EVIDENCE OF SOCIAL WITHDRAWAL IN GROUP-HOUSED DAIRY CALVES IN RELATION TO DISBUDDING AND INDIVIDUAL PERSONALITY TRAITS

By

KATIE N. GINGERICH

A THESIS PRESENTED TO THE GRADUATE SCHOOL OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE

UNIVERSITY OF FLORIDA

2020
To my parents, Dave and Linda Gingerich.
For their unwavering support towards helping me achieve anything I set out to do.
ACKNOWLEDGMENTS

I would first like to thank my advisor, Dr. Emily Miller-Cushon for her guidance and enthusiasm for helping me become a better researcher, and for giving me the undergraduate research opportunity that changed the trajectory of my career. I have always appreciated our long discussions which spark new ideas and leave me more confident and motivated.

I would also like to thank the other members of my advisory committee. Dr. Albert De Vries and Dr. Carissa Wickens have both contributed their time and thoughtful feedback throughout the duration of my program.

I must thank the farm staff at the University of Florida Dairy Unit for their assistance not only with calf care, but for helping me to grow in my understanding of calf husbandry. I would also like to thank all of my undergraduate research assistants for their help with data collection, for taking a vested interest in my research, and for growing with me as scientists. I must also thank my fellow graduate students, particularly Emily Lindner, for her friendship, and for making long days at the farm feel a bit shorter.

Finally, I would like to thank my friends and family for their support and guidance. Thank you to my dad, Dave, for continuing to answer my questions about adulthood and taking an interest in my research. Thank you to my mom, Linda, for inspiring my interest in science, continuing to help me with homework in graduate school, and having long conversations about animal behavior. Thank you to Marley, for making my life innumerably fluffier and happier. Lastly, thank you to Justus Lewis, for navigating life with me and not tuning me out when I start complaining about my battles with SAS.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>4</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>7</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>8</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>9</td>
</tr>
<tr>
<td>CHAPTER</td>
<td></td>
</tr>
<tr>
<td>1 LITERATURE REVIEW</td>
<td>11</td>
</tr>
<tr>
<td>Social Behavior of Dairy Calves</td>
<td>11</td>
</tr>
<tr>
<td>Physiological Cues and Internal State</td>
<td>12</td>
</tr>
<tr>
<td>Neurobiology of Social Behavior</td>
<td>14</td>
</tr>
<tr>
<td>Previous Experience and Social Bonding</td>
<td>17</td>
</tr>
<tr>
<td>Personality</td>
<td>20</td>
</tr>
<tr>
<td>Pain</td>
<td>21</td>
</tr>
<tr>
<td>External Factors Influencing Social Behavior</td>
<td>25</td>
</tr>
<tr>
<td>Consequences of Social Isolation</td>
<td>26</td>
</tr>
<tr>
<td>Disbudding</td>
<td>29</td>
</tr>
<tr>
<td>Popularity and Consumer Perspectives</td>
<td>29</td>
</tr>
<tr>
<td>Effects of Disbudding on Behavior</td>
<td>32</td>
</tr>
<tr>
<td>Pain and Pain Mitigation</td>
<td>37</td>
</tr>
<tr>
<td>Personality and Individual Variability</td>
<td>38</td>
</tr>
<tr>
<td>The Relationship Between Personality and Behavior</td>
<td>38</td>
</tr>
<tr>
<td>Individual Variability and Livestock Management</td>
<td>42</td>
</tr>
<tr>
<td>Summary</td>
<td>43</td>
</tr>
<tr>
<td>2 DISBudding EFFECTS USE OF A SHELTER PROVIDED TO GROUP-HOUSED DAIRY CALVES</td>
<td>45</td>
</tr>
<tr>
<td>Abstract</td>
<td>45</td>
</tr>
<tr>
<td>Introduction</td>
<td>46</td>
</tr>
<tr>
<td>Materials and Methods</td>
<td>48</td>
</tr>
<tr>
<td>Animals and Management</td>
<td>48</td>
</tr>
<tr>
<td>Experimental Design</td>
<td>50</td>
</tr>
<tr>
<td>Data Collection</td>
<td>51</td>
</tr>
<tr>
<td>Statistical Analyses</td>
<td>52</td>
</tr>
<tr>
<td>Results</td>
<td>54</td>
</tr>
<tr>
<td>Discussion</td>
<td>56</td>
</tr>
<tr>
<td>Conclusion</td>
<td>63</td>
</tr>
</tbody>
</table>
# LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-1</td>
<td>Individual and social use of a shelter providing visual isolation in group-housed calves subjected to disbudding (DB) or handling only (CON), for 3 days following treatment.</td>
</tr>
<tr>
<td>2-2</td>
<td>Activity and resting behavior within an area providing visual isolation (shelter) in group-housed calves (4/pen) subjected to disbudding (DB) or handling only (CON), for 3 days following treatment.</td>
</tr>
<tr>
<td>3-1</td>
<td>Ethogram of behaviors recorded during the four behavioral tests when calves were tested in open field, novel object, unfamiliar calf, and unfamiliar human tests.</td>
</tr>
<tr>
<td>3-2</td>
<td>Coefficients (loadings) of the eigenvalues for the first two factors extracted by principal component analysis of behavioral measures in the open field and novel object tests (OFNO) in order to assess calves’ (n=32) response to novel environments.</td>
</tr>
<tr>
<td>3-3</td>
<td>Coefficients (loadings) of the eigenvalues for the first two factors extracted by principal component analysis of behavioral measures in the unfamiliar calf and unfamiliar human tests (UHUC) in order to assess calves’ (n=32) response to novel social environments.</td>
</tr>
<tr>
<td>3-4</td>
<td>Relationships between factor scores from the UHUC principal component analysis and use of the shelter and other pen features (hay and brush use) during the baseline day.</td>
</tr>
<tr>
<td>3-5</td>
<td>Activity and use of a shelter providing visual isolation in group-housed calves (8 calves/pen) subjected to disbudding (DB) or handling only (CON) for 3 days following treatment.</td>
</tr>
</tbody>
</table>
## LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-1</td>
<td>Image of calf using the shelter, a three-sided, open-top barrier made out of corrugated plastic (1.2 × 1.2 × 1.2 m; width × depth × height) ..................................................66</td>
</tr>
<tr>
<td>2-2</td>
<td>Schematic of the pen, depicting the shelter (three-sided, open-top barrier made out of corrugated plastic; 1.2 × 1.2; width × depth) and its location within the pen relative to other resources... ........................................................................................................67</td>
</tr>
<tr>
<td>2-3</td>
<td>Individual and day-to-day shelter use (h/d) for 3 consecutive days following either disbudding (DB) or sham handling only (CON) for each of 6 pens (4 calves/pen; pens labelled alphabetically) ........................................................................................................68</td>
</tr>
<tr>
<td>2-4</td>
<td>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 h to 1159 h) beginning at time of disbudding (DB) or sham handling only (CON).................69</td>
</tr>
<tr>
<td>3-1</td>
<td>Overhead view of the pen, depicting both shelters (three-sided, open-top barriers made out of corrugated plastic; 1.2 × 1.2; width × depth) and their location within the pen relative to other resources... ........................................................................................................89</td>
</tr>
</tbody>
</table>
EVIDENCE OF SOCIAL WITHDRAWAL IN GROUP-HOUSED DAIRY CALVES IN RELATION TO DISBUDDING AND INDIVIDUAL PERSONALITY TRAITS

By
Katie N. Gingerich

August 2020

Chair: Emily Miller-Cushon
Major: Animal Sciences

Evidence across species points to changes in social behavior as a potential indicator of individual welfare. The aim of this thesis was to investigate social withdrawal in dairy calves and how it relates to pain. We characterized use of a shelter offering visual and physical separation from the rest of the pen in group-housed calves following disbudding, and how expression of social withdrawal in the home pen varied between individuals and related to personality.

In a first experiment characterizing shelter use following disbudding (Chapter 2), disbudded calves were found to use the shelter more, spent less time lying, and spent a greater proportion of their lying time inside the shelter. Disbudded calves also entered an empty shelter, and left an occupied shelter more frequently, suggesting a possible preference for social isolation. A second experiment (Chapter 2) was conducted to further explore effects of disbudding on shelter use and the role of individual personality traits on shelter use. Personality traits, as determined using behavioral tests, were associated with use of the shelter prior to disbudding; ‘calf-avoidant’ calves tended to use the shelters more, whereas ‘exploratory active’ calves used the shelters less. Additionally, characterization of shelter use by disbudded calves supported results from Experiment 1.
These results provide the first evidence that calves may prefer to withdraw when in pain, and that this behavior may be subject to individual personality. Further research is needed to understand motivations for social withdrawal and the expression of this behavior as a welfare indicator.
CHAPTER 1
LITERATURE REVIEW

The rearing of dairy calves in intensive farming systems differs from natural social dynamics, having implications for calf welfare. In recent years, research has identified broad benefits of housing dairy calves in groups of similarly aged conspecifics, compared to socially isolated housing which remains the industry norm. This review will discuss the ontogeny of social behaviors, as well as the proximate and ultimate mechanisms driving these behaviors in gregarious species. In addition, understanding changes in social behavior may have important welfare implications, and this review examines how social behaviors are modified in response to external stimuli such as pain. This review also examines the effects of disbudding on behavior of dairy calves and the different methods of pain relief. The use of this procedure in different geographic regions and consumer opinions toward this procedure and animal welfare are addressed. Finally, this review discusses the role of individual variability in social behavior expression in dairy calves and other species, and the importance of allowing for more variable expression of behavior in modern farming systems.

Social Behavior of Dairy Calves

Dairy cattle are naturally social animals who live in matriarchal herds of various ages. However, it is standard practice within the dairy industry to rear calves in isolation until after weaning (USDA, 2008). This lack of social contact during early life broadly affects development of social behavior. Studying the behavior of dairy calves raised in social groups of similarly aged conspecifics can provide insight into the natural social behaviors and social networks of these animals. Additionally, investigating the impact that the expression of social behaviors can have on cognition, feeding behavior, growth, and health later in life, can aid in developing strategies and management practices aimed at improving the dairy calf welfare. This section will provide
an overview of how social behavior is subject to experience and internal state, such as genetics, physiology, health, personality, and mood. The neurobiological underpinnings governing the expression of social behavior will be discussed. Examples will be drawn across species to illustrate concepts where research in calves is limited, but the focus will be on applying these concepts to understand behavior and welfare of calves.

**Physiological Cues and Internal State**

Differences among individuals will influence how animals assimilate within social groups and how they initiate and perceive social behavior. For example, different breeds of cattle tend to behave differently in social situations. The Herens and Brunes des Alpes are two breeds of cattle that originated in the same mountain region and were bred for milk production, but the Herens breed was also selected for its dominance and aggression. Social behavior, fear reactions, ease of handling, and physiological components have been compared between these two breeds, revealing a genetic basis for some differences in these behavioral responses. The Brunes des Alpes, the species that had not been selected for dominance and aggression, were found to be easier to handle, more socially motivated, and had lower blood testosterone levels than the Herens cows (Plusquellec and Bouissou, 2001).

Individual social interactions are also influenced by physiological cues and may depend on other needs such as hunger and health. For example, calves that were fed an increased milk allotment (9 L/d) exhibited more play behavior than calves that were fed a standard milk allotment (5 L/d) (Jensen et al., 2015a). This suggests that hunger or nutritional status may be a limiting factor in the expression of play behavior which is considered a pro-social indicator of positive welfare. The health of the animal also impacts social behavior in dairy calves. Calves experiencing a state of mild respiratory disease engaged in fewer competitive displacements
surrounding milk feeding, suggesting that sick calves may be less likely to feed at peak feeding times (Hixson et al., 2018). These sick calves also had a lower frequency of head butting, which is associated with play, and were less likely to initiate social grooming (Hixson et al., 2018). This could indicate either general lethargy associated with febrile disease or a motivation for social isolation. There is evidence for the latter; when given the option, dairy cows will isolate themselves prior to parturition and when exhibiting signs of disease or infection such as mastitis or pneumonia (Proudfoot et al., 2014a).

The hormonal state of the animal can also impact the expression of social behaviors. For example, social behavior is affected by the estrous cycles of female hamsters. Non-estrous females exhibited aggression towards both male and female social companions, while estrous females were not aggressive and spent more time in lordosis, which is an indicator of sexual receptivity (Floody and Pfaff, 1977). Additionally, while females who had undergone both adrenalectomy and ovariectomy procedures behaved more similarly to the non-estrous females, the altered females who were injected with both estradiol and progesterone exhibited a reduction in aggressive behaviors (Floody and Pfaff, 1977). This indicates that in some contexts, hormones play a crucial role in the expression of social behaviors such as aggression. Inversely, social interaction itself can affect the estrous cycles of hamsters. Entrainment of estrous cycles, in which the cycles of socially housed individuals synchronize, occurs in many species of social mammals. Hamsters that either lived together or in close proximity established estrous cycle synchrony and social interaction tended to accelerate this entrainment process (Handelmann et al., 1980). Interestingly, the cycles of the submissive animals adjusted to obtain synchrony with the dominant animals (Handelmann et al., 1980), suggesting a bidirectional relationship between
dominance hierarchy position and hormonal control of behavior. Further research is necessary to evaluate the hormonal control of social behavior in dairy cattle.

**Neurobiology of Social Behavior**

Social behavior is processed in specific brain regions including the amygdala and the prefrontal cortex, and depends on neurotransmitters such as oxytocin, dopamine, and serotonin. While oxytocin serves to increase social bonding, the neurotransmitters involved in regulation of agonistic behaviors, serotonin and dopamine, work to decrease these behaviors. Many of the processes involved in the regulation and performance of social behaviors are also implicated in emotional regulation, suggesting a relationship between social behavior and emotional state.

While much of the work investigating sensory processing involves the regions of the brain designated for processing specific sensory stimuli, there are also critical brain regions dedicated to the interpretation and judgement of this sensory stimuli. The amygdala influences memory, attention, decision-making, and interpretation of socially relevant stimuli (Adolphs, 2003). The amygdala is most famous for its role in emotion regulation and contributes to processing social stimuli with emotional valence. Patients with bilateral amygdala damage show impaired ability to judge trustworthiness in others (Winston et al., 2002), and processing fearful human facial expressions (Adolphs et al., 1995). Additionally, removal of the prefrontal and anterior cortex causes dramatic changes in social and maternal behavior, including decreased self-grooming and social grooming, more time spent farther away from conspecifics, decreased frequency of facial expressions, gestures, and vocalizations, and the failure to rejoin family associates upon reintroduction to the group. This implicates the prefrontal and anterior cortex in the processing of both social and emotional stimuli (Franzen and Myers, 1973).
The neuropeptide oxytocin has a broad impact on social behavior, including pro-social affiliative behaviors such as empathy and cooperation, which strengthen social bonds and establish preference for certain individuals. In addition to promoting social preferences, oxytocin promotes and initiates maternal behavior and lactation, and promotes sexual behaviors in both males and females (Lukas et al., 2011). To evaluate the effects of oxytocin on social preferences, rats were given either an oxytocin receptor antagonist, a vasopressin receptor antagonist, or a placebo, and then subjected to social preference tests. These preference tests were performed in both a novel arena and in their home environment. The rats that received the oxytocin receptor antagonist had a significant decrease in investigation during both social preference tests (Lukas, et al. 2011), implicating oxytocin in the mechanism underlying social bonding. The mechanism for social preference and bonding is closely tied to the mechanism for social avoidance. To examine the role of oxytocin in social avoidance, rats were exposed to a larger, aggressive unfamiliar male rat and experienced social defeat. Rats that had been defeated showed significant decrease in social stimulus investigation in a social preference test, indicating social avoidance. To test the role of oxytocin in this pathway, rats were given either intracerebroventricular (i.c.v.) oxytocin or a placebo after the social defeat and before the preference test. In this context, i.c.v. oxytocin eliminated social avoidance, and conversely, reinstated social preference for the former defeater. (Lukas, et al. 2011). This further indicates that oxytocin is a critical component of social bonding.

In addition to oxytocin, dopamine and serotonin are also important neurotransmitters in the expression of agonistic behaviors in male rats. Researchers depleted dopamine in the orbitofrontal cortex by infusion of 6-hydroxydopamine and observed the agonistic behavior of rats in dyadic interactions. They reported enhancement of aggressive behaviors, indicating that
dopamine is a mediator of agonistic behaviors; lesions of the orbitofrontal cortex generate similar results (De Bruin, 1990). Serotonin inhibits aggressive behavior in a variety of species including fish and primates. To determine whether aggression as a result of serotonergic dysfunction is correlated with indicators of stress, blood and cerebrospinal fluid were collected from rhesus monkeys, and behavior was observed during social interactions. There was a negative correlation between high rankings for aggression and serotonin concentrations (Higley, et al. 1992). This suggests that serotonin acts as an inhibitor of aggressive behavior. There was also a positive correlation between norepinephrine, plasma corticotropin, and cortisol concentrations and high rankings of aggression, indicating that aggression and stress are related, and that serotonin may have an additional role in mediating the stress response system.

Environmental complexity in general impacts brain and neural development, and social isolation can lead to various abnormalities in social behavior. Sensory stimulation caused by environmental complexity leads to neuronal changes, including increased dendritic branching and synaptogenesis in the cortex and hippocampus, leading to increased neuronal plasticity (reviewed by van Praag et al., 2000). There is also evidence to suggest that mice reared in social isolation develop behavioral abnormalities that are similar to human psychiatric disorders such as attention-deficit hyperactivity disorders, obsessive-compulsive disorder, autism, and schizophrenia (Koike et al., 2009). Spontaneously hyperactive rats, a well-established animal model of ADHD, interacted more frequently with an unfamiliar social companion than controls, suggesting changes in social behavior are associated with this pathology. (Hopkins et al., 2009). There is evidence to suggest that impaired dopamine function, a neurotransmitter involved in the regulation of social behavior, may cause symptoms related to ADHD. Insufficient dopamine release during development may impair the strength of synaptic connections related to recognition of rewarding
stimuli. This suggests that abnormal brain development induced by social isolation may cause brain abnormalities that are similar to ADHD and other human psychiatric disorders, and that these disorders can have broad effects on social behavior expression. More research is necessary to investigate the effects of social isolation on brain development in dairy calves.

**Previous Experience and Social Bonding**

Previous experience and the environment in which animals are raised have a critical influence on the development of social behaviors. Social experience is critical for the development of social relationships, including the formation of stable dominance hierarchies, and informs social preferences and bonding. Further, early social experience is critical for development of social behaviors. For example, calves housed in different degrees of social contact respond differently to social situations later in life. This indicates a relationship between early social housing and the development of the social behaviors necessary to cope with changing social environments later in life.

Experience helps animals to make effective decisions about future social interactions and results in the development of stable social relationships. Following their first exposure to a new social group the frequency of physical agonistic (bunting, pushing, and fighting) interactions is highest (Kondo and Hurnik, 1990). These interactions serve to establish social relationships, but over time, these physical agonistic interactions decrease, and are replaced with less severe non-physical agonistic interactions (threatening and avoiding) (Kondo and Hurnik, 1990). This shows that individuals remember their previous interactions with conspecifics and that the outcome of these social interactions; whether they result in agonistic or affiliative interactions, can help the animal to make decisions about future interactions with conspecifics. This information can also be useful in decision making when interacting with unfamiliar animals. Establishment of
dominant and subordinate individuals relies on the memories of previous interactions to be preserved. It also serves to reduce the frequency of potentially harmful physical confrontations and allows a social group of animals to devote their cognitive energy to other areas related to fitness such as predator avoidance.

Lasting social interaction can also aid in the formation of preferential relationships in dairy cattle. When presented with a choice between a familiar social companion and an unfamiliar social companion in a Y-maze, calves spent significantly more time in the area where the familiar conspecific was located (Færevik et al., 2006). Additionally, familiar social partners seem to reduce stress when placed together in an unfamiliar environment (Færevik et al., 2006). Calves separated by themselves vocalized more, were more immobile, and explored less than calves that were separated with either type of companion (Færevik et al., 2006). Further, calves that spent the most time with the familiar calf during the Y-maze vocalized more when they were separated with an unfamiliar calf than when they were separated with a familiar calf (Færevik et al., 2006). This provides evidence for the formation of preferred social relationships as well as the presence of individual differences in the significance of these social bonds.

The amount of time and degree of social contact also affects the formation of social bonds. Dairy cattle that had long-term (shared youth experience and shared adult experience) and short term (shared dry period and synchronized group entry) familiarity were more likely to interact and be direct neighbors when feeding, social grooming, and when resting (Gutmann et al., 2015). Additionally, cows with a long-term shared youth experience had more intense social relationships (Gutmann et al., 2015). This suggests that dairy cows remember previous social interactions and can maintain dyadic relationships, and that shared experiences serve to promote bond formation and maintenance in these relationships. Bond formation through shared
experiences also affects social relationships in weaned dairy calves. Calves were either housed individually or in pairs at day 5 or day 28 of life. All calves were then grouped together after weaning and social networks, as determined based on social proximity, showed stability in a 4-week observation period and was highly differentiated (Bolt et al., 2017). Calves that were pair housed from day 5 had a stronger association with their ex-pen mate than individually housed calves in the first week (Bolt, et al. 2017). Over time these associations weakened, and by week 4 there were no significant differences between treatments (Bolt et al., 2017). This provides additional evidence for the formation of preferential social companions in early life, but that these preferences are subject to change with experience. This also suggests that dairy calves require social interaction to develop effective social behaviors, and that once that requirement for social contact is met, calves are able to express social behaviors and develop dyadic relationships.

Social grooming is an important affiliative behavior in gregarious species that serves to initiate and maintain social bonds, as hormones released during social grooming bouts reinforce this behavior. Extensive research into primate sociality and grooming has proposed two hypotheses for the functional purposes of social grooming; to remove ectoparasites from fur and to aid in the formation of social bonds. In support of a socially facilitated motivation for grooming, research suggests that social grooming bouts in the rhesus monkey are longer, and are performed in different locations on the body, and with different methods than they perform self-grooming (Boccia, 1983). This discrepancy in the location and method of social grooming suggests that although utilitarian functional consequences of social grooming are obtained, the behavior itself is at least partially socially motivated. Further, opiates and endorphins are released during social grooming bouts, giving rise to a proximate consequence of social
grooming (Keverne et al., 1989). This hormonal response seems to operate as positive reinforcement to continue this behavior.

In cattle, although dominance rank does not impact social grooming, competition for feed affects the expression of this behavior whereby social grooming declines when competition between animals increased (Val-Lailet et al., 2009). This effect was seen more predominantly in low-ranking, primiparous animals. These naïve animals are more susceptible to socio-negative interactions which suggests that prior experience may help animals develop effective coping strategies to stressful and changing environments.

**Personality**

Individual personality traits are stable across time and govern how animals cope with various external stimuli. Individual personalities of animals play a role in the way in which they interact with conspecifics and their position within their social network. A relationship between network centrality and personality has been demonstrated in wild songbirds. ‘Proactive,’ fast exploring birds associated weakly with a greater number of individuals and moved quickly between flocks (Aplin et al., 2013). ‘Reactive,’ slow-exploring birds, however, formed synergistically stable relationships (Aplin et al., 2013). Further, personality is related to differences in social behavior, including response to novel, socially relevant stimuli, and position within a social network in snakes (Skinner and Miller, 2020), and individual differences in response to social interactions remain stable over time in mice (Malloy et al., 2005). This suggests that social strategies are subject to individual variability and are mediated by individual personality.

Additionally, there is evidence to suggest that a bidirectional relationship exists between position within a social network and an individuals’ personality, such that an animal’s
personality will be influenced by its position within its social network, and that position will in-
turn, be influenced by their individual personality (reviewed by Krause et al., 2010). In socially
housed livestock, a better understanding of the relationship between personality and social
behavior or preferences for social withdrawal may have important management and welfare
implications.

Behavioral variability among individuals in social species is thought to have adaptive
consequences. The social niche specialization hypothesis suggests that individuals in a group
occupy specific social roles which maintains individual differences in personality (Montiglio et
al., 2013). Further, the ecological niche theory suggests that behavioral diversification is adaptive
because social niches and structures, such as dominance hierarchies, reduce daily conflict among
conspecifics (Bergmüller and Taborsky, 2010). This suggests that individual variability of
behavioral expression is natural and advantageous and that differences in social strategies
evolved as a mechanism to allow unrelated conspecifics to live together in groups while sharing
responsibilities related to acquisition of food and predator avoidance. Further research is needed
to determine the extent of naturally varying behavior and personality traits, and to evaluate how
we can allow for broader expression of individual differences in intensive livestock systems.

Pain

Pain, an unpleasant sensory and emotional experience associated with actual or perceived
tissue damage (Merskey and Bogduk, 1994) is associated with poor welfare. Pain has been
shown to alter social behaviors in gregarious species in complex ways, as animals may be
affected by the painful experience of a conspecific in the form of emotional empathy, social
contact may reduce response to stressors, and social interactions or social preferences may be
altered when an animal is experiencing pain.
Modification of social behaviors in response to pain is important in cohabitating social species because it serves to transmit information about the painful stimulus and the health status of the individual (Martin et al., 2014). For example, humans communicate pain through both verbal and nonverbal expression that serves to help them escape the painful stimulus, communicate information about the stimulus, and acquire help from uninjured persons nearby (Hadjistavropoulos et al., 2011). The perception of these painful experiences and elicitation of an emotional response by other humans is known as empathy, whereas the perception of pain among conspecifics in animals is known as emotional contagion (de Waal, 2008). Both empathy and emotional contagion, a form of empathy that does not require a higher evolved “theory of mind” (Hatfield et al., 1993), serve to aid in the communication of pain and other negative states among members of a group.

Although empathy was once thought to be a uniquely human characteristic, studies have shown that animals display behaviors that mimic this human quality. Rats will learn to open a door for a trapped cage mate, freeing them from a distressing situation (Bartal et al., 2011). Further, when provided with chocolate chips, a palatable reward, free rats chose to share the reward with their trapped cage mate after freeing them from restraint about half of the time. Interestingly, the rats who eventually became ‘door-openers’ also had a lower latency to approach the ledge of a half-opened cage. This suggests that freeing trapped cage mates is related to boldness scores, and that individual differences in behavior may be correlated with pro-social behaviors. Calves spent more time in proximity and engaging in social interaction with pen-mates who had just been disbudded compared to calves who had been sham disbudded (Ede et al., 2020), indicating that dairy calves may experience some degree of emotional contagion in response to painful stimuli. Further, female mice not experiencing pain are more likely to
approach familiar same sex conspecifics that are in pain than they are to approach unaffected and affected unfamiliars, and unaffected familiar conspecifics (Langford, et al., 2010). This effect was not seen in male mice. This suggests that responses to conspecifics in pain and pro-social behaviors are evident in mice and may be mediated by sex differences. Additionally, increased frequency of contact initiated by the unaffected mouse was correlated with lower frequency of expression of pain-specific behaviors, suggesting that the proximity of a social companion ameliorated some experience of pain.

Social contagion and the elicitation of attention and help that it brings to individuals in pain allows for the effects of social buffering to take place. Social buffering refers to the reduction of pain sensitivity and pain-specific behaviors when in the presence of a social companion. This phenomenon has been well studied in rodents. Mice have been shown to decrease pain-specific behaviors in the presence of a familiar partner (Gioiosa et al., 2009), and decrease frequency of freezing after being exposed to a non-fearful partner (Guzmán et al., 2009). Although social buffering effects have not been demonstrated in cattle in a direct response to pain, pair housed dairy calves vocalize less at weaning compared to individually housed calves (Bolt et al., 2017), indicating that calves experience the effects of social buffering in response to some stimuli.

Furthermore, lame cows were less likely than healthy cows to initiate an agonistic interaction and received more social grooming from conspecifics (Galindo and Broom, 2010). This suggests that not only do lame cattle modify their social behavior when experiencing pain, conspecifics also change their behavior by directing more licking behavior towards members of their social group who are in pain. This increased grooming may act as an analgesic to alleviate some of the pain experienced. Additionally, dairy calves were less likely to initiate social
grooming bouts but tended to receive more social grooming when experiencing a mild disease state (Hixson et al., 2018). This further suggests that social grooming may serve to ameliorate some experiences of discomfort or pain in cattle. Although specific effects of social buffering in response to pain has not been well described in calves, pair housed calves vocalized less than individually housed calves during weaning (De Paula Vieira et al., 2010). This suggests that a social companion somewhat reduced the negative effects of weaning.

In addition to animals in pain soliciting attention from conspecifics as a result of a painful experience, animals will modify their social strategies and the intensity and frequency of social behaviors while in pain. Male rabbits who were determined to be dominant within their social groups were exposed to a painful formalin injection. These rabbits decreased their general activity levels and social behavior after this painful experience (Farabollini et al., 1988). Additionally, this reduction of motor and social behavior was correlated with the intensity of pain, as assessed by the amount of time the rabbit spent licking its injured foot. Interestingly, despite the dominant male expressing modified social behaviors, the dominance hierarchy remained unchanged; the dominant animal did not develop submissive behaviors, and the submissive animals did not alter their social behavior or the frequency of affiliative and agonistic interactions. This suggests that dominance hierarchies remain intact when dominant members experience short term, acute pain.

Cattle vocalizations have been shown to encode information related to individual identity (Padilla de la Torre et al., 2015) and are utilized to communicate experiences of pain to members of their social group (Schwartzkopf-Genswein et al., 1998). Interestingly, Watts and Stookey (1999) have shown that the vocalizations emitted during painful procedures such as branding, differ in acoustic quality to vocalizations emitted in response to restraint only. Further, Green et
al., (2019) demonstrated that cattle retain individuality in their vocalizations during both positive (anticipation of feed) and negative (denied access to feed and isolation from conspecifics) situations. This suggests that vocalizations are used to communicate among conspecifics in a specific way, and that the specificity of these vocalizations may be utilized by members of the group to determine the identity and state of the caller. Additionally, this provides further evidence of cattle altering their social behavior when they are in pain.

**External Factors Influencing Social Behavior**

Expression of social behavior in cattle is subject to various external factors related to management, including stocking density, frequent regrouping, and feed bunk design, as well as characteristics of the social group such as age range.

In dairy cattle, as stocking density at the feed bunk increases, the number of displacements also increases (Huzzey et al., 2006). The type of feed barrier also affected the amount of displacements. The cows in the headlock feed barrier were displaced less than the cows in the post-and-rail barrier (Huzzey et al., 2006). The headlock barriers provide a physical separation between cows at adjacent feed station, and this suggests that this separation affects the way cows interact with each other at the feed bunk, and that specific housing and management factors can have implications for the expression of social behavior.

Age can also affect dairy calf social behavior. Age heterogeneity in groups of calves increases competition for feed with the older calves in the group performing more displacements at the feed bunk (Færevik et al., 2006). Calves in these heterogeneous groups also had a lower average daily gain than calves housed in homogenous age groups (Færevik et al., 2006). Age at introduction to large social groups early in life also affects the ease with which dairy calves assimilate into the group. Calves housed individually for 6 days before grouping licked and
sniffed less, changed posture more, and spent less time laying down than calves that were
introduced at 14 days of age (Rasmussen et al., 2006). Additionally, the calves that were
introduced at 14 days of age spent more time engaging in social lying than the calves introduced
at 6 days of age (Rasmussen et al., 2006). These results suggest that calves respond to social
introduction differently at different ages, and that calves grouped at 14 days of age may be able
to integrate into their group better than the calves group-housed at 6 days of age.

Consequences of Social Isolation

Cattle are naturally social, gregarious animals and they are motivated to perform social
behaviors and to access social companions. It is common practice for most dairy operations to
house their dairy calves individually, which disallows natural development of social behaviors in
early life. Social housing affects response to novelty, feeding behavior, and cognition in dairy
calves. Further, investigation into natural social relationships, such as the cow-calf bond, may
provide insight into highly motivated social behaviors during early life.

Social experience in early life broadly affects behavior and emotional regulation
throughout the life span. Much of the research in this area examines the effects of maternal
caregiving in rats. Rat pups that were removed from their nest after 10 days of life had deficits in
their ability to learn and to regulate their emotional states as adults (Padoin et al., 2001). The
presence of the dam in early life is also important in regulated stress response of the pup. To
examine the organizational effect that maternal separation has on the developing stress response,
rat pups were removed from their mother for 180 minutes each day from days 2-14 of life. These
rats showed increased pituitary-adrenal responses to acute stress and resulted in decreased
glucocorticoid receptor binding in the hippocampus, frontal cortex, and hypothalamus (Padoin et
al., 2001). Overall, this heightened response to acute stress and reduced efficiency in the pathway
designated to cope with these stress responses may lead to the elicitation of an emotional response that the animal is not equipped to cope with.

Calves housed individually are more reactive to novelty. In a social novelty test, calves housed individually had a longer latency to approach the unfamiliar social companion, kicked and defecated more frequently, and spent more time running than calves housed in pairs (De Paula Vieira et al., 2012). Reactivity to novelty may be detrimental to welfare because calves will be frequently exposed to novel social situations as they develop and experience management transitions. Group housed dairy calves also experience reduced food neophobia, which is the reluctance to consume novel food sources; group-housed calves not only had a lower latency to approach the novel feed, but they also consumed more (Costa et al., 2014). Decreased food neophobia would be beneficial to animals as they experience dietary transitions.

Social isolation can also lead to cognitive deficits in dairy calves. Group-housed calves performed better in a reversal learning task than calves that were raised individually. In this study, calves learned to discriminate two colors in a Y-maze. Once the initial learning criteria was met, the training stimuli was reversed (the previously unrewarded color became the rewarded color). Although the rate of learning in the initial phase was similar, individually housed calves performed worse than group housed calves in the reversal phase (Gaillard et al., 2014). These results suggest that lack of social enrichment is detrimental to the cognitive function of dairy calves.

Social contact affects feeding behavior, and subsequently, intake and growth of dairy calves. Social learning is an important factor in feed selection as information about safe, high-quality feed sources is transmitted through groups of animals. Social learning is the most efficient form of learning because it does not require the animal to perform the activity itself; in
this case sampling the new feed source. This allows the observing animal to learn from the outcome of the experience without incurring any of the possible costs such as inadequate energy intake, sickness, or death. Calves housed in pairs starting at 6 days of age had increased dry matter intake and weight gain compared to calves housed in pairs starting at 43 days of age and calves housed individually (Costa et al., 2015; De Paula Vieira et al., 2010). Calves housed in pairs also appear to have a reduced stress response to weaning as indicated by reduced vocalizations (De Paula Vieira et al., 2010; Bolt et al., 2017). These results suggest that social support promotes feed intake as well as offering support in stressful situations.

Evaluating the specific consequences of cow-calf separation is important for promoting positive welfare for dairy calves. In general, the relationship between mammalian mothers in their offspring can be characterized by affiliative behaviors such as social grooming, synchronization of behaviors, and provision of resources (reviewed by Newberry and Swanson, 2008). In addition, this relationship is crucial for transmission of information about adequate food sources (V et al., 1990) and predator avoidance tactics (Mateo and Holmes, 1997). The role of the dam in highly social species that live in large groups, such as cattle, is even more poignant, as the mother must initiate individual recognition between the dam and the offspring and facilitate social bonding through high frequencies of contact during early lift to ensure continued care (reviewed by Newberry and Swanson, 2008). The neurotransmitters involved in developing the maternal bond include oxytocin, dopamine, opioids (reviewed by Newberry and Swanson, 2008), which are also associated with social bonding in unrelated conspecifics, as previously discussed. Despite research describing the functional consequences and mechanisms of action of maternal bonding, and the effects of maternal separation in many other mammalian species, the long-term effects of maternal separation in cattle has not been well described.
However, in dairy calves who were reared with their dams, separation from the dam causes a negative judgement bias, as assessed using a cognitive bias test (Daros et al., 2014), indicating that separation from the dam may induce a negative affective state. Further evaluating the impacts of cow-calf separation and designing management practices to mitigate potential negative effects, including provision of social partners to promote adequate development of social behaviors, is crucial to ensure the sustainability of the industry.

**Disbudding**

Hot-iron disbudding is a common procedure in the dairy industry in which the horn bud and surrounding tissue are burned off and cauterized to prevent future horn development. In general, disbudding and dehorning is done to prevent injury to conspecifics and human handlers, however, the pain associated with this procedure makes this a relevant animal welfare concern. This section will discuss the popularity of this procedure in the United States, Canada, and Europe, the effects of disbudding on behavior, and how pain following disbudding is evaluated and managed.

**Popularity and Consumer Perspectives**

Disbudding remains a popular procedure on dairy farms around the world, but there is still considerable variability in disbudding method and pain mitigation protocol. In the United States, 94% of dairy farms perform a disbudding or dehorning procedure of some kind, and 50% of these calves are undergoing hot-iron disbudding (USDA, 2018). Only 70% of the calves experiencing hot-iron disbudding received pain relief of any kind (USDA, 2018).

In Canada, surveys indicate that while most farms implement some kind of disbudding procedure, veterinarian involvement and use of analgesics remains varied (Winder et al., 2016). 73% of producers reported that a member of their staff performed disbudding procedures, while 27% of producers indicated that a veterinarian or veterinarian technician disbudded calves on
their farm. This discrepancy in who performs this procedure is significant; while 97% of veterinarians report use of a local anesthetic, only 62% of producers use this form of pain relief (Winder et al., 2016). This indicates that despite considerable efforts being made to develop effective pain mitigation protocols, many farmers are continuing to perform this procedure themselves without the use of these protocols.

Despite directives from The Council of Europe, which represents all European Union member states, recommending the use of pain mitigation in calves disbudded after 4 weeks of age, disbudding practices remain widely varied throughout Europe. Although the prevalence of farms with disbudded cattle was lower than in the United States and Canada with 80% of dairy farms performing the procedure, hot-iron disbudding remained the most popular method (Cozzi et al., 2015). Additionally, only 35.5% of farms performing a disbudding procedure used any kind of pain mitigation medication (Cozzi et al., 2015). Overwhelmingly, the main reason why producers chose to disbud their cattle was to reduce the risk of injury among herd mates (Cozzi et al., 2015). Similarly to practices in Canada, 71.7% of European farms rely on a stockman to perform the disbudding procedure (Cozzi et al., 2015). In Finland, more than 70% of dairy farmers disbud their calves, and veterinarian involvement and use of pain mitigation strategies remains varied (Hokkanen et al., 2015). In Italy, only 10% of disbudded calves receive local anesthetics, 5% receive analgesics, and 4% receive a sedative prior to disbudding, with the majority of these decisions about pain mitigation surrounding disbudding related to an unwillingness to pay for these added costs (Gottardo et al., 2011).

Public concern for farm animal welfare has risen rapidly over the last two decades. Increasingly, people are becoming more concerned about the care that animals in captivity receive. A 2002 survey indicated that 86% of respondents were either ‘very concerned’ or
‘somewhat concerned’ about livestock welfare. 61% of people altered their purchasing decisions to align with these concerns, by purchasing products that they viewed to be more ‘animal welfare friendly’ (Bennett and Blaney, 2002). This indicates that consumers have been changing their animal product buying habits to align with their beliefs about animal welfare since at least the early 2000’s. A 2015 study indicated that 90% of respondents with varying degrees of involvement in the dairy industry stated that pain relief should be provided during disbudding (Robbins et al., 2015), with females being more supportive of the use of pain mitigation than males. Additionally, only 83% of dairy producers supported pain mitigation following disbudding, whereas 93% of animal advocates stated their favor for pain mitigation (Robbins et al., 2015). There was no effect of education, participant age, or country on support for pain mitigation. This suggests that across demographics, public support for pain relief following disbudding remains high.

This increase in public concern is mirrored by a lack of public knowledge about animal agriculture and farm animal welfare. Despite the discrepancy between concern for animal welfare and knowledge about the industry, a study conducted by the European Commission in 2009 found that 58% of respondents wanted further information about farm animal welfare, and the more a person claims to know about animal welfare, the more likely they are to desire more information. This suggests that although modern society has left many people disconnected with current agricultural practices, there is still concern for how livestock are treated. Troublingly, a survey conducted in 2014 indicated that while 56% of households in the United States did not cite a source for their animal welfare information, those that did cite a source commonly cited information from animal rights organizations. This indicates a need for evidence-based, public animal welfare education.
These world-wide discrepancies in disbudding method and pain mitigation protocol indicate further need for research into the negative effects of the procedure and the development of a more adequate pain mitigation protocol. Additionally, there is a need for increased extension efforts aimed at providing producers the information necessary to make informed decisions about the practices that cause their animals pain and impact animal welfare, and for public animal welfare education.

**Effects of Disbudding on Behavior**

Hot-iron disbudding elicits various behavioral changes including changes in resting, feeding, and pain-specific behaviors. Additionally, this procedure has been associated with behavioral indicators of negative emotional state including a conditioned place aversion for the location of the disbudding procedure, and induction of a negative judgement bias, indicative of states of pessimism and anhedonia.

The effects of disbudding on lying behavior is dependent upon the methods used to measure this behavior and the pain mitigation protocol provided. Decreased lying time has been observed following disbudding for varying amounts of time. Sutherland, et al. (2018) described decreased lying times in the hour following disbudding in calves disbudded without pain relief, and in the calves disbudded with only a non-steroidal anti-inflammatory drug (NSAID). Calves that received a sham disbudding procedure, were disbudded with a local anesthetic, or were disbudded with both a local anesthetic and an NSAID did not change their resting behavior, suggesting that local anesthetics mitigate this change in behavior following disbudding. However, the results of this study only examine the acute effects of disbudding on lying behavior, as behavior was only recorded for one hour following disbudding.
Similarly to resting behaviors, the effect of disbudding on feeding behavior in dairy calves varies depending on how feeding behavior is measured and the pain mitigation protocol used. Sutherland et al. (2018) found that calves receiving a sham disbudding procedure performed more unrewarded visits to an automatic milk feeder than calves disbudded without provision of analgesics or disbudded with either only a local anesthetic or only an NSAID pain reliever. Interestingly, there was no difference in unrewarded visits between the sham calves and calves that received both a local anesthetic and an NSAID in addition to disbudding. This suggests that impacts of disbudding on feeding behavior can be mediated by the combinatory effects of the local anesthetic and the NSAID, but neither medication was able to mitigate these effects on its own.

Conditioned place preference has been used to evaluate the reinforcing effects of conditioned stimuli. Conversely, conditioned place aversion is used to evaluate the reinforcing effects of negative stimuli. Both conditioned place preference and avoidance occur when the subject learns to associate a location with a stimulus. Dairy calves who learned the location of lidocaine cornual nerve block administration demonstrated conditioned place preference for this location 3 weeks after the disbudding procedure (Adcock and Tucker, 2020). Calves who received only a sham disbudding procedure in addition to the local anesthetic did not demonstrate this preference. Cornual nerve blocks have been shown to be painful and aversive procedures, and a preference for this procedure in the weeks following disbudding indicates that calves are still experiencing pain. Additionally, calves demonstrate a conditioned place aversion to the location in which they were previously disbudded 48, 72, and 96 hours after the procedure, even when they were provided both a local anesthetic (Ede et al., 2019). Calves will work for access to procedures that ameliorate pain in the weeks following disbudding, and that calves
remember the location in which they were disbudded and preferentially avoid it. This suggest that disbudding is both a salient and aversive experience for calves.

Judgement bias tasks have been hailed as the gold standard of evaluating affective states in animals. In judgement bias tasks, animals are operantly conditioned to perform a task in which one cue is associated with a reward, and an opposing cue is associated with either no reward, or an aversive stimulus. Subsequently, animals are presented with ambiguous stimuli, and the assumptions that the animal makes about whether the ambiguous stimuli will lead to positive outcomes demonstrates whether an animal is ‘optimistic’ or ‘pessimistic’ while performing the test. Animals are then subjected to either a negative or a positive experience to evaluate the effects of that experience on the animal’s mood. Animals who judge ambiguous stimuli more negatively are considered pessimistic, and experiencing a negative affective state and reduced welfare, whereas animals who perceive ambiguous stimuli more positively are considered more optimistic. Following disbudding with a local anesthetic, calves are more likely to judge ambiguous stimuli as negative, which indicates that a negative change in affective state is associated with this procedure (Neave et al., 2013).

**Pain and Pain Mitigation**

In addition to behavioral indicators of pain, disbudding elicits physiological indicators of pain, including increasing heart rate variability, respiratory rates, and cortisol levels. Additionally, wound sensitivity and measures of evoked pain can be used to characterize some aspects of the duration of local pain. Further, disbudding has long-lasting effects on behavior, and has been shown to cause chronic pain in some calves. Various pain mitigation protocols have proven to be effective at mitigating some of the effects of short term, acute pain, however further
research is necessary to evaluate how pain can be ameliorated in the days and weeks following the procedure.

Heart rate and heart rate variability (HRV) has been used to assess autonomic nervous system activity in cattle. Calves that were disbudded without a local anesthetic increased their heart rate, with their heart rate remaining elevated compared to controls receiving a sham disbudding, and disbudded calves receiving a local anesthetic (Stewart et al., 2008). These calves disbudded without pain relief also had increased heart rate variability. These changes in heart rate and HRV could be due to increased autonomic stimulation, which is indicative of acute pain. This elevation in heart rate and changes in HRV was confirmed by (Stewart et al., 2009), who determined that these changes were most pronounced 2 and 3 hours following disbudding in calves receiving local anesthetic. However, calves that received both a local anesthetic and an NSAID pain reliever did not experience this elevation in heart rate or HRV at this time, suggesting that the combination of a local anesthetic and a NSAID is sufficient for mitigating the effects of disbudding on this parameter in the hours following the procedure. There is also evidence to suggest that disbudding without pain relief results in elevated heart rates for at least 24 hours when compared to calves who received NSAID after the procedure (Heinrich et al., 2009). However, heart rate and HRV do not serve as direct measurement for the intensity of pain experienced, and caution should be taken when interpreting these results.

Cortisol is a hormone that has been commonly used to evaluate the impact of stressful experiences in animals, as plasma cortisol concentrations serve as a reflection of hypothalamus-pituitary-adrenal activation (Stafford and Mellor, 2005). A meta-analysis of the effects of local anesthetics and analgesics following disbudding on blood cortisol levels found that local anesthetics reduced blood cortisol levels in the first hour following the procedure, but no effect
of treatment was found 2 or 3 hours post-disbudding. Interestingly, treatment with local anesthetic resulted in increased blood cortisol concentrations at 4 hours following disbudding. NSAIDs were found to have no effect on cortisol concentrations 2- or 3-hours post-disbudding but were shown to decrease cortisol concentrations 4 hours after the procedure. Additionally, calves receiving both local anesthetic and NSAIDs were found to have higher cortisol levels at 24 hours following disbudding. This suggests that the combination of local anesthetic and NSAID pain relievers work together to buffer the effects of disbudding on blood cortisol levels during the first 24 hours after the procedure, but are insufficient for mitigating this effect for longer than this period. However, similarly to heart rate and HRV, plasma cortisol levels do not serve as a direct measurement of the intensity of pain experienced, but rather as a measure of the overall aversiveness of the experience. Therefore, caution should be taken when interpreting the effects of painful stimuli on plasma cortisol concentrations, and these measurements should be paired with other physiological or behavioral indicators of pain.

Pressure sensitivity around the wound can be used to assess the level of invoked pain that is being experienced at the wound site. A meta analyses of the effects of pain mitigation on pain following disbudding concluded that while there was no effect of treatment on wound sensitivity 2 hours following disbudding, at 4 and 6 hours following disbudding, calves treated with NSAID and a local anesthetic tolerated more pressure to the wound than calves not receiving analgesic (Winder et al., 2018). Additionally, there was no difference between treatments at any later time points (8, 12, 24, 48, 72, or 96 h) following disbudding. This suggests that current pain mitigation protocols consisting of and NSAID and local anesthetic is most effective for reducing wound sensitivity between 4 and 6 hours following the procedure, but further work is needed to determine tactics for long-term pain mitigation. Additionally, wound sensitivity persists for at
least 14 weeks following disbudding when provided a local anesthetic, NSAID, and a sedative around their procedure (Casoni et al., 2019a), suggesting that the effects of the disbudding procedure may be persistent and examining pain relief in the days and weeks following the procedure may be worthwhile.

Pain specific behaviors can vary based on the location of the wound. Following disbudding, head shaking, ear flicking, and head rubbing are behaviors that are commonly examined to determine the amount of pain being experienced and the efficacy of treatments. A meta-analysis revealed that the effects of a local anesthetic and NSAID pain reliever were most effective at mitigating ear-flicking behavior at 3 and 4 h, and head shaking behavior at 4 and 6 h following disbudding, but there was no effect of pain mitigation on frequency of head rubbing (Winder et al., 2018). This further suggests that current pain mitigation protocols are most effective between 3- and 6-hours following disbudding, and that further research is needed to determine how persistent pain can be managed in dairy calves.

Chronic pain refers to pain that persists past normal wound healing time and is typically classified as such when it lasts for more than three to six months (Treede et al., 2015). Burn injuries, the type of wound inflicted during hot-iron disbudding, has been linked to considerable chronic pain in humans (reviewed by Summer et al., 2007). In humans, chronic pain due to burns is estimated to affect 36% of burn patients (Malenfant et al., 1996). The mechanism and prevalence of chronic pain has not been well characterized in animals, however there is evidence to suggest that dairy calves experience chronic pain in the months following hot-iron disbudding. Approximately 38% of calves were determined to be affected by chronic pain, as assessed using clinical pain assessment, wound pain pressure thresholds, and mechanical allodynia (Casoni et
al., 2019a). This suggests that animals develop symptoms of chronic pain, and that current pain mitigation protocol for disbudding may be insufficient.

To date, the most commonly recommendation protocol for relieving pain associated with disbudding involves the use of a local cornual nerve block (a local anesthetic), a non-steroidal anti-inflammatory (NSAID), and a sedative. These drugs work best when provided in combination with one another, but do not relieve all experiences of pain. Additionally, there is currently no recommended treatment protocol for ameliorating persistent pain in the days and weeks following disbudding.

**Personality and Individual Variability**

Animal personalities are referred to as within-population differences in individual behavior which are relatively stable over time, and the behavior of an individual in one context correlates with its behavior in a different context. In this section we will discuss the proximate mechanisms driving differences in personality, including differences in metabolism and stress physiology, as well as ultimate mechanisms, including the development of dominance hierarchies and the importance of behavioral variability in gregarious species. We will then discuss the importance of understanding individual variability in livestock husbandry.

**The Relationship Between Personality and Behavior**

Differences in personality have a wide range of effects on behavioral expression. At the individual level, differences in personality have been correlated with differences in stress physiology, metabolism, and cognition, providing a proximate mechanism for personality development. These individual differences in personality allow for the establishment of dominance hierarchies in social species. Personality has been correlated with differences in foraging and feeding behavior, social behavior strategies, cognitive ability, and behavioral
flexibility and mood. Finally, there is a genetic component to differences in animal personalities that may offer the opportunities for artificial selection that enhances welfare.

Both environmental stimuli and internal biological feedback act to develop personalities within an individual. There is evidence to suggest that energy metabolism effects personality development, in which fast-growing animals with a high metabolic rate become more aggressive, active, and bold (Biro and Stamps, 2008). This is based on the hypothesis that individual differences in life history productivity that further develop consistent differences in personality relevant to acquisition of resources. There is evidence to suggest that coping styles, the theoretical precursor to animal personalities, is related to differences in stress physiology. Animals that were found to be either proactive in response to novel, stressful, or painful stimuli exhibited low hypothalamo-pituitary-adrenal axis (HPA) activity and parasympathetic activity, and high sympathetic and testosterone activity (reviewed by Koolhaas et al., 1999). Conversely, reactive individuals displayed normal HPA activity, high HPA axis and parasympathetic reactivity, and low sympathetic and testosterone activity. This suggests that individual differences in behavior is mediated by stress hormone activity. Further, differences in personality have been correlated with cerebral lateralization, the preferential use of one brain hemisphere to execute cognitive functions. Animals who were determined to be more ‘bold’ during a behavioral tests in a novel environment displayed more cerebral lateralization when exploring a familiar environment (Reddon and Hurd, 2009). Overall, at the proximate level, it appears that personality development is controlled by metabolism, stress physiology, and brain morphology.

Individual differences in personality are also related to natural history, as they appear to be highly conserved in many species and adaptive. As previously discussed,
the social niche specialization hypothesis suggests that individuals in a group occupy specific social roles which maintains individual differences in personality (Montiglio et al., 2013), whereas the ecological niche theory suggests that behavioral diversification is adaptive because social niches and structures reduce daily conflict among conspecifics (Bergmüller and Taborsky, 2010). Additionally, variations in personality due to energy metabolism suggest that the animal with the highest fitness expresses behaviors such as boldness and aggressiveness that further perpetuate this disparity in fitness levels, thus assuring that the individuals higher fitness gain the most access to resources (Biro and Stamps, 2008). Therefore, the retention of individual personalities within animal populations is inherently connected to differences in social behavior expression.

Although personality traits are defined as maintaining stability over time, early life experience may affect personality ontogeny. Differences in the photoperiod during early life influence personality in mature guinea pigs, whereby animals born in the spring developed a more ‘fearless’ personality type, had a lower metabolic rate, and higher levels of cortisol than animals who were born in the fall (Guenther et al., 2014). This implicates early life experience in not only personality development, but also on the development of the proximate causes of personality. In rats, individuals who were larger at birth were bolder and more explorative (Rödel and Meyer, 2011). Additionally, rats who were part of either a small or large litter were more anxious than rats born into medium sized litters. This implicates the early life social environment in the development of personality traits. Further research is needed to determine the role of early life experience on the development of personality in dairy cattle.

In addition to early life experience, other critical periods of development such as sexual maturation impact personality development. Dairy cattle demonstrate consistency for personality
traits related to boldness between pre- and post-weaning, post-weaning and puberty, and puberty and lactation (Neave et al., 2020). However, these boldness scores were not stable between pre-weaning and lactation, or between post-weaning and lactation. Similarly, factor scores for exploratory-active personality traits followed a similar developmental trajectory, with stability being maintained over adjacent developmental periods (between pre- and post-weaning) but showing discontinuity between staggered developmental periods (between pre-weaning and lactation.) This indicates that although personality traits may follow stable developmental trajectories, these traits do not remain stagnant in their stability over time and continue to change as an animal matures. Although the mechanisms driving this change in personality development are not yet well understood, changes in both the internal state of the animal and their social and physical environment are implicated in driving these changes (Trillmich et al., 2018).

Personality is related to different methods than an animal employs to find food, and how willing the animal is to try new sources of feed. This relationship between personality and differences in feeding behavior has been demonstrated in dairy calves. Calves that were more exploratory and active in response to novelty began consuming solid feed at an early age and ate more grain during the entire pre-weaning period (Neave et al., 2018). Additionally, these exploratory and active calves visited the automatic milk feeder less frequently during weaning, suggesting that they were less negatively affected by stress during weaning. Interestingly, calves that were more vocal and inactive in response to novelty visited the milk feeder more frequently during weaning. This suggests that these calves may have experienced more stress as a result of weaning and chose to continue unsuccessfully approaching the automatic milk feeder for food instead of searching for new feed sources. For dairy cattle, the development of feeding behavior and early consumption of solid feed is important to ensure long-term productivity.
Individual Variability and Livestock Management

Animals with differing personalities may have different needs which contribute to their individual welfare. Offering choice within the environment allows for increased animal agency, and the opportunity for animals to make choices that enhance their individual welfare. Understanding these differences in personality in livestock may allow us to design improved housing and management systems that allow for more variable behavioral expression.

Preference tests, in which an animal may choose one environment or stimulus over another, have long been utilized to gain information about what is important to animals and how we can maximize welfare. However, as previously discussed, personality differences result in variable expression of behavior. Results of preference tests are often interpreted at the population level, potentially masking wide individual variability. Further, preference and motivation to perform different behaviors may vary with external and internal factors. For example, dairy cows choose to preferentially calve behind a barrier that offers isolation from pen-mates at night, but not during the day (Proudfoot et al., 2014a). This indicates that there may be differences in preference based on the time of day. Additionally, both sick dairy calves (Hixson et al., 2018) and lame dairy cattle (Galindo and Broom, 2010) alter their social behavior, suggesting that preferences for social contact may change when an animal is experiencing pain or disease. Early life experience may also alter decision making; dairy-calves housed in pairs during the pre-weaning period chose to spend more time feeding in synchrony with their pen-mate after weaning, while calves housed individually did not display this preference for social feeding synchrony (Miller-Cushon and DeVries, 2016).

Providing the opportunity for choice in the environment allows animals to make choices that enhance their individual welfare. Despite the recent shift toward social housing, dairy calves
are still housed in barren environments that do not allow for much variability in behavioral expression. The inclusion of pen features, such as brushes and physical barriers may allow for more variable behavioral expression and allow animals to promote their individual welfare by making choices that are beneficial for them. Accommodating individual preferences through environmental complexity and provision of opportunities for social seclusion may have a range of welfare implications. Providing animals with the ability to make choices in their environment is an important way to ensure individual welfare needs are met (Śpinka, 2019). Further research into how additional pen complexity impacts livestock welfare is needed and provides substantial opportunity for enhancing animal welfare.

**Summary**

Social behavior expression is a highly motivated natural behavior that is important to gregarious species of animals including cattle. Dairy calves form social bonds early in life, develop preferred social companions, and will work for access to a social partner. Access to social partners and the development of social behaviors has numerous benefits, including increased solid feed intake, improved cognition, and reduced neophobia. Personality and individual variability are an important aspect of social behavior, whereby individuals living together in a group occupy different positions within their social network and their dominance hierarchies. Individual differences in behavior and personality are responsible for this distribution of behavior. Social behavior is a natural behavior that is highly susceptible to changes when animals experience disease and pain, as well as particularly salient life events such as disbudding. Social behaviors, and the preference for withdrawal from a social group may be related to both experience of pain and to differences in personality.

Disbudding has been shown to induce a negative judgement bias, which is indicative of reduced welfare, decrease lying behavior, and elevate cortisol levels and heart rate variability.
Further, disbudding pain lasts for at least 14 weeks following the procedure, and approximately 38% of disbudded calves develop symptoms of chronic pain. Although current pain mitigation protocols, including the use of local anesthesia, non-steroidal anti-inflammatory medications, and sedatives have proven effective at ameliorating acute, short-term experiences of pain, there is currently no recommendation for pain mitigation in the days and weeks following the procedure. Additionally, despite behavioral and physiological indicators of pain and welfare have been well studied in the 24 hours following the procedure, further research is necessary to characterize the long-term effects of disbudding on behavior in the days and weeks following the procedure. Despite the popularity and longevity of this procedure, pain mitigation protocol and disbudding technique remains varied.

Animal personalities are relative behavioral frameworks that cause individual variation in behavior within a population. Personality is mediated by metabolism, stress physiology, and brain morphology. In addition, the development of personality is dependent upon early life experience, and can change throughout the lifespan, particularly during times of broad changes in physiology such as sexual maturation. Further, animals with different personalities or experiencing stressful vents that elicits changes in behavior, such as pain and disease, may benefit from added environmental complexity that gives them the opportunity to make choices in their environment and express a broader range of behaviors. Despite evidence in other species, there are considerable gaps in our knowledge surrounding the extent to which social behavior of calves may be subject to experiences such as pain or individual personality traits.
CHAPTER 2
DISBUDDING AFFECTS USE OF A SHELTER PROVIDED TO GROUP-HOUSING DAIRY
CALVES

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 x 8.0 m). Each pen contained a three-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 non-steroidal anti-inflammatory. 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). 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), 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.

**Introduction**

Measuring behavioral changes can be a means to identify and alleviate individual experiences of pain, an unpleasant sensory and emotional experience (Merskey and Bogduk, 1994). For pre-weaned 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 weeks (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 judgement 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. Across species, there is evidence that social withdrawal may occur when an individual is experiencing a negative emotional 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 to indicate that patients who suffer from chronic pain develop depression (Fishbain et al., 1997; Brown, 1990). This suggests a relationship between pain, affective state, and sociality. There are well established neurobiological links between emotional state and social behavior, as the amygdala processes information related to both social (Mihara et al., 2017; Grabenhorst et al., 2019) and emotional stimuli (Sergerie et al., 2008). 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 out 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 post-partum metritis (Proudfoot et al., 2014a). 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, thus improving the social environment (Stoye et al., 2012).

In this experiment, we provided group-housed dairy calves with a shelter, consisting of a three-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, USA; 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). As there was no previous data 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 two daily feedings of 4 L each.

At approximately 2 weeks 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, USA) via an automated starter feeder (DeLaval, CF 1000X, 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 Clarifly; Southeast Milk, Inc., Mayo, FL, USA) fed through an automated milk feeder (DeLaval CF1000X, DeLaval, Kansas City, MO). The feeder was set to limit milk intake per visit to a maximum of 2.5 L, such that calves were required to wait for 2 hours after their previous meal of 2.5 L to receive additional milk. 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 2-1) was built with 3 sides (1.2 by 1.2; 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 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 2-2). 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 week 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 a 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 1200h). Calves were disbudded by a trained veterinarian, or by trained veterinary students under direct supervision of the veterinarian. Calves received a non-steroidal anti-inflammatory drug for analgesia 3-4 h prior to disbudding (meloxicam provided orally; 0.5 mg/kg; Unichem Pharmaceuticals USA Inc., Rochelle Park, NJ) and a local cornual nerve block 10-15 min prior to 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 pre-heated for at least 10 min prior to cautery disbudding of calves. Hair was clipped over the horn buds, and the calf manually restrained with the head held to the side. The disbudding iron tip was applied firmly over the horn bud for 8-10 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 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 (inter-observer reliability was calculated for one day of video collection, with Cohen’s kappa ≥ 0.85, as calculated in BORIS, for all observer comparisons).

Statistical Analyses

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 prior to analysis using the PROC UNIVARIATE procedure within SAS (version 9.4; SAS Institute Inc., Cary, NC, USA), 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 % of total lying time. Hourly shelter use duration was transformed using the two-parameter Box-Cox transformation (Box and Cox, 1964), where \( g(y;\lambda_1,\lambda_2) = \log(y+\lambda_2) \) with \( \lambda_1 = 0 \) and \( \lambda_2 = 1 \), 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 days. 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. However, visual inspection of these data indicated that hourly shelter use followed a similar pattern by treatment on each observation day and this was confirmed by examination of average hourly shelter use by daytime and night on each observation day, prior to summarizing data across days. 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 square 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
2-3. 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/day and the maximum shelter use was 20.7 h/day (overall arithmetic mean of 4.5 h/day and
median of 2.5 h/d). Calves showed individual consistency in their use of the shelter over time
(between days 0 and 1: $R^2 = 0.40$, $P < 0.001$; between days 1 and 2: $R^2 = 0.43$, $P < 0.001$; and
between days 0 and 2: $R^2 = 0.24$, $P = 0.009$).

Individual and social use of the shelter is reported in Table 2-1. 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 2-2). 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 brackets: 6.5 (2.9, 13.5) vs. 2.7 (0.9, 6.1) min/hour; DB vs. CON; F₁,₁₁₇ = 7.51; P = 0.014] and d 1 [9.6 (4.8, 18.5) vs. 4.6 (2.0, 9.2) min/h; F₁,₁₁₇ = 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; F₁,₁₁₇ = 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 brackets: 4.0 (0.7, 13.6) vs. 3.4 (0.5, 11.7) min/hour; DB vs. CON; F₁,₁₁₇ < 0.94; P > 0.34].

Diurnal patterns of shelter use (Figure 2-4) were examined in more detail by averaging hourly shelter use across consecutive observation days. However, hourly patterns of shelter use did not differ between treatments (no treatment by hour interaction; F₁,₁₂₈₁ = 0.99; P = 0.47). Overall hourly duration of shelter use was greater for disbudded calves (F₁,₅₂₃ = 5.39; P = 0.02), consistent with day-level data, and differed by hour (F₂₃,₅₂₃ = 2.09; P = 0.0043). 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 (F₁₂,₂₈₁ = 0.95; P = 0.50) or night (F₁₀,₂₃₇ = 1.33; P = 0.22). However, consistent with results of average hourly shelter use, disbudded calves used the shelter more overall during
the daytime ($F_{1,281} = 11.90; P < 0.001$) whereas hourly use did not differ overall during the night ($F_{1,237} = 0.41; P = 0.52$).

**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 persistent 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, similarities in duration of social shelter use, as a % of total time in the shelter, suggests a need for further research to evaluate effects of pain on social behavior.

While 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., 2014a; b). Generally, 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. Increased shelter use following disbudding was evident during daylight hours, whereas shelter use did not differ during the night. 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. It is possible that shelter use in disbudded calves was motivated by a specific avoidance of humans, due to association with the painful procedure. Increased shelter use during the 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 pen-mates, 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 utilize 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 affiliative behaviors inside the shelter may provide more information about how calves are choosing to utilize the shelters, and how this 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. While we hypothesized that animals in pain may seek out social isolation, there is also evidence to suggest 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, 2010). 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, there has been limited examination of 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, whereas reduced social contact has been observed in sick calves (e.g. Hixson et al., 2018). 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. 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 non-steroidal anti-inflammatory 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, particularly beyond d 0 of observation. 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 to a baseline period during which calves received a sham procedure only, suggesting that calves may still be experiencing pain despite analgesia.

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. Changes in behavior following disbudding were relatively consistent for our 3-day observation period following the procedure, suggesting that shelter use may have been affected by the persistent experience of pain, even with provision of analgesia on d0. 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 had a preference for lying 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 to a sham procedure or full pain mitigation (Sutherland et al., 2018), and provision of meloxicam similarly resulted in greater lying time compared to calves disbudded with local anesthetic only (Heinrich et al., 2010) or no anesthetic (Theurer et al., 2002). These results suggest that the experience of pain post-disbudding 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 may be insufficient. This evidence of prolonged changes in aspects of social behavior and rest contributes to our somewhat limited knowledge of non-evoked 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 days (with and without analgesic; Heinrich et al., 2010), and wound sensitivity for at least 14 weeks (Casoni et al., 2019). The timeframe and severity of post-disbudding 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 weeks (Casoni et al., 2019). Adcock and Tucker (2020) also demonstrated that, at 20 days 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 related to the emotional experience of 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 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 as, 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 2-2) 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 judgement 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 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 (Špinka, 2019). This added level of pen complexity may also differentially improve the welfare of animals experiencing pain, facilitating seclusion when preferred.

**Conclusion**

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 day experimental period.
Table 2-1. 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 days (D) following treatment (T) at 36 d of age. Data are reported as (back-transformed) least square means with 95% confidence intervals (CI) pooled by treatment.

<table>
<thead>
<tr>
<th>Item</th>
<th>DB</th>
<th></th>
<th></th>
<th></th>
<th>CON</th>
<th></th>
<th></th>
<th></th>
<th>P-value</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>95 % CI</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>95 % CI</td>
<td>T</td>
<td>D</td>
<td>TxD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shelter use, min/d(^1)</td>
<td>120.2</td>
<td>244.9</td>
<td>191.3</td>
<td>(74.5, 425.2)</td>
<td>86.5</td>
<td>90.2</td>
<td>109.3</td>
<td>(39.7, 226.7)</td>
<td>0.031</td>
<td>0.23</td>
<td>0.37</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Social use, % of use(^2)</td>
<td>54.7</td>
<td>58.5</td>
<td>72.0</td>
<td>(49.6, 73.9)</td>
<td>63.1</td>
<td>62.3</td>
<td>63.2</td>
<td>(50.7, 75.0)</td>
<td>0.86</td>
<td>0.34</td>
<td>0.37</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Social use within treatment, % of use(^1,2)</td>
<td>23.6</td>
<td>41.5</td>
<td>29.2</td>
<td>(12.3, 59.6)</td>
<td>12.0</td>
<td>6.1</td>
<td>10.2</td>
<td>(1.0, 26.9)</td>
<td>0.017</td>
<td>0.95</td>
<td>0.36</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shelter visits, no/d(^3)</td>
<td>13.8</td>
<td>14.9</td>
<td>16.4</td>
<td>(11.8, 18.3)</td>
<td>10.5</td>
<td>12.3</td>
<td>11.5</td>
<td>(8.2, 14.7)</td>
<td>0.063</td>
<td>0.48</td>
<td>0.75</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Solitary entrance, no/d(^3)</td>
<td>7.6</td>
<td>8.5</td>
<td>8.3</td>
<td>(6.1, 10.2)</td>
<td>5.3</td>
<td>6.8</td>
<td>4.5</td>
<td>(3.5, 7.6)</td>
<td>0.02</td>
<td>0.45</td>
<td>0.65</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Social entrance, no/d(^1,3)</td>
<td>5.4</td>
<td>5.7</td>
<td>7</td>
<td>(3.7, 9.0)</td>
<td>4.7</td>
<td>3.9</td>
<td>5.9</td>
<td>(2.8, 7.4)</td>
<td>0.25</td>
<td>0.23</td>
<td>0.87</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Solitary exit, no/d(^3)</td>
<td>6.3</td>
<td>7.8</td>
<td>6.3</td>
<td>(4.1, 9.6)</td>
<td>5.7</td>
<td>6.8</td>
<td>4.7</td>
<td>(2.9, 8.4)</td>
<td>0.38</td>
<td>0.33</td>
<td>0.92</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Social exit, no/d(^1,3)</td>
<td>6.8</td>
<td>7.1</td>
<td>8.8</td>
<td>(4.9, 8.7)</td>
<td>4.4</td>
<td>4.6</td>
<td>5.7</td>
<td>(3.8, 7.6)</td>
<td>0.02</td>
<td>0.36</td>
<td>0.99</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^1\)Reported means and CI are back-transformed from data that were log-transformed (shelter use) or square root-transformed (frequency of social exits and entrances, and social use within treatment, % of total use).

\(^2\)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.

\(^3\)Solitary entrances or exits defined when the focal calf entered or exited the shelter when it was otherwise unoccupied. Social entrances or exists defined when the focal calf entered or exited the shelter when another calf was occupying it.
Table 2-2. 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 days (D) following treatment (T) at 36 d of age. Data are reported as (back-transformed) least square means with 95% confidence intervals (CI) pooled by treatment.

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

^1Reported means and CI are back-transformed from data that were square root-transformed.
Figure 2-1. Image of calf using the shelter, a three-sided, open-top barrier made out of corrugated plastic (1.2 × 1.2 × 1.2 m; width × depth × height).
Figure 2-2. Schematic of the pen, depicting the shelter (three-sided, open-top barrier made out of corrugated plastic; 1.2 × 1.2; width × depth) and its location within the pen relative to other resources.
Figure 2-3. 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 labelled alphabetically). The dashed line represents the overall mean (back-transformed from the log-transformed data set).
Figure 2-4. 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 h 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 least square means. Confidence intervals for daytime hours pooled by treatment: [3.0 (min 0.9), 16.4 (max 31.40)] for DB calves and [0.6 (min 0), 6.1 (max 10.3] for CON calves, and for nighttime hours pooled by treatment: [0.9 (min 0.01), 13.3 (max 27.7)] for DB calves and [0.6 (min 0), 10.5 (max 19.1)] for CON calves.
CHAPTER 3
EXAMINING THE EFFECTS OF INDIVIDUAL PERSONALITY TRAITS AND DISBUDDING ON BEHAVIORAL EXPRESSION IN SOCIALY HOUSED CALVES

Abstract

Social behavior may generally reflect emotional state in gregarious animals, and individual differences in expression of social behavior may be related to individual differences in personality. In this experiment, we provided group-housed calves with shelters providing physical seclusion from the rest of the pen and examined behavioral expression within the home pen. Our objectives were 1) to evaluate the development of individual personality traits in relation to early social contact and their association with social and other behavioral expression following group-housing, and 2) to examine effects of disbudding on behavior of group-housed calves provided two shelters, intended to reduce potential competition from pen-mates. Holstein heifer and bull calves were housed in either individual pens (n = 16), or in pairs (n = 8 pairs) at birth before they were moved to group pens at approximately 2 weeks of life (8 calves/pen; 7.4 × 16.0 m). During week 4 of life, calves were tested in a series of standardized behavioral tests used to measure personality, including an open field test, a novel object test, an unfamiliar calf test, and an unfamiliar human test. During week 5 of life, a subset of calves were randomly assigned to be either disbudded (DB; n = 8; 10 bulls and 2 heifers; 36.2 ± 3.9 d of age) or receive handling only (CON; n = 8; 9 bulls and 3 heifers; 36.3 ± 4.2 d of age). Disbudded calves received a local corneal nerve block, non-steroidal anti-inflammatory, and a sedative. Behavior was recorded continuously from video for 24 hours in the week preceding disbudding treatment, and immediately following disbudding treatment for 3 consecutive days. Four factors were identified from principal component analysis of responses during the behavioral tests and were interpreted as “exploratory active”, “inactive avoidant”, “calf-avoidant”, and “human-directed”.

70
Calves housed in pairs before grouping had higher scores for the ‘exploratory active’ and ‘calf avoidant’ factor whereas calves housed individually calves were more ‘inactive avoidant’. The factor scores for ‘calf avoidant’ tended to be associated with shelter use and frequency of entering an occupied shelter. Calves tended to use the shelter more following disbudding (132.19 vs 93.01 min/day; DB vs CON), spent less time lying (16.85 vs 17.24 h/day; DB vs CON) and spent a greater proportion of their total lying time located in the shelter (13.62 vs 8.31 %; DB vs CON). Our results suggest that social contact in early life has persistent effects on behavioral traits reflective of personality after social grouping, and that both personality factors and disbudding affect decisions to use environmental features including a shelter offering visual and physical separation from the rest of the pen.

**Introduction**

Increasing environmental complexity for group-housed calves may be a means to facilitate expression of natural behaviors, including more variant expression of social behaviors, improving the social environment (Stoye et al., 2012). For example, Proudfoot et al. (2014a; b) found that periparturient cows prefer to calve behind a barrier, facilitating natural motivations for self-isolation at parturition. In results summarized in Chapter 2 of this thesis, we found that pre-weaned calves increase use of a barrier providing physical separation from the rest of the pen following disbudding, suggesting a potential preference for social withdrawal when in pain. However, considerable individual variability in shelter use in calves indicates that factors that contribute to individual differences in behavioral expression, such as personality and early life experience, may be a mediating factor in preference for social withdrawal.

Personality is defined as ‘a set of consistent individual differences in behavior across contexts and time’ (Sih et al., 2004). It has been established across species that personality traits in animals, as assessed using standardized behavioral tests, have stability across time and are
associated with individual behavioral expression. In particular, differences in personality have been related to expression of social behavior in wild songbirds (Aplin et al., 2013) and pigs (Friel et al., 2016). In dairy calves, Lecorps et al. (2019) demonstrated that calves that rated as more pessimistic, as assessed using a judgement bias task, were more selective with their social partners. Although differences in pessimism can be mediated by personality (Asher et al., 2016), pessimism is also related to mood states which vary over time. Therefore, further research is needed to determine the relationship between personality and social behavior in group-housed dairy calves. 

Early life experience also plays a critical role in behavioral development and personality traits, having potential longer-term effects on individual stress response and coping mechanisms. Early social contact affects personality traits in great tits (Aplin et al., 2013) as well as response to novelty (De Paula Vieira et al., 2012) and social behavior (Duve and Jensen, 2011) in dairy calves. Early social experiences may have longer-term implications for development of personality traits and expression of social behaviors in group-housed calves.

Based on our previous findings reported in Chapter 2 of this thesis, while social behavior is variable between individuals, social withdrawal may depend on the experience of pain. Hot-iron disbudding is a painful procedure that is associated with the elicitation of pain-specific behaviors (e.g. ear flicks and head shakes; Heinrich et al., 2010), wound sensitivity lasting at least 14 weeks (Casoni et al., 2019a), negative judgement biases (Neave et al., 2013), and reduced play behaviors (Mintline et al., 2013). Despite considerable research into describing the long-term effects that disbudding has on dairy calves, including the development of chronic pain (Casoni et al., 2019a), methods of pain mitigation remain insufficient to alleviate the long-term effects of this procedure. Additionally, the changes in shelter use reported in Chapter 2 suggest that understanding changes in social behavior at disbudding can provide insight into individual experiences of pain.
However, social dynamics surrounding shelter use raised questions of possible competition for access to the single shelter.

To further examine causation of behavior within social housing, including social withdrawal as assessed based on use of a shelter providing visual and physical seclusion from the rest of the pen, the objectives of this study were to 1) evaluate the development of individual personality traits in relation to early social contact and their association with behavioral expression following group-housing, and 2) examine effects of disbudding on behavior of group-housed calves provided two shelters, intended to reduce potential competition from pen-mates. We hypothesized that, if differences in personality are a mediating factor in motivation for social withdraw, then we would see associations between personality traits and characteristics of shelter use as well as other behaviors expressed in a social environment. Second, we hypothesized that, if disbudded calves prefer to withdraw socially when experiencing the negative affective state of pain, then calves would increase use of the shelter following disbudding, specifically preferring to enter an unoccupied shelter rather than an occupied one.

**Materials and Methods**

**Animals and Management**

Holstein heifer and bull calves (n = 32; 16 heifers and 16 bulls) were enrolled at the University of Florida Dairy Unit (Hague, FL, USA). 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. 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 randomly assigned to either individual (1.9 x 0.8 m; n = 8 calves) or pair-housing (1.9 x 1.6 m, n = 8 calves/4 pairs) within wire mesh pens allowing visual but not
physical contact with other calves. Calves were provided 8 L/d of pasteurized waste milk via teat buckets in two daily feedings.

At approximately 2 weeks of age (15.7 ± 2.2 d of age; mean ± SD) calves were moved to groups of 4 (age range of 4.6 ± 1.4 d; mean ± SD), comprised of a balanced number of calves from pre-grouping housing treatment (2 calves housed individually prior to grouping and 2 calves housed in a paired pen). After 1-week habituation to group-housing, gates were opened to the adjacent pen of 4 calves to form a group of 8 calves (7.4 × 16.0 m). These pens were deep-bedded with sand and located under an open-sided barn equipped with overhead fans for air circulation. 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, USA) via an automated starter feeder (DeLaval, CF 1000X, DeLaval, Kansas City, MO). All calves received a maximum of 12 L/d milk replacer (28 % CP and 15 % fat with Bovatec and Clarify; Southeast Milk, Inc., Mayo, FL, USA) fed through an automated milk feeder (DeLaval CF1000X, DeLaval, Kansas City, MO). The feeder was set to limit milk intake per visit to a maximum of 2.5 L.

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. All calves were determined to be healthy for the duration of the experiment, based on daily health checks by study staff and weekly clinical exams.

Each group of 8 calves had access to two shelters built out of dark green corrugated plastic to allow visual and physical separation from the rest of them pen. The shelter (shown in Figure 3-1) was built with 3 sides (1.2 by 1.2; width by depth), with the opening side was half-
enclosed to provide an entrance area (0.6 m), and was high enough (1.2 m) to prevent calves looking in over the wall, as described in Chapter 2. The shelters remained in the pens for the duration of the experiment. All calves also had *ad libitum* access to hay that was replaced once daily, and rotating brushes (DeLaval mini swinging brush; DeLaval, Tumba, Sweden).

**Behavioral Tests**

In week 4 of life (27.2 ± 3.5 d of age) calves were exposed to a series of standardized behavioral tests used to assess individual personality traits (adapted from previously developed tests: Neave et al., 2018; Lecorps et al., 2018). All tests began at approximately the same time each morning (0900h) and lasted for 10 minutes. A start box (2 m x 2.5 m) was drawn on the ground at the point of calf entry with spray paint. A stimulus marker in the shape of an ‘X’ was drawn with spray paint 2.54 m away from the edge of the start box in the center of the arena to ensure consistent placement of novel stimuli for each test. Calves were tested in a random order within pen for every test, and each calf received the series of four tests in the same order. Calves were haltered in their home pen, placed into an individual pen, and calmly led to the testing arena. The test began once the halter was removed and the calf had all four legs in the testing arena, within the start box.

On the first day of testing we conducted an open field test in which no stimulus was placed on the marker. For the second test, a novel object test, a green kick ball (22 cm in diameter), was placed on the stimulus marker. The third test was an unfamiliar calf test: two calves that had been habituated to the testing arena through testing in the previous week were placed in individual pens on either side of the stimulus marker, 15 cm apart, such that the front of the pens were flush with the edge of the stimulus marker. The fourth test was an unfamiliar human test: a person who was unfamiliar to the calves stood motionless on the stimulus marker. For the first 5 minutes of the test, she kept her feet together, arms by her side, and held her gaze
looking forward and down towards the ground. After 5 minutes had passed, she slowly approached the calf, wherever it was located within the arena, while looking towards the calf’s hooves and keeping her facial expression neutral. Once she approached the calf, she extended a hand toward the calf. The same person wearing the same clothes was used for every testing every calf.

A camera (Hero 7 Black; GoPro, Inc.; San Mateo, CA) was mounted to a corner post in the arena, 2 m from the ground, such that the entire testing arena was within view. Behavior was recorded continuously from video for the duration of the test using Behavioral Observation Research Interactive Software (BORIS; Friard and Gamba, 2016) according to the ethogram described in Table 3-1. 1 observer characterized all behavior from video (intra-observer reliability was calculated for one day of video collection, with Cohen’s kappa ≥ 0.95, as calculated in BORIS).

**Disbudding Procedure**

During week 4 of life, a subset of calves (4 calves/pen) were randomly assigned to be either disbudded (n = 8; 10 bulls and 2 heifers; 36.2 ± 3.9 d of age) or receive handling only (n = 8; 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 1200h). Calves were disbudded by a trained veterinarian, or by trained veterinary students under direct supervision of the veterinarian. Calves received a non-steroidal anti-inflammatory drug for analgesia 3-4 h prior to disbudding (meloxicam provided orally; 0.5 mg/kg; Unichem Pharmaceuticals USA Inc., Rochelle Park, NJ), a local corneal nerve block 10-15 min prior to disbudding (5 mL of 2% lidocaine hydrochloride; Bimeda-MTC, Cambridge, ON, Canada; injected on each side over the cornual nerve), and a sedative (xylazine hydrochloride; 0.02 mg/kg; Vedco; St Joseph, MI; injected intramuscularly in the neck) approximately 5 minutes
prior to the administration of local anesthesia. 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 pre-heated for at least 10 min prior to cautery disbudding of calves. Hair was clipped over the horn buds, and the calf manually restrained with the head held to the side. The disbudding iron tip was applied firmly over the horn bud for 8-10 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 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.

**Behavioral Observation in the Home Pen**

We recorded behavior for 24 hours during the week before disbudding after all calves in the pen had undergone behavioral testing, to establish baseline measurements for all behaviors. Additionally, behavior was recorded continuously from video for 3 consecutive days for the subset of calves that we observed following disbudding or handling only. Behavioral observation began immediately following the disbudding procedure or handling only (1100 h – 1200 h) according to the ethogram described in Table 3-1. Calves were recorded by a single video camera per pen (Axis M2026-LE Network Camera; Axis Communications, Lund, Sweden) mounted in the center of each pen approximately 3 m from the ground such that the entire double pen could be seen from one camera. 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. A total of 5 observers were used to characterize behavior from video (inter-observer reliability was calculated for one day of video collection, with Cohen’s kappa ≥ 0.90, as calculated in BORIS, for all observer comparisons).
Standing and lying behavior was recorded using accelerometers (HOBO Pendant G Logger, Onset Computer Corp., Pocasset, MA). Following the protocol developed by UBC AWP (2013), data loggers were affixed to the back-rear leg using a bandage (Vetrap Bandaging Tame, #M, Bracknell, UK). Loggers were placed upon entry to the group pens, were replaced as necessary, and remained for the duration of the experiment. Total daily lying time and lying bout frequency were recorded for each calf on every focal day.

**Statistical Analyses**

Data from the behavioral tests were analyzed using a principal component analysis (Proc Factor in SAS). All behaviors recorded as durations were calculated as a percentage of test duration. Latency to contact the unfamiliar calf and unfamiliar human were log-transformed, and duration of contact with the unfamiliar human was square-root transformed to meet assumptions of normality. All variables were standardized to mean of 0 and standard deviation of 1 (Proc Standard in SAS). These data were divided into two principal component analyses.

First, we analyzed responses from the open field and the novel object tests as these tests indicated reactivity to novelty within the environment. We included 5 variables: standing still (duration; averaged across tests), squares crossed (count; averaged across tests), self-grooming (duration; averaged across tests), contact with the novel object (duration; from novel object test only), and the latency to contact the novel object (duration; from novel object test only). These 5 behaviors were subjected to a principal component analysis with varimax rotation to condense correlated measures into principal components. Two principal components with eigenvalues equal to or larger than 1 accounted for 72% of the variance; these were retained for further analyses.

Responses in the unfamiliar calf and the unfamiliar human tests were included in the second analysis to assess features of sociality and included 6 variables: standing still (duration;
averaged across tests), licking and sniffing the wall of the arena (duration; averaged across tests), latency and duration of contact with the unfamiliar calves (durations; unfamiliar calf test), the latency and duration of contact with the unfamiliar human (durations; unfamiliar human test). These 6 behaviors were subjected to a principal component analysis with varimax rotation to condense correlated measures into principal components. Two principal components with eigenvalues equal to or larger than 1 accounted for 61% of the variance; these were retained for further analyses. We calculated factor scores for each calf and initially analyzed the effects of pre-grouping housing treatment on factor scores using general linear mixed models (Proc Mixed in SAS), with early housing treatment (pair-housed or individually-housed during the first two weeks of life) as a fixed effect and group pen as a random effect.

For behavioral data of calves in their home pen (recorded according to ethogram in Table 3-1), we summarized duration and frequency of behavioral data by day (24 h observation period beginning at 1200 h of each calendar day). We additionally calculated lying in the shelter as a percentage of total shelter use, lying in the shelter as a percentage of total lying time. Data were screened for normality prior to analysis using the PROC UNIVARIATE procedure within SAS (version 9.4; SAS Institute Inc., Cary, NC, USA). Daily duration of shelter use was log-transformed, and duration and percentage of lying time were square root-transformed.

To analyze the association between personality traits and behavior, we examined the correlation between factor scores from the principal component analysis and behavior in the home pen (using linear regression (Proc Reg in SAS).

To analyze the effects of disbudding on shelter use, data were analyzed using the MIXED procedure of SAS, in a model including day as a repeated measure and the fixed effects of disbudding treatment, pre-grouping housing treatment, day, and treatment by day interactions,
with calf (or pair of calves, for analysis of social use within treatment) as the subject and pen as a random effect. Pre-grouping housing treatment referred to housing during the first 2 weeks, prior to introduction to the group pen for this trial, and these treatments were balanced across disbudding treatments. In addition, behavior during the baseline day (duration and frequency of shelter use) was included as a covariate for each behavior. The variance-covariance matrix structure was selected on the basis of best fit according to Schwarz’s Bayesian information criterion. 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 = 4 pairs of calves/treatment). For all other analysis, the experimental unit was the individual calf. If data were subject to a treatment by day interaction that was significant or bordering on significant (P ≤ 0.15), we explored differences between treatments by day (using the SLICE command). Consistency in shelter use between observation days was further assessed using linear regression (using PROC REG in SAS).

Values for effects of disbudding on use of the shelter are reported as least squares means. Significance was declared at P < 0.05, and trends were reported if 0.05 ≤ P ≤ 0.10.

Results

Characterization of Personality Traits

Based on data collected during our series of behavioral tests, the principal component analysis revealed factor scores reflective of different personality traits. The factor loadings for the open field and novel object tests are reported in Table 3-2. The first factor from the open field and novel object tests explained 43.7% of the variance and contained high positive loadings for time spent in contact with the novel object, and activity. It also contained a high negative loading for the latency to touch the novel object. We interpreted this factor as representing
exploratory behaviors, with calves loading high on this factor termed “exploratory active”. The second factor from the open field and novel object tests explained 28.9% of the variance and contained high positive loadings for inactivity, time spent self-grooming, and latency to contact the novel object, and a high negative loading for activity. We interpreted this factor as representing inactivity and avoidance of the novel object, with calves loading highly on this factor termed “inactive avoidant”.

The factor loadings for the unfamiliar human and unfamiliar calf tests are reported in Table 3-3. The first factor from unfamiliar human and unfamiliar calf tests explained 40.3% of the variance and contained high positive loadings for time spent licking and sniffing the arena, and latency to contact the unfamiliar calves, with high negative loadings for the duration of contact with the unfamiliar calf and the amount of time spent in contact with the unfamiliar human. We interpreted this factor to represent calf-avoidance and pen-directed behavior, with calves loading highly on this factor termed “calf-avoidant”. The second factor from unfamiliar human and unfamiliar calf tests explained 21.1% of the variance and contained high positive loadings for inactivity and time spent in contact with the unfamiliar human, with high negative loadings for latency to contact the unfamiliar calves and the latency to contact the unfamiliar human. We interpreted this factor to represent human directed and less active behavior, with calves loading highly on this factor termed “human-directed”.

**Development of Personality Traits and Association with Behavior in Social Housing**

Pre-grouping housing affected three out of the four personality factors revealed in the principal component analysis. Results of the analysis of the open field and novel object test indicated that calves housed in pairs rather than individually prior to grouping had higher scores for the factor interpreted as ‘exploratory active (0.75 vs -0.67; previously pair-housed vs individually-housed; SE = 0.44; P = 0.03) and lower scores for the factor interpreted as ‘inactive
avoidant’ (-0.83 vs 0.74; PH vs IH; SE = 0.61; P = 0.004). Results of the analysis of the unfamiliar calf and human test indicated that calves housed in pairs rather than individually prior to grouping had higher scores for the factor interpreted as ‘calf-avoidant’ (0.77 vs -0.68; PH vs IH; SE = 0.50; P = 0.05) and there was no effect of pre-grouping social contact on scores for the factor interpreted as ‘human-directed’ (-0.44 vs 0.39; PH vs IH; SE = 0.44; P = 0.20).

The relationships between personality factors and behavior within the home pen are reported in Table 3-4. Factor scores for ‘exploratory active’ were positively correlated with the amount of time spent lying in the shelter but negatively correlated with total shelter use and time spent standing in the shelter. Factor scores for ‘Inactivate avoidant’ were positively correlated with the amount of time spent standing in the shelter. Factor scores for ‘calf avoidant’ were positively correlated with total shelter use, shelter visit duration, and the amount of time spent lying in the shelter. These factor scores were also negatively correlated with the number of times they chose to lay down in the closed corner side of the shelter, and both social entrances and exits to the shelter. Factor scores for ‘human-directed’ were not related to any measurements related to use of the shelter but were positively correlated with the time spent using the rotating brushes.

Effect of Disbudding on Activity and Shelter Use

Characterization of shelter use and activity are reported in Table 3-5. Overall, calves tended to use the shelter more following disbudding, with no interaction between treatment and day. Shelter visit duration was also greater for the disbudded calves. Time spent sharing the shelter with the calf on the same treatment as a percentage of total shelter use did not differ between treatments. There was no difference in solitary or social entries to the shelter. Control calves exited empty shelters more than disbudding calves, but there was no difference between
treatments for exiting an occupied shelter. There were differences inactivity and resting location preference following disbudding. Overall, disbudded calves spent less time lying than control calves. Disbudded calves also spent greater percentage of their total lying time located in the shelter. This was also subject to a treatment by day interaction, with the greatest difference between treatments on day 2 following disbudding.

**Discussion**

The objective of this study was to evaluate individual differences in calf behavior within social housing, and particularly the use of a shelter offering visual and physical separation from the rest of the pen, and to further investigate findings from Chapter 2 related to how disbudding affects these behaviors. Our results indicate an influence of early life experience and personality traits on various aspects of behavior in group-housed calves and provide further evidence that disbudding affects social behavior in group-housed calves.

**Personality Development and Associations with Behavior**

Development of personality traits and association with behavior in social housing. Social contact prior to grouping influenced factor scores indicative of personality traits, calves housed in pairs in the first two weeks of life were more exploratory and active, whereas individual housed calves were more inactive and avoidant of the novel object. These results can be interpreted as reduced fearfulness in calves with previous social experience are consistent with previous findings of the effect of social housing on response to novelty in dairy calves. Social housing has been shown to reduce food neophobia (Costa et al., 2014), and lessen reactivity to environmental novelty (De Paula Vieira et al., 2012). De Paula Viera et al. (2012) found that calves housed individually were more active, as indicated by increased time spent running, and more reactive, as indicated by increased rates of backing-off events. However, De Paula Vieira et al. (2012) examined calves who had been exposed to varying levels of social contact for the
entire experimental period, whereas our findings are novel in demonstrating an effect of social contact during only the first weeks of life on subsequent behavior after adaptation to social groups. We also found that calves previously raised in pairs had responses during behavioral testing indicative of increased avoidance of the unfamiliar calves. Interestingly, De Paula Vieira et al. (2012) found that individually housed calves were more persistent in their attempts at social interaction. The authors suggest that these differences are indicative of deficits in social behavior in individually housed calves. The greater tendency to contact the unfamiliar calf exhibited by the individually housed calves in the present study could similarly be interpreted as deficits in social behavior. This is supported by spontaneously hypertensive rats, a well-established animal model of ADHD, interacting more frequently with an unfamiliar social companion, compared to controls (Hopkins et al., 2009). Overall, these findings suggest that early social contact influenced the development of aspects of personality in group housed dairy calves. Further research is needed to determine the duration of the effects of social isolation during early life on response to novelty and personality factors.

We found evidence that individual personality traits, as assessed based on responses in the behavioral tests, were associated with various aspects of behavioral expression in the home pen. The tests that we conducted are similar to tests performed in similar assessments of personality in dairy calves (Neave et al., 2018; Lecorps et al., 2018). Interestingly, the ‘exploratory active’ factor which likely reflected reduced fearfulness, was negatively correlated with shelter use duration. There is evidence across species to suggest that antipredator behaviors, such as vigilance behavior in dairy cattle (Welp et al., 2004), are correlated with personality (Jones and Godin, 2010; Andersson et al., 2014; Favreau et al., 2014). The decreased utilization of the shelters by calves who scored more highly as ‘exploratory active’ could be related to
differences in antipredator behavior strategies, and more specifically, expression of vigilance behavior, as the shelter would have limited the calves’ ability to surveil their environment. We also found that the ‘calf avoidant’ factor was positively correlated with shelter use duration, which could be due to a greater preference for social withdrawal. Lecorps (2019) demonstrated that calves that were more pessimistic were more selective in their social relationships, suggesting a link between personality and social preferences in dairy calves. Additionally, the ‘calf-avoidant’ factor score tended to be negatively correlated with frequency of entering an occupied shelter, further suggesting that this personality factor may be related to broader expression of social behavior and social withdrawal.

Personality factors were also correlated with activity surrounding shelter use and use of the rotating brushes during the baseline day. This suggests that personality traits may influence individual interaction with features of their environment. This is supported by calves with higher exploratory active scores tending to spend more time lying, and less time standing in the shelter, whereas those with higher inactive avoidant scores spent more time standing in the shelter. Similarly, calf avoidant calves tended to enter and leave occupied shelters more frequently. Although the human directed factor score did not correlate with behaviors related to shelter use, the more human directed calves spent more time using the rotating brushes. Although the mechanism underlying these changes in behavior relative to personality is unknown, this suggests that calves with different personalities are making different choices about how to interact with their environment. These findings contribute to a currently limited understanding of how personality differences may relate to individual decisions to interact with features of the environment in intensively housed livestock.
The correlations between personality and use of a shelter and interaction with other pen features suggests that personality influences how individuals interact with their environment, and possibly, how they change their behaviors to cope with stressors. Further, mood and personality interact to determine cognitive biases in pigs (Asher et al., 2016). Pigs that displayed a more proactive personality were more likely to approach ambiguous stimuli in a cognitive bias task, regardless of the level of enrichment in their environments. However, pigs with a more reactive personality type were heavily influenced by environmental enrichment, with the enriched reactive pigs behaving more optimistically, and the less enriched reactive pigs behaving more pessimistically. This suggests that environmental enrichment may differentially improve the welfare of animals with certain personalities. Future work should aim to describe the relationship between environmental enrichment, personality, and behavioral changes following stressful events. In Chapter 2 we discuss the importance of providing environmental complexity that allows for more variable behavioral expression. The relationship between personality factors and differences in behavior in the home pen further suggests that accommodating individual preferences within the environment is important to welfare.

**Effects of Disbudding on Activity and Shelter Use**

We found that calves tended to use shelter more per day following disbudding and had longer visit durations. These findings support our results from Chapter 2 of this thesis in which dairy calves were housed in groups of 4 were provided one shelter. In the present study, calves were housed in groups of 8 and were provided 2 shelters, suggesting that this preference for increased shelter use is also present despite changes in social dynamics and physical aspects of the pen in the present study.

In Chapter 2, we observed an increased frequency of entering an empty shelter and leaving an occupied shelter following disbudding, whereas we did not find any difference in
these measures of shelter use in the present study. Instead, we observed that the control calves left an empty shelter more frequently than disbudded calves. Differences in these responses between studies could be due to the increased space and availability of shelters for calves in the present study. Although the calves had the same space per calf as that previously described in Chapter 2, we anecdotally observed that most of the calves in the group would choose to spend their time in the same half of the pen. This would allow for individual expression of social withdrawal in other areas of the group pen that did not require use of the shelter, and evaluation of social behavior outside the shelter would provide further insight into potential preferences for social withdrawal. Space allowance has been shown to alter calf behavior in a variety of ways including increased play behavior (Jensen and Kyhn, 2000) and diurnal patterns of behavior (Tapki et al., 2006).

Decisions to use the shelter may be due to environmental factors. Specifically, the present study was performed during the summer months, from May to September. The ambient temperatures and humidity that is common during summers in Florida could have modified decisions to use the shelters. Although the shelters were placed in positions within the pen where the air flow from the fans would enter, the enclosed nature of the shelters could have still reduced air flow and been a less desirable location.

It is important to note that the calves in this study were provided with a local cornual nerve block, a local analgesic, and a sedative to mitigate the pain associated with disbudding. This pain management protocol has proven to mitigate some of the specific pain behaviors associated with disbudding, including ear flicks and head shaking (Heinrich et al., 2010). However, the changes that we observed in undisturbed resting behavior following disbudding are consistent with previous findings from Chapter 2 which demonstrated that calves reduced their
lying time for at least 3 days following disbudding, even when treated with meloxicam and a local cornual nerve block. In the present study, we observed that disbudding had a persistent effect on lying time, with calves spending less time lying, and lying in the shelter for a greater percentage of their total lying time. Interestingly, in the present study calves were given a xylazine sedative. Xylazine is a2-adrenoceptor agonist that causes central nervous system depression, typically resulting in reduced activity in calves (Scholtysik et al., 1998). Therefore, the retained reduction in lying time after disbudding in calves receiving this sedative is further evidence that disbudding severely impacts resting behavior in calves.

**Conclusion**

Our results suggest that individual behavioral expression of group-housed calves within their home pen, including use of a shelter offering social seclusion, is associated with personality traits, which are subject to early social experience. These results also provide further evidence that calves may use this barrier more following disbudding and spend a greater proportion of their total resting time located in the shelter. Additionally, disbudded calves spent less time resting following the procedure, and the preference for rest in the shelter in individuals who have disturbed resting following disbudding should be examined further.
Table 3-1. Ethogram of behaviors recorded during the four behavioral tests (n=32), in the home pen during the baseline day (n=32) and in the 3 days following disbudding (n=16).

<table>
<thead>
<tr>
<th>Behavior</th>
<th>Description</th>
<th>Measure</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Behavioral Tests</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inactive</td>
<td>Standing still without sniffing or licking the walls or the ground</td>
<td>Duration</td>
</tr>
<tr>
<td>Licking and sniffing</td>
<td>Calf muzzle within 5 cm of the walls of the arena</td>
<td>Duration</td>
</tr>
<tr>
<td>Self-grooming</td>
<td>Contacting any part of the body with muzzle or using a back foot to contact any part of head/neck</td>
<td>Duration</td>
</tr>
<tr>
<td>Activity</td>
<td>Crossing into a new quadrant (test arena divided into 4 equal quadrants)</td>
<td>Frequency</td>
</tr>
<tr>
<td>Latency to contact</td>
<td>Time until the calf touches the object, calf, or human (muzzle within 5 cm)</td>
<td>Duration</td>
</tr>
<tr>
<td>Contacting the stimulus</td>
<td>Calf muzzle within 5 cm of the unfamiliar stimulus</td>
<td>Duration and Frequency</td>
</tr>
<tr>
<td>Calf, human, or object contact</td>
<td>Calf muzzle within 5 cm of object, calf, or human</td>
<td>Duration</td>
</tr>
<tr>
<td>Play initiation</td>
<td>Head butting (head in contact with human), or mock butt where the calf approaches the unfamiliar calf or human with head oriented downward but does not come in contact</td>
<td>Frequency</td>
</tr>
<tr>
<td><strong>Behavior in the home pen</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shelter use</td>
<td>Half or more of the body located within the shelter; calf’s body position (lying or standing) was noted</td>
<td>Duration and Frequency</td>
</tr>
<tr>
<td>Solitary entrance or exit</td>
<td>Entering or leaving a shelter that is unoccupied by another calf</td>
<td>Frequency</td>
</tr>
<tr>
<td>Social entrance or exit</td>
<td>Entering or leaving a shelter that is occupied by at least one other calf</td>
<td>Frequency</td>
</tr>
<tr>
<td>Brush use</td>
<td>Any part of the body in contact with rotating brush</td>
<td>Frequency</td>
</tr>
<tr>
<td>Lying on the shelter’s closed side</td>
<td>Lying down with at least half of the body on the enclosed side of the shelter</td>
<td>Frequency</td>
</tr>
</tbody>
</table>
Table 3-2. Coefficients (loadings) of the eigenvalues for the first two factors extracted by principal component analysis of behavioral measures in the open field and novel object tests to assess calves’ (n=32) response to novel environments.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Factor 1</th>
<th>Factor 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contact with the novel object (% of time)</td>
<td>0.91</td>
<td>-0.2</td>
</tr>
<tr>
<td>Inactive (% of time)</td>
<td>-0.15</td>
<td>0.88</td>
</tr>
<tr>
<td>Self-grooming (% of time)</td>
<td>0.15</td>
<td>0.80</td>
</tr>
<tr>
<td>Latency to contact the novel object (s)</td>
<td>-0.89</td>
<td>0.9</td>
</tr>
<tr>
<td>Activity (number of squares crossed)</td>
<td>0.41</td>
<td>-0.60</td>
</tr>
<tr>
<td>Eigenvalues</td>
<td>2.18</td>
<td>1.45</td>
</tr>
<tr>
<td>Variance explained (%)</td>
<td>43.7</td>
<td>28.9</td>
</tr>
<tr>
<td>Interpretation</td>
<td>Exploratory active</td>
<td>Inactive avoidant</td>
</tr>
</tbody>
</table>
Table 3-3. Coefficients (loadings) of the eigenvalues for the first two factors extracted by principal component analysis of behavioral measures in the unfamiliar calf and unfamiliar human tests to assess calves’ (n=32) response to novel social stimuli.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Factor 1</th>
<th>Factor 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inactive (% of time)</td>
<td>0.21</td>
<td>0.66</td>
</tr>
<tr>
<td>Licking and sniffing (% of time)</td>
<td>0.87</td>
<td>-0.18</td>
</tr>
<tr>
<td>Latency to contact the unfamiliar calves (% of time)</td>
<td>0.52</td>
<td>-0.35</td>
</tr>
<tr>
<td>Contact with the unfamiliar calf (% of time)</td>
<td>-0.85</td>
<td>-0.10</td>
</tr>
<tr>
<td>Latency to contact the unfamiliar human (s)</td>
<td>0.23</td>
<td>-0.75</td>
</tr>
<tr>
<td>Contact with the unfamiliar human (% of time)</td>
<td>-0.36</td>
<td>0.73</td>
</tr>
<tr>
<td>Eigenvalues</td>
<td>2.42</td>
<td>1.15</td>
</tr>
<tr>
<td>Variance explained (%)</td>
<td>40.3</td>
<td>21.1</td>
</tr>
<tr>
<td>Interpretation</td>
<td>Calf avoidance</td>
<td>Human-directed behavior</td>
</tr>
</tbody>
</table>
Table 3-4. Relationships between factor scores from the principal component analysis and use of the shelter and other pen features the baseline day during week 4 of life (n=32).

<table>
<thead>
<tr>
<th>Measure</th>
<th>Social Novelty Factor 1 (calf avoidant)</th>
<th></th>
<th>Social Novelty Factor 2 (human-directed)</th>
<th></th>
<th>Environmental Novelty Factor 1 (exploratory active)</th>
<th></th>
<th>Environmental Novelty Factor 2 (inactivity and avoidance)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Effect Direction</td>
<td>F-value</td>
<td>P-value</td>
<td>Effect Direction</td>
<td>F-value</td>
<td>P-value</td>
<td>Effect Direction</td>
<td>F-value</td>
</tr>
<tr>
<td>Shelter use</td>
<td>+</td>
<td>2.66</td>
<td>0.11</td>
<td>0.09</td>
<td>0.77</td>
<td>-</td>
<td>2.79</td>
<td>0.10</td>
</tr>
<tr>
<td>Shelter visit duration</td>
<td>+</td>
<td>8.58</td>
<td>0.007</td>
<td>0.03</td>
<td>0.85</td>
<td>-</td>
<td>0.60</td>
<td>0.44</td>
</tr>
<tr>
<td>Lying in shelter, min/d</td>
<td>+</td>
<td>3.48</td>
<td>0.07</td>
<td>0.02</td>
<td>0.89</td>
<td>+</td>
<td>3.37</td>
<td>0.10</td>
</tr>
<tr>
<td>Standing in the shelter</td>
<td></td>
<td>0.13</td>
<td>0.72</td>
<td>1.12</td>
<td>0.29</td>
<td>-</td>
<td>3.14</td>
<td>0.08</td>
</tr>
<tr>
<td>Lying down in the closed side of the shelter no/d</td>
<td>-</td>
<td>3.67</td>
<td>0.08</td>
<td>0.01</td>
<td>0.90</td>
<td>0.02</td>
<td>0.88</td>
<td>0.16</td>
</tr>
<tr>
<td>Solitary entrance, no/d³</td>
<td></td>
<td>0.92</td>
<td>0.35</td>
<td>0.03</td>
<td>0.86</td>
<td>0.00</td>
<td>0.96</td>
<td>1.06</td>
</tr>
<tr>
<td>Social entrance, no/d¹</td>
<td>-</td>
<td>3.60</td>
<td>0.06</td>
<td>0.73</td>
<td>0.40</td>
<td>0.01</td>
<td>0.93</td>
<td>0.73</td>
</tr>
<tr>
<td>Solitary exit, no/d¹</td>
<td></td>
<td>0.57</td>
<td>0.46</td>
<td>0.07</td>
<td>0.79</td>
<td>0.33</td>
<td>0.57</td>
<td>1.74</td>
</tr>
<tr>
<td>Social exit, no/d¹</td>
<td>-</td>
<td>3.19</td>
<td>0.08</td>
<td>0.89</td>
<td>0.35</td>
<td>0.38</td>
<td>0.53</td>
<td>0.58</td>
</tr>
<tr>
<td>Time spent using the brushes</td>
<td>-</td>
<td>0.09</td>
<td>0.77</td>
<td>6.87</td>
<td>0.01</td>
<td>0.01</td>
<td>0.93</td>
<td>0.41</td>
</tr>
</tbody>
</table>

1Solitary entrances or exits defined when the focal calf entered or exited the shelter when it was otherwise unoccupied. Social entrances or exits defined when the focal calf entered or exited the shelter when another calf was occupying it.
Table 3-5. Activity and use of a shelter providing visual isolation in group-housed calves (8 calves/pen) subjected to disbudding (DB; 
n = 8, 2 calves observed/pen) or handling only (CON; n = 8, 2 calves observed/pen), for 3 days (D) following treatment (T) 
at 36 d of age.

<table>
<thead>
<tr>
<th>Item</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>95% CI</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>95% CI</th>
<th>T</th>
<th>D</th>
<th>TxD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shelter use, min/d</td>
<td>82.3</td>
<td>130.0</td>
<td>215.4</td>
<td>(51.0, 340.0)</td>
<td>78.3</td>
<td>92.0</td>
<td>111.6</td>
<td>(35.9, 238.4)</td>
<td>0.085</td>
<td>0.09</td>
<td>0.59</td>
</tr>
<tr>
<td>Shelter visit frequency, no/d</td>
<td>8.2</td>
<td>7.5</td>
<td>9.0</td>
<td>(5.6, 10.9)</td>
<td>8.0</td>
<td>10.0</td>
<td>9.5</td>
<td>(6.6, 11.8)</td>
<td>0.508</td>
<td>0.678</td>
<td>0.42</td>
</tr>
<tr>
<td>Lying time, h/d</td>
<td>16.7</td>
<td>16.6</td>
<td>17.3</td>
<td>(16.3, 17.8)</td>
<td>17.0</td>
<td>17.3</td>
<td>17.4</td>
<td>(16.7, 17.8)</td>
<td>0.039</td>
<td>0.067</td>
<td>0.57</td>
</tr>
<tr>
<td>Lying in the shelter, % of total lying</td>
<td>7.3</td>
<td>10.7</td>
<td>26.0</td>
<td>(4.8, 27.0)</td>
<td>9.1</td>
<td>6.3</td>
<td>7.3</td>
<td>(1.9, 19.1)</td>
<td>0.059</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>lying time</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Solitary entrance, no/d</td>
<td>2.7</td>
<td>2.8</td>
<td>4.0</td>
<td>(2.2, 4.2)</td>
<td>2.2</td>
<td>4.1</td>
<td>4.3</td>
<td>(2.5, 4.5)</td>
<td>0.887</td>
<td>0.053</td>
<td>0.36</td>
</tr>
<tr>
<td>Social entrance, no/d</td>
<td>4.8</td>
<td>4.3</td>
<td>4.8</td>
<td>(2.6, 6.7)</td>
<td>3.9</td>
<td>5.5</td>
<td>4.6</td>
<td>(2.5, 6.8)</td>
<td>0.371</td>
<td>0.846</td>
<td>0.54</td>
</tr>
<tr>
<td>Solitary exit, no/d</td>
<td>3.1</td>
<td>2.8</td>
<td>3.3</td>
<td>(1.8, 4.3)</td>
<td>5.9</td>
<td>6.4</td>
<td>6.9</td>
<td>(4.5, 8.4)</td>
<td>0.044</td>
<td>0.782</td>
<td>0.87</td>
</tr>
<tr>
<td>Social exit, no/d</td>
<td>3.8</td>
<td>2.9</td>
<td>5.0</td>
<td>(2.1, 6.2)</td>
<td>4.0</td>
<td>4.7</td>
<td>4.4</td>
<td>(2.4, 6.9)</td>
<td>0.71</td>
<td>0.477</td>
<td>0.49</td>
</tr>
</tbody>
</table>

1Reported means and CI are back-transformed from data that were log-transformed (shelter use) or square root-transformed (frequency social exits, lying in the shelter and social use within treatment, % of total use, lying in the shelter, % of total lying time, and frequency of lying on the closed side).

2Solitary entrances or exits defined when the focal calf entered or exited the shelter when it was otherwise unoccupied. Social entrances or exists defined when the focal calf entered or exited the shelter when another calf was occupying it.
Figure 3-1. Overhead view of the pen, depicting both shelters (three-sided, open-top barriers made out of corrugated plastic; 1.2 × 1.2; width × depth) and their location within the pen relative to other resources. Photo courtesy of author.
CHAPTER 4
GENERAL DISCUSSION AND SUMMARY

Group-housing of dairy calves has gained popularity in recent years and provides numerous welfare benefits (reviewed by Costa et al., 2016) including increased solid feed intake (Miller-Cushon and DeVries, 2016), reduced neophobia (Costa et al., 2014), and increased competitive success after weaning (Duve and Jensen, 2012). However, despite the clear benefits of social housing, there is a need for better understanding of the preferences and social behavior of calves in these environments. Gregarious animals, including cattle, express highly complex social behaviors which serve to initiate and maintain social bonds and establish dominance hierarchies. Dairy calves form preferential social bonds with conspecifics and will work for access to social partners, indicating that expression of social behaviors is highly motivated. However, evidence of social withdrawal in certain situations, including prior to parturition in dairy cattle (Proudfoot et al., 2014a) suggests that social needs are complicated and perhaps not well accommodated in typical production settings.

A major component of this thesis was the investigation of changes in social behavior, including social withdrawal, following disbudding. Previous research surrounding disbudding has focused primarily on behavioral and physiological indicators of acute pain. While previous findings indicate that disbudding causes pain and elicits pain-specific behaviors in the hours and days following the procedure, there is less knowledge of how disbudding affects natural behavior in the home pen over time.

The primary aim of this thesis was to determine if dairy calves make use of a shelter providing the opportunity for social withdrawal, and if use of this shelter would change following disbudding. Calves made use of this novel pen feature, with every calf entering the shelter at least once (Chapter 2). However, use of the shelter was highly variable, with calves
using the shelters from a range of 10.8 min/d to 20.7 h/day (Chapter 2). There was also significant pen-level variability in shelter use, suggesting that this behavior may be socially facilitated. Social facilitation is common in dairy cattle, and has been observed in lying behavior (Stoye et al., 2012), and in feeding behavior (Stoye et al., 2012).

Calves used the shelters more following disbudding when provided one shelter and housed in groups of 4 and tended to use the shelters more following disbudding when provided 2 shelters and housed in groups of 8. This treatment effect despite considerable individual and pen-level variability suggests that there was some feature of this space that was preferred by calves after the aversive experience of hot-iron disbudding. Although the specific mechanism driving this increased use of the shelters is unclear, it could be due to specific avoidance of other calves. This avoidance of pen-mates could be driven by an avoidance of jostling due to the increased play that has been observed in non-disbudded calves (Mintline et al., 2013). Disbudded calves display wound sensitivity of disbudding wounds for at least 14 weeks following the procedure (Casoni et al., 2019a), suggesting that in the days following the procedure, their disbudding wounds would be sensitive to physical stimulation. Further, in Chapter 2 we observed that calves entered an empty shelter more frequently and left an occupied shelter more frequently, indicating that preference for social seclusion may be motivating increased shelter use. However, this characterization of entries and exits to the shelter was not observed in Chapter 3. Therefore, we cannot definitively determine if increased shelter use was motivated by a specific avoidance of conspecifics. Future work should include observations of social behavior outside of the shelters to more thoroughly characterize social behavior expression following disbudding and ascertain specific motivations driving these changes in behavior.
Alternatively, increased use of the shelters in disbudded calves could be due to a specific motivation for social support, as pairs of disbudded calves spent more time sharing the shelter than pairs of control calves (Chapter 2). Although the shelter was originally designed for one calf to be able to enter and turn around comfortably, we frequently observed social use of the shelter. In Chapter 2, we also observed that calves spent the same amount of time sharing the shelter with another calf regardless of treatment. Effects of social buffering has been observed in dairy calves at weaning, in which pair housed calves vocalize less than individually housed calves (Bolt et al., 2017). Additionally, across species, social buffering serves to reduce stress in response to novel stimuli and environments (Beery and Kaufer, 2015; Hennessy et al., 2008). Therefore, caution should be taken when interpreting results from Chapters 2 and 3 that discuss use of the shelter as an indicator of preference for social withdrawal. Future research should utilize different sized shelters and observations of behaviors within the shelters to better characterize decisions to utilize the shelter socially or alone.

In Chapter 2 we found that disbudded calves used the shelters more during the day. This diurnal effect on shelter use has been demonstrated in dairy cattle at parturition, whereby cows preferred to calve behind a barrier during the day, but not at night (Proudfoot et al., 2014a). This increased use of protective structures during the day could be driven by a specific motivation for human avoidance. In the case of disbudding, calves could be seeking protection from the humans who caused them pain and stress. Dairy cattle are able to distinguish between individual humans, and learned fear of specific handlers has negative consequences for production (reviewed by Rushen et al., 1999). Further, hourly use of the shelter by disbudded calves increased during the morning hours that coincided with the morning milk feeding and the return of farm and research
personnel to the unit. This suggests that human activity may affect the undisturbed behavior of dairy calves.

We also found that disbudded calves spent less time lying than control calves. Effects of disbudding on lying behavior are inconsistent across the literature, which could in part be due to the different methods used in each experiment. Doherty et al. (2007), using 10-min scan sampling, found no effect of disbudding, with varying doses of local anesthetic, on lying time compared to calves that were not disbudded. However, the more sensitive methods used in both Theurer et al. (2012), using accelerometers recording body position every second, Henrich et al. (2010), using accelerometers recording every 30 s, described decreased lying time in calves disbudded without pain relief, compared to calves receiving meloxicam. Similarly, in Chapter 2 we watched behavior continuously for 72 and in Chapter 3 of this thesis we used accelerometers that recorded body position every minute hours to characterize lying behavior. This suggests that a decrease in lying time may be expected in calves experiencing pain post-disbudding and could be related to elevated levels of pain and discomfort that prevented quality rest. Further, we found that calves spent a greater proportion of their lying time located within the shelter following disbudding. Resting location preferences in dairy cattle have not been well described, however Senft et al. (1985) observed that grazing heifers chose to rest in areas with high grass cover at night, which may suggest some natural preference for shelter. The decision to spend more time resting within the shelters in calves who display disturbed resting behavior after disbudding warrants further investigation into resting location preference, particularly after disbudding or other similar stressful events.

An additional objective of this thesis was to investigate the role of personality in decisions to utilize the shelter, and to evaluate the role of early life social contact on personality
development. Due to the degree of individual variability observed in Chapter 2, we further examined how individual differences in personality mediate decisions to use the shelters and interact with other pen features such as the rotating brushes. We found that social contact in early life affected personality traits after grouping. Calves that were housed individually in the first two weeks of life had higher factor scores for the ‘inactive avoidant’ factor, whereas calves housed in pairs had higher scores for the ‘exploratory active’ and ‘calf avoidant’ factors. This indicates that social deprivation in early life has a carry-over effect on personality development, as the behavioral tests used to assess personality were performed after the calves had been housed in groups for two weeks. This also indicates that there may be a sensitive period of development during the first two weeks of life in which social contact is necessary to adequately develop aspects of personality. Further research is needed to evaluate the longevity of these effects of early life experience on personality.

We found that behavior within the home pen was affected by differences in personality, with each of the four personality factors that we described effecting at least one behavioral measurement. Differences in personality among conspecifics is unsurprising: it is thought that behavioral variability has adaptive consequences because social structures such as dominance hierarches reduce daily conflict among conspecifics (Bergmüller and Taborsky, 2010). Personality differences have been described in dairy cattle (Neave et al., 2018; Lecorps et al., 2018; Neave et al., 2020), however further research is needed to determine how we can allow for broader behavioral expression in intensive farming systems. The opportunity for more variable behavioral expression may differentially improve the welfare of animals of certain personality types.
The timeframe of changes in behavior following disbudding is an area where further research is recommended. Behavioral responses to disbudding may be evidence in shorter timeframes coinciding with stages of pain relief. However, the Experiments described in Chapter 2 and 3 were not designed to detect pain-specific behaviors, but rather to investigate subtle changes in behavior following disbudding, including those which exhibited diurnal patterns. Observation of these normal patterns of behavior, such as activity and feeding, which are also subject to external factors, may not yield meaningful indicators of pain on shorter time frames. However, further understanding of the role of pain severity in the behavioral changes we observed may be better understood through inclusion of calves likely to experiencing various levels of pain. In the present study, we are unable to determine if changes in behavior relate to pain associated with disbudding, or a more general stress response. Further research evaluating the effects of disbudding pain on social behavior should include varying degrees of pain mitigation and the inclusion of other indicators of pain, such as pain-specific behaviors.

The benefits of providing choice within the environment has implications for improving welfare for animals experiencing pain and for animals of different personality types. It has been argued that providing choice within the environment improves welfare because it allows animals to make choices that benefit them and allows for broader behavioral expression. In Chapters 2 and 3 we observed that, although they spent less time lying following disbudding, calves spent a greater proportion of their lying time located in the shelter. Although the mechanism driving rest in the shelters is unclear, choices of specific resting location in calves experiencing pain and exhibiting altered resting behavior may have important welfare consequences and should be explored further. In Chapter 3 we observed that calves with higher scores for the ‘calf avoidant’ factor spent more time using the shelters, while calves with higher scores for the ‘inactive
avoidant’ factor spent less time using the shelters. This indicates that the availability of the shelter may differentially affect the welfare of the calves depending on personality traits. This also speaks to the importance of evaluating individual variability when investigating the welfare implications of novel pen features or procedures. Further research is needed to determine the role of personality on behavioral expression when experiencing pain.

It is important to note that the calves in Chapter 2 were given a local anesthetic and analgesic, and the calves in Chapter 3 were given a local anesthetic, an analgesic, and a sedative. Our findings of persistent behavioral changes in disbudded calves, even with provision of local anesthetic, analgesic, and a sedative, suggest that current approaches to mitigate pain following disbudding may be insufficient. This evidence of prolonged changes in aspects of social behavior and rest contributes to our somewhat limited knowledge of non-evoked behavioral states following disbudding with analgesia. Research concerning long-term 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.

This research has identified a need for further investigation into methods for pain mitigation in the days and weeks after disbudding, and individual assessments of pain in livestock. More interest has been given to individual variability in the experience of pain and expression of related behaviors in veterinary medicine. Veterinarians regularly use behavior-based scales to evaluate pain levels in animals to inform their decisions about pain management protocol on the individual level. The utilization of these pain scales to assist in pain management strategies in clinical settings has been proven effective in horses and donkeys (Ashley et al., 2010), and in dogs (Hansen, 2003). Additionally, Lush and Ijichi (2018) have demonstrated that differences in
personality (measured using Monash Canine Personality Questionnaire- Revised) in dogs are related to pain score (as assessed using the Short-Form Glasgow Composite Measure Pain Scale), with dogs that were rated as highly “extraverted” having significantly higher pain scores. The authors of this study conclude that personality may be a useful tool in the clinical assessment of pain. Despite the promising research in companion animals that suggests differences in personality may be an effective tool for customizing pain mitigation protocol, this relationship between expression of pain and personality type has not been evaluated in dairy calves. Further research should explore this relationship between personality and expression of pain-related behaviors to develop effective pain mitigation protocol that is tailored to the individual.


BIOGRAPHICAL SKETCH

Katie Nicole Gingerich was born in Clearwater, Florida, USA in 1994 to Dave and Linda Gingerich. She attended St. Paul’s School from kindergarten through middle school. For high school she attended Palm Harbor University High School and was enrolled in the medical magnet program. During her undergraduate studies she took classes at Santa Fe College and St. Petersburg College before transferring to the University of Florida where she earned her bachelor’s degree in animal sciences in 2018. She began her Master of Science degree at the University of Florida under the supervision of Dr. Emily Miller-Cushon. During her graduate program she studied dairy calf behavior and welfare with a special interest in understanding the connection between social behavior and affective state. She earned her Master of Science degree in the Department of Animal Sciences in Summer 2020.