Disbudding affects use of a shelter provided to group-housed dairy calves

K. N. Gingerich,1 V. Choulet,2 and E. K. Miller-Cushon1*
1Department of Animal Sciences, University of Florida, Gainesville 32611
2Institut national supérieur des sciences agronomiques de l’alimentation et de l’environnement, AgroSup Dijon, Dijon Cedex, 21079, France

ABSTRACT

Disbudding in dairy calves is well established as a painful procedure with broad effects on behavior. The hypothesis of this experiment was that group-housed calves subjected to hot-iron disbudding would exhibit social withdrawal, based on use of a shelter providing physical and visual seclusion from the rest of the pen. We examined effects of hot-iron disbudding on use of this shelter, including individual and shared use, and resting behavior within the shelter. Holstein heifer and bull calves (n = 24) were housed in group pens (4 calves/pen; 3.7 × 8.0 m). Each pen contained a 3-sided open-top shelter (1.2 m square, and 1.2 m high) with an additional half-enclosed wall to allow entry, built out of corrugated plastic. Calves were randomly assigned within pen to be disbudded (n = 12; 10 bulls and 2 heifers; 36.2 ± 3.9 d of age) or receive sham handling only (n = 12; 9 bulls and 3 heifers; 36.3 ± 4.2 d of age). Disbudded calves received a local cornual nerve block and a nonsteroidal antiinflammatory medication before the procedure. Behavior was recorded continuously from video for 3 consecutive days, beginning immediately following the disbudding procedure or handling. Shelter use was highly variable between calves (ranging from 10.8 min/d to 20.7 h/d), but calves showed individual consistency in their use of the shelter over time. Disbudded calves spent more time in the shelter (4.6 vs. 1.6 h/d; disbudded vs. sham). Social use of the shelter as a percentage of shelter use was not affected by disbudding, but disbudded calves spent more time in the shelter together (31 vs. 9% of shelter use; disbudded vs. sham). Social use of the shelter as a percentage of shelter use was not affected by disbudding, but disbudded calves spent more time in the shelter together (31 vs. 9% of shelter use; disbudded vs. sham). Disbudded calves entered the shelter more frequently when it was unoccupied (8.1 vs. 5.5 entries; disbudded vs. sham) and similarly left it more frequently when it was occupied. Disbudded calves used the shelter more during daylight hours (0700 to 2000 h; 8.9 vs. 4.1 min/h) on each day, including d 0 when pain mitigation was effective, whereas use did not differ during the night. Disbudded calves spent approximately 40 min less time lying/d and spent a greater percentage of their lying time inside the shelter. These results suggest that disbudded calves make greater use of environmental features that offer seclusion, with use of the shelter possibly reflecting an increased preference for social withdrawal or for some other aspect of this area of visual and physical separation. Further, these results suggest that disbudding even with recommended pain mitigation affects behavior for at least several days.

Key words: dairy calf, group housing, disbudding, social behavior

INTRODUCTION

Measuring behavioral changes can be a means to identify and alleviate individual experiences of pain, an unpleasant sensory and emotional experience (Raja et al., 2020). For preweaned dairy calves, a common painful procedure is hot-iron disbudding, where the horn bud and surrounding tissue are burned and removed to prevent horn growth later in life. Indications of pain associated with this procedure include behavioral changes such as increased wound-directed behavior (e.g., ear flicks and head shaking; Heinrich et al., 2010) and wound sensitivity lasting at least 14 wk (Casoni et al., 2019). Disbudding also elicits a stress response indicated by increased plasma cortisol levels (Stilwell et al., 2010; Allen et al., 2013) and respiratory and heart rates (Heinrich et al., 2009) and causes behavioral changes indicative of a negative affective state, such as reduced play (Mintline et al., 2013) and negative judgment bias (Neave et al., 2013).

Whereas a range of individual behavioral responses to pain in disbudded calves have been evaluated (reviewed by Herskin and Nielsen, 2018), social interactions may provide novel insight into affective state, referring to emotions and other feelings with positive or negative valence, and varying arousal (Ede et al., 2019). Across species, evidence indicates that social withdrawal may occur when an individual is experiencing a negative affective state. For example, reduced social contact is evident in humans experiencing depression (Girard et al., 2014; Visentini et al., 2018) and chronic pain (in childhood through to adulthood; reviewed by Forgeron...
et al., 2010). Additionally, there is evidence that patients who suffer from chronic pain develop depression (Fishbain et al., 1997; Brown, 1990), suggesting a relationship between pain, affective state, and sociality. However, effects of disbudding on social behavior, or preference for seclusion following this procedure, have not been evaluated in dairy calves.

A better understanding of changes in social behavior may provide insight into animal welfare in different circumstances. There are several examples of gregarious animals seeking isolation, suggesting that social separation may reflect aspects of an individual’s state, and that providing opportunity for seclusion in intensive housing, particularly during key events in an individual’s life, may improve welfare. Dairy calves exposed to an experimental disease challenge had reduced frequency of social lying, and initiated less social grooming (Hixson et al., 2018). Additionally, changes in social behavior and self-isolation behaviors are evident around parturition. Dairy cows have been found to preferentially calve behind a physical barrier that separates them from their penmates, and this self-isolation is more pronounced in cows who develop postpartum metritis (Proudfoot et al., 2014). To date, little is known about the motivation, or lack thereof, of gregarious animals to socially isolate themselves when experiencing pain.

More generally, the social environment of intensively raised, gregarious livestock species can be a major determinant of their welfare. Dairy calves are motivated to obtain social contact (Holm et al., 2002), and group-housing of pre-weaned dairy calves is becoming more common, providing a range of welfare benefits, including opportunity for early social development (Duve and Jensen, 2012). However, aspects of intensive housing, including limited space, may create challenges such as competition and social instability (Proudfoot and Habing, 2015). Increasing environmental complexity, such as provision of opportunities for social withdrawal and seclusion, may be a means to facilitate expression of natural social behaviors and improve the social environment (Stoye et al., 2012), yet limited investigation has been done of how group-housed dairy calves may use environmental features that accommodate social withdrawal.

In this experiment, we provided group-housed dairy calves with a shelter, consisting of a 3-sided visual and physical barrier, to examine whether calves may use this opportunity for seclusion and to evaluate if disbudding affects use of the shelter, including duration and frequency of time inside, diurnal patterns of shelter use, and resting behavior within the shelter. We speculated that disbudded calves may prefer to withdraw socially when experiencing the negative affective state of pain, and therefore we hypothesized that calves would increase use of the shelter following disbudding.

**MATERIALS AND METHODS**

**Animals and Management**

Holstein heifer and bull calves (n = 24; 5 heifers and 19 bulls) were enrolled at the University of Florida Dairy Unit (Hague, FL; a dairy research facility housing Holstein cattle from birth to lactation) during the fall, from September to December 2018. Our distribution of heifers and bulls was restricted by a concurrent trial at this facility that was enrolling heifers, such that we consecutively enrolled all available calves. Calves were managed according to the standard operating procedures for this facility with all study procedures reviewed and approved by the University of Florida Institutional Animal Care and Use Committee (IACUC Study #201609416). Because no previous data were available describing our primary response variable (shelter use) with which to perform a power calculation, sample size was estimated based on previous experiments describing changes in social behavior associated with disease in calves under similar housing management as the present study (Hixson et al., 2018) and evaluating changes in feeding and activity associated with disbudding (Sutherland et al., 2018).

Calves received 4 L of quality-controlled colostrum by bottle within 1 h of birth and were uniquely identified with radiofrequency identification ear tags. At birth, calves were initially housed in individual wire mesh pens, allowing visual but not physical contact with other calves. While they were individually housed, calves were provided 8 L/d of pasteurized waste milk via teat buckets in 2 daily feedings of 4 L each.

At approximately 2 wk of age (15.7 ± 2.2 d of age; mean ± SD), calves were moved to group pens (4 calves/pen; 3.7 × 8.0 m) that were deep-bedded with sand and located under an open-sided barn equipped with overhead fans for air circulation. Groups were formed of consecutively enrolled calves, and calves within the same group were placed in the group pen on the same day, with an age range of 4.6 ± 1.4 d (mean ± SD) within the group. All pens had ad libitum access to water and pelleted calf starter (22% CP and 2% fat; Ampli-Calf Starter Warm Weather, Purina Animal Nutrition LLC, Shoreview, MN) via an automated starter feeder (DeLaval CF1000X, DeLaval, Kansas City, MO). Upon introduction to the group pen, all calves received a daily maximum of 12 L/d milk replacer (28% CP and 15% fat with Bovatec and Clarifyl; Southeast Milk Inc., Mayo, FL) fed through an automated milk feeder (DeLaval CF1000X, DeLaval). The feeder was
set to allow a maximum meal size of 2.5 L and minimum meal size of 1 L. Following the conclusion of the experiment, calves were weaned gradually over 10 d, beginning at 43 ± 2 d of age. Calves received a weekly veterinary exam and were monitored daily for signs of illness by farm and research personnel. Only calves that were clinically healthy at the point of entry to group housing were enrolled in the study. Calves were determined to be healthy for the duration of the experiment, based on daily health checks by study staff and weekly clinical exams.

**Experimental Design**

Each group calf pen contained a shelter built out of dark green corrugated plastic to allow visual and physical separation from the rest of the pen. The shelter (shown in Figure 1) was built with 3 sides (1.2 by 1.2 m; width by depth), with the opening side half-enclosed to provide an entrance area (0.6 m), and was high enough (1.2 m) to prevent calves from looking in over the wall. The size of the shelter was designed to be large enough for one calf to lie down and turn around comfortably. The shelter was built in the corner of the pen, configured such that it was on the side of the pen farthest from the automated feeder, and located at the center wall of the open-sided barn (pen configuration illustrated in Figure 2). In each pen, the opening of the shelter was positioned with a consistent orientation relative to location of overhead fans in the barn. The shelters remained in the pens for the duration of the experiment.

During wk 4 of life, calves were randomly assigned within pen (2 calves/pen) to be either disbudded (n = 12; 10 bulls and 2 heifers; 36.2 ± 3.9 d of age) or receive handling only (n = 12; 9 bulls and 3 heifers; 36.3 ± 4.2 d of age). Within each pen, treatment (disbudding or handling only) was provided on the same day and approximate time (between 1100 and 1200 h). Calves were disbudded by a trained veterinarian, or by trained veterinary students under direct supervision of the veterinarian. Calves received a nonsteroidal antiinflammatory drug for analgesia 3 to 4 h before disbudding (meloxicam provided orally; 0.5 mg/kg; Unichem Pharmaceuticals USA Inc., Rochelle Park, NJ) and a local

*Figure 1.* Image of calf using the shelter, a 3-sided, open-top barrier made out of corrugated plastic (1.2 × 1.2 × 1.2 m; width × depth × height).

*Figure 2.* Schematic diagram of the pen, depicting the shelter (3-sided, open-top barrier made out of corrugated plastic; 1.2 × 1.2 m; width × depth) and its location within the pen relative to other resources.
cornual nerve block 10 to 15 min before disbudding (5 mL of 2% lidocaine hydrochloride; Bimeda-MTC, Cambridge, ON, Canada; injected on each side over the cornual nerve). An electric disbudding iron fitted with a 1.3 cm internal diameter and 1.9 cm external diameter tip (Rhinehart X-50A, Rhinehart Development Corporation, Spencerville, IN) was preheated for at least 10 min before cautery disbudding of calves. Hair was clipped over the horn buds, and the calf was manually restrained with the head held to the side. The disbudding iron tip was applied firmly over the horn bud for 8 to 10 s to achieve dark copper rings around the base of each horn bud. A topical bandage (AluSpray Aerosol Bandage, Neogen Animal Safety, Lexington, KY) was applied over the disbudding sites around the base of each horn bud. A topical bandage (AluSpray Aerosol Bandage, Neogen Animal Safety, Lexington, KY) was applied over the disbudding sites after disbudding was completed.

For handling only, calves were restrained for approximately the same duration of time as disbudded calves, for a standard veterinary clinical exam.

Data Collection

Each pen was recorded by a digital video camera (Axis M2026-LE Network Camera; Axis Communications, Lund, Sweden) mounted in the center of the outside wall of the pen, approximately 3 m from the ground. Behavior was recorded continuously from video using Behavioral Observation Research Interactive Software (BORIS; Friard and Gamba, 2016). Each calf was individually identified based on coat markings. We recorded behavior for 3 consecutive days, beginning immediately following the disbudding procedure or handling (1200 h). We defined shelter use as half or more of the calf’s body located within the shelter. Preliminary observation revealed frequent social use of the shelter, and so we recorded social use with identity of companion noted. Transitions into and out of the shelter were recorded as events, with entrances specified as either a solitary entrance (focal calf entering the shelter when it was not occupied) or social entrance (focal calf entering the shelter when at least one other calf was already inside, regardless of whether shared use was initiated by the focal calf), and exits from the shelter specified as either a solitary exit (focal calf leaving the shelter when no other calf was located inside) or social exit (focal calf leaving the shelter when it was occupied by another calf). We also recorded lying time from video, defined as the body of the focal calf resting on the ground, and noted location of lying as inside or outside the shelter. Observers were blind to treatment (disbudding wounds were not visible on video) and most were not aware of our specific hypothesis. A total of 5 observers were used to characterize behavior from video (interobserver reliability was calculated for 1 d of video collection, with Cohen’s kappa ≥ 0.85, as calculated in BORIS, for all observer comparisons).

Statistical Analysis

From behavioral data, we summarized duration and frequency by day (24-h observation period beginning at 1200 h of each calendar day). We calculated social use of the shelter as a percentage of total shelter use, social use within treatment (in shelter with another calf on the same treatment) as a percentage of total shelter use, lying in the shelter as a percentage of total shelter use, and lying in the shelter as a percentage of total lying time. Additionally, hourly shelter use duration was calculated for analysis of diurnal patterns.

Data were screened for normality before analysis using the PROC UNIVARIATE procedure within SAS (version 9.4, SAS Institute Inc., Cary, NC), and transformed to meet assumptions of normality if needed. Daily duration of shelter use was log-transformed and the following variables were square root-transformed: frequency of entering and exiting the shelter when it was occupied (social entrance and social exit), social use within treatment as a percentage of shelter use, frequency of lying bouts in the shelter, lying in the shelter as a percentage of total lying time. Hourly shelter use duration was transformed using a Box-Cox transformation (Box and Cox, 1964), where \( \log(y + 1) \), rather than the log-transformation used for daily shelter use duration, due to the presence of zero values in the hourly data set.

Data summarized daily were analyzed using the MIXED procedure of SAS, with day as a repeated measure. For the analysis of duration of social shelter use within treatment (time that the focal calf spent with the other calf on the same treatment), data were summarized by pair within pen, such that the experimental unit for analysis was pair \((n = 6\) pairs of calves/treatment\). For all other analysis, the experimental unit was the individual calf. The model included the fixed effects of treatment, day (categorical variable), treatment by day interaction, with calf (or pair of calves, for analysis of social use within treatment) as the subject and pen as a random effect. The variance-covariance matrix structure on the basis of best fit according to Schwarz’s Bayesian information criterion (compound symmetry or autoregressive were selected for all data). Consistency in shelter use between observation days was assessed using linear regression (using PROC REG in SAS).

To assess shorter-term treatment effects within each day, hourly shelter use duration was averaged by day across daytime hours (between 0700 and 2000 h) and nighttime hours (between 2000 and 0700 h; based on times of sunrise and sunset). The effect of treatment
on hourly average shelter use during the daytime and night was analyzed separately by day.

To characterize diurnal patterns of shelter use, hourly duration of shelter use was averaged by hour across consecutive observation d 1 and 2, excluding d 0 during which the anesthetic and analgesic were effective for part of the day. Evaluation of diurnal patterns of shelter use on individual days was not possible due to insufficient data at this time scale; many calves did not enter the shelter each hour. Hourly data were analyzed in a similar model to daily data, with hour as the repeated measure (categorical variable, to assess hourly differences between treatments).

Homogeneity of variance was verified by plotting residuals from the model against predicted values. Data are reported as (back-transformed) least squares means and 95% confidence intervals. Significance was declared at $P < 0.05$, and trends were reported if $0.05 \leq P \leq 0.10$.

**RESULTS**

Shelter use is illustrated for individual calves over consecutive observation days in Figure 3. Although all calves used the shelter at least once during each 24-h observation period, duration of shelter use was highly variable between calves. The minimum shelter use was 10.8 min/d and the maximum shelter use was 20.7 h/d (overall arithmetic mean of 4.5 h/d and median of 2.5 h/d). Calves showed individual consistency in their use of the shelter over time (between d 0 and 1: $R^2 = 0.40$, $P < 0.001$; between d 1 and 2: $R^2 = 0.43$, $P < 0.001$; and between d 0 and 2: $R^2 = 0.24$, $P = 0.009$).

Individual and social use of the shelter is reported in Table 1. Overall, calves used the shelter more after disbudding, with no interaction between treatment and day. Social use as a percentage of total shelter use did not differ between treatments, but disbudded calves spent a greater proportion of their shelter use with the calf on the same treatment. Disbudded calves tended to enter the shelter more frequently overall, driven by a greater frequency of entrances into the shelter when it was unoccupied, whereas the frequency of entering the shelter when it was occupied with another calf did not differ between treatments. In contrast, disbudded calves left the shelter more frequently when it was occupied, whereas the frequency of leaving the shelter when it was unoccupied did not differ between treatments.

Disbudded calves spent less time lying than non-disbudded calves, with no effect of day or interaction between treatment and day (Table 2). The frequency of total daily lying bouts and daily lying bouts within the shelter did not differ between treatments. The percentage of time in the shelter spent lying also did not differ between treatments, but disbudded calves were located inside the shelter for a greater percentage of their daily lying time.

Analysis of hourly shelter use during shorter timeframes within each day indicated that treatment effects differed with time of day but followed a consistent trend across observation days. Average hourly shelter use was greater for disbudded calves during daytime hours on d 0 [back-transformed means with 95% CI in parentheses: 6.5 (2.9, 13.5) vs. 2.7 (0.9, 6.1) min/h; disbudded vs. control; $F_{1,17} = 7.51$; $P = 0.014$] and d 1 [9.6 (4.8, 18.5) vs. 4.6 (2.0, 9.2) min/h; $F_{1,17} = 5.6$; $P = 0.030$] and tended to be greater on d 2 [10.6 (5.3, 20.5) vs. 4.9 (2.2, 9.8) min/h; $F_{1,17} = 3.65$; $P = 0.073$]. However, average hourly shelter use did not differ during the night on any observation day [back-transformed overall mean with pooled 95% CI in parentheses: 4.0 (0.7, 13.6) vs. 3.4 (0.5, 11.7) min/h; disbudded vs. control; $F_{1,17} < 0.94$; $P > 0.34$].

Diurnal patterns of shelter use (Figure 4) were examined in more detail by averaging hourly shelter use across consecutive observation days, including d 1 and

![Figure 3](https://example.com/figure3.png)

**Figure 3.** Individual and day-to-day shelter use (h/d) for 3 consecutive days following either disbudding (DB; n = 12 calves; black diagonal line-filled bars) or sham handling only (CON; n = 12 calves; gray bars) for each of 6 pens (4 calves/pen; pens labeled alphabetically). The dashed line represents the overall mean (back-transformed from the log-transformed data set).
Table 1. Individual and social use of a shelter providing visual isolation in group-housed calves (4/pen) subjected to disbudding (DB; n = 12, 2/pen) or handling only (CON; n = 12, 2/pen), for 3 d (D) following treatment (T) at 36 d of age.

<table>
<thead>
<tr>
<th>Item</th>
<th>DB</th>
<th>95% CI</th>
<th>CON</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Item</td>
<td>0 1 2</td>
<td>0 1 2</td>
<td></td>
<td></td>
<td>T  D</td>
</tr>
<tr>
<td>Shelter use, 2 min/d</td>
<td>120.2 244.9 191.3</td>
<td>(74.5, 425.2)</td>
<td>86.5 90.2 109.3</td>
<td>(39.7, 226.7)</td>
<td>0.031  0.23 0.37</td>
</tr>
<tr>
<td>Social use, % of use</td>
<td>54.7 58.5 72.0</td>
<td>(49.6, 73.9)</td>
<td>63.1 62.3 63.2</td>
<td>(50.7, 75.0)</td>
<td>0.86   0.34 0.37</td>
</tr>
<tr>
<td>Social use within treatment, % of use</td>
<td>23.6 41.5 29.2</td>
<td>(12.3, 59.6)</td>
<td>12.0 6.1 10.2</td>
<td>(1.0, 26.9)</td>
<td>0.017  0.95 0.36</td>
</tr>
<tr>
<td>Shelter visits, no./d</td>
<td>13.8 14.9 16.4</td>
<td>(11.8, 18.3)</td>
<td>10.5 12.3 11.5</td>
<td>(8.2, 14.7)</td>
<td>0.063  0.48 0.75</td>
</tr>
<tr>
<td>Social entrance, no./d</td>
<td>7.6 8.5 8.3</td>
<td>(6.1, 10.2)</td>
<td>5.3 6.8 4.5</td>
<td>(3.5, 7.6)</td>
<td>0.02   0.45 0.65</td>
</tr>
<tr>
<td>Solitary entrance, no./d</td>
<td>5.4 5.7 7</td>
<td>(3.7, 9.0)</td>
<td>4.7 3.9 5.9</td>
<td>(2.8, 7.4)</td>
<td>0.25   0.23 0.87</td>
</tr>
<tr>
<td>Social exit, no./d</td>
<td>6.3 7.8 6.3</td>
<td>(4.1, 9.6)</td>
<td>5.7 6.8 4.7</td>
<td>(2.9, 8.4)</td>
<td>0.38   0.33 0.92</td>
</tr>
<tr>
<td>Social exit, no./d</td>
<td>6.8 7.1 8.8</td>
<td>(4.9, 8.7)</td>
<td>4.4 4.6 5.7</td>
<td>(3.8, 7.6)</td>
<td>0.02   0.36 0.99</td>
</tr>
</tbody>
</table>

1 Data are reported as (back-transformed) LSM with 95% CI pooled by treatment.
2 Social use defined when the focal calf was in the shelter with at least 1 other calf present, regardless of whether shared shelter use was initiated by the focal calf. Social use within treatment defined when the focal calf was in the shelter at the same time as the pen mate assigned to the same treatment.

Table 2. Activity and resting behavior within an area providing visual isolation (shelter) in group-housed calves (4/pen) subjected to disbudding (DB; n = 12, 2/pen) or handling only (CON; n = 12, 2/pen), for 3 d (D) following treatment (T) at 36 d of age.

<table>
<thead>
<tr>
<th>Item</th>
<th>DB</th>
<th>95% CI</th>
<th>CON</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Item</td>
<td>0 1 2</td>
<td>0 1 2</td>
<td></td>
<td></td>
<td>T  D</td>
</tr>
<tr>
<td>Lying time, h/d</td>
<td>16.6 17.0 17.3</td>
<td>(16.3, 17.7)</td>
<td>17.5 17.7 17.8</td>
<td>(16.9, 18.4)</td>
<td>0.036  0.35 0.85</td>
</tr>
<tr>
<td>Lying bout frequency, no./d</td>
<td>21.3 20.8 21.7</td>
<td>(19.6, 22.9)</td>
<td>21.8 23.3 23.6</td>
<td>(21.2, 24.6)</td>
<td>0.18   0.65 0.66</td>
</tr>
<tr>
<td>Lying in shelter bout frequency, no./d</td>
<td>2.4 4.7 4.7</td>
<td>(1.9, 6.5)</td>
<td>2.3 3.2 2.2</td>
<td>(1.0, 4.8)</td>
<td>0.18   0.34 0.56</td>
</tr>
<tr>
<td>Lying in shelter, % of total shelter use</td>
<td>36.1 57.9 61.5</td>
<td>(27.3, 76.4)</td>
<td>42.9 52.1 52.4</td>
<td>(24.6, 73.7)</td>
<td>0.72   0.05 0.55</td>
</tr>
<tr>
<td>Lying in shelter, % of total lying time</td>
<td>12.0 17.8 19.0</td>
<td>(4.6, 34.5)</td>
<td>4.1 8.6 8.8</td>
<td>(0.6, 20.3)</td>
<td>0.048  0.18 0.99</td>
</tr>
</tbody>
</table>

1 Data are reported as (back-transformed) LSM with 95% CI pooled by treatment.
2 Reported means and CI are back-transformed from data that were square root-transformed.
in this analysis and excluding d 0 during which the anesthetic and analgesic were effective for part of the time. Hourly patterns of shelter use did not differ between treatments (no treatment by hour interaction; $F_{23,523} = 0.89; P = 0.62$). Overall hourly duration of shelter use was greater for disbudded calves ($F_{1,523} = 4.84; P = 0.028$), consistent with day-level data, and differed by hour ($F_{23,523} = 1.78; P = 0.015$). Analysis of hourly patterns of shelter use separately by daytime and nighttime hours also suggested that patterns of shelter use were similar between treatments, with no treatment by hour interaction during the day ($F_{12,281} = 0.70; P = 0.76$) or night ($F_{10,237} = 1.31; P = 0.22$). However, consistent with results of average hourly shelter use, disbudded calves used the shelter more overall during the daytime ($F_{1,281} = 8.62; P = 0.004$), whereas hourly use did not differ overall during the night ($F_{1,237} = 0.64; P = 0.42$).

**DISCUSSION**

The objective of this study was to determine how group-housed calves make use of a barrier offering visual and physical separation from the rest of the pen, and how use of this shelter may be affected by disbudding, a painful procedure. Given evidence in humans of reductions in social contact when experiencing chronic pain (Brown, 1990), we hypothesized that calves may increase use of this secluded area while experiencing pain in the days following disbudding. In support of this hypothesis, we observed that disbudding resulted in increased shelter use. We also found that calves were more likely to enter the shelter when it was unoccupied and leave the shelter when another calf was present in it, suggesting that the disbudded calves may have preferred use of the empty shelter. However, the extent of social shelter use suggests a need for further research to evaluate effects of pain on social behavior.

Although little work to date has evaluated effects of pain on social behavior in cattle, there is evidence of social withdrawal in cattle surrounding particularly salient life events, such as sickness in calves (Hixson et al., 2018) and parturition in cattle (Proudfoot et al., 2014). A range of factors may motivate social withdrawal in gregarious species. It has been speculated that social isolation in sick animals may serve an evolutionary function of reducing disease transmission (Dantzer and Kelley, 2007), whereas social isolation during parturition may facilitate maternal bonding (Nowak et al., 2000). In the present study, shelter use may have been motivated by factors apart from a specific choice to alter social contact or severity of pain following disbudding. Increased shelter use following disbudding was evident during daylight hours on each day of observation, including d 0 during the time period when the local block was effective, whereas shelter use did not differ during the night on any day. Proudfoot et al. (2014) found that dairy cow preference to calve within a shelter was increased during the day and speculated that shelter use may depend on light and human activity. Although the cause of social withdrawal at calving may easily differ from the present study, shelter use in disbudded calves may have also been due to avoidance of commotion associated with management activities. Farm and research staff were active only during the day, and the shelter provided visual separation and possibly some auditory reduction. Considering that shelter use was affected by disbudding during the daytime hours

![Figure 4. Diurnal patterns of shelter use, with hourly shelter use (min/h) averaged across 3 consecutive 24-h observations (starting at noon on each calendar day; 1200 to 1159 h) beginning at time of disbudding (DB; n = 12 calves; black diagonal line-filled bars) or sham handling only (CON; n = 12 calves; gray bars). Hours of the night (from 2000 to 0700 h, as defined based on sunrise and sunset) are indicated by the gray background. Hourly shelter use is shown as back-transformed LSM. Confidence intervals for daytime hours by treatment: [2.2 (minimum 0.2), 18.4 (maximum 49.7)] for DB calves and [0.5 (minimum 0), 7.7 (maximum 14.4) for CON calves, and for nighttime hours by treatment: [1.0 (minimum 0), 10.8 (maximum 20.1)] for DB calves and [0.4 (minimum 0), 7.5 (maximum 10.6)] for CON calves.](image-url)
of d 0 when the local block was effective, it is possible that shelter use in disbudded calves may have been motivated by a specific avoidance of humans who were present during the day, due to association with the procedure of disbudding, rather due to a motivation for social withdrawal when in pain. Increased shelter use during daytime hours of each day, and the greater preference to lie within the shelter in disbudded calves, may also have been partially motivated by avoidance of potentially painful physical contact with penmates, as the shelter provided some protection from the movement and jostling of active calves.

We observed that disbudded calves often used the shelter together. This may suggest that both individuals preferred features of this secluded space, such as the physical shelter from active penmates, regardless of preference for social contact or isolation. The presence of multiple disbudded calves in each group may also have created social dynamics within the group that affected individual ability to use the shelter. Provision of separate shelters in future work may provide clarity on factors motivating shelter use and social isolation more specifically. The greater frequency of leaving the occupied shelter in disbudded calves suggests that there may have been competition for shelter access, or preference for solitary use. Observation of agonistic behaviors inside the shelter, such as displacements, may shed light on potential competition for this resource. Additionally, observation of other behavior within the shelter, including affiliative and agonistic behaviors, may provide further insight into factors motivating shelter use, and why shelter use may change after events like disbudding.

Alternatively, the extent of social use of the shelter between disbudded calves could suggest a specific motivation for social support. Although we hypothesized that animals in pain may seek out social isolation, evidence also suggests that social contact during painful or stressful events may improve welfare. For example, rhesus macaques displayed reduced abnormal behavior during exposure to stressful events when they were pair-housed versus housed individually (Gilbert and Baker, 2011). Additionally, the presence of a familiar social partner reduces plasma cortisol concentrations in female guinea pigs when exposed to a novel environment (Hennessy et al., 2008). Further, this social buffering effect was not observed when the guinea pigs were tested with an unfamiliar companion, suggesting that the familiarity of the social companion is critical. Further evaluation of social behavior beyond shelter use would be needed to evaluate potential social buffering effects in disbudded calves. To date, limited examination has been done on the effects of pain, or other negative affective states, on motivation for either social contact or social isolation in dairy cattle. However, Jensen et al. (2015) found that dairy cows with hoof ulcers in individual hospital pens spent more time in an area of the pen that was closer in proximity and within visual contact of neighboring animals, both when resting and active. These results suggest that the cows did not seek social isolation when experiencing certain types of pain in the absence of systemic symptoms. We encourage further research to examine the effects of individual welfare on social relationships in group-housed dairy cattle, as this understanding may provide insight into improved housing design as well as useful indicators of welfare.

It is important to note that disbudded calves in the present study received both local anesthetic and analgesic, which was sufficient pain relief in previous work to mitigate various acute behavioral responses to disbudding in calves receiving local anesthetic only, such as an increase in ear flicks and head shaking (Heinrich et al., 2010) and decreased lying time (Theurer et al., 2012; Sutherland et al., 2018). As such, we did not expect the calves to experience acute pain, particularly immediately following the procedure. While we did not have a specific hypothesis concerning day to day changes in shelter use following disbudding, we expected disbudded calves to experience some degree of persistent pain during the days following the procedure. It is important to note that differences in shelter use by day and time of day are intertwined with the changing experience of pain following the procedure, as the local anesthetic wore off during d 0 and the intensity of the pain experienced may have fluctuated over the observation period. However, changes in behavior following disbudding, and effects of time of day on shelter use, were relatively consistent for our 3-d observation period following the procedure, suggesting that shelter use may have been affected by persistent pain, even with provision of the local block and analgesia on d 0, or some other aspect of the disbudding experience, as we discuss above. We also cannot definitively say whether increased use of the shelter, particularly on the day of the procedure, is a direct result of pain, rather than a more generalized stress response associated with the experience. A clearer relationship between the experience of pain and shelter use may have been established through inclusion of calves that were disbudded without analgesia entirely. Additionally, we could have provided different levels of nonsteroidal antiinflammatory medication following the procedure. However, given the novelty of the shelter provided and exploratory nature of this study, we did not see justification to withhold analgesia or deviate from the current protocol developed by the veterinarians at our research station. Further, we did not expect the standard anesthetic and analgesia provided.
in the present study to relieve all experiences of pain in disbudded calves. Heinrich et al. (2010) noted an increase in behavioral indicators of pain (ear flicking and head shaking) following disbudding in calves treated with analgesia, compared with a baseline period during which calves received a sham procedure only, suggesting that calves may still be experiencing pain despite analgesia.

In addition to effects on shelter use, we found that disbudding had ongoing effects on lying time, where disbudded calves spent less time lying but preferred to lie within the shelter. Previous findings suggest lying time may decrease when calves are experiencing pain. Disbudding with either local anesthetic or meloxicam only resulted in less lying time compared with a sham procedure or full pain mitigation (Sutherland et al., 2018), and provision of meloxicam similarly resulted in greater lying time compared with calves disbudded with local anesthetic only (Heinrich et al., 2010) or no anesthetic (Theurer et al., 2012). These results suggest that the experience of pain postdisbudding may negatively affect rest, yet in most previous work these effects on lying time were mitigated by provision of meloxicam. Our findings of persistent behavioral changes in disbudded calves, even with provision of local anesthetic and analgesic, may suggest that current approaches to mitigate pain following disbudding are insufficient. This evidence of prolonged change in aspects of social behavior and rest contributes to our somewhat limited knowledge of nonevoked behavioral states following disbudding with analgesia. Research concerning persistent pain and changes in undisturbed behavior of calves disbudded with analgesic is limited (as reviewed by Herskin and Nielsen, 2018), and our results also suggest a need for longer-term observation of behavior following disbudding.

The duration of pain experienced following disbudding remains unclear. Previous findings suggest that hot-iron disbudding affects wound-directed behavior in calves for at least 2 d (with and without analgesic; Heinrich et al., 2010), and wound sensitivity for at least 14 wk (Casoni et al., 2019). The timeframe and severity of postdisbudding pain when calves are provided analgesic has not been well defined, and the longevity of effects on shelter use arising after disbudding may depend on the mechanism driving this change in behavior. If shelter use is driven by avoidance of jostling due to wound sensitivity, effects may be long lasting. Recent findings also suggest that disbudding may result in ongoing chronic pain, as assessed using a variety of measures including clinical pain assessment, quantitative sensory tests, nociceptive reflexes, and conditioning pain modulation, for at least 14 wk (Casoni et al., 2019). Adcock and Tucker (2020) also demonstrated that, at 20 d after disbudding, calves did not display an aversion for a lidocaine cornual nerve block, an analgesic that is painful upon administration. This indicates that calves were willing to trade off a painful experience for longer-term benefits of analgesia, providing further evidence that disbudding results in pain that persists for some weeks. If shelter use is specifically related to pain, we could expect the increase in shelter use to be similarly ongoing. Further research is needed to evaluate longer-term behavioral changes following disbudding to elucidate the duration of persistent pain following disbudding.

In addition to evaluating effects of disbudding on shelter use as a possible indicator of social withdrawal, we were interested in generally characterizing shelter use because, to our knowledge, this approach to increasing pen complexity for group-housed dairy calves has not been evaluated previously. Use of the shelter was subject to considerable individual and pen variability, yet all calves used the shelter at least once on each observation day. It is interesting to note that treatment effects were seen only during the day. This suggests that this pen feature may be preferred and provide benefit to calves during certain times of day, or in response to some environmental factors, apart from the disbudding event that we focused on in this study. In particular, the shelter may have provided a place to rest without interruption; on average, half of the time in the shelter was spent resting. Preferences for resting locations have not been well studied in intensively housed dairy calves and cattle. Interestingly, Senft et al. (1985) observed that grazing heifers selected resting sites with high grass cover at night, which may suggest a preference for some shelter, which may function as a predator avoidance strategy.

Pen-level variability in shelter use (as illustrated in Figure 2) may suggest that shelter use is socially facilitated. Social facilitation, or the elicitation of previously learned behavior by a demonstrator, is evident in synchronized lying behavior in cattle (Stoye et al., 2012) and feeding behavior in calves (Costa et al., 2015), and may explain other effects in group-housed dairy calves, such as increased feeding time (Miller-Cushon and DeVries, 2016). Further, variability in shelter use between pens, such as location in the barn and environmental factors such as air quality and flow, may have influenced shelter use between pens. To limit this effect, the shelter was placed in the same position in each pen relative to position of fans and air flow. As discussed above, consideration of social influences on shelter use may be important in future work, as pen-level dynamics may be likely to either enhance or alter preferences for shelter use in relation to pain or other individual attributes.
In addition to pen-level variability, we observed individual differences in shelter use worth exploring in future work. For example, personality traits are associated with social behavior across species, which may relate to shelter use by dairy calves. In recent work, Lecorps et al. (2019) found that dairy calves that were more pessimistic, as determined by a judgment bias test, formed more preferential dyadic relationships to other calves in their social group. This suggests that personality differences may contribute to individual variability in social behavior in dairy calves and may partially explain differences in the use of environmental features that offer opportunity for seclusion. Evaluation of shelter use over time, apart from disbudding, in relation to other behavioral outcomes may further explain individual variability.

Accommodating individual preferences through environmental complexity and provision of opportunities for social seclusion may have a range of welfare implications. Interestingly, regardless of treatment, every calf entered the shelter at least once during each focal day. This pen feature allowed calves the opportunity to behave in more variable ways and to make choices that may enhance their individual welfare. Providing animals with the ability to make choices in their environment is an important way to ensure individual welfare needs are met (Spinka, 2019). This added level of pen complexity may also differentially improve the welfare of animals experiencing pain, facilitating seclusion when preferred.

**CONCLUSIONS**

Our results suggest that group-housed dairy calves make use of a barrier offering seclusion from the rest of the pen, and that the use of this area may depend on individual experiences, including pain following disbudding, that are reflective of welfare. Preference for shelter use was increased in disbudded calves, which may reflect a preference for social isolation or for some other aspect of this area of visual and physical separation. Additionally, we demonstrated that disbudding, even when accompanied by analgesia, has persistent effects on the undisturbed behavior of group-housed dairy calves, as demonstrated by changes in social behavior and resting time that did not return to the levels of the control calves by the end of our 3-d experimental period.

**ACKNOWLEDGMENTS**

We thank the staff at the University of Florida Dairy Unit and our undergraduate research assistants Lisette Coll-Roman, Emily Lindner, Alejandro Forero, Alexis Brocious, Bianca Hofmann, Harrison Warman, Margaret Faut, Megan McDowell, Lauren Peter, and Samantha Stella for their technical assistance. Thank you to Fiona Maunsell (College of Veterinary Medicine, University of Florida, Gainesville) for her guidance and oversight of the disbudding procedures. Thank you also to Dave Gingerich (Bear Design LLC, Clearwater, FL) for contributing the illustrations of the pen layout and shelter design. KG was supported on a research assistantship by funds awarded through the Department of Animal Sciences, University of Florida. This research was supported by the USDA National Institute of Food and Agriculture (Washington, DC), Hatch Multistate project 1012277. The authors declare no conflicts of interest.

**REFERENCES**


ORCIDs

K. N. Gingerich https://orcid.org/0000-0003-1170-9579
V. Choulet https://orcid.org/0000-0002-8809-8772
E. K. Miller-Cushon https://orcid.org/0000-0003-1876-807X