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# Indicators of Good Welfare in Horses

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# 1 Introduction

## 1.1 THE ISSUE

Traditional welfare standards have focused on the absence of negative affective (mental) experiences e.g. pain, fear, distress. The ability to identify situations that elicit negative emotional responses would allow animal caretakers to prevent or minimise them, to reduce poor welfare, and improve human and animal safety.

Animal welfare science now regards positive or optimal welfare, rather than simply the absence of negative welfare states or indices, as a desirable outcome. The ability to identify and measure affective experience (emotion) in animals that have improved welfare, beyond that of simply the absence of stress, may provide a novel approach to welfare assessment and the promotion of good welfare.

It is argued that if an animal is experiencing pleasure or happiness, or is in a happy emotional state, then its needs are being met, and its welfare is good (Boissy et al. 2007). This approach may render the assessment of other factors such as the Five Freedoms, domains or needs welfare frameworks unnecessary. Ignoring positive aspects of welfare not only disregards significant features of animal behaviour and physiology but also affects how animal caretakers make decisions about animal management practices and impacts the quality of life as well as the animal's longevity (Yeates & Main 2008).

Although positive emotion is suggested to be a core component of animal welfare, there is a lack of agreement on how to assess positive experiences in animals. Animal welfare science has begun to explore the existence, detection and measurement of positive emotions, such as pleasure, in order to inform discussion and provide methods for practical application (Reefmann et al. 2009a). Objective and preferably non-invasive indicators of positive emotion are needed to assess animal welfare status, verify assumptions regarding environmental enrichment, and promote positive welfare.

This report outlines work that was commissioned to investigate affective experience in horses. The scientific literature regarding theories on the existence and nature of emotion in animals was reviewed. The scientific literature on experimental research of emotion in horses and other animals was examined. From this knowledge base, two experiments were designed and conducted to investigate potential indicators of emotion in horses.

## 1.2 KEY CONCEPTS

There is no unified agreement on key terms and concepts or their definition. They are introduced below and their usage in this report is clarified.

### 1.2.1 Emotion

In the context of this report affect and feeling have the same meaning as emotion. Emotion, or affective experience, is an elusive concept to define. An emotion can be broadly defined as, an innate, intense but short-lived response to an event (internal or external) that has behavioural, physiological (autonomic), subjective (sometimes referred to as the feeling) and cognitive (appraisal) components (Panksepp 2005; Paul et al. 2005; Boissy et al. 2007; Garland et al. 2010; de Waal 2011). Whilst not universal, this componential approach appears commonly in the literature. Two factors stand out, the immediacy and brevity of emotions. Paul et al. (2005) categorised emotion in animals as being object (or stimulus) associated, that is they do not result from internal stimuli. The existence of the subjective and cognitive components of emotion in animals is subject to debate (Paul et al. 2005). Animal sentience, the ability to perceive, feel or be conscious, is closely related to emotion and also subject to debate.

Scientists have been uncomfortable with using the term emotion due to concerns about anthropomorphism when regarding the existence of the subjective component of emotion in animals. Various terms such as emotional or affective process and affective experience, have arisen to avoid using the term emotion or to differentiate the behavioural, physiological, and cognitive components from the subjective component (Paul et al. 2005).

Identifying variables that can be used as indicators of emotion in animals is an ultimate goal. Unlike research in humans, self-report cannot be used as a gold-standard for determination and measurement of emotion. In humans, self-report is linked to observable changes in physiology, behaviour, and decision making/cognitive function. In animals, it may be possible to infer emotion based on observation and measurement of changes in physiology, behaviour, and for emotional states, cognition.

#### *1.2.1.1 Behavioural Component of Emotion*

Emotions can be characterised by the behaviours that accompany them. Behaviours may involve non-verbal expression via body language, facial expression and vocalisation as well as motor action patterns such as those associated with fight or flight responses, or approach and investigation behaviour patterns.

#### *1.2.1.2 Physiological Component of Emotion*

Emotions are accompanied by changes in physiological arousal which is mainly mediated via the autonomic nervous system (Weiten 1992). There may be changes in heart rate, blood pressure, respiratory rate, piloerection, pupil size, sweat, cortisol level, catecholamine levels, and other neuroendocrinological changes. Many physiological variables have been investigated in studies of pain, fear and distress in animals (Fraser 2008).

#### *1.2.1.3 Subjective Component of Emotion*

In humans, the experience of emotion is subjective, personalised, and peculiar to the being feeling it. Whilst emotion may be categorised, typed and graded based on indicators such as self-report, physiological changes, behaviour, and cognitive appraisal, the exact way an emotion is perceived by a subject is intangible and irreproducible between individuals. This accounts for differences in the intensity and type of emotional response by individuals to the same stimuli. It may reflect the differences in experience, learning and memory, genetics, neural processing and neural plasticity, self-awareness, language and other communication skills, health status, and temperament that make subjects individual. The existence or nature of the subjective component in animals is debateable (see Paul et al. 2005).

#### *1.2.1.4 Cognitive Component of Emotion*

According to appraisal theory, emotions result from an individual's evaluation of a situation, which may be conscious or sub-conscious. Evaluation results in broad classification of the situation as pleasant or unpleasant (Weiten 1992). Humans can experience emotions when thinking about a situation. It is unknown whether animals can do this, although anticipation of fearful or painful situations may provide some insight.

### 1.2.2 Emotional States

Some authors have used the term emotional state interchangeably with emotion. For clarity the term is used in this report to refer to the longer, over-arching mood or outlook, for example depression and happiness. An animal may have other emotions during an emotional state for example the overall state may be happy but an animal may experience feelings of short-term pain or fear, conversely a depressed emotional state may have moments of pleasure.

Less is known about emotional states than emotions in animals. Research in humans suggests that the emotional state may be a reflection of the balance or ratio of positive to negative emotional experiences during the medium to long term and can be intentionally manipulated to improve wellbeing (Garland et al. 2010). Boissy et al. (2007) suggest that research on positive emotion is necessary to inform research on positive emotional states.

## 1.3 NATURE OF EMOTION IN ANIMALS

It is plausible that animals experience emotions broadly similar to humans. Based on homology, evolutionary benefit, analogous anatomy, physiology and behaviour, and philosophical arguments, most scientists agree that it is likely that animals experience emotion, including positive emotion. Whether or not or to what degree they are cognitively aware (conscious) is debateable. Other issues, such as the exact degree of consciousness and cognitive ability in each species compared to humans, whether emotion has a subjective component in animals, specific types of emotion experienced by animals, and which emotions involve conscious cognitive input and when, will continue to be the subject of debate. Future studies on animal emotion should consider emotion in broad terms of valence (positive or negative psychological value) and arousal. To progress experimental research on the measurement of positive emotion in animals several premises, for which there is scientific support, must be accepted. These include: that animals, at least non-human mammals, can experience emotion; emotion experienced by animals can be positive or negative and vary in level of arousal; emotional valence may be detected and measured by behavioural, physiological and cognitive means. Additionally, adoption of the concept that minimisation of negative emotion and provision of positive emotion inducing experiences is necessary to safeguard an animal’s welfare, allows for practical application of research on animal emotion.

## 1.4 POTENTIAL STIMULI FOR INDUCING AFFECTIVE EXPERIENCE IN HORSES

Various stimuli that could be considered for inducing emotion in horses are given in Table 1, with presumed positive emotion on the left and a similar type of stimulus to induce presumed negative emotion where possible on the right. When choosing stimuli to use, many factors, such as the social and ethical responsibility not to cause unreasonable or unnecessary pain or distress, repeatability and standardisation, ease of use, the duration of the stimulus and response, likely arousal level, risks and potential confounding of variables to be measured, need to be considered.

Stimuli such as grooming or massage of different sites, head lowering, warm water immersion, human interaction, and food have been used as putatively pleasant experiences in horses and may be useful for inducing positive emotion in future studies.

Studies on indicators of emotion should validate how the putatively positive or negative emotion inducing experience is perceived by the individual. Thus any physiological, behavioural or cognitive changes seen with such treatments can be said to indicate the emotion without forming a circular argument. Pain and the consumption of food should be used with caution in studies on emotion. The behavioural and physiological responses of horses may be altered by anticipation (excitement, increase in heart rate, head shaking, pawing) (Innes & McBride 2008), ingestion (involves head and neck movement, facial expressions may be obscured or altered by eating), and digestion (altered autonomic and cardiac activity, neuroendocrine changes, endorphin release) which may or may not reflect changes in emotion. Ideally, positive and negative emotion inducing experiences should be similar in terms of arousal or intensity, be based on the same substrate, and include a neutral or intermediate treatment for comparison.

## 1.5 EXPERIMENTAL RESEARCH ON POTENTIAL INDICATORS OF AFFECTIVE EXPERIENCE

Despite the lack of clear agreement on approaches to animal emotion, terminology, definitions, or indeed what specific emotions animals are capable of, much progress has been made on assessment of negative emotions such as pain and fear in many species. Less progress has been made on assessment of positive emotion and only in a few species (Boissy et al. 2007). The little research that has been conducted on emotion in horses has mostly focused on pain, fear and the stress responses.

Changes due to positive emotional responses are generally considered more subtle and variable and thus present a challenge for scientific study (Boissy et al. 2007). Only one study has explicitly focused on positive emotion in horses (Stratton et al. 2010a). However, examination of the limited studies on the behavioural and physiological responses of horses undergoing experiences which may be considered pleasant may also help direct future work on markers of positive emotion in horses. Physiological, behavioural and cognitive parameters that have been measured in horses and other animals were evaluated for their usefulness as potential indicators of emotion in horses.

Physiological parameters that may be considered for use as potential indicators of emotion that have been measured in horses are given in Table 2 and those that have been used other species are given in Table 3. Heart rate variability may be a useful measure. Eye temperature warrants further investigation. Both are non-invasive measures. More invasive measures may be affected by sampling and may indicate arousal rather than emotional valence. Research in humans suggests other physiological parameters such as immune function and glucose utilisation by the brain may be affected by positive and negative emotion (Steptoe et al. 2005; Boissy et al. 2007; Matsunaga et al. 2008; Matsunaga et al. 2009). However, there is little research in animal species. The measurement of urinary oxytocin also looks promising but requires further investigation before application in horses.

Behaviours and behaviour assessments that may be considered for use as potential indicators of emotion are given in Table 4. Parameters such as body posture or movement, facial expression, vocalisation and investigation could be useful as potential indicators of positive or negative emotion in horses. Animals displaying abnormal stereotypic behaviour may respond differently and should be considered separately from normal animals when assessing emotion (Lebelt et al. 1998; Normando et al. 2007).

Some parameters, whilst giving valuable information, would be impractical, too invasive or costly to investigate in horses at this time. These include self-stimulation behaviour, electrical stimulation, immune function, neurochemical changes, and brain imaging or perfusion measures. Other parameters, such as assessment of cognitive bias, stress coping behaviour, and goal directed behaviour, may be more suited to the study of emotional states.

Once valid, non-invasive indicators of emotion have been identified, qualitative behavioural assessment methods might be useful for verifying the suitability for use by horse caretakers.

Consideration also needs to be taken with the timing of stimuli and measurements (duration of stimuli, when to start and stop recording), and factors such as time of day effects on baseline of individual and diurnal patterns. Interpretation can sometimes be difficult. The same physiological response, for example increased heart rate, has been shown in humans with emotions of differing valence or with activity. Physiological changes may simply reflect the intensity of arousal or physical activity, not necessarily emotional valence (Paul et al. 2005).

There may also be problems with the use of behavioural measures with regard to recording error, bias, interpretation and reliability. Cognitive bias measures have been most useful in elucidating emotional states rather than emotions, however, equivocal and unexpected results have been seen in some studies and care needs to be taken with interpretation of results as well as attention to confounding issues.

It seems likely that no single measure will be suitable for assessing emotion in animals and the use of a single measure will likely lead to erroneous conclusions. It may be that characteristic patterns are apparent when several measures are considered together. This level of analysis is also challenging.

Table 1 Potential emotion inducing experiences in horses

| Stimulus category | Pleasant application of stimulus | | Unpleasant application of stimulus | |
|---|---|---|---|---|
| | Method of application | Considerations for use | Method of application | Considerations for use |
| Grooming by human | Massage or grooming to mimic mutual grooming by horses<br>Preferred areas: withers, dorsal neck/mane, dorsal rump | Horses need to be still in order to apply stimuli<br>Previous studies used it<br>Inexpensive equipment<br>Associated with a person<br>Standardisation of stimulus<br>Low safety risk | Human massage, groom, scratch or scrub<br>Flank/cranial thigh fold, inguinal/ventral abdominal region, under tail/between thighs | Habituation might occur (as with most of the unpleasant stimuli) |
| Food sight and/or smell | Anticipation of food e.g. associate with predictor object/sound/light | Depends on current motivation for food and satiety level<br>Neurophysiological changes may be due to food which may not have anything to do with emotional valence<br>Thwarted anticipation may lead to frustration<br>May get high arousal | Hunger or withdrawal of presented food/food taken away, inability to access food | May induce frustration |
| Food, consumption | Consumption of desired food e.g. carrot, grain/pellets, molasses soaked pellets<br>Food toy/cognitive task e.g. Equiball | Positive emotion induced by meeting or exceeding expectation<br>Depends on current motivation for food and satiety level<br>Neurophysiological changes may be due to food which may not have anything to do with emotional valence<br>Investigation and prehension of | Consumption of aversive food e.g. quinine soaked grain, wooden pellets | Negative emotion may be induced if animals' expectations are underwhelmed<br>Depends on current motivation for food and satiety level |

| Stimulus category | Pleasant application of stimulus | | Unpleasant application of stimulus | |
|---|---|---|---|---|
| | Method of application | Considerations for use | Method of application | Considerations for use |
| | | food alters behaviour and may obscure potentially useful behavioural indicators (e.g. lip/chin movements) | | |
| Social contact sight, hearing or smell | Access to conspecific or other companion<br>Conspecific: real horse (familiar), mirror, picture, audio/video recording, smell (faeces/urine) e.g. studies on housed horses, stereotypies<br>Non-interacting human<br>Other species | Social relationships may be complex<br>May need to establish who is a preferred affiliate first<br>Other species have shown a preference for picture of peers' face<br>Some evidence that horses seek human company/contact<br>Associated with particular person | Isolation or unfamiliar companion Complete social isolation, mixing with unfamiliar conspecifics, mixing with other species | Isolation of prey species likely to be highly unpleasant<br>May get confounding of other stimuli if all are applied under conditions of social isolation |
| Thermal comfort | Choice of ambient temperature and humidity, shade options | Access to temperature controlled facilities | Cold/wet or hot conditions | Access to temperature controlled facilities that reach extremes |
| Reproduction sight, smell, touch or taste | Sexual stimulation<br>Males – sight or smell/lick urine from mare in estrus<br>Tactile stimulation of genitalia | May induce frustration<br>Social and ethical responsibility not to cause unreasonable or unnecessary pain or distress | Sexual inhibition<br>Cold water squirted on genitalia Unfulfilled sexual stimulation | Human safety an issue<br>Social and ethical responsibility not to cause unreasonable or unnecessary pain or distress |
| Sounds | Rhythmic/repetitive noise – predictability<br>Certain type of music? | Unknown if any music or rhythms preferred by horses | Sudden loud noises – unpredictable, fear<br>Recording of gunshots, fireworks, intermittent banging/hammering | Habituation might occur |
| Insects | Mutual insect swatting | Difficult to standardise | Irritation from flies/ insects | Difficult to standardise |

| Stimulus category | Pleasant application of stimulus | | Unpleasant application of stimulus | |
|---|---|---|---|---|
| | Method of application | Considerations for use | Method of application | Considerations for use |
| | Person swats insects from around head | May provide relief or may represent a return to neutral state but not pleasant | Release flies around head/feet | |
| Space | Freedom, choice over location<br>Space to run, escape route/door. | May represent a return to neutral state/homeostasis but not pleasant<br>Horses are able to move so limits possible measures and exercise confounds | Confined space<br>Low roof, walk between narrow walls | Horses able to move |
| Exercise | Warm water immersion | Likely to be unpleasant until habituated as initially novel Facilities/cost an issue<br>Horses moving | Forced exercise e.g. walker, swimming, lunge with pessoa type system | Facilities, cost<br>Habituate then may be pleasant Horses are moving so limits possible measures and exercise confounds |
| Autogrooming | Choice of grooming tools<br>Provide brushes/objects to rub against | Other species have displayed preference<br>Horse may not choose to experience anything | | |
| Pheromones | Equine Appeasing Pheromone | Studies show calming effect<br>Not available in New Zealand | | |
| Pain | | | SC or IM Injection of stinging substance, minor skin damage e.g. thermal/laser | Social and ethical responsibility not to cause unreasonable or unnecessary pain or distress<br>Behaviour of injected body part altered<br>Horses may become 'needle shy' |

| Stimulus category | Pleasant application of stimulus | | Unpleasant application of stimulus | |
|---|---|---|---|---|
| | Method of application | Considerations for use | Method of application | Considerations for use |
| | | | | and future use or welfare (if need injections) may be compromised |
| Spray | | | Aerosol spray e.g. remote spray collar, insect spray<br>Scented or unscented | Sudden, novel, remotely activated/unassociated with a person, possibly safer<br>Unlikely to be pleasant<br>Used in temperament tests<br>Habituation might occur |
| Predator threat sight, hearing or smell | | | Real, poster, recording, smell (faeces/urine) e.g. dog/wolf, pig, large cats, bear | Unknown if would elicit any reaction |
| Restraint | | | Hold leg up/stand on 3 legs, fore leg or hind leg | Induced fatigue may be the unpleasant experience<br>Safety a concern |
| Visual challenge | | | Crossing thresholds e.g. light to dark, shadows | Involves the horse moving<br>May not be aversive enough |
| Object sound and/or sight | | | Sudden movements e.g. flapping things – flag, umbrella opening | Habituation might occur<br>Dependent on previous experience of objects |
| | | | Novel objects<br>Bright colours, 2D or 3D e.g. road cone, opened umbrella | Habituation might occur<br>Dependent on previous experience of objects |
| Water splash | | | Urinating on hard surface | May not be unpleasant enough |

| Stimulus category | Pleasant application of stimulus | | Unpleasant application of stimulus | |
|---|---|---|---|---|
| | Method of application | Considerations for use | Method of application | Considerations for use |
| | | | Mimic by pouring water from a height | May be pleasant to some |

Table 2 Potential physiological indicators of emotion that have been used in horses

| Parameter | Used for positive emotion? | Used for negative emotion? | Strengths | Limitations | References |
|---|---|---|---|---|---|
| Heart rate | Yes | Yes | Used in a number of species and different situations<br>Useful for negative emotion<br>May be useful for positive emotion with low arousal<br>Duration of heart rate elevation may differ between positive and negative emotion | Changes may be due to arousal or physical activity rather than emotional valence<br>Not as useful as heart rate variability in determining balance of autonomic activity or emotional component<br>Does not correlate well with pain in horses | (Feh & de Mazieres 1993; Brosschot & Thayer 2003; Normando et al. 2003; McBride et al. 2004; Jansen et al. 2009; Sankey et al. 2010a; Stratton et al. 2010a) |
| Heart rate variability | Yes | Yes | Better measure of stress than heart rate, cortisol or adrenaline/noradrenaline<br>Non-invasive way to determine sympathetic and parasympathetic responses<br>Increased parasympathetic nervous system activity may indicate relaxation, a positive emotion<br>Good stability over time within individual horses | Changes may be due to arousal or physical activity rather than emotional valence<br>May not see a change when positive emotion is associated with increased arousal<br>Prone to errors/artefact<br>Calculation and interpretation of heart rate variability is difficult<br>Large variation between individuals<br>Possible gender effect<br>Warrants further investigation | (Kuwahara et al. 1996; Visser et al. 2002; Bachmann et al. 2003; Kato et al. 2003; Rietmann et al. 2004a; Rietmann et al. 2004b; von Borell et al. 2007; Reefmann et al. 2009c; Reefmann et al. 2009a; Kok & Fredrickson 2010; Zebunke et al. 2011) |
| Respiratory rate | Yes | Yes | Non-invasive<br>Useful for negative emotion | Changes may be due to arousal or physical activity rather than emotional valence | (McCann et al. 1988; Hamra et al. 1993; Kuwahara et al. 1996; |

| Parameter | Used for positive emotion? | Used for negative emotion? | Strengths | Limitations | References |
|---|---|---|---|---|---|
| | | | | Inconsistent results across studies<br>Difficult to measure continuously in horses<br>Prone to errors/artefact | Reefmann et al. 2009c; Reefmann et al. 2009a; Stratton et al. 2010b; Reefmann et al. 2012) |
| Skin temperature | Yes | Yes | Non-invasive<br>May be an association between ear pinna temperature and stress in horses | No association with emotion in sheep<br>Changes may be due to arousal or physical activity rather than emotional valence | (Kay & Hall 2009; Reefmann et al. 2009c; Reefmann et al. 2009a; Moe et al. 2012) |
| Blood pressure variability | | Yes | Correlates with some measures of heart rate variability in horse | Invasive and does not offer further information than that obtained from heart rate variability | (Kuwahara et al. 1996; Poletto et al. 2011) |
| Eye temperature | | Yes | Non-invasive<br>Useful for negative emotion | Emerging area of research<br>Only been applied to the study of negative emotion<br>Warrants further investigation | (Stewart et al. 2005; Stewart et al. 2007; Stewart et al. 2008b; Stewart et al. 2008a; Stewart et al. 2009; Stubsjoen et al. 2009; McGreevy et al. 2012) |
| Rectal temperature | | Yes | | Measurement may be unpleasant/irritating and difficult to continuously record in conscious horse | (Hamra et al. 1993; Stull 1999) |
| Glucocorticoid (e.g. cortisol) and catecholamine (e.g. | | Yes | Useful for evaluating acute stress | Can be difficult to measure<br>Invasive if measured from blood samples<br>The act of sampling may cause changes | Many<br>(Rietmann et al. 2004a) |

| Parameter | Used for positive emotion? | Used for negative emotion? | Strengths | Limitations | References |
|---|---|---|---|---|---|
| adrenalin) levels | | | | Inconsistent results when animals experience chronic stress<br>Heart Rate Variability may be a better measure of stress<br>Changes may be due to arousal rather than emotional valence and may not correlate with behaviour | |

Table 3 Potential physiological indicators of emotion that have not been used in horses

| Parameter | Used for positive emotion? | Used for negative emotion? | Strengths | Limitations | References |
|---|---|---|---|---|---|
| Humidity above skin or skin conductance | Yes | Yes | Non-invasive<br>Variability of humidity may indicate emotion in sheep | Reliability is questionable as the measure can be variable | (Reefmann et al. 2009c; Reefmann et al. 2009a) |
| Neuroendocrine levels (e.g. oxytocin) | Yes | Yes | Urinary oxytocin may be useful non-invasive measure in future | Can be difficult to measure<br>Invasive (except for urinary oxytocin)<br>Expensive | (Agren et al. 1997b; Agren et al. 1997a; Odendaal & Meintjes 2003; Holst 2007; Matsunaga et al. 2008; Bateson et al. 2011; Mitsui et al. 2011) |
| Brain activity – imaging (e.g. functional MRI, PET) | Yes | Yes | Novel but showing promise in dogs that can be trained to lie still during a scan | Not suitable for horses at this point as recumbency for long periods not desirable<br>Expensive and equipment is not readily available | (Burgdorf & Panksepp 2006; Wager et al. 2008; Berns et al. 2012; Tettamanti et al. 2012) |
| Brain activity - functional near-infrared spectroscopy to estimate oxyhaemoglobin concentration | Yes | Yes | Potential to assess emotions and shows promise in sheep | Novel and further validation is required<br>Expensive and equipment is not readily available | (Muehlemann et al. 2011) |

Table 4 Potential behavioural indicators of emotion in animals

| Behaviour or Behaviour Test | Used in Horses? | Used for positive emotion? | Used for negative emotion? | Comment | References |
|---|---|---|---|---|---|
| Facial expression including ears | Yes | Yes | Yes | Form of communication may be reliant on an audience Sheep ear position looks promising for positive emotion<br>Eye aperture in sheep also useful<br>Grimace scales for pain developed in mice and rats<br>Similarity of facial expressions between rats, mice, chimpanzees and humans with pleasant and unpleasant tastes<br>Potential indicator of emotion in horses | (Schilder et al. 1984; Paul et al. 2005; Boissy et al. 2007; Warren-Smith et al. 2007; Reefmann et al. 2009b; Reefmann et al. 2009a; Stubsjoen et al. 2009; Flecknell 2010; Langford et al. 2010; de Waal 2011; Leach et al. 2011; Sotocinal et al. 2011) |
| Body posture/movement | Yes | Yes | Yes | Tail postures in sheep may indicate emotion<br>Leg movement may indicate emotion in horses | Many. (McBride et al. 2004; Hausberger et al. 2008; Fureix et al. 2009a; Fureix et al. 2009b; Sankey et al. 2010c; Sankey et al. 2010a; Stratton et al. 2010b; Fureix et al. 2011; Zimmerman et al. 2011; Schutz et al. 2012) |
| Play behaviour | Yes | Yes | Yes | Seen in young animals<br>Interpretation reliant on context as occurs when other needs have been satisfied but also can occur under adverse welfare conditions<br>Possibly indicator of opioid mediated reward mechanisms | (Jensen et al. 1998; Jensen & Kyhn 2000; McDonnell & Poulin 2002; Paul et al. 2005; Fraser 2008; Hall et al. 2008; Held & Spinka 2011; Hausberger et al. 2012) |
| Behaviour score | Yes | Yes | Yes | Subjective, qualitative or semi quantitative but may correlate with other quantitative measures<br>Can be difficult to replicate | (LeScolan et al. 1997; McBride et al. 2004; Minero et al. 2009) |
| Vocalisations | Yes | Yes | Yes | Form of communication, may be reliant on an audience | (Knutson et al. 2002; McDonnell 2003; |

| Behaviour or Behaviour Test | Used in Horses? | Used for positive emotion? | Used for negative emotion? | Comment | References |
|---|---|---|---|---|---|
| | | | | Rats and primates laugh when tickled<br>Potential indicator of emotion in horses | Burgdorf & Panksepp 2006; Stubsjoen et al. 2009; Gogoleva et al. 2010; de Waal 2011; Imfeld-Mueller et al. 2011) |
| Licking and chewing | Yes | Yes | Yes | Calm horses display more than aroused horses | (Warren-Smith et al. 2007) |
| Investigative or approach/avoidance behaviour | Yes | Yes | | Similar to human-animal interaction, may indicate emotional state<br>May just reflect differences in temperament and experience<br>Warrants further investigation | (Forkman et al. 2007; Fureix et al. 2009a; Westerath et al. 2009) |
| Affiliative behaviour/social interaction | Yes | Yes | | Being licked by a conspecific in cattle may indicate positive emotion<br>Potential indicator of positive emotion in horses | (Feh & de Mazieres 1993; Rousing & Wemelsfelder 2006; Laister et al. 2011) |
| Aggressive behaviour | Yes | | Yes | May indicate negative emotion | (Fureix et al. 2009b) |
| Stereotypic behaviour | Yes | | Yes | Abnormal behaviour<br>Indicator of welfare compromise at first initiation of behaviour<br>Animals displaying stereotypic behaviour have altered responses and should be considered separately | (Lebelt et al. 1998; Moe et al. 2006; Normando et al. 2007) |
| Visible eye white | | Yes | Yes | Care with method of measuring, can be labour intensive<br>Useful in sheep and cattle<br>Potential indicator of emotion in horses | (Sandem et al. 2002; Sandem et al. 2006; Reefmann et al. 2009c) |
| Grooming Behaviour | | Yes | | May occur with anticipation of positive event in chickens<br>Potential indicator of positive emotion in horses | (Zimmerman et al. 2011) |

| Behaviour or Behaviour Test | Used in Horses? | Used for positive emotion? | Used for negative emotion? | Comment | References |
|---|---|---|---|---|---|
| Anticipatory behaviour | | | Yes | May vary with positive or negative event<br>Short-term stress leads to increase<br>Long-term stress leads to decreased response (anhedonic-like) | (Moe et al. 2006; Van der Harst & Spruijt 2007; Greiveldinger et al. 2011) |
| Operant resource testing | Yes | Yes | Yes | Strength of motivation for a stimulus e.g. lever press | |
| Human-animal interaction | Yes | Yes | Yes | Motionless person test<br>May be a useful summative indicator of overall welfare in horses<br>May indicate emotional state<br>Based on approach/avoidance behaviour | (Henry et al. 2005; Hausberger et al. 2008; Fureix et al. 2009a; Minero et al. 2009; Sankey et al. 2010b; Sankey et al. 2010c; Sankey et al. 2010a) |
| Laterality of visual field test | Yes | | Yes | May be useful for negative emotion in horses, left eye/ear predominates | (Larose et al. 2006; Des Roches et al. 2008; Farmer et al. 2010) |
| Behavioural tests – open field, elevated maze, hole board, black and white box, social interaction | Yes | | Yes | Standardised tests, most often used in psychopharmacology, problems with interpretation, precise time period over which to monitor behaviour | |
| Startle response test | | Yes | Yes | May reflect emotional state<br>Startle increases with negative state, decreases with positive state | (Forkman et al. 2007) |
| Place preference/avoidance test | | Yes | Yes | Value of stimulus to animal<br>Conditioned place preferences then experimentally challenge | |

| Behaviour or Behaviour Test | Used in Horses? | Used for positive emotion? | Used for negative emotion? | Comment | References |
|---|---|---|---|---|---|
| Sucrose consumption test | | Yes | Yes | Emotional state<br>Correlates with humans as a measure of depression | |
| Qualitative Behavioural Assessment (QBA) | Yes | Yes | Yes | Also known as free choice profiling (FCP)<br>Measures how people interpret behaviour which may or may not be accurate<br>Has shown correlation with other measures of behaviour and physiology<br>Potential as indicator of positive emotion in horses needs to be explored | (Wemelsfelder 2000; Wemelsfelder et al. 2000; Wemelsfelder et al. 2001; Wemelsfelder & Farish 2004; Rousing & Wemelsfelder 2006; Wemelsfelder 2007; Napolitano et al. 2008; Minero et al. 2009; Wemelsfelder et al. 2009; Walker et al. 2010) |

# 2 Determining a horse's preferred stimulus

## 2.1 OBJECTIVE

One of the problems with research on emotion in animals has been the need to assume that the applied stimulus is causing a positive emotion such as pleasure, or a negative emotion, such as pain or fear, as animals are unable to self-report like humans can. Animal's inability to self-report is a major weakness when determining behavioural and physiological responses to different emotional states. This experiment was designed to address this issue by using a novel application of preference testing. The experiment was designed to allow horses to indicate their preferred stimulus by choosing to spend more time in an area associated with that stimulus. A preference for a stimulus suggests that the stimulus is more pleasurable i.e. induces a more positive emotion (Humphrey 1972; Dawkins 2008).

In the current study, stimuli likely to induce positive or negative emotion were chosen based on previous research. Grooming or massage of the withers region was thought to be desirable (Feh & de Mazieres 1993; McBride et al. 2004; Reefmann et al. 2009a; Stratton et al. 2010a). The presence of a non-interacting person was predicted to be desirable for some horses and the absence of an additional stimulus (no interference) desirable for other horses (Fureix et al. 2009a; Stratton et al. 2010a). The spray was predicted to be aversive for all horses and has been used in studies of avoidance task learning, temperament and reactivity (Seaman et al. 2002; Visser et al. 2003; Lansade & Simon 2010).

The aim of this experiment was to identify horses with a preference for one of four stimuli. These horses would then be used in the next experiment on identifying indicators of emotion.

## 2.2 METHODS

Twenty horses from a teaching herd maintained at the Massey University Veterinary Large Animal Teaching Unit, Palmerston North, New Zealand were used. The experiment was conducted in accordance with the Massey University Code of Ethics for Animals (MUAEC approval 10/92). Horses were habituated to the experimental setup and equipment then trained to associate a visual cue and spatial location with each stimulus. The stimuli were:

- An unfamiliar person standing, not interacting or looking at the horse for four minutes (Person)
- An unfamiliar person grooming the left wither area for four minutes (Groom)
- A lightly scented aerosol spray emitted behind the right elbow of the horse via remote control every 20 seconds for 90 seconds (Spray)
- No stimulus for four minutes (No-stimulus)

Horses were given free choice of the four physically similar locations during four tests. No stimuli were given during tests. Video recordings were made. The proportion of time that each horse spent in each area during each test was calculated. The stimulus associated with the area where the horse spent the most time was determined to be the horse's preferred stimulus for that test. The results from each test were examined to determine if a horse displayed a consistent preference in the majority of tests (i.e. in three out of four tests).

## 2.3 RESULTS

Eleven horses displayed a consistent preference. Of the remaining nine horses, one was excluded due to display of stereotypic behaviour. Stereotypic behaviour is abnormal and may affect learning as well as emotion. One horse chose to be in the start box semi-circle during all four tests and was deemed not to make a choice of stimuli. Seven horses did not display a consistent preference.

Of those horses that displayed a consistent preference, 6/11 preferred the person stimulus, 3/11 preferred the grooming stimulus, and 2/11 preferred the no stimulus situation. For horses that displayed a consistent preference, the spray stimulus was not preferred in any tests.

Due to weather constraints the number of tests was reduced to three for four horses. This was accommodated by analysing results as a proportion of the number of tests.

## 2.4 DISCUSSION

Just over 50% of horses (11/19) displayed a consistent preference for a stimulus. Horses' preferences differed. The majority preferred having a person nearby, to wither grooming or being left alone. This may be related to the need for safety or companionship, past experience, or other individual differences. Contrary to previous research, wither grooming by a person may be overestimated as an enjoyable stimulus for all horses (Feh & de Mazieres 1993; McBride et al. 2004). What horses like cannot be assumed and varies on an individual basis.

This experiment used a novel design of open choice preference testing to identify a stimulus that induced positive emotion for each horse. A limitation of this experiment is the inability to rank the stimuli for each horse. Pairwise presentation of the stimuli would have enabled ranking. However, repeated pairwise testing may not provide consistent results and would have taken 24 times longer and cost more. The current study design enabled the aim to be accomplished in an efficient way.

There may be other factors that influenced horses' choices apart from preference for the stimulus. Although the stimulus location was randomised across horses, a location effect cannot be ruled out in an individual horse. No stimuli were given during test sessions to avoid any influence on the horse's choices by the presence of people in the arena and to prevent the spray stimulus from overshadowing everything else. However, this meant a heavy reliance on the association of the visual and spatial cues with the stimuli. Therefore the association was reaffirmed by a training session after each test.

Although unlikely, it could be argued that if a horse did not find any of the stimuli pleasant, then its preference may actually be for the least aversive stimulus (aversion test). The underlying emotion may not be an absolute positive, but more positive than that experienced during the other stimuli.

Future research on indicators of emotion should not assume that animals will like or dislike the given stimuli. A method which identifies whether individual animals actually perceive the putatively pleasant stimulus as pleasurable prior to measuring potential indicators of emotion is required.

Eleven horses and their preferred stimulus were identified for use in the next experiment. Furthermore, the spray was considered likely to be aversive to these horses and suitable for inducing negative emotion in the next experiment.

# 3 Identifying indicators of emotion in horses

In this experiment, the horses that were identified in experiment one as having a preference for one of the stimuli were used to investigate potential indicators of emotion. A preferred stimulus is more desirable than the other stimuli and induces a more positive emotion (valence or arousal). The spray stimulus was never preferred and is likely to be aversive and induce negative emotion. It was assumed that the horse's preferences were stable over time and location.

The aim of this study was to investigate how behavioural and physiological responses of horses changed whilst undergoing a pleasant experience compared to a mildly aversive stimulus and neutral or intermediate experiences.

## 3.1 METHODS

For each of the eleven horses, their preferred stimulus from experiment one was used to induce positive emotion and the spray stimulus was used to induce negative emotion. It was anticipated that the other two stimuli would induce emotion intermediate in valence between the preferred and aversive ones. Selected behavioural and physiological variables were measured and analysed to determine if they may be used as indicators of emotion in horses. The experiment was conducted in accordance with the Massey University Code of Ethics for Animals (MUAEC approval 10/116).

Each experiment session consisted of repetitions of four different treatments of three minutes duration and had a total duration of approximately 50 minutes. The stimuli used were the same as for the previous experiment but delivered with the horse standing in stocks to enable behavioural and physiological measurements (Table 5).

Table 5 Name and description of treatments

| Treatment | Description of stimulus |
|---|---|
| Person (P) | A person was standing 1m in front of the horse, and was not interacting or looking at the horse. The person was the same for each horse and was previously unfamiliar to the horses. The horses were able to sniff/lick the person. The person would step back out of reach if the horse tried to bite. |
| Groom (G) | A person groomed the left withers area using a rubber grooming tool in a circular motion with consistent pressure. The person was the same for each horse. |
| Spray (S) | A lightly scented aerosol spray was emitted behind the right elbow every twenty seconds for three sprays (if deemed safe to do so) from a remotely controlled spray training collar (Petsafe SRT200 Remote Spray Trainer, Radio Systems Corporation, Knoxville, USA) . The collar was placed under an elastic surcingle on the right side of the chest midway between ventrum and dorsum. |
| No-stimulus (C) | Horses were standing in stocks. No additional stimulus was present. |

The Spray treatment was applied once per session while the Person and Groom treatments were applied two times, once before and once after the Spray treatment. The No-stimulus treatment was applied seven times, three times before and four times after the Spray treatment, as it was interspersed between the other treatments. The treatment order was fixed to allow for the possibility of a negative carryover effect from the Spray treatment. However, the order in which the Person and Groom treatments were given was randomised for each horse. The session was repeated three times for each horse, with at least 24 hours between sessions.

Horses were habituated to the facilities and equipment. A companion horse was always present so horses were not socially isolated. Figure 1 and Figure 2 show a horse in the experimental setting.

Figure 1 View of the horse as recorded by the side camera with a fisheye lens.

Figure 2 View of the horse as recorded by the front camera.

### 3.1.1 Behavioural Measures

Video recordings from the side view camera (Figure 1; Sony DCR-SR85E, Auckland, New Zealand) and head view camera (Figure 3; Canon Legria HFM31, Sydney, Australia) were examined using Interact software (version 9, Mangold International GmbH, Arnstorf, Germany) to record all occurrences and duration of behaviours pertaining to the body of the horse and the head of the horse including facial expression (Table 6). After recording behaviour for two horses the decision was made to remove behaviours that were not reliable (e.g. due to subtlety of changes or relative camera angle) or where performance was dependent on another behaviour (e.g. head tucked/extended with investigation). On this basis the following behaviours were not scored: cheek concave or convex, temporal fossa flat or convex, head extended, neutral or tucked, head left, centre or right, head rotation, top lip extended, eyebrow angle sub-categorisation to forward, mid or back. The ethogram for body behaviours is described in

Table 6 (modified from McDonnell 2003) and the ethogram for head behaviours is described in Table 7.

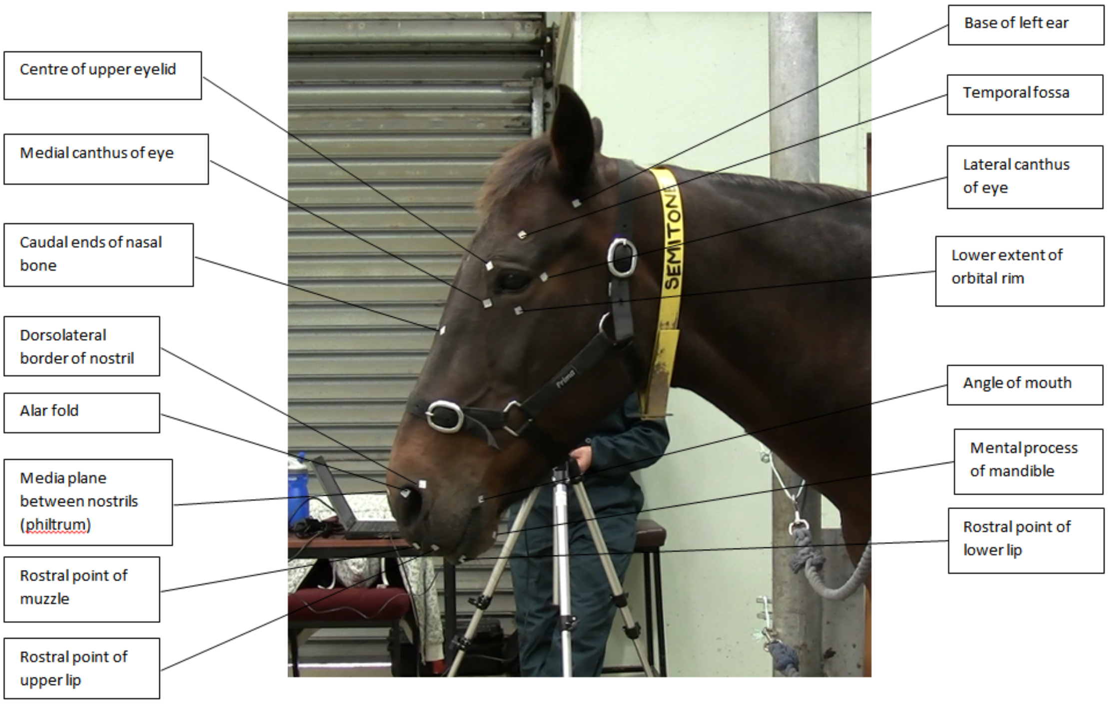


Figure 3 View of the horse as recorded by the head camera. The position of white markers on prominent head features is shown.

Table 6 Ethogram of body behaviours recorded from video.

| Body part | Behaviour | Description | Data type* |
|---|---|---|---|
| Foreleg | Lift | Complete lift of foreleg hoof from ground in a vertical direction | Rate |
| | Strike | Complete lift of foreleg hoof from ground also with leg movement forward | Yes/No |
| | Rear | Both front legs lift from ground | - |
| Hindleg | Lift | Complete lift of hindleg hoof from ground in a vertical direction | Rate |
| | Kick | Complete lift of hindleg hoof from ground surface also with leg movement backwards | Yes/No |
| | Rest | Heel bulb of hindleg removed from ground while toe of hoof remains in contact with ground | - |
| | Stand | Soles of both hooves in full contact with ground | - |
| Neck position | Very low | Neck lowered approximately -30 to -90º from horizontal through vertebral column | Rate |
| | Low | Neck lowered approximately -10 to -30º from horizontal through vertebral column | Duration |
| | Neutral | Neck is horizontal with the vertebral column +/-10º | - |
| | High | Neck approximately 10-45º above the horizontal through vertebral column | Duration |
| | Very high | Neck approximately 45-90º above horizontal through vertebral column | Rate |
| Tail | Lift | Movement of dock in vertical plane resulting in dock placement higher than horizontal to spine/pelvis - including tail lift before elimination | Yes/No |
| | Swish | Mild dock movement defined as lateral movement of the dock without moving in a vertical plane away from the hindquarters | Rate |
| | Thrash | Strong repeated vertical dock movement from the hindquarters with a gap clearly visible between the dock and hindquarters, with or without lateral movement. | Yes/No |
| | Neutral | Tail held in typical position, neither clamped nor raised | - |
| Vocalisation | Snort | Loud, harsh sound made by quick forceful movement of air | Yes/No |
| | Whinny (neigh) | Loud, prolonged call, beginning high pitched, ending lower pitched | Yes/No |
| | Nicker | Low pitched, gutturally pulsated sound | - |

| Body part | Behaviour | Description | Data type* |
|---|---|---|---|
| Elimination | Urination | Horse urinates, leg position not counted during urination | - |
| | Defecation | Horse defecates, leg position not counted during defecation | Yes/No |

*Rate data were analysed using a mixed effects Poisson model, Yes/No data were analysed using a mixed effects logistic regression model, Duration of time as a proportion data were analysed using a mixed linear regression model. Neck low and very low were amalgamated, as were neck high and very high.

- Data were not analysed as either included for calculation of proportion of time, were neutral positions, did not occur often enough to analyse or due to irregularities in the data.

Table 7 Ethogram of head behaviours recorded from video.

| Class | Behaviour | Description | Data Type |
|---|---|---|---|
| Head position | Shake | Repeated head rotation around cervical vertebrae | Yes/No |
| | Tossing | Repeated, rapid, forceful vertical flicking of head/nose | Yes/No |
| | Weaving | Repeated lateral movements of head from one side to the other | - |
| Ears# | Forward | Ear rotated forward | Rate |
| | Side | Ear rotated to side | - |
| | Back | Ear rotated backward but meatus still visible | Duration |
| | Flat | Ear flat back against neck, meatus flattened or not visible | Yes/No |
| | Flicking | Rapid movement of ear back and forth | - |
| Eye | Blink | Momentary eyelid closure | Rate |
| | Sclera | Sclera/white of eye visible | Rate |
| | Aperture small | Small area of eyeball visible. Narrowed palpebral fissure | Yes/No |
| | Aperture neutral | Eye aperture intermediate, neither large or small | - |
| | Aperture large | Large area of eyeball visible. Widened palpebral fissure | Yes/No |
| | Eyebrow wrinkle | Wrinkling of skin above eyebrow | Yes/No |
| | Eyebrow angle | Angulation in eyebrow | Yes/No |
| Mouth | Contracted lips | Decreased distance from corner of lips to end of muzzle | Yes/No |
| | Drooping lip | Lower lip not apposing upper lip and projects forward | Yes/No |

| Class | Behaviour | Description | Data Type |
|---|---|---|---|
| | Lip licking/chewing | Tongue flicked over lips, or chewing movements with mouth. Excluded when had access to food | Rate |
| | Face pulling | Extended or repetitive contortion of face that does not fit into other discrete categories | Yes/No |
| | Investigation | Sniffing, mouthing (grasping with lips or teeth) an object or person. Excluding where access to food. | Yes/No |
| | Yawn | Large opening of mouth | - |
| | Eating | Chewing, lip licking with food in mouth, prehending food | - |
| Chin | Chin wobble | Repeated, rapid movement of chin excluding other mouth movements (yawn, eating, lip licking/chewing, investigation) | Rate |
| | Parrot mouth | Upper and lower lips puckered. Upper lip extends beyond lower lip similar to a parrot's beak. Excluding other mouth movements (yawn, eating, lip licking/chewing, investigation) | Yes/No |
| | Puckered chin | Mentum more pointed, skin puckers under chin | Yes/No |
| Cheek | Cheek muscle contraction | Masseter muscle moving. Excluding when access to food, when licking and chewing, vocalising or yawning | - |
| Nares | Flared | Increased distance between medial and lateral alar fold | Duration |
| | Neutral | Nares in typical position neither narrowed or flared | - |
| | Narrowed | Nares narrowed or flattened | Yes/No |

*Rate data were analysed using a mixed effects Poisson model, Yes/No data were analysed using a mixed effects logistic regression model, Duration of time as a proportion data were analysed using a mixed linear regression model.

[#] Left and right ear recorded and analysed separately

- Data were not analysed as either included for calculation of proportion of time, were neutral positions, did not occur often enough to analyse or due to irregularities in the data.

### 3.1.2 Physiological Measures

Heart rate, heart rate variability and eye temperature were used as physiological measures because they are thought to be non-invasive indicators of the autonomic nervous system.

The two branches of the autonomic system, the sympathetic and the parasympathetic branches are in balance. The relationship between the two branches is complex, with heart rate representing the net effect. Assessment of the autonomic nervous system may yield information about emotional experience. Emotional arousal or excitement, whether due to positive or negative emotion, is indicated by increased activity of the sympathetic branch of the autonomic nervous system. Positive emotion may be indicated by increased activity of the parasympathetic (or vagal) branch (Stewart et al. 2011). Parasympathetic tone is a useful indicator of psychological and physiological flexibility and may be useful for assessing ability to cope with stress.

Heart rate is controlled by the autonomic nervous system. Analysis of the variation in time between heart beats (heart rate variability) is a non-invasive measure which yields more information about the balance of sympathetic and parasympathetic nervous tone. Heart rate variability is also affected by other conditions such as physical exercise and physical as well as mental health (Boissy et al. 2007; von Borell et al. 2007).

Increased arousal is usually associated with increased heart rate and decreased overall variability in the time between heart beats (standard deviation of normal-to-normal heart beat interval – SDNN), which is affected by both the sympathetic and parasympathetic branches. Sudden increases in heart rate are usually due to decreased parasympathetic tone, with sustained increased heart rate a function of increased sympathetic tone. Positive emotional valence is associated with an increase in the short term variability of the difference in time between heart beats (root mean square of successive differences – RMSSD) with an increase or no change to SDNN and is due to the effect of the parasympathetic branch. The ratio of RMSSD:SDNN (reported by some as SDNN:RMSSD) reflects the balance of parasympathetic and sympathetic tone and may increase with positive emotion and decrease with stress (Visser et al. 2002; Boissy et al. 2007; von Borell et al. 2007; Zebunke et al. 2011; Wang & Huang 2012).

The interval between heart beats (IBI) was measured using Polar Equine heart rate monitors (RS800CX, Polar Electro Oy, Helsinki, Finland). The mean interval, mean heart rate, SDNN, RMSSD and RMSSD:SDNN ratio were calculated from error corrected data, over 10 second periods, using Microsoft Excel 2010 (Zebunke et al. 2011).

Maximum eye temperature has been validated in cattle and sheep as a non-invasive indicator of pain and fear (Stewart et al. 2008b; Stewart et al. 2008a; Stewart et al. 2010). A drop in eye temperature is thought to be caused by vasoconstriction due to a predominance of the sympathetic nervous system (Stewart et al. 2008a). It was hypothesised that a predominance of the parasympathetic nervous system with positive emotion may lead to vasodilation and an increase in eye temperature. To the authors' knowledge this is the first research on eye temperature in horses and the first investigation of eye temperature and positive emotion in any species.

Thermal images of the horse's right eye were recorded continuously using an infrared thermography (IRT) video camera (ThermaCam S60, FLIR Systems AB, Danderyd, Sweden) located to one side of the horse. The maximum (at medial canthus) and average eye temperature (ET) was calculated two to three times per minute using image analysis software (ThermaCAM Researcher Pro version 2.10). Clear images that contained a minimum of five pixels were used (Figure 4).

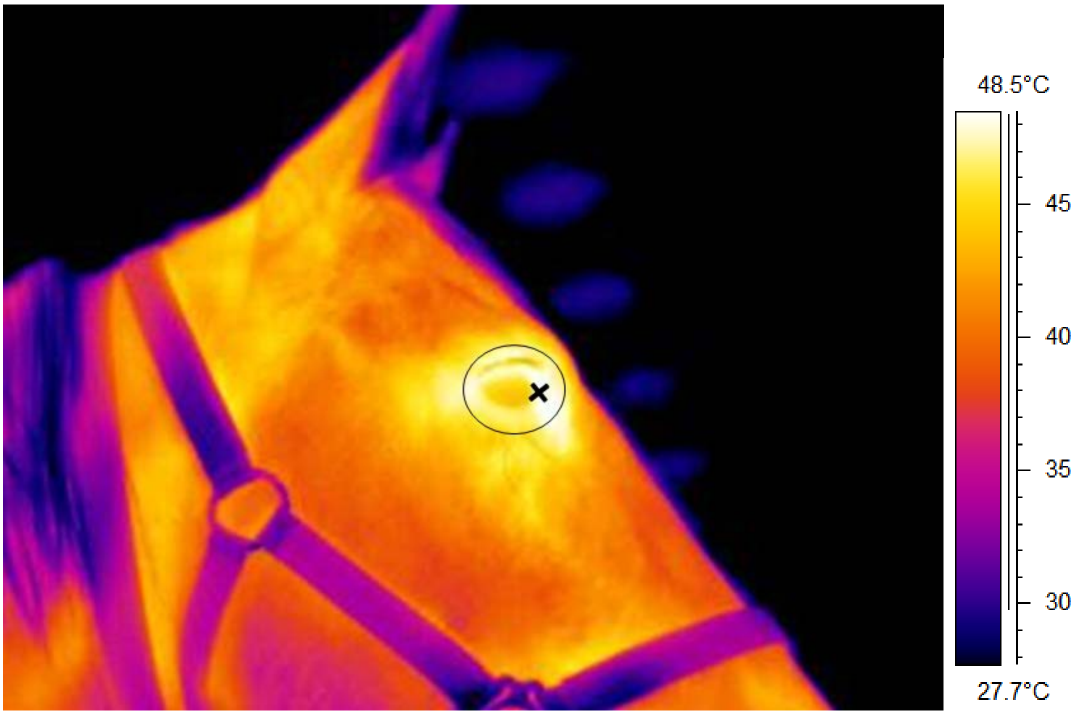


Figure 4 Thermal image of a horse's head showing position where maximum eye temperature was taken (x) and the area over which average eye temperature was calculated (oval).

### 3.1.3 Data analysis

The results from the two Groom, two Person, two No-stimulus and one Spray treatments for each replicate were analysed. The periods used for analysis of the No-stimulus treatment were randomly selected for each replicate, with one occurring before and one after spray. For each horse, the treatments were categorised as preferred, intermediate or aversive (spray).Statistical models were used that accounted for repeated measures on each horse and correlation between successive physiological measurements. Results from the preferred category were compared to those from the intermediate category and to those from the aversive category.

Statistical analysis was performed in the software programme 'R' (R Development Core Team, 2012). The significance of relationships was assessed using the log likelihood ratio test and Wald test statistics. Statistical significance was set at $p<0.05$.

## 3.2 RESULTS

A simplified overview of the results is presented in Table 8.

Compared to the preferred treatment, the aversive treatment resulted in an increase in the following behaviours: foreleg lift, hindleg lift, hindleg kick, neck very low, duration of neck above neutral (high/very high), neck very high rate, tail swish, left ear forward, left ear back, right ear back, blink, sclera visible, large eye aperture, contracted lips, and nares flared. There was a decrease in the duration of time the neck was below neutral (low/very low), and the tail lift, right ear forward, small eye aperture, eyebrow wrinkle, eyebrow angle, lower lip droop, lip licking and chewing, face pulling, investigation, chin wobble, and nares narrowed behaviours. There was also a tendency towards a decrease in head shaking ($p=0.06$).

The intermediate preference, compared to the preferred treatment, resulted an increase in the rate of left ear forward, a decrease in the rate of neck very high and tendencies towards a decrease in the rate of sclera visible ($p=0.10$), tail swish ($p=0.10$) and an increase in the rate of neck very low ($p=0.07$) behaviours.

Intra-observer reliability was assessed by comparison of results from a repeated view of randomly chosen horses/replicates. The Pearson's correlation between the repeats demonstrated very good reliability (Petrie & Watson 2006). They averaged 0.99 (±0.004), range 0.86 to 1 for the body behaviour, and averaged 0.96 (±0.009), range 0.83 to 1 for the head behaviour.

During the preferred treatment the average heart rate was 40 beats per minute. The heart rate and the interval between heart beats did not differ between the intermediate and preferred treatments. During the aversive treatment the heart rate increased by 10 beats per minute and the interval between heart beats decreased by 284 milliseconds compared to the preferred treatment.

After adjusting for ambient air temperature, the average eye temperature was 0.07$^{o}$C lower during the aversive treatment than when a horses' preferred treatment was applied. There was no significant difference in average eye temperature between the intermediate and preferred treatments. There was no significant impact of preference on the maximum eye temperature.

Table 8 Summary of indicators of affective experience in horses showing effect of preference for a treatment on physiological and behavioural measures. $p<0.05$

| Category | Variable | Change in Aversive V Preferred | Change in Intermediate V Preferred |
|---|---|---|---|
| Leg | Rate of foreleg lifts | ↑↑↑ | - |
| | Likelihood of displaying a foreleg strike | - | - |
| | Rate of hindleg lifts | ↑↑↑↑ | - |
| | Likelihood of displaying a hindleg kick | ↑↑↑↑ | - |
| Neck | Rate of moving neck to very low position | ↑ | ↑# |
| | Duration of time with neck below neutral | ↓ | - |
| | Duration of time with neck above neutral | ↑↑ | - |
| | Rate of moving neck to very high position | ↑↑ | ↓ |
| Tail | Likelihood of displaying a tail lift | ↓↓ | - |
| | Rate of tail swishes | ↑↑↑ | ↓# |
| | Likelihood of displaying a tail thrash | - | - |
| Vocal | Likelihood of snorting | - | - |
| | Likelihood of whinnying | - | - |
| Eliminate | Likelihood of defecating | - | - |
| Head | Likelihood of displaying a head shake | ↓↓ | - |
| | Likelihood of displaying a head toss | - | - |
| Ears | Rate of moving left ear to forward position | ↑ | ↑ |
| | Rate of moving right ear to forward position | ↓ | - |
| | Duration of time with left ear back | ↑↑ | - |
| | Duration of time with right ear back | ↑↑ | - |
| Eye | Rate of blinking | ↑ | - |
| | Rate of eye white (sclera) visible | ↑ | ↓# |
| | Likelihood of displaying a small eye aperture | ↓↓ | - |
| | Likelihood of displaying a large eye aperture | ↑↑↑ | - |
| | Likelihood of displaying a wrinkled eyebrow | ↓↓ | - |
| | Likelihood of displaying an angled eyebrow | ↓↓ | - |
| Mouth | Likelihood of displaying contracted mouth | ↑↑↑↑ | - |
| | Likelihood of displaying a drooping lip | ↓↓ | - |
| | Rate of lip licking or chewing | ↓ | - |
| | Likelihood of displaying a face pull | ↓↓ | - |
| | Likelihood of displaying oral investigation behaviour | ↓↓ | - |

| | | | |
|---|---|---|---|
| Chin | Rate of chin wobbles | ↓ | - |
| | Likelihood of displaying a parrot mouth | - | - |
| | Likelihood of displaying a puckered chin | - | - |
| Nares | Duration of time with nares flared | ↑↑↑ | - |
| | Likelihood of displaying narrowed nares | ↓↓ | - |
| Heart | Average heart rate | ↑ | - |
| | Average interval between heart beats | ↓ | - |
| | Variance in the interval between heart beats (SDNN) | ↑ | - |
| | Short term variance in the interval between heart beats (RMSSD) | - | ↓[#] |
| | Ratio of short term to overall variation in the interval between heart beats (RMSSD:SDNN) | ↓ | - |
| Eye temp | Average eye temperature | ↓ | - |
| | Maximum eye temperature | - | - |

- No significant findings
[#] $p<0.10$
↑ 0-50% increase, ↑↑ 51-100% increase, ↑↑↑ 101-200% increase, ↑↑↑↑ >200% increase
↓ 0-50% decrease, ↓↓ 51-100% decrease, ↓↓↓ 101-200% decrease, ↓↓↓↓ >200% decrease

## 3.3 DISCUSSION

To the authors' knowledge, this is the first report detailing investigation of facial expression for positive or negative emotion in horses. Previous work in other species suggested that ear position, eye aperture, nares and lips may indicate emotion (Reefmann et al. 2009b; Reefmann et al. 2009a; Langford et al. 2010; Sotocinal et al. 2011). In the current study, few differences were seen in head behaviour and facial expressions between the preferred and intermediate treatments. However, a non-aversive stimulus (i.e. preferred or intermediate treatment) was more likely to result in the occurrence of some behaviours and facial expressions than an aversive stimulus. Such behaviours were small eye aperture, drooping lower lip, face pulling, narrowed nares, investigation using the muzzle, an angled eyebrow, and wrinkling of the eyebrow and a tendency towards the occurrence of a head shake. Sheep undergoing a putatively positive emotion inducing stimulus were also found to have decreased eye aperture (Reefmann et al. 2009a). Additionally, the rate of chin wobbles, and lip licking and chewing were higher with a non-aversive stimulus in the current study.

Ear position has been suggested to be indicative of emotional valence in sheep (Reefmann et al. 2009b; Reefmann et al. 2009a). In horses, it has been suggested that the ears forward position is an indicator that a stimulus is perceived as positive (Sankey et al. 2010c). In contrast, the ears are forward proportionately less in sheep experiencing a putatively positive stimulus (Reefmann et al. 2009a). In the current study the left ear was in the forward position less often with the preferred treatment compared to either the aversive or the intermediate treatments. This supports the results from the sheep study and suggest that at least one ear is forward less often with a preferred stimulus. Alternatively, ear position could indicate attention to auditory stimuli and thus reflect arousal. It may be that the arousal level is lower with a putatively positive experience compared to that with a putatively negative experience. It is also possible that animals experiencing positive emotion are less attentive to auditory environmental stimuli and perhaps less vigilant.

More of the behaviours were affected by the aversive treatment than the intermediate preference when compared to the preferred treatment. Most of the changes seen with the aversive treatment were logical, for example, the increase in the rate of fore and hind leg lifts, the higher probability of the occurrence of a kick, increase in number of times sclera/eye white was visible and the time spent with ears back or nares flared compared to the preferred treatment. These changes may indicate an increase in arousal with a negative emotional valence.

There is interplay between arousal level and emotional valence which is difficult to separate. The neck position might be expected to indicate arousal level (McGreevy 2004). High positions might be expected to reflect high arousal, with increased attention to the environment (hypervigilance) or increased attention to a specific stimulus. Low positions might be expected to indicate low arousal. However, this may not be such a simple linear relationship. In the current study, horses experiencing a preferred stimulus tended to move their neck to the very low position fewer times than with the intermediate or aversive treatments. Thus the neck very low position may not necessarily be a sign of low arousal (relaxation) or positive emotion.

In contrast, horses moved their necks to the very high position more with a preferred stimulus than an intermediate one, but less than with an aversive stimulus. Horses undergoing a preferred stimulus may be more attentive to a meaningful stimulus, thus more aroused than with the intermediate stimuli. Horses displayed an increased rate of movements to the neck very high position as well as an increase in the time spent with the neck above neutral with the aversive treatment. The increase in time spent with the neck above neutral may be due to hypervigilance resulting from anxiety due to an aversive experience. We suggest that the rate of movements to the neck very high position may indicate arousal, and the time spent with the neck above neutral may indicate negative emotion.

Tail swish behaviour was affected similarly to the neck very high behaviour with respect to preference. The rate was markedly increased with the aversive relative to the preferred treatment but tended to be moderately increased with the preferred relative to the intermediate treatment. The rate of tail swish behaviour may also indicate arousal.

The cardiac parameters, heart rate and heart rate variability, differed between an aversive stimulus and a preferred or intermediate stimulus. The increase in heart rate, decrease in interval between heart beats and decrease in RMSSD:SDNN ratio with the aversive preference indicated a predominance of the sympathetic nervous system over the parasympathetic nervous system which may be due to mental stress or arousal. These findings were consistent with previous research (von Borell et al. 2007).

The observed increase in SDNN with the aversive treatment contradicted what might be expected based on the literature. It may be an artefact due to the intermittent nature of the stimulus and the recording length, or there may have been a spurious impact from the results of one horse. The effect of artefacts on data needs to be considered as a significant effect on heart rate variability parameters can be erroneously seen, particularly with automated error correction methods (von Borell et al. 2007). In this study, artefacts were replaced with missing values rather than interpolated using the Polar software and equipment manufacturer's undisclosed proprietary methods. Further research is required to examine the effect of different methods of artefact determination and data processing on heart rate variability outcomes. Future research should give details on the method of error correction.

There was a tendency toward a decrease in RMSSD with the intermediate treatment which suggests that there was more parasympathetic activity with the preferred treatment. It may be that horses do not experience large reactions to non-aversive stimuli. Thus differentiating small differences between emotionally positive and more neutral stimuli may require larger numbers of horses than used in this study. It could be that horses' preferences are not stable over long periods of time such that preferences changed between the first and second experiments. The time intervals over which the heart rate variability parameters were calculated could also be considered as a factor.

The average temperature of the eye and surrounding tissue was found to vary significantly with the stimuli. In contrast, the maximum eye temperature was not found to differ between stimuli, which differs from studies investigating response to pain in sheep and cattle (Stubsjoen et al. 2009; Stewart et al. 2010). The average eye temperature of horses was lower with an aversive stimulus. This decrease most likely reflects vasoconstriction mediated by stimulation of the sympathetic nervous systems. In cattle undergoing aversive handling, the use of an electric prod resulted in a decrease in maximum eye temp of 0.42$^{o}$C on average in the first 20seconds following prod (Stewart et al. 2008b). The Spray treatment used as the aversive treatment in this study is likely to be less aversive in comparison, thus the lower magnitude of response. Additionally, an effect of lesser magnitude might be expected when looking at an average rather than a maximum temperature and the length of recording may have reduced the average due to the intermittent nature of the aversive stimulus. This is the first time such a drop in eye temperature has been documented in horses.

It was hypothesised that the preferred treatment would increase parasympathetic nervous system activity, assessed using eye temperature and heart rate variability, and induce behaviours associated with a relaxed state. Horses experiencing a preferred stimulus did not display behaviours associated with a relaxed state (decreased arousal). Some of the behaviours displayed may indicate increased arousal. Although changes to heart rate variability showed a tendency suggesting more parasympathetic activity with the preferred treatment, insufficient evidence was found in this study to support this hypothesis.

The intra-observer reliability of behavioural observations was extremely high (Petrie & Watson 2006; Martin & Bateson 2007). Future studies should also include measures of observer reliability.

An attempt was made to include a control treatment (the No-stimulus treatment) as a reference point for the changes seen with the other treatments. A control treatment would ideally be of neutral emotional valence, that is, it wouldn't elicit a positive or a negative emotion and would elicit the same response from all horses. However, a control treatment is not possible as a No-stimulus treatment may not elicit the same type and strength of emotion across all animals because animals' emotional responses are specific to the individual. There are no gold standard treatments for this type of research with which comparison can be made. Additionally, in this study, the No-stimulus treatment was the preferred treatment for some of the horses. Future research could include a no-stimulus treatment and exclude horses that preferred this treatment over other putatively pleasant experiences. However, future research should recognise that a control situation does not exist.

A number of behaviours were observed rarely or only in a small number of horses. When interpreting the results from events that occurred rarely it is important to note that non-significance should not be view as evidence that the behaviour is not linked to emotion as the power to detect significance would be low. Given that these behaviours tended to occur in only a small number of horses, future studies to investigate these less common behaviours should focus on including a larger number of horses rather than increasing the replicates in a smaller number of horses.

The nature of the treatment may limit or encourage certain behaviours. For example, horses were observed to extend their head and neck upwards during the Groom treatment, perhaps in attempt at mutual grooming (McGreevy 2004), and an increase in the neck very high behaviour was found. This was not seen with the other treatments when analysed by treatment rather than preference. It is possible that the behaviour changes are specific to the type of stimulus and thus not universally applicable. Fear or anxiety caused by a sudden, novel and unpredictable stimulus, in this case the Spray, might cause a different behavioural reaction than another type of aversive stimulus, for example pain from a needle/injection. Future research should aim to use positive, intermediate and negative stimuli that use the same substrate, elicit a similar level of physical reaction (exercise), with constant rather than intermittent application and response.

The aim of this study was to investigate how physiological and behavioural responses of horses change whilst undergoing a pleasant experience compared to a mildly aversive stimulus and a neutral/intermediate experience. The aversive treatment resulted in changes to many behavioural and physiological measures. However, few differences were seen between the intermediate and preferred treatments. It is possible to differentiate between a horse experiencing an aversive stimulus and one experiencing a non-aversive stimulus based on physiological and behavioural responses. Observation of the rate of neck movements to the very high position, tail swishing behaviour and physiological responses may provide information about arousal. The duration of time spent with the neck above neutral may indicate negative emotion.

It should be acknowledged that, in terms of welfare, the aversive stimulus (spray) is not considered by scientists to be extremely unpleasant because it is applied over a short time period and is not painful. However, there was sufficient contrast in emotion between the preferred and aversive treatments to enable behavioural and physiological differences to be detected. How pleasant the positive treatments were, as perceived by the horses, is not able to be determined. There may not have been sufficient emotional contrast between the intermediate and preferred treatments to enable many behavioural and physiological differences to be detected.

It was also not possible in this study, to use a large number of pleasant or aversive stimuli, and so further research is required to determine if the findings of this study are particular to the stimuli used or generalised to all aversive or pleasant stimuli.

This experiment highlighted the difficulties encountered with conducting a study using sufficiently powerful pleasant and aversive treatments that are ethically acceptable and practical in the experimental setting.

# 4 Indicators of affective experience

If we accept that a horse undergoing an aversive stimulus is experiencing negative emotion, then indicators of negative emotion include: a higher average heart rate, lower average interval between heart beats and a lower RMSSD:SDNN ratio for heart rate variability. Horses exposed to an aversive stimulus also demonstrate an increase in the number of moves to the neck very low position but a decrease in time spent with neck below neutral, an increase in the number of moves to the neck very high position with an increase in the time spent with neck above neutral. Horses will have a decreased average eye temperature, an increased time with left or right ear back or nares flared, and an increased rate of leg lifts, tail swish, and blinking. The number of times the eye white (sclera) is visible or left ear is in the forward position will also be increased. The likelihood of a hindleg kick, a large eye aperture or a contracted mouth being displayed is also increased. Other indicators are a decrease in the rate of lip licking and chewing, chin wobble, or right ear in the forward position.

A horse experiencing a non-aversive stimulus and non-negative emotion may have their neck below neutral for a longer time but display less moves to the neck very low position, have their neck above neutral for less time, and show more lip licking and chewing or chin wobble behaviours. Behaviours more likely to be seen in horses experiencing a non-aversive stimulus include tail lift, small eye aperture, wrinkled eyebrow, angled eyebrow, drooping lower lip, face pulling, investigation with the mouth, and narrowed nares.

It may be possible to differentiate between a horse experiencing positive emotion due to a preferred stimulus from a horse experiencing a less positive emotion due to an intermediate stimulus based on the following behaviours: a smaller increase in the number of moves to the neck very high position than that seen with an aversive stimulus or a decrease in the number of times the left ear is forward. There is also a tendency towards a decrease in the number of moves to the neck very low position, a smaller increase in tail swish rate or number of times the eye white is visible, and an increase in the RMSSD measure of heart rate variability in horses experiencing positive emotion.

The number of neck movements to the very high positions, tail swishing and possibly ear forward position may reflect arousal level.

# 5 Conclusion

A review of the literature concluded that both positive and negative emotions are likely to be experienced by animals and it identified potential indicators of emotion and emotion - inducing experiences. This information was used to conduct two experiments to investigate potential indicators of emotional experience in horses.

The first experiment was designed to allow horses to indicate their preferred stimulus by choosing to spend more time in an area associated with that stimulus. This was deemed necessary because one of the fundamental problems with research on emotion in animals is the need to assume what the animal is feeling as animals are unable to self-report. What horses like cannot be assumed and varies on an individual basis. The identification of each horse's preferred stimulus then allowed the comparison of physiological and behavioural responses between horses experiencing a preferred, intermediate or aversive treatment, in order to identify indicators of the underlying emotion.

In the second experiment a number of potential physiological and behavioural indicators of emotion were investigated. The following may be useful indicators for identifying horses' emotional experiences: heart rate, heart rate variability, eye temperature, and behaviours involving legs, neck, tail, ears, eyes, mouth, chin, and nares.

Behaviour may be useful as an indicator of horses undergoing negative or non-negative emotional experience. Weak indicators were found that differentiate a more positive emotional experience from a less positive or intermediate one. There may be more subtle changes with positive emotion than with negative emotion. These may not be reliably detectable, or may require more subjects in order to find statistically significant differences.

Whether changes in indicators represent changes in arousal or emotional valence needs to be considered. It is possible that animals experiencing positive emotion are more attentive to the stimulus than during an intermediate emotional experience, but are less attentive to auditory environmental stimuli, and perhaps less vigilant, than during a negative emotional experience.

Further research is suggested to validate and expand on the findings, including assessment of the use of behavioural observation by horse carers for welfare applications. Repetition with a larger number of horses that have the same treatment preferences, with a larger difference in value between the preferred and intermediate treatments might be useful for future research. Further research investigating the attenuation of a negative emotional response by co-administration of a positive emotion inducing stimulus (counter-conditioning) could have practical implications for horse welfare and human safety.

It is difficult to separate changes that are due to variation in arousal level versus those due to variation in emotional valence. Analysis of results from different treatments and preferences is challenging. The previous experiences of horses may influence their preferences. Responses to non-aversive stimuli may be subtle. To help address these issues, it is suggested future investigations should use horses with the same preferences, and more horses be included. Ideally the stimulus used to induce the negative, intermediate and positive emotion would have the same level of physical arousal, and use the same type of stimulus. Horses would ideally have the same background/experience. A higher value preferred treatment or larger difference in value between preferred and intermediate treatments may result in larger, more measurable changes.

These studies explored the assessment of emotion, including positive emotion, an emerging area of animal welfare science, in a novel way in a species that has not been investigated previously. Compared to many studies, they used a relatively large number of horses (20 and 11) and gathered a substantial amount of information, using a large number of novel parameters including eye temperature and facial expressions. Various indicators of negative and non-negative emotion and arousal were identified. The studies form a foundation for further research and can be used to inform welfare policy. The indicators could be used in the assessment of the welfare of horses (Stewart et al. 2011). Situations that induce signs of negative emotion could be minimised and situations that elicit signs of intermediate or positive emotion could be encouraged, thus improving the welfare of horses.

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