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Assessing the emotional states of dairy cows housed with or without their calves

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ABSTRACT

The practice of keeping dairy cows with their calves continues to gain interest. Cow-calf contact, or lack thereof, is expected to affect emotional states, but this requires empirical testing. Different types of cow-calf contact may also affect the emotional states of cows. The primary objective of this study was to assess the emotional state of dairy cows with full-time (23 h/d), part-time (10 h/d), or no-contact with their calves (separated 48 h after birth), using a visual judgment bias test (JBT) about one month after calving; JBT is the current gold-standard method to assess emotional state in animals by evaluating optimism or pessimism (illustrated by the proverbial half-full or half-empty water glass). The secondary objective was to compare outcomes of color- versus shape-based visual JBT. Fifty dairy cows were trained to approach a positive image on a screen (rewarded with food) and to avoid a negative image (else punished with waving bag). Once learned (>80% correct over 2 consecutive days), cows were presented with 3 ambiguous images (each presented once per day among 4 positive and 3 negative images, repeated over 4 d), and their approach responses recorded. For the color method (10 full-time, 9 part-time and 11 no-contact cows), positive and negative images were a solid red or white background; ambiguous images were shades of pink. For the shape method (8 full-time, 6 part-time and 6 no-contact cows), positive and negative images were a white circle or cross on a black background; ambiguous images were overlaid circle and cross in varying shades of gray. Cows learned to discriminate colors quicker than shapes (7.3 d, confidence limits [CL]: 6.6–8.2 d; vs. 9.3 d, CL: 8.1–10.6 d). Approaches to ambiguous colors followed a generalization curve (81.0, 33.1, and 5.0 ± 3.7% for near-positive, middle, and near-negative images, respectively), but not approaches to ambiguous shapes (31.9%, 25.7%, and 21.9% ± 4.8%, respectively), indicating colors over shapes should be used in visual JBT for cattle. Part-time cows approached fewer ambiguous color images than full-time cows (23.5%, CL: 13.4%–34.4%; vs. 44.8%, CL; 32.8%–57.1%) whereas no-contact cows were intermediate, but not different from full-time or part-time cows (37.8%, CL; 26.8%–49.5%). The color JBT results show a pessimistic bias (indicating a negative emotional state) in cows with part-time calf contact, possibly due to repeated daily separation from her young calf, relative to cows with full-time calf contact. Thus, cow-calf contact systems appear to influence the emotional state of cows depending on the practice. Cows without calf contact showed no difference in judgment bias between cows with full- or part-time calf contact, suggesting these cows probably do not experience a pervasive negative emotional state (relative to those with calf contact) approximately 30 d after calf separation. However, individual variability in judgment bias was evident for all treatments. The visual judgment bias test is a useful methodology for assessing emotional states of dairy cows; future research should prioritize understanding the emotional states of dairy cows in alternative management systems.
Key words: cognitive bias, affective state, mood, cow-calf contact, maternal behavior

INTRODUCTION

A long-standing practice in the dairy industry that is receiving increased scrutiny is the separation of cow and calf at birth; the main cited reasons for this practice are to reduce stress at separation, increase saleable milk, and protect cow and calf health (e.g., reduce likelihood of mastitis or calf scours; Sumner and von Keyserlingk, 2018; Beaver et al., 2019). The unnaturalness of cow-calf separation is the primary objection from some stakeholders (especially the public; Placzek et al., 2020), which has stimulated some interest in maintaining cow-calf contact for extended periods after birth

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(Johnsen et al., 2016). Management systems that permit cow-calf contact provide cows with opportunities to engage in maternal care and form social bonds with their calves (Wenker et al., 2021), which are known to be rewarding in other species (Newberry and Swanson, 2008). Separation from the calf leads to strong negative behavioral responses by the cow (see review by Meagher et al., 2019). Citizens have expressed concern that this could lead to lasting negative emotional states, whereas in contrast, prolonged cow-calf contact would be positive for the animals (Ventura et al., 2013; Busch et al., 2017). The emotional states of cows housed with or without their calves have not been empirically tested.

Recently there is interest in providing cow-calf contact for only part of the day (part-time contact), which may improve saleable milk yield and facilitate cow and calf management, while still achieving the behavioral and health benefits reported from full-time cow-calf contact (see reviews by Beaver et al., 2019; Meagher et al., 2019). However, part-time contact involves repeated daily separations of the cow and calf for a period of time (e.g., during the day or night hours), which may induce negative emotional states (even if temporary) that may overshadow any potential positive effect of cow-calf contact. Therefore, it is necessary to explore whether cow-calf contact of different daily durations (full- or part-time) affects the emotional state of cows compared with those without any calf contact.

The assessment of emotional states in animals is challenging, given the subjective nature of emotion, which can only be directly determined by asking the subjects how they feel. This is not possible in animals, so indirect methods are necessary. Emotions exert a strong influence on cognitive processes, or the way that we think and interpret information (Paul et al., 2005). This phenomenon, called cognitive bias, has been widely tested in animals; one form of cognitive bias, called judgment bias, involves differences in interpretation of ambiguous information (Mendl et al., 2009). For example, consider the proverbial glass that is half-empty or half-full of water. Individuals in a more negative emotional state (such as depression) are more likely to interpret uncertain information as negative (a pessimistic judgment bias, i.e., the glass is half-empty), whereas those in a positive emotional state (such as excited) will interpret the same information more positively (an optimistic judgment bias, i.e., the glass is half-full; Wright and Bower, 1992). Judgment bias is now considered the gold-standard validated method of assessing emotional states in animals, including livestock (Lagisz et al., 2020).

Judgment bias methods essentially ask animals whether the glass is half-empty or half-full by repeatedly presenting rewarded and unrewarded/punished stimuli (such as a bucket in a particular location, or seeing a particular image), most commonly in a go/no-go discrimination task. Then, the animal is presented with intermediate (ambiguous) stimuli that have never been seen before. How the animal responds to these ambiguous stimuli reflects their interpretation of uncertain information: do they see them as likely to be rewarded (by approaching, showing optimism), or likely to be unrewarded/punished (by avoiding, showing pessimism). In adult dairy cattle, judgment bias has been used to assess the emotional state of cows managed on pasture (compared with indoor housing; Crump et al., 2021) and cows experiencing different positive and negative indoor-housing conditions (such as variations in stocking density, social stability, and enrichment provision; Kremer et al., 2021). Interestingly, neither study observed a judgment bias, meaning either that the conditions did not induce differences in emotional state, or that the method was not sensitive enough to detect these differences. These studies employed a spatial judgment bias task (e.g., where left and right sides of an arena are rewarded and unrewarded, and middle locations are tested for biases in approach or avoidance responses), following studies in dairy calves that successfully detected differences in emotional state (e.g., Lecorps et al., 2018; Bučková et al., 2019).Others have used a visual judgment bias task in dairy calves (e.g., where red and white colors are rewarded and unrewarded, and shades of pink are tested for biases in approach or avoidance responses). The studies in dairy calves identified a negative (pessimistic) judgment bias when calves experienced dehorning (Neave et al., 2013), or separation from the cow (Daros et al., 2014). Taken together, these studies indicate that some judgment bias methodologies may be more sensitive to changes in emotional state in dairy cattle than others. Therefore, the visual judgment bias task is worthy of testing in adult dairy cows given its success in dairy calves. Additionally, the use of visual cues in these tasks provides an opportunity to explore visual processing and visual discrimination by cows, for which our knowledge is limited in dairy cattle (e.g., Entsu et al., 1992; Rehkämper and Görlach, 1997). Our companion study found that cows attend to different parts of an image composed of shapes (Neave et al., 2023), so the outcomes of visual judgment bias tests (**JBT**) in dairy cattle may be affected by choice of image, such as colors or shapes.

The primary objective of this study was to assess the relative emotional state of dairy cows with full-time (23 h/d), part-time (10 h/d), or no calf contact (separated 48 h after birth), using a visual judgment bias task. We hypothesized that cows with full-time calf contact would show a more optimistic bias, indicative of a more positive emotional state, compared with cows with part-time or no calf contact. A secondary objective was to determine if the type of visual cue (colors or shapes) affects training and testing performance of cows in the judgment bias task. We hypothesized that cows would take longer to learn the shape task, but that both color and shape would be suitable judgment bias methods.

MATERIALS AND METHODS

This study was conducted from September 2021 to August 2022 at the Danish Cattle Research Centre, Aarhus University (Tjele, Denmark). All animal procedures were approved by the Danish Animal Experiments Inspectorate (Permit No. 2021-15-0201-00989) in accordance with the Danish Ministry of Environment and Food Act No. 474 (May 15, 2014).

Animal Management and Treatment Groups

Eighty-four Danish Holstein dairy cows and their calves were enrolled at calving in 7 blocks of 12 cows each. A subset of these cows were part of a concurrent cognitive experiment (reported in Neave et al., 2023). All cows calved in an individual maternity pen where they remained with their calf for approximately 48 h (range: 42 to 66 h). Eligibility criteria for study enrolment required the cow and calf to be healthy (i.e., cow does not have signs of milk fever and calf is vital, assessed by farm staff twice per day), no twin births or calving complications, and that the calf could suckle from the cow without assistance within 48 h. Cows and their calves were then moved from the maternity pen to one of 3 group housing treatments: (1) full-time contact between the cow and calf, apart from milking times (total 23 h of cow-calf contact per day); (2) part-time contact between the cow and calf, between morning and afternoon milking at 0530 and 1530 (total 10 h of cow-calf contact per day); (3) no-contact, where the cow and calf were separated after leaving the maternity pen and had no further cow-calf contact. Assignment to treatments occurred in pairs (i.e., 2 cow-calf pairs to avoid stress of entering a pen alone) until all 12 cows of a block were assigned to a treatment pen. Order of dam-contact treatment assignment was pre-determined for each block, on a rotational basis: part-time, full-time, no-contact (block 1); full-time, no-contact, part-time (block 2); no-contact, part-time, full-time (block 3), and so on until 7 blocks were filled. Each treatment within a block was balanced for 2 primiparous and 2 multiparous cows whenever possible. Average lactation of multiparous cows in full- and part-time treatments was 2.3 ± 0.6 (range 2 to 4) and 2.7 ± 0.9 (range 2 to 5) lactations, respectively.

No-contact cows were housed in the main barn facility in a pen of 12 cows, which included 8 experimental cows (4 per block) and 4 nonexperimental cows. These cows had no visual or auditory contact with their calves. The pen contained 12 computerized feed bins (Insentec B.V., Marknesse, the Netherlands) in which cows were fed a TMR twice daily at 1030 and 2000 h, and 12 lying stalls equipped with mattresses and topped with sawdust daily. The pen was equipped with an automated rotating brush.

Full-time and part-time cows and their calves were housed in a dedicated barn in straw-bedded pens (one treatment per pen; 7.5 × 9 m) containing 4 cows and 4 calves. Calves had dedicated access to 2 creep areas (3 × 3 m and 1.5 × 1.5 m), one in each of the back corners of the main pen; each creep contained ad libitum concentrate from a bowl and hay from a rack, and the larger calf creep area contained a self-filling water bowl. Both cows and calves had access to 2 feed troughs (each 2 × 0.75 m) with ad libitum TMR (approximately 50:50 concentrate to roughage ratio) that was refreshed twice daily at 0800 and 2000 h. Cows also had access to 2 rotating grooming brushes and 2 self-filling water bowls, mounted on opposite sides of the pen. Straw bedding was added daily and completely cleaned out approximately every 4 wk.

All cows were milked in a double-12 parallel milking parlor twice daily. Full-time and part-time cows were milked at 0500 and 1530 h, and no-contact cows were milked 30 min later (the next milking rotation) at 0530 and 1600 h. After each afternoon milking, part-time cows were redirected via sorting gates to a pen adjacent to the no-contact cows, without visual or auditory contact with their calves. This pen contained 14 lying stalls (identical to no-contact cows), and TMR was delivered at a feed bunk with headlocks, refreshed at 2000 h. After morning milking, part-time cows returned to their home pen to be reunited with their calves. Full-time and no-contact cows always returned to their home pen after each milking.

Overview of the Judgment Bias Task

Cows were first trained in a visual go/no-go discrimination task, followed by testing in a judgment bias task, using methods adapted from Neave et al. (2013) for dairy calves. Cows began training the day after entering the treatment pen (at 48 h postcalving) and had 25 d to complete testing (due to the start of a concurrent experiment). Briefly, cows were clicker-trained to approach an image on a screen to receive a food reward (positive image), and to avoid approaching a different image or else the cow received a punishment

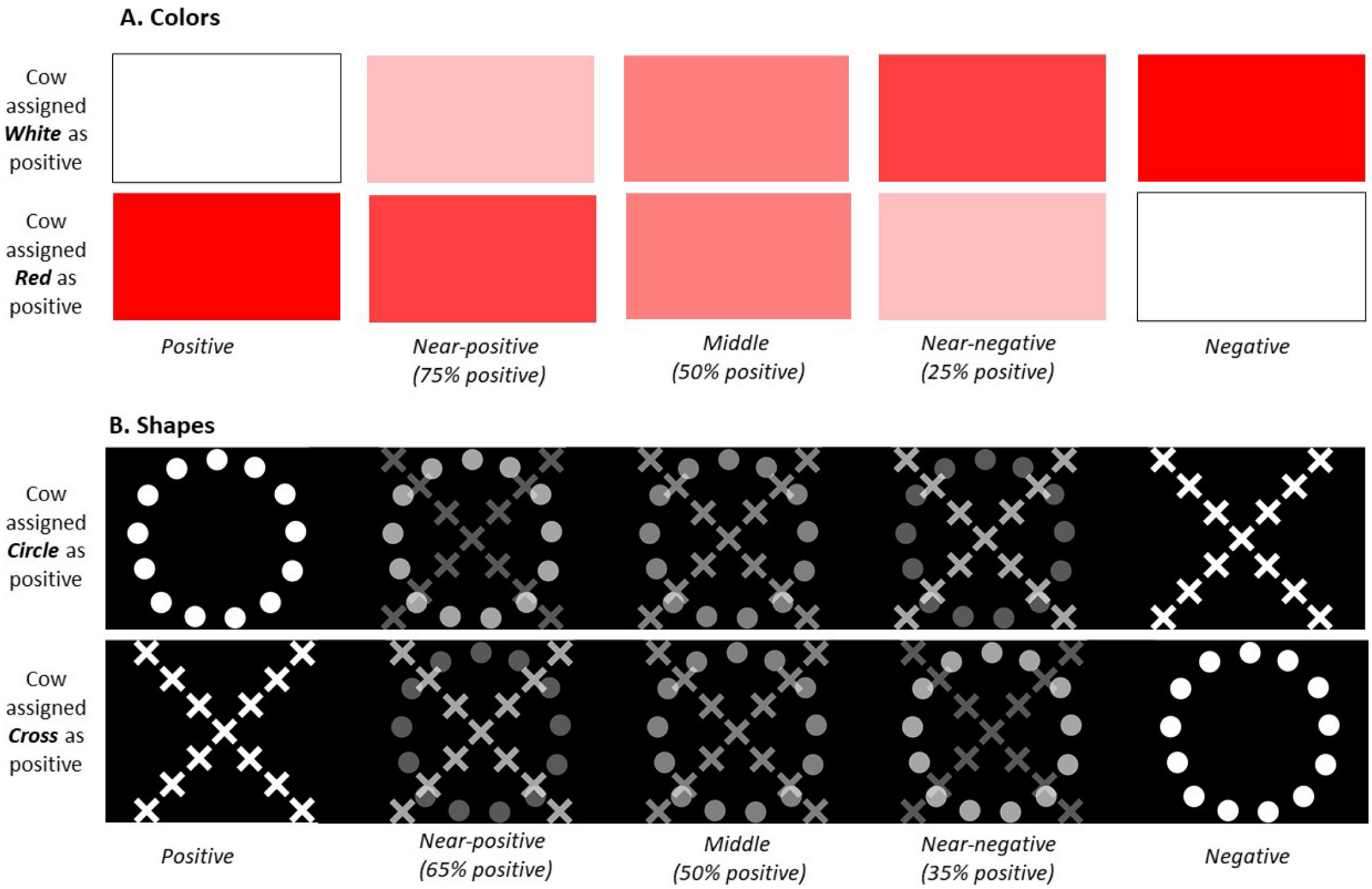

Figure 1. Images used in training and testing periods of the (A) color-based and (B) shape-based visual judgment bias task. Cows were assigned to a positive and negative image, and the 3 intermediate images reflected 75%, 50%, and 25% of the original positive image (for colors), or 65%, 50%, and 35% of the original positive image (for shapes). The black outline of the white image was not seen by the cows.

(waving plastic bag; negative image). These positive and negative images were either colors (solid red or white background; Figure 1a) or shapes (13 identical white circles or crosses arranged in an overall circle or cross on a black background; Figure 1b). Once these associations were learned, cows were presented with 3 ambiguous images that were intermediate between the positive and negative images. These were either 3 shades of pink (light, medium and dark pink) or 3 versions of overlaid circle and cross that were 3 shades of gray. Cows were expected to approach an ambiguous image if they interpreted it to be similar to the original trained positive image. Approaches to the ambiguous images were never rewarded nor punished. We compared how frequently cows in different cow-calf contact conditions approached these ambiguous images.

Table 1 summarizes the training and testing steps, and Table 2 provides the number of cows in each treatment and for each visual method that completed each step. Of the 84 cows enrolled in the study at calving, 72 cows were enrolled for judgment bias training (block 1 to 7: n = 11, 8, 11, 6, 12, 12, and 12 cows, respectively), with n = 24 cows per treatment. Cows from block 1 to 3 were assigned to shapes (n = 10 per treatment), and cows from blocks 4 to 7 were assigned to colors (n = 14 per treatment). This confound in method assignment to block was because the original methodology was only a shape-based task, but preliminary examination of data up to block 3 revealed no judgment bias generalization curve (see the Results section), so subsequent blocks were assigned to a color-based task. Assignment to the positive and negative images (red or white; circle or cross) were balanced between treatments and arenas. Blinding of experimenter to cow-calf contact treatment assignment was not possible for training and testing of cows. Cows were assessed twice daily by milkers for mastitis, and behavior during training and testing were monitored for changes suggestive of illness, which were reported to farm staff and treated accordingly. Unhealthy cows were not trained or tested (described below). Cows that were in heat resumed training after 2 d.

Table 1. Summary of training and testing steps for the color- or shape-based visual judgment bias task in dairy cows

Phase (step)	Purpose	Image(s) presented	Criterion to proceed
Initial training			
Habituation	To familiarize cows to the arena and to presentation of food reward underneath the screen	None	Cow does not move backward when tray is presented and eats comfortably from tray
Shaping for approach and nose-touch image	To train cows to pay attention to image on screen, and to approach and nose-touch the positive image	Positive	Cow walks without stopping to nose-touch image on screen 10 times
Discrimination training			
40% negative rate	To train cows to approach and nose-touch the positive image, and to avoid nose-touching the negative image, at a rate of 40% negative and 60% positive images	Positive Negative	≥80% correct in a single day, to a maximum of 4 d
50% negative rate	To train cows to approach and nose-touch the positive image, and to avoid nose-touching the negative image, at a rate of 50% negative and positive images	Positive Negative	Average >80% correct over 2 consecutive days
Judgment bias testing	To test cows' judgment bias by presenting 3 ambiguous (intermediate) images among positive and negative images	Positive Near-positive Middle Near-negative Negative	Completed 4 test days within 25 d since habituation start (due to concurrent experiment)

Training and Testing Arena

The training and testing procedures occurred in a separate barn in 2 adjacent rectangular arenas, each consisting of a start box and door allowing access to the main arena (Figure 2a–c). Visual and auditory contact with other cows, but not calves, was possible from within the arena. A pair of cows from the same treatment pen were held simultaneously in the 2 start boxes, with physical contact possible over the dividing fence. A 108 cm display screen was mounted at the front of the main arena, 6.2 m from the start box. An operator sat behind the screen (Figure 2d) to deliver rewards and punishments and to control presentation of the images from a laptop computer (using Microsoft PowerPoint for Microsoft 365 MSO). The operator moved the food tray in and out from under the screen, allowing the cow access to the food reward. Further details of the training and testing arena can be found in the supplemental material (https://data.mendeley.com/datasets/tg3bx7xs27/1).

The color images displayed on the screen are shown in Figure 1a; positive and negative images were white and red, and ambiguous colors were 75%, 50%, and 25% red saturation (i.e., shades of pink). The shape images are shown in Figure 1b; positive and negative images were a series of small circles or crosses arranged in a larger overall circle or cross, and ambiguous shapes were created by overlaying and fading the circle or cross to create different ratios of positive and negative. A pilot study revealed that 75% and 25% ratios of cross and shape did not generate expected intermediate approach responses, so the final ambiguous shapes were composed of 65%, 50%, and 35% ratios of cross and

Table 2. Number of dairy cows that were enrolled and completed each phase of judgment bias training and testing, for each visual method (colors or shapes) and each treatment (full-time, part-time, or no calf contact)

	Treatment			
Visual method or phase	Full-time	Part-time	No contact	Total
Color (white or red)				
Enrolled	14	14	14	42
Completed habituation	13	14	14	41
Completed shaping training	12	11	12	35
Completed discrimination training	10	9	11	30
Completed judgment bias testing	10	9	11	30
Shape (circle or cross)				
Enrolled	10	10	10	30
Completed habituation	9	8	10	27
Completed shaping training	9	8	10	27
Completed discrimination training	8	6	6	20
Completed judgment bias testing	8	6	6	20

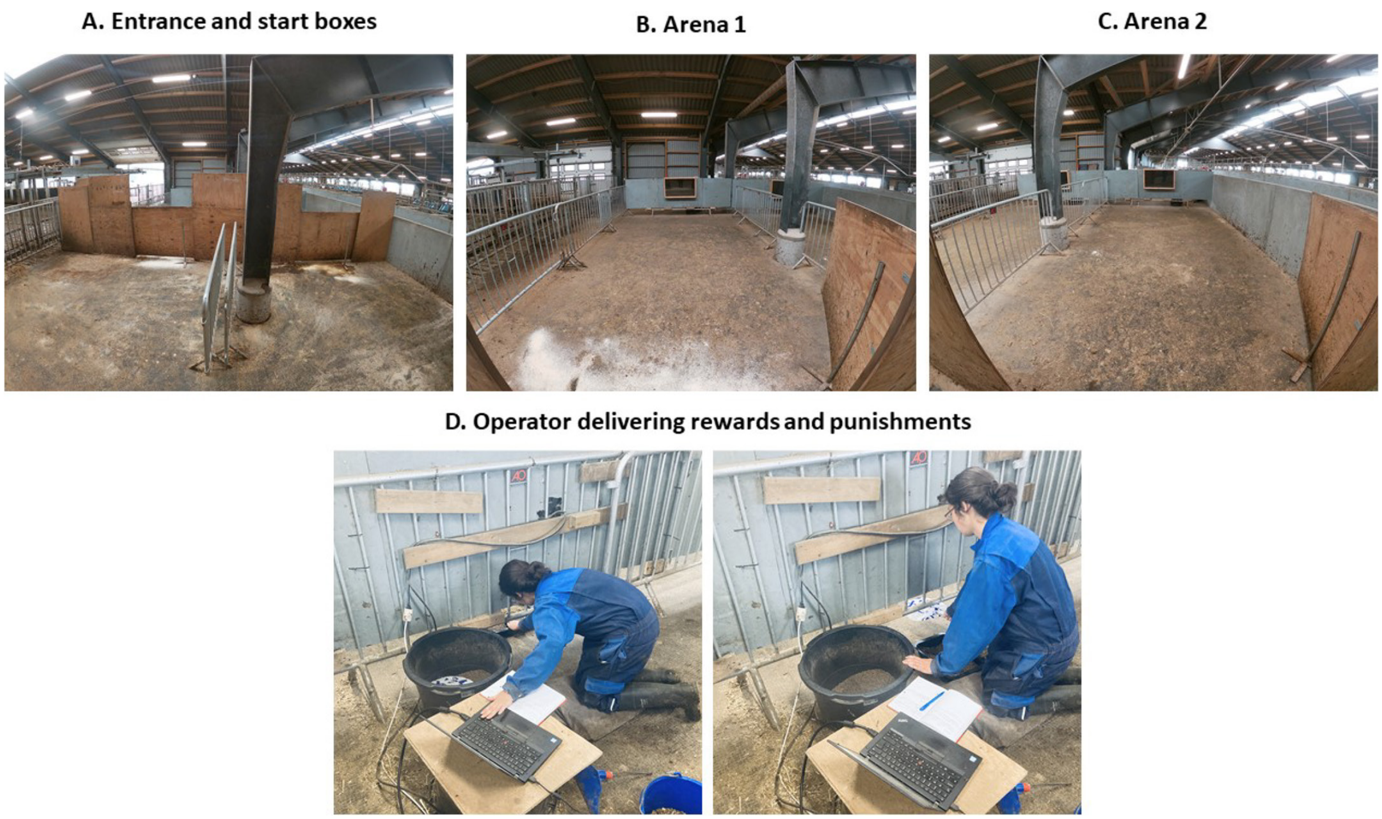

Figure 2. Training and testing facility used for visual judgment bias procedures. (A) A pair of cows entered through the far-left gate into the start box of arena 1, and one cow continued to walk into the arena 2 start box (inside fences are moveable). One cow was held in each start box in between image presentations using a wooden door. (B) View of arena 1 and (C) arena 2, from the cow's perspective. When the wooden door from the start box opened, the TV screen displayed the positive, negative, or ambiguous images, 6.2 m from the start box door. (D) An operator sat behind the screen to control the computer, which displayed images on the screen. The operator also delivered the food reward in a tray by sliding it underneath the screen, and delivered the punishment by waving a plastic bag.

shape, by progressively making the shape less white. The circle and cross images contained identically sized smaller elements (6 × 6 cm) arranged in the same-sized overall larger element (42 × 42 cm).

Initial Training

A detailed description of initial training is provided in the supplemental material (https://data.mendeley.com/datasets/tg3bx7xs27/1). Each cow was trained once per weekday between 0800 and 1200 h for a maximum of 10 min and experienced approximately 1 h of feed restriction beforehand to maintain motivation for the feed reward in the task. Part-time contact treatment had been reunited with their calves for at least 3.5 h before training began. Training order within treatment was maintained, and treatment order (e.g., no-contact cows first, part-time cows second, full-time cows third) rotated between blocks. The experimenter stood inside the arena with the cow during training.

Habituation Phase. A pair of cows were habituated simultaneously to adjacent arenas. Food rewards (familiar TMR and pelleted concentrate in 2 sides of a baking tray) were initially placed at the center of the arena, then moved closer and closer to the display screen until the cow was comfortably eating from the tray as it moved in and out from underneath the display screen (therefore familiarizing the cow to tray movement and the operator's hand). Four cows were excluded at this habituation stage due to fear of the arena or tray (2 full-time and 2 part-time).

Shaping Phase. Cows were individually clicker-trained to nose-touch the positive image on the display screen using a shaping procedure (see Supplemental Video S1: https://data.mendeley.com/datasets/tg3bx7xs27/1). As cows stood directly in front of the display screen, cows were first conditioned to the sound of the clicker device by pairing a click with immediate presentation of the food reward under the screen. Over subsequent training days, the cow was rewarded with a click and food reward for successive approximations of the desired behavior: any small head movement upward, then eyes level with the bottom of the screen, then nose-touch the center of the screen. Finally, cows had to nose-touch the positive image on the screen by walking from the start box, without stopping, repeated 10 times. Six cows were excluded at this shaping stage (1 full-time, 3 part-time, and 2 no-contact) after at least 5 d of refusal to eat the food reward during clicker training.

Discrimination Training

A detailed description of discrimination training is provided in the supplemental material (https://data.mendeley.com/datasets/tg3bx7xs27/1). Discrimination training was conducted in pairs (in most cases, the same pair as their habituation day partner), with one cow in each arena, for approximately 30 min. Order of training was the same as for initial training phase, and cows experienced approximately 1 h of feed restriction before training. A training day always began with 3 refresher positive images (to evaluate food motivation), followed by a randomly selected sequence of 10 pseudorandomly alternating positive or negative images (initially 40% negative and 60% positive images, and then increased to 50% negative and positive images). Each image was displayed on the screen, and the cow was released from the start box. If the cow correctly approached and touched the positive image within 30 s, the food reward was delivered for 5 s (Supplemental Video S2: https://data.mendeley.com/datasets/tg3bx7xs27/1); if not, the experimenter encouraged her to approach and touch (during the 40% negative sequence, up to 4 training days to facilitate learning) or the cow was returned to the start box (during the 50% training sequence). If the cow correctly avoided the negative image for 30 s, the experimenter called "Good girl!" and the image changed to black (Supplemental Video S3: https://data.mendeley.com/datasets/tg3bx7xs27/1); however, if the cow approached the negative image, the experimenter called "No!" and a punishment was delivered (the operator vigorously waved a small plastic bag attached to a wooden handle 4 times underneath the screen). In between each image presentation in the sequence, the cow was returned to the start box where she waited for approximately 1 min as the cow in the adjacent arena was trained in the same manner. This intertrial interval (i.e., wait time between stimuli) is known to increase an animal's learning speed in discrimination tasks (Ward et al., 2013). Although there was a risk of inducing frustration or boredom with waiting times, the presence of a partner cow in the adjacent arena was expected to reduce this risk. In addition, there was no wait time in between the 3 refresher images, which also served to reduce possible frustration to obtain a food reward at the beginning of the training day.

Cows were considered trained and ready for testing when they averaged >80% correct responses (with 50% negative images in the training sequence) over 2 consecutive training days. Cows needed to complete training (inclusive of initial and discrimination training phases) within 25 d due to enrolment in a concurrent experiment. Five cows trained with colors were excluded (2 full-time, 2 part-time, and 1 no-contact) and 7 cows trained with shapes were excluded (1 full-time, 2 part-time, and 4 no-contact). All exclusions were due to failure to meet the discrimination learning criterion by the deadline, except for one no-contact cow that was euthanized due to an injury (unrelated to training or housing treatment) that required a humane endpoint. Seven cows required corrective training (following Hintze et al., 2017), which was applied if approach responses to the positive were extinguished or if negative images were repeatedly approached (see supplemental material); all but one of these cows met the learning criteria afterward.

In summary, of the 72 cows enrolled for training, a total of 6 full-time, 9 part-time, and 7 no-contact cows were excluded before judgment bias testing. Thus, a total of 30 cows trained with colors (10 full-time, 9 part-time, and 11 no-contact), and 20 cows trained with shapes (8 full-time, 6 part-time, and 6 no-contact) met the learning criterion and were eligible for judgment bias testing. This sample size per treatment was similar to the sample size reported by Neave et al. (2013) who detected statistical differences in proportion of ambiguous images approached with a sample size of 9 calves.

Judgment Bias Testing

At the time of testing, cows had experienced their housing treatment for about one month, which was similar across treatments (full-time: 29.1 ± 6.8, range: 16 to 42 d; part-time: 30.5 ± 4.5, range: 21 to 40 d; no-contact: 33.1 ± 4.7, range: 24 to 42 d) and method (colors: 30.8 ± 5.1 d, range: 17 to 40 d; shapes: 30.5 ± 6.8 d, range: 16 to 42 d). Cows were tested in pairs (same partner as during discrimination training) for 4 consecutive days, with each test day lasting approximately 30 min per pair. Cows had obstructed view of the adjacent arena, so it is unlikely cows could learn by observing their partner responses. To maintain consistency with training, test days always began with 3 consecutive refresher positive images (with no intertrial interval in the start box, identical to training). This was followed by the 10-image test sequence containing 4 positive images (**P**), 3 negative images (**N**), and one each of the 3 ambiguous images: near-positive (**NP**), middle (**M**), and near-negative (**NN**; see Figure 1).

Thus, over the 4 test days, cows were presented a total of 16 P, 12 N, and 4 each of NP, M, and NN images. The ambiguous images were always presented third, sixth, and ninth in the sequence, and the sequence always began and ended with a positive image. Test sequences were fixed for each day and for all cows: P-N-M-P-N-NP-N-P-NN-P (d 1), P-N-NN-P-N-M-N-P-NP-P (d 2), P-N-NP-P-N-NN-N-P-M-P (d 3), P-N-M-P-N-NN-N-P-NP-P (d 4). The cow was returned to the start box in between each image presentation, where she had the same 1 min intertrial interval as during training. Positive and negative images continued to be reinforced, but the ambiguous images were neither rewarded nor punished if the cow approached and touched; in this case, the experimenter called "OK" in a neutral voice, the image changed to black, and the cow was returned to the start box (see Supplemental Video S4: https://data.mendeley.com/datasets/tg3bx7xs27/1). This also occurred if the cow did not approach the ambiguous images within 30 s.

The cow's response to each image was recorded as go (approached and touched, coded as 1) or no-go (did not approach and touch, coded as 0), and latency to touch each image was recorded from the moment the cow's front hoof crossed the start line to the moment the cow nose-touched the screen (using a hand-held electronic timer). No-go responses received a censored latency of 30 s. Data were recorded by the experimenter in a notebook immediately after each image presentation. The 3 consecutive refresh positive images that preceded a test sequence were not included for analysis. Any uncertainty about response or latency records were verified from the video camera (Hikvision DS-2DE2A204IW-DE3) mounted above each arena.

Statistical Analysis

All statistical analyses were performed using SAS Studio (OnDemand for Academics, SAS Institute Inc.), with cow as the experimental unit. All outcome variables (proportion of images approached; latency to approach) were assessed for approximation of a normal distribution using PROC UNIVARIATE and examining model residuals. For 3 cows, a JBT day was excluded from analysis (1 full-time on shapes, 1 full-time on colors, and 1 no-contact on colors, who became scared due to an unexpected noise in the barn and would not approach any image part-way through test d 4, 3, and 1, respectively; behavior on subsequent test days was normal and these test days were included for analysis). For all models described below, the degrees of freedom were calculated using the Satterthwaite approximation and backward elimination of explanatory variables from the full model was performed until all remaining variables in the model were $P < 0.3$; the final models are reported.

Cows that completed judgment bias testing (n = 50 cows) were tested for differences in their learning time during the training period. The effect of treatment on number of days to complete initial training (for each of habituation and shaping phases) was tested in a mixed regression model (PROC MIXED) with the fixed effects of treatment (full-time, part-time or no-contact), parity (primiparous or multiparous), method (colors or shapes), arena (1 or 2), and testing order; cow and block were included as random effects. The final models for the habituation phase included the fixed effects of treatment, parity, and testing order, whereas the final model for the shaping phase included only treatment as fixed effect. In addition, the effect of treatment and method on the number of days to complete discrimination training was tested using an identical initial model, except that the outcome variable was transformed using the natural logarithm ($\log_{10} + 1$) to achieve an approximate normal distribution of residuals. The final model contained the fixed effects of treatment, method and arena.

A requirement for a valid JBT is that (1) animals continue to respond to the positive and negative images as trained, (2) animals show generalized responses to the ambiguous images (i.e., show an intermediate level of approach responses compared with positive and negative images), and (3) approach responses to images do not decline with repeated test days (i.e., animals have not learned to avoid approaching the ambiguous images due to lack of reward; Gygax, 2014). These assumptions were tested for each method (colors or shapes) with a binary logistic regression model with logit link and binomial distribution (PROC GLIMMIX). The outcome variable was the logit of go responses (1 = approached; 0 = did not approach) with fixed effects of image (P, NP, M, NN, and N), treatment (full-time, part-time and no-contact), parity (primiparous or multiparous), positive image (cross or circle for shape method; red or white for color method), test day (1 to 4), arena (1 or 2), testing order, days to complete discrimination training, and days after entering treatment. The interactions of image × positive image, and treatment × positive image, were also included. Cow and block were random effects, and included repeated observations of image and test day. The final models for color method included the fixed effects of image, treatment, positive image, order, image × positive image, and treat × positive image; for shape method the fixed effects were image, treatment, positive image, test day, days to complete discrimination training, arena, and image × positive image.

The key question in judgment bias analyses is whether treatment affects approach responses to the ambiguous images, and if this depends on the relative location of the ambiguous image (i.e., NP, M, or NN). This requires testing the interaction of image × treatment, but the logistic regression model described above did not permit this. This likely occurred because part-time cows never approached the NN color image (see the Results section). A solution was to aggregate responses over the 4 test days, given we observed no differences in approach responses over test days (see the Results section); this permitted testing for differences in the proportion of images approached. The sum of go responses to each image, divided by the total number of images presented (16 positive, 12 negative, and 4 each of ambiguous NP, M, and NN) was calculated. For cows that had test days removed from analysis (described above), the total number of images presented was adjusted accordingly. The proportion of images approached was transformed using arcsine square-root (Gotelli and Ellison, 2004).

First, a mixed regression model (PROC MIXED) tested whether treatment affected the proportion of positive and negative images approached (i.e., suggesting differences in motivation to obtain a reward or avoid a punishment). For each method and each image (P and N), the outcome variable was the transformed proportion of images approached, and fixed effects were: treatment (full-time, part-time and no-contact), parity (primiparous or multiparous), positive image (cross or circle; red or white), arena (1 or 2), testing order, days to complete discrimination training, days after entering treatment, and the interaction of treatment × positive image. Cow and block were random effects. Final models for color method were (P image: treatment, positive image, parity, and order; N image: treatment, positive image, days to complete discrimination training, and treatment × positive image) and for shape method (P image: treatment, positive image, parity, days to complete discrimination training, days after entering treatment, and treatment × positive training image; N image: treatment, positive image, parity, arena, days to complete discrimination training, days after entering treatment, and treatment × positive image).

Second, a mixed regression model (PROC MIXED) tested whether treatment affected the proportion of ambiguous images approached, and if this depended on location of the ambiguous image (i.e., NP, M, or NN). For each method, the outcome variable was the transformed proportion of images approached, and fixed effects were ambiguous image (NP, M, and NN), treatment (full-time, part-time and no-contact), parity (primiparous or multiparous), positive image (cross or circle; red or white), arena (1 or 2), testing order, days to complete discrimination training, days after entering treatment, and the 2- and 3-way interactions of image × treatment × positive image. Cow and block were random effects, and included the repeated observations of ambiguous image. The final models for color method were image, treatment, positive image, image × positive image, and treatment × positive image; and for shape method were image, treatment, positive image, arena, and days to complete discrimination training.

The latencies to approach images were also tested for differences between treatments using survival analysis with a Cox's proportional hazards frailty model (PROC PHREG), and graphically presented using PROC LIFETEST. To account for the multiple observations per cow, latencies to each image were first averaged within a test day, then over the 4 test days, to create an aggregated latency per image per cow. Cows that did not approach an image were assigned a censored latency of 30 (corresponding to the maximum of 30 s allowed in the test). First, latencies to approach the positive and negative images were tested separately for differences between treatments, as an indicator of approach and avoidance motivation; for each method and image (P and N), the model included the censored latencies as the outcome, and explanatory variables were: treatment (full-time, part-time and no-contact), parity (primiparous or multiparous), positive image (cross or circle; red or white), arena (1 or 2), block (1 to 7), testing order, days to complete discrimination training, days after entering treatment, and treatment × positive image. Cow was included as a random effect. The final models for color method were (P image: treatment, positive image, parity, and days to complete discrimination training; N image: treatment, parity, block, and order) and for shape method were (P image: treatment and parity; N image: treatment, positive image, days to complete discrimination training, and treatment × positive image).

Second, latencies to approach the ambiguous images were tested for differences between treatments, and whether cows showed intermediate (generalized) latencies to the ambiguous images that differed from each other. For each method, the model included censored latencies as the outcome, and explanatory variables were: image (NP, M, and NN), treatment (full-time, part-time and no-contact), positive image (cross or circle; red or white), arena (1 or 2), parity (primiparous or multiparous), block, testing order, days to complete discrimination training, days after entering treatment, and the 2- and 3-way interactions of image × treatment × positive image. Cow was included as a random effect. Treatment and positive image were always retained in the models, and other effects were removed during backward elimination if $P > 0.3$ to obtain final models for color method (image, treatment, positive image, block, and treatment × positive image) and shape method (image, treatment, positive image, block, order, days to complete discrimination training, and image × treatment).

Results are reported as odds ratios (**OR**) and confidence limits (**CL**) from the logistic regression models; least squares means ± standard error or back-transformed means and confidence limits from the mixed regression models; and hazard ratios (**HR**) from the Cox proportional hazards model, where HR <1 indicates a lower probability of approaching the image within 30 s, and HR >1 indicates a higher probability of approaching the image within 30 s. Significance level was declared at $P \leq 0.05$.

RESULTS

Training and Discrimination

Cows required between 2 to 8 d to complete the habituation phase; no-contact cows habituated sooner (2.4 ± 0.3 d) than full-time and part-time cows (3.6 ± 0.3 d and 3.2 ± 0.3 d, respectively; $F_{2,45} = 4.2$; $P = 0.02$), and multiparous cows habituated sooner than primiparous cows (2.7 ± 0.2 d and 3.4 ± 0.3 d, respectively; $F_{1,45} = 3.9$; $P = 0.05$). To complete the shaping phase, cows required between 4 and 11 d with no differences between treatments (full-time: 6.4 ± 0.4 d; part-time: 6.3 ± 0.5 d; no-contact: 6.5 ± 0.4 d; $F_{2,47} = 0.05$; $P = 0.95$). To complete discrimination training, cows required between 5 and 11 d (color method) and between 4 and 18 d (shape method). Cows took longer to discriminate between the shapes (9.3 d, CL: 8.1–10.6 d) than between the colors (7.3 d, CL: 6.6–8.2 d; $F_{1,42} = 7.6$; $P = 0.01$), with no differences between treatments (full-time: 7.9 d, CL: 6.9–9.1 d; part-time: 8.7 d, CL: 7.4–10.1 d; no-contact: 8.4 d, CL: 7.0–9.4 d; $F_{2,45} = 0.4$; $P = 0.67$).

Approach Responses to Images

For the color method, when presented the ambiguous images (shades of pink), cows showed a generalization in approach responses that were intermediate to the positive and negative images; cows were more likely to approach the NP than the middle color (OR = 15.0, CL: 6.5–34.9; $t_{1,201.7} = 3.8$; $P < 0.001$), and were more likely to approach the middle than the NN color (OR = 9.2, CL: 3.1–26.8; $t_{1,247.2} = 4.4$; $P < 0.001$). However, for the shape method, we observed no generalization to the ambiguous images; cows were equally likely to approach the NP and middle shapes (OR = 1.4, CL: 0.5–3.8; $t_{1,65.8} = 0.8$; $P = 0.43$), and were equally likely to approach the middle and NN shapes (OR = 1.2, CL: 0.4–3.2; $t_{1,69.6} = 0.6$; $P = 0.56$). Approach responses to images did not change over repeated test days for neither color ($F_{3,82.0} = 0.04$; $P = 0.99$) nor shape methods ($F_{3,68.1} = 1.5$; $P = 0.23$).

The percentage of approach responses to each image (aggregated over all test days) for each treatment are shown for visualization purposes in Figure 3a (color method) and Figure 3b (shape method). Cows nearly always approached the positive images, with no differences between treatments for either color ($F_{2,24} = 1.5$; $P = 0.24$) or shape method ($F_{2,11} = 1.2$; $P = 0.34$). Cows mostly avoided the negative images, but full-time and part-time cows avoided more than no-contact cows, for both color ($F_{2,23} = 4.7$; $P = 0.02$) and shape methods ($F_{2,10} = 13.8$; $P < 0.01$).

For the color method (Figure 3a), approach responses to ambiguous colors were affected by treatment ($F_{2,24} = 3.5$; $P = 0.05$), but did not depend on the shade of pink (treatment × ambiguous image: $F_{4,52} = 0.5$; $P = 0.75$). Full-time cows approached more ambiguous images (44.8%, CL: 32.8–57.1%) than part-time cows (23.5%, CL: 13.4–35.4%; $t_{1,24} = 2.6$; $P = 0.02$), whereas no-contact cows were intermediate (37.8%, CL: 26.8–49.5) but not significantly different from either full-time ($t_{1,24} = 0.85$; $P = 0.40$) and part-time cows ($t_{1,24} = 1.8$; $P = 0.08$). Cows assigned red as the positive image approached fewer ambiguous images (20.6%, CL: 12.8–29.7%) than cows assigned white (51.1%, CL: 41.3–60.1%; $F_{1,24} = 21.8$; $P < 0.01$).

For the shape method (Figure 3b), approach responses to ambiguous shapes were not affected by treatment (full-time: 20.9%, CL: 10.0–34.5; part-time: 12.1%, CL: 2.9–26.4; no-contact: 23.9%, CL: 11.0–39.9; $F_{2,14} = 1.0$; $P = 0.41$), or interaction of treatment × ambiguous image ($F_{4,34} = 1.2$; $P = 0.32$). However, cows assigned the circle as the positive image approached fewer ambiguous images (4.5%, CL: 0.36–13.3%) than cows assigned the cross (39.6%, CL: 26.1–53.8%; $F_{1,14} = 18.8$; $P < 0.01$).

Latencies to Approach Images

Figure 4 shows the survival curves for latencies to approach images in the color (Figure 4a) and shape (Figure 4b) tasks. For the color method, we observed no treatment differences in latency to approach the positive image (Wald $\chi^2_{2,24.9} = 5.6$; $P = 0.06$) or negative image (Wald $\chi^2_{2,19.0} = 5.3$; $P = 0.07$). Latencies to approach the ambiguous colors were significantly different from each other (about 12, 23 and 29 s to NP, M, and NN, respectively; Wald $\chi^2_{2,10.9} = 65.7$; $P < 0.001$), but we observed no image × treatment interaction (Wald $\chi^2_{4,17.1} = 4.7$; $P = 0.31$). Part-time cows were slower to approach ambiguous colors than full-time (Wald $\chi^2_{1,10.9} = 5.2$; $P = 0.02$; HR = 0.24, 95% CI: 0.07–0.86) and no-contact cows (Wald $\chi^2_{1,10.9} = 3.9$; $P = 0.05$; HR = 0.23,

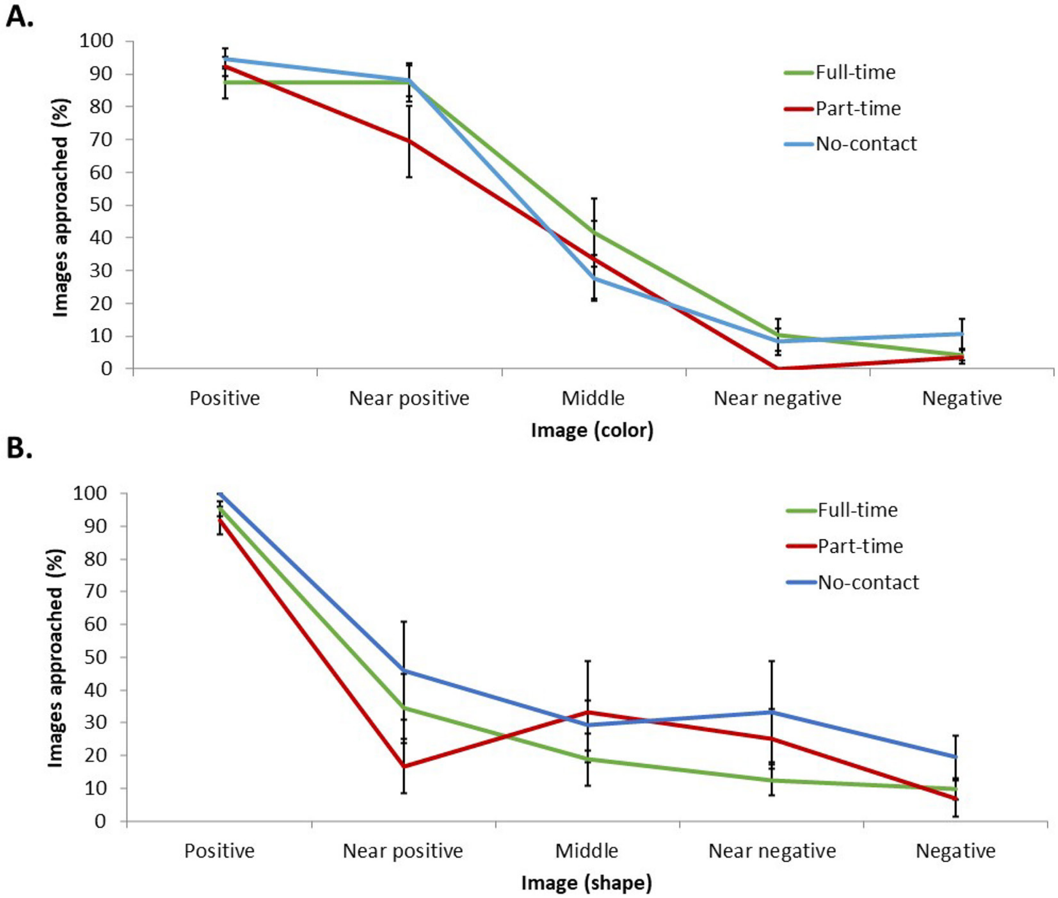

Figure 3. Raw mean (±SE) percentage of images approached during the judgment bias test period, for (A) color-based task (10 full-time, 9 part-time, and 11 no-contact cows); (B) shape-based task (8 full-time, 6 part-time, and 6 no-contact cows). On each test day, for 4 d, cows were presented a pseudorandom sequence of 10 images: 4 positive, 3 negative, and one of each ambiguous image. The percentages of images approached were aggregated over the 4 test days for each treatment.

95% CI: 0.07–0.80), but only when they were assigned red as their positive image. For the shape method, we observed no treatment differences for latency to approach the positive image (Wald $\chi^2_{2,15.7} = 0.04$; $P = 0.98$) or negative image (Wald $\chi^2_{2,7.9} = 0.34$; $P = 0.84$). Latencies to the ambiguous shapes were significantly different from each other (about 24, 26, and 27 s to NP, M, and NN, respectively; Wald $\chi^2_{2,20.6} = 10.2$; $P < 0.01$), but we observed no treatment effect (Wald $\chi^2_{2,20.6} = 1.7$; $P = 0.42$) or treatment × image interaction (Wald $\chi^2_{4,20.6} = 5.4$; $P = 0.25$).

DISCUSSION

In the color-based JBT, we observed a negative pessimistic judgment bias in cows housed part-time with their calves, indicative of a negative emotional state relative to cows housed full-time with their calves. Cows that were separated from their calves showed no difference in judgment bias compared with cows with either full- or part-time cow-calf contact. These findings shed new light on our understanding of the emotional states of dairy cows managed with or without their calves, and have implications for the management of cow-calf contact systems.

Negative Judgment Bias in Cows with Part-Time Compared with Full-Time Calf Contact

An important result of our study is that the type of cow-calf contact system may affect the emotional state of dairy cows. Cows with only part-time calf contact showed a negative (pessimistic) judgment bias in approach responses in the color task, indicating these cows may experience a negative emotional state relative to cows with full-time calf contact. This negative bias was not due to lack of motivation to access food rewards in the task (as indicated by high approach responses and short latencies to reach the positive image) and was not due to learning that the ambiguous cues

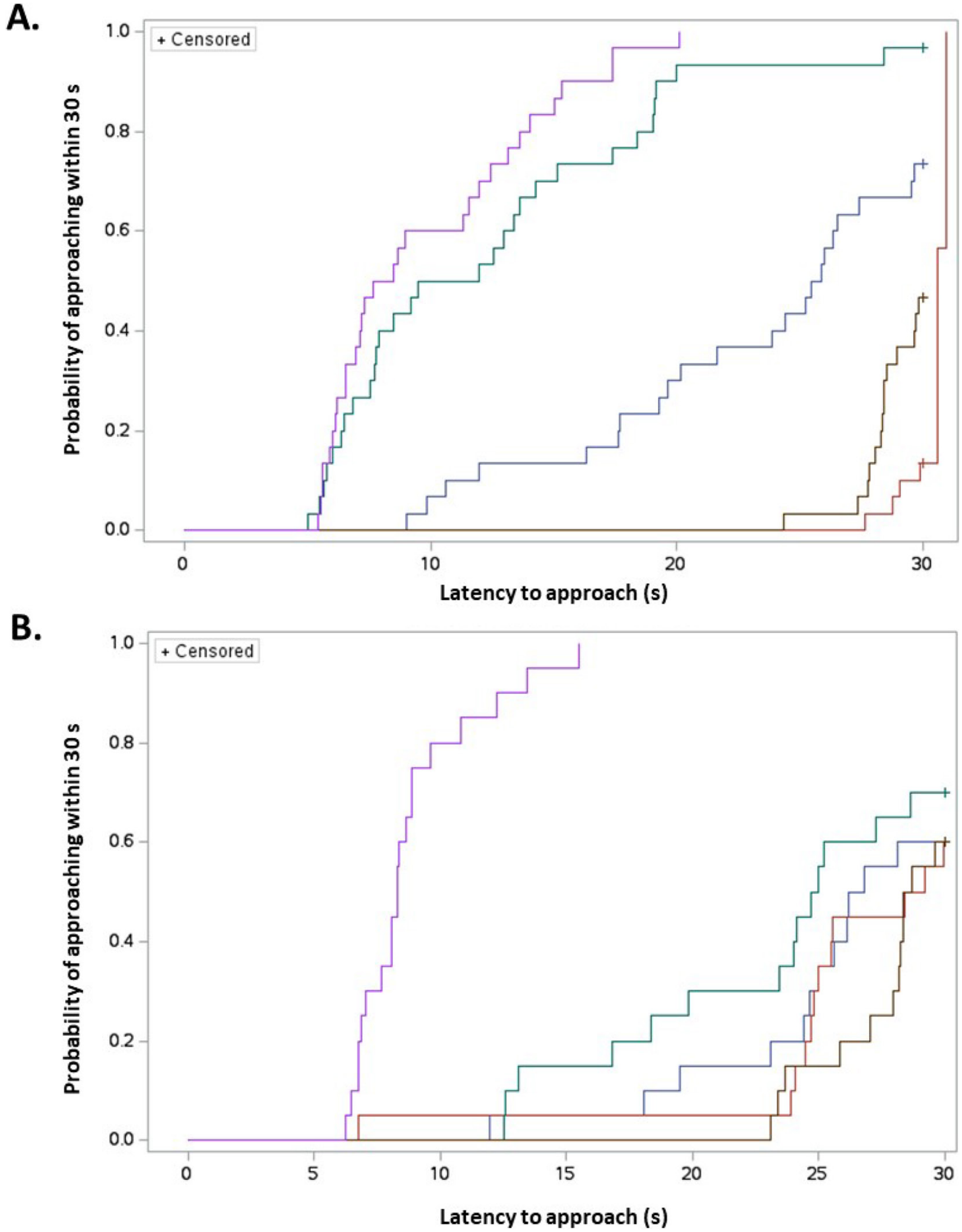

Figure 4. Survival curves of the cumulative probabilities of approaching images in the (A) color-based or (B) shape-based judgment bias task. The y-axis indicates the probability of a cow approaching the image within the 30 s time limit, and the x-axis indicates the latency for a cow to approach the image. Positive image = purple line; near-positive image = green line; middle image = blue line; near-negative image = brown line; negative image = red line. If a cow did not approach within 30 s, this cow received a censored latency of 30 s (indicated by +).

were unrewarded (as indicated by no decrease in approach responses over test days). Part-time calf contact systems involve repeated daily temporary separations of the cow and calf; in this study, cows were housed overnight in a separate barn without visual and with limited auditory contact to their calves, which may have been experienced as negative. Cows are known to show strong behavioral responses to separation from their calves just a few days after birth (Flower and Weary, 2001; Stěhulová et al., 2008) and after a prolonged suckling period (Johnsen et al., 2015, 2018; Wenker et al., 2022). These negative behavioral responses reduce substantially when cow and calf are reunited (Boissy and Le Neindre, 1997; Solano et al., 2007; Schnaider et al., 2022), and grooming of calves also increases (Roadknight et al., 2022); these responses suggest that repeated separations may be experienced negatively. A recent study of cows with only part-time access to their calves during the nighttime (i.e., experience repeated temporary separations and reunion after milking, similar to our study) observed nursing avoidance behavior and agonistic interactions toward cows and calves upon reunion with calves (Roadknight et al., 2022). These behaviors may be due to discomfort when calves suckle from an empty udder, and frustration when hungry calves attempt to suckle from cows other than their dam (Roadknight et al., 2022; Bertelsen and Jensen, 2023a). These behaviors may also have occurred in our study and contributed to the observed negative emotional state in these cows.

There are 2 other possible explanations for this relative negative judgment bias in cows with part-time compared with full-time calf contact. The repeated changes and differences in housing environment could have affected emotional state. For instance, dairy cows will choose to lie in an open pack over a freestall area (Fregonesi et al., 2007) and are more motivated to access straw-bedded than mattress lying surfaces (Shewbridge Carter et al., 2022), which were provided during calf contact and separation periods, respectively. However, cows with no calf contact (who did not show a negative, pessimistic judgment bias) experienced the same housing and lying conditions as part-time cows during the separation periods, suggesting that these conditions alone did not contribute to differences in emotional state. Second, participation in the JBT required separation from the calf to go to the test arena; this meant full-time cows experienced a total of 3 (short, maximum 30 min) separation events, whereas part-time cows experienced only 2 separation events. Because both treatments experienced a similar period of calf separation for testing, we suggest it is more likely the negative bias in part-time cows is related to the daily extended period of separation, rather than separation necessary for testing.

The type of negative emotional state that part-time contact cows may be experiencing is unclear. Many JBT designs, including our own, include 3 ambiguous stimuli to determine if biases emerge closer to the positive or negative stimuli; if so, this would permit interpretation of a depressive-like state (negative bias at the NP image) versus an anxiety-like state (negative bias at the NN image; Mendl et al., 2009). We did not observe either bias (i.e., no image × treatment interaction), suggesting that individual cows might emotionally experience part-time contact in different ways. Overall, our results suggest there may be a negative emotional effect of part-time calf contact for the cow. A recent study found that part-time cows vocalized when separated from the calves, but that this response declined as calves grew older (Bertelsen and Jensen, 2023b), so further research is necessary to better understand the positive and negative trade-offs of this type of management system and when it may be applied.

No Difference in Judgment Bias Between Cows with Full-Time or No Calf Contact

Wenker et al. (2020) observed that some cows who were separated from their calves at birth were motivated to reunite with their calves about 1 wk after separation and engaged in calf-directed behaviors, which may suggest a negative state during prolonged separation. The results of our study showed no difference in judgment bias in approach responses in the color task between cows without calf contact (i.e., separated 48 h after birth) and cows with full-time calf contact. On a positive note, this finding suggests that cows without calf contact (and under these study conditions: freestall housing with 12 mattress-bedded lying stalls at 1:1 stocking density and provided brushes) are probably not experiencing longer-term negative emotional states, which have been reported in calves after about 30 d of social isolation (Bučková et al., 2019). However, on a cautionary note, a lack of negative judgment bias should not be taken as evidence that no-contact cows do not experience anything negative about separation from their calf shortly after birth. We observed plenty of behavioral evidence that cows show strong negative reactions to separation from their calves (see review, Meagher et al., 2019; Neave et al., 2024), which presumably result in a negative emotional state at that time. For instance, calves show a negative judgment bias at 24 h after separation from the dam at 6 wk of age (Daros et al., 2014), suggesting a negative emotional response to cow-calf separation at least in calves. Our results herein suggest that such negative states do not appear to persist, at least when separation occurs at 48 h after birth and tested on average 30 d later (although this range was 17 to 40 d). However, notably we observed individual variation in the responses of no-contact cows, where some showed more negative or more positive responses. It is unknown if separation of cow and calf after extended periods of contact (as in full-time or part-time calf contact systems) leads to longer-term negative emotional states in these cows. Judgment bias should reflect longer-term mood, rather than short-term emotions, but appears to assess a combination of the two (Kremer et al., 2020). The question remains how we assess the effect of accumulated positive and negative emotional states (e.g., due to cow-calf contact and separation, respectively) on animal welfare at the time of measurement. Webb et al. (2019) proposed weighing the sum of the positive experiences against the sum of the negative experiences in a simple ratio. Future research is encouraged to develop and validate this approach.

An initial interpretation of the lack of difference in judgment bias between cows with full-time or no calf contact (interpreted as similar emotional state) might be that housing cow and calf together for a prolonged period offers no additional positive emotional benefits to the cow. We posit this is unlikely given that cows develop strong bonds with their calves (von Keyserlingk and Weary, 2007; Jensen, 2011; Johnsen et al., 2015), characterized by affiliative behaviors that are known to be calming and rewarding in nature in other species (Newberry and Swanson, 2008), and cows are motivated to reunite with their calves (Wenker et al., 2020). Thus, contact with offspring should have positive hedonic value for the cow.

Another possible explanation is that the test may not have been sensitive enough to detect subtle differences in emotional state between full-time and no-contact cows. Previous studies of judgment bias in dairy cows found no bias (and thus similar emotional states) among cows experiencing pastured versus indoor-housing systems (Crump et al., 2021), and among cows experiencing different manipulations of indoor conditions (Kremer et al., 2021). It may be that the JBT is better able to detect negative biases, whereas differences in positive biases may be more difficult. Unfortunately, the use of judgment bias to examine positive emotional states is rare, even across species (Baciadonna and McElligott, 2015; Roelofs et al., 2016), so future research is strongly encouraged to explore the sensitivity of judgment bias to different positive states.

No-contact cows more often approached the negative trained image compared with full- or part-time cows, suggesting they may be less sensitive to negative images and more likely to take risks. This could also explain why these cows did not show the predicted negative judgment bias relative to cow-calf contact cows; from an evolutionary perspective, cows that rear their calves may refrain from taking risks to ensure their calves are protected from possible environmental dangers (Lima and Dill, 1990).

Visual Judgment Bias—A New Method in Dairy Cows and New Insights for Cattle Vision

A second objective of this study was to test two visual judgment bias tasks, which have never been used in adult dairy cows. Thus, our study offers an important step forward in assessment methodologies for emotional state and also sheds light on the visual processing of cattle in general. The color-based task, originally developed for use in dairy calves (Neave et al., 2013), successfully identified judgment biases in dairy cows, but the shape-based task did not. Cows took longer to discriminate the shapes than the colors (about 2 to 3 extra days), suggesting the shape images were more complex to learn. The ambiguous shapes were also quite complex, with the 2 overlaid shapes that were faded to 65, 50, or 35% of white. The similar response rates to all 3 ambiguous shapes suggests they were not distinguishable, and the low response rates suggests they may require more visual processing time, resulting in cows more often avoiding than approaching. Consequently, low approach responses to all ambiguous images makes it more difficult to detect biases related to treatment. Furthermore, the lower sample size of the shape-based task (6 part-time and no-contact cows, 8 full-time cows) resulted in lower power to detect treatment differences; for instance, the NP shape image had numerically lower responses in part-time cows, which complements the pattern observed in the color-based task. Nonetheless, cows are clearly able to visually process and learn to discriminate complex images involving shapes, as also seen in other studies that presented cattle with geometric figures (Baldwin, 1981; Entsu et al., 1992; Rehkämper and Görlach, 1997).

A surprising finding was that cows more often approached the ambiguous images if they were assigned white or cross as their positive-trained image. This suggests that these cows were more likely to interpret white or cross elements in the ambiguous images and respond optimistically. In contrast, those assigned to red or circle were less likely to interpret these elements as positive in the ambiguous images; furthermore, part-time cows were slower to approach ambiguous images when assigned red as the positive training image, suggesting greater hesitation when faced with uncertain information. In dairy calves, Neave et al. (2013) reported no difference in responses to images depending on white or red color assignment, but Daros et al. (2014) only trained calves on the white image because the authors also noted reduced response rates when calves were trained on the red image (personal communication; Daros et al.). It is not clear why these biases toward a particular color or shape occurred in adult dairy cows, but perhaps we observed differences in associative learning between the positive and negative stimuli depending on the color or pattern. For instance, work that explored how animals learn color versus patterned stimuli suggested the most salient stimulus can overshadow learning of other components (Mackintosh, 1976), which may explain why domestic chicks learned a bright color better than a pattern (Aronsson and Gamberale-Stille, 2008). It has also been suggested that positive stimuli may be learned more effectively if the stimulus is more contrasting or salient (Gamberale-Stille and Guilford, 2003), which could explain reduced responding to ambiguous images when trained on the red versus white image. We also observed differences in how cattle visually process images with colors and shapes. Visual processing of red color requires activation of the retinal cones to process long-wavelengths of light which is not necessary for white light (Jacobs, 1993), and it is not understood how different color saturations (i.e., the shades of pink) affect this process in cattle. Although cattle can discriminate different intensities of long-wavelength (red) light (Phillips and Lomas, 2001), and can discriminate between short- and long-wavelengths of light (Gilbert and Arave, 1986), there is surprisingly little understanding of color vision and use of spectral information in animals (Kelber and Osorio, 2010). These explanations for biases in positive training image remain highly speculative, and rely on assumptions that animals see and process images in ways that humans have designed them. We cannot know for certain how cattle process these images during discrimination learning, and there may be individual differences in this process.

Based on results of the current study, we recommend using a color-based over a shape-based task. Although equipment and more complex training are necessary (e.g., a screen, clicker and shaping training techniques), total training time averaged 18 d per cow, which is comparable to both reports of spatial judgment bias methods in dairy cows (18 d: Crump et al., 2021; 24–27 d: Kremer et al., 2021). We included an intertrial interval (wait time in between image presentations) which may have reduced training time (Ward et al., 2013). This intertrial interval allowed us to train and test cows in pairs, for time efficiency as well as to reduce possible negative emotional states by avoiding social isolation in the test arena. However, a social companion can also introduce some uncontrollable distractions (e.g., sounds from the partner cow). Overall, the visual judgment bias task is a promising method for detecting cognitive biases related to emotional state in dairy cattle, and is supported by 2 previous reports using the same methods in dairy calves (Neave et al., 2013; Daros et al., 2014).

Limitations

Training and testing of cows in judgment bias takes considerable time and effort, and not all cows learn the task; 30 of 42 cows completed the color-based task and 20 of 30 cows completed the shape-based task, resulting in an average drop-out rate of 30%. Therefore, our test results are biased to the population of cows that could learn the task, and within our 25-d time frame (due to a concurrent study). It is possible that cows failing to complete training were in a more negative emotional state, or that training in the cognitive task itself was rewarding for cows, which may be alternative explanations for the biases observed in our study. Both issues regarding sample bias and training being cognitive enrichment have been raised as potentially confounding factors in judgment bias testing (Roelofs et al., 2016). Furthermore, this 30% drop-out rate resulted in some low sample sizes, especially in the shape-based task where we observed only 6 cows in the part-time and no-contact treatments. The lack of treatment effects in this shape-based task may be related to lack of power, but in any case, the low response rate (no generalization curve) to the ambiguous images does not support the use of this method approach. Sample size and drop-out rate could possibly be improved with refined training techniques, such as active trial initiation that have been used in calves (Neave et al., 2013) and horses (Hintze et al., 2018). Finally, we cannot be certain that cow-calf contact (or lack thereof) alone led to the judgment biases observed in our study; other management factors could have contributed to differences in emotional states, such as lying surface (straw or mattress), housing design (open pack or freestall), and group size (4 or 12 cows).

CONCLUSIONS

Cows with only part-time calf contact, who experience repeated daily separations from their approx. one-month-old calves, showed a negative judgment bias suggestive of a negative emotional state, compared with cows with full-time calf contact; cows with full-time and no calf contact showed no difference in judgment bias. Thus, cow-calf contact systems may differentially influence the emotional state of cows depending on their design. No difference in judgment bias was evident between cows with full-time contact and cows separated from their calves at 48 h after birth; however, other differences in housing management may have affected emotional states. We strongly encourage future research to test how alternative management systems affect the emotional states of dairy cows before they are adopted into practice; the color-based visual JBT is a suggested methodology.

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