint during the procedure), and that trained animals are less distressed by the procedure (as evidenced by lower cortisol levels than control animals that were not trained and thus required restraint). To our knowledge, there are no reports on the effects of such training on cattle.

The objectives of the present study were to (1) describe a positive reinforcement regimen used to train dairy heifers to voluntarily stand still for a sham injection, and (2) assess the effects of this training on the responses to an actual injection. We hypothesized that trained heifers would show reduced behavioral responses to the injection and reduced aversion to return to the location of injection relative to heifers that were not trained. For the untrained heifers, we hypothesized that heifers that were habituated to the training area (but not trained) would react to the injection similarly to inexperienced heifers, but that habituated heifers would show reduced aversion to return to the injection location.

MATERIALS AND METHODS

Data for this study were collected from July to December 2019. All procedures were approved by the University of British Columbia Animal Care Committee (protocols A18-0174-A003 and A15-0117).

Animals, Management, and Housing

Twenty-four Holstein heifers were followed from July to December 2019. At the time of enrollment, animals ranged in age from 6 to 9 mo old [mean ± standard deviation (**SD**): 7.7 ± 0.8 mo] and were clinically healthy. Heifers were housed in a freestall barn group pen with access to the feedbunk through 26 headlocks and to 26 lying stalls deep-bedded with sand. Heifers had ad libitum access to a TMR, fed once daily at 0830 h and pushed up 3 times daily (1100, 1800, and 2200 h).

All heifers had some experience with restraint in the headlock feed barrier (headlock width center-to-center: 41 cm) during the study period, including when vaccinated (twice when heifers were between 8 and 10 mo old), when treated with a fly repellant (twice), and—for 7 of the 24 heifers—when they were bred. Two animals in the habituation treatment were lame for short periods during the training phase; these animals were not brought into the treatment area at these times.

Treatment During Training Period

Each heifer was allocated to 1 of 3 treatments: agency, habituation, and naïve. Agency heifers were trained to accept a sham injection without restraint. Habituation heifers were exposed to the treatment area to a similar extent as agency heifers. Naïve heifers were handled only minimally during the training period and only introduced to the treatment area at the time of injection. Blocks of 3 heifers ("trios") were formed based on age similarity; within each trio, heifers were randomly assigned to one of the 3 treatments without replacement, such that treatments were similar in age at the time of enrollment (mean ± SD: 7.8 ± 0.9, 7.7 ± 0.8, and 7.6 ± 0.8 mo, for agency, habituation, and naïve heifers, respectively).

Structure of Training Sessions. An area of the alley adjacent to the heifers' home pen was assigned as the treatment area, located between the 2 pen gates (6 m from one and 1.4 m from the other gate of the home pen; see Figure 1). Two gates blocked the heifers' access to the remaining alley, one of them with an integrated headlock (headlock width center-to-center: 48 cm). The fourth side of the treatment area was defined by a 1.2-m-high wall and railing, bordering onto a cattle handling area with a squeeze chute where the assistant was standing for the injection. The concrete flooring of the alley was covered with a thin layer of sand to improve traction.

Training took place on average 4 times per week (1 to 7 sessions per week, with 1 wk without training; 21 weeks in total). Sessions started at approximately 1100 h. For training sessions, agency and habituation heifers were divided into 2 groups, with both groups composed of 4 of the agency heifers and their respective habituation partners. These groups were handled separately, and the group handled first was alternated daily.

To facilitate animal handling during training sessions, all 24 heifers were briefly locked in the headlocks at the feedbunk until the first group (4 agency and 4 habituation heifers) had been sorted and brought to a waiting area within the home pen. From this group, one heifer at a time was brought to the treatment area. The order in which heifers were handled within a training session was based on their willingness to leave the waiting area of the home pen; when the gate to the alley was opened, the first heifer who exited the waiting area without being prompted was allowed to come. When none of the remaining heifers voluntarily left the pen, they were brought up individually in no specific order but rather by ease of handling (e.g., a standing heifer was brought before a heifer who was lying). After the training session, the heifer was brought to a return area within the home pen where she remained until all 8 animals in the group had completed their training. At this point, the second group of 8 heifers was brought to the waiting area and the training sequence repeated.

Agency Heifers. The goal behavior for agency heifers was defined as "standing calmly with the head in the open headlock while a sham injection is given"; once heifers were successful at the goal behavior, they were deemed ready for the injection. Training of the agency heifers used positive reinforcement training and desensitization in combination with counterconditioning (see Table 1 for definitions). Grain was used as reinforcer during conditioning and was offered to heifers in a handheld black rubber bucket. For the last 2 mo of training, grain was mixed with the regular feed of the heifers (taken from the feedbunk; 1 kg of grain mixed with approximately 0.2 kg of TMR) to calm responses of the heifers. During the course of a training session, heifers consumed 0.7 ± 0.3 kg (as fed) of grain and, once mixed with the TMR, 1.0 ± 0.3 kg (as fed) of the feed mix. Training sessions were led by one trainer (J.L.), who made all training decisions, delivered the reinforcer, and directed the assistant (A.M.), who was responsible for all manipulations, including the injection.

A detailed training plan was implemented for all agency animals, but training steps within the plan were dynamic, based on the individual animals' performance (e.g., a training step was made easier for the heifer if she chose not to engage). In short, the training steps for "standing calmly in headlock" consisted of (1) habituating the heifer to the training arena and to eating out of the handheld bucket; (2) teaching the heifer to step forward and with her head through the headlock; and (3) teaching her to remain calmly in the headlock for an increasing amount of time. The headlock remained

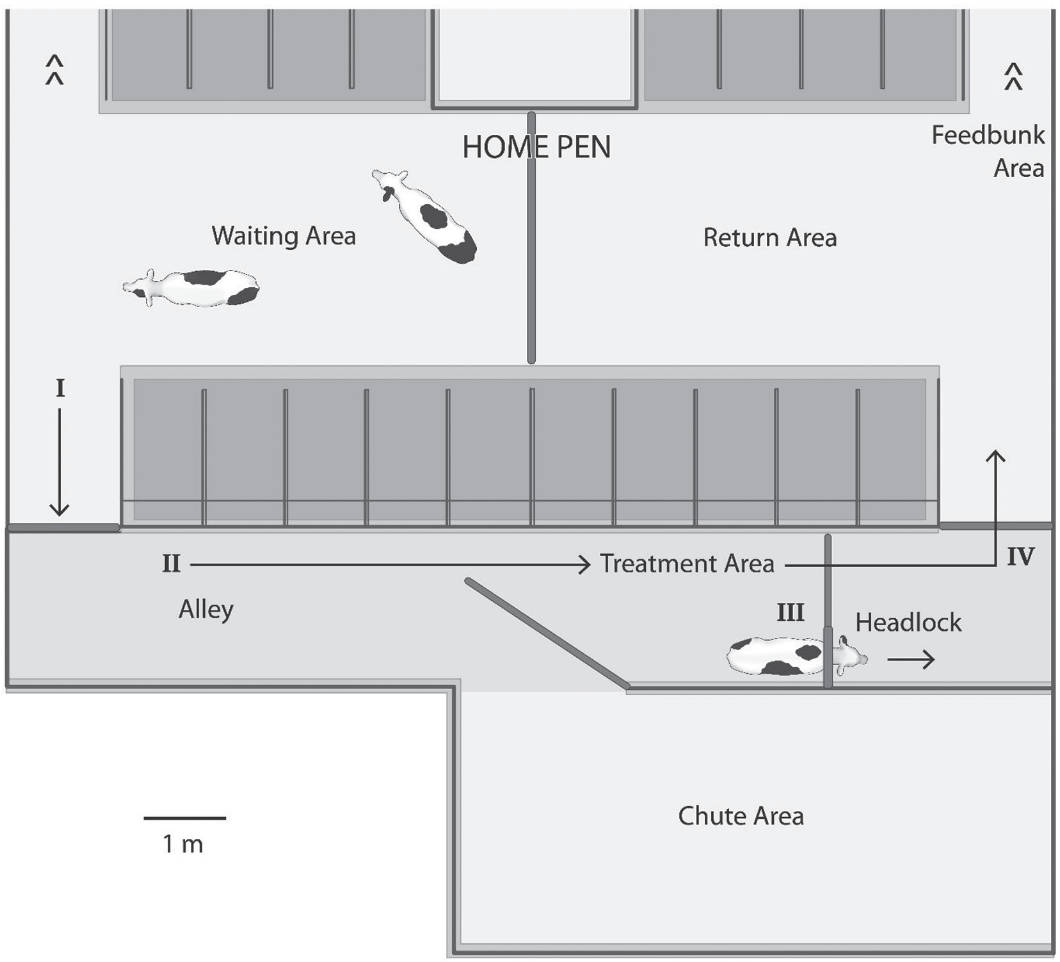

Figure 1. Schematic of the home pen and treatment area. To facilitate handling of the heifers during training sessions, the home pen was temporarily divided into 3 parts (waiting area, return area, and feedbunk alley). At the beginning of a session, a group of 8 heifers (4 agency and 4 habituation heifers) was brought to the waiting area; from there, one heifer at a time was brought to the treatment area. After the training session, the heifer was brought to a return area within the home pen. Once the first group of 8 heifers completed their training, the second group was moved to the waiting area and training was continued. I to IV indicate latency measures taken in the 3 d before and 3 d after the injection: I = voluntary participation; II = latency to arrive in treatment area; III = headlock (push) latency; IV = latency to leave.

open at all times, allowing heifers to withdraw and disengage at any time. The trainer stood in the alley at the opposite side of the headlock. Once a heifer was standing calmly in the headlock for approximately 10 s between reinforcers, the training plan progressed to initiating the desensitizing and counterconditioning to the presence and touch of the assistant. The counterconditioning process was started with the assistant standing motionless, and in the position for the injection (i.e., in the chute area and next to the heifer's shoulder). Initially, the assistant only moved her arms. Once the heifer remained calm, training progressed to touching the animal at the injection site (behind the right shoulder, above the rib cage) with the back of 2 fingers, to pressure with a capped pen, tenting of the skin, a poke with the end of a paperclip, and the combination of skin tent with a paperclip poke and a poke with a dulled needle (without perforating the skin; see Figure 2 for progression through these steps by each heifer). The overall goal during the training process was to not provoke behavioral responses by the heifer (desensitization), even as the intensity of the sensation increased; thus, the decision whether to proceed to the next sensation was made based on the heifer's response to the current sensation. For example, if a heifer reacted to being touched by stepping away or withdrawing from the headlock, or if she reacted more subtly (e.g., skin twitch, head movement) in 2 successive repetitions of being touched, the next sensation was made less intensive. All progressions were combined with food such that for the first repetitions of a new step, food and touch were delivered simultaneously (with food delivery extending beyond the touch), but then in subsequent repetitions food was presented after the heifer was touched. During the last month of training, food was delivered simultaneously to the touch (and extending beyond), adjusting the process to the animals who progressed more slowly; this practice was continued for the actual injection. Agency heifers experienced on average 85 ± 4.6 (range: 79 to 91) training sessions and spent an average of 8 ± 2.0 (3 to 17) min per session in the treatment area.

Habituation and Naïve Heifers. The goal for the habituation group was to familiarize the animals with all aspects of the handling and to habituate them to the treatment area and restraint in the headlock. The experience of each habituation heifer was matched to the experience of the agency heifer within her trio, in that she was brought to the treatment area as often and was handled for the same duration as her agency partner. Handling duration was measured from the time a heifer exited the home pen and entered the alley to the time when the gates from the treatment area were opened for her to return to the home pen. At the beginning of the training period, habituation heifers were allowed to move in the treatment area freely, but once agency heifers had learned to enter and stand in the headlock voluntarily, habituation heifers were gently pushed and restrained in the headlock upon entering the treatment area. The movement of habituation heifers was further restricted by closing a side gate perpendicular to the headlock gate, preventing heifers from side stepping away from where the assistant would stand for the injection. The trainer and the assistant immediately moved away from the heifer once she was locked in the headlock, and until it was time to bring the heifer back to the home pen. Habituation heifers experienced, on average, 82 ± 5.2 (75 to 89) habituation sessions and spent an average of 8 ± 2.1 (2 to 20) min per session in the treatment area.

Table 1. Definitions of operant and classical conditioning procedures used for training 8 dairy heifers aged 7.8 ± 0.9 mo (mean ± SD) to enter a headlock and to stand calmly with the head in the open headlock while a sham subcutaneous injection was given

Procedure	Definition
Positive reinforcement	The likelihood for a behavior to occur in the future is increased by the addition of a (desirable) consequence to the learner
Classical conditioning[1]	Associative learning, in which pairing of a neutral stimulus (conditioned stimulus, CS) with a stimulus that has negative or positive valence to the learner (unconditioned stimulus, US) leads to a conditioned response to the initially neutral stimulus
Counterconditioning[1]	Associative learning where negative expectations of a stimulus-conditioned outcome are replaced with a positive one. A stimulus with negative or positive valence is paired with a stimulus of the opposite valence to change the original stimulus' valence and behavioral response to it.
Systematic desensitization	Gradual and progressive exposure to a stimulus of negative valence at a level that does not elicit a behavioral response by the individual

[1]Even though the training situation may have been new to the heifers (e.g., they had never been touched with a paperclip), given the initial avoidance response of most heifers, we argue that we countered this emotional response and therefore use the term "counterconditioning" throughout this text, being aware that some aspects of the training steps may have reflected classical conditioning for some of the animals and stimuli.

During the training period, handling of the naïve group was limited to the times that they were locked in the feedbunk headlocks together with their pen mates while animals in the other treatments were sorted for the training sessions.

Treatment During Testing and Outcome Measures

Latencies Before the Injection. From the day an agency heifer first successfully accepted a sham injection, morning sessions were continued daily for her and her habituation partner, and the injection was scheduled for 3 d after. The sessions and handling procedures for agency and habituation heifers continued identically to previous training sessions, but now heifers were given a set amount of time (as described below) to voluntarily move from the waiting area to the treatment area and into the headlock. If animals did not proceed voluntarily within the allowed time, they were gently pushed. Each session was now video recorded with a camera (Samsung Galaxy 8 phone camera) placed in the chute area and adjusted to give a view of the home pen, exit gate, alley, and treatment area including headlock.

Heifer behaviors coming into the treatment area were video recorded; the entire process of moving a heifer from the home pen to the treatment area and into the headlock was divided and defined as follows. Voluntary participation was defined (as a binary outcome) when heifers exited the waiting area of the home pen with all 4 feet within 15 s of opening the gate to the alley. Further, latencies were measured in 3 intervals: (1) latency to arrive in treatment area: the time heifers walked along the alley from the pen gate to the treatment area (maximum of 60 s); (2) headlock latency: the time from opening the headlock to the heifer entering the headlock (maximum of 15 s); and (3) headlock push latency: the time from opening the second gate of the treatment area (behind the heifer) to when the heifer was pushed into and restrained in the headlock (this only applied to heifers who did not enter the headlock within 15 s).

Naïve heifers were brought to the treatment area for the first time on the day of injection. First, a naïve heifer was individually brought to the waiting area within the home pen and given 4 min to adjust. Thereafter, starting with opening the gate to the alley, their handling procedure was identical to that of habituation heifers, and they were also gently pushed into the headlock if they had not entered voluntarily within the allowed time.

Injections. For agency heifers, the injection session unfolded identically to the training sessions except that the sham injection was replaced with an actual injection. Habituation and naïve heifers were approached by the assistant and were injected after they had been

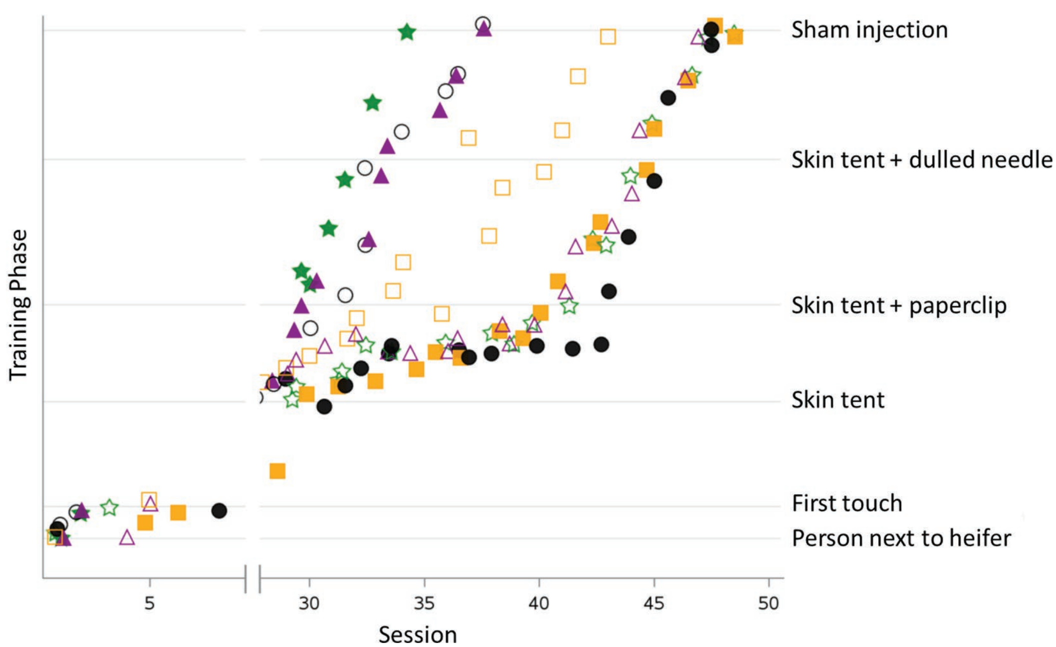

Figure 2. Progress over training session of the counterconditioning process for a sham (subcutaneous) injection in 8 agency heifers (each shown with a different color-shape combination symbol), where the skin was tented and the heifer was poked with a dulled needle with pressure but without perforating the skin. A sensation was always paired with food, and a new sensation was introduced with low intensity. Intensity was gradually increased for each sensation, but only when the heifer did not react to the current intensity by stepping away or with head movements or skin twitches. Session 1 represents the first session of the counterconditioning process where the assistant stood next to the heifer; sessions 8 to 28 are not shown.

restrained in the headlock for approximately 1 min. All heifers received a single subcutaneous injection of 1 mL of 0.9% NaCl solution (Hospira) behind their right shoulder above the ribcage. Behavioral reactions were scored from video, with a lateral view of the heifer (recorded with Vixia HF R82, Canon). Each heifer was scored twice: once for the entire procedure (starting at the first touch for tenting of skin) and once for the injection only (starting at the time of needle perforation). In both instances, behaviors were scored until 10 s after the needle was removed. A camera set up in a lateral angle behind the heifer allowed for a detailed view of the needle perforating the skin (HDC-TM 41, Panasonic). The behaviors scored were (1) leg lift (when a heifer lifted one front or rear hoof partially or completely off the ground), (2) head toss (when a heifer moved her head in a jerky upward movement), and (3) backing up (when a heifer shifted her body position backward). The number of occurrences for each behavior were summed into one reactivity score per heifer (Table 2). The duration of the procedure (from tenting the skin to withdrawal of both hands) and the duration of the injection (from perforating the skin to withdrawal of the needle) were both assessed from the front-angle video recordings.

In one habituation heifer, the needle bent at the first injection attempt before perforating the skin; a new needle and syringe were used for the actual injection, and behavioral reactions were recorded only for the attempt that led to the injection. One heifer of the naïve group was excluded from injection following her violent escape attempts to tenting of the skin.

Latencies After the Injection. On each of the 3 d following the injection, heifers were brought to the treatment area in the same manner as on the day of injection. Agency heifers were subjected to the routine procedures, including sham injection, and habituation and naïve heifers were handled for the same amount of time as the agency heifer of their trio, and restrained in the headlock once in the treatment area. Latencies to reach the treatment area and enter the headlock were recorded and scored as in the 3 d before the injection. If an animal could not be pushed into the headlock within the time her agency partner had spent in the treatment area, the time from opening the second gate of the treatment area (behind the heifer) to the time when the agency heifer's duration was matched was recorded as headlock push latency.

In addition, starting on the day of injection, animals were allowed 3 min to voluntarily return to the home pen after the gates to the return area in the home pen were opened; this was recorded as latency to leave (time from when gates to the home pen were opened until the heifer entered the home pen with all 4 feet).

Statistical Analyses

The number of heifers enrolled was determined on the basis of the size of pen available for the study and the maximum number of animals that could be trained with the research staff available. We did not perform a power analysis as our primary aim was descriptive; because this is the first work published in this area, we had no basis for estimating effect size or variance.

All statistical analyses were performed using SAS (version 9.4; SAS Institute Inc.), with heifer as the experimental unit. Given the multiple observations per animal, latency measures for each treatment were assessed for time effects with a mixed linear regression model (PROC MIXED), separately for the days before the injection and the days after the injection. Both models contained day (relative to the injection), treatment, and the interaction of day and treatment as fixed effects, with day identified as a repeated measure. As time effects were not evident (interaction of day and treatment with $P > 0.1$ for all outcome variables), preinjection data were only considered for the day of injection (as this was available for all 3 treatments), and the 3 data points recorded after the injection were averaged to 1 data point per animal. Summarized variables were scrutinized using PROC UNIVARIATE.

Table 2. Behavioral reactions (median and interquartile range) of 8 agency, 8 habituation, and 7 naïve heifers to a subcutaneous injection

			Treatment group		
Behavior	Definition	Type[1]	Agency	Habituation	Naïve
Steps (no.)	Heifer lifts one front or rear hoof off the ground	P	6 (2–12.5)	12 (8–22.5)	3 (3–5)
		I	6 (2–12.5)	9.5 (7.5–14)	3 (0–3)
Head toss (no.)	Heifer moves head in a jerky upward movement	P	0 (0–0)	2.5 (0.5–3)	0 (0–1)
		I	0 (0–0)	1.5 (0.5–2.5)	0 (0–1)
Backing up (no.)	Heifer shifts body position backward; agency only: heifer backs out of the headlock	P	0 (0–1)	2 (2–2.5)	2 (0–3)
		I	0 (0–1)	1 (1–15)	1 (0–2)

[1]Behaviors were recorded from touching the heifer for the skin tent (procedure, P) and starting with piercing of the skin (injection, I), and, for both P and I, until 10 s after the needle was removed.

All latencies were tested for differences between treatments before and after injection separately. To assess treatment differences for voluntary participation before the injection (binary outcome), a Fisher's exact test was employed. Most variables were non-normally distributed (i.e., alley latency, headlock latency, push latency and latency to leave); we thus analyzed all continuous data with Kruskal-Wallis tests (PROC NPAR1WAY Wilcoxon). When results indicated treatment differences ($P < 0.05$), a Dwass, Steel, Critchlow-Fligner post hoc test was performed to determine which pairs of treatments differed. The significance level was set at $P \leq 0.05$, and a tendency at $P \leq 0.10$. All behaviors were scored by one observer (intraclass correlation coefficient >0.9 for all behaviors) who was blind to treatment and the objectives of the study.

RESULTS

Latencies Before the Injection

Agency heifers voluntarily exited the waiting area of the home pen more often than habituation heifers or naïve heifers before the injection [$P < 0.001$; agency 7 (of 8); habituation 1 (of 8); naïve 0 (of 8)]. We observed a difference in latency to arrive in the treatment area [χ^2(df = 2) = 11.02, $P < 0.01$; Figure 3] and to enter the headlock [χ^2(df = 2) = 22.59, $P < 0.001$]; agency heifers had lower latencies than habituation and naïve heifers. All agency heifers entered the headlock voluntarily; habituation heifers had lower latencies to be pushed into the headlock than naïve heifers ($P < 0.001$).

Immediate Responses to the Injection

Of the agency heifers, 5 remained in the headlock position and kept eating immediately following the injection. The other 3 heifers backed out of the headlock for 1, 3, and 5 s, respectively. Two of these 3 heifers approached the assistant before reentering the headlock, and the other heifer reentered the headlock immediately. Videos of all heifers during the injection can be viewed at https://youtu.be/FOnlIVwdnoE.

There was a treatment difference in reactivity score for the entire procedure [χ^2(df = 2) = 9.24, $P < 0.01$] and for the injection alone [χ^2(df = 2) = 10.5, $P < 0.01$; Figure 4]. When scored for the entire procedure, habituation heifers showed more reactions than agency and naïve heifers. When scored for the injection only, habituation heifers showed more reactions than naïve heifers but not agency heifers. Habituation and naïve heifers both had higher reactivity scores for the entire procedure than for the injection alone. None of the agency heifers reacted to tenting of the skin, so, for this treatment, the reactivity score for the entire procedure was equal to the score for the injection alone.

There was no treatment difference in the duration of injection and procedure (injection: $P = 0.64$; procedure: $P = 0.19$), and neither the duration of the injection nor the procedure was correlated with the reactivity of the heifer (procedure: r = 0.33, df = 21, $P = 0.1$; injection: r = 0.15, df = 21, $P = 0.47$).

Latencies on the Days Following Injection

On the 3 d after the injection, agency heifers voluntarily exited the waiting area more often than habituation ($P = 0.001$) and naïve ($P < 0.01$) heifers [χ^2(df = 2) = 16.3, $P < 0.001$; mean rank score = 19.4 for agency heifers, 6.9 for habituation heifers, and 9.4 for naïve heifers].

Similar to the results obtained before the injection, agency heifers came into the treatment area [χ^2(df = 2) = 30.69, $P < 0.001$] and entered the headlock [χ^2(df = 2) = 62.11, $P < 0.001$] more rapidly than habituation and naïve heifers. Only one heifer from the habituation and naïve treatments ever entered the headlock within 15 s (and did so only once); all others had to be pushed into the headlock. Habituation heifers tended to have lower latencies to be pushed into the headlock than naïve heifers ($P = 0.09$; Figure 3). On the second day after the injection, 2 heifers of the naïve group could not be pushed into the headlock within the time allotted (to match the respective agency heifers' time in the treatment area).

Naïve heifers returned to the home pen more quickly than did heifers in the agency ($P < 0.01$) and habituation ($P < 0.01$) treatments [χ^2(df = 2) = 16.79, $P < 0.001$; mean rank score = 15.4 for agency heifers, 15.3 for habituation heifers, and 4.3 for naïve heifers].

DISCUSSION

A simple injection, as commonly experienced by cattle, may consist of multiple aversive components, including isolation from the social group (Herskin et al., 2007), novelty of the environment (Forkman et al., 2007), restraint (Chen et al., 2016), proximity to humans and human touch (Müller et al., 2008), and pain of the injection (Ede et al., 2018). The results of the current study show that heifers provided the opportunity to freely enter and leave the headlock and trained to accept all elements except the injection showed few behavioral responses and were more willing to return to the treatment area on days following the procedure. We argue below that several aspects of the conditioning procedure contributed to this outcome.

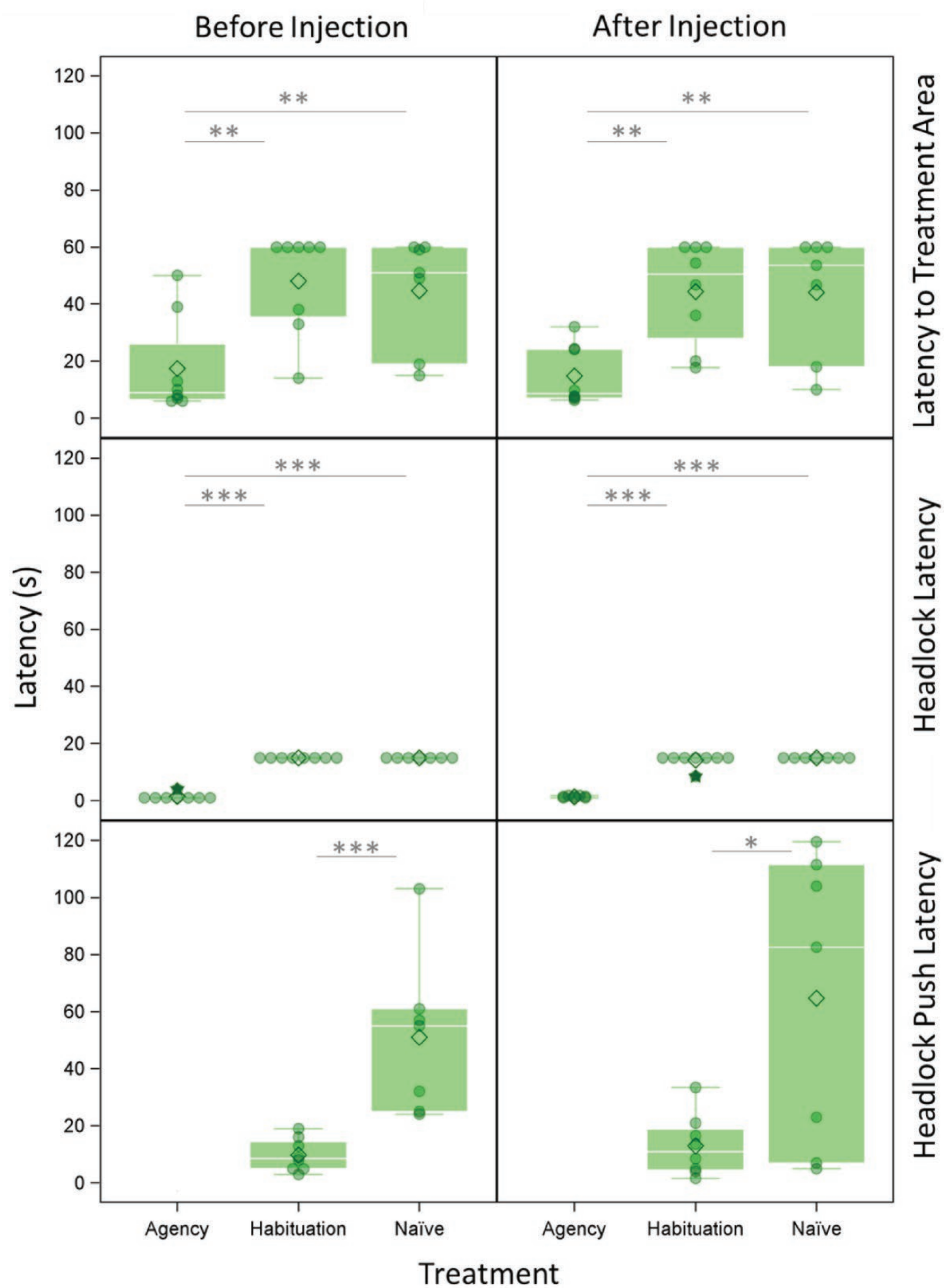

Figure 3. Latency of agency (n = 8), habituation (n = 8), and naïve (n = 7) heifers from leaving the waiting area to arriving in the treatment area (maximum allowed time: 60 s), headlock latency (15 s), and headlock push latency after heifers did not enter the headlock voluntarily. Data are shown for d 0 (before the injection) and d 1 to 3 (after the injection, averaged across 3 d) separately. Dots represent data of individual heifers; stars represent outliers. Horizontal lines of the boxes indicate lower quartile, median, and upper quartile, and whiskers represent 1.5 × IQR (interquartile range) from the upper and lower quartile, respectively. $*P < 0.1$, $**P < 0.05$, $***P < 0.01$.

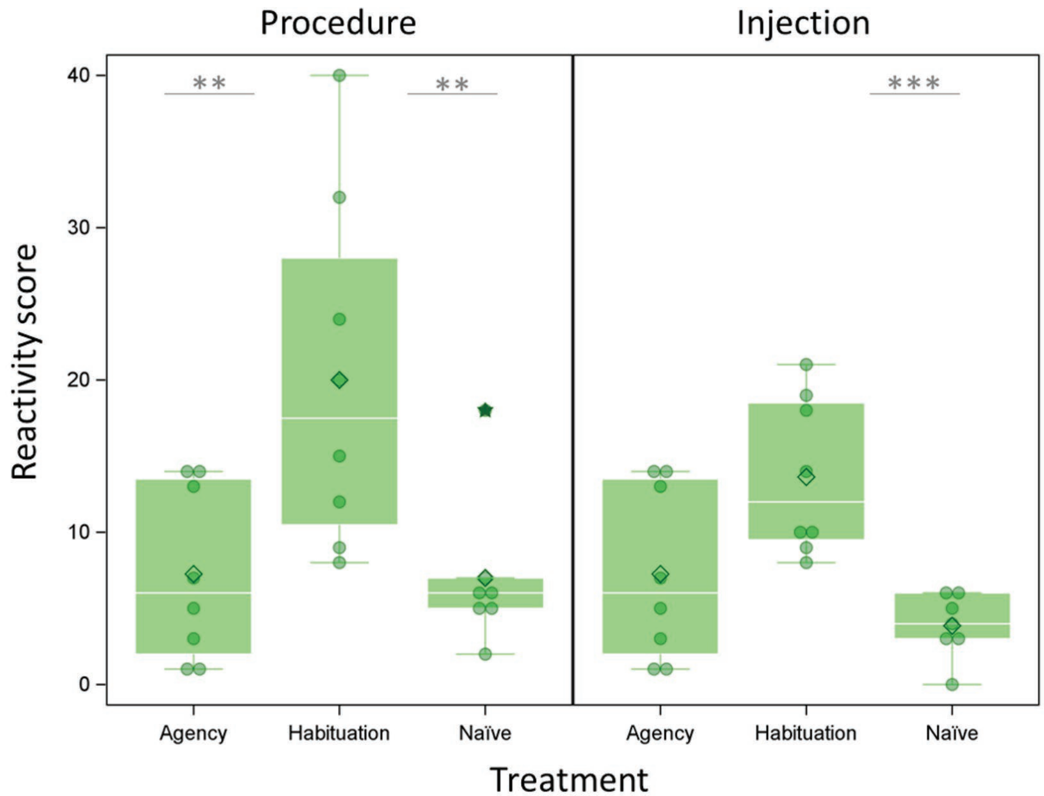

Figure 4. Reactions of agency (n = 8), habituation (n = 8), and naïve (n = 7) heifers to a subcutaneous injection of 1 mL of saline solution. Reactions were scored starting with tenting of the skin (Procedure) and when the needle pierced the skin (Injection). The reactivity score constituted of number of leg lifts, number of backing up attempts (or backing out of headlock for agency heifers), and number of head tosses. Dots represent data of individual heifers; stars represent outliers. Horizontal lines of the boxes indicate lower quartile, median, and upper quartile, and whiskers represent 1.5 × IQR (interquartile range) from the upper and lower quartile, respectively. $^{**}P < 0.05$, $^{***}P < 0.01$.

Positive reinforcement training for aversive procedures, such as blood draws, has been shown to reduce stress responses in other species, including bonobos (Behringer et al., 2014) and grizzly bears (Joyce-Zuniga et al., 2016). Positive reinforcement training allows for control and predictability during the learning process and when performing a task (Bassett and Buchanan-Smith, 2007). In our study, agency heifers could exert control (i.e., directly influence outcomes through their behavior; Sambrook and Buchanan-Smith, 1997) to access grain (by entering the headlock) and control proximity to the assistant and the (sham) injection. This level of control may have influenced how pain of the injection was perceived by agency heifers; studies on humans have shown that enhanced control can decrease pain sensitivity (Müller and Netter, 2000; Müller 2012).

In conjunction with positive reinforcement training, a counterconditioning approach (typically in conjunction with systematic desensitization) is frequently used for overcoming fear responses when training voluntary behaviors in animals (Heidenreich 2012). Counterconditioning modifies an individual's behavioral response through associative learning, whereby negative expectations of a stimulus-conditioned outcome are replaced with positive ones (Keller et al., 2020). Our goal was that the agency heifers would remain unresponsive but calm to the visual and tactile stimuli associated with the sham injection, by pairing these stimuli with food. In addition to a lowered fear response, conditioning procedures alter pain perception in humans (reviewed by Zhang et al., 2019). Some argue that the Bayesian Mind theorem explains the mechanisms of classical conditioning (Prévost et al., 2013; Tzovara et al., 2018); the Bayesian Mind paradigm suggests that how stimuli, including pain and its intensity, are perceived (the affect part of pain) is influenced by previous experiences and expectations (Ongaro and Kaptchuk, 2019). Repeated exposure to the treatment area, as well as the gradual increase of unpleasant sensations in combination with food, allowed heifers to know what to expect during a training session and to form overall positive expectations for the session. These positive expectations, together with the provision of control, may have led to a reduced experience of fear and pain in these animals.

Feeding during the session may also have functioned as a distractor, in this way reducing responsiveness of heifers in the agency treatment. Using distraction to decrease pain is well established in humans, especially children (e.g., Sparks, 2001; Canbulat et al., 2014), although the effects diminish with more painful procedures (e.g., kidney stone removal; Gezginci et al., 2018). Reports of the effects of distraction on pain responses in animals are rare; one study demonstrated that rats distracted with a novel object showed fewer pain responses than controls when tested with a standard pain-inducing procedure (intraplantar formalin injection; Ford et al., 2008). More work is required to specifically assess the effect of distractors on pain responses in animals. Drawing from the knowledge provided in the human literature, feeding agency heifers during the injection may have contributed to a lowered experience of pain through distraction, and thus also to a reduced behavioral response. However, food as a distraction is not sufficient to explain the differences in response between agency heifers and the other treatments. At the beginning of the counterconditioning process, agency heifers reacted to a simple touch even while being fed, and some heifers were slow to progress to more intense stimuli (also while being fed) as evidenced by the slow improvements made over time.

The combined evidence from the reaction to the injection and latency to approach the treatment area and headlock suggest that heifers in the habituation and naïve treatments perceived different aspects of the procedure as aversive. The habituation heifers appeared to have perceived the injection itself as especially aversive, given that animals in this treatment showed the highest number of behavioral reactions to the needle. This pronounced reaction may have been due to the negative contrast between expectation of the procedure (as based upon their experience in the previous habituation sessions) and their actual experience during the injection; during the training period, habituation heifers had experienced that humans retreat when stepping into the headlock, and they may have connected human approach with the consequent opening of the headlock and return to the home pen. In addition, habituation heifers may have attended more to the injection than naïve heifers, as this was the only novel aspect for these animals; focusing on pain has shown to lead to increased pain perception in humans (Ruscheweyh et al., 2011). That said, the injection experience may have not been so aversive as to override the positive effects of repeated exposure to the handling and treatment area, as latencies to be pushed into the headlock remained low after the injection (compared with those for naïve heifers) and, like agency heifers, the habituated heifers remained in the treatment area for the allowed time after being released.

For the naïve heifers, the entire experience was novel, making it impossible to separate the response to specific aversive stimuli associated with the procedure. Naïve heifers may have been sufficiently stressed by the social isolation, restraint and novelty, that the injection per se was less salient. Stress-induced analgesia (e.g., Miguez et al., 2014) is more pronounced (in rats) when stressors are not controllable (Maier et al., 1982). The increased time needed to push at least some of naïve heifers into the headlock on the days following injection suggests conditioned place aversion, indicating that even though these animals showed few behavioral reactions to the injection, they found the entire procedure to be aversive. This interpretation is consistent with the results of Pajor et al. (2000), who used approach latencies to assess the aversion of different handling procedures in cattle. Including a naïve group without injection may have helped differentiate between the effects of the handling (i.e., novelty, social isolation and restraint) and the direct effects of the injection.

Training dairy with positive reinforcement to participate in at least some routine management procedures may also have a positive influence on the human–animal relationship, including fear of and confidence in humans (reviewed by Waiblinger et al., 2006). Much work on improving the relationship between humans and cattle has focused on the frequency (Lensink et al., 2000; Parham et al., 2019) and quality of handling, particularly "gentle handling," which often evolves some level of tactile interaction; for example, gently stroking the animal (e.g., Lürzel et al., 2018; Lange et al., 2020). In our study, the positive effect of frequent handling might have been reflected in habituation heifers remaining in the treatment area for as long as agency heifers did. Agency heifers voluntarily approached the treatment area (with humans present) more often, suggesting that using food to decrease fear of humans was helpful. In a series of experiments, Pajor et al. (2003) found that dairy heifers chose food but not gentle handling over a neutral control treatment, indicating that food was of more value to the animals. Similarly, in horses, training with food versus grooming resulted in better learning outcomes and improved measures of the human–horse relationship (Sankey et al., 2010). Manually feeding cattle reduced fear responses (Ebinghaus et al., 2018), likely because it was possible for animals to associate the presence of humans with the delivery of food (classical conditioning). We suggest that future research investigates the use of food, as compared with gentle interactions, on the human–animal bond and fear reactions.

The human–animal relationship is further influenced by the attitudes and behaviors of the farmer (Waiblinger et al., 2002). Caretakers' attitudes can be positively influenced by the ease with which an animal is handled (reviewed by Waiblinger et al., 2006). In our study, agency heifers were much faster to move to the treatment area and into the headlock, whereas habituation alone did not lead to overall short latencies. Worker injury is an important risk associated with handling cattle (Sorge et al., 2014; Lindahl et al., 2016), and this risk was presumably less for the agency heifers, given that handlers could remain on the opposite side of the fence and there was no need to for physical contact. Others have reported a reduction in handling times associated with positive reinforcement training. For example, horses trained with positive reinforcement loaded into a trailer faster than nontrained animals (Dai et al., 2019), and sheep that were offered food after moving through a chute were more easily pushed than nontrained sheep (Hutson, 1985). Together, these findings suggest that training animals with positive reinforcement may lead to easier and more efficient handling and potentially improve the human–animal relationship on farms; we suggest studying these aspects in more detail in the future.

To address our objectives, we used a single injection that likely caused only mild pain (Ede et al., 2018); how our results can be extrapolated to more aversive or repeated treatment, as animals experience within a farm setting, is unclear. Other research indicates that this approach could work. For example, Callealta et al. (2020) reported that captive lionesses, once trained with positive reinforcement, could be subjected to repeated blood draws and vaginal swabs without restraint and while maintaining their cooperation.

A limitation of our study is the small sample size, increasing the risk of type II error (e.g., Garamszegi, 2016). Although the current sample size was sufficient to detect treatment differences in latency measures, a larger sample may have allowed us to better detect differences in the behavioral reactions to the injection.

This study provides a starting point on how to introduce positive reinforcement training to farms, but the described procedures are not feasible for many commercial dairies. We suggest that, with further research, (1) the training process could potentially be improved and shortened, and (2) technology might be used for at least some of the training; for example, teaching cattle with positive reinforcement to move to handling areas on a farm. Several technologies are already being used for training cattle, such as that used for virtual fencing (e.g., Campbell et al., 2019; Lomax et al., 2019), and to motivate cows to enter automatic milking systems (Jacobs and Siegford, 2012). These technologies could be modified to support a positive reinforcement regimen for dairy cattle training, including aspects of husbandry training.

CONCLUSIONS

The results of our study suggest that agency heifers had a less negative experience during injection compared with heifers in other treatments, and that the agency heifers were motivated to participate in the training procedure. We conclude that positive reinforcement training and counterconditioning can make husbandry procedures for dairy cows less aversive.

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