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Facial expression of pain in Nellore and crossbred beef cattle

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ABSTRACT

Although the science of facial expression of pain in humans is advanced, it has not been extensively explored in nonhuman animals. The study of facial expression as an indicator of pain might represent a substantial advance in pain recognition and management in other species, not yet studied. The main objective of this work was to investigate whether specific facial action units (AUs), previously related to painful facial expressions in human and some nonhuman animals, are also activated in beef cattle during acute painful stimulation. The study also investigated whether specific facial AUs of beef cattle in acute pain differed by sex and two genotypes. The activation of AUs was examined through pictures of a total of 35 beef cattle before and during branding with a hot iron, characterizing moments of no-pain (N) and pain (P). There were 17 female and 18 male beef cattle of two different genotypes: Nellore and crossbred (1/2 Nellore, 1/4 Bonsmara, 1/8 Red Angus, and 1/8 Aberdeen Angus). Results showed no differences in activation of AUs between males and females, and a higher frequency of mouth opening in the crossbred animals. The activation of the AUs backward ears, dilated nostril, open mouth, raised inner brow, and raised outer brow were significantly associated with the presence of the painful stimulus and should be considered for the development of further pain assessment methods using facial expressions for this species. The higher proportion of open mouth reactions in crossbred compared with Nellore cattle in response to an acutely painful stimulus indicates the importance of considering genotype when assessing pain by facial expression in cattle. As more detailed information becomes available, the on-farm use of facial expression to recognize pain in cattle may override some of the difficulties in animal pain diagnosis and, consequently, allow for rapid and consistent intervention to ameliorate suffering.

Introduction

Facial expression is an effective evolutionary tool for the externalization of emotions in a wide variety of animal species (Darwin, 1872). Facial expressions are considered central for communicating emotions (Awasthi & Mandal, 2015). The subtleties and meanings of the facial features in humans have been studied since the days of Aristotle (Russell, 1994), and a set of basic emotions has long been described as universally recognizable through facial expression, including happiness, surprise, fear, anger, disgust, and sadness (Duchenne, 1990). More recently, 22 categories of human facial expression of emotions have been described (Du et al., 2014), demonstrating the complexity of this communication model and its potential in assessing subjective feelings. Aan Het Rot et al. (2017) investigated which behavioral responses may be elicited by emotion. The limitation is that although emotional expressions are usually interpreted in the social context in which they occur, the hypothetical situation and cultural differences may interfere the assessment. Some studies have shown that, compared with the observation of other emotions, observations of pain cues are preferentially processed (Coll et al., 2016).

Because facial expressions are completely dependent on muscle tension and relaxation, the anatomy of facial features has been detailed in an effort to determine the relationship between the involuntary activation of particular facial muscles and specific emotions (Grant, 1969; Ekman and Friesen, 1976). As a result of this

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effort, the Facial Action Coding System was developed (Ekman and Friesen, 1978). The Facial Action Coding System establishes 44 fundamental anatomical components of facial movements, called action units (AUs), allowing the description of muscles activated during a multitude of facial expressions and, therefore, of emotions (Ekman et al., 1980; Ekman, 1993).

The possibility of objectively assessing emotions through facial expressions has created a new field within pain research. Pain is an important animal welfare problem, not least in cattle. Gleerup et al. (2015a) suggested that one reason for the inconsistence of pain relief for cattle is the inadequate ability to assess pain.

Pain is recognized as an extremely aversive subjective experience, involving emotional components such as anger, sadness, and agony (Zubieta, 2010). The benefits of externalizing pain through facial expressions are believed to be evolutionary (Williams, 2002) and might effective at increasing survival chances by inducing empathy in other individuals (Jackson et al., 2005). The facial expressions have been shown to be consistent during the induction of pain by several modalities of nociceptive stimulation in humans, and five AUs are described as comprising a basic universal signal of pain: brow lowering, lid tightening, wrinkled nose, raised upper lip, and eye closure (Prkachin, 1992).

Although the science of facial expression of pain in humans is advanced, it has not been extensively explored on nonhuman animals (Waller and Micheletta, 2013). This lack of exploration might be due to an assumption that other animals may not exhibit the same range of facial expressions as humans (Flecknell, 2010). Recent studies in monkeys, sheep (Tate et al., 2006), and dogs (Bloom and Friedman, 2013) demonstrate that they may express their emotions through facial movements and that mice (Langford et al., 2010) and horses (Dalla Costa et al., 2014) display specific facial expressions, activating similar AUs as humans when experiencing painful situations.

The exploration of facial expression as an indicator of pain might represent a substantial advance in pain recognition and management in other species not yet studied. Facial expression has the potential to become a very useful tool especially for use on farm animals, which are often submitted to painful procedures. Although facial expression in farm animals has received little attention, there are evolutionary similarities with other tested animals, like the activation of analogous AUs, that are worth exploring (Millman, 2013).

The main objective of this work was to investigate whether specific facial AUs, previously related to painful facial expressions in human and some nonhuman animals, are also activated in beef cattle during acute painful stimulation. The study also investigated whether specific facial AUs of beef cattle in acute pain are different between sex and two cattle genotypes.

Material and methods

For this study, hot iron branding was used as a model of acute painful stimulation because it has been scientifically described as a painful procedure with long-lasting inflammatory reactions and is still a common procedure used in beef cattle farms all over the world (Lindegaard and Andersen, 2012). Cattle from a commercial farm located in the town of Guairaçá, North of the state of Paraná, Southern Brazil were selected. The farm was selected for reasons of proximity and presence of excellent handling facilities and also because branding with hot iron is adopted as a standard identification procedure. No animals were branded exclusively for the purposes of this study.

The pain assessment of 35 beef cattle, 17 females and 18 castrated males, was carried out. Cattle weighed 209.9 kg ± 33.5 kg and were Nellore (20 animals) or crossbred (1/2 Nellore, 1/4 Bonsmara, 1/8 Red Angus, and 1/8 Aberdeen Angus) (15 animals). At the age of 8 months, cattle were brought to the handling chute for branding, as the regular procedure on the farm. During this procedure, each animal was filmed with a digital camera (Sony SteadyShot DSC-W320) pointed to their face. Each video was one minute long and captured frames from moments before, during, and after the application of the hot iron. Every time the hot iron touched the animal, a fingertip was placed in the video frame to indicate the exact moment of branding.

The videos of periods before and during hot iron branding were analyzed to compare the activation of facial AUs. All videos were uploaded to a computer, and frames of moments before and during the application of the hot iron were cropped using the Windows Media Player software, so each animal had a “pain” and “no-pain” picture to be investigated. The same observer classified all pictures into expressions of pain using facial AUs established in the literature (Table 1).

During the analysis of the AUs (Table 1), four additional AUs were added: raised inner brow, raised outer brow, eye closure, and tongue show. The number of animals observed for each facial AU was different because of the structure of the chute and frame capture. The clear visualisation of each AU was used as criterium for data inclusion when describing and comparing the situations of no-pain (N) and pain (P). When an AU was not clearly visible, it was not scored in that animal. If a determined AU was activated on both N and P frames, but there was an obvious difference in intensity of activation, the less intense activation was scored as “less active” and the most intense was scored as “active” to demonstrate the potential use of that indicator. Therefore, active AUs either represent activation versus nonactivation or a more intensely activated AU as compared with a less intensely activated AU.

Association between the acute painful stimulus and the activation of the AUs was determined by applying the McNemar test. Animals with respective AUs visible on only one of the N or P frames were not included in the statistical analysis. Proportions of activation of AUs between sexes and breeds on both N and P moments were tested with the binomial proportion test. Statistical analysis was performed using the BioEstat 5.3 statistical software.

Results

Data corresponding to the number of cattle observed and those showing activation of facial AUs at nonpainful and painful stimulus moments are presented in Table 2. Only five of 15 AUs were observable for all 35 animals: orbital tightening, tension above eye, brow lowering, eye closure, and raised inner brow. From these AUs, the activation of the raised inner brow was associated with the painful stimulus ($P = 0.0074$). Orbital tightening, tension above eye, brow lowering, and eye closure AUs were not present for both N and P frames ($P = 1.0000$).

The AU backward ears was visible in 15 of 35 animals, mainly due to the structure of the chute, which could trap the ears of the cattle with the neck bars, making spontaneous expression of ear position impossible. Statistical analysis of the 15 animals with visible ears showed high association between backward position of the ears and the painful stimulus ($P = 0.0078$).

In cattle, the upper lip forms an extension of the skin that covers the lower lip, hampering the visualization of these components of the AU strained mouth on this species. In our experiment, this AU was visible only in two animals, which held their heads in a higher position, allowing frame capture of this AU during N and P moments. In both cases, no AU activation was shown, but observations were limited. Moreover, the AUs strained chewing muscle, raised cheek, wrinkled nose, and raised upper lip were inactive before and

Table 1
Description of action units related to facial expression of pain on different species in previous researches and on beef cattle in this study

Species	Research	Action units	Description
Mice	Langford et al., (2010)	Backward ears	Characterized by the animal positioning its ears with the distal end pointed caudally.
Horse	Dalla Costa et al., (2014); Gleerup et al., (2015b)		
Dairy cattle	Gleerup et al., (2015a)		
Sheep	McLennan et al., (2016)		
Human	Prkachin (1992)	Orbital tightening	The narrowing of the orbital area, with a closed eyelid.
Mice	Langford et al., (2010)		
Horse	Dalla Costa et al., (2014)		
Sheep	McLennan et al., (2016)		
Horse	Dalla Costa et al., (2014)	Tension above eye	Represented by the increased visibility of the underlying bone surfaces in the area above the eye
Mice	Langford et al., (2010)	Strained chewing muscle	Characterized by the increased tension of muscles above the mouth
Horse	Dalla Costa et al., (2014)		
Horse	Dalla Costa et al., (2014)	Strained mouth	Visible when the upper lip is drawn caudally and the lower lip is drawn cranially forming a prominent chin
Horse	Dalla Costa et al., (2014)	Dilated nostril	When nostrils are clearly strained and slightly dilated
Human	Prkachin (1992)	Lowered brow	Characterized by the straining of the frontal area, with eyes drawn together
Human	Prkachin (1992)	Raised cheek	Represented by the convex appearance of the cheek
Mice	Langford et al., (2010)		
Sheep	McLennan et al., (2016)		
Human	Prkachin (1992)	Wrinkled nose/Raised upper lip	Strained portion of skin on the bridge of the nose is visible
Mice	Langford et al., (2010)		
Human	Prkachin (1992)	Open Mouth	When mouth is opening in some level
Human	Hadjistavropoulos and Craig (1994)	Raised inner brow raise	Characterized by the elevation and straining of medial brow area[a]
Human	Hadjistavropoulos and Craig (1994)	Raised outer brow raise	Characterized by the elevation and straining of lateral brow area[a]
Rat	Lorenzini et al., (2010)	Eye closure	When eyes are completely closed[a]
Cattle	This study	Tongue show	Exposure of tongue to outside of the mouth[a]

[a] Description of facial action units by this study.

during acute painful stimulation on all animals and did not differ statistically between N and P frames ($P = 1.0000$).

The AUs dilated nostril, open mouth, and raised outer brow (Figure) have all shown a statistical association between their activation and an acute pain stimulation ($P < 0.0001$). Tongue exposure was observed in five of 27 animals during branding ($P = 0.0625$).

There was no difference between male and female animals for the number of AUs activated. When activation of AUs was compared between breeds, crossbred animals had a higher proportion of animals with their mouths open when in pain ($P < 0.05$), but no other differences were found (Table 3).

Discussion

Hot iron branding was chosen as a model for acute painful stimulation in our study because it has long been related to increased escape-avoidance reaction as well as increased heart rate and increased epinephrine release in beef cattle, indicating important acute pain sensation (Lay et al., 1992). In this study, systematic measurements of the facial expression responses to pain were made.

Acute pain is known to increase the head shaking behavior (Heinrich et al., 2010) and general activity (Millman, 2013) in cattle, all of which negatively interfere with filming. To improve video characteristics, Dalla Costa et al. (2014) filmed horses hours after surgical intervention when images were clearer and more easily obtained. Our objective was to evaluate acute pain responses, so video capture had to be simultaneous with the painful event. Recognition of acute responses to pain may be of great value to animal welfare as it allows for rapid management of pain and thus acts to reduce the duration of suffering (Flecknell and Roughan, 2004), especially for those animals reared extensively without close care. The number of pictures used in this study is similar to other studies that identified and described AUs related to pain in other species (Langford et al., 2010; Keating et al., 2012; Dalla Costa et al., 2014).

Cattle are often described as stoic, in that they do not display obvious pain behavior; however, during the last decade, research in a number of supposedly stoic prey species (horses, rats, mice, and rabbits) has shown that changes in behavior are good predictors of pain, among which facial expressions are frequently cited (Leach et al., 2012; Gleerup et al., 2015). In this regard, some of the AUs previously related to pain expression in other species (Prkachin, 1992; Langford et al., 2010; Keating et al., 2012; Dalla Costa et al., 2014), such as orbital tightening, tension above eye, strained chewing muscle, strained mouth, lowered brow, raised cheek, wrinkled nose, raised upper lip, eye closure, and tongue exposure,

Table 2
Total number of animals observed for each facial action unit (AU) and number of beef cattle showing activation of the facial action units when in no-pain (N) and acute pain (P) situations

Activation of AUs[a]	BE	OT	TAE	SCM	SM	DN	LB	RC	WN	RUL	OM	EC	RIB	ROB	TS
N and P	4	0	0	0	0	0	0	0	0	0	0	0	9	3	0
Only N	0	0	0	0	0	1	1	0	0	0	0	0	2	0	0
Only P	8[b]	0	0	0	0	20[b]	0	0	0	0	17[b]	1	14[b]	16[b]	5
None	3	35	35	32	2	4	34	31	32	30	13	34	10	14	22
Total *n* observed	15	35	35	32	2	25	35	31	32	30	30	35	35	33	27

[a] Backward ears (BE), orbital tightening (OT), tension above eye (TAE), strained chewing muscles (SCM), strained mouth (SM), dilated nostril (DN), lowered brow (LB), raised cheek (RC), wrinkled nose (WN), raised upper lip (RUL), open mouth (OM), eye closure (EC), raised inner brow raise (RIB), raised outer brow raise (ROB), and tongue show (TS).
[b] Association between the acute painful stimulus and the activation of the respective AUs by the McNemar test with $P < 0.01$.

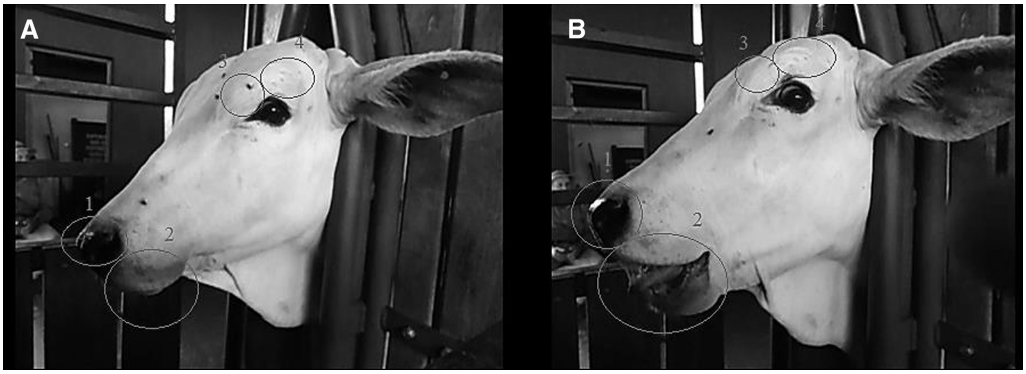

Figure. Actions units: dilated nostril (1), open mouth (2), inner (3) and outer (4) brow raise on the same animal moments before (A) and during (B) branding with a hot iron.

have not shown a pain-specific response in this experiment. This might be explained by evolutionary reasons. It may not be adaptive for prey animals like cattle to show a range of pain expressions to a predator (Davidson et al., 2002). In addition, behavior in response to pain varies greatly between species and this also includes facial expressions. Similar facial expressions might express distinct emotions depending on the species, so care must be taken when interpreting them (Waller and Micheletta, 2013).

Five of the AUs studied showed high association between their activation and the acute pain caused by branding: backward ears, dilated nostril, open mouth, and raised inner and outer brow. The AUs backward ears and dilated nostril have been studied before in other animal species and proved to be reliable pain indicators in mice submitted to a 0.9% acetic acid abdominal constriction test (Langford et al., 2010), in rabbits undergoing ear tattooing (Keating et al., 2012) and in horses after surgical castration (Dalla Costa et al., 2014). The AU open mouth has not been described as a pain indicator in animals, but it is intensely activated when humans experience shock and cold pain assays (Prkachin, 1992). The opening of the mouth might also be related to vocalization, which also increases in frequency when cattle are under pain (Watts and Stookey, 2000). Watts and Stookey (1999) found that the application of a hot branding iron to beef cattle yielded a much higher rate of vocal response than did sham branding. Further investigation using images together with audio should help determining the possible relationships. To our knowledge, the other two AUs associated with pain in our study, that is, raised inner and outer brow, have never been reported as pain indicators in facial expression studies before. This might be related to interspecific differences; however, the experimental design may have influenced our results as well. Previous studies have focused in responses to pain up to eight hours after stimulation (Dalla Costa et al., 2014), not on immediate responses to acute pain as in the case of our work. Inner and outer brow raising has been related to the expression of other

Table 3
Proportion and statistical significance (*P*) of active facial units on beef cattle of different gender and breeds before (no pain) and during branding with hot iron (pain)

Action units	Moment	Gender (active/observed)		*P*	Breed (active/observed)		*P*
		Male	Female		Nellore	Crossbreed	
Backward ears	No pain	2/9	2/6	0.73	2/10	2/5	0.40
	Pain	6/9	6/6	0.18	7/10	5/5	0.17
Orbital tightening	No pain	0/18	0/17	-	0/20	0/15	-
	Pain	0/18	0/17	-	0/20	0/15	-
Tension above eye	No pain	0/18	0/17	-	0/20	0/15	-
	Pain	0/18	0/17	-	0/20	0/15	-
Strained chewing muscle	No pain	0/15	0/17	-	0/19	0/13	-
	Pain	0/15	0/17	-	0/19	0/13	-
Strained mouth	No pain	0/1	0/1	-	-	0/2	-
	Pain	0/1	0/1	-	-	0/2	-
Dilated nostril	No pain	1/13	0/12	0.58	1/16	0/9	0.58
	Pain	9/13	11/12	0.16	13/16	7/9	0.83
Lowered brow	No pain	1/18	0/17	0.58	1/20	0/15	0.58
	Pain	0/18	0/17	-	0/20	0/15	-
Raised cheek	No pain	0/14	0/17	-	0/18	0/13	-
	Pain	0/14	0/17	-	0/18	0/13	-
Wrinkled nose	No pain	0/15	0/17	-	0/19	0/13	-
	Pain	0/15	0/17	-	0/19	0/13	-
Raised upper lip	No pain	0/14	0/16	-	0/18	0/12	-
	Pain	0/14	0/16	-	0/18	0/12	-
Open mouth	No pain	0/14	0/16	-	0/18	0/12	-
	Pain	6/14	11/16	0.15	7/18	10/12	0.02
Eyes closed	No pain	0/18	0/17	-	0/20	0/15	-
	Pain	1/18	0/17	0.58	1/20	0/15	0.58
Raised inner brow	No pain	6/18	5/17	0.80	5/20	6/15	0.34
	Pain	11/18	12/17	0.55	13/20	10/15	0.92
Raised outer brow	No pain	2/16	1/17	0.50	2/19	1/14	0.74
	Pain	9/16	10/17	0.88	11/19	8/14	0.97
Tongue show	No pain	0/14	0/13	-	0/17	0/10	-
	Pain	4/14	1/13	0.11	3/17	2/10	0.88

emotions, such as fear and surprise, in studies with humans (Williams, 2002; Du et al., 2014). In fact, the sudden onset and the early stages of pain might produce a compounded experience of pain and startle that may culminate in this facial expression (Prkachin, 1992).

Behavioral parameters including lowered ears, tension of the muscles alongside the head (mimic muscles and chewing muscles), dilated nostrils, tense stare, and tension above the eyes (Dalla Costa et al., 2014; Gleerup et al., 2015) in horses and dairy cattle, along with lowered head and back arching, were also observed in Nellore cattle after castration (De Oliveira et al., 2014). However, the pain scale for dairy cattle used by Gleerup et al., 2015a did not include changes in ear position and facial expressions.

In the cattle, we studied there were no differences in facial expression associated with pain between males and females, but this finding has been reported for humans (Kunz et al., 2006). There may be a similar nociceptive response between male and female cattle, as has been reported by Chesler et al. (2002) for mice, where sex accounted for only 0.4% of the overall variance in nociceptive responses in a typical research laboratory. However, further research is needed to understand whether the absence of difference between male and female cattle is specific to facial responses or may be generalized to all pain responses.

Considering other factors which affected nociceptive responses, Chesler et al. (2002) reported that genotype accounted for 27% and the interaction between genotype and environment contributed with 18% of total variance. Our results show the influence of genotype on the activation of facial AUs during acute pain. Crossbred cattle had a higher proportion of open mouth responses during pain than Nellore cattle. Effects of genotype on temperament are well known, but this new information has practical relevance. It is known that *Bos indicus* presents more excitable temperament than *Bos taurus* (Grandin, 1998; Burrow, 2001). However, the lower proportion of Nellore expressing open mouth during pain in this study suggests this breed may be more stoic than crossbred cattle in response to acute pain. In addition, considering the trends for mechanical nociceptive threshold differences between horse genotypes (Haussler and Erb, 2006), it is possible that cattle genotypes may respond at different nociceptive thresholds as well.

The description of the five specific AUs related to acute pain identified in this study is of great value for the development of new methods for pain assessment using facial expressions in cattle and might, consequently, positively affect the welfare of these animals. The establishment of new pain assessment methods that are noninvasive, low cost, and practical will allow for more effective animal pain management than it is possible today (Flecknell and Roughan, 2004). Assessment of pain through facial expression comprises all these criteria, and thus, its on-field application in cattle and other farm animal species seems warranted.

Conclusion

The activation of the AUs backward ears, dilated nostril, open mouth, raised inner brow, and raised outer brow in beef cattle was associated with the presence of an acute painful stimulus, here represented by hot iron branding, and should be considered for the development of further pain assessment methods using facial expressions for this species. Facial responses tend to be stable for male and female animals; however, the higher proportion of open mouth reactions in crossbred compared with Nellore animals in response to acute painful stimulus indicates the importance of considering genotype when assessing pain by facial expression in cattle. As more detailed information becomes available, the on-farm use of facial expression to recognize pain in cattle may override some of the difficulties in animal pain diagnosis and, consequently, allow for rapid and consistent intervention, which may decrease animal suffering.

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Authors' contributions: The idea for the paper was conceived by Bruno Roberto Müller and Carla Forte Maiolino Molento. The experiments were designed by Bruno Roberto Müller and Carla Forte Maiolino Molento. The experiments were performed by Bruno Roberto Müller. The data were analyzed by Bruno Roberto Müller. The paper was written by Bruno Roberto Müller, Vanessa Souza Soriano, Jennifer Cristina Biscarra Bellio, and Carla Forte Maiolino Molento.

Ethical considerations

Bioethics and Biosecurity Committee Approval: The methodology of this experiment was approved by the Animal Use Ethics Committee of Agricultural Sciences Campus of the Universidade Federal do Paraná under Protocol 074/2013.

Conflict of interest

The authors declare no conflict of interest.

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