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Qualitative Behavioural Assessment and Quantitative Physiological Measurement of Cattle Naïve and Habituated to Road Transport

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KEYWORDS

free choice profiling, physiology, stress, welfare

ABSTRACT

The present study examined whether observers could distinguish between cattle that are naïve to road transport and the same cattle after becoming more habituated to transport. The behavioural expression of cattle was assessed through the method of qualitative behavioural assessment (QBA), and these assessments were correlated with various physiological parameters. Fourteen Angus steers were assessed during their first road trip and then again on their ninth trip, 15 days later. Blood samples were collected immediately before and after transport, and heart rate and core body temperature were measured continuously throughout each trip. Video footage recorded during each trip was edited and clips showing each individual within the first 30 min of departure were randomly ordered and shown to observers for QBA. There was significant ($P < 0.001$) consensus among 40 observers in their assessment of behavioural expression of the cattle. Transport-naïve cattle were described as more 'agitated', while transport-habituated were described as more 'calm'. Core body temperature ($P < 0.01$), plasma glucose ($P < 0.05$) and the neutrophil : lymphocyte ratio ($P < 0.01$) were higher for the first trip than for the habituated trip ($P < 0.01$). QBA were significantly correlated with core body temperature ($P < 0.01$), heart rate ($P < 0.01$), plasma glucose ($P < 0.05$) and the neutrophil : lymphocyte ratio ($P< 0.01$).QBA appears to be a valid and integrative method of assessing cattle welfare under the conditions tested within the present study. There was significant consensus in the ability of human observers to interpret behavioural expression of cattle during this experiment. In addition, observers could identify differences in behavioural expression between cattle that were naïve versus habituated to transport, and these differences were supported by physiological measurements.

Introduction

Improvement of animal welfare has become increasingly important to animal production industries over recent years, and consequently much research has investigated the validity of various measurements of welfare state (Verbeke and Viaene 2000). The need to improve welfare has been driven to improve production (Ferguson and Warner 2008), as well as in response to increasing consumer pressure for improvement of quality of life for production animals. This concept of quality of life not only includes the absence of suffering, but also the quality of an animal's relationship with its whole environment in terms of whether or how it can address its preferential needs (Wemelsfelder 2007).

An animal's quality of life and therefore its welfare must be based on a wide range of measures in addition to health and production indices (Hewson 2003). As a result these measures are often complex, with normal ranges difficult to establish or interpret due to individual differences and context-specific responses. Physiological responses are reasonably widely regarded as informative measures of response to stress, and therefore indicators of animal welfare (Dawkins 2003). However, physiological responses as measures of welfare are often expensive, invasive, and need to be evaluated under carefully controlled conditions. Furthermore, sampling itself can negatively affect animal welfare. Measuring welfare via physiological responses may therefore be difficult to implement in commercial situations. Many physiological parameters also vary with time of day, temperature and reproductive cycle. Finally, physiological responses are able to indicate levels of arousal, but not the valence (positive or negative response) of the animal's state. For example, sympathetic activation is observed under fearful situations but is also seen during positive physical activities such as play (Barber 1991). The above reasons make interpretation of physiological parameters extremely difficult under some circumstances and these measures therefore should not be considered in isolation.

One way of providing context for physiological responses is the assessment of an animal's behavioural response to a particular situation. For example, behavioural responses in pigs, such as aggressive interaction in animal housing systems, can give context to physiological responses such as increased plasma cortisol, glucose and immunosuppression (Barnett *et al*. 1985, 1987). Behaviour has traditionally been assessed quantitatively, most commonly in categories of physical movement (e.g. count or percentage of time carrying out particular behaviours, i.e. head bunting, lying down and running). For example, common behavioural responses to the transport process include decreased lying (Kent and Ewbank 1983*b*) and increased aggression (Knowles 1999). These behavioural responses provide a fragmented assessment of behaviour that can be reintegrated together to identify underlying dimensions of responsiveness to a situation (Banks 1982; Spoolder *et al*. 2003). However, such a retrospective evaluation is unlikely to account for subtle variations in social patterns of behaviour, and is not well equipped to evaluate measures that occur with low incidence or are difficult to quantify (Rousing and Wemelsfelder 2006). Therefore there is potential for important information to be omitted in such ethograms. A different approach is qualitative behavioural assessment (QBA) used as an indicator of animal welfare in conjunction with traditionally retrospective, quantified judgements (Wemelsfelder *et al*. 2000, 2001; Rousing and Wemelsfelder 2006; Napolitano *et al*. 2008).

Wemelsfelder *et al*. (2000, 2001) were the first to suggest that QBA may provide an integrative assessment tool for use in animal welfare studies. This method quantifies dimensions of an animal's behavioural expression in response to its environment by instructing human observers to integrate perceived details of behaviour and its context into judgements of an animal's overall style of behaviour (e.g. timid, bold, friendly, hostile). Qualitative behavioural assessment studies show that observers can reach significant agreement in their assessment of behavioural expression in pigs (Wemelsfelder *et al*. 2000, 2001), cattle (Rousing and Wemelsfelder 2006), horses (Napolitano *et al*. 2008; Minero *et al*. 2009), poultry (Wemelsfelder 2007) and dogs (Walker *et al*. 2010). This suggests that these assessments were based on commonly perceived and systematically applied criteria. Rousing and Wemelsfelder (2006) also found that qualitative assessments of cattle correlated significantly with quantitative assessments of behaviour recorded by researchers during the same time periods. For example, cows that showed responses such as pushing and head-butting through quantitative assessment were characterised as aggressive and bullying through qualitative assessment. Napolitano *et al*. (2008) similarly found a significant correlation between quantitative and qualitative assessments of behaviour in horses and ponies exposed to an environmental challenge, with horses assessed as more quiet and calm, with a lower frequency of bucking and kicking than ponies.

Additional studies are still needed to validate QBA against widely accepted welfare measures (e.g. physiological responses) within specific animal production contexts. The aim of the present study was to validate QBA as an assessment of the welfare of cattle, and correlate qualitative behavioural assessment to a range of physiological measures. As part of the process of validating QBA as a measure of welfare state, we sought to undertake QBA on animals that represented a range of behaviour that could be linked with known physiological states. Road transport of cattle was selected as the model because it is a well known stressor for livestock (Kent and Ewbank 1983*a*, 1983*b*; Murata and Hirose 1990, 1991; Tarrant *et al*. 1992), and the treatment can be manipulated and is therefore to some degree controllable.

Materials and methods

Animals and transportation

Fourteen Angus steers (12 months of age; 347 ± 11 kg) were randomly selected from a transport-naïve herd that had the same sire. Throughout the study, cattle were housed in a single paddock and provided with a feedlot ration, group fed at 3% liveweight/head per day (Easy Beef, Milne Agrigroup, Welshpool, WA, Australia; 14.5% crude protein and 11.0 MJ/kg metabolisable energy).

The challenge applied in the present study was to compare these cattle on their first exposure to transport (‘naïve’) with the same exposure once they had become more habituated to the transport conditions (their 9th 90-min trip over 15 days). Due to logistical constraints, cattle were transported in two groups of seven (transport Groups 1 and 2), with the same individuals making up each group on successive days. For each transport trip, cattle were transported by the same driver, in a car-drawn, double-axel trailer with a stock cage (3.66 × 2.05 m). The trailer had a solid roof and the walls were constructed from steel bars, allowing plentiful ventilation. The trailer had metal-grate flooring that was cleaned following each trip. Stocking rate on the trailer was within industry recommendations (1.07 m^2/head) (Standing Committee on Agricultural and Resource Management 2002).

Groups 1 and 2 were transported on different days but at the same time of day for the naïve and habituated transport trips. Both groups were transported on the same day for the habituation trips at alternate times (one group in the morning and the other in the afternoon, swapping on alternate days). Prior to each transport event, cattle were left to settle for 1 h in a holding yard adjoining the crush and loading ramp. During this time feed was withdrawn but water was available *ad libitum*. Environmental temperature (°C) and relative humidity (%) were recorded (every 2 s) during transport (Onset HOBO H8 Pros, #H08-032-IS, OneTemp Pty Ltd, Parramatta, NSW, Australia). Each logger was positioned at cattle head height, with one at the front and one at the rear of the trailer.

Physiology

Temperature loggers (iButtons, Maxim Integrated Products, Sunnyvale, CA, USA; accuracy ± 0.1°C) were surgically implanted into the peritoneal cavity in the region of the right paralumbar fossa, as described in Beatty *et al*. (2006). The surgery took place 16 days before the naïve transport trip, allowing time for recovery. Retrieval of the loggers took place at slaughter, following completion of the experiment. The loggers were set to record core body temperature (T_{core}) every 2 min for the duration of the experiment. Body temperature data was analysed for the first 30 min after departure during naïve and habituated transport treatment ('during transport'), during which time core temperature responses to the new environment may be most marked (Jacobson and Cook 1998; Pettiford *et al*. 2008). This was compared with the same time period on eight non-transport, non-handling days during the study period ('non-transport'). Blood was collected by the same person, using jugular venapuncture while the animal was held in a crush before and after the naïve and habituated transport trips. Samples were not taken during the transport trips as the sampling process would have potentially influenced the behavioural response of the animals. The 'before' blood sample was taken once cattle had been left for 1 h to settle and moved from the holding yard to the adjoining the crush. Once the 'before' blood sample was taken, the animal was moved into the holding yard until the whole group had been sampled; cattle were then loaded as a group onto the truck without the use of electric prods for immediate departure. The 'after' sample was collected as soon as the cattle exited the truck after transport. Whole blood was collected in EDTA, immediately refrigerated and used for analysis of complete haematological profile within 24 h of collection, using a Roche Cobas Minos Haematology machine (Castle Hill, NSW, Australia). A second blood sample in EDTA was centrifuged for 15 min at 300*g*, and the plasma removed and frozen before batch analysis for glucose, β-hydroxyl-butyrate, cortisol, prolactin and insulin-like growth factor (IGF)-1.

Heart rate (HR, beats per min) was recorded (every 5 s) during naïve and habituated transport with external heart rate monitors (Polar Equine S625X, Polar Electro Oy, http://www.pursuitperformance.com.au/). Custom-made elastic belts were used for fixing of electrodes, depending on the animal's size. The first electrode was located ~10 cm to the left of the central back line, immediately behind the withers. The second electrode was located in the pericardium area. The receiver was located on the central back line in a purpose-built pocket attached to the belt. Heart rate monitors were fitted immediately after before-transport blood sampling and were removed following after-transport blood sampling. Prior to each habituation trip, cattle were fitted with the heart rate belts while in the crush. The belts were removed immediately following transport. HR was analysed for the first 30 min after departure during the naïve and habituated transport treatment ('during transport'), during which time heart rate response to the new environment may be most marked (Jacobson and Cook 1998).

Qualitative behavioural assessment

Video footage was recorded during transport with four digital cameras (Panasonic SDR-H250, Belrose, NSW, Australia) fixed to the front and back of the trailer, above cattle head height. The first available suitable clip was selected for each individual (a clip was required to be of sufficient duration, with head and shoulders visible) to represent its behaviour during transport. The cattle often held their heads down during the transport and therefore, for most animals, there was only one suitable clip of adequate length and quality available. The clips were 15–30 s long and all fell within the first 30 min after departure, during which time physiological and behavioural response to the new environment may be most marked. (Jacobson and Cook 1998; Knowles 1999; Pettiford *et al*. 2008). Individuals were identified using numbers printed on the outside of the heart rate belts.

The 28 clips (14 naïve and 14 habituated) were edited to highlight individual focal cattle by increasing the opacity of the surrounding animals in the same frame (Adobe Premiere Pro CS3 and Adobe After Effects CS3, Chatswood, NSW, Australia). Observers were recruited from University staff and students and members of the public by advertising on notice boards and email and accepting all 40 persons that responded. Each observer was required to attend two sessions on campus or by correspondence. Observers were given detailed instructions on completing the sessions but were not told about the experimental treatments or that the cattle were on a truck. The two sessions are detailed below.

1. *Session 1: term generation*. Observers were shown 15 video clips of the experimental cattle demonstrating a wide range of behavioural expressions to allow observers to describe as many

aspects of the cattle's expressive repertoire as possible (11 of these clips were the experimental footage and also used in Session 2). After watching each clip, observers were given 2 min to write down any words that they thought described that animal's behavioural expression. There was no limit imposed to the number of terms an observer could generate, but terms needed to describe not what the animal was doing (i.e. physical descriptions of the animal such as vocalising, chewing, tail flicking), but how the animal was doing it. Subsequent editing of the observer terms was carried out to remove terms that described actions, whereas terms that were in the negative form were transformed to the positive for ease of scoring (e.g. 'unhappy' became 'happy'). Terms were subsequently arranged so that terms with a similar meaning were not listed together and within these constraints the order of terms in the list was effectively random.

2. *Session 2: quantification*. Each of the observer terms was printed in a list, with each observer having a list of their own individual terms. The terms were attached to a 100-mmvisual analogue scale ranging from minimum to maximum for quantification during Session 2. Observers used their own terms to quantitatively score (by marking on the visual analogue scale) the behavioural expression of individual cattle shown in the 28 video clips from the naïve and habituated transport trips (shown in random order). Each of the cattle was scored on every term generated by that observer.

Statistical analyses

Physiological measures

Average and maximum T_{core}, average HR and hormones, metabolites and haematological parameters were analysed by repeated-measures ANOVA for treatment (independent variable; naïve *v.* habituated) and group (random factor; Transport groups 1 and 2) effects. The repeated dependent measures included measurements collected either during transport or at the same time of day over non-transport, non-handling days for average and maximum T_{core}, or before transport *v.* after transport for hormones, metabolites and haematological parameters. Due to lost contact and interference of heart rate monitors, reliable data were not obtained for all animals for both naïve and habituated trips [therefore, for HR data, n = 5 (naïve trip) and n = 4 (habituated trip)]. Retrieval of core temperature data was unsuccessful for two individuals (therefore n = 12 for temperature data) and for the remaining physiological measurements (metabolites and haematological parameters) n = 14.

Generalised Procrustes analysis of cattle scores

The observer scores generated from the 28 video clips were analysed with generalised Procrustes analysis (GPA), using a specialised software edition written for Françoise Wemelsfelder (GENSTAT 2008, VSN International, Hemel Hempstead, Hertfordshire, UK). For a detailed description of its procedures, see Wemelsfelder *et al*. (2000). Briefly summarised, GPA calculates a consensus or 'best fit' profile between observer assessments through complex pattern matching. This consensus profile has several main dimensions (usually 2 or 3) explaining the variation between animals. Each animal receives a quantitative score on each of these dimensions, so that the animal's position in the consensus profile can be graphically represented in two- or three-dimensional plots. Each plot represents each of the cattle twice (once for each treatment) where the position of the cattle indicates its scores on each GPA axis. To compare treatments, the GPA scores for each dimension were analysed using repeated-measures ANOVA (Statistica 9.0, StatSoft, North Melbourne, Vic., Australia), with the scores for each individual for the two transport events compared as the repeated-measures.

GPA dimensions were interpreted by correlating the animals' scores to the observers' individual scoring patterns, producing word charts describing the consensus for individual observers that can be compared for linguistic consistency. From these word charts, a list of terms describing the consensus dimensions was produced, by selecting terms for each observer that correlated strongly with those dimensions (r > 0.7 on GPA dimension 1, r > 0.5 on GPA dimension 2, r > 0.5 on GPA dimension 3). The time point when video clips were taken during the naïve and habituated transport trips was compared with behavioural assessment scores (individual animals' GPA scores for each dimension) by Pearson's correlation (Microsoft Excel 2003, North Ryde, NSW, Australia).

<u>Analysis of treatment effects on QBA scores</u>

To compare experimental treatments, the average scores for each GPA dimension for each animal were compared using repeated-measures ANOVA, with the repeated dependent measures being the GPA scores measured for each experimental treatment (naïve *v.* habituated). Transport group was included as a random factor (Transport groups 1 and 2).

<u>Correlation of GPA cattle scores to other measures</u>

The physiological responses to transport were compared with behavioural assessment scores (individual animals' GPA scores for each dimension) by Pearson's correlation (Excel 2003). Individual scores for both naïve and habituated trips were used for the present analysis. The physiological response was expressed as the change in each parameter due to transport. For hormones, metabolites and haematological parameters, the change due to transport was expressed as a proportion of after-transport values : before-transport values. The change in core body temperature due to transport was calculated as during-transport values (encompassing the first 30 min of transport)/means of eight non-transport, non-handling days at the same time of day for the same individual. The change in heart rate due to transport was calculated as values during transport (5–10 min after departure)/before-transport values (recorded 5–10 min before departure).

GPA was carried out with GENSTAT, and repeated-measures analyses and *post hoc* analyses using Tukey's honest significant difference test were carried out with Statistica 9.0. Values are presented throughout as means ± 1 s.d.

Results

The 40 observers participating in the study generated a total of 178 different terms to describe the cattle they were shown, with an average of 17±7 (minimum 9, maximum 48) terms per observer. The Procrustes statistic was 47% and this differed significantly from a mean randomised profile (t_{99} = 69.4, P < 0.001). Three main GPA dimensions were identified, explaining 54.0, 8.5 and 5.2% of the variation between animals for GPA dimensions 1, 2 and 3 respectively.

Word charts were produced for each of the 40 observers and Fig. 1 shows an example of one observer's terms graphed against the three GPA dimensions. Terms from all 40 observers were pooled and terms with the strongest correlation with each of the GPA dimensions are shown in Table 1. Low values for GPA dimension 1 were associated with terms such as 'calm', 'comfortable', 'relaxed', 'bored' and 'indifferent'. High values were associated with high-energy terms such as 'agitated', 'restless', 'stressed' and 'anxious'. There were similarities in the terms that correlated with QBA dimensions 2 and 3. Low values for GPA dimension 2 were associated with terms such as 'sedate', 'weary' and 'fatigued' and high values were associated with terms such as 'alert', 'curious' and 'aware'. Low values for GPA dimension 3 were associated with terms such as 'weary', 'soothed' and 'exhausted' and high values were associated with terms such as 'alert' and 'questioning' (Table 1). The three highest weighting terms for each GPA dimension, as shown in Table 1, were selected for purposes of labelling the dimensions (Fig. 2) and describing the dimensions in relation to physiological responses.

The positions of individual cattle on the first two GPA dimensions are shown in Fig. 2. Naïve cattle had a significantly higher GPA score on dimension 1 than habituated cattle ($F_{1,13}$ = 12.07, P < 0.01, mean ± s.d. of 0.069 ± 0.082 for naïve and –0.069 ± 0.049 for habituated). Naïve cattle were scored as more ‘agitated’, ‘restless’ and ‘stressed’ than when they had become habituated to transport, when they were scored as more ‘calm’, ‘comfortable’ and ‘relaxed’. There were no treatment effects on GPA dimension 2 ($F_{1,13}$ = 0.01, P = 0.99, mean ± s.d. of 0.001 ± 0.033 for naïve and –0.001 ± 0.045 for habituated) or GPA dimension 3 ($F_{1,13}$ = 1.63, P = 0.239, mean ± s.d. of –0.007 ± 0.037 for naïve and 0.0071 ± 0.021 for habituated). There was no effect of time that clips were taken on GPA dimensions 1, 2 or 3 for the naïve or habituated animals.

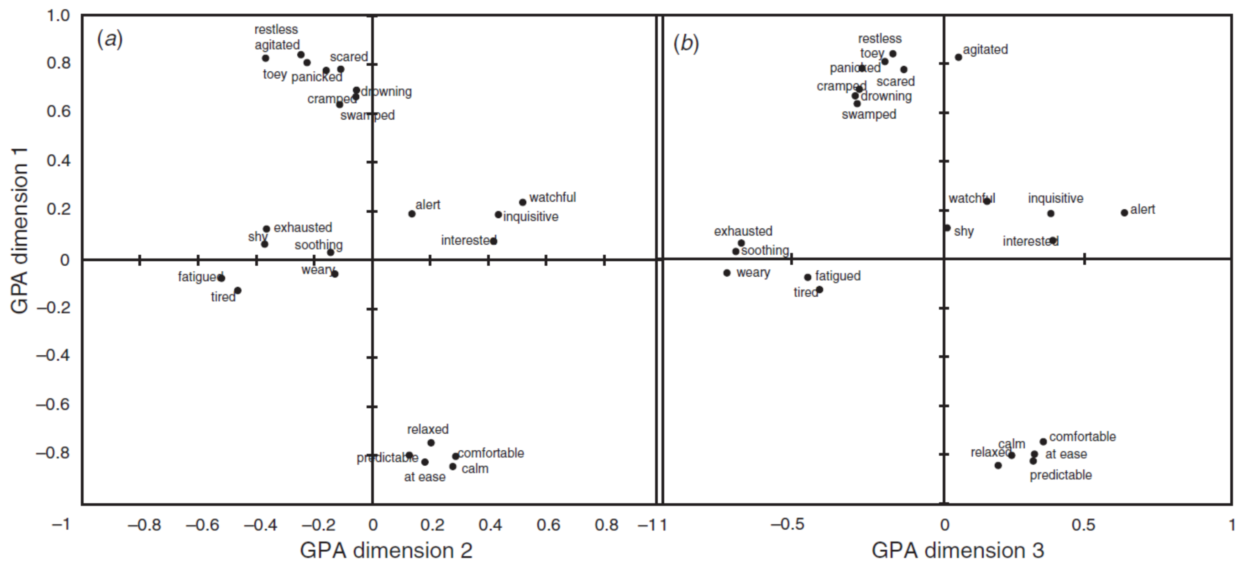

Fig. 1. Word map of consensus profile for generalised Procrustes analysis (GPA) for one observer viewing cattle from both naive and habituated treatments. (*a*) GPA dimensions 1 and 2; (*b*) GPA dimensions 1 and 3.

Physiological parameters

Asignificant treatment × time interaction was found for red blood cell count, haematocrit and haemoglobin levels (P < 0.05; Table 2), which were more depressed in naïve animals following transport compared with the same animals once they had become habituated to transport (Table 2). There was also a treatment × time interaction for glucose (P < 0.05; Table 2), with an elevation following transport for naïve animals, but no rise for habituated animals. The significant treatment × time interaction for neutrophil : lymphocyte ratio, shown by a larger increase following transport in naïve compared with habituated animals (P < 0.001; Table 2), similarly indicated a differential impact on the immune system. White blood cell count demonstrated a marginal treatment · time effect (P = 0.055), with a higher white blood cell count in naïve cattle compared with habituated cattle (treatment: P < 0.05) and in response to transport of naïve cattle (time: P < 0.01; Table 2).

Transport (i.e. a significant time effect: before transport *v.* after transport) resulted in an increase in plasma cortisol (P < 0.05), whereas naïve cattle also had higher plasma cortisol compared with habituated cattle (treatment: P < 0.01; Table 2). Transport also resulted in a decreased monocyte count (time: P < 0.05; Table 2) and neutrophil : lymphocyte ratio (time: P < 0.01; Table 2), indicating a significant immune system response.

Table 1: Terms used by observers to describe cattle behavioural expression during transport. Terms for all observers, showing the highest negative and positive correlation with generalised Procrustes analysis (GPA) dimensions 1, 2 and 3 of the consensus profile. Terms shown have a correlation of >0.7 (high values) and <–0.7 (low values) for GPA dimension 1 and >0.5 (high values) and <–0.5 (low values) for GPA dimensions 2 and 3. Order of terms is determined first by number of observers to use that term (in parentheses if >1) and second by weighting of each term

GPA dimension	Low values	High values
1 (54%)	Calm (13), comfortable (7), relaxed (7), content (4), at ease (3), bored (2), settled (2), quiet (1), indifferent (1), predictable (1), happy (1), subdued (1), accepting (1), composed (1), fearful (1), controlled (1)	Agitated (11), restless (7), stressed (7), anxious (6), flighty (5), nervous (5), alert (3), frightened (3), scared (3), worried (3), alarmed (2), concerned (2), fearful (2), frustrated (2), panicked (2), unsure (2), wants to escape (2), claustrophobic (1), confused (1), content (1), distressed (1), evasive (1), excitable (1), fidgety (1), hemmed in (1), impatient (1), inquisitive (1), lively (1), on edge (1), perplexed (1), tense (1), terrified (1), toey (1), trapped (1), twitchy (1), unnerved (1), wants to leave (1)
2 (8.5%)	Sedate (1), upset (1), annoyed (1), frightened (1), weary (1), nervous (1), fatigued (1), sad (1), bored (1), happy (1)	Alert (5), curious (4), aware (4), inquisitive (3), interested (2), focussed (1), quiet (1), relaxed (1), wary (1), shy (1), watchful (1)
3 (5.2%)	Weary (1), soothed (1), exhausted (1), depressed (1), irritated (1), alert (1), threatened (1), sad (1)	Alert (3), questioning (1)

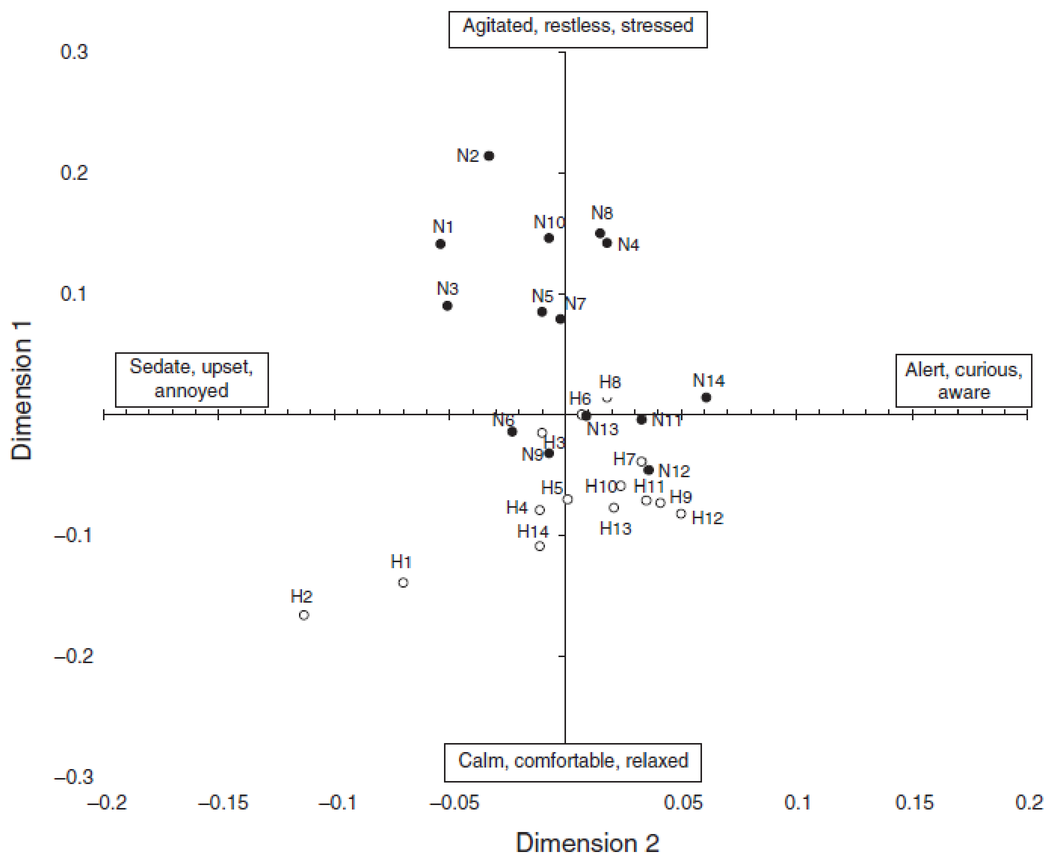

Fig. 2. Positions of individual cattle (represented by numbers) on generalised Procrustes analysis dimensions 1 and 2 obtained from qualitative behavioural assessment. Each animal is represented twice, once as naïve (N, closed circles) and second as habituated (H, open circles).

Platelet count was higher in naïve cattle than in habituated cattle (treatment: $P < 0.01$; Table 2), and also demonstrated a significant response to transport (time: $P<0.05$). Plasma prolactin (time: $P < 0.01$; Table 2) and b-hydroxy butyrate (time: $P < 0.05$; Table 2) were lower following transport. IGF-1 was elevated in the habituated compared with the naïve cattle (treatment: $P < 0.01$).

The mean and maximum body temperature (T_{core}) was elevated during the first and last 30 min of transport, lairage and the first blood sample compared with when animals were in home pens (non-transport) in naïve and habituated cattle. However, during transport the elevation was greater in naïve cattle compared with habituated cattle (treatment × time: $P < 0.001$; Fig. 3, Table 2).

Correlation of physiology to behavior

GPA dimension 1 was positively correlated with the difference between non-transport and during-transport values for maximum core temperature ($P < 0.05$), heart rate ($P < 0.01$) and the difference between before and after transport for plasma glucose ($P < 0.05$), white blood cell count ($P < 0.01$) neutrophils ($P < 0.01$) and neutrophil : lymphocyte ratio ($P < 0.01$) (Table 2). This indicated that these parameters were significantly higher in cattle also assessed as more 'agitated', 'restless' and 'stressed' (as opposed to 'calm', 'comfortable' and 'relaxed'). The differences between before- and after-transport haemoglobin ($P < 0.05$), heamatocrit ($P < 0.05$) and lymphocyte count ($P < 0.05$) were negatively correlated with GPA dimension 1 (Table 2), indicating that these parameters were significantly lower in cattle also assessed as more 'agitated', 'restless' and 'stressed'. The difference between before- and after-transport plasma glucose was negatively correlated with GPA dimension 3 ($P < 0.05$), indicating that glucose was higher in cattle assessed as being more 'weary', 'soothed' and 'exhausted'. The differences between before- and after-transport red blood cell and platelet counts were negatively correlated with GPA dimension 2 ($P < 0.05$), indicating that both red blood cell and platelet counts were higher in cattle assessed as being more 'sedate', 'upset' and 'annoyed'.

Discussion

Using a QBA approach, observers were able to distinguish between cattle that were naïve and those that were habituated to transport based on their behavioural expression. In addition to observers being able to distinguish between treatment groups, there were significant correlations between observers' scores for behavioural expression and physiological measures that reflect the physiological response to this experimental treatment. Transport resulted in significantly altered physiological variables typically associated with the stress response. The correlations between qualitative behavioural assessment scores and physiology serve to validate QBA scores as a measure of the animals' integrated state.

There was significant consensus between observers in their assessment of the behavioural expression of the cattle, with the GPA consensus profile explaining around half the variation in scores between the observers. This indicates that a reasonable degree of variability in the behavioural assessments was left unaccounted for, which may be due to several underlying reasons, or combination of reasons, which cannot be easily identified without further investigation. Given significant consensus, however, an important measure of the consistency of observer assessments is the ease and clarity with which its main dimensions can be interpreted, and can be used to distinguish between experimental treatments. The observers used terms in a similar way, and it was possible to identify distinct clusters of words with similar meanings on each dimension. Based on the qualitative assessments of cattle behaviour during transport, three main dimensions of behavioural expression were found (dimension 1: 'calm'/'comfortable' versus 'agitated'/'restless'; dimension 2: 'sedate'/'weary' versus 'alert'/'curious'; dimension 3 'weary/soothed' versus 'alert'/'questioning').

Table 2. Values (mean ± s.d.) and repeated measures *P*-values of blood parameters and body temperature for treatment (naïve *v.* habituated) and sampling time (before transport *v.* after transport for blood values; before transport *v.* during transport for body temperature) and correlation of values with qualitative behavioural assessment (QBA) scores. For raw values with significant treatment × time interactions, different letters indicate significant differences (at *P*=0.05). Correlations between physiological parameters and generalised Procrustes analysis (GPA) dimensions are indicated in the right-hand columns. Significant effects are indicated in bold (P* < 0.05, ***P* < 0.01) for correlations with blood parameters (r_{26}), body temperature (r_{24}) and heart rate (r_7)**

Parameter	Raw value				*P*-value (repeated-measures ANOVA)			Pearson's *r* (correlation with QBA scores)		
	Naïve		Habituated		Treatment (naïve *v.* habituated)	Time (before *v.* after)	Treatment × time interaction	GPA 1	GPA 2	GPA 3
	Before	After	Before	After						
Hormones										
Cortisol (ng/mL)	68.5±56.3	119±48.6	38.5±27.5	43.5±28.6	**<0.001**	**0.021**	0.074	0.182	-0.007	-0.054
Insulin-like growth factor (ng/mL)	39.6±12.6	39.6±13.8	48.1±15.5	51.87±14.19	**<0.001**	0.258	0.345	-0.305	-0.23	0.17
Prolactin (ng/mL)	33.4±29.5	7.42±5.34	25.9±18.7	17.7±15.6	0.462	**<0.001**	0.078	-0.034	-0.105	-0.304
Metabolites										
β-Hydroxy butyrate (mmol/L)	0.219±0.105	0.199±0.084	0.219±0.064	0.181±0.048	0.655	**0.014**	0.38	0.24	-0.127	-0.153
Glucose (mmol/L)	5.37±0.521ac	6.04±0.356b	5.66±0.389a	5.60±0.457c	**0.016**	**<0.001**	**0.022**	**0.428***	0.282	**-0.408***
Haematological parameter										
Red blood cells ($\times 10^9$/L)	8.89±0.57a	8.52±0.53b	8.21±0.37c	8.15±0.42c	**<0.001**	**0.027**	**0.016**	0.031	**0.342***	0.035
Haematocrit (%)	0.383±0.023a	0.365±0.023b	0.351±0.017c	0.348±0.019c	**<0.001**	**0.011**	**0.023**	**0.333***	0.184	0.205
Haemoglobin (g/L)	130±7.49a	122±6.98b	122±6.09b	120±6.50b	**0.006**	**0.003**	**0.011**	**0.400***	0.202	0.126
White blood cells ($\times 10^9$/L)	9.20±1.32	12.6±1.83	9.69±0.797	11.4±1.90	**0.026**	**<0.001**	0.055	**0.649****	0.132	0.161
Eosinophils ($\times 10^9$/L)	0.10±0.04	0.10±0.03	0.08±0.02	0.09±0.03	0.054	0.825	0.387	0.031	0.224	0.199
Monocytes ($\times 10^9$/L)	0.48±0.48	0.33±0.28	0.24±0.10	0.20±0.11	0.118	**0.017**	0.096	0.109	0.171	0.305
Neutrophils ($\times 10^9$/L)	2.47±0.38a	6.38±1.71b	3.01±0.55a	4.62±1.34d	0.074	**<0.001**	**<0.001**	**0.535****	0.157	0.009
Lymphocytes ($\times 10^9$/L)	6.33±1.14a	5.05±0.79b	5.46±1.00c	5.39±0.83c	0.245	**0.006**	**<0.001**	**0.392***	0.147	0.013
Neutrophil : lymphocyte ratio	0.40±0.07a	1.30±0.43b	0.59±0.22c	0.88±0.31d	0.177	**<0.001**	**<0.001**	**0.525****	0.167	0.038
Platelet ($\times 10^9$/L)	729±136	784±166	565±176	581±174	**<0.001**	**0.030**	0.176	0.001	**0.331***	0.183
Body temperature										
Mean T_{core} (°C)	38.68±0.206a	39.63±0.488b[A]	38.60±0.15c[A]	39.12±0.238d[A]	**<0.001**	**<0.001**	**<0.001**	0.316	0.002	-0.108
Maximum T_{core} (°C)	39.70±0.513b	39.70±0.513b[A]	38.64±0.138c[A]	39.17±0.229d[A]	**<0.001**	**<0.001**	**<0.001**	**0.333***	-0.007	-0.091
Average heart rate (0–30 min)		127.3±45.4[A]		107.8±42.3[A]				**0.783****	0.569	-0.546

[A]During.

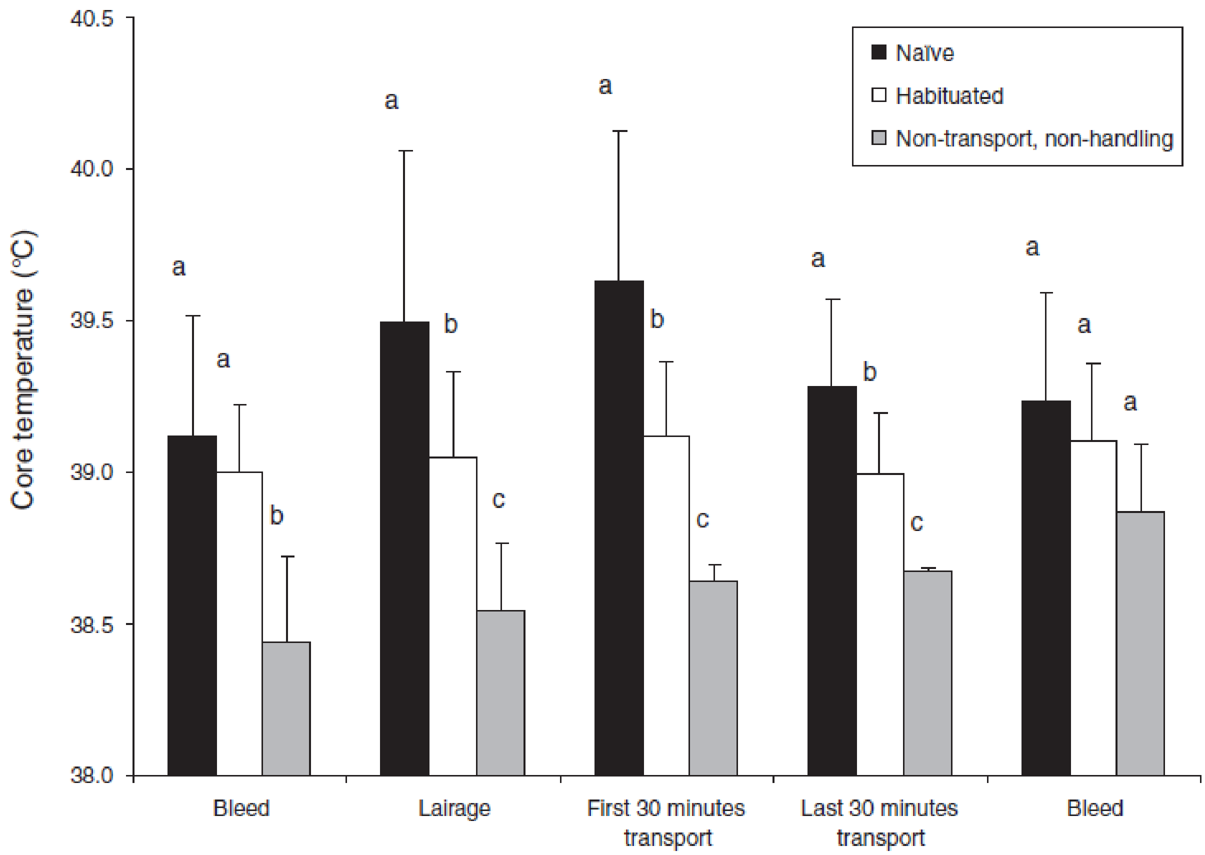

Fig. 3. Mean (±s.d.) body temperature (T_{core}) of cattle naïve and habituated to transport, compared with the same time of day on eight non-transport, non-handling days. Five time points during the process of handling and transport are indicated. Within each experimental activity, different letters indicate significant differences between the treatments (P < 0.05).

The relative position of the cattle on the QBA dimensions varied depending whether they were on their naïve or habituated trip, with the majority of naïve animals scored highly on dimension 1 (i.e. observed to be more 'agitated', 'restless', 'stressed') and the habituated animals scored as low on dimension 1 (i.e. observed to be more 'calm', 'comfortable', 'relaxed'). In previous studies, similar qualitative terms have been used informally to describe cattle behaviour during transport. For example, cattle have been perceived as more anxious and restless during the initial stages of road transport (Knowles 1999). The use of qualitative behavioural assessment supports these perceptions, therefore enhancing models of animal welfare assessment.

Transport-naïve cattle had increased mean and maximum core temperatures, increased concentrations of cortisol and glucose and increased neutrophil : lymphocyte ratios compared with samples taken from the animals habituated to transport. These physiological responses were similar to those recorded in cattle in response to transport in previous studies (Murata and Hirose 1990, 1991; Tarrant *et al*. 1992; Schaefer *et al*. 1997; Jacobson and Cook 1998; Knowles 1999). Furthermore, similar results were found in habituation studies on cattle, where repeated transport resulted in decreased cortisol levels (Locatelli *et* *al*. 1989) and heart rate (Jacobson and Cook 1998). Repeated handling also resulted in a decrease in cortisol response (Andrade *et al*. 2001; Solano *et al*. 2004) and repeated exposure to noise during handling resulted in decreased heart rate and level of movement (Waynert *et al*. 1999). A study by Uetake *et al*. (2009) also found that rectal temperature, measured following road transport, was reduced (although not significantly) after 1 week of habituation to transport. This core temperature response is a reflection of increased heat production, possibly through activation of the sympathetic nervous system (Sjaastad *et al*. 2003).

Physiological responses of cattle in the present study were also similar to those in sheep in a parallel study where sheep were both naïve and habituated to road transport (S. Wickham, T. Collins, A. Barnes, D. Miller, D. Beatty, D. Blache, F. Wemelsfelder and T. Fleming, unpubl. data). Sheep had an increased core temperature, heart rate, plasma cortisol, white blood cell count and neutrophil : lymphocyte ratio in response to transport and the naïve treatment compared with the habituated treatment, indicating an activation of the hypothalamic–pituitary–adrenal axis and an immune system response due to stress. Hydration status, as indicated by haematocrit, was affected by transport and treatment in both sheep (S. Wickham, T. Collins, A. Barnes, D. Miller, D. Beatty, D. Blache, F. Wemelsfelder and T. Fleming, unpubl. data) and cattle in the present study. Interestingly, the response of haematocrit to transport in both sheep and cattle indicated haemodilution rather than dehydration. Previous studies have found that a cortisol response can interfere with mechanisms that control hydration status (Parker *et al*. 2004).

There were significant correlations between the physiological responses and the assessed behavioural expressions in the present study. Maximum core temperature, heart rate, glucose, white blood cell count and neutrophil : lymphocyte ratio were correlated positively with dimension 1, on which naïve cattle received significantly higher scores than habituated cattle, suggesting that the QBA process was detecting behavioural manifestations of stress in the naïve cattle. Cortisol is commonly used to measure stress response, and in the present study, there was a significant treatment and time interaction; however, cortisol response was not correlated with QBA. It is likely that the cortisol half-life in cattle under stress was shorter than the transport period. Locatelli *et al*. (1989) found that the cortisol response peaked at 30 min during transport and was reduced from this point at 60 min of transport. In the present study, where our after-transport blood sample was carried out after 90 min of transport, the peak cortisol response may have been missed. It is likely that because the cortisol response was only short-term there was not a decrease in b-hydroxyl-butyrate. Changes in b-hydroxyl-butyrate are usually associated with longer-term nutritional stressors in cattle (Shaw and Tume 1992); however, a previous study with bulls found no effect of transportation stress on b-hydroxyl-butyrate, although there was an acute increase in plasma cortisol (Buckham Sporer *et al*. 2008).

Road transport was a useful model that provided significant manifestations of stress, indicated by both physiological and behavioural measures. However, the present study did not investigate the capacity for use of QBA within the road transport industry since we could not adequately replicate commercial transport conditions within the logistical constraints of our experimental design. The extension of the present study to commercial conditions would require further investigation. We note that it is recommended by industry that ‘livestock handlers should have experience in animal handling to ensure welfare of cattle in their charge’ (Standing Committee on Agricultural and Resource Management 2002). This stockmanship reflects the handlers’ ability to undertake assessment of behavioural expressions of animals in much the same manner as we have quantified in the present study.

QBA allows whole-animal assessment in an integrative sense. It is a quick and non-invasive assessment that correlates with commonly used physiological measures of welfare in a road transport scenario. It may, therefore, be useful as an aide to interpretation of more detailed welfare measures or to highlight situations that require more intensive welfare assessment, particularly in animal production scenarios where more invasive welfare assessments are difficult to implement. To facilitate practical application in commercial farming contexts, it will be necessary to develop pre-fixed QBA scoring lists and test their validity in the specific situations they are meant to address. A start with such work has been made in relation to general on-farm welfare inspection (Wemelsfelder *et al*. 2008; Wemelsfelder and Millard 2009), and, given the encouraging results of the present study, could be extended to include welfare assessment during transportation.

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