UC Davis
UC Davis Previously Published Works

Title

Conditioned place preference reveals ongoing pain in calves 3 weeks after disbudding

Permalink

https://escholarship.org/uc/item/5gb4g514

Journal

Scientific Reports, 10(1)

ISSN

2045-2322

Authors

Adcock, Sarah JJ
Tucker, Cassandra B

Publication Date

2020

DOI

10.1038/s41598-020-60260-7

Copyright Information

Peer reviewed

OPEN

Conditioned place preference reveals ongoing pain in calves 3 weeks after disbudding

Sarah J. J. Adcock[1,2] & Cassandra B. Tucker [1]*

Hot-iron disbudding, a routine procedure that prevents horn bud growth through cauterization, is painful for calves. The resulting burns remain sensitive to touch for weeks, but it is unknown whether calves experience ongoing, non-evoked pain. We evaluated conditioned place preference for analgesia in 44 calves disbudded or sham-disbudded 6 hours (Day 0) or 20 days (Day 20) before testing (n = 11/treatment). Calves were conditioned to associate the effects of a lidocaine cornual nerve block with the location and pattern of a visual stimulus, and a control injection of saline with the contrasting stimulus. On Day 0, disbudded calves tended to prefer the lidocaine-paired stimulus over the saline-paired one, suggesting that they found analgesia rewarding. On Day 20, sham calves avoided the lidocaine-paired stimulus, consistent with humans' experience of this drug being painful. Disbudded calves on Day 20 did not show this aversion, suggesting that they traded off the short-term pain of the lidocaine with the longer-term analgesia provided. Day 0 sham calves did not avoid the lidocaine-paired stimulus, likely because they received less than half the dose of Day 20 calves during conditioning. Thus, higher doses of lidocaine are aversive to uninjured animals, but disbudded calves are willing to engage in this cost. We conclude that calves experience ongoing pain 3 weeks after disbudding, raising additional welfare concerns about this procedure.

Disbudding, a husbandry procedure to prevent horn growth, is performed on 94% of U.S. dairies to reduce injuries to humans and other animals[1]. A common disbudding practice is to cauterize the horn-growing tissue with a heated iron when calves are 0 to 2 months of age. This procedure is unequivocally painful; the post-operative period is associated with wound-directed behaviors such as ear flicks, head shakes, and head rubbing, as well as physiological changes including increased plasma cortisol and heart rate. These responses are dampened by administration of local anesthetics and non-steroidal anti-inflammatory drugs[2,3].

The resulting burns from disbudding remain sensitive to mechanical stimulation until they have re-epithelialized, which takes from 6 to 13 weeks[4,5]. Others reported increased sensitivity for at least 14 weeks, after re-epithelialization had concluded[6]. However, it is unknown whether pain persists when the wounds are not being stimulated, as the mechanisms underlying hypersensitivity and ongoing pain are dissociable[7]. Given its important implications for cattle welfare, research is needed to address whether calves experience ongoing pain in the weeks after disbudding.

Operant measures, such as the conditioned place preference paradigm, have been developed to unmask ongoing pain in rodent injury models. This paradigm is based on the premise that an animal will choose to spend more time in an environment in which a previously rewarding experience (e.g., administration of pain relief) occurred[8]. When given the choice between an environment paired with analgesic treatment and another paired with a control vehicle, injured rodents, but not their sham-treated counterparts, prefer the drug-paired environment[9]. A place conditioning paradigm has been used to demonstrate that calves will avoid an area where they experienced disbudding without post-operative pain control compared to an area where they received a sham procedure[10] or were disbudded with the use of an NSAID[11]. Place conditioning has not been used to assess ongoing pain in the weeks after the procedure.

Our objective was to test conditioned place preference for a lidocaine cornual nerve block 6 hours and 20 days after disbudding calves. We chose lidocaine as the analgesic agent because it has a quick onset (1–5 min), short duration of action (90 min), negligible side effects, and established antinociceptive efficacy in calves[12]. The selected timepoints after disbudding, 6 hours and 20 days, represent respectively the acute post-operative period

[1]Center for Animal Welfare, Department of Animal Science, University of California, Davis, 95616, USA. [2]Animal Behavior Graduate Group, University of California, Davis, 95616, USA. *email: cbtucker@ucdavis.edu

and a period of transition when the initial necrotic tissue begins to peel away from the scalp and eventually falls off, leaving an open wound[4,5]. Since wounds vary in healing time[4,5], we also documented the tissue types present in the wound bed to verify the healing stage at these timepoints.

Pre-conditioning baselines are often included in conditioned place preference tests to account for an untrained individual's potential bias towards one stimulus over another in the absence of conditioning. However, pre-exposure to the conditioned stimuli before conditioning can diminish subsequent learning, a process known as latent inhibition[13]. Moreover, as noted by Dixon *et al.*[14], there is no guarantee that a single baseline measure accurately represents an animal's bias or that it remains consistent through time. Thus, rather than including a baseline, we compared the proportion of time disbudded calves spent in contact with the lidocaine-paired stimulus to: (1) the proportion of time that sham calves spent in contact with the lidocaine-paired stimulus to determine whether disbudding altered conditioned place preference; and (2) chance expectation (i.e. 0.5) to determine whether disbudded calves had a preference, aversion, or neutral stance towards the drug. We predicted that, regardless of time since disbudding, disbudded calves would: (1) spend a greater proportion of time in contact with the lidocaine-paired stimulus than sham-operated controls, and (2) spend more than 50% of the time with the lidocaine-paired stimulus.

Our secondary objective was to confirm findings from previous studies that the wounds are sensitive during healing. We predicted that disbudded calves would be more sensitive to mechanical stimulation of the wounds than sham calves regardless of time since disbudding, consistent with previous findings[4,6].

Materials and Methods

This work was undertaken at the University of California Davis Dairy Facility from June to October 2017. All experimental protocols were approved by and carried out in accordance with the University of California Davis Institutional Animal Care and Use Committee (protocol # 19901).

Animals and housing. We enrolled all female calves born between May 26 and September 13, for a total of 35 Holsteins and 9 Jerseys. Immediately after birth, calves were housed individually in outdoor enclosures consisting of a plastic hutch (2.0 m long × 1.5 m wide) and a wire-fenced pen (2.0 m long × 1.5 wide × 0.9 m high). The enclosures were spaced 0.5 m apart and bedded with rice hulls approximately 15–20 cm deep. Calves were bottle-fed milk replacer (26% CP, 16% fat, 15% total solids; Calva Products Inc., Acampo, CA) at 930 h and 1630 h each day and had ad libitum access to water and starter (18.3% CP, 2.8% fat, 4% crude fat; Associated Feed & Supply Co., Turlock, CA). Holstein and Jersey calves received 4.8 or 3.8 L/d at the start of the experiment, respectively. At 24 d of age, the amount was increased by 1 L for both breeds, and then again at 31 d of age for Jerseys only (Supplementary Fig. S1). Calves were switched to bucket feeding at 35 to 38 days of age, and a dry total mixed ration was introduced. We note that although this plane of nutrition is conventional industry practice and follows the facility's standard operating procedure, it is below that needed to meet calves' nutritional requirements for maintenance, growth, and development[15].

Treatments. Calves were blocked by birth order and randomly allocated to 1 of 4 treatment groups balanced for breed when they were 21 to 28 days of age: disbudded or sham-disbudded 6 hours (Day 0) or 20 days (Day 20) before preference testing (n = 11/treatment). This sample size was determined from rodent studies of conditioned place preference[16,17]. All calves underwent a conditioning phase for 3 days before the preference test. For Day 0 calves, we disbudded or sham-disbudded the left horn bud 3 days before the conditioning phase to make the experience of pain relief salient for the disbudded calves. We allotted 3 days between disbudding the left horn bud and conditioning to ensure the meloxicam, a non-steroidal anti-inflammatory drug given at the time of the procedure, was eliminated from the plasma before conditioning began[18]. The right horn bud was disbudded or sham-disbudded the day of the preference test. Day 20 calves began the conditioning phase 17 days after both horn buds were disbudded or sham-disbudded (Fig. 1). All Day 20 calves had transitioned to bucket-feeding by the start of the conditioning phase.

Between 21–28 days of age, Day 0 and Day 20 calves were disbudded/sham-disbudded on the left or both sides of the head, respectively. Body weights were similar among treatment groups the day before disbudding (mean ± SD; Day 0 Sham: 44 ± 6 kg; Day 0 Disbud: 45 ± 7 kg; Day 20 Sham: 43 ± 7 kg; Day 20 Disbud: 43 ± 5 kg). On the day of disbudding, the calf was restrained in a head device in her home enclosure, as previously depicted[19]. A 5 × 5 cm patch of hair was clipped with a size 40 electric razor blade on each side of the head to locate the horn bud. We used a 20 gauge × 25 mm needle to administer a cornual nerve block consisting of 5.5 mL buffered lidocaine (2% lidocaine hydrochloride diluted with 8.4% sodium bicarbonate in a 10:1 ratio). If the horn bud was not numb after 10 min, as assessed by pinprick, we gave an additional 2 mL of buffered lidocaine. An electric cautery iron (X50, Rhinehart Development Corp., Spencerville, IN) was fitted with a 1.3 cm tip and heated to 424 ± 18 °C. It was applied to the horn bud for 20 ± 4 seconds (mean ± SD). After disbudding, the calf received 1 mg/kg of meloxicam tablets in a gelatin capsule (3.5 g; Torpac Inc., Fairfield, NJ). Sham-disbudded calves received the same treatment, with the exception that the iron was ambient temperature and the gelatin capsule was empty. Sham calves did not receive meloxicam because the Animal Medicinal Drug Use Clarification Act prohibits nontherapeutic off-label use of this drug[20]. SJJA performed all disbudding procedures.

Conditioning phase. We conditioned each calf to associate the analgesic effect of a lidocaine cornual nerve block with the location (left or right) and pattern (black with white stripes or solid black) of a plywood board (90 cm high × 150 cm long) attached to the side of the wire-fenced pen in the calf's enclosure. The board of the contrasting location and pattern was paired with a saline injection as the control. We attached a rubber nipple to the board to increase the biological relevance of the conditioned stimulus; calves are motivated to suck, even when no milk is provided[21].
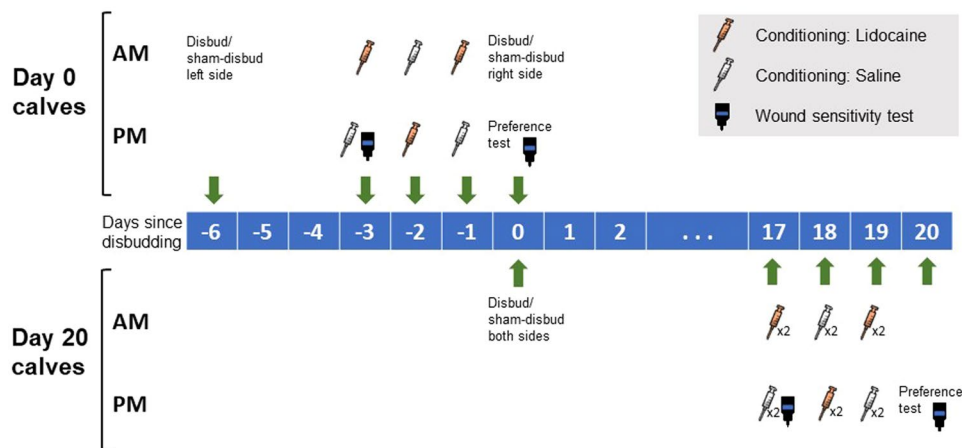
Figure 1. Timeline for evaluating conditioned place preference in calves disbudded or sham-disbudded 6 hours (Day 0) or 20 days (Day 20) before preference testing. During each conditioning trial, Day 0 and 20 calves received an injection on the left or both sides of the head (indicated by *x2*), respectively, followed by presentation of the conditioned stimulus. The substance injected in each conditioning trial alternated between lidocaine and saline. Only half of the calves in the Day 0 and Day 20 groups were conditioned with lidocaine first, as illustrated here.

Thirty minutes before the first conditioning trial, we clipped the injection site behind the lateral canthus of the calf's eye and applied a lidocaine-prilocaine cream (Hi-Tech Pharmacal Co., Inc. Amityville, NY). Twenty-five minutes later, the calf was restrained in a head device in her enclosure and blindfolded. We injected buffered lidocaine or saline solution around the cornual nerve on the disbudded/sham-disbudded side of the head. Thus, Day 0 calves received an injection on the left side only, whereas Day 20 calves received injections on both sides. The injection consisted of 5.5 mL for Day 0 calves, whereas Day 20 calves received 6.6 mL on each side to account for the larger body size. During the injection, the striped or solid board was attached to the left or right side of the wire-fenced pen. Immediately after the injection, the blindfold was removed, the calf was released from the head device and allowed 10 minutes to interact with the board and nipple. For the first conditioning trial only, we guided the calf to the nipple within 1 minute after she was released. In the second trial, we repeated the procedure with the opposite pairing: calves that received lidocaine in the first trial now received saline, and vice versa, and were exposed to the opposite pattern and location. Lidocaine and saline administration alternated between the morning and evening for 3 days, such that each calf received 3 conditioning trials with both the lidocaine-paired and saline-paired stimuli (Fig. 1). The evening trial occurred approximately 6 hours after the morning one to ensure the calf had returned to a drug-free state, if she received lidocaine earlier. The pattern and location of the conditioned stimulus, as well as the drug used in the first conditioning trial, were balanced across treatments.

Preference test. The morning after the last conditioning trial, the right horn bud for Day 0 calves was disbudded or sham-disbudded in the same manner as previously described, with the exception that meloxicam was given after the preference testing occurred (maximum 12 hours later), to ensure the calf was in a drug-free state during the test. We did not perform any procedures on Day 20 calves in the morning. In the evening, we tested all calves' preference for the lidocaine- or saline-paired stimulus (Fig. 1). The calf was briefly confined to her hutch while both boards were set up in their respective locations. The test began when we removed the opaque barrier from in front of the hutch, allowing the calf to freely access both boards and nipples for 5 minutes (Fig. 2).

Behavioral scoring. During the conditioning trials and preference test, behavioral activity was recorded with a video camera (HC-V180, Panasonic, Kadoma, Japan) positioned approximately 120 cm in front of the pen. Two trained observers, blinded to the drug-stimulus pairings, analysed all videos for the duration of time the calf spent in contact with the conditioned stimulus (inter-observer reliability: intraclass correlation coefficient = 0.96). We defined contact as the muzzle or tongue touching the board or nipple for at least 1 s. Videos were analyzed using BORIS (Behavioral Observation Research Interactive Software[22]).

Wound healing and sensitivity. We measured sensitivity around the horn bud area immediately before the second conditioning trial and immediately after the preference test (Fig. 1) using a digital algometer fitted with a 4-mm-diameter round rubber tip (ProdPlus; TopCat Metrology Ltd., Little Downham, UK). The calf was restrained in the head device and blindfolded. We then applied an increasing amount of force to the edge of the disbudding wound, or intact horn bud for sham calves, as described previously[4]. The test ended when the calf moved her head or a maximum cut-off point of 10 N was reached. We repeated the test if a fly landed on the head, a loud noise occurred, or the calf urinated or defecated. If 3 interruptions occurred we abandoned the test (3% of tests).

Wound sensitivity tests occurred at the lateral and caudal edges of each wound or the equivalent location on sham calves. The order of test sites was: left lateral, left caudal, right caudal, and right lateral. To ensure force was applied at a consistent rate, personnel operating the algometer were trained to meet a set of criteria

Figure 2. During the preference test, the calf has access to both the lidocaine- and saline-paired stimuli over a 5-minute period.

before performing the tests[4]. We calculated the rate that force was applied in each test from video recordings (0.35 ± 0.12 N/s; 2% of videos missing). If force was increased at a rate <0.1 or> 0.6 N/s, the data were excluded (10% of tests). Due to the nature of the tests, the operator of the algometer was not blind to treatment.

We took digital photographs of the wound with a DSLR camera (D5300; Nikon Corp., Tokyo, Japan) after sensitivity testing was completed. Photos were taken 15 cm from the wound. One person scored the photos for tissues present in the wound bed, as described previously[4].

Statistical analysis. All analyses described below were conducted separately for Day 0 and Day 20 calves, as methodological and age differences between the two groups prevented meaningful comparison.

Conditioning trials. To check whether disbudding affected engagement with the conditioned stimulus during the conditioning trials, we ran a linear mixed model with disbudding (disbudded vs sham) as the fixed effect and calf as a random effect. Duration of contact with the conditioned stimulus in each trial was the dependent variable.

Preference test. We used a zero-inflated beta regression model to assess the effect of disbudding on the proportion of time spent in contact with the lidocaine-paired stimulus. Disbudding was a fixed effect, with proportion of time in contact with the lidocaine-paired stimulus as the dependent variable. We used one-sample Wilcoxon tests to compare the proportion of time spent in contact with the lidocaine-paired stimulus to chance expectation (0.5).

Wound sensitivity. A preliminary analysis indicated that there was no effect of side (left vs right) or location (caudal vs lateral) on wound sensitivity, so we averaged data for each calf into two scores: one for the first day of conditioning and one for the day of the preference test. For each day, we ran a linear model to test the effect of disbudding on wound sensitivity.

Model fitting. Data were analyzed in R, version 3.4.1[23]. Linear mixed models were fitted using the REML method implemented in the *nlme* package[24]. Degrees of freedom were estimated using the containment method. We confirmed homogeneity of variance and normality using residuals vs fits plots and Q-Q plots, respectively. Zero-inflated beta regression was performed with the *gamlss* package[25]. Estimated marginal means and SE were calculated with the *emmeans* package[26].

Results
Conditioning phase. Calves spent a mean $\pm$ SD of 227 ± 147 seconds in contact with the conditioned stimulus during each 10-minute trial. No contact with the conditioned stimulus occurred in 1% of trials, and only on the third day of conditioning. Disbudded and sham calves did not differ in the amount of time they spent in contact with the conditioned stimulus for both Day 0 and Day 20 groups ($F_{1,20} \leq 0.83$, $P \geq 0.374$).

Preference test. On Day 0, disbudded calves tended to spend more than 50% of the time in contact with the lidocaine-paired stimulus (Wilcox test: median proportion of time (IQR): 0.62 (0.11); $P = 0.083$), whereas sham calves showed no preference (0.62 (0.61), $P = 1$). However, the proportion of time in contact with the lidocaine-paired stimulus did not differ between sham and disbudded calves (Fig. 3; $t = -0.19$, $P = 0.848$).
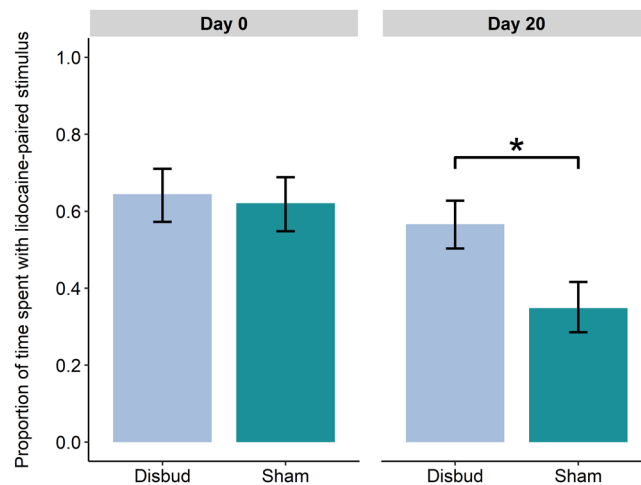
Figure 3. Proportion of time disbudded and sham calves spent with the lidocaine-paired stimulus 0 or 20 days after the procedure (estimated marginal means ± SE). Values are back-transformed from the logit scale. *P = 0.034.

Tissue	Day 0 calves		Day 20 calves	
	First day of conditioning	Preference test	First day of conditioning	Preference test
Attached necrotic	100	100	0	0
Burns outside the ring	18	23	0	0
Detaching necrotic	0	0	100	71
Exudate	0	0	0	5
Granulation	0	0	0	29
Crust	0	0	0	14

Table 1. The percentage of disbudding wounds in which the tissue was present on the first day of conditioning and immediately after the preference test. Day 0 calves (n = 11) had the left horn bud cauterized 3 days before the first day of conditioning and the right horn bud cauterized the same day as the preference test. Day 20 calves (n = 11) were cauterized on both sides 17 days before the first day of conditioning.

On Day 20, sham calves avoided the lidocaine-paired stimulus (0.27 (0.46), $P = 0.045$), whereas the disbudded calves showed no preference (0.57 (0.22), $P = 0.240$). Disbudded calves spent a greater proportion of time in contact with the lidocaine-paired stimulus compared to sham calves (Fig. 3; $t = -2.32$, $P = 0.033$).

Wound healing and sensitivity. The necrotic tissue was still attached to the scalp in all wounds after the preference test in Day 0 calves. On the first conditioning day for Day 20 calves, the tissue was detaching from the scalp in all wounds. By the day of the preference test, the necrotic tissue had fallen off in 29% of wounds (Table 1).

Day 0 disbudded calves were more sensitive to pressure algometry compared to sham calves on both the first day of conditioning and on the day of the preference test ($F_{1,19-20} \geq 10.29$; $P \leq 0.005$; Fig. 4). Day 20 disbudded calves tended to be more sensitive compared to sham calves on the first day of conditioning ($F_{1,20} = 3.85$; $P = 0.064$; Fig. 4). However, sham calves subsequently became more sensitive ($F_{1,10} = 6.37$; $P = 0.030$) such that they did not differ from disbudded calves on the day of the preference test ($F_{1,20} = 0.45$; $P = 0.512$; Fig. 4).

Discussion

We assessed whether a conditioned place preference test would reveal ongoing pain in the hours immediately after disbudding (Day 0), and 3 weeks later, during healing (Day 20). We found limited evidence that disbudding produced conditioned place preference for a lidocaine cornual nerve block on Day 0. Disbudded calves, but not shams, tended to spend more time with the lidocaine-paired stimulus, suggesting that they found analgesia rewarding. However, this effect is not particularly robust given that we observed no differences in the proportion of time spent in contact with the lidocaine-paired stimulus between disbudded and sham calves on Day 0. Interestingly, 20 days after disbudding, sham calves demonstrated conditioned place aversion to local anesthesia, implying that they experienced an unpleasant state after receiving the nerve block during the conditioning trials and learned to associate it with the lidocaine-paired stimulus. The disbudded calves on Day 20 did not avoid the lidocaine-paired stimulus, suggesting that they traded off the short-term aversiveness of the lidocaine to receive the longer-term benefits of analgesia. The disbudded calves' willingness to engage in this trade-off suggests they were experiencing ongoing pain 3 weeks after the procedure.
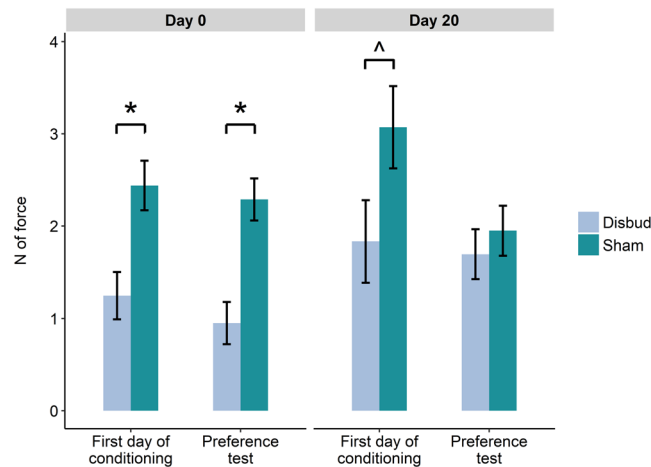
Figure 4. Disbudding wound sensitivity (measured as force, N; estimated marginal means ± SE) for calves tested on the first day of conditioning and immediately after the preference test. Day 0 calves were disbudded/sham-disbudded on the left side 3 days before the first day of conditioning and disbudded/sham-disbudded on the right side the same day as the preference test. Day 20 calves were disbudded/sham-disbudded on both sides 17 days before the first day of conditioning. *P ≤ 0.005; ^P = 0.064.

The results we observed on Day 20 partially supported our predictions; the disbudded calves spent a greater proportion of time with the lidocaine-paired stimulus compared to sham calves, but they did not show a preference for it over the saline-paired stimulus. The lower proportion of time spent interacting with the lidocaine-paired stimulus than we had predicted is consistent with the well-documented painful effects of lidocaine injections in humans[27]. We took steps to reduce this aversiveness by buffering the lidocaine with sodium bicarbonate to decrease its acidity and by applying a topical anesthetic to numb the skin before injection. Unfortunately, we have since found that the anesthetic cream we used may worsen injection pain rather than alleviate it[19]. However, the cream was used for both lidocaine and saline injections and so cannot explain the sham calves' aversion to lidocaine. In humans, buffering lidocaine decreases the pain of injection for non-oral procedures, but does not eliminate it[27,28]. A strategy that we did not consider was warming lidocaine to body temperature, as this has been shown to decrease pain in humans, compared to when it is given at room temperature[29]. Although we did not measure the temperature of the lidocaine, it was certainly below the calf's body temperature (38–39 °C). It would be of interest to investigate whether buffering and warming lidocaine are effective strategies for reducing pain from lidocaine injections in calves.

The aversion to lidocaine in sham calves was only observed on Day 20, and not on Day 0. We suspect this is because Day 20 calves received two injections in each conditioning trial, whereas Day 0 calves received one on the left side only. Moreover, each injection consisted of 6.6 ml for Day 20 calves, compared to 5.5 ml in Day 0 calves. Dose-dependent conditioned place aversions to drugs are commonly observed in rodents[30–32].

It is surprising that we did not find stronger evidence of conditioned place preference on Day 0, as calves unequivocally experience ongoing pain in the hours after disbudding[2]. Thus, the weak evidence for ongoing pain on Day 0 in our study likely reflects a limitation of our methodology to detect it. Pain is a dynamic process, and it is possible that a lidocaine cornual nerve block may be more effective at managing pain occurring in the later vs earlier stages of healing. In severely burned humans and rodents, the inflammatory response is most elevated in the first week after injury[33,34] and pain may not be localized to the site of the injury. Thus, it is possible a non-steroidal anti-inflammatory drug (NSAID) may have provided more effective relief for ongoing pain in the first week after disbudding compared to a local anesthetic targeting just the immediate vicinity of the wound. We chose lidocaine as our pain-relieving agent since it has a quicker onset and shorter duration of action than the shortest-acting approved NSAID in cattle, flunixin meglumine, which has a half life of 5 hours when administered intravenously[35].

It is also possible that the pain was severe enough during conditioning or the preference test in Day 0 calves that it interfered with their ability to attend to cues. Pain impairs performance on cognitive tasks in rodents and humans[36]. Other stressors, such as hunger due to the restricted milk volume, may also have limited the calves' ability to learn the conditioned place preference task, as has been suggested in chickens[14]. The Day 0 calves' performance may also be related to a less developed cognitive ability compared to Day 20 calves. Day 0 calves were 24–31 days of age on the first day of conditioning, whereas Day 20 calves were 38–45 days of age. Calves between 1–5 weeks of age can learn discrimination tasks[37,38], but an extensive training period was required. We limited conditioning to 6 sessions for ethical reasons (i.e., to minimize the number of injections each calf received), but it is possible that more sessions may have improved the Day 0 calves' ability to discriminate between the saline- and lidocaine-paired stimuli.

On the first day of conditioning for Day 0 and Day 20 calves, wounds were more sensitive compared to non-disbudded tissue, as demonstrated previously[4,6]. However, Day 20 sham calves became more sensitive from the first day of conditioning to the preference test. This increase in sensitivity in the sham animals contrasts

6

with other studies in which injured calves are consistently more responsive than their non-injured counterparts throughout healing[4,6,39]. We believe this difference in sensitivity reflects conditioned hyperalgesia[40], consistent with the sham calves' lidocaine-induced conditioned place aversion. As we performed the pressure algometry immediately after the preference test, the recent presence of the lidocaine-paired stimulus may have cued the sham calves to expect an unpleasant state, causing a heightened response to pressure.

On Day 20, the disbudded calves, but not their sham counterparts, were willing to pay a cost (i.e., short-term pain from a lidocaine injection) in order to access the longer-term analgesia provided, suggesting they experienced ongoing pain. The presence of ongoing pain 3 weeks after disbudding indicates that a combination of local anesthetic and a single dose of NSAID at the time of the procedure, as is current best practice, is not enough. The longest-acting NSAIDs, meloxicam and carprofen, have a half-life of 22 or 37 h, respectively[41]. To date, no published studies have evaluated strategies for mitigating disbudding pain beyond the acute period. An effective medication protocol will require an understanding of the progression of symptoms through healing. For example, it is possible that pruritus (e.g., itch) develops at some point in the weeks after disbudding[3]. Pruritus is a common and distressing complication after burns in humans, and can have a profound impact on quality of life[42]. In human burn patients, moderate to severe pruritus began 2 to 3 weeks into healing[43]. Indeed, it is possible the cornual nerve block was relieving pruritus in the Day 20 disbudded calves as local anesthetics have anti-pruritic actions[44]. The necrotic tissue on all wounds was peeling by 17 days after disbudding, and had started to fall of by 20 days, similar to previous descriptions[4,5]. It would be of interest to know whether calves experience pruritus at this stage in order to provide appropriate medication; antihistamines, for example, are commonly prescribed for pruritus in human burn patients[42].

Conclusion

Calves experience ongoing pain 3 weeks after disbudding, raising additional concerns about the welfare implications of this procedure. This finding indicates that current recommended pain management practices are not sufficient, and longer-term strategies are needed.

Data availability

Data are available in the Dryad Digital Repository: https://doi.org/10.25338/B8NS56.

References

1. USDA. *Health and Management Practices on U.S. Dairy Operations, 2014*. USDA–APHIS–VS–CEAH–NAHMS, Fort Collins, CO, https://www.aphis.usda.gov/animal_health/nahms/dairy/downloads/dairy14/Dairy14_dr_PartIII.pdf (2018).
2. Stock, M. L., Baldridge, S. L., Griffin, D. & Coetzee, J. F. Bovine dehorning: Assessing pain and providing analgesic management. *Vet. Clin. North Am. Food Anim. Pract.* **29**, 103–133, https://doi.org/10.1016/j.cvfa.2012.11.001 (2013).
3. Herskin, M. S. & Nielsen, B. H. Welfare effects of the use of a combination of local anesthesia and NSAID for disbudding analgesia in dairy calves-reviewed across different welfare concerns. *Front. Vet. Sci.* **5**, 117, https://doi.org/10.3389/fvets.2018.00117 (2018).
4. Adcock, S. J. J. & Tucker, C. B. The effect of disbudding age on healing and pain sensitivity in dairy calves. *J. Dairy Sci.* **101**, 10361–10373, https://doi.org/10.3168/jds.2018-14987 (2018).
5. Adcock, S. J. J., Vieira, S. K., Alvarez, L. & Tucker, C. B. Iron and laterality effects on healing of cautery disbudding wounds in dairy calves. *J. Dairy Sci.* **102**, 10163–10172, https://doi.org/10.3168/jds.2018-16121 (2019).
6. Casoni, D., Mirra, A., Suter, M. R., Gutzwiller, A. & Spadavecchia, C. Can disbudding of calves (one versus four weeks of age) induce chronic pain? *Physiol. Behav.* **199**, 47–55, https://doi.org/10.1016/j.physbeh.2018.11.010 (2019).
7. Mogil, J. S. & Crager, S. E. What should we be measuring in behavioral studies of chronic pain in animals? *Pain* **112**, 12–15, https://doi.org/10.1016/j.pain.2004.09.028 (2004).
8. Mogil, J. S. Animal models of pain: Progress and challenges. *Nat. Rev. Neurosci.* **10**, 283–294, https://doi.org/10.1038/nrn2606 (2009).
9. Navratilova, E. & Porreca, F. Reward and motivation in pain and pain relief. *Nat. Neurosci.* **17**, 1304–1312, https://doi.org/10.1038/nn.3811 (2014).
10. Ede, T., Lecorps, B., von Keyserlingk, M. A. G. & Weary, D. M. Calf aversion to hot-iron disbudding. *Sci. Rep.* **9**, 5344, https://doi.org/10.1038/s41598-019-41798-7 (2019).
11. Ede, T., von Keyserlingk, M. A. G. & Weary, D. M. Assessing the affective component of pain, and the efficacy of pain control, using conditioned place aversion in calves. *Biol. Lett.* **15**, 20190642, https://doi.org/10.1098/rsbl.2019.0642 (2019).
12. Fierheller, E. E., Caulkett, N. A., Haley, D. B., Florence, D. & Doepel, L. Onset, duration and efficacy of four methods of local anesthesia of the horn bud in calves. *Vet. Anaesth. Analg.* **39**, 431–435, https://doi.org/10.1111/j.1467-2995.2012.00717.x (2012).
13. Bardo, M. T., Rowlett, J. K. & Harris, M. J. Conditioned place preference using opiate and stimulant drugs: A meta-analysis. *Neurosci. Biobehav. Rev.* **19**, 39–51, https://doi.org/10.1016/0149-7634(94)00021-R (1995).
14. Dixon, L. M. *et al.* Conditioned place preference or aversion as animal welfare assessment tools: Limitations in their application. *Appl. Anim. Behav. Sci.* **148**, 164–176, https://doi.org/10.1016/j.applanim.2013.07.012 (2013).
15. Khan, M. A., Weary, D. M. & von Keyserlingk, M. A. Invited review: Effects of milk ration on solid feed intake, weaning, and performance in dairy heifers. *J. Dairy Sci.* **94**, 1071–1081, https://doi.org/10.3168/jds.2010-3733 (2011).
16. Davoody, L. *et al.* Conditioned place preference reveals tonic pain in an animal model of central pain. *J. Pain* **12**, 868–874, https://doi.org/10.1016/j.jpain.2011.01.010 (2011).
17. He, Y., Tian, X., Hu, X., Porreca, F. & Wang, Z. J. Negative reinforcement reveals non-evoked ongoing pain in mice with tissue or nerve injury. *J. Pain* **13**, 598–607, https://doi.org/10.1016/j.jpain.2012.03.011 (2012).
18. Mosher, R. A., Coetzee, J. F., Cull, C. A., Gehring, R. & KuKanich, B. Pharmacokinetics of oral meloxicam in ruminant and preruminant calves. *J. Vet. Pharmacol. Ther.* **35**, 373–381, https://doi.org/10.1111/j.1365-2885.2011.01331.x (2012).
19. Jimenez, R. E., Adcock, S. J. J. & Tucker, C. B. Acute pain responses in dairy calves undergoing cornual nerve blocks with or without topical anesthetic. *J. Dairy Sci.* **102**, 3431–3438, https://doi.org/10.3168/jds.2018-15445 (2019).
20. U.S. Food and Drug Administration. *Animal Medicinal Drug Use Clarification Act (AMDUCA) of 1994.*, https://www.fda.gov/animalveterinary/guidancecomplianceenforcement/actsrulesregulations/ucm085377.htm (1994).
21. Rushen, J. & de Passillé, A. M. The motivation of non-nutritive sucking in calves, *Bos taurus*. *Anim. Behav.* **49**, 1503–1510, https://doi.org/10.1016/0003-3472(95)90071-3 (1995).

22. Friard, O. & Gamba, M. BORIS: A free, versatile open-source event-logging software for video/audio coding and live observations. *Methods Ecol. Evol.* **7**, 1325–1330, https://doi.org/10.1111/2041-210X.12584 (2016).
23. R Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria (2017).
24. Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D. & R Core Team. nlme: Linear and nonlinear mixed effects models. R package version 3.1-131, http://CRAN.R-project.org/package=nlme. (2017).
25. Rigby, R. A. & Stasinopoulos, D. M. Generalized additive models for location, scale and shape. *J. Roy. Stat. Soc. Ser. C. (Appl. Stat.)* **54**, 507–554, https://doi.org/10.1111/j.1467-9876.2005.00510.x (2005).
26. Lenth, R. V. emmeans: Estimated marginal means, aka least-squares means. R package version 1.3.0., https://CRAN.R-project.org/package=emmeans. (2018).
27. Cepeda, M. S. *et al.* Adjusting the pH of lidocaine for reducing pain on injection. *Cochrane Database Syst. Rev.* **12**, CD006581, https://doi.org/10.1002/14651858.CD006581.pub2 (2010).
28. Aulestia-Viera, P. V., Braga, M. M. & Borsatti, M. A. The effect of adjusting the pH of local anaesthetics in dentistry: A systematic review and meta-analysis. *Int. Endod. J.* **51**, 862–876, https://doi.org/10.1111/iej.12899 (2018).
29. Lundbom, J. S. *et al.* The influence of lidocaine temperature on pain during subcutaneous injection. *J. Plast. Surg. Hand Surg.* **51**, 118–121, https://doi.org/10.1080/2000656X.2016.1194281 (2017).
30. Barr, G. A., Paredes, W. & Bridger, W. H. Place conditioning with morphine and phencyclidine: Dose dependent effects. *Life Sci.* **36**, 363–368, https://doi.org/10.1016/0024-3205(85)90122-5 (1985).
31. Brockwell, N. T., Eikelboom, R. & Beninger, R. J. Caffeine-induced place and taste conditioning: Production of dose-dependent preference and aversion. *Pharmacol. Biochem. Behav.* **38**, 513–517, https://doi.org/10.1016/0091-3057(91)90006-N (1991).
32. van der Kooy, D., O'Shaughnessy, M., Mucha, R. F. & Kalant, H. Motivational properties of ethanol in naive rats as studied by place conditioning. *Pharmacol. Biochem. Behav.* **19**, 441–445, https://doi.org/10.1016/0091-3057(83)90117-X (1983).
33. Finnerty, C. C. *et al.* Cytokine expression profile over time in severely burned pediatric patients. *Shock* **26**, 13–19, https://doi.org/10.1097/01.shk.0000223120.26394.7d (2006).
34. Gauglitz, G. G. *et al.* Characterization of the inflammatory response during acute and post-acute phases after severe burn. *Shock* **30**, 503–507, https://doi.org/10.1097/SHK.0b013e31816e3373 (2008).
35. Kleinhenz, M. D. *et al.* The pharmacokinetics of transdermal flunixin meglumine in Holstein calves. *J. Vet. Pharmacol. Ther.* **39**, 612–615, https://doi.org/10.1111/jvp.12314 (2016).
36. Low, L. A. The impact of pain upon cognition: What have rodent studies told us? *Pain* **154**, 2603–2605, https://doi.org/10.1016/j.pain.2013.06.012 (2013).
37. Neave, H. W., Daros, R. R., Costa, J. H. C., von Keyserlingk, M. A. G. & Weary, D. M. Pain and pessimism: Dairy calves exhibit negative judgement bias following hot-iron disbudding. *Plos One* **8**, e80556, https://doi.org/10.1371/journal.pone.0080556 (2013).
38. Gaillard, C., Meagher, R. K., von Keyserlingk, M. A. G. & Weary, D. M. Social housing improves dairy calves' performance in two cognitive tests. *Plos One* **9**, e90205, https://doi.org/10.1371/journal.pone.0090205 (2014).
39. Tucker, C. B. *et al.* Pain sensitivity and healing of hot-iron cattle brands. *J. Anim. Sci.* **92**, 5674–5682, https://doi.org/10.2527/jas.2014-7887 (2014).
40. Madden, V. J. *et al.* Can pain or hyperalgesia be a classically conditioned response in humans? A systematic review and meta-analysis. *Pain Med.* **17**, 1094–1111, https://doi.org/10.1093/pm/pnv044 (2016).
41. Stock, M. L. & Coetzee, J. F. Clinical pharmacology of analgesic drugs in cattle. *Vet. Clin. North Am. Food Anim. Pract.* **31**, 113–138, https://doi.org/10.1016/j.cvfa.2014.11.002 (2015).
42. Nedelec, B. & LaSalle, L. Postburn itch: A review of the literature. *Wounds* **30**, E118–E124 (2018).
43. Casaer, M., Kums, V., Wouters, P. J., Van den kerckhove, E. & Van den Berghe, G. Pruritus in patients with small burn injuries. *Burns* **34**, 185–191, https://doi.org/10.1016/j.burns.2007.03.004 (2008).
44. Elmariah, S. B. & Lerner, E. A. Topical therapies for pruritus. *Semin. Cutan. Med. Surg.* **30**, 118–126, https://doi.org/10.1016/j.sder.2011.04.008 (2011).

Acknowledgements

Author contributions

S.J.J.A. and C.B.T. conceived and designed the experiment. S.J.J.A. performed the experiment and statistical analyses. S.J.J.A. and C.B.T. interpreted the results. S.J.J.A. wrote the manuscript and C.B.T. revised it.

Competing interests

The authors declare no competing interests.

Additional information

Supplementary information is available for this paper at https://doi.org/10.1038/s41598-020-60260-7.

Correspondence and requests for materials should be addressed to C.B.T.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.