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# Heart rate variability: a biomarker of dairy calf welfare

*J. B. Clapp*[A,B], *S. Croarkin*[A], *C. Dolphin*[A] *and S. K. Lyons*[A]

[A]School of Agriculture, Food and Rural Development, Newcastle University, Newcastle upon Tyne, UK.
[B]Corresponding author. Email: jim.clapp@ncl.ac.uk

**Abstract.** Dairy calf welfare is recognised to be compromised from common management practices. In this study heart rate variability (HRV) was used to measure stress in 25 young dairy calves to quantify the degraded welfare they experienced from weaning separation and isolation and also the painful disbudding procedure. It was shown the time spent on the cow before separation had a significant negative correlation to HRV ($r^2 = -0.68$, $P = 0.03$). The longer a calf spent in isolation the lower its HRV 3 days after joining a group pen ($P = 0.037$). The removal of a dummy teat elicited a significant drop in HRV ($P = 0.05$), identifying the addictive properties of sucking in calves. Post disbudding stress, reflected by declining HRV values, was only partly alleviated by the non-steroidal anti-inflammatory drug meloxicam after 48 h. The findings showed calf welfare would be improved by reducing the time between birth and separation and also the days spent in single pens. Providing dummy teats for individually housed calves showed potential as a positive environmental enrichment. Meloxicam may improve welfare by alleviating some chronic pain following hot iron disbudding. We conclude these findings illustrate that HRV, as a science-based animal-centric biomarker of animal welfare, may be used to help improve farmed animal practice.

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## Introduction

Heart rate variability (HRV) is a measure of autonomic nervous system balance between parasympathetic and sympathetic activity and as such is purported to reflect the stress level of farm animals (von Borell *et al.* 2007) and work stress in humans (Jarczok *et al.* 2013). More specifically, a reduced parasympathetic or vagal tone is physiologically linked to stress, making depressed HRV a potential marker for stress and poor health (Thayer *et al.* 2012). Furthermore a low HRV is a recognised risk factor for pathophysiology and psychopathology (Thayer and Lane 2009). Enhanced vagal activity, associated with higher HRV, has been shown to prevent body tissue damage through regulating cytokines associated with inflammation and autoimmune disease also with tumour necrosis factor, which is linked to endotoxaemia (Olofsson *et al.* 2012).

HRV is quantified in several ways; the most informative parameter is the root mean square of successive differences (RMSSD) in inter heart beat intervals (von Borell *et al.* 2007). This is a time domain measure of the beat-to-beat variations associated with vagal heart regulation (von Borell *et al.* 2007). Frequency domain measures of HRV can also be used, with the high frequencies (HF) being associated with vagal activity and low frequencies (LF) predominantly sympathetic in origin; such measures are best calibrated to the animals' resting respiratory rate (von Borell *et al.* 2007). A persistent drop in RMSSD or increased LF to HF ratio, measured in a resting animal, is taken to indicate the individual is stressed (von Borell *et al.* 2007). Stress in the context of this study is defined by Koolhaas *et al.* (2011) as when an animal is not adapting to a stressor from the environmental demand exceeding its capacity to respond. HRV in cattle can be used to measure such stress from physical, pathological and emotional origins (von Borell *et al.* 2007).

Human twin studies have shown RMSSD and HF measures of HRV, including stress-induced HRV changes, to be significantly heritable also low HRV may be an endophenotype for a broad range of dysfunctions (Thayer and Lane 2009). Furthermore between-subject variation in HRV, in addition to heritability, can depend on age, sex and environmental factors (Task Force of the European Society of Cardiology the North American Society of Pacing Electrophysiology 1996). Individually, dairy cattle have been shown to have developmentally stable traits relating to their behaviour and stress responsiveness (Van Reenen *et al.* 2005), consequently it is expected that dairy calf HRV will show significant inter-individual variation. This may preclude numerically small studies comparing HRV between treatment and control animals, making longitudinal cross over designs more preferable.

In a chronic pain study in children, it was shown applying additional pain failed to reduce their HRV further, resulting in a characteristic static HRV response (Evans *et al.* 2013). This finding may limit the value of measuring HRV changes in already chronically stressed dairy calves however they should be identifiable by having a significantly lower resting HRV than unstressed calves.

Typically the management of young dairy calves is inherently stressful and compromises their welfare, from early weaning (both nutritional and separation), isolation and routine surgical procedures such as dehorning (Phillips 2002; Weary *et al.* 2008). The aim of this study was to explore dairy calf HRV as a biomarker of their stress levels and thus welfare, with the objective of identifying how the care and management could be changed to improve their future welfare.

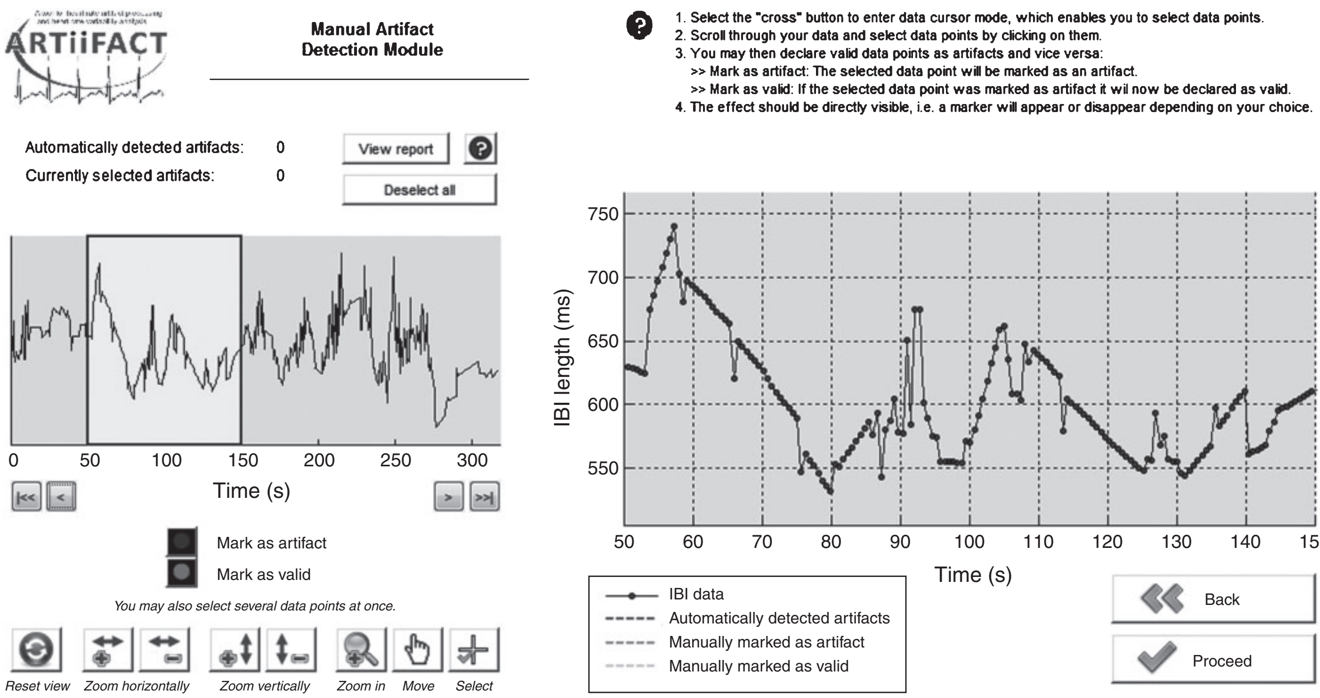
## Materials and methods

### *Animals and management*

A total of 25 young Friesian dairy calves of mixed sex (8 entire bulls and 17 heifers) were studied over a 4-week period in July 2013 at the Newcastle University Nafferton farm. At the start of the study 13 calves were already separated from their mothers and housed in the solid-sided individual pens (150 by 73 cm with 90-cm-high sides); over subsequent weeks the remaining calves became available, following separation, after they were born. The mean (±s.d.) age at separation was 2.68 ± 0.90 days and varied because of management and post calving factors such as milk fever or lameness in the cows. The separated calves were trained by farm staff to drink fresh milk from a bucket provided twice daily at 0900 hours and 1500 hours. After a few days in the single pens a home mix concentrate (beans, wheat and multi-mineral 40 : 60 : 2.5 by weight respectively) was provided *ad libitum*. Calves remained in the single pens for on average (±s.d.) 13.3 ± 5.0 days, depending on when group pens became available for them to be transferred to. The group pens were 315 cm wide and 325 cm deep with 105-cm-high solid sheet metal sides, each housing four calves. In the group pens the twice daily milk ration also the home mix concentrate were continued with in addition *ad libitum* hay and water. Calves in both single and group pens were deep bedded on barley straw.

### *Heart monitor*

Between 1000 hours and 1200 hours each weekday, Polar Equine RS800CX Science (Polar Electro UK Ltd, Heathcote Way, Warwick, UK) heart monitors were applied to each calf for a minimum of 30 min. To accustom the calves to wearing the monitors for the first time this period was extended to 2–3 h. Body hair was not shaved but a copious amount of electrode gel (Signagel, Parker Laboratories Ltd, Fairfield, NJ, USA) was applied to ensure a good electrical contact (von Borell *et al.* 2007). The heart data was downloaded from the watch receiver each day and processed using both the Polar and Artiifact (Kaufmann *et al.* 2011) software to produce the RMSSD value of HRV. The section of the heart trace used for analysis in accustomed calves was the 5–10-min time segment after the monitor had been applied; when the undisturbed calves had resting heart rates. For each calf the segment of trace used was kept between 500 and 520 beats, similar to Stewart *et al.* (2009) who used 512 beats. The time period varied with heart rate, younger calves being higher, but this was typically around the recommended 5-min duration (Task Force of the European Society of Cardiology the North American Society of Pacing Electrophysiology 1996). Error corrections to the trace were carried out using the default Polar setting of moderate filter power with a minimum beat protection of six beats per minute. Although Stewart *et al.* (2009) rejected any trace requiring an error correction exceeding 5%; we rejected those over 2% because above this value we found that the Polar and Artiifact RMSSD values became divergent. The Artiifact software both automatically removed anomalous HRV spikes also allowed the detection and manual removal of 'flats' and 'stairs' (Fig. 1), which represented a loss of signal. Although the time domain HF was not used in this study, this could only be achieved using the Artiifact software because the Polar high frequency upper limit of 0.5 Hz is lower than the 0.83 Hz appropriate for calves (von Borell *et al.* 2007).



Manual Artifact Detection Module

Automatically detected artifacts: 0

Currently selected artifacts: 0

1. Select the "cross" button to enter data cursor mode, which enables you to select data points.
2. Scroll through your data and select data points by clicking on them.
3. You may then declare valid data points as artifacts and vice versa:
   >> Mark as artifact: The selected data point will be marked as an artifact.
   >> Mark as valid: If the selected data point was marked as artifact it wil now be declared as valid.
4. The effect should be directly visible, i.e. a marker will appear or disappear depending on your choice.

You may also select several data points at once.

**Fig. 1.** Screen shot of Artiifact manual detection module. Multiple 'flats' and 'stairs' are seen that require manual removal (the program does not automatically detect them), this trace was rejected because little remained after correction.

### Dummy teats

The concept of using dummy teats to reduce weaning stress in calves was derived from the common use of pacifiers to calm human infants. Dummy teats were constructed from a 30-cm$^2$ board and an auto-feeder teat (Mole Valley Farmers, South Molton, Devon, UK). The teat was secured to the board in a centrally drilled hole and the board attached by cable ties to the front gate of each individual pen. Dummy teats were provided to isolated calves for a 5-day period.

### Hot iron disbudding

Three days before the end of the study, hot iron disbudding was carried out on the calves under local anaesthetic (Adrenacaine, Norbrook, Newry, Northern Ireland) using a standard electric disbudding iron (Mole Valley Farmers). In brief the calves were caught while still wearing a heart monitor and 3–5 mL of Adrenacaine (5% procaine hydrochloride with 1 : 50 000 adrenaline) injected beneath the right and left temporal ridge to block the respective cornual nerves. Eight of the 18 calves were randomly selected to concurrently receive a single dose (0.5 mg/kg) of the non-steroidal anti-inflammatory drug (NSAID) meloxicam (Metacam, Boehringer Ingelheim, Ingelheim am Rhein, Germany), given subcutaneously in the mid neck region. Hair was clipped from around the horn buds to make them clearly visible. Disbudding proceeded ~10–15 min later, satisfactory anaesthesia was assessed first by the observed dropped upper eye lashes and then from being unresponsive to touching the skin-horn margin with the hot iron (700°C). If the calf reacted at this point a further 3 mL of local anaesthetic was administered to the sensitive side and the calf reassessed 10 min later and only disbudded if unresponsive. All calves were under 2 months old, so defined as disbudding (Stock *et al.* 2013), with no significant difference ($P$ = 0.506) using a two sample $t$-test, between the mean age of treated (32.5 days old) and control calves (36.0 days old).

### Approach score

In order to measure fearfulness in the 18 group-penned calves before and after disbudding an approach score was used (Welfare Quality® 2009). The score, on a scale from 0 to 4, relates to how close the tester's right hand was (held out in front of them at calf head height) when the calf backed away. A score of '0' equates to the tester being able to touch the calf's nose and '4' the calf backs away at 2 m or more; the rate of approach was ~1 m per second. The approach score was not carried out by the researchers who disbudded the calves.

### Statistical analyses

All data was analysed using Minitab 16 (Minitab Ltd, Brandon Court, Coventry, UK). To determine if parametric techniques could be used, data was tested for normality and equal variance using the Kolmogorov–Smirnov and Levene's tests respectively. Logarithmic transformation was used where appropriate. Parametric tests included Pearson's correlation, paired and two sample $t$-tests with equivalent non-parametric tests being Spearman's rank, Wilcoxon's and Mann–Whitney tests respectively. $P$-values less than 0.05 at a 95% confidence interval were taken to show a statistically significant difference.

## Results

### Separation stress and weaning age

Log-transformation of the RMSSD values was used to achieve a normal distribution for statistical analysis. The time spent on the cow before separation had a significant negative correlation to log RMSSD values, $r^2$ = –0.68, $P$ = 0.03 and $r^2$ = –0.35, $P$ = 0.045, on the day of separation and 3 days after respectively (Fig. 2).

### Single pen isolation stress

Using Spearman's rank order correlation a significant negative correlation ($r_s$ = –0.61, $P$ = 0.037) was shown between the RMSSD value in calves 3 days after joining a group pen and the number of days they had spent in the single pens beforehand (Fig. 3).

### Dummy teat dependence

There was no significant difference in RMSSD values between calves with and without dummy teats, with both categories of calf showing a significant increase in HRV over a 5-day period. However, a significant ($P$ = 0.05) decline in HRV was shown following the removal of the dummy teats from seven single-penned calves.

### Hot iron disbudding

Following disbudding RMSSD values showed an overall non-significant declining trend in both the 8 meloxicam-treated and 10 untreated calves, this became significant after 2 days post disbudding in calves not given meloxicam (Fig. 4). When both groups were combined there was a highly significant decrease ($P$ = 0.008) in HRV between 1 day before and 2 days after disbudding.

### Approach score

Approach scores showed a significant increase ($P$ < 0.001) on both 1 and 2 days following disbudding compared with the day

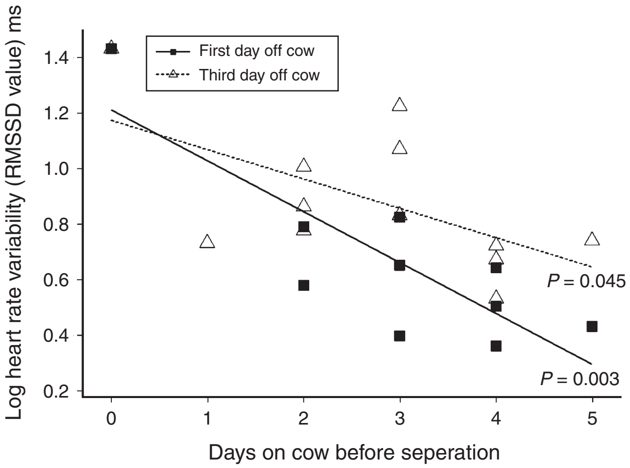


**Fig. 2.** The significant negative effect of the number of days spent with the cow following birth on heart rate variability (using the log RMSSD value), both on the day ($r^2$ = –0.68, $P$ = 0.003, $n$ = 10) and 3 days ($r^2$ = –0.35, $P$ = 0.045, $n$ = 12) after separation.

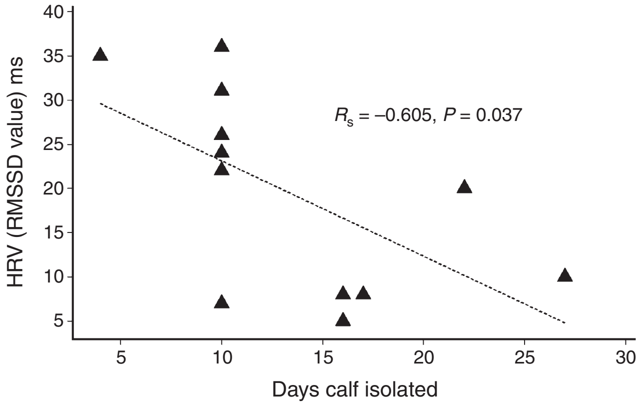


**Fig. 3.** The significant correlation (Spearman's rank order, $n = 12$) between the number of days spent by a calf in single pen isolation and its subsequent heart rate variability (HRV) 3 days after joining a group pen.

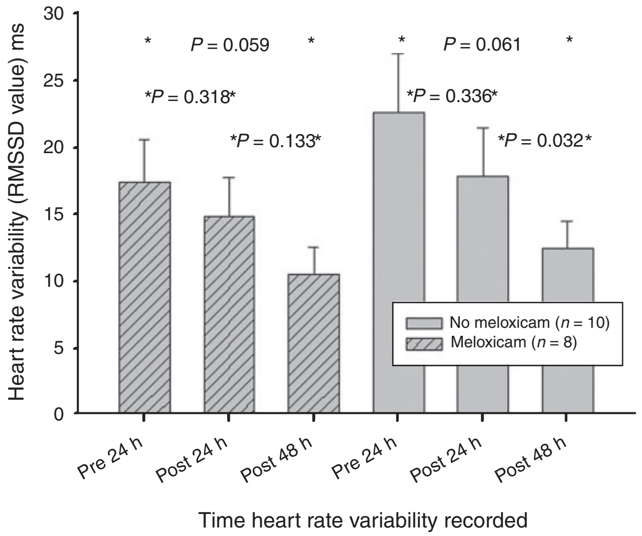


**Fig. 4.** Heart rate variability in calves the day before and on the 2 days following hot iron disbudding. Eight calves were given subcutaneous meloxicam (0.5 mg/kg) at the same time as the local anaesthetic and 10 calves were controls, receiving just the local anaesthetic. Columns show the mean with a standard error bar, $P$-values derived from paired $t$-test between columns identified with *.

before disbudding, signifying their increased fearfulness. There was no significant difference ($P = 0.272$) using a two sample $t$-test, in approach scores between the 8 meloxicam-treated (mean 1.63) and 10 untreated (mean 1.20) calves on the second day after disbudding.

## Discussion

### Separation stress and weaning age

The finding of this study using HRV to measure stress agrees with published work showing that separation stress increases in dairy calves with time after birth (Weary and Chua 2000; Stěhulova *et al.* 2008). The results (Fig. 2) show a clear disadvantage, in terms of increased stress, from keeping the calf on the cow beyond 2 days. Although Weary and Chua (2000) argue there could be health advantages from colostrum by keeping the calf with its mother, calves could still be fed colostrum after separation. It is important to note that the calf stress levels, here measured using HRV, still remained significantly affected by weaning age 3 days after separation, showing the lasting detrimental influence of later separation.

### Single pen isolation stress

From these results the longer a calf spent in the single pens the more stressed it was, in terms of a lower HRV, after joining a group pen. This agrees with a long held view that social isolation reduces a calf's ability to appropriately interact in group situations, including being dominated by conspecifics (Broom and Leaver 1978; Phillips 2002). Furthermore it would be expected such isolation stress would be greater the longer the time difference between it and the other group members, however this study was too small to show such a relationship.

### Dummy teat dependence

Non-nutritive sucking behaviour in calves has been associated with post weaning feeding deficiencies including the absence of the cow (Phillips 2010; Veissier *et al.* 2013) also teat sucking in calves is stress reducing shown by the fact that it lowers their blood levels of the stress hormone cortisol (Lupoli *et al.* 2001). Furthermore weaning stress in calves was reduced using a teat without it being a source of milk (Budzynska and Weary 2008). In our study calves with and without teats both showed a significant increase in HRV over a 5-day period implying both groups had declining stress levels. However, the calves with teats became stressed, shown by a significant drop in HRV, when the teats were removed from their pens. An addiction to sucking milk has been described in the literature and may be linked to both the milk containing opioids and the physical action of sucking causing endogenous opioid release in the calf (Weary *et al.* 2008). Consequently it is proposed these calves were showing signs of opiate addiction withdrawal symptoms, brought on by the removal of the dummy teats. There was some concern before the study that providing dummy teats may interfere with the transition from sucking the cow to bucket feeding milk. This was allayed by the farm staff who reported having no more difficulty in this respect with calves that had dummy teats.

### Hot iron disbudding

Hot iron disbudding produces both acute and chronic pain in calves, which is only partly mitigated by using local anaesthetics (Petrie *et al.* 1996), although current thinking is that concomitant use of NSAID such as meloxicam (Heinrich *et al.* 2009) and sedatives would provide more complete analgesia (Stock *et al.* 2013). In this study RMSSD values were lower than those reported in a similar study on hot iron dehorning of dairy calves (Stewart *et al.* 2009). This may have been the result of concomitant stress in our calves with the added effect of producing a relatively static HRV response (Evans *et al.* 2013) to disbudding. Alternatively the different data correction limits set between this and the Stewart *et al.* (2009) study may have been partly responsible. It is proposed that in this study because we used two different software programs to determine RMSSD values, that were effectively identical, this validated our methodology. A further explanation for the non-significant effect comparing before and after disbudding and treatment with or without meloxicam, is the widely reported inter-individual variation of HRV (Task Force of the European Society of Cardiology the North American Society of Pacing Electrophysiology 1996). To resolve this, numerically larger longitudinal studies should be carried out in future.

### *Approach score*

Approach score results showed a significantly increased fearfulness of the calves to human contact following disbudding. This was not mitigated by the NSAID meloxicam, which may have been because it was given too soon beforehand to have an effect at disbudding. Future work should investigate if approach score is influenced by earlier meloxicam administration and the added use of a sedative (Stock *et al.* 2013).

## Conclusions

Human studies show HRV analysis to be a reliable index of emotionality and homeostatic regulation (Appelhans and Luecken 2008), which respectively could be taken to equate with an animal's perception of its situation and how well it is physiologically adapting to it. Consequently HRV may be linked to welfare as defined by Dawkins (2012); a state of good welfare being when an animal 'has what it wants' reflected in a calm emotionality and 'is healthy' shown by a high vagal tone.

In this study we have demonstrated that HRV meets the definition of a biomarker (Atkinson *et al.* 2001) for dairy calf stress on three counts; it provides objective measures for a normal biological process (separation and isolation stress), a pathogenic process (disbudding tissue damage) and a pharmacological response to a therapeutic intervention (meloxicam administration). Consequently we propose that HRV could be a biomarker of welfare in dairy calves experiencing the stressors used in this study.

By using the RMSSD value of HRV as a measure of stress, this study has shown that reducing the time before separation following birth will reduce dairy calf stress. Limiting the number of days in single pens before being group housed will promote less stressful social interactions between calves. The provision of dummy teats to individually housed calves was not detrimental to management and may constitute a positive environmental enrichment. The administration of meloxicam may alleviate some chronic pain following hot iron disbudding, however a further study is required to determine if this effect is maintained beyond 48 h post disbudding.

HRV as a science-based animal-centric measurement offers the potential, through stress detection, for monitoring dairy calf welfare and should be more widely used to drive forward reforms in the dairy industry.

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## References

Appelhans BM, Luecken LJ (2008) Heart rate variability and pain: associations of two interrelated homeostatic processes. *Biological Psychology* **77**, 174–182. doi:10.1016/j.biopsycho.2007.10.004

Atkinson AJ, Colburn WA, DeGruttola VG, DeMets DL, Downing GJ, Hoth DF, Oates JA, Peck CC, Schooley RT, Spilker BA, Woodcock J, Zeger SL (2001) Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. *Clinical Pharmacology and Therapeutics* **69**, 89–95. doi:10.1067/mcp.2001.113989

Broom DM, Leaver JD (1978) Effects of group-rearing or partial isolation on later social behaviour of calves. *Animal Behaviour* **26**, 1255–1263. doi:10.1016/0003-3472(78)90116-1

Budzynska M, Weary DM (2008) Weaning distress in dairy calves: effects of alternative weaning procedures. *Applied Animal Behaviour Science* **112**, 33–39. doi:10.1016/j.applanim.2007.08.004

Dawkins MS (2012) 'Why animals matter: animal consciousness, animal welfare and human well-being.' (Oxford University Press: New York, NY)

Evans S, Seidman LC, Tsao JC, Lung KC, Zeltzer LK, Naliboff BD (2013) Heart rate variability as a biomarker for autonomic nervous system response differences between children with chronic pain and healthy control children. *Journal of Pain Research* **6**, 449–457.

Heinrich A, Duffield TF, Lissemore KD, Squires EJ, Millman ST (2009) The impact of meloxicam on postsurgical stress associated with cautery dehorning. *Journal of Dairy Science* **92**(2), 540–547. doi:10.3168/jds.2008-1424

Jarczok MN, Jarczoka M, Maussc D, Koenigd J, Lie J, Herra RM, Thayer JF (2013) Autonomic nervous system activity and workplace stressors – a systematic review. *Neuroscience and Biobehavioral Reviews* **37**, 1810–1823. doi:10.1016/j.neubiorev.2013.07.004

Kaufmann T, Sütterlin S, Schulz SM, Vögele C (2011) ARTiiFACT: a tool for heart rate artifact processing and heart rate variability analysis. *Behavior Research Methods* **43**(4), 1161–1170. doi:10.3758/s13428-011-0107-7

Koolhaas JM, Bartolomucci A, Buwalda B, de Boer SF, Flügge G, Korte SM, Meerlo P, Murison R, Olivier B, Palanza P, Richter-Levin G, Sgoifo A, Steimer T, Stiedl O, van Dijk G, Wöhr M, Fuchs E (2011) Stress revisited: a critical evaluation of the stress concept. *Neuroscience and Biobehavioral Reviews* **35**, 1291–1301. doi:10.1016/j.neubiorev.2011.02.003

Lupoli B, Johansson B, Uvnas-Moberg K, Svennersten-Sjaunja K (2001) Effect of suckling on the release of oxytocin, prolactin, cortisol, gastrin, cholecystokinin, somatostatin and insulin in dairy cows and their calves. *The Journal of Dairy Research* **68**, 175–187. doi:10.1017/S0022029901004721

Olofsson PS, Rosas-Ballina M, Levine YA, Tracey KJ (2012) Rethinking inflammation: neural circuits in the regulation of immunity. *Immunological Reviews* **248**, 188–204. doi:10.1111/j.1600-065X.2012.01138.x

Petrie NJ, Mellor DJ, Stafford KJ, Bruce RA, Ward RN (1996) Cortisol responses of calves to two methods of disbudding used with or without local anaesthetic. *New Zealand Veterinary Journal* **44**, 9–14. doi:10.1080/00480169.1996.35924

Phillips CJC (2002) 'Cattle behaviour and welfare.' 2nd edn. (Blackwell Science Ltd: Oxford, UK)

Phillips CJC (2010) 'Principles of cattle production.' 2nd edn. (CABI: Wallingford, UK)

Welfare Quality® (2009) 'Welfare Quality® assessment protocol for cattle.' (Welfare Quality® Consortium: Lelystad, The Netherlands)

Stěhulova I, Lidfors L, Spinka M (2008) Response of dairy cows and calves to early separation: Effect of calf age and visual and auditory contact after separation. *Applied Animal Behaviour Science* **110**, 144–165. doi:10.1016/j.applanim.2007.03.028

Stewart M, Stookey JM, Stafford KJ, Tucker CB, Rogers AR, Dowling SK, Verkerk GA, Schaefer AL, Webster JR (2009) Effects of local anesthetic and nonsteroidal anti-inflammatory drug on pain responses of dairy calves to hot-iron dehorning. . *Journal of Dairy Science* **92**(4), 1512–1519. doi:10.3168/jds.2008-1578

Stock ML, Baldridge SL, Griffin D, Coetzee JF (2013) Bovine dehorning assessing pain and providing analgesic management. *Veterinary Clinics of North America: Food Animal Practice* **29**, 103–133. doi:10.1016/j.cvfa.2012.11.001

Task Force of the European Society of Cardiology the North American Society of Pacing Electrophysiology (1996) Heart rate variability: standards of measurement, physiological interpretation and clinical use. *Circulation* **93**, 1043–1065. doi:10.1161/01.CIR.93.5.1043

Thayer JF, Lane D (2009) Claude Bernard and the heart–brain connection: further elaboration of a model of neurovisceral integration. *Neuroscience & Biobehavioral Reviews* **33**(2), 81–88. doi:10.1016/j.neubiorev.2011.11.009

Thayer JF, Ahs F, Fredrikson M, Sollers JJ 3rd, Wager TD (2012) A meta-analysis of heart rate variability and neuroimaging studies: implications for heart rate variability as a marker of stress and health. *Neuroscience & Biobehavioral Reviews* **36**(2), 747–756. doi:10.1016/j.neubiorev.2011.11.009

Van Reenen CG, O'Connell NE, Van der Werf JTN, Korte SM, Hopster H, Jones RB, Blokhuis HJ (2005) Responses of calves to acute stress: individual consistency and relations between behavioural and physiological measures. *Physiology & Behavior* **85**, 557–570. doi:10.1016/j.physbeh.2005.06.015

Veissier I, Care S, Pomies D (2013) Suckling, weaning and the development of oral behaviour in dairy calves. *Applied Animal Behaviour Science* **147**, 11–18. doi:10.1016/j.applanim.2013.05.002

von Borell E, Langbein J, Despres G, Hansen S, Leterrier C, Marchant-Forde J, Marchant-Forde R, Minero M, Mohr E, Prunier A, Valance D, Veissier I (2007) Heart rate variability as a measure of autonomic regulation of cardiac activity for assessing stress and welfare in farm animals – a review. *Physiology & Behavior* **92**, 293–316. doi:10.1016/j.physbeh.2007.01.007

Weary DM, Chua B (2000) Effects of early separation on the dairy cow and calf 1. Separation at 6 h, 1 day and 4 days after birth. *Applied Animal Behaviour Science* **69**, 177–188. doi:10.1016/S0168-1591(00)00128-3

Weary DM, Jasper J, Hotzel MJ (2008) Understanding weaning distress. *Applied Animal Behaviour Science* **110**, 24–41. doi:10.1016/j.applanim.2007.03.025