



Review Article

Facial Expression: An Under-Utilized Tool for the Assessment of Welfare in Mammals

Kris A. Descovich^{1,2,3}, Jennifer Wathan⁴, Matthew C. Leach⁵, Hannah M. Buchanan-Smith¹, Paul Flecknell⁶, David Farningham⁷ and Sarah-Jane Vick¹

¹Psychology, Faculty of Natural Sciences, University of Stirling, Stirling, United Kingdom; ²Environmental and Animal Sciences, Unitec Institute of Technology, Auckland, New Zealand; ³Centre for Animal Welfare and Ethics, University of Queensland, Gatton, Australia; ⁴School of Psychology, University of Sussex, Brighton, United Kingdom; ⁵School of Agriculture, Food & Rural Development, University of Newcastle, Newcastle, United Kingdom; ⁶Comparative Biology Centre, University of Newcastle, Newcastle, United Kingdom; ⁷Centre for Macaques, Medical Research Council, Salisbury, United Kingdom

Summary

Animal welfare is a key issue for industries that use or impact upon animals. The accurate identification of welfare states is particularly relevant to the field of bioscience, where the 3Rs framework encourages refinement of experimental procedures involving animal models. The assessment and improvement of welfare states in animals depends on reliable and valid measurement tools. Behavioral measures (activity, attention, posture and vocalization) are frequently used because they are immediate and non-invasive, however no single indicator can yield a complete picture of the internal state of an animal. Facial expressions are extensively studied in humans as a measure of psychological and emotional experiences but are infrequently used in animal studies, with the exception of emerging research on pain behavior. In this review, we discuss current evidence for facial representations of underlying affective states, and how communicative or functional expressions can be useful within welfare assessments. Validated tools for measuring facial movement are outlined, and the potential of expressions as honest signals is discussed, alongside other challenges and limitations to facial expression measurement within the context of animal welfare. We conclude that facial expression determination in animals is a useful but underutilized measure that complements existing tools in the assessment of welfare.

Keywords: refinement, animal welfare, facial expressions, emotion, communication

1 Introduction

The promotion of good animal welfare is a prominent issue for society at large and in particular for industries that use or impact upon animals. This includes the keeping of pets or wildlife, farming of livestock, and even anthropogenic effects on wild species. The duty to offer animals adequate welfare standards is now legislated in many countries, with requirements for environmental, nutritional, and social conditions, and protection from pain, injury and suffering, e.g., the Indian Prevention of Cruelty to Animals Act of 1960, amended 1982¹; the US Ani-

mal Welfare Act of 1966, last amended 2013²; the UK Animal Welfare Act of 2006³, and the New Zealand Animal Welfare Act, 1999, last amended 2015⁴. The issue of animal welfare is particularly pertinent for the biosciences, where there is both an ethical and legal duty to minimize the impact of experimentation on animal models through refinement (e.g., EU Directive 2010/63/EU⁵, UK Animals (Scientific Procedures) Act of 1986, consolidated 2014⁶) although such legislation does not cover all experimental animal models, for example, in the US, rats, mice, birds and farm animals used in bioscience are not covered by the US Animal Welfare Act². This duty also extends beyond

¹ <http://www.envfor.nic.in/legis/awbi/awbi01.pdf>

² <https://www.nal.usda.gov/awic/animal-welfare-act>

³ <http://www.legislation.gov.uk/ukpga/2006/45/contents>

⁴ <http://legislation.govt.nz/act/public/1999/0142/latest/DLM49664.html>

⁵ <http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32010L0063&from=EN>

⁶ <https://www.gov.uk/government/publications/consolidated-version-of-aspa-1986>





experimental protocols to include all aspects of the laboratory animal's life, including transportation, housing and husbandry (Rennie and Buchanan-Smith, 2006). In biomedical research, it is also critical that high welfare standards are maintained, including the minimization or prevention of pain, as data validity may be compromised when taken from animal models with impaired welfare (Würbel, 2001; Poole, 1997; Everds et al., 2013; Hall et al., 2015).

Promoting animal welfare is generally considered by society as a moral duty, with the expectation that those who use animals will protect their welfare as far as possible. For example, society is more accepting of animal use in biomedical research when it is considered humane, as outlined in a recent MORI poll, where 69% of people surveyed accepted animal research "as long as there is no unnecessary suffering to the animals and there is no alternative" (Leaman et al., 2014, p. 6). Welfare states also impact the quality of service that animals provide to humans. For example, in agricultural industries poor health and stress can reduce livestock meat quality, and in biomedical science stress may contribute to the collection of unreliable or unrealistic data from animal models (Würbel, 2001; Klumpp et al., 2006; Ferguson and Warner, 2008; Schwartzkopf-Genswein et al., 2012; Hall et al., 2015). Therefore, animal-oriented industries can also benefit directly from good animal welfare.

The assessment and improvement of animal welfare depends on reliable and valid measurement tools, which may include behavioral, physiological, clinical and psychological indicators (Mason and Mendl, 1993; Dawkins, 2004; Mormède et al., 2007; Mendl et al., 2009). No single indicator can yield a completely accurate picture of an animal's welfare state, and multiple indicators may not result in agreement (Mason and Mendl, 1993). Behavioral measures such as activity, attention and vocalizations are valuable and commonly used indicators of welfare state, as they are immediate, non-invasive and require a relatively short training period for observers (Mason and Latham, 2004; Manteuffel et al., 2004; Bethell, 2015).

Animals show individualized responses to their internal, external and social environments, including variables that are introduced to improve welfare, such as socialization, training and enrichment (Izzo et al., 2011; Coleman, 2012). Individual responses may be predicted by factors such as age, sex and life history while others may be more aligned to variables such as temperament (Izzo et al., 2011; Coleman, 2012). It follows that achieving good welfare in animals requires understanding of predictable and generalized patterns, as well as modifications to account for the experiences and needs of the individual. Traditionally, welfare assessment has focused on the adequacies of physical resources (e.g., nutrition, space), however it is now well recognized that animal welfare is intrinsically linked to psychological wellbeing. Unfortunately, the psychological experiences of non-human animals and the behavioral manifestations of these experiences are still not well understood, making them challenging to identify. For instance, stereotypy performance, self-directed behavior, and reproductive failure may indicate poor welfare states, however they also lack temporal or stimulus specificity and so cannot be easily attributable to a direct cause (Mason, 2006; von Borell et al., 2007; Novak and Meyer, 2009).

Stereotypies and self-directed behavior may develop as coping mechanisms, and therefore individuals that perform these may experience better welfare states than those in comparable environments that lack coping strategies (Mason and Latham, 2004; Mohiyeddini and Semple, 2013). Furthermore, animals that perform stereotypies are resistant to behavioral extinction and therefore the existence of stereotypies do not necessarily indicate current welfare state (Mason and Latham, 2004; Mason, 2006). As a further complication, diverse animal internal states may manifest behaviorally in similar ways. For example, a reduced behavioral reaction to repeated stimuli may indicate either desensitization or learned helplessness, with polar opposite ramifications for interpreting welfare (Overall, 2013). In summary, behavior is essential for the assessment of welfare in animals but some limitations exist in terms of accurately interpreting internal states, or indicating triggering stimuli.

One observational tool that may strengthen the assessment of welfare by complementing current behavioral or other measures is the use of facial expressions (Tab. 1). In humans, facial expressions have been extensively studied as a measure of the psychological and emotional experience (Darwin, 1872; Ekman, 1993; Hole and Bourne, 2010). Despite this, the systematic use of facial expression in animal welfare science is rare, with the notable exception of emerging research on pain indicators (e.g., Langford et al., 2010; Leach et al., 2011; Gleerup et al., 2015a). Facial expressions in mammals are widespread with many facial movements conserved across species (Darwin, 1872; Diogo, 2009; Waller and Micheletta, 2013). Facial expressions have the potential to reliably indicate psychological and emotional experiences in animals, and can provide information on temporal or stimuli-specific reactions. Facial expressions also have social and reproductive functions (e.g., Moehlman, 1998; Parr et al., 2005) and can therefore be more broadly relevant to welfare assessment than exclusively as indicators of affective state. Facial expressions can determine generalized, species-specific patterns, as well as accommodate individual variation, and reliable systems for the recording and measurement of facial expressions with high validity already exist for several taxa (e.g., Parr et al., 2010; Wathan et al., 2015). Humans have an innate observational bias to focus on the facial region, even when instructed to monitor other body areas (Leach et al., 2011), which may facilitate the use of facial expressions in welfare monitoring programs. Moreover, animals appear to have less voluntary control over facial expressions in comparison to motor behavior, although the current evidence is restricted to primate species (Jürgens, 2009; Hopkins et al., 2011). This is similar to the amount of volition over vocalizations (Jürgens, 2009). In humans, voluntary control of facial expression weakens as emotional intensity heightens leading to "emotional leakage" (Porter et al., 2012), suggesting that facial expressions in animals may, at least in some circumstances, be "honest" signals of welfare states, and useful as adjunct measures alongside existing indicators.

In this paper, we review the current literature on facial expression function and modulation in mammalian species, and discuss potential applications to the empirical determination of welfare. Only mammals are included in this review due to the

homology of facial musculature across this taxonomic group. These data may be irrelevant for other taxa (e.g., birds, reptiles) with comparatively reduced mimetic structure (Cooke, 2015). For the purpose of this discussion we define facial expressions as any movements derived from individual or combined muscle activation. We include eye widening, rolling or blinking, and tongue movements when visible, but not gaze direction / attention or ingestive behavior. The utility of facial expressions first-ly will be discussed in relation to negative and positive affective states. Pain will be examined in a separate section due to its specific, well-defined contribution to poor welfare states, as well as the comparatively large body of literature on facial indicators of pain. In mammals, facial expressions have been extensively

used to study social communication, particularly amongst primate species, leading to key insights about animal cognition (e.g., Parr et al., 1998). Conflict between facial expression as a communicative tool and as an expression of emotion (Fridlund, 1991) may contribute to its underutilization in animal welfare science, although we argue that both are useful for the interpretation of welfare states. Therefore, in this review, the relevance of facial expression to welfare assessment will be discussed in the context of communication as well as in relation to affective states, with each providing explanatory power to identify the internal animal experience. Finally, measuring methods for facial expressions will be outlined, and potential challenges of using facial expression as a welfare indicator will be discussed.

Tab. 1: The contribution of facial expression to welfare assessment of mammals under the Five Domains Model
(Mellor and Beausoleil, 2015)

Domain	Experience	Evidence that facial expressions have the potential to indicate animal experiences	Example references
1. Nutrition	Hunger / thirst	Indicator of satiety	Cabanac and Lafrance, 1990
2. Environment	Taste aversion	Indicator of taste aversion (disgust)	Cabanac and Lafrance, 1990
	Thermal comfort	Species-specific thermoregulatory expressions, e.g., panting, tooth grinding, gaping	Spotila et al., 1977; Wells, 1978; Robertshaw, 2006
	Strong odors	Indicator of olfactory action, e.g., flehmen	Gaughwin, 1979; Stahlbaum and Houpt, 1989; Weeks et al., 2002; Charpentier et al., 2013
	Loud noises	Indicator of arousal, vigilance, startle response or fear	Fox, 1970; Sandem and Braastad, 2005; Kaiser et al., 2006; Bennett et al., 2012
3. Health	Health	Indicator of overall health by degree of asymmetry	Sefcek and King, 2007; Knierim et al., 2007
4. Behavioral restriction	Expression of social behaviors	Indicator of social communication, intent signaling and perception	Partan, 2002; Parr et al., 2005, 2007; Bethell et al., 2012
	Expression of other behaviors	Dependent on the behavior. Frustration of motivation may be evident from displacement behaviors	Baker and Aureli, 1997; Sandem et al., 2002; De Marco et al., 2010; Vick and Paukner, 2010
	Human-animal relationship quality	Indicator of animal-human relationship	Nagasawa et al., 2013
	Expression of coping or abnormal behaviors	Performance of oral stereotypy	Redbo, 1998; Mason et al., 2007; Fernandez et al., 2008; Mason, 2010; Fureix et al., 2011; Tan et al., 2013
5. Affective experience	Positive emotional states	Indicator of general positive welfare state, play intentions, and affiliation behavior	Fox, 1970; Waller and Dunbar, 2005; Judge and Bachmann, 2013; Yanagi and Berman, 2014
	Pain states	Indicator of pain	Craig et al., 1991; Langford et al., 2010; Sotocinal et al., 2011; Leach et al., 2012; Dalla Costa et al., 2014; Wathan et al., 2015; McLennan et al., 2016
	Negative emotional states	Potential indicator of fear, aggressive intent, disgust, frustration	Fox, 1970; Cabanac and Lafrance, 1990; Beerda et al., 1997; Steiner et al., 2001; Casey, 2007; Parr et al., 2005, 2007; Leiner and Fendt, 2011; Defensor et al., 2012



2 Affective state, welfare and facial expressions

It is increasingly accepted in the general and scientific communities that animals lead emotional lives, despite the inherent difficulties of measuring affective components in animals (Désiré et al., 2002; Mendl et al., 2010; Panksepp et al., 2011). Emotions are “unlearned response systems” that are experienced as “intense but short-living affective responses to an event” (de Waal, 2011). Emotions are considered to serve an adaptive function because they reinforce behavior that enhances fitness (Dawkins, 1990; Fredrickson, 2004; Fraser and Duncan, 1998; Panksepp, 1998). Moods are long-term responses arising from the cumulative experience of short-term emotional responses, and both moods and emotions are encompassed in the term “affective state” (Mendl et al., 2010).

Affective states are often described in terms of a valence / intensity model, with valence ranging between negative and positive, and intensity referring to the level of arousal (Désiré et al., 2002). Conscious affective states are integral to individual experience and central to understanding animal welfare (Boissy and Erhard, 2014). Within an affective state framework, adequate animal welfare can be defined as the absence of long-term or severe negative emotions or moods, in combination with the opportunity to experience positive emotions and moods (Boissy et al., 2007). In humans, conscious emotional states (“feelings”) can be self-reported using language (e.g., Au et al., 1994). In animals, vocalizations may differ dependent on affect (e.g., ultrasonic vocalizations in rodents: Knutson et al., 1998; Portfors, 2007), however the reliability of these measures is in some doubt (Jourdan et al., 2001; Wallace et al., 2005). Although there are other methods that can be used with animals in order to determine preferences or needs of individuals (e.g., conditioned place preferences, Bardo and Bevins, 2000), a self-report comparable with humans is impossible. Therefore, assessment of affective states in animals is reliant on measurable proxy indicators.

Facial expressions are temporally relevant, measurable and sensitive indicators of emotional valence (Dimberg and Thunberg, 1998). This is true even in response to subliminal triggering stimuli, or when attempts are made to suppress the emotional response (Dimberg et al., 2000, 2002; Porter and ten Brinke, 2008). For these reasons, the observation of facial expressions in animals has significant potential for the assessment of internal states, and therefore welfare, of animals.

3 Can facial expression indicate negative affective states?

The avoidance of long-term negative affect is a defining requirement of adequate animal welfare (Boissy et al., 2007). In humans, negative emotional states have prototypical facial configurations (Ekman and Rosenberg, 2005; Waller et al., 2008a). From a social context, negative facial expressions convey adaptive advantages to both signalers and observers. They draw more attention than positive expressions and interrupt task performance in observers (Vuilleumier et al., 2001; Eastwood et

al., 2003). Here, current evidence for animal facial expressions during negative contexts, and how these may relate to negative affective states, is discussed by facial region.

3.1 Eye region

The adjustment of eyelid aperture is a common element in emotional display, with increasing aperture and eye white visibility associated with negative emotion in both humans and other animals (Sandem et al., 2002, 2006; Lee et al., 2013). Eyelid aperture is predominantly controlled by elevation of the upper eyelid from the levator palpebrae superioris muscle, found in the facial structure of most mammals (Spencer and Porter, 2006). In humans, eyelid aperture increases in the fear, anger and surprise expressions (Williams, 2002; Waller et al., 2008a). Widening of the eyes improves the peripheral visual field resulting in greater sensory intake and more effective vigilance (Susskind and Anderson, 2008). In sheep (*Ovis aries*), eyelid aperture increases in aversive contexts (e.g., isolation from the social group) and negatively correlates with cardiac measures of parasympathetic nervous system activation (Reefmann et al., 2009a, 2010). Similarly, increased eyelid aperture, along with panting, is a sign of anxiety in dogs (*Canis familiaris*) during intra-venous catheter placement, and was reduced by a sedative (acepromazine), an analgesic (oxymorphone), a placebo, and by restraint (Light et al., 1993), although pharmacological muscular relaxation may have contributed to some of these effects. Increased visibility of eye white sclera may present alongside widened eyes in fearful and/or stressful situations in humans, horses (*Equus caballus*), and cows (Sandem et al., 2002, 2004; Whalen et al., 2004; Sandem and Braastad, 2005; Sandem et al., 2006; von Borstel et al., 2009), and the administration of the anti-anxiety drug diazepam reduces this response in cows (Sandem et al., 2006). Exposure of the sclera is caused by movement of the eyeball within the eye socket and so may present independently of changes in eyelid aperture (Wathan et al., 2015).

Eyebrow raising through activation of the medial portion of the frontalis muscle is associated with the negative states of surprise and fear in humans (Waller et al., 2008b). Primates, horses, and dogs also have the capacity for a similar expression (Vick et al., 2007; Parr et al., 2010; Caeiro et al., 2012; Waller et al., 2012, 2013; Gleeup et al., 2015a). There is some evidence that brow raising is activated by pain states in horses (Gleeup et al., 2015a), although this action is caused by activation of the levator anguli oculi medialis muscle in this species (Wathan et al., 2015). This facial action increases the perceived size of the eye region, although it does not increase the actual aperture of the eyes. Proportionally large eyes are infantile characteristics in many mammals, and induce a care-giving response from humans (Glocker et al., 2009; Archer and Monton, 2011). In line with this, shelter dogs that display high rates of eyebrow raising are re-homed sooner than those that do so at a lower rate (Waller et al., 2013). This suggests that this facial movement may result in improved fitness through social recruitment.

In contrast to eye widening, mice (*Mus musculus*) in aggressive social situations may “tighten” their eyes by reducing eyelid aperture in combination with ear flattening, and nose and cheek swelling (Defensor et al., 2012). This constricted expression is

observed in resident mice exposed to intruding conspecifics and is assumed to protect sensitive areas of the face from attack, a hypothesis supported by differences in attack style between residents and intruders. Resident mice received more bites to their face and intruders (who do not exhibit the constricted face) received more bites to the back and flank (Defensor et al., 2012). In humans, eyelid aperture reduction is associated with anger and may signal dominance or impending threat (Waller et al., 2008a; Shariff and Tracy, 2011). Threat signaling in some species (e.g., primates / canids) incorporates a fixed stare (Fox, 1970; Partan, 2002; Oettinger et al., 2007). Facial expressions that are precursors of agonistic encounters are highly relevant to welfare assessment because poor welfare can lead to increased aggression; and conversely, social instability can lead to psychological and/or physiological stress (Broom et al., 1995; Beerda et al., 1999; Tamashiro et al., 2005; Broom, 2008). This will be further discussed in Section 6.

3.2 Nose and cheek region

In humans, several nose and cheek actions contribute to negative emotional expressions. Nose wrinkling (procerus contraction) is a component of disgust and engagement of the cheek's zygomatic minor muscle is used in sadness expressions, commonly resulting in a deepening of the nasolabial furrow (Vrana, 1993; Waller et al., 2008a). As many species are equipped with the relevant facial musculature (Diogo et al., 2009), it seems likely that contraction of muscles in the nose and cheek regions may also indicate negative affect in some other mammals, although it is infrequently mentioned in the literature. Nose and cheek swelling in mice was noted in combination with tightened eyes as a protective mechanism in aggressive encounters and a similar expression occurs when experiencing pain states (discussed in more detail in Section 4) (Langford et al., 2010; Defensor et al., 2012).

3.3 Mouth and jaw region

Many mammalian species frequently engage mouth and jaw movements in displays of affective states, in social communication, and as displacement or stereotypical behaviors; all of these are useful for determining welfare states. Fearful expressions in humans are sometimes accompanied by lip stretching, in chimpanzees (*Pan troglodytes*) by lip corner pulling (zygomatic major, a similar retraction of lip corners may be generated by contraction of the platysma in some species), lip parting and funneling, in horses by upper lip elongation, and in dogs by extended tongue and snout licking (Beerda et al., 1997; Williams, 2002; Casey, 2007; Parr et al., 2007; Waller et al., 2008a; Leiner and Fendt, 2011). In social communication, a fearful expression may act as an appeasement signal to mitigate conflict, however fear experiences are also associated with increased performance of aggressive behavior, which may be characterized by or combined with other facial components (Hsu and Sun, 2010; Bloom and Friedman, 2013; Beisner and McCowan, 2014; Ley et al., 2016). Dogs, for example, may raise the lips, expose the teeth and gape the jaw to indicate a threat (Fox, 1970; Goodwin et al., 1997). Pursing of the lips by funneling, tightening and pressing are associated with anger in humans, while an analogous ex-

pression of a "bulging lip face" has been found in chimpanzees, and an open mouth with a direct stare is used to signal threat in rhesus macaques (*Macaca mulatta*) (Partan, 2002; Parr et al., 2007; Waller et al., 2008b).

"Disgust" expressions are reflexive behaviors present even in neonates. They occur in response to aversive tastes or visual or emotive stimuli, and are important for individual and group fitness (Steiner et al., 2001; Erickson and Schulkin, 2003; Chapman et al., 2009). Lip retraction as a disgust response is common to both humans and non-human primates, as are other facial responses of mouth gaping and downward tongue extension (Vrana, 1993; Steiner et al., 2001). Disgust in other species has been less frequently studied although it is known that rats (*Rattus norvegicus*) show facial expressions in response to taste, with the valence of the expression dependent on satiety, innate taste preferences and learned experiences (Garcia et al., 1974; Grill and Norgren, 1978; Pelchat et al., 1983; Cabanac and Lafrance, 1990). Taste aversion in rats is demonstrated by mouth opening (gaping) into a triangle shape along with forward protrusion of the head (Grill and Norgren, 1978; Cabanac and Lafrance, 1990).

Many animals (including humans) also perform mouth movements as displacement activities; (behavior apparently irrelevant in the context performed that may offer insight into the internal state) (Maestripietri et al., 1992). Displacement activities appear when conflicting motivations are experienced simultaneously or when an animal is frustrated in performing a motivated action (Maestripietri et al., 1992). Displacement activities may present as a wide range of actions including licking, yawning, chewing and mouth twisting (Baker and Aureli, 1997; De Marco et al., 2010; Vick and Paukner, 2010; Mohiyeddin and Semple, 2013). Displacement yawning is broadly recognized to increase with anxiety or social conflict in primates (e.g., *Macaca nigra*, Hadidian, 1980; *M. mulatta*, Graves and Wallen, 2006; *Pan troglodytes*, Vick and Paukner, 2010) but has also been observed in other species including non-mammals, e.g., ostriches (*Struthio camelus*, Sauer and Sauer, 1967), dogs (Buttner and Strasser, 2014), fish (*Microspathodon chrysurus*, Rasa, 1971), and horses (Fureix et al., 2011). In horses, the frequency of yawning correlates positively with the performance of stereotypic behavior (Fureix et al., 2011). Like displacement behaviors, stereotypies appear functionless in the context in which they occur, but are "repetitive behaviours induced by frustration, repeated attempts to cope, and/or central nervous system dysfunction" (Mason, 2006, p. 326). Oral stereotypies occur across many mammal species including giraffe (*Giraffa camelopardalis tippelskirchi*, Fernandez et al., 2008), cows (*Bos taurus*, Redbo, 1998), bears (*Helarctos malayanus*, Tan et al., 2013), walrus (*Odobenus rosmarus*, Bergeron et al., 2006), primates (e.g., *Macaca silenus*, Mallapur et al., 2005), and horses (Fureix et al., 2011), and can result in serious oral injuries (Mason et al., 2007; Mason, 2010). Oral stereotypies manifest as a variety of mouth behaviors. In the horse for example, these may include lip snapping, crib-biting, and chewing of inedible substrates (Bergeron et al., 2006; Benhajali et al., 2010). In primates, oral stereotypies commonly present as repetitive mouth movements, lip smacking, tongue thrusting, coprophagy, or regurgitation (Lewis et al., 1990; Bour-



geois and Brent, 2005; Bloomsmithe et al., 2007; Hill, 2009). Stereotypies are commonly used as indicators of welfare; however, they lack specificity to causal variables, resist modification once established, and act as a coping mechanism to facilitate better welfare states in challenging environments (Mason, 2006).

3.4 Ear movements

In animals with mobile ears, ear position is an important indicator for both social communication and internal states (Andrew, 1963; Parr et al., 2005; Diogo et al., 2009; Defensor et al., 2012; Wathan and McComb, 2014). As ear position is controlled by the facial muscles, movement of the ears is classified as a facial expression. In horses, backward ears are associated with fear or a non-specific negative affective state, and forward-facing ears may represent arousal or attention; however, both backward and forward ear postures have been observed during agonistic encounters, indicating a need for further study to differentiate these responses (McDonnell, 2003; Waring, 2003; Kaiser et al., 2006; von Borstel et al., 2009; Reefmann et al., 2009b; Boissy et al., 2011). A study on positive and negative reinforcement training found that horses exposed to negative reinforcement training used the ears back position more commonly than those that were positively reinforced for behavior (Briefer Freymond et al., 2014). Negative emotional experiences in sheep are expressed by ear position, with backward positioned ears performed in negative situations over which the sheep has no control (Boissy et al., 2011). In negative, but controllable contexts the ears are pointed up (hypothesized by the authors to represent anger) and in situations when the animals were exposed to unexpected stimuli the ears were up but asymmetrical (Boissy et al., 2011). In some species (e.g., chimpanzees and mice) flattened ears are associated with the performance or anticipation of aggressive behavior (Parr et al., 2005; Defensor et al., 2012). Canids (e.g., foxes (*Vulpes vulpes*) and domestic dogs) hold their ears in a low position during anxious or fearful emotional states (Fox, 1970; Beerda et al., 1997).

4 Can facial expressions indicate pain states?

Recognition of pain in animals is clearly of significance to animal welfare. If pain cannot be adequately identified, nor its severity and nature assessed, then it cannot be alleviated optimally and those procedures that cause pain cannot be refined. In many countries where animals are utilized by humans, there is a legal requirement for effective pain assessment and alleviation, e.g., in the UK since 2013 for pets⁷ and in the EU Directive 2010/63/EU⁸. Evidence suggests that pain alleviation opportunities are under-utilized both in routine veterinary practice and regulated research, although this appears to be improving for companion animals (Lascelles et al., 1999; Capner et al., 1999; Hewson et al., 2006a,b; Coulter et al., 2009; Stokes et al., 2009; Keown et al., 2011; Kongara et al., 2016). One explanation for

this underutilization is that pain, like any internal state, can be challenging to recognize in animals (Sneddon et al., 2014). This is unsurprising when it is impossible to directly measure any internal state (Bateson, 1991; Flecknell et al., 2011). However, we pragmatically assume animals experience pain, as demonstrated by the implementation of animal protection and welfare legislation, e.g., in the UK⁸. In humans, pain is routinely assessed using self-report (e.g., visual analogue scale, McGill pain questionnaire (Hawker et al., 2011)), an option not currently available for the communication of animal pain experience to caregivers. Consequently, the assessment of pain in animals is reliant on proxy pain indices, with many advances in the development and validation of such measures (see Rutherford, 2002; Weary et al., 2006; Sneddon et al., 2014).

Pain assessment indices have limitations to their efficacy in assessing animal pain, including a lack of specificity in identifying pain over other negative internal states, a requirement for expertise on species-specific behavior, innate biases of observers, and in some cases, being time consuming to develop and implement (Weary et al., 2006; Rutherford, 2002; Leach et al., 2011; Sneddon et al., 2014). For humans that are unable to verbally or diagrammatically express their pain (i.e., pre-lingual children and patients with dementia) proxy assessment measures using facial expression are routinely used (Williams, 2002). Humans have a prototypical “pain face” (Fig. 1) that changes with aging but is generally characterized by a lowered brow, raised cheeks, tightened eyelids, wrinkled nose, raised upper lip and closed eyes (Prkachin, 2009). Recent advances in this area have identified facial expressions associated with pain in several mammalian species. Grimace scales (scale comprising different expressions that are considered to be associated with pain) (Fig. 1) have been developed to identify when animals are in pain and to potentially assess its severity in mice (Langford et al., 2010), rats (Sotocinal et al., 2011), rabbits (*Oryctolagus cuniculi*, Keating et al., 2012), horses (Dalla Costa et al., 2014; Glerup et al., 2015a), cows (Glerup et al., 2015b) and sheep (McLennan et al., 2016).

The study of Langford et al. (2010) in laboratory mice was the first to systematically demonstrate that mouse facial expression changes in response to noxious stimuli that are potentially painful. This culminated in the development of the Mouse Grimace Scale (MGS), which is comprised of five facial configurations: Orbital tightening, nose bulge, cheek bulge, ear position, and whisker change (Langford et al., 2010). An important potential feature of the MGS is that it can identify not only the presence or absence of pain but also the intensity of the pain experienced, with more extreme pain experiences correlating with more intense facial configurations. This seminal study has led to the development of similar scales for other species. The Rat Grimace Scale was developed by Sotocinal et al. (2011), with further validation by Oliver et al. (2014), and incorporates four facial configurations: orbital tightening, nose/cheek flattening, ear changes and whisker changes. The Rabbit Grimace Scale incor-

⁷ doi:10.14573/altex.1608251s

⁸ doi:10.14573/altex.1608251s

porates five facial configurations: orbital tightening, cheek flattening, nose shape, whisker position, and ear position (Keating et al., 2012). The Horse Grimace Scale incorporates six facial configurations: Stiffly backward ears, orbital tightening, tension above the eye area as determined by visibility of the temporal crest bone, prominent chewing muscles, strained mouth with a prominent chin, and strained nostrils with flattening of the profile (Dalla Costa et al., 2014). Prior to the Horse Grimace Scale, several studies suggested individual features in the horse were associated with pain: lip curling and an “abnormal facial expression” in synovitis (Bussi eres et al., 2008); lip curling in colic (J ochle, 1989); and nostril flaring in the respiratory disease heaves (Courouc e-Malblanc et al., 2008). Recently, the Sheep Pain Facial Expression Scale (SPFES) was developed to assess pain responses to footrot and mastitis (McLennan et al., 2016). The SPFES uses six facial changes: Orbital tightening, cheek tightening, rotation of the ear, lip and jaw profile changes, and shortening and narrowing of the philtrum (McLennan et al., 2016). Lip curling has also been reported in response to castration, where it intermittently occurred in pain states but was absent in control lambs and those treated with analgesia (Molony et al., 2002). In cows, facial configurations associated with pain include a tense ear position in a backwards or low profile, a tense stare or a withdrawn appearance, furrow lines above the eyes, muscle tension on the side of the head, strained nostrils, dilated nostrils, “lines” above the nostrils, and increased tonus of the lips (Gleerup et al., 2015b).

Facial grimace scales may have advantages over the use of other proxy measures of pain in animals. Grimaces are comprised of a few key indicators, resulting in a potentially more practical scale for implementation even in real-time application (Leach et al., 2012; Leung et al., 2016). Furthermore, the grimace scale indicators are concentrated in the facial area and exploit the tendency of human observers to focus on animal faces (Leach et al., 2011). Facial expressions are widespread in

mammals and many facial movements are evolutionarily conserved across mammalian species, including humans (Diogo, 2009; Waller and Micheletta, 2013). The consequence of this may be that facial expressions are easier for humans to identify and score due to a degree of universality / generalizability. Facial expressions provide a means for studying the affective component of pain in animals over nociception. From humans, it is known that the affective pain experience has a significant impact on welfare, and is expressed through prototypical facial configurations and this is likely to be also true for animals (Williams, 2002). In human studies, lesioning of the rostral anterior insula (associated with the affective component of pain) can result in pain asymbolia: the disassociation of the unpleasant experience and the nociceptive response to pain (e.g., Berthier et al., 1987). In the study by Langford et al. (2010), the lesioning of the rostral anterior insula in mice eliminated performance of the “pain face”, but not behavioral reactions, e.g., abdominal writhing. Although this study was conducted with a small number of animals ($n = 6$), the results suggest that the pain face may be representative of the affective component of pain in this species (Langford et al., 2010).

Despite significant advances in identifying “pain faces” in several species of mammals, the use of facial expression scales for the assessment of pain has limitations. There is the potential for false positives (i.e., indicating pain when none is present) in animals that are asleep, sedated or anesthetized (e.g., Langford et al., 2010; Sotocinal et al., 2011; Miller et al., 2015). In mice, some of the individual facial actions in the MGS have been observed during agonistic encounters, indicating they are not pain specific (Defensor et al., 2012). In order to apply grimace scales in a clinical context we need to better understand what a normal or non-pain facial expression looks like, and there is evidence in mice that this is influenced by strain and gender (Miller and Leach, 2015b). Therefore, facial expressions should only be used to assess pain in animals that are awake, caution

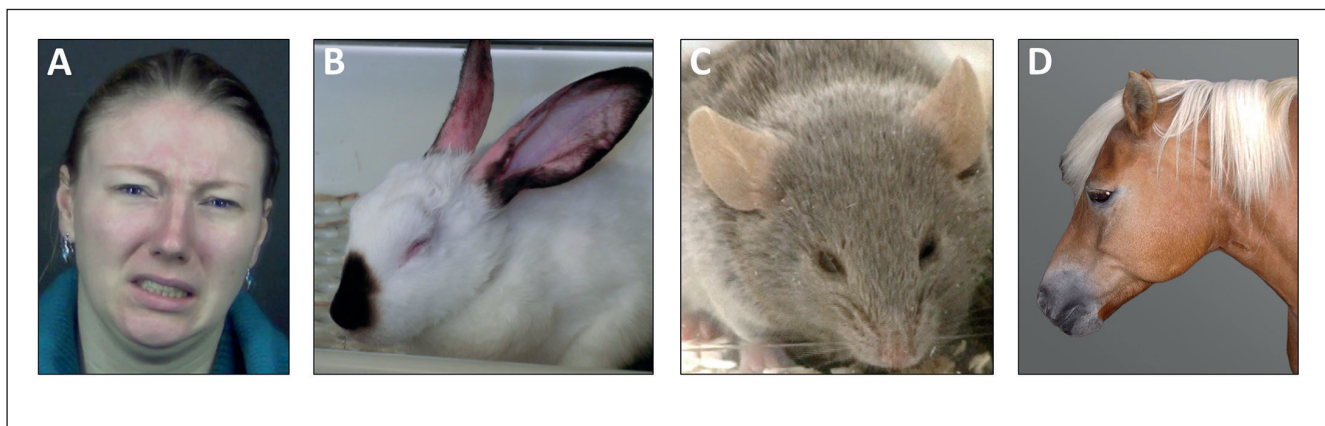


Fig. 1: Example of pain facial expression

a) Human (  University of Stirling 2013, pics.stir.ac.uk): lowered brow, orbital tightening, nose wrinkled, lip corner pulled, eyelid tightened and lips parted; b) rabbit (  M. Leach): orbital tightening, cheek flattening, ear and whisker position changes. Rabbit grimace scale also includes pointed nose which is unclear in this rabbit. c) mouse (  M. Leach): orbital tightening, nose and cheek bulge, ear and whisker position changes, d) horse (Dalla Costa et al., 2014): Ear position, orbital tightening, strained chewing muscles and nostrils, tension above eye, strained mouth and pronounced chin

should be used in their interpretation with respect to the environmental context, and they currently should be used alongside other validated indices of pain assessment (e.g., Dalla Costa et al., 2014) to ensure they are not specific to one type of pain or painful procedure and to minimize the potential for false negatives or positives in detecting pain states. Facial expressions of pain may also only indicate pain of a particular severity or duration, and be less useful, for example, in the identification of very acute or chronic pain (Langford et al., 2010; Miller and Leach, 2015a). These aspects should be incorporated into future studies on facial expressions of pain.

5 Can facial expression indicate positive affective states?

In the study of animal emotions and animal welfare, positive states have received less empirical attention than negative ones, however awareness of the importance of positive experiences is increasing, as is characterization of what constitutes a positive experience of an animal (Burgdorf and Panksepp, 2006; Boissy et al., 2007; Mellor and Beausoleil, 2015).

Play behavior is generally accepted to indicate positive affect (Panksepp, 2005; Burgdorf and Panksepp, 2006; Bekoff, 2015) as it reduces in frequency when conditions are challenging, energetic availability is low, or as a consequence of poor health, deprivation or reduced parental care (Loy, 1970; Lawrence, 1987; Thornton and Waterman-Pearson, 2002; Krachun et al., 2010; Held and Špinka, 2011). Play behavior is intrinsically rewarding (Boissy et al., 2007) and has been described as an “opioid-mediated pleasurable emotional experience” (Held and Špinka, 2011, p. 891). Play has both immediate and future benefits for psychological and long-term fitness, and as a contagious behavior can promote welfare at the group level (Bekoff, 2001;

Held and Špinka, 2011). Play behavior varies in its expression between species (Bekoff and Byers, 1998; Špinka et al., 2001) with many mammals using a play face: a ritualized facial expression that communicates a playful intent (e.g., canids, Fox, 1970; Rooney et al., 2001; chimpanzees, Parr and Waller, 2006; rhesus macaques, Yanagi and Berman, 2014; humans, Young and Décarie, 1977). Play faces are used with both conspecifics and heterospecifics, for example between dogs and their owners (Rooney et al., 2001), and may help others to interpret gross motor behavior as playful, because play can be rough and may resemble some aspects of aggression (Shyan et al., 2003).

In addition to specific facial configurations, generalized facial relaxation may also indicate positive affect. In humans, contentment is characterized by a relaxed facial expression (Burton and Crossley, 2003). Similarly, the play face in many primate species has been generally described as a relaxed expression with an open mouth (Andrew, 1963; Parr et al., 2005; Waller and Dunbar, 2005; Judge and Bachmann, 2013). In the horse, relaxation of the muzzle, upper eyelids and ears has been described as indicating a “well state” (Gleerup et al., 2015a).

5.1 Eye region

A reduction in eyelid aperture is associated with some negative emotions, however it is also associated with positive affect or playful situations in humans (Fig. 2), cats (*Felis catus*), and canids (Fox, 1970; Ekman et al., 1990; Ley, 2016). However, in humans the narrowing of the eyes seen in negative and positive situations is quantitatively different and this difference is perceivable by observers (Ekman et al., 1990; Waller et al., 2008a; Meletti et al., 2012). In some positive situations (e.g., happiness) eye narrowing can involve raising of the infraorbital area, while in others a relaxed or contented state can lead to relaxation or contraction of the eye area or the eyelids (Hietanen, 1998; Waller et al., 2008a). This is absent in the eye-narrowing

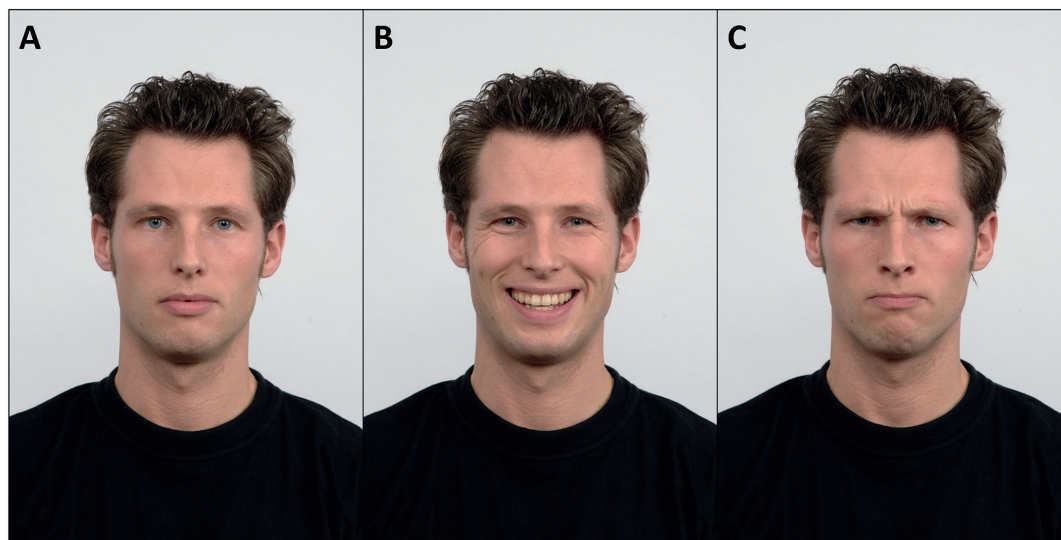


Fig. 2: Prototypical facial expressions in humans
a) Neutral, b) happy and c) angry (Langner et al., 2010)

configuration performed in negative situations (e.g., anger), which arises from contraction of the eyelids and sometimes by lowering of the eyebrow (Waller et al., 2008a). However, to what extent this might also apply to non-human animals has yet to be examined.

5.2 Nose and cheek region

In humans, raising of the cheeks, which leads to changes in the eye area (see Section 3.1), is associated with positive emotions and can differentiate between “enjoyment” and “social” smiles (Ekman et al., 1990; Waller et al., 2008a). This facial movement has not previously been reported as an indicator of positive affect in other species.

5.3 Mouth and jaw region

In humans, happiness is expressed via relaxed facial muscles and the affiliative facial expressions of laughing and smiling, which configures as a lip corner retraction caused by action of the zygomatic major muscles (Ekman et al., 1990; Ruch, 1995; Waller et al., 2008a). Analogous expressions occur in primate species such as chimpanzees, also characterized by lip corner retraction, however with the upper teeth covered and lower lip relaxed (“relaxed open mouth expression”), often used as a “play face”, or with the lips retracted from the teeth (“bared teeth display”), although the latter is also used as an appeasement signal (van Hooff, 1972; Preuschoft and van Hooff, 1997; de Waal, 2003). The mouth and jaw region are common components in play face configuration, and have also been observed in non-primates such as in canids (e.g., *C. aureus*, *C. lupus*), equids (e.g., *Equus quagga*), mustelids (*Mustela putorius*), and domestic cats (Poole, 1978; Martin, 1984; Schilder et al., 1984; Feddersen-Petersen, 1991). Common features include an open mouth, relaxed or stretched jaw, teeth covered to varying degrees and some lip corner retraction (Darwin, 1872; Fox, 1970; Schilder et al., 1984; Rooney, 2001). Some features may resemble aggression (e.g., nose wrinkling and teeth baring in wolves, *Canis lupus*) but are distinguishable when combined with other signals such as posture or body tension (Feddersen-Petersen, 1991). Mouth movements are made in response to pleasant taste stimuli such as sweet foods. In rats, this behavior is seen as licking or movement of the upper lip and tongue protrusion (Grill and Norgren, 1978; Cabanac and LaFrance, 1990). Humans and non-human primates also protrude the tongue in response to sweet foods and may also smack their lips (Steiner et al., 2001).

Sexual motivation may also be indicated by some facial expressions and is relevant to welfare assessment as reproduction can be suppressed when welfare is poor (Broom, 2008). One such facial expression is flehmen, characterized by movement around the mouth, jaw and nose, and thought to be functional in monitoring estrous cycles of females from their urine, although it may also serve other communicative functions (Stahlbaum and Houpt, 1989; Weeks et al., 2002). In the donkey (*Equus asinus*), for example, this has been described as “raising the head with the muzzle pointed toward the sky, the upper lip drawn back extensively and puckered, with the upper teeth and gums exposed, and nostrils wrinkled into a longitudinal and closed position” (Moehlman, 1998, p. 136). Flehmen has been observed

in a wide range of mammals including marsupials (e.g., wombat, *Lasiorhinus latifrons*, Gaughwin, 1979), ungulates (e.g., horse, Weeks et al., 2002; Arabian camel, *Camelus dromedaries*, Fatnassi et al., 2014), primates (e.g., mandrill, *Mandrillus sphinx*, Charpentier et al., 2013), and felids (e.g., puma, *Puma concolor*, Allen et al., 2014). Recognizing sexual motivation by flehmen expression may also assist with the interpretation of other behavioral changes that occur during reproduction or courting, such as increased locomotion or aggression, which can confound interpretations of welfare (Morgan et al., 2004). It is important to note that equids may show a similar expression when in pain (Pritchett et al., 2003), highlighting the need for multi-modal tools that allow for different welfare states to be differentiated, for example using facial expressions to complement behavioral or physiological measures. Increased investigation of these signals may improve differentiation between similar expressions performed in different contexts.

5.4 Ears

Ear position may be useful indicators of emotional valence or intensity in animals with ear mobility, for example, relaxed ears correspond with a neutral emotional state in sheep and a positive one in cows (Schmied et al., 2008; Boissy et al., 2011). In horses, front-oriented, pricked ears indicate attention or alertness, and although this is commonly considered to indicate positive emotional valence, this has not yet been empirically determined (Innes and McBride, 2008; Heleski et al., 2009; Proctor and Carder, 2014). However, in animals with mobile ears the neutral ear position can vary both between and within species, and therefore it is important that a baseline position be established for each species, and that individual differences are also taken into account (Andrew, 1963; Wathan et al., 2015). As with negative welfare states, ear position may provide important information on positive states in animals but further research is needed to classify ear position responses in detail.

6 Can facial expression as a social signal indicate welfare?

In many species, the opportunity for positive social interaction is a key component of maintaining good captive welfare (Mason, 1991; Olsson and Westlund, 2007). Communication between conspecifics is an important component of social stability, particularly in gregarious animal societies such as primates (Sussman et al., 2005). Group communication and social stability has health benefits for individuals within those groups (Silk et al., 2009, 2010; Nunez et al., 2014). Communication is multi-modal and may contain auditory, visual or olfactory components, dependent on context and distance between signaler and receiver (Parr et al., 2005; Burrows, 2008; da Cunha and Byrne, 2009; Waller et al., 2013). Signaling is important for social information transfer, and facilitates affiliation, spacing, agonistic intent, or predator avoidance (Partan, 2002; da Cunha and Byrne, 2009; Kiriazis and Slobodchikoff, 2006; Micheletta et al., 2013). Facial expressions are most important for communicating in close range interactions and may indicate signaler



intent and impending behavior to the receiver (Partan, 2002; Parr and Waller, 2006; Oettinger et al., 2007).

The relevance of facial signaling to animal welfare assessment is illustrated by the use of facial displays to replace or precede aggressive intent. Threatening facial expressions benefit both aggressor and receiver by allowing direct aggression and its potential consequences to be avoided (Judge and de Waal, 1993). Aggression, which may include facial signals, can also indicate perceived threat by the signaler, which may be directed towards within-group conspecifics, humans, or heterospecifics (e.g., neophobia) (Mitchell et al., 1992; Partan, 2002; Leonardi et al., 2010; Peiman et al., 2010). Aggressive behavior is associated with fearful or anxious affective states and stress (Galac and Knol, 1997; Boissy, 1995; Honess and Marin, 2006) that are relevant within a welfare framework. Agonistic facial expressions in reaction to ambiguous stimuli may also be useful as an indicator of cognitive bias, a measure of the animal's perceptual valence that ranges from an optimistic to a pessimistic bias (Bar-Haim et al., 2007; Bethell et al., 2012). Rates of agonistic and submissive facial expressions can indicate changes in social dynamics or escalation of aggressive interactions, which are normal in a natural context but are undesirable at elevated frequencies or intensities because of the potential for injury and distress (Kikusui and Mori, 2009; Akre et al., 2011). In golden-bellied mangabeys (*Cercocebus galeritus*), for example, aggressive facial displays were measured in a zoo setting (Mitchell et al., 1992). It was found that zoo visitor numbers had a significant effect on the frequency of facial displays; lower visitor numbers were associated with fewer aggressive facial displays both towards humans and conspecifics. Although the authors regarded these changes as within the parameters of normal behavior, it supports the premise that facial displays can reflect environmental conditions. Although the majority of studies incorporating facial expression in non-primate species use few facial features, one study in donkeys detailed expressions under numerous contexts (Moehlman, 1998), suggesting that more comprehensive accounts of situational facial configurations are achievable in other species. Donkeys use an open-mouth face as a social threat (Moehlman, 1998). In males, a protruding and downward pulled upper lip is displayed when courting a female, and occasionally in response to threats by another male (Moehlman, 1998). A jawing mouth movement (repetitive opening and closing of the jaw) is displayed by females during copulation as well as by males when mounted by other males, or when approached by a more dominant animal (Moehlman, 1998).

Appeasement and affiliative signaling can similarly indicate internal state, social conflict, and the presence or perception of threat, all of which are relevant for animal welfare. In chimpanzees, for example, affiliation is characterized by a silent bared-teeth display as a signal of benign intention or submission (Preuschoft and van Hooff, 1997; Waller and Dunbar, 2005). Fearful expressions serve a communicative role in appeasing potential or actual threat from conspecifics (Marsh et al., 2005a; Shariff and Tracy, 2011). In dogs, for example, appeasement and "stress" signals include panting, lip or nose licking, and tongue flicking (Kuhne et al., 2012). These signals increase when dogs are exposed to uncomfortable situations such as inappropriate

petting, and are performed more towards familiar people than those who are unfamiliar. Therefore, facial communication can provide insight into internal states in mammals and allow for interpretations on welfare and environmental effects.

The contingency of using social signals as an indicator of welfare is dependent upon the "honesty" of the signaler (Krebs and Dawkins, 1984; Fridlund, 1991; Weary and Fraser, 1995). In some cases, a given signal may be actively deceptive in that the signaler actively attempts to mislead the observer, or passively deceptive where a genuine signal is suppressed by the presence of an observer. If expression of a signal increases an animal's vulnerability, for example, pain vocalization in a prey species, the signal may be suppressed. In this case it would be incorrect to assume that a lack of signal implies a lack of need. The hiding of pain responses is considered prevalent by vets (Fenwick et al., 2014), however pain behavior may also serve as a strategy to recruit altruistic assistance from others (Langford et al., 2006; de Waal, 2008), and concealment when assistance could be expected would be maladaptive. Signal suppression is most likely to occur in the presence of either a threat or a competitor, and has direct relevance to human-animal interactions including but not limited to observer effects and learned helplessness (Overmier and Seligman, 1967; Seligman and Maier, 1967; Weary and Fraser, 1995; Jack et al., 2008; Crofoot et al., 2010). Signals are most likely to be honest when the signaler and receiver are related, the animals have shared interests compared to competing interests, the degree or intensity of the signal varies with the need, and the production of the signal has a cost to the signaler (Weary and Fraser, 1995). However, these issues are not specific to the study of facial expression but are true of all animal signals including vocalizations and posture, and strategies that avoid behavioral alteration from observer or competitor effects may be equally applied to facial displays as to other behavior. In fact, evidence from human studies suggests that facial expressions are subject to "emotional leakage" when suppression is attempted, and in some cases, are more reliable indicators of internal states and motivations than body motor movements (Craig et al., 1991; Williams, 2002; Ekman and Rosenberg, 2005; ten Brinke et al., 2012). This suggests that facial expressions can be a useful and honest measure that can be applied to the identification of underlying affective states in animals.

7 Methods of measurement

Facial expression has been measured using both "bottom-up" and "top-down" techniques. Facial action coding systems (FACS) are a bottom up method of identifying and recording facial expressions based on the underlying facial musculature and muscle movement (Ekman and Friesen, 1978). Rather than categorizing gestalt expressions associated with one specific context, FACS documents all the observable facial movements for a species, accommodating all potential facial configurations and making this method suitable for use across a wide range of settings. The original FACS was developed for use in humans (Ekman and Rosenberg, 2005), and this framework has

since been applied to a number of different nonhuman primates and domesticated species, i.e., chimpanzees (Vick et al., 2007), orangutans (*Pongo pygmaeus*, Caeiro et al., 2012), rhesus macaques (Parr et al., 2010), gibbons and siamangs (*Hylobatidae*, Waller et al., 2012), horses (Wathan et al., 2015), dogs (Waller et al., 2013) and cats (Caeiro et al., 2013). This methodology allows direct comparisons using identical techniques across species with a different facial morphology (e.g., Waller et al., 2014). Frequencies and intensities of individual action units and configurations for multiple muscle actions can be analyzed. Grimace scales for pain identification use a simplified version of the FACS approach, with muscle movements defined by changes in appearance of key facial features occurring during pain states (e.g., Sotocinal et al., 2011; Dalla Costa et al., 2014). These appearance changes may be created by individual or grouped muscle actions and grimace scales often incorporate a 3-point intensity scale to better assess pain intensity.

In contrast to FACS, facial expressions used in social communication research are categorized according to multiple simultaneous muscle movements that have commonly accepted configurations such as “fear grimace” and “relaxed open mouth display” (Parr et al., 2005; Waller and Dunbar, 2005; Parr and Waller, 2006; De Marco et al., 2008). This is a “top-down” system of coding, with expressions then counted or timed for analysis. This protocol is useful for characterizing social communication in well-studied species such as primates, however, pre-determined labels risk becoming misnomers when applied to studies of emotion or welfare, and may thus incorrectly guide interpretation in a welfare context. For example, the “fear grimace” in primates may not necessarily reflect an internal fearful state but has other communicative functions such as submission, appeasement or affiliation (de Waal, 2003; Waller and Dunbar, 2005; Beisner and McCowan, 2014).

An alternative method of assessing emotion or welfare by facial expression is by measuring laterality in expression production (Fernández-Carriba et al., 2002; Wallez and Vauclair, 2012). The phenomenon of laterality, or asymmetry, in motor activity, auditory processing, and visual attention is widespread across vertebrates and is caused by an imbalanced contribution of the cerebral hemispheres to cognitive processing (Rogers, 2014). Presence or strength of lateralization is affected by variables such as species and individual differences, however it has also been proposed as a useful welfare indicator by Rogers (2010) because stressed animals can become more active in their right hemisphere, correlating ipsilaterally to greater dominance on the left side of the body. An alternate hypothesis is that strength in laterality is less affected by emotional valence and more by level of arousal or emotional intensity. In humans, for example, the production of emotional facial expressions is stronger on the left side of the face (Sackeim et al., 1978) and dogs show more left facial activation when reunited with their owners than when reacting to strangers (Nagasawa et al., 2013). Asymmetrical ear position may indicate pain in horses (Gleerup et al., 2015a) and a startle response in sheep (Boissy et al., 2011). Rhesus macaques exhibit some asymmetry in the production of facial expression and vocalizations although this is thought to be unrelated to emotional valence (Hauser and Akre, 2001). A recent

review of both body and facial lateralization in response to emotional stimuli concluded that across the vertebrates, a generalized pattern exists for processing negative emotional contexts (e.g., fear, aggression) with the right cerebral hemisphere and positively associated experiences (e.g., food rewards) with the left (Leliveld et al., 2013). More empirical evidence is needed in a range of species to determine generalized patterns specifically in facial lateralization that have the potential to be applied to welfare contexts.

8 When is facial expression not a reliable indicator for welfare?

The reliability of using facial expression as a welfare indicator is reliant on several assumptions.

Firstly, that the species of interest has the facial structure that allows sufficient facial mobility to generate observable expressions (Chevalier-Skolnikoff, 1973; Cooke, 2015). The use of facial signals by mammals is related to taxa with those species characterized by gregariousness involving intricate social environments, a factor which is thought to have adaptively increased facial muscle structure and facial expression use (Byrne and Whiten, 1985; Burrows, 2008). It may be possible that as the capacity to generate facial expression becomes more complex it can be used for greater specificity in detection of emotions, while in less social or visual mammals it may only be reliable in indicating either valence (negative/positive) or intensity.

Secondly, changes in facial expression must be observable. Overt or sustained expressions may be noted by direct observation, however subtle or fleeting facial changes are captured more easily using technology. Still images from video footage have been used with success in grimace pain scales (Sotocinal et al., 2011; Leach et al., 2012; Miller et al., 2015), and advances in technology yield high quality still and video footage. Stills are taken when the face is clearly visible, and coding is then conducted on a random selection of this pool of images (Miller et al., 2015). Live coding of grimace scales has been attempted with some success (Leung et al., 2016), however in other studies the results were found to be significantly different to those obtained by still images (Miller and Leach, 2015b). Both photographs and video have been used for FACS, however this method of fine-grained measurement can be challenging and time-consuming (Ekman and Rosenberg, 2005; Vick et al., 2007; Parr et al., 2010). Video footage allows movement to be detected, which facilitates detection of facial changes. For ease and accuracy of FACS style coding, close range, high quality, high definition video is necessary. Poor filming conditions and the physical appearance of the animal or human may also affect how observable facial configurations are (Marsh et al., 2005b; Dalla Costa et al., 2014). For example, rhesus macaques have individual differences in brow size that may contribute to an open-eyed “surprised” appearance, or a lowered-brow “angry” appearance, and therefore an accurate neutral expression should be obtained prior to coding of muscle activation. Shadows can also be cast on the face during different head positions and this may mimic the changes in appearance resulting from muscle



action. Coding of reduced speed video using FACS can assist in overcoming this issue (Ekman and Rosenberg, 2005; Parr et al., 2010).

Thirdly, different affective states must be sufficiently differentiated in the face, or contribute significantly to the interpretation of gross level behavior. In the development of the MGS it was observed that sleeping and sick mice show similarities in some of the grimace muscle actions (Langford et al., 2010). Similarly, Defensor et al. (2012) described a similar facial expression in mice that were exposed to intruders in their territory. In horses, an upper lip curl can be due to both flehmen (Stahlbaum and Houpt, 1989) and abdominal pain (Pritchett et al., 2003), and in nonhuman primates yawning indicates both threat and displacement behavior (Andrew, 1963; Vick and Paukner, 2010). These examples suggest that facial expressions of similar appearance may derive from multiple etiologies. However, these may be differentiated by closer examination of facial changes, or by combining this information with other behavior, vocalizations and context. Displacement activities are often fragmented, incomplete versions of the “source” behavior (Russell and Russell, 1985; Maestripieri et al., 1992) and this may assist in distinguishing between similar behaviors with different functions. For example, Vick and Paukner (2010) demonstrated that displacement yawning in chimpanzees could be differentiated from other yawning types by facial configuration, intensity, and by the succeeding behavior. Alternatively, in some circumstances it may only be possible to identify valence, without further specification.

An additional methodological consideration is that the production of vocalization results in facial muscle actions. Facial and vocal communications are motivationally linked, and are combined for multi-modal social expression (Andrew, 1963; Chevalier-Skolnikoff, 1973; Lehner, 1978; Partan, 2002; Micheletta et al., 2013). Both may be important measures of welfare and facial expressions created in the production of sound should be differentiated rather than disregarded. In rhesus macaques, the mouth creates fixed movements when producing vocalizations, while non-vocal mouth expressions are more flexible in movement and shape (Partan, 2002), and again subtleties or multimodal information may assist in differentiating affect or motivation (e.g., Slocombe et al., 2011).

Finally, interpreting facial expression or behavior as a signal of welfare state relies on the honesty of that signal in reflecting the internal condition. Animals may suppress honest signals when it is advantageous, for example when they are vulnerable to attack or to protect available resources (Weary and Fraser, 1995). The animal’s affective state may also be influenced by external circumstances. For example, environmental and social conditions modulate pain experiences in rodents (Rivat et al., 2007; Sorge et al., 2014). In humans, facial expressions can be voluntarily generated or suppressed, which can result in observer deception (Bartlett et al., 2014). However, voluntary and genuine facial expressions (e.g., smiling) in humans have subtle defining differences, and suppression of expression, for example hiding of the pain face, is often incomplete (Craig et al., 1991; Ekman, 1992; Erickson and Schulkin, 2003). In humans, falsified facial expressions tend to be more inconsistent in

their production and are also accompanied by a higher blink rate (Porter and ten Brinke, 2008; ten Brinke et al., 2012). In practice, a combination of facial expression and somatic movement is likely to provide the most reliable indicator of internal states. However, further research into signal honesty and audience effects on production is required to assess the potential impact of these factors on the reliability and validity of facial expressions as a welfare measure.

9 Summary and conclusions

Identification of the internal state of animals has inherent challenges that impair our ability to measure welfare states, and restrain opportunities for experimental refinement when animal models are utilized. Although facial expressions are infrequently used as a measure of welfare in animals, evidence suggests that such expressions, in mammals at least, may provide important insights into internal states. Facial expressions can potentially indicate psychological and emotional experiences in animals, as well as temporal and stimuli specific reactions. Robust, objective systems for the recording and measurement of facial expressions already exist for several species, and may take advantage of the innate human observational bias to focus on the facial area. Furthermore, evidence from primates suggests that facial expression may be a more honest signal of internal state than general behavior. While facial displays cannot replace other behavioral or physiological indicators of welfare, emotion or health, they are a largely untapped resource with considerable potential to enhance our understanding of affective states and experiences in animals and subsequently to underpin improvements in applied animal welfare.

References

- Akre, A. K., Bakken, M., Hovland, A. L. et al. (2011). Clustered environmental enrichments induce more aggression and stereotypic behaviour than do dispersed enrichments in female mice. *Appl Anim Behav Sci* 131, 145-152. doi:10.1016/j.applanim.2011.01.010
- Allen, M. L., Wittmer, H. U. and Wilmers, C. C. (2014). Puma communication behaviours: Understanding functional use and variation among sex and age classes. *Behaviour* 151, 819-840. doi:10.1163/1568539X-00003173
- Andrew, R. J. (1963). The origin and evolution of the calls and facial expressions of the primates. *Behaviour* 20, 1-107. doi:10.1163/156853963X00220
- Archer, J. and Monton, S. (2011). Preferences for infant facial features in pet dogs and cats. *Ethology* 117, 217-226. doi:10.1111/j.1439-0310.2010.01863.x
- Au, E., Loprinzi, C. L., Dhodapkar, M. et al. (1994). Regular use of a verbal pain scale improves the understanding of oncology inpatient pain intensity. *J Clin Oncol* 12, 2751-2755.
- Baker, K. C. and Aureli, F. (1997). Behavioural indicators of anxiety: An empirical test in chimpanzees. *Behaviour* 134, 1031-1050. doi:10.1163/156853997X00386

- Bardo, M. T. and Bevins, R. A. (2000). Conditioned place preference: What does it add to our preclinical understanding of drug reward? *Psychopharmacology* 153, 31-43. doi:10.1007/s002130000569
- Bar-Haim, Y., Lamy, D., Pergamin, L. et al. (2007). Threat-related attentional bias in anxious and nonanxious individuals: A meta-analytic study. *Psychol Bull* 133, 1-24. doi:10.1037/0033-2909.133.1.1
- Bartlett, M. S., Littlewort, G. C., Frank, M. G. and Lee, K. (2014). Automatic decoding of facial movements reveals deceptive pain expressions. *Curr Biol* 24, 738-743. doi:10.1016/j.cub.2014.02.009
- Bateson, P. (1991). Assessment of pain in animals. *Anim Behav* 42, 827-839. doi:10.1016/S0003-3472(05)80127-7
- Beerd, B., Schilder, M. B. H., van Hooff, J. A. R. A. M. and de Vries, H. W. (1997). Manifestations of chronic and acute stress in dogs. *Appl Anim Behav Sci* 52, 307-319. doi:10.1016/S0168-1591(96)01131-8
- Beerd, B., Schilder, M. B. H., van Hooff, J. A. R. A. M. et al. (1999). Chronic stress in dogs subjected to social and spatial restriction. I. Behavioral responses. *Physiol Behav* 66, 233-242. doi:10.1016/S0031-9384(98)00289-3
- Beisner, B. A. and McCowan, B. (2014). Signaling context modulates social function of silent bared-teeth displays in rhesus macaques (*Macaca mulatta*). *Amer J Primatol* 76, 111-121. doi:10.1002/ajp.22214
- Bekoff, M. and Byers, J. A. (1998). *Animal Play: Evolutionary, Comparative and Ecological Perspectives*. Cambridge University Press. doi:10.1017/cbo9780511608575
- Bekoff, M. (2001). The evolution of animal play, emotions, and social morality: On science, theology, spirituality, personhood, and love. *Zygon* 36, 615-655. doi:10.1111/0591-2385.00388
- Bekoff, M. (2015). Playful fun in dogs. *Curr Biol* 25, R4-7. doi:10.1016/j.cub.2014.09.007
- Benhajali, H., Richard-Yris, M.-A., Ezzaouia, M. et al. (2010). Reproductive status and stereotypies in breeding mares: A brief report. *Appl Anim Behav Sci* 128, 64-68. doi:10.1016/j.applanim.2010.09.007
- Bennett, S. L., Litster, A., Weng, H.-Y. et al. (2012). Investigating behavior assessment instruments to predict aggression in dogs. *Appl Anim Behav Sci* 141, 139-148. doi:10.1016/j.applanim.2012.08.005
- Bergeron, R., Badnell-Waters, A. J., Lambton, S. and Mason, G. (2006). Stereotypic oral behaviour in captive ungulates: Foraging, diet and gastrointestinal function. In G. Mason and J. Rushen (eds.), *Stereotypic Animal Behaviour: Fundamentals and Applications to Welfare* (19-57). 2nd edition. Trowbridge, UK: CABI. doi:10.1079/9780851990040.0019
- Berthier, M., Starkstein, S. and Leiguarda, R. (1987). Behavioral effects of damage to the right insula and surrounding regions. *Cortex* 23, 673-678. doi:10.1016/S0010-9452(87)80057-6
- Bethell, E. J., Holmes, A., MacLarnon, A. and Semple, S. (2012). Cognitive bias in a non-human primate: Husbandry procedures influence cognitive indicators of psychological well-being in captive rhesus macaques. *Anim Welfare* 21, 185-195. doi:10.7120/09627286.21.2.185
- Bethell, E. J. (2015). A "how-to" guide for designing judgment bias studies to assess captive animal welfare. *J Appl Anim Welf Sci* 18, Suppl 1, S18-S42. doi:10.1080/10888705.2015.1075833
- Bloom, T. and Friedman, H. (2013). Classifying dogs' (*Canis familiaris*) facial expressions from photographs. *Behav Process* 96, 1-10. doi:10.1016/j.beproc.2013.02.010
- Bloomsmith, M. A., Marr, M. J. and Maple, T. L. (2007). Addressing nonhuman primate behavioral problems through the application of operant conditioning: Is the human treatment approach a useful model? *Appl Anim Behav Sci* 102, 205-222. doi:10.1016/j.applanim.2006.05.028
- Boissy, A. (1995). Fear and fearfulness in animals. *Q Rev Biol* 70, 165-191. doi:10.1086/418981
- Boissy, A., Manteuffel, G., Jensen, M. B. et al. (2007). Assessment of positive emotions in animals to improve their welfare. *Physiol Behav* 92, 375-397. doi:10.1016/j.physbeh.2007.02.003
- Boissy, A., Aubert, A., Désiré, L. et al. (2011). Cognitive sciences to relate ear postures to emotions in sheep. *Anim Welfare* 20, 47-56.
- Boissy, A. and Erhard, H. W. (2014). How studying interactions between animal emotions, cognition, and personality can contribute to improve farm animal welfare. In T. Grandin and M. J. Deesing (eds.), *Genetics and the Behaviour of Domestic Animals* (81-113). 2nd edition. Elsevier. doi:10.1016/b978-0-12-394586-0.00003-2
- Bourgeois, S. R. and Brent, L. (2005). Modifying the behaviour of singly caged baboons: Evaluating the effectiveness of four enrichment techniques. *Anim Welfare* 14, 71-81.
- Briefer Freymond, S., Briefer, E. F., Zollinger, A. et al. (2014). Behaviour of horses in a judgment bias test associated with positive or negative reinforcement. *Appl Anim Behav Sci* 158, 34-45. doi:10.1016/j.applanim.2014.06.006
- Broom, D. M., Mendl, M. T. and Zanella, A. J. (1995). A comparison of the welfare of sows in different housing conditions. *Anim Sci* 61, 369-385. doi:10.1017/S1357729800013928
- Broom, D. (2008). Welfare assessment and relevant ethical decisions: Key concepts. *Annu Rev Biomed Sci* 10, T79-T90. doi:10.5016/1806-8774.2008.v10pT79
- Burgdorf, J. and Panksepp, J. (2006). The neurobiology of positive emotions. *Neurosci Biobehav R* 30, 173-187. doi:10.1016/j.neubiorev.2005.06.001
- Burrows, A. M. (2008). The facial expression musculature in primates and its evolutionary significance. *Bioessays* 30, 212-225. doi:10.1002/bies.20719
- Burton, C. and Crossley, M. (2003). Examining the utility of the Saskatchewan mood inventory for individuals with memory loss. *Can J Aging* 22, 297-310. doi:10.1017/S0714980800003913
- Bussi res, G., Jacques, C., Lainay, O. et al. (2008). Development of a composite orthopaedic pain scale in horses. *Res Vet Sci* 85, 294-306. doi:10.1016/j.rvsc.2007.10.011
- Buttner, A. P. and Strasser, R. (2014). Contagious yawning, social cognition, and arousal: An investigation of the processes underlying shelter dogs' responses to human yawns. *Anim Cogn* 17, 95-104. doi:10.1007/s10071-013-0641-z



- Byrne, R. W. and Whiten, A. (1985). Tactical deception of familiar individuals in baboons (*Papio ursinus*). *Anim Behav* 33, 669-673. doi:10.1016/S0003-3472(85)80093-2
- Cabanac, M. and Lafrance, L. (1990). Postingestive alliesthesia: The rat tells the same story. *Physiol Behav* 47, 539-543. doi:10.1016/0031-9384(90)90123-L
- Caeiro, C. C., Waller, B. M., Zimmermann, E. et al. (2012). OrangFACS: A muscle-based facial movement coding system for orangutans (*Pongo* spp.). *Int J Primatol* 34, 115-129. doi:10.1007/s10764-012-9652-x
- Caeiro, C. C., Burrows, A. M. and Waller, B. M. (2013). CatFACS. University of Portsmouth. <http://www.catfacs.com>
- Capner, A., Lascelles, B. D. X. and Waterman-Pearson, A. E. (1999). Current British veterinary attitudes to perioperative analgesia for dogs. *Vet Rec* 145, 95-99. doi:10.1136/vr.145.4.95
- Casey, R. A. (2007). Clinical problems associated with the intensive management of performance horses. In N. K. Waran (ed.), *The Welfare of Horses* (19-44). Dordrecht, The Netherlands: Springer Netherlands.
- Chapman, H. A., Kim, D. A., Susskind, J. M. and Anderson, A. K. (2009). In bad taste: Evidence for the oral origins of moral disgust. *Science* 323, 1222-1226. doi:10.1126/science.1165565
- Charpentier, M. J. E., Mboumba, S., Ditsoga, C. and Drea, C. M. (2013). Nasopalatine ducts and flehmen behavior in the mandrill: Reevaluating olfactory communication in Old World primates. *Amer J Primatol* 75, 703-714. doi:10.1002/ajp.22146
- Chevalier-Skolnikoff, S. (1973). Facial expression of emotion in nonhuman primates. In P. Ekman (ed.), *Darwin and Facial Expression: A Century of Research in Review* (11-89). New York, NY: Academic Press.
- Coleman, K. (2012). Individual differences in temperament and behavioral management practices for nonhuman primates. *Appl Anim Behav Sci* 137, 106-113. doi:10.1016/j.applanim.2011.08.002
- Cooke, G. M. (2015). Concern regarding the use of the term "nonhuman animals" in the article "ontogeny and phylogeny of facial expression of pain". *Pain* 156, 1828. doi:10.1097/j.pain.0000000000000235
- Coulter, C. A., Flecknell, P. A. and Richardson, C. A. (2009). Reported analgesic administration to rabbits, pigs, sheep, dogs and non-human primates undergoing experimental surgical procedures. *Lab Animal* 43, 232. doi:10.1258/la.2008.008021
- Couroucé-Malblanc, A., Fortier, G., Pronost, S. et al. (2008). Comparison of prednisolone and dexamethasone effects in the presence of environmental control in heaves-affected horses. *Vet J* 175, 227-233. doi:10.1016/j.tvjl.2006.12.006
- Craig, K. D., Hyde, S. A. and Patrick, C. J. (1991). Genuine, suppressed and faked facial behavior during exacerbation of chronic low back pain. *Pain* 46, 161-171. doi:10.1016/0304-3959(91)90071-5
- Crofoot, M. C., Lambert, T. D., Kays, R. and Wikelski, M. C. (2010). Does watching a monkey change its behaviour? Quantifying observer effects in habituated wild primates using automated radiotelemetry. *Anim Behav* 80, 475-480. doi:10.1016/j.anbehav.2010.06.006
- da Cunha, R. G. T. and Byrne, R. (2009). The use of vocal communication in keeping the spatial cohesion of groups: Intentionality and specific functions. In P. Garber, A. Estrada, J. Bicca-Marques et al. (eds.), *South American Primates* (341-363). New York: Springer. doi:10.1007/978-0-387-78705-3_13
- Dalla Costa, E., Minero, M., Lebelt, D. et al. (2014). Development of the horse grimace scale (HGS) as a pain assessment tool in horses undergoing routine castration. *PLoS One* 9, e92281. doi:10.1371/journal.pone.0092281
- Darwin, C. (1872). *The Expression of the Emotions in Man and Animals*. London, UK: John Murray. doi:10.1037/10001-000
- Dawkins, M. S. (1990). From an animal's point of view: Motivation, fitness, and animal welfare. *Behav Brain Sci* 13, 1-9. doi:10.1017/S0140525X00077104
- Dawkins, M. S. (2004). Using behaviour to assess animal welfare. *Anim Welfare* 13, Suppl, S3-S7.
- De Marco, A., Petit, O. and Visalberghi, E. (2008). The repertoire and social function of facial displays in *Cebus capucinus*. *Int J Primatol* 29, 469-486. doi:10.1007/s10764-007-9174-0
- De Marco, A., Cozzolino, R., Dessi-Fulgheri, F. and Thierry, B. (2010). Conflicts induce affiliative interactions among bystanders in a tolerant species of macaque (*Macaca tonkeana*). *Anim Behav* 80, 197-203. doi:10.1016/j.anbehav.2010.04.016
- de Waal, F. B. M. (2003). Darwin's legacy and the study of primate visual communication. *Ann NY Acad Sci* 1000, 7-31. doi:10.1196/annals.1280.003
- de Waal, F. B. M. (2008). Putting the altruism back into altruism: The evolution of empathy. *Annu Rev Psychol* 59, 279-300. doi:10.1146/annurev.psych.59.103006.093625
- de Waal, F. B. M. (2011). What is an animal emotion? *Ann NY Acad Sci* 1224, 191-206. doi:10.1111/j.1749-6632.2010.05912.x
- Defensor, E. B., Corley, M. J., Blanchard, R. J. and Blanchard, D. C. (2012). Facial expressions of mice in aggressive and fearful contexts. *Physiol Behav* 107, 680-685. doi:10.1016/j.physbeh.2012.03.024
- Désiré, L., Boissy, A. and Veissier, I. (2002). Emotions in farm animals: A new approach to animal welfare in applied ethology. *Behav Process* 60, 165-180. doi:10.1016/S0376-6357(02)00081-5
- Dimberg, U. and Thunberg, M. (1998). Rapid facial reactions to emotional facial expressions. *Scand J Psychol* 39, 39-45. doi:10.1111/1467-9450.00054
- Dimberg, U., Thunberg, M. and Elmehed, K. (2000). Unconscious facial reactions to emotional facial expressions. *Psychol Sci* 11, 86-89. doi:10.1111/1467-9280.00221
- Dimberg, U., Thunberg, M. and Grunedal, S. (2002). Facial reactions to emotional stimuli: Automatically controlled emotional responses. *Cognition Emotion* 16, 449-471. doi:10.1080/02699930143000356
- Diogo, R., Wood, B. A., Aziz, M. A. and Burrows, A. (2009). On the origin, homologies and evolution of primate facial muscles, with a particular focus on hominoids and a suggested unifying nomenclature for the facial muscles of the Mammalia. *J Anat* 215, 300-319. doi:10.1111/j.1469-7580.2009.01111.x
- Eastwood, J. D., Smilek, D. and Merikle, P. M. (2003). Negative facial expression captures attention and disrupts performance.

- Percept Psychophys* 65, 352-358. doi:10.3758/BF03194566
- Ekman, P. and Friesen, W. V. (1978). *Facial Action Coding System (FACS): A Technique for the Measurement of Facial Action* (22). Palo Alto, CA: Consulting Psychologists Press.
- Ekman, P., Davidson, R. J. and Friesen, W. V. (1990). The Duchenne smile: Emotional expression and brain physiology. II. *J Pers Soc Psychol* 58, 342-353. doi:10.1037/0022-3514.58.2.342
- Ekman, P. (1992). An argument for basic emotions. *Cognition Emotion* 6, 169-200. doi:10.1080/02699939208411068
- Ekman, P. (1993). Facial expression and emotion. *Am Psychol* 48, 384-392. doi:10.1037/0003-066X.48.4.384
- Ekman, P. and Rosenberg, E. L. (eds.). (2005). *What the Face Reveals: Basic and Applied Studies of Spontaneous Expression Using the Facial Action Coding System (FACS)*. 2nd edition. Oxford University Press.
- Erickson, K. and Schulkin, J. (2003). Facial expressions of emotion: A cognitive neuroscience perspective. *Brain Cogn* 52, 52-60. doi:10.1016/S0278-2626(03)00008-3
- Everds, N. E., Snyder, P. W., Bailey, K. L. et al. (2013). Interpreting stress responses during routine toxicity studies: A review of the biology, impact, and assessment. *Toxicol Pathol* 41, 560-614. doi:10.1177/0192623312466452
- Fatnassi, M., Padalino, B., Monaco, D. et al. (2014). Evaluation of sexual behavior of housed male camels (*Camelus dromedarius*) through female parades: Correlation with climatic parameters. *Trop Anim Health Pro* 46, 313-321. doi:10.1007/s11250-013-0489-x
- Feddersen-Petersen, D. (1991). The ontogeny of social play and agonistic behaviour in selected canid species. *Bonn Zool Beitr* 42, 97-114.
- Fenwick, N., Duffus, S. E. G. and Griffin, G. (2014). Pain management for animals used in science: Views of scientists and veterinarians in Canada. *Animals* 4, 494-514. doi:10.3390/ani4030494
- Ferguson, D. M. and Warner, R. D. (2008). Have we underestimated the impact of pre-slaughter stress on meat quality in ruminants? *Meat Sci* 80, 12-19. doi:10.1016/j.meatsci.2008.05.004
- Fernandez, L. T., Bashaw, M. J., Sartor, R. L. et al. (2008). Tongue twisters: Feeding enrichment to reduce oral stereotypy in giraffe. *Zoo Biol* 27, 200-212. doi:10.1002/zoo.20180
- Fernández-Carriba, S., Loeches, Á., Morcillo, A. and Hopkins, W. D. (2002). Asymmetry in facial expression of emotions by chimpanzees. *Neuropsychologia* 40, 1523-1533. doi:10.1016/S00283932(02)00028-3
- Flecknell, P. A., Leach, M. C. and Bateson, M. (2011). Affective state and quality of life in mice. *Pain* 152, 963-964. doi:10.1016/j.pain.2011.01.030
- Fox, M. W. (1970). A comparative study of the development of facial expressions in canids; wolf, coyote and foxes. *Behaviour* 36, 49-73. doi:10.1163/156853970X00042
- Fraser, D. and Duncan, I. J. (1998). "Pleasures", "pains", and animal welfare: Toward a natural history of affect. *Anim Welfare* 7, 383-396.
- Fredrickson, B. L. (2004). The broaden-and-build theory of positive emotions. *Philos T Roy Soc B* 359, 1367-1378. doi:10.1098/rstb.2004.1512
- Fridlund, A. J. (1991). Evolution and facial action in reflex, social motive, and para-language. *Biol Psychol* 32, 3-100. doi:10.1016/0301-0511(91)90003-Y
- Fureix, C., Gorecka-Bruzda, A., Gautier, E. and Hausberger, M. (2011). Cooccurrence of yawning and stereotypic behaviour in horses (*Equus caballus*). *ISRN Zool* 2011, 1-10. doi:10.5402/2011/271209
- Galac, S. and Knol, B. W. (1997). Fear-motivated aggression in dogs: Patient characteristics, diagnosis and therapy. *Anim Welfare* 6, 9-15.
- Garcia, J., Hankins, W. G. and Rusiniak, K. W. (1974). Behavioral regulation of the milieu interne in man and rat. *Science* 185, 824-831. doi:10.1126/science.185.4154.824
- Gaughwin, M. D. (1979). The occurrence of flehmen in a marsupial – the hairy-nosed wombat (*Lasiorhinus latifrons*). *Anim Behav* 27, 1063-1065. doi:10.1016/0003-3472(79)90054-X
- Gleerup, K. B., Forkman, B., Lindegaard, C. and Andersen, P. H. (2015a). An equine pain face. *Vet Anaesth Analg* 42, 103-114. doi:10.1111/vaa.12212
- Gleerup, K. B., Andersen, P. H., Munksgaard, L. and Forkman, B. (2015b). Pain evaluation in dairy cattle. *Appl Anim Behav Sci* 171, 25-32. doi:10.1016/j.applanim.2015.08.023
- Glocker, M. L., Langleben, D. D., Ruparel, K. et al. (2009). Baby schema in infant faces induces cuteness perception and motivation for caretaking in adults. *Ethology* 115, 257-263. doi:10.1111/j.1439-0310.2008.01603.x
- Goodwin, D., Bradshaw, J. W. S. and Wickens, S. M. (1997). Paedomorphosis affects agonistic visual signals of domestic dogs. *Anim Behav* 53, 297-304. doi:10.1006/anbe.1996.0370
- Graves, F. C. and Wallen, K. (2006). Androgen-induced yawning in rhesus monkey females is reversed with a nonsteroidal anti-androgen. *Horm Behav* 49, 233-236. doi:10.1016/j.yhbeh.2005.07.005
- Grill, H. J. and Norgren, R. (1978). The taste reactivity test. I. Mimetic responses to gustatory stimuli in neurologically normal rats. *Brain Res* 143, 263-279. doi:10.1016/0006-8993(78)90568-1
- Hadidian, J. (1980). Yawning in an old world monkey, *Macaca nigra* (Primates: Cercopithecidae). *Behaviour* 75, 133-147. doi:10.1163/156853980X00375
- Hall, L. E., Robinson, S. and Buchanan-Smith, H. M. (2015). Refining dosing by oral gavage in the dog: A protocol to harmonise welfare. *J Pharmacol Toxicol* 72, 35-46. doi:10.1016/j.vascn.2014.12.007
- Hauser, M. D. and Akre, K. (2001). Asymmetries in the timing of facial and vocal expressions by rhesus monkeys: Implications for hemispheric specialization. *Anim Behav* 61, 391-400. doi:10.1006/anbe.2000.1588
- Hawker, G. A., Mian, S., Kendzerska, T. and French, M. (2011). Measures of adult pain: Visual analog scale for pain (VAS pain), numeric rating scale for pain (NRS pain), McGill pain questionnaire (MPQ), short-form McGill pain questionnaire (SF-MPQ), chronic pain grade scale (CPGS), short form-36 bodily pain scale (SF). *Arthrit Care Res* 63, Suppl S11, S240-S252. doi:10.1002/acr.20543
- Held, S. D. E. and Špinka, M. (2011). Animal play and animal welfare. *Anim Behav* 81, 891-899. doi:10.1016/j.anbehav.



- 2011.01.007
- Heleski, C. R., McGreevy, P. D., Kaiser, L. J. et al. (2009). Effects on behaviour and rein tension on horses ridden with or without martingales and rein inserts. *Vet J* 181, 56-62. doi:10.1016/j.tvjl.2009.03.011
- Hewson, C. J., Dohoo, I. R. and Lemke, K. A. (2006a). Perioperative use of analgesics in dogs and cats by Canadian veterinarians in 2001. *Can Vet J* 47, 352-359.
- Hewson, C. J., Dohoo, I. R. and Lemke, K. A. (2006b). Factors affecting the use of postincisional analgesics in dogs and cats by Canadian veterinarians in 2001. *Can Vet J* 47, 453-459.
- Hietanen, J. K., Surakka, V. and Linnankoski, I. (1998). Facial electromyographic responses to vocal affect expressions. *Psychophysiology* 35, 530-536. doi:10.1017/S0048577298970445
- Hill, S. P. (2009). Do gorillas regurgitate potentially injurious stomach acid during 'regurgitation and reingestion?'. *Anim Welfare* 18, 123-127.
- Hole, G. and Bourne, V. (2010). *Face Processing: Psychological, Neuropsychological, and Applied Perspectives*. Oxford University Press.
- Honess, P. E. and Marin, C. M. (2006). Behavioural and physiological aspects of stress and aggression in nonhuman primates. *Neurosci Biobehav R* 30, 390-412. doi:10.1016/j.neubiorev.2005.04.003
- Hopkins, W. D., Tagliatela, J. P. and Leavens, D. A. (2011). Do chimpanzees have voluntary control of their facial expressions and vocalizations? In A. Vilain, J. Schwartz, and C. Abry (eds.), *Primate Communication and Human Language: Vocalisation, Gestures, Imitation and Deixis in Humans and Non-Humans* (71-88). John Benjamins Publishing Company. doi:10.1075/ais.1.05hop
- Hsu, Y. and Sun, L. (2010). Factors associated with aggressive responses in pet dogs. *Appl Anim Behav Sci* 123, 108-123. doi:10.1016/j.applanim.2010.01.013
- Innes, L. and McBride, S. (2008). Negative versus positive reinforcement: An evaluation of training strategies for rehabilitated horses. *Appl Anim Behav Sci* 112, 357-368. doi:10.1016/j.applanim.2007.08.011
- Izzo, G. N., Bashaw, M. J. and Campbell, J. B. (2011). Enrichment and individual differences affect welfare indicators in squirrel monkeys (*Saimiri sciureus*). *J Comp Psychol* 125, 347-352. doi:10.1037/a0024294
- Jack, K. M., Lenz, B. B., Healan, E. et al. (2008). The effects of observer presence on the behavior of *Cebus capucinus* in Costa Rica. *Amer J Primatol* 70, 490-494. doi:10.1002/ajp.20512
- Jöchle, W. (1989). Field trial evaluation of detomidine as a sedative and analgesic in horses with colic. *Equine Vet J* 21, 117-120. doi:10.1111/j.2042-3306.1989.tb05669.x
- Jourdan, D., Ardid, D. and Eschalié, A. (2001). Analysis of ultrasonic vocalisation does not allow chronic pain to be evaluated in rats. *Pain* 95, 165-173. doi:10.1016/S0304-3959(01)00394-3
- Judge, P. G. and de Waal, F. B. (1993). Conflict avoidance among rhesus monkeys: Coping with short-term crowding. *Anim Behav* 46, 221-232. doi:10.1006/anbe.1993.1184
- Judge, P. G. and Bachmann, K. A. (2013). Witnessing reconciliation reduces arousal of bystanders in a baboon group (*Papio hamadryas hamadryas*). *Anim Behav* 85, 881-889. doi:10.1016/j.anbehav.2013.02.011
- Jürgens, U. (2009). The neural control of vocalization in mammals: A review. *J Voice* 23, 1-10. doi:10.1016/j.jvoice.2007.07.005
- Kaiser, L., Heleski, C. R., Siegford, J. and Smith, K. A. (2006). Stress-related behaviors among horses used in a therapeutic riding program. *J Am Vet Med Assoc* 228, 39-45. doi:10.2460/javma.228.1.39
- Keating, S. C., Thomas, A. A., Flecknell, P. A. and Leach, M. C. (2012). Evaluation of EMLA cream for preventing pain during tattooing of rabbits: Changes in physiological, behavioural and facial expression responses. *PLoS One* 7, e44437. doi:10.1371/journal.pone.0044437
- Keown, A. J., Farnworth, M. J. and Adams, N. J. (2011). Attitudes towards perception and management of pain in rabbits and guinea pigs by a sample of veterinarians in New Zealand. *New Zeal Vet J* 59, 305-310. doi:10.1080/00480169.2011.609477
- Kikusui, T. and Mori, Y. (2009). Behavioural and neurochemical consequences of early weaning in rodents. *J Neuroendocrinol* 21, 427-431. doi:10.1111/j.1365-2826.2009.01837.x
- Kiriazis, J. and Slobodchikoff, C. N. (2006). Perceptual specificity in the alarm calls of Gunnison's prairie dogs. *Behav Process* 73, 29-35. doi:10.1016/j.beproc.2006.01.015
- Klumpp, A., Trautmann, T., Markert, M. and Guth, B. (2006). Optimizing the experimental environment for dog telemetry studies. *J Pharmacol Toxicol* 54, 141-149. doi:10.1016/j.vascn.2006.03.010
- Knierim, U., Van Dongen, S., Forkman, B. et al. (2007). Fluctuating asymmetry as an animal welfare indicator – a review of methodology and validity. *Physiol Behav* 92, 398-421. doi:10.1016/j.physbeh.2007.02.014
- Knutson, B., Burgdorf, J. and Panksepp, J. (1998). Anticipation of play elicits high-frequency ultrasonic vocalisations in young rats. *J Comp Psychol* 112, 65-73. doi:10.1037/0735-7036.112.1.65
- Kongara, K., Squance, H. E., Topham, I. A. and Bridges, J. P. (2016). Attitudes and perceptions of veterinary paraprofessionals in New Zealand to postoperative pain in dogs and cats. *New Zeal Vet J* 64, 112-116. doi:10.1080/00480169.2015.1111172
- Krachun, C., Rushen, J. and de Passillé, A. M. (2010). Play behaviour in dairy calves is reduced by weaning and by a low energy intake. *Appl Anim Behav Sci* 122, 71-76. doi:10.1016/j.applanim.2009.12.002
- Krebs, J. R. and Dawkins, R. (1984). Animal signals: Mind-reading and manipulation. In J. R. Krebs and N. B. Davies, *Behavioural Ecology: An Evolutionary Approach* (380-402). 2nd edition. Oxford University Press.
- Kuhne, F., Höbner, J. C. and Struwe, R. (2012). Effects of human-dog familiarity on dogs' behavioural responses to petting. *Appl Anim Behav Sci* 142, 176-181. doi:10.1016/j.applanim.2012.10.003
- Langford, D. J., Crager, S. E., Shehzad, Z. et al. (2006). Social modulation of pain as evidence for empathy in mice. *Science*

- 312, 1967-1970. doi:10.1126/science.1128322
- Langford, D. J., Bailey, A. L., Chanda, M. L. et al. (2010). Coding of facial expressions of pain in the laboratory mouse. *Nature Med* 7, 447-449. doi:10.1038/nmeth.1455
- Langner, O., Dotsch, R., Bijlstra, G. et al. (2010). Presentation and validation of the radboud faces database. *Cognition Emotion* 24, 1377-1388. doi:10.1080/02699930903485076
- Lascelles, B. D. X., Capner, C. A. and Waterman-Pearson, A. E. (1999). Current British veterinary attitudes to perioperative analgesia for cats and small mammals. *Vet Rec* 145, 601-604. doi:10.1136/vr.145.21.601
- Lawrence, A. (1987). Consumer demand theory and the assessment of animal welfare. *Anim Behav* 35, 293-295. doi:10.1016/S0003-3472(87)80236-1
- Leach, M. C., Coulter, C. A., Richardson, C. A. and Flecknell, P. A. (2011). Are we looking in the wrong place? Implications for behavioural-based pain assessment in rabbits (*Oryctolagus cuniculi*) and beyond? *PLoS One* 6, e13347. doi:10.1371/journal.pone.0013347
- Leach, M. C., Klaus, K., Miller, A. L. et al. (2012). The assessment of post-vasectomy pain in mice using behaviour and the mouse grimace scale. *PLoS One* 7, s35656. doi:10.1371/journal.pone.0035656
- Leaman, J., Latter, J. and Clemence, M. (2014). Attitudes to animal research in 2014. A report by Ipsos MORI for the Department for Business, Innovation and Skills. UK.
- Lee, D. H., Susskind, J. M. and Anderson, A. K. (2013). Social transmission of the sensory benefits of eye widening in fear expressions. *Psychol Sci* 24, 957-965. doi:10.1177/0956797612464500
- Lehner, P. N. (1978). Coyote vocalizations: A lexicon and comparisons with other canids. *Anim Behav* 26, 712-722. doi:10.1016/0003-3472(78)90138-0
- Leiner, L. and Fendt, M. (2011). Behavioural fear and heart rate responses of horses after exposure to novel objects: Effects of habituation. *Appl Anim Behav Sci* 131, 104-109. doi:10.1016/j.applanim.2011.02.004
- Leliveld, L. M., Langbein, J. and Puppe, B. (2013). The emergence of emotional lateralization: Evidence in non-human vertebrates and implications for farm animals. *Appl Anim Behav Sci* 145, 1-14. doi:10.1016/j.applanim.2013.02.002
- Leonardi, R., Buchanan-Smith, H. M., Dufour, V. et al. (2010). Living together: Behavior and welfare in single and mixed species groups of capuchin (*Cebus apella*) and squirrel monkeys (*Saimiri sciureus*). *Am J Primatol* 72, 33-47. doi:10.1002/ajp.20748
- Leung, V., Zhang, E. and Pang, D. S. (2016). Real-time application of the rat grimace scale as a welfare refinement in laboratory rats. *Sci Rep* 6, 31667. doi:10.1038/srep31667
- Lewis, M. H., Gluck, J. P., Beauchamp, A. J. et al. (1990). Long-term effects of early social isolation in *Macaca mulatta*: Changes in dopamine receptor function following apomorphine challenge. *Brain Res* 513, 67-73. doi:10.1016/0006-8993(90)91089-Y
- Ley, J. M. (2016). Feline communication. In I. Rodan and S. Heath (eds.), *Feline Behavioral Health and Welfare* (24-33). Saint Louis, US: Elsevier. doi:10.1016/B978-1-4557-7401-2.00003-9
- Light, G. S., Hardie, E. M., Young, M. S. et al. (1993). Pain and anxiety behaviors of dogs during intravenous catheterization after premedication with placebo, acepromazine or oxymorphone. *Appl Anim Behav Sci* 37, 331-343. doi:10.1016/0168-1591(93)90122-6
- Loy, J. (1970). Behavioral responses of free-ranging rhesus monkeys to food shortage. *Am J Phys Anthropol* 33, 263-271. doi:10.1002/ajpa.1330330212
- Maestriperi, D., Schino, G., Aureli, F. and Troisi, A. (1992). A modest proposal: Displacement activities as an indicator of emotions in primates. *Anim Behav* 44, 967-979. doi:10.1016/S0003-3472(05)80592-5
- Mallapur, A., Waran, N. and Sinha, A. (2005). Factors influencing the behaviour and welfare of lion-tailed macaques in Indian zoos. *Appl Anim Behav Sci* 91, 337-353. doi:10.1016/j.applanim.2004.10.002
- Manteuffel, G., Puppe, B. and Schön, P. C. (2004). Vocalization of farm animals as a measure of welfare. *Appl Anim Behav Sci* 88, 163-182. doi:10.1016/j.applanim.2004.02.012
- Marsh, A. A., Ambady, N. and Kleck, R. E. (2005a). The effects of fear and anger facial expressions on approach- and avoidance-related behaviors. *Emotion* 5, 119-124. doi:10.1037/1528-3542.5.1.119
- Marsh, A. A., Adams, R. B. and Kleck, R. E. (2005b). Why do fear and anger look the way they do? Form and social function in facial expressions. *Pers Soc Psychol B* 31, 73-86. doi:10.1177/0146167204271306
- Martin, P. (1984). The (four) whys and wherefores of play in cats: A review of functional, evolutionary, developmental and causal issues. In *Play in Animals and Humans* (71-94). Oxford, UK: Blackwell Publishing.
- Mason, G. (1991). Stereotypies: A critical review. *Anim Behav* 41, 1015-1037. doi:10.1016/S0003-3472(05)80640-2
- Mason, G. and Mendl, M. (1993). Why is there no simple way of measuring animal welfare? *Anim Welfare* 2, 301-319.
- Mason, G. J. and Latham, R. (2004). Can't stop, won't stop: Is stereotypy a reliable animal welfare indicator? *Anim Welfare* 13, Suppl 1, 57-69.
- Mason, G. (2006). Stereotypic behaviour in captive animals: Fundamentals and implications for welfare and beyond. In *Stereotypic Animal Behaviour: Fundamentals and Applications to Welfare* (325). CABI. doi:10.1079/9780851990040.0325
- Mason, G., Clubb, R., Latham, N. and Vickery, S. (2007). Why and how should we use environmental enrichment to tackle stereotypic behaviour? *Appl Anim Behav Sci* 102, 163-188. doi:10.1016/j.applanim.2006.05.041
- Mason, G. J. (2010). Species differences in responses to captivity: Stress, welfare and the comparative method. *Trends Ecol Evol* 25, 713-721. doi:10.1016/j.tree.2010.08.011
- McDonnell, S. (2003). *The Equid Ethogram: A Practical Field Guide to Horse Behavior*. ECLIPSE Press. Retrieved from: <http://books.google.co.uk/books?id=-Mvm9NjH0WUC>
- McLennan, K. M., Rebelo, C. J. B., Corke, M. J. et al. (2016). Development of a facial expression scale using footrot and mastitis as models of pain in sheep. *Appl Anim Behav Sci* 176, 19-26. doi:10.1016/j.applanim.2016.01.007



- Meletti, S., Cantalupo, G., Benuzzi, F. et al. (2012). Fear and happiness in the eyes: An intra-cerebral event-related potential study from the human amygdala. *Neuropsychologia* 50, 44-54. doi:10.1016/j.neuropsychologia.2011.10.020
- Mellor, D. and Beausoleil, N. (2015). Extending the “five domains” model for animal welfare assessment to incorporate positive welfare states. *Anim Welfare* 24, 241-253. doi:10.7120/09627286.24.3.241
- Mendl, M., Burman, O. H. P., Parker, R. M. A. and Paul, E. S. (2009). Cognitive bias as an indicator of animal emotion and welfare: Emerging evidence and underlying mechanisms. *Appl Anim Behav Sci* 118, 161-181. doi:10.1016/j.applanim.2009.02.023
- Mendl, M., Burman, O. H. and Paul, E. S. (2010). An integrative and functional framework for the study of animal emotion and mood. *P Roy Soc Lond B Bio* 277, 2895-2904. doi:10.1098/rspb.2010.0303
- Micheletta, J., Engelhardt, A., Matthews, L. et al. (2013). Multi-component and multimodal lipsmacking in crested macaques (*Macaca nigra*). *Amer J Primatol* 75, 763-773. doi:10.1002/ajp.22105
- Miller, A., Kitson, G., Skalkoyannis, B. and Leach, M. (2015). The effect of isoflurane and buprenorphine on the mouse grimace scale and behaviour in CBA and DBA/2 mice. *Appl Anim Behav Sci* 172, 58-62. doi:10.1016/j.applanim.2015.08.038
- Miller, A. L. and Leach, M. C. (2015a). Using the mouse grimace scale to assess pain associated with routine ear notching and the effect of analgesia in laboratory mice. *Lab Animal* 49, 117-120. doi:10.1177/0023677214559084
- Miller, A. L. and Leach, M. C. (2015b). The mouse grimace scale: A clinically useful tool? *PLoS One* 10, e0136000. doi:10.1371/journal.pone.0136000
- Mitchell, G., Herring, F., Tromborg, C. et al. (1992). Targets of aggressive facial displays by golden-bellied mangabeys (*Cercocebus galeritus chrysogaster*) at the Sacramento zoo. *Appl Anim Behav Sci* 33, 249-259. doi:10.1016/S0168-1591(05)80012-7
- Moehlman, P. D. (1998). Behavioral patterns and communication in feral asses (*Equus africanus*). *Appl Anim Behav Sci* 60, 125-169. doi:10.1016/S0168-1591(98)00162-2
- Mohiyeddini, C. and Semple, S. (2013). Displacement behaviour regulates the experience of stress in men. *Stress* 16, 163-171. doi:10.3109/10253890.2012.707709
- Molony, V., Kent, J. E. and McKendrick, I. J. (2002). Validation of a method for assessment of an acute pain in lambs. *Appl Anim Behav Sci* 76, 215-238. doi:10.1016/S0168-1591(02)00014-X
- Morgan, M. A., Schulkin, J. and Pfaff, D. W. (2004). Estrogens and non-reproductive behaviors related to activity and fear. *Neurosci Biobehav R* 28, 55-63. doi:10.1016/j.neubiorev.2003.11.017
- Mormède, P., Andanson, S., Auperin, B. et al. (2007). Exploration of the hypothalamic-pituitary-adrenal function as a tool to evaluate animal welfare. *Physiol Behav* 92, 317-339. doi:10.1016/j.physbeh.2006.12.003
- Nagasawa, M., Kawai, E., Mogi, K. and Kikusui, T. (2013). Dogs show left facial lateralization upon reunion with their owners. *Behav Process* 98, 112-116. doi:10.1016/j.beproc.2013.05.012
- Novak, M. A. and Meyer, J. S. (2009). Alopecia: Possible causes and treatments, particularly in captive nonhuman primates. *Comparative Med* 59, 18-26. <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2703143/>
- Nunez, C. M. V., Adelman, J. S. and Rubenstein, D. I. (2014). Sociality increases juvenile survival after a catastrophic event in the feral horse (*Equus caballus*). *Behav Ecol* 26, 138-147. doi:10.1093/beheco/aru163
- Oettinger, B. C., Crockett, C. M. and Bellanca, R. U. (2007). Communicative contexts of the LEN facial expression of pig-tailed macaques (*Macaca nemestrina*). *Primates* 48, 293-302. doi:10.1007/s10329-007-0046-1
- Oliver, V., De Rantere, D. D., Ritchie, R. et al. (2014). Psychometric assessment of the rat grimace scale and development of an analgesic intervention score. *PLoS One* 9, e97882. doi:10.1371/journal.pone.0097882
- Olsson, I. A. S. and Westlund, K. (2007). More than numbers matter: The effect of social factors on behaviour and welfare of laboratory rodents and non-human primates. *Appl Anim Behav Sci* 103, 229-254. doi:10.1016/j.applanim.2006.05.022
- Overall, K. (2013). *Manual of Clinical Behavioral Medicine for Dogs and Cats*. Saint Louis, US: Elsevier Health Sciences.
- Overmier, J. B. and Seligman, M. E. (1967). Effects of inescapable shock upon subsequent escape and avoidance responding. *J Comp Physiol Psych* 63, 28-33. doi:10.1037/h0024166
- Panksepp, J. (1998). *Affective Neuroscience: The Foundations of Human and Animal Emotions*. Oxford University Press.
- Panksepp, J. (2005). Affective consciousness: Core emotional feelings in animals and humans. *Conscious Cogn* 14, 30-80. doi:10.1016/j.concog.2004.10.004
- Panksepp, J., Fuchs, T. and Iacobucci, P. (2011). The basic neuroscience of emotional experiences in mammals: The case of subcortical FEAR circuitry and implications for clinical anxiety. *Appl Anim Behav Sci* 129, 1-17. doi:10.1016/j.applanim.2010.09.014
- Parr, L. A., Hopkins, W. D. and de Waal, F. (1998). The perception of facial expressions by chimpanzees, Pan troglodytes. *Evol Comm* 2, 1-23. doi:10.1075/eoc.2.1.02par
- Parr, L. A., Cohen, M. and Waal, F. (2005). Influence of social context on the use of blended and graded facial displays in chimpanzees. *Int J Primatol* 26, 73-103. doi:10.1007/s10764-005-0724-z
- Parr, L. A. and Waller, B. M. (2006). Understanding chimpanzee facial expression: Insights into the evolution of communication. *Soc Cogn Affect Neurosci* 1, 221-228. doi:10.1093/scan/nsi031
- Parr, L. A., Waller, B. M., Vick, S. J. and Bard, K. A. (2007). Classifying chimpanzee facial expressions using muscle action. *Emotion* 7, 172-181. doi:10.1037/1528-3542.7.1.172
- Parr, L. A., Waller, B. M., Burrows, A. M. et al. (2010). Brief communication: MaqFACS: A muscle-based facial movement coding system for the rhesus macaque. *Am J Phys Anthropol* 143, 625-630. doi:10.1002/ajpa.21401

- Partan, S. R. (2002). Single and multichannel signal composition: Facial expressions and vocalizations of rhesus macaques (*Macaca mulatta*). *Behaviour* 139, 993-1027. doi:10.1163/15685390260337877
- Peiman, K., Robinson, B. and Agrawal, A. (2010). Ecology and evolution of resource-related heterospecific aggression. *Q Rev Biol* 85, 133-158. doi:10.1086/652374
- Pelchat, M. L., Grill, H. J., Rozin, P. and Jacobs, J. (1983). Quality of acquired responses to tastes by *Rattus norvegicus* depends on type of associated discomfort. *J Comp Psychol* 92, 140-153. doi:10.1037/0735-7036.92.2.140
- Poole, T. B. (1978). An analysis of social play in polecats (*Mustelidae*) with comments on the form and evolutionary history of the open mouth play face. *Anim Behav* 26, 36-49. doi:10.1016/0003-3472(78)90006-4
- Poole, T. (1997). Happy animals make good science. *Lab Animal*, 31, 116-124. doi:10.1258/002367797780600198
- Porter, S. and ten Brinke, L. (2008). Reading between the lies: Identifying concealed and falsified emotions in universal facial expressions. *Psychol Sci* 19, 508-514. doi:10.1111/j.1467-9280.2008.02116.x
- Porter, S., ten Brinke, L. and Wallace, B. (2012). Secrets and lies: Involuntary leakage in deceptive facial expressions as a function of emotional intensity. *J Nonverbal Behav* 36, 23-37. doi:10.1007/s10919-011-0120-7
- Portfors, C. V. (2007). Types and functions of ultrasonic vocalizations in laboratory rats and mice. *J Am Assoc Lab Anim Sci* 46, 28-34.
- Preuschhof, S. and van Hooff, J. A. (1997). The social function of "smile" and "laughter": Variations across primate species and societies. In U. C. Segerstråle and P. Molnár (eds.), *Non-verbal Communication: Where Nature Meets Culture* (171-190). Hillside, NJ, USA: Lawrence Erlbaum Associates.
- Pritchett, L. C., Ulibarri, C., Roberts, M. C. et al. (2003). Identification of potential physiological and behavioral indicators of postoperative pain in horses after exploratory celiotomy for colic. *Appl Anim Behav Sci* 80, 31-43. doi:10.1016/S0168-1591(02)00205-8
- Prkachin, K. M. (2009). Assessing pain by facial expression: Facial expression as nexus. *Pain Res Manag* 14, 53-58. doi:10.1155/2009/542964
- Proctor, H. S. and Carder, G. (2014). Can ear postures reliably measure the positive emotional state of cows? *Appl Anim Behav Sci* 161, 20-27. doi:10.1016/j.applanim.2014.09.015
- Rasa, O. A. E. (1971). The causal factors and function of "yawning" in *Microspathodon chrysurus* (Pisces: Pomacentridae). *Behaviour* 39, 39-57. doi:10.1163/156853971X00168
- Redbo, I. (1998). Relations between oral stereotypies, open-field behavior, and pituitary-adrenal system in growing dairy cattle. *Physiol Behav* 64, 273-278. doi:10.1016/S0031-9384(98)00059-6
- Reefmann, N., Wechsler, B. and Gygas, L. (2009a). Behavioural and physiological assessment of positive and negative emotion in sheep. *Anim Behav* 78, 651-659. doi:10.1016/j.anbehav.2009.06.015
- Reefmann, N., Bütikofer Kaszás, F., Wechsler, B. and Gygas, L. (2009b). Ear and tail postures as indicators of emotional valence in sheep. *Appl Anim Behav Sci* 118, 199-207. doi:10.1016/j.applanim.2009.02.013
- Reefmann, N., Muehleemann, T., Wolf, M. et al. (2010). Simultaneous measurement of brain activity, physiology and behavior in large animals. In A. Spink, F. Grieco, O. Krips et al. (eds.), *Proceedings of Measuring Behavior* (38-40). Eindhoven, The Netherlands.
- Rennie, A. E. and Buchanan-Smith, H. M. (2006). Refinement of the use of non-human primates in scientific research. Part II: Housing, husbandry and acquisition. *Anim Welfare* 15, 215-238.
- Rivat, C., Laboureyras, E., Laulin, J. P. et al. (2007). Non-no-ciceptive environmental stress induces hyperalgesia, not analgesia, in pain and opioid-experienced rats. *Neuropsychopharmacology* 32, 2217-2228. doi:10.1038/sj.npp.1301340
- Robertshaw, D. (2006). Mechanisms for the control of respiratory evaporative heat loss in panting animals. *J Appl Physiol* 101, 664-668. doi:10.1152/jappphysiol.01380.2005
- Rogers, L. J. (2010). Relevance of brain and behavioural lateralization to animal welfare. *Appl Anim Behav Sci* 127, 1-11. doi:10.1016/j.applanim.2010.06.008
- Rogers, L. J. (2014). Asymmetry of brain and behavior in animals: Its development, function, and human relevance. *Genesis* 52, 555-571. doi:10.1002/dvg.22741
- Rooney, N. J., Bradshaw, J. W. S. and Robinson, I. H. (2001). Do dogs respond to play signals given by humans? *Anim Behav* 61, 715-722. doi:10.1006/anbe.2000.1661
- Ruch, W. (1995). Will the real relationship between facial expression and affective experience please stand up: The case of exhilaration. *Cognition Emotion* 9, 33-58. doi:10.1080/02699939508408964
- Russell, C. and Russell, W. (1985). Conflict activities in monkeys. *Soc Biol Hum Aff* 50, 26-48.
- Rutherford, K. M. D. (2002). Assessing pain in animals. *Anim Welfare* 23, 31-53.
- Sackeim, H. A., Gur, R. C. and Saucy, M. C. (1978). Emotions are expressed more intensely on the left side of the face. *Science* 202, 434-436. doi:10.1126/science.705335
- Sandem, A. I., Braastad, B. O. and Bøe, K. E. (2002). Eye white may indicate emotional state on a frustration-contentedness axis in dairy cows. *Appl Anim Behav Sci* 79, 1-10. doi:10.1016/S0168-1591(02)00029-1
- Sandem, A. I., Janczak, A. M. and Braastad, B. O. (2004). A short note on effects of exposure to a novel stimulus (umbrella) on behaviour and percentage of eye-white in cows. *Appl Anim Behav Sci* 89, 309-314. doi:10.1016/j.applanim.2004.06.011
- Sandem, A. I. and Braastad, B. O. (2005). Effects of cow-calf separation on visible eye white and behaviour in dairy cows – a brief report. *Appl Anim Behav Sci* 95, 233-239. doi:10.1016/j.applanim.2005.04.011
- Sandem, A. I., Janczak, A. M., Salte, R. and Braastad, B. O. (2006). The use of diazepam as a pharmacological validation of eye white as an indicator of emotional state in dairy cows. *Appl Anim Behav Sci* 96, 177-183. doi:10.1016/j.applanim.2005.06.008



- Sauer, E. G. F. and Sauer, E. M. (1967). Yawning and other maintenance activities in the South African ostrich. *Auk* 84, 571-587. doi:10.2307/4083337
- Schilder, M. B. H., van Hooff, J. A. R. A. M., van Geer-Plesman, C. J. and Wensing, J. B. (1984). A quantitative analysis of facial expression in the plains zebra. *Z Tierpsychol* 66, 11-32. doi:10.1111/j.1439-0310.1984.tb01352.x
- Schmied, C., Waiblinger, S., Scharl, T. et al. (2008). Stroking of different body regions by a human: Effects on behaviour and heart rate of dairy cows. *Appl Anim Behav Sci* 109, 25-38. doi:10.1016/j.applanim.2007.01.013
- Schwartzkopf-Genswein, K. S., Faucitano, L., Dadgar, S. et al. (2012). Road transport of cattle, swine and poultry in North America and its impact on animal welfare, carcass and meat quality: A review. *Meat Sci* 92, 227-243. doi:10.1016/j.meatsci.2012.04.010
- Sefcek, J. A. and King, J. E. (2007). Chimpanzee facial symmetry: A biometric measure of chimpanzee health. *Amer J Primatol* 69, 1257-1263. doi:10.1002/ajp.20426
- Seligman, M. E. and Maier, S. F. (1967). Failure to escape traumatic shock. *J Exp Psychol* 74, 1-9. doi:10.1037/h0024514
- Shariff, A. F. and Tracy, J. L. (2011). What are emotion expressions for? *Curr Dir Psychol Sci* 20, 395-399. doi:10.1177/0963721411424739
- Shyan, M. R., Fortune, K. A. and King, C. (2003). "Bark parks" – a study on interdog aggression in a limited-control environment. *J Appl Anim Welf Sci* 6, 25-32. doi:10.1207/S15327604JAWS0601_02
- Silk, J. B., Beehner, J. C., Bergman, T. J. et al. (2009). The benefits of social capital: Close social bonds among female baboons enhance offspring survival. *P Roy Soc Lond B Bio* 276, 3099-3104. doi:10.1098/rspb.2009.0681
- Silk, J. B., Beehner, J. C., Bergman, T. J. et al. (2010). Strong and consistent social bonds enhance the longevity of female baboons. *Curr Biol* 20, 1359-1361. doi:10.1016/j.cub.2010.05.067
- Slocombe, K. E., Waller, B. M. and Liebal, K. (2011). The language void: The need for multi-modality in primate communication research. *Anim Behav* 81, 919-924. doi:10.1016/j.anbehav.2011.02.002
- Sneddon, L. U., Elwood, R. W., Adamo, S. A. and Leach, M. C. (2014). Defining and assessing animal pain. *Anim Behav* 97, 201-212. doi:10.1016/j.anbehav.2014.09.007
- Sorge, R. E., Martin, L. J., Isbester, K. A. et al. (2014). Olfactory exposure to males, including men, causes stress and related analgesia in rodents. *Nat Methods* 11, 629-632. doi:10.1038/nmeth.2935
- Sotocinal, S. G., Sorge, R. E., Zaloum, A. et al. (2011). The rat grimace scale: A partially automated method for quantifying pain in the laboratory rat via facial expressions. *Mol Pain* 7, 55. doi:10.1186/1744-8069-7-55
- Spencer, R. F. and Porter, J. D. (2006). Biological organization of the extraocular muscles. *Prog Brain Res* 151, 43-79. doi:10.1016/S0079-6123(05)51002-1
- Spinka, M., Newberry, R. C. and Beckoff, M. (2001). Mammalian play: Training for the unexpected. *Q Rev Biol* 76, 141-168. doi:10.1086/393866
- Spotila, J. R., Terpin, K. M. and Dodson, P. (1977). Mouth gaping as an effective thermoregulatory device in alligators. *Nature* 265, 235-236. doi:10.1038/265235a0
- Stahlbaum, C. C. and Houpt, K. A. (1989). The role of the flehmen response in the behavioral repertoire of the stallion. *Physiol Behav* 45, 1207-1214. doi:10.1016/0031-9384(89)90111-X
- Steiner, J. E., Glaser, D., Hawilo, M. E. and Berridge, K. C. (2001). Comparative expression of hedonic impact: Affective reactions to taste by human infants and other primates. *Neurosci Biobehav R* 25, 53-74. doi:10.1016/S0149-7634(00)00051-8
- Stokes, E. L., Flecknell, P. A. and Richardson, C. A. (2009). Reported analgesic and anaesthetic administration to rodents undergoing experimental surgical procedures. *Lab Animal*, 43, 149-154. doi:10.1258/la.2008.008020
- Susskind, J. M. and Anderson, A. K. (2008). Facial expression form and function. *Commun Integr Biol* 1, 148-149. doi:10.4161/cib.1.2.6999
- Sussman, R. W., Garber, P. A. and Cheverud, J. M. (2005). Importance of cooperation and affiliation in the evolution of primate sociality. *Am J Phys Anthropol* 128, 84-97. doi:10.1002/ajpa.20196
- Tamashiro, K. L. K., Nguyen, M. M. N. and Sakai, R. R. (2005). Social stress: From rodents to primates. *Front Neuroendocrinol* 26, 27-40. doi:10.1016/j.yfrne.2005.03.001
- Tan, H. M., Ong, S. M. and Langat, G. (2013). The influence of enclosure design on diurnal activity and stereotypic behaviour in captive Malayan Sun bears (*Helarctos malayanus*). *Res Vet Sci* 94, 228-239. doi:10.1016/j.rvsc.2012.09.024
- ten Brinke, L., Porter, S. and Baker, A. (2012). Darwin the detective: Observable facial muscle contractions reveal emotional high-stakes lies. *Evol Hum Behav* 33, 411-416. doi:10.1016/j.evolhumbehav.2011.12.003
- Thornton, P. and Waterman-Pearson, A. (2002). Behavioural responses to castration in lambs. *Anim Welfare* 11, 203-212.
- van Hooff, J. A. R. A. M. (1972). A comparative approach to the phylogeny of laughter and smiling. In R. Hinde (ed.), *Non-Verbal Communication* (209-241). Cambridge University Press.
- Vick, S. J., Waller, B. M., Parr, L. A. et al. (2007). A cross-species comparison of facial morphology and movement in humans and chimpanzees using the facial action coding system (FACS). *J Nonverbal Behav* 31, 1-20. doi:10.1007/s10919-006-0017-z
- Vick, S. J. and Paukner, A. (2010). Variation and context of yawns in captive chimpanzees (*Pan troglodytes*). *Amer J Primatol* 72, 262-269. doi:10.1002/ajp.20781
- von Borell, E., Dobson, H. and Prunier, A. (2007). Stress, behaviour and reproductive performance in female cattle and pigs. *Horm Behav* 52, 130-138. doi:10.1016/j.yhbeh.2007.03.014
- von Borstel, U. U., Duncan, I. J. H., Shoveller, A. K. et al. (2009). Impact of riding in a coercively obtained Rollkur posture on welfare and fear of performance horses. *Appl Anim Behav Sci* 116, 228-236. doi:10.1016/j.applanim.2008.10.001
- Vrana, S. R. (1993). The psychophysiology of disgust: Differ-

- entiating negative emotional contexts with facial EMG. *Psychophysiology* 30, 279-286. doi:10.1111/j.1469-8986.1993.tb03354.x
- Vuilleumier, P., Armony, J. L., Driver, J. and Dolan, R. J. (2001). Effects of attention and emotion on face processing in the human brain: An event-related fMRI study. *Neuron* 30, 829-841. doi:10.1016/S0896-6273(01)00328-2
- Wallace, V. C. J., Norbury, T. A. and Rice, A. S. C. (2005). Ultrasound vocalisation by rodents does not correlate with behavioural measures of persistent pain. *Eur J Pain* 9, 445-452. doi:10.1016/j.ejpain.2004.10.006
- Waller, B. M. and Dunbar, R. I. M. (2005). Differential behavioural effects of silent bared teeth display and relaxed open mouth display in chimpanzees (*Pan troglodytes*). *Ethology* 111, 129-142. doi:10.1111/j.1439-0310.2004.01045.x
- Waller, B. M., Cray, J. J. and Burrows, A. M. (2008a). Selection for universal facial emotion. *Emotion* 8, 435-439. doi:10.1037/1528-3542.8.3.435
- Waller, B. M., Parr, L. A., Gothard, K. M. et al. (2008b). Mapping the contribution of single muscles to facial movements in the rhesus macaque. *Physiol Behav* 95, 93-100. doi:10.1016/j.physbeh.2008.05.002
- Waller, B. M., Lembeck, M., Kuchenbuch, P. et al. (2012). GibbonFACS: A muscle-based facial movement coding system for Hylobatids. *Int J Primatol* 33, 809-821. doi:10.1007/s10764-012-9611-6
- Waller, B. M. and Micheletta, J. (2013). Facial expression in nonhuman animals. *Emot Rev* 5, 54-59. doi:10.1177/1754073912451503
- Waller, B. M., Peirce, K., Caeiro, C. C. et al. (2013). Paedomorphic facial expressions give dogs a selective advantage. *PLoS One* 8, e82686. doi:10.1371/journal.pone.0082686
- Waller, B. M., Misch, A., Whitehouse, J. and Herrmann, E. (2014). Children, but not chimpanzees, have facial correlates of determination. *Biol Lett* 10, 20130974. doi:10.1098/rsbl.2013.0974
- Wallez, C. and Vauclair, J. (2012). First evidence of population-level oro-facial asymmetries during the production of distress calls by macaque (*Macaca mulatta*) and baboon (*Papio anubis*) infants. *Behav Brain Res* 234, 69-75. doi:10.1016/j.bbr.2012.06.004
- Waring, G. H. (2003). *Horse Behavior*. Noyes Publications/William Andrew Publishing.
- Wathan, J. and McComb, K. (2014). The eyes and ears are visual indicators of attention in domestic horses. *Curr Biol* 24, R677-679. doi:10.1016/j.cub.2014.06.023
- Wathan, J., Burrows, A. M., Waller, B. M. and McComb, K. (2015). EquiFACS: The equine facial action coding system. *PLoS One* 10, e0131738. doi:10.1371/journal.pone.0131738
- Weary, D. M. and Fraser, D. (1995). Signalling need: Costly signals and animal welfare assessment. *Appl Anim Behav Sci* 44, 159-169. doi:10.1016/0168-1591(95)00611-U
- Weary, D. M., Niel, L., Flower, F. C. and Fraser, D. (2006). Identifying and preventing pain in animals. *Appl Anim Behav Sci* 100, 64-76. doi:10.1016/j.applanim.2006.04.013
- Weeks, J. W., Crowell-Davis, S. L. and Heusner, G. (2002). Preliminary study of the development of the flehmen response in *Equus caballus*. *Appl Anim Behav Sci* 78, 329-335. doi:10.1016/S0168-1591(02)00110-7
- Wells, R. T. (1978). Thermoregulation and activity rhythms in the hairy-nosed wombat, *Lasiorhinus latifrons* (Owen) (Vombatidae). *Aust J Zool* 26, 639-651. doi:10.1071/ZO9780639
- Whalen, P. J., Kagan, J., Cook, R. G. et al. (2004). Human amygdala responsivity to masked fearful eye whites. *Science* 306, 2061. doi:10.1126/science.1103617
- Williams, A. C. de C. (2002). Facial expression of pain: An evolutionary account. *Behav Brain Sci* 25, 439-488. doi:10.1017/s0140525x02000080
- Würbel, H. (2001). Ideal homes? Housing effects on rodent brain and behaviour. *Trends Neurosci* 24, 207-211. doi:10.1016/S0166-2236(00)01718-5
- Yanagi, A. and Berman, C. M. (2014). Body signals during social play in free-ranging rhesus macaques (*Macaca mulatta*): A systematic analysis. *Amer J Primatol* 76, 168-179. doi:10.1002/ajp.22219
- Young, G. and Décarie, T. G. (1977). An ethology-based catalogue of facial/vocal behaviour in infancy. *Anim Behav* 25, 95-107. doi:10.1016/0003-3472(77)90071-9

Conflict of interest

No author has any conflict of interest to report in relation to this research.

Acknowledgements

The authors would like to thank the technical and care staff who assisted in the conduct of these studies. This research was funded by the NC3Rs (NC/K001159/1).

Correspondence to

Kris Descovich, PhD
Environmental and Animals Sciences
Unitec Institute of Technology
Private Bag 92025, Victoria Street West, Auckland 1142
New Zealand
Phone: +64 9 815 4321 (ext 7321)
e-mail: kdescovich@unitec.ac.nz