Review

Defining and assessing animal pain

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The detection and assessment of pain in animals is crucial to improving their welfare in a variety of contexts in which humans are ethically or legally bound to do so. Thus clear standards to judge whether pain is likely to occur in any animal species is vital to inform whether to alleviate pain or to drive the refinement of procedures to reduce invasiveness, thereby minimizing pain. We define two key concepts that can be used to evaluate the potential for pain in both invertebrate and vertebrate taxa. First, responses to noxious, potentially painful events should affect neurobiology, physiology and behaviour in a different manner to innocuous stimuli and subsequent behaviour should be modified including avoidance learning and protective responses. Second, animals should show a change in motivational state after experiencing a painful event such that future behavioural decision making is altered and can be measured as a change in conditioned place preference, self-administration of analgesia, paying a cost to access analgesia or avoidance of painful stimuli and reduced performance in concurrent events. The extent to which vertebrate and selected invertebrate groups fulfil these criteria is discussed in light of the empirical evidence and where there are gaps in our knowledge we propose future studies are vital to improve our assessment of pain. This review highlights arguments regarding animal pain and defines criteria that demonstrate, beyond a reasonable doubt, whether animals of a given species experience pain.

Bateson's (1991) seminal review on the assessment of pain has been influential in inspiring numerous researchers investigating pain in animals. Bateson set out a clear framework upon which hypothesis-driven research questions could be derived regarding the capacity for pain in any species. Indeed, the criteria suggested have been applied to numerous species, particularly non-mammalian vertebrates (e.g. fish, Sneddon, 2011) and more recently invertebrates (e.g. crustaceans, Barr, Laming, Dick, & Elwood, 2008). Well-defined criteria were proposed and it was suggested that animals that fulfilled all criteria should be considered capable of pain. These criteria were: possession of nociceptors, receptors that detect damaging stimuli on or in the body; pathways from nociceptors to the brain; brain structures analogous to the human cerebral cortex that process pain; opioid receptors and endogenous opioid substances in a nociceptive neural system; a reduction in adverse behavioural and physiological effects after administration of analgesics or painkillers; learning to avoid potentially painful stimuli and that this learning is rapid and inelastic. Sneddon (2004) added that animals should suspend normal behaviour for a prolonged period rather than show a reflex response, with adverse changes in behaviour reflective of signs of 'discomfort' as shown by long-term motivational change. These robust scientific approaches can provide evidence strongly suggesting that an animal is capable of experiencing pain and we can then seek to reduce or ameliorate that condition by reducing the invasiveness of any procedures to which we subject animals or, when this is unavoidable, providing pain relief. However, Bateson's review has been recently criticized as being outdated (Rose et al., 2014). Given the advances made in the scientific study of pain, the technologies now at our disposal and more recent evidence from a wider variety of taxonomic groups, this review provides a timely update on the definition, assessment and importance of animal pain.

PAIN: A COMPLEX ISSUE

Nociception, the capacity to respond to potentially damaging stimuli, is a basic sensory ability (Purves et al., 2012), and even

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occurs in bacteria (Berg, 1975). Testing whether animals are able to respond to noxious stimuli is typically straightforward, even though many nociceptors are multifunctional (Tsagareli, 2011). Philosophers and scientists, however, make a distinction between pain and nociception (Allen, 2011) because pain is primarily a subjective experience of anguish, despair and other negative affective states (e.g. see Allen, Fuchs, Shriver, & Wilson, 2005). The difficulty in demonstrating whether animals feel pain, as opposed to just nociception, lies in our ability to recognize negative internal mental states in other species (Broom, 1998).

Animals have both physiological and behavioural responses to nociception that parallel those that accompany the experience of pain in humans and this is the basis for the argument by analogy (Allen et al., 2005; Sherwin, 2001). However, there are weaknesses to this concept. Clearly animal pain behaviour differs from human pain behaviour, as does the underlying neuroanatomy. When are these differences important (i.e. rendering the argument by analogy invalid) and when are they inconsequential? Understanding the biology of a given species may be helpful here. Some suggest animals may behave as though they are in pain, but this behaviour may reflect nociception without suffering (e.g. Allen, 2004). Thus, analogous behavioural and physiological responses need not imply identical mechanisms. Allen et al. (2005) reviewed the evidence for pain in rodents and compared it with data from humans, concluding that the evidence is not conclusive. However, Shriver (2006) reviewed similar evidence and concluded that it was 'beyond a reasonable doubt' that most mammals feel pain. We review here data that has led to a consensus that it is beyond a reasonable doubt that pain can be experienced in animals (Allen, 2011). This review presents a combination of behavioural, physiological and evolutionary evidence and arguments, which taken together demonstrate, beyond a reasonable doubt, that animals from different phyla experience pain.

The opposition to the idea that animals experience pain has sparked fierce debates over the capacity of nonprimate animals for pain (e.g. Bermond, 1997, 2001; Rose, 2002; Rose et al., 2014). However, although it cannot be proven that animals experience pain, it also cannot be proven that they do not. We propose that if animals fulfil our criteria below then they should be considered capable, beyond a reasonable doubt, of experiencing pain with implications for their health and welfare.

FUNCTION OF PAIN

Nociception is a fundamental sensory system that alerts an animal or human to potential damage. Nociceptive pathways connect with brain areas important for motivation, and animals are motivated to avoid the injurious stimulus and protect themselves from further damage (Bateson, 1991). Therefore, it would be adaptive to evolve such a system and many diverse taxa possess specific receptors, i.e. nociceptors that detect damaging stimuli, for example *Drosophila melanogaster* and *Caenorhabditis elegans* (Im & Galko, 2012; Neely et al., 2010; Wittenburg & Baumeister, 1999). However, different species are likely to show specific differences in how these nociceptors operate.

Evolutionary heritage and life history place very different pressures on animal groups and they are exposed to different types of nociceptive stimuli (e.g. high mechanical pressure, extremes of temperature, noxious chemicals). Therefore, animals will have evolved their nociceptive and possible pain systems to meet the demands of their environment (Broom, 2001; Rutherford, 2002).

The advantage of nociception seems clear. However, some animals also have an associated aversive motivational state similar to many of the aspects of pain in humans. It is the existence of this aversive motivational state that leads us to propose that, beyond a reasonable doubt, at least some animals experience pain. We should consider the function of this aversive motivational state because it might guide us in establishing how pain might be better defined and shown to be likely in particular taxa. The key function appears to be that the aversive experience of pain creates a strong and lasting motivation that enables the animal to avoid getting into a similar situation in the future. That is it increases fitness by assisting long-term protection from further damage (Bateson, 1991; Elwood, 2011; Sneddon, 2004). Thus, while nociception typically allows for an immediate reduction of tissue damage, pain typically allows for longer-term protection. Unfortunately this single criterion, on its own, does not prove that an animal experiences pain. Nociception can also have long-lasting effects without invoking higher-order neural processes (e.g. long-term nociceptive sensitization, Chase, 2002; Smith & Lewin, 2009). Therefore, such long-term behavioural changes, although consistent with the concept of pain, require further evidence as we discuss below.

DEFINITION OF ANIMAL PAIN

Because it is impossible to know how other humans feel when they are in pain, we rely upon their ability to communicate their experience of pain. This illustrates how difficult it is to measure pain in humans that cannot speak (e.g. neonates) or animals that do not share our language. Therefore, the commonly used definition of human pain cannot be directly applied to animals because it relies on either knowing how animals feel or requiring them to be able to communicate their subjective experiences to us. The International Association for the Study of Pain defined human pain as 'An unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage' (IASP, 1979, p. 249). However, the IASP (1979, p. 249) also refers to adults unable to communicate, neonates and infants and adds that 'The inability to communicate verbally does not negate the possibility that an individual is experiencing pain' and so we believe this can be applied to animals.

It is vital that an animal-based definition of pain allows rigorous scientific investigation of disparate species and also allows us to detect, assess and alleviate pain in animals where possible. The most commonly used definition for animals is 'an aversive sensory experience caused by actual or potential injury that elicits protective and vegetative reactions, results in learned behaviour, and may modify species specific behaviour' (Zimmerman, 1986, p. 2). Sneddon (2009, p. 338) refined this definition suggesting that animals in pain should 'quickly learn to avoid the noxious stimulus and demonstrate sustained changes in behaviour that have a protective function to reduce further injury and pain, prevent the injury from recurring, and promote healing and recovery.' We use these definitions as the foundation for our criteria by which possible pain experience might be judged.

Pain provides strong motivation for animals to learn to avoid damaging stimuli within a few trials (Carlsson et al., 2006). The aversive experience associated with pain is probably an important driver in ensuring that animals survive in a dangerous habitat avoiding injury that may otherwise lead to ill health and mortality. Instead of considering pain to be a special property of humans, it is likely that pain, and its associated motivational state, has an adaptive survival function for animals. We believe that the aversive affective component of pain, therefore, is integral to its evolutionary function (Dawkins, 1980, 2012), otherwise animals would frequently damage themselves in the same manner and be incapable of altering their behavioural decisions to learn to avoid injury. A negative internal state can produce robust and repeatable changes in behaviour induced by damaging stimuli in animals. However, other mechanisms might also produce some similar

effects (e.g. nociceptor sensitization, Smith & Lewin, 2009). Further, animal pain may not be identical to the internal subjective experience that humans have but it does have the same protective function (Rutherford, 2002).

Clever experimentation can yield insights into the animal's experience (e.g. self-administration of analgesia, Danbury et al., 1997; selective attention, Ashley, Ringrose, Edwards, McCrohan, & Sneddon, 2009; Sneddon, Braithwaite, & Gentle, 2003a; paying a cost to access analgesia). Examples of potentially painful events leading to motivational changes suggest the potential for a negative affective state associated with injury. Here we list criteria that animals can be tested against to determine their potential capacity for pain. Determining whether a specific species experiences pain will typically require species-specific behavioural and physiological tests. These are based upon the mechanisms to detect, react and respond to pain and have two key sets of evidence (Table 1): (1) whole animal responses to noxious stimuli such as physiological change and effects of analgesics and local anaesthetics which differ from those to innocuous stimuli and (2) evidence of long-term motivational change that might include rapid learning. These criteria must be considered as a whole and not as indicators in isolation (Table 1). For many species specific data are lacking and for the future of the field it is imperative scientists continue to test the evidence for pain experience in animals (Table 2). Further, we accept that the distinction between these two sets may mean responses can be considered to belong to both criteria. Before we review this evidence, however, we examine another criterion suggested by Bateson (1991), i.e. that an animal requires the neural apparatus to detect, possess and respond to tissue damage for it to feel pain. That is the animal must have an effective nociceptor system to enable a neural input allowing perception of tissue damage. However, having that system does not mean that pain will follow.

THE NEURAL APPARATUS

Nociceptors (A and C fibres) are found in most groups of vertebrates, including mammals (Carstens & Moberg, 2000; Weary, Neil, Flower, & Fraser, 2006), birds (Breward & Gentle, 1985; Gentle, Bradbury, & Wilson, 2003; Gentle & Tilston, 2000; Gentle, Tilston, & McKeegan, 2001; Hothersall et al., 2011; McKeegan, 2004; McKeegan, Demmers, Wathes, Jones, & Gentle, 2002), reptiles (Liang & Terashima, 1993; Terashima & Liang, 1994), amphibians (review in Guénette, Giroux, & Vachon, 2013) and fish (e.g. Roques, Abbink, Geurds, van de Vis, & Flik, 2010; Sneddon, 2002). However, the proportion of A and C fibres may differ between groups. In mammals these fibres link to CNS structures and pathways (at least at the subcortical level; Carstens & Moberg, 2000; Weary et al., 2006), and so are capable of the sensory (i.e. nociceptive) component of pain. For example, Hess, Sergejeva, Budinsky, Zeilhofer, and Brune (2007) demonstrated, using functional magnetic resonance imaging (fMRI), that nociceptive activation with an inflamed paw in rats, *Rattus norvegicus*, induced activation of the primary somatosensory areas (areas in humans associated with affective experience): the insula, anterior cingulate cortex and medial thalamus. Avian nociceptive afferents also project to the brainstem and ascend to the primary presumed pain centres in the forebrain (Dubbeldam, 2009). A key difference between mammals and birds, however, is a substantial divergence in the sequence of the vanilloid receptor 1 (VR1) that binds capsaicin (ca. 68%; Jordt & Julius, 2002). Whereas mammals find the burning sensation noxious and avoid eating chilli peppers, capsaicin does not activate the avian receptor so birds can ingest these and act as an aid to dispersal of the seeds. This is a convincing example of how evolution shapes nociceptors.

In amphibians the ascending tracts reach the brainstem and the thalamus and project to the cortex (Kicliter & Ebbesson, 1976; Vesselkin, Agayan, & Nomokovona, 1971). Within the teleost brain there are various connections to the thalamus and cortical areas (Rink & Wullimann, 2004). Furthermore, teleost forebrain and midbrain areas are active during potentially painful stimulation and this differs from that shown during innocuous treatments (e.g. gene expression in common carp, *Cyprinus carpio*, and rainbow trout, *Oncorhynchus mykiss*, Reilly, Quinn, Cossins, & Sneddon, 2008b; electrical activity in Atlantic salmon, *Salmo salar*, Nordgreen, Horsberg, Ranheim, & Chen, 2007; goldfish, *Carassius auratus*, and rainbow trout, Dunlop & Laming, 2005; activity using fMRI in common carp, Sneddon, 2013); thus activity is not restricted to merely the hindbrain and spinal cord nociceptive reflex centres (Rose, 2002). Further, nociceptors in teleost fish are strikingly similar to mammalian nociceptors (Ashley, Sneddon, & McCrohan, 2006; Ashley, Sneddon, & McCrohan, 2007; Mettam et al., 2012; Sneddon, 2003a, 2004, 2011, 2013). However, rainbow trout nociceptors are not responsive to cold temperatures below 4 °C (Ashley et al., 2007). This is intuitive since these fish may frequently encounter such low temperatures and it would not be adaptive to perceive them as noxious. In elasmobranchs unmyelinated C fibres are lacking but small myelinated fibres are in abundance and could be A-delta fibres (Cameron, Plenderleith, & Snow, 1990; Kitchener, Fuller, & Snow, 2010; Snow, Plenderleith, & Wright, 1993). However, electrophysiological studies are needed to determine whether nociceptors occur in this group.

Table 1
The key principles and criteria for pain in animals

Key principles	Criteria
1. Whole animal responses that differ from innocuous stimulation	Evidence of central processing of nociception involving brain areas that regulate motivated behaviour (including learning and fear)
	Nociceptive processing sensitive to endogenous modulators (e.g. opioids in vertebrates)
	Nociception activates physiological responses (one or a combination of the following: change in respiration, heart rate or hormonal levels (e.g. cortisol in some vertebrates)
	Evidence that responses are not just a nociceptive reflex (i.e. Not simply moving away)
	Alterations in behaviour over longer term that reduce encounters with the stimulus
	Protective behaviour such as wound guarding, limping, rubbing or licking
	All of the above reduced by analgesia or local anaesthetics
2. Change in motivation	Self-administration of analgesia
	Pay a cost to access analgesia
	Selective attention whereby the response to the noxious stimulus has high priority over other stimuli; the animal does not respond appropriately to concurrent events (e.g. presentation of predator; reduced performance in learning and memory tasks)
	Altered behaviour after noxious stimulation where changes can be observed in conditioned place avoidance and avoidance learning paradigms
	Relief learning
	Long-lasting change in a suite of responses especially those relating to avoidance of repeat noxious stimulation
	Avoidance of the noxious stimulus modified by other motivational requirements as in trade-offs
	Evidence of paying a cost to avoid the noxious stimulus

Table 2
Criteria for pain perception

Vertebrates	Mammalia	Aves	Amphibia/Reptilia	Agnatha/Osteichthyes	Cephalopoda	Decapods	Insecta
Nociceptors	√	√	√	√	√	√	√
Pathways to CNS	√	√	√	√	√	√	√
Central processing in brain	√	√	√	√	√	√	√
Receptors for analgesic drugs	√	√	√	√	√	√	?[a]
Physiological responses	√	√	√	√	√	√	?
Movement away from noxious stimuli	√	√	√	√	√	√	√
Behavioural changes from norm	√	√	√	√	√	√	√
Protective behaviour	√	√	√	√	√	√	No
Responses reduced by analgesic drugs	√	√	√	√	√	√	√[a]
Self-administration of analgesia	√	√	?	√	?	?	?
Responses with high priority over other stimuli	√	√/?[b]	?	√	√	√	No
Pay cost to access analgesia	√	?	?	√	?	?	?
Altered behavioural choices/preferences	√	√	?	√	√	√	√
Relief learning	√	?	?	?	?	?	√
Rubbing, limping or guarding	√	√	?	√	√	√	?
Paying a cost to avoid stimulus	√	√	?	√	?	√	?
Trade-offs with other requirements	√	√	?	√	?	√	?

(√) in selected taxa indicates that at least one species within an animal class fulfils the criterion, while (?) denotes that more evidence is needed or that it is inconclusive. The more criteria an individual species satisfies, the more likely it is that the species experiences pain as defined in this review.

[a] Pharmacological evidence that opioids work as analgesics in cockroaches, *Periplaneta americana* (Gritsai, Dubynin, Pilipenko, & Petrov, 2004).

[b] Some studies show pain is imperative whereas others demonstrate reduced pain behaviour when birds are starved or placed in novel circumstances.

These studies demonstrate that most vertebrates not only have nociceptors but also that they link to the brain so they at least have the capacity for some sort of 'central experience' of the noxious stimulus and this is essential for pain to be considered as a possibility. The situation in arthropods and molluscs, however, is not so clear cut. Certainly, as previously noted, they have nociceptors that allow for perceptual input (reviewed by Crook, Lewis, Roger, Hanlon, & Walters, 2011; Dyuizen, Kotsyuba, & Lamash, 2012; Elwood, 2011). Indeed, much is known about the functioning of nociceptors from the elegant work employing specific mutants with specific nociceptor variants (Tobin & Bargmann, 2004) but our knowledge of the brains of these animals is not so established as that for vertebrates. Nevertheless, long-term changes in central nervous activity have been noted in shore crabs, *Hemigrapsus sanguineus*, following a noxious stimulus (Dyuizen et al., 2012) and thus information from nociceptors must be conveyed to central areas. Further, there are sustained increases in nociceptor firing following tissue damage in cephalopods, coupled with long-term alteration of motivational state (Crook, Hanlon, & Walters, 2013). Thus there is the potential for central processing of information about noxious stimuli in some invertebrates. Here we examine evidence that might indicate that at least some animals fulfil our criteria for pain, starting with the five main groups within the Phylum Chordata, then the Mollusca and finally the Arthropoda.

MAMMALS

Whole Animal Response

Stimuli that are considered painful in humans have been shown to induce similar physiological and behavioural changes in other nonhuman mammals. The majority of physiological changes associated with potentially painful stimuli are mediated by the sympathetic nervous system and hypothalamic–pituitary–adrenal axis (HPA). The sympathetic responses can be determined either directly by measuring the circulating catecholamines, adrenaline and noradrenaline (e.g. Mellor et al., 2002; Raekallio, Taylor, & Bloomfield, 1997), or the resulting autonomic changes such as heart rate (e.g. Arras, Rettich, Cinelli, Kasermann, & Burki, 2007; Peers, Mellor, Wintour, & Dodic, 2002), blood pressure (Keating, Thomas, Flecknell, & Leach, 2012; Peers et al., 2002), body temperature (e.g. Hellebrekers, Kemme, & vanWandelen, 1994),

respiratory rate (e.g. Hellebrekers et al., 1994) and body weight change (e.g. Liles, Flecknell, Roughan, & Cruz-Madoran, 1998). HPA changes in response to painful stimuli are most commonly assessed by measuring production of glucocorticoids such as in rodents (e.g. *R. norvegicus*, Goldkuhl, Hau, & Abelson, 2010; Kalliokoski et al., 2010), horses, *Equus ferus caballus* (e.g. Pritchett, Ulibarri, Roberts, Schneider, & Sellon, 2003), sheep, *Ovis aries* (e.g. Kent, Molony, & Robertson, 1993), and cattle, *Bos primigenius* (e.g. Robertson, Kent, & Molony, 1994). These physiological changes are universally considered to reflect negative states that are inevitably associated with pain, for example, fear (Sapolsky, Romero, & Munck, 2000).

Behaviour represents the most commonly used index of animal pain and can be categorized by its level of complexity. At the simplest level it is a single reflex response to noxious stimuli (nociception) that does not require higher processing (i.e. experience). However, as the complexity of a behavioural response increases the likelihood of it requiring higher processing also increases. Painful stimuli cause changes in general behaviour, pain-specific behaviours and facial expressions that occur beyond acute application of the noxious stimulus. However, these responses have been argued to represent 'complex' reflex responses (e.g. Rose et al., 2014), and they can be mimicked by robots (e.g. Breazeal, 2011; Lee-Johnson & Carnegie, 2010). However, when potentially painful stimuli alter decisions and choices made by the animal (e.g. preference for pain relief, reaction to other nonpain-related stimuli etc.) then they are demonstrating a level of behavioural complexity that is likely to require some negative internal experience (i.e. pain).

Measuring both changes in general behaviour and the development of abnormal behaviour are often used to assess pain, including demeanour (e.g. Stanway, Taylor, & Brodbelt, 1996), reaction to handling (e.g. Thornton & Waterman-Pearson, 1999), posture (e.g. Slingsby & Waterman-Pearson, 1998), activity (Roughan & Flecknell, 2000), vocalization (e.g. Hellebrekers et al., 1994), food and water intake (e.g. Leach et al., 2009), gait (Sprecher, Hostetler, & Kanneene, 1997), rearing (Matson et al., 2007), etc. As Weary et al. (2006) proposed, often the most effective behavioural indicators of pain are those that animals are highly motivated to perform; for example, rodents are highly motivated to rear up in their cages, but this declines significantly after abdominal surgery (Roughan & Flecknell, 2001). Reviews by Carstens and Moberg (2000), Rutherford (2002) and Weary et al. (2006) provide a comprehensive overview of behavioural-based indicators

and their validation in mammals. As a consequence, pain-specific behavioural indices have been identified and constructed into assessment schemes in a range of species, including rodents (e.g. Roughan & Flecknell, 2001, 2003; Wright-Williams, Courade, Richardson, Roughan, & Flecknell, 2007), rabbits, *Lepus curpaeums* (e.g. Leach et al., 2009), lambs, *O. aries* (e.g. Molony & Kent, 1997), cattle (Faulkner & Weary, 2000; Molony, Kent, & Robertson, 1995), pigs, *Sus scrofa domesticus* (Leslie, Hernández-Jover, Newman, & Holyoake, 2010; Taylor & Weary, 2000), and horses (Ashley, Waterman-Pearson, & Whay, 2005).

Facial expressions are routinely used to assess pain in humans, particularly in those who are unable to communicate (Williams, 2002), and are considered to offer an effective method with a limited range of indicators that can be used rapidly and easily with minimal training. Facial expressions are scored using a facial action coding scheme (FACS) that measures the individual movements or 'action units' of the face that comprise an expression (e.g. Ekman & Friesen, 1978). Recently, similar schemes ('grimace scales') have been developed for a limited number of mammalian species including rodents (*Mus musculus*, Langford, Bailey, Chanda, Clarke, & Drummond, 2010; Leach et al., 2012, *R. norvegicus*, Sotocinal et al., 2011), rabbits (Keating et al., 2012) and horses (e.g. Costa et al., 2014). Each grimace scale comprises a number of anatomically based 'action units' (e.g. changes in the shape of the eyes, nose, cheeks, mouth and ears).

The exhibition of both behavioural and facial indicators has been shown to change from before to after a painful event, and these changes can be reduced by the administration of routinely used pain-relieving drugs or simply by time (i.e. recovery). Although many authors believe that such complex responses that are observed alongside other potential indices of pain demonstrate higher processing (i.e. experience), we currently have limited objective evidence that the behaviour reflects an integrated response to external stimuli and relates directly to an affective state. However, a study by Langford et al. (2010) may provide such evidence. In this study, mice, *M. musculus*, underwent lesioning of the rostral anterior insula (implicated in the affective component of pain in humans) and this prevented changes in facial expression but not abdominal writhing (the behavioural marker of abdominal pain or nociception). A similar effect is observed in humans with insular lesions that are associated with pain asymbolia (the disassociation of the affective [unpleasant experience] and the sensory component [nociceptive response] of pain; Langford et al., 2010). In these patients the emotional responses to pain decline significantly without the associated reduction in nociceptive response or pain thresholds (Berthier, Starkstein, & Leiguarda, 1987).

Long-term Motivational and Behavioural Change

Among some of the most complex behavioural responses to pain are those in preference and avoidance studies. These demonstrate that animals are able to use their internal 'state' (i.e. apparent experience of pain) to learn, make decisions and then perform behaviours that ameliorate that pain state. For example, mammals show avoidance of places in which potentially painful stimuli are delivered (Gao, Ren, Zhang, & Zhao, 2004) and will pay a cost to avoid such stimuli. Rats will cover electrodes in their cages with bedding so shock can be avoided (Pinel, Symons, Christensen, & Tees, 1989). Further, there are numerous examples of long-term directed licking or rubbing of the body area damaged by a noxious stimulus (e.g. Wheeler-Aceto & Cowan, 1991). Mammals also show 'pain relief learning' in which stimuli that are temporally associated with the termination of a noxious stimulus, e.g. electric shock, have a positive valence and are preferred over neutral stimuli (Gerber et al., 2014). Such responses are much more difficult

to account for as being simply a complex reflex or nociceptive sensitization as they require considerably higher processing (Bateson, 1991). Further behavioural tests commonly assess how animals respond when given a choice between avoiding a situation that is associated with pain and/or taking a drug that relieves pain. For example, Colpaert et al. (1980) demonstrated that rats given a choice between sugar solution and solution containing pain relief chose the sugar solution if healthy (nonpainful), but the pain relief-containing solution when experiencing a potentially painful condition (arthritis). These studies on mammals provide the benchmark by which other animals are judged, and certainly provide a basis for testing species for which pain has not been explored.

In the future, 'cognitive bias' testing may offer a more direct means of assessing the affective component of pain in mammals. This technique has only been applied to animals relatively recently and involves measuring cognitive or judgement biases in the interpretation of ambiguous information (e.g. Brydges et al., 2011; Douglas et al., 2012; Harding et al., 2004; Mendl, Burman, Parker, & Paul, 2009). This technique has focused on the impact of environmental and husbandry procedures on affective state; however, such measures could be directly applicable to the assessment of the affective component of pain. It could be argued that such measures would offer the most valid indicators of pain as they could determine the significance of the pain to the animals. Therefore the potential merits of these techniques warrant their inclusion in this review, even though they have not been used in this context.

BIRDS

Whole Animal Response

Potentially painful stimuli influence a range of physiological responses in birds (review in Prunier et al., 2013), for example plasma corticosterone and heart rate increase after beak trimming and feather removal (Davis, Anderson, & Jones, 2004; Gentle & Hunter, 1991; Glatz, 1987; Glatz & Lunam, 1994). Birds also exhibit withdrawal responses to a variety of noxious treatments that are used as standard in mammalian pain studies, for example foot withdrawal in response to high temperature in parrots, *Amazona ventralis*, kestrels, *Falco sparverius*, and chickens, *Gallus gallus domesticus* (Geelen et al., 2013; Hothersall et al., 2011; Roach & Sufka, 2003; Sanchez-Migallon Guzman et al., 2013), instantaneous removal of the foot from hot water in Japanese quail, *Coturnix japonica* (Evrard & Balthazart, 2002), as well as movement away from mechanical stimuli (Evrard & Balthazart, 2002; Hothersall et al., 2011). Application of analgesics increased the thermal threshold for foot withdrawal in kestrels and parrots (Geelen et al., 2013; Guzman et al., 2011). Morphine significantly reduced responsiveness to noxious heat and mechanical pressure in quail (Evrard & Balthazart, 2002) and the NSAID dexamethasone significantly diminished the inflammation and hyperalgesia to carrageenan in chickens (Roach & Sufka, 2003). Further, some analgesic drugs are administered by veterinarians and experimenters to ameliorate apparent pain, suggesting a high evolutionary conservation of receptors for drugs such as opioids and NSAIDs (Jordt & Julius, 2002; Nasr, Nicol, & Murrell, 2012).

Long-term Motivational and Behavioural Change

Self-selection of analgesic-dosed food has been demonstrated in chickens: lame birds selectively chose food drugged with carprofen (Danbury, Weeks, Chambers, Waterman-Pearson, & Kestin, 2000). This approach has had mixed results in other pain models: beak-trimmed birds did consume more carprofen-dosed food but did not resume normal pecking rates, although the maximum force

they exerted while pecking was higher than in groups not receiving carprofen (Freire & Glatz, 2008). Putting chickens in a novel situation or starving them to elicit a motivational shift to feed reduces pain-related responses (Wylie & Gentle, 1998; Gentle & Tilston, 1999). This may mean that pain is not as important as satiating hunger or exploring a new habitat. However, this shows that the reactions to pain are not simple reflexes, otherwise the birds would perform the same behaviour regardless of context. The behaviours seen after a painful event are indicative of abnormal behaviours and certainly of guarding behaviour in which an animal does not use a painful area or limb. Birds with keel fractures substantially reduce their movement to new perches as well as taking longer to reach a food reward in runway tests (Nasr et al., 2012). Flight from a perch to the ground may require more complex motivational decisions as well as integration of movement and decision making that is impaired by keel fractures but substantially improved by the administration of butorphanol (Nasr et al., 2012). Thus, behavioural decisions are demonstrably affected by pain in birds.

As with mammals, cognitive bias testing may also offer a more direct means of assessing the affective component of pain in birds. This technique has so far been focused mainly on the impact of environmental conditions on affective state by measuring cognitive biases in the interpretation of ambiguous information (e.g. Brilot, Asher, & Bateson, 2010; Matheson et al., 2008). As for mammals, however, it has considerable potential utility for the assessment of pain in birds (see the Mammal section for more detail).

AMPHIBIANS AND REPTILES

Whole Animal Response

Amphibians show a classic wiping response to application of acetic acid as well as a withdrawal response to noxious heat and mechanical stimulation (Willenbring & Stevens, 1996). Both responses are attenuated by administration of compounds with analgesic properties (Kanetoh et al., 2003; Mohan & Stevens, 2006; Stevens, Martin, & Stahlheber, 2009). Similarly, reptiles display characteristic responses to painful stimulation (e.g. limb retraction in response to formalin in Speke's hinged tortoise, *Kinixys spekii* Wambugu, Towett, Kiama, Abelson, & Kanui, 2010; withdrawal from high temperatures in bearded dragons, *Pogona vitticeps*, corn snakes, *Elaphe guttata*, Sladky, Kinney, & Johnson, 2008, and turtles, *Trachemys scripta*, Sladky et al., 2007; withdrawal from a strong mechanical pressure in red-eared slider turtles, *T. s. elegans*, Kischinovsky, Duse, Wang, & Bertelsen, 2013) that are again reduced by analgesia.

Further, four opioid receptors have been identified in amphibians including the mu, delta and kappa opioid receptors but also an opioid receptor-like protein (Stevens et al., 2009). Sequence comparisons have demonstrated that the amphibian opioid receptors are highly conserved (70–84% similar to mammals) and are expressed in the CNS areas apparently involved in pain experience (Stevens, 2004; Stevens et al., 2007). Therefore, fulfilling one of the criteria for pain that Bateson (1991) suggested, amphibians and reptiles share with mammals an opioid and endogenous opioid system involved in pain mechanisms.

Long-term Motivational and Behavioural Change

Generally, studies on pain in these animal taxa are sparse and much more research is required to understand fully the implications of potentially painful events on their biology, behaviour and welfare (Table 2; Mosley, 2006, 2011). Given the lack of empirical evidence Mosley (2011) suggested clear criteria when assessing pain in reptiles that could be applied to amphibians as well.

Parameters such as gait, unwillingness to perform normal behaviours, exaggerated flight response, closure of eyes, decreased appetite, colour change and abnormal respiration may act as key indicators to assessing affective state (Mosley, 2011). Caution should also be applied in light of life history and ecological differences, for example some reptile species live in deserts where they would regularly experience extreme heat that would be nociceptive to mammals. When applying hot or cold noxious temperatures it may be important to understand whether cooling or warming the test species is a more relevant pain test (Mosley, 2006). For example, red-eared slider turtles acclimated to 20 °C lost nociceptive sensation compared with those held at 35 °C, which were fully responsive to mechanical pinching (Kischinovsky et al., 2013). Thus, an intelligent understanding of what the species will experience in nature should be used to inform meaningful experimental studies.

FISH

Whole Animal Response

Teleost fish move away from noxious stimuli that would cause pain in mammals. For example, koi carp, *C. carpio*, move away from a clamp exerting high mechanical pressure to the lip and tail and this withdrawal response is decreased when the fish are lightly anaesthetized (Stockman, Weber, Kass, Pascoe, & Paul-Murphy, 2013). Classical conditioning studies using the negative reinforcement of electric shock is a popular paradigm in fish experiments (e.g. Yoshida & Hirano, 2010). Rainbow trout and goldfish learn to avoid an area where electric shock is given (Dunlop, Millsopp, & Laming, 2006) but trade off the risk of entering the shock zone when they are fed there to satiate their hunger after 3 days of food deprivation (Millsopp & Laming, 2008). Ehrensing, Michell, and Kastin (1982) demonstrated that responses to electric shock were reduced by the opioid painkiller morphine and that in turn the effect of morphine was blocked by the antagonists MIF-1 and naloxone.

In vivo administration of potentially painful stimuli results in prolonged, complicated responses (reviews in Sneddon, 2009). Physiologically, opercular beat rate (ventilation of the gills) is enhanced by subcutaneous injection of noxious chemicals in trout, *O. mykiss*, and zebrafish, *Danio rerio*, as well as an increase in plasma cortisol in trout (Ashley et al., 2009; Reilly, Quinn, Cossins, & Sneddon, 2008a; Sneddon, 2003b). Concomitantly, trout and zebrafish reduce swimming activity (Correia, Cunha, Scholze, & Stevens, 2011; Reilly et al., 2008a; Sneddon, 2003b). When injected with noxious chemicals into the frontal lips, trout suspend feeding behaviour for 3 h and only resume feeding when their behaviour and physiology return to normal (Sneddon, 2003b); sham-handled and saline-injected controls resume feeding after 80 min as do acid-injected fish when administered with morphine. Thus this suspension in feeding is similar to the guarding behaviour of affected parts of the body by mammals and birds. Fish are the most diverse vertebrate group and show obvious species differences in pain-related behaviour, as do mammals (Flecknell, Gledhill, & Richardson, 2007). Piaçu, *Leporinus macrocephalus*, injected with formalin and Nile tilapia, *Oreochromis niloticus*, that have had the tail fin severed actually increase swimming after the painful treatment (Alves, Barbosa Júnior, & Hoffmann, 2013; Roques et al., 2010). In contrast, Atlantic salmon experiencing abdominal peritonitis due to vaccination decreased swimming and suspended feeding for up to 2 days (Bjørge et al., 2011). Therefore, these disparate responses highlight that pain indicators will have to be quantified on a species by species basis and to different modes of pain in fish. Adverse changes in behaviour last from 3 h up to 2 days and are not simple instantaneous nociceptive reflexes.

Long-term Motivational and Behavioural Change

Anomalous behaviours such as tail beating in zebrafish with acid injected near the tail fin (Maximino, 2011), rocking to and fro on the substrate by rainbow trout and common carp injected with noxious chemicals and rubbing of the injection site by rainbow trout and goldfish (Newby, Wilkie, & Stevens, 2009; Reilly et al., 2008a; Sneddon, 2003b; Sneddon, Braithwaite, & Gentle, 2003b) are only seen in fish given a potentially painful treatment and not observed in sham-handled controls, saline-injected fish or reported in any other toxicological studies using fish. Therefore, these are likely to be specific to pain and to be ameliorated when painkillers are given; thus they may be valid indicators of discomfort and suffering (Mettam, Oulton, McCrohan, & Sneddon, 2011; Sneddon, 2003b).

As described above, fish are able to learn to avoid noxious stimuli and the experience affects subsequent behaviour. For example, goldfish and rainbow trout avoid an area where they received an electric shock (Dunlop et al., 2006). As many studies demonstrate, fish do not feed when given painful stimuli, so it is difficult to attempt the type of self-administration approaches that have been used in birds and mammals which will consume food or water dosed with a painkiller (e.g. Pham et al., 2010). However, understanding how important the experience is to fish can be tackled by determining whether fish will pay a cost to access analgesia. Zebrafish given access to a barren, brightly lit chamber or an enriched chamber repeatedly choose the enriched area (Sneddon, 2013). When these fish are subcutaneously injected with acetic acid or saline as a control they still choose the same favourable, enriched chamber. However, if an analgesic is dissolved in the barren, unpreferred chamber zebrafish injected with noxious acid lose their preference for the favourable area and spend over half their time in the unfavourable, barren chamber (Sneddon, 2013). This suggests they are willing to pay a cost to enter a less preferred environment to access pain relief.

Selective attention approaches have been employed to understand the importance of the pain experience to fish. For example, trout will ignore novel objects in fear tests rather than show neophobia when in apparent pain; however, this is reversed when morphine is administered (Sneddon et al., 2003b). Noxiously stimulated trout also do not show appropriate antipredator responses by seeking cover and performing escape behaviour (Ashley et al., 2009). Therefore, in the context of fear and predation pain is the imperative. Piaçu exposed to a predator stimulus show an enhanced stress response and endogenous analgesia in which endorphins reduce the impact of painful treatment (Alves et al., 2013). These studies combined demonstrate that painful stimuli appear to take priority over competing stimuli and that central mechanisms are activated to reduce pain.

MOLLUSCS

Molluscs include bivalves, gastropods, nudibranchs and cephalopods, which differ markedly in morphology, behaviour and neural complexity (Crook & Walters, 2011). Various species respond to noxious stimuli and show associative learning (Kavaliers, 1988; Crook & Walters, 2011). Cephalopods are highly mobile with a large, complex brain and good learning ability (Mather, 2011) and they have recently been included in the European Union Directive (2010/63/EU) that provides protection from suffering in animal experimentation. The responses of squid, *Loligo pealeii*, to localized injury are difficult to explain without invoking long-term changes within central processing centres in the brain (Crook et al., 2011). Injured squid showed a greater visual responsiveness to approaching stimuli if tested more than 10 min after

injury. In mammals, this increased defensive response to visual stimuli would typically have been interpreted as indicating increased fearfulness. It certainly demonstrates a long-term change in motivational state after injury, which is consistent with the concept of pain. Additionally, injured animals show increased sensitivity after injury. Squid with a small area of a fin crushed with forceps showed more firing of nociceptors when that area was subsequently touched. This enhanced sensitivity had a rapid onset and lasted for approximately 70 min (Crook et al., 2013). However, squid do not appear to show targeted wound-tending behaviour (Crook et al., 2011), although increased sensitivity and prolonged behaviour directed at the site of a wound has been observed in the octopus *Abdopus aculeatus* (Alupay et al., 2014). Long-term sensitization of nociceptors (Crook et al., 2013) and defence responses (Crook et al., 2011) have been interpreted as indicating ongoing pain in vertebrates, but possibly do not require complex neural processing (Chase, 2002). The function of the sensitization was tested by Crook, Dickson, Hanlon, and Walters (2014) by injuring squid, some with and some without anaesthetic, and comparing them with uninjured controls, again some with and some without anaesthetic. The squid were then exposed to predatory fish which showed increased attention to the injured animals regardless of anaesthetic treatment. However, injured squid initiated defensive responses earlier than did controls but this effect was blocked by the anaesthetic. The anaesthetic also blocked the sensitization that normally follows injury and these squid had a lower survival from predatory attempts than did those not given anaesthetic. However, anaesthetic without injury did not reduce survival indicating that there was a positive fitness effect from the sensitization. These results are important as they are consistent with the idea that pain-like states function to promote future survival.

Observations of changed predatory tactics of octopus, *Octopus joubini*, when hermit crab prey, *Pagurus pollicaris*, had stinging anemones, *Calliactis tricolor*, on their shells are also consistent with the idea of responses not explained by nociception (review in Elwood, 2011). These long-term behavioural and neuronal changes should be viewed in the context of the advanced learning ability of cephalopods (Mather, 2011).

ARTHROPODS: DECAPODS

Various decapod crustaceans have been investigated to determine whether responses to noxious stimuli are merely nociceptive reflexes, with no short-term or long-term effects on CNS function. Shore crabs, *Carcinus maenas*, show rapid (two-trial) discrimination avoidance learning when shocked in one of two dark shelters (Magee & Elwood, 2013). Further, hermit crabs, *Pagurus bernhardus*, that received a single shock within their shell showed a prolonged increase in motivation to leave that shell and move into a new one (Appel & Elwood, 2009a; Elwood & Appel, 2009). They approached and investigated the new shell more quickly indicating an increased motivation for shell change (Elwood, 1995; Elwood & Stewart, 1985).

Decapods also show prolonged rubbing or guarding of an affected area as seen in vertebrates (Weary et al., 2006). Glass prawns, *Palaemon elegans*, perform prolonged rubbing and grooming of the specific antenna brushed with either sodium hydroxide or acetic acid (Barr et al., 2008). However, if the antenna is pretreated with a local anaesthetic, the grooming and rubbing are much reduced. Prolonged abdominal grooming also occurs in hermit crabs, *P. bernhardus*, after shock on the abdomen (Appel & Elwood, 2009a, 2009b). Further, edible crabs, *Cancer pagurus*, with a cheliped (claw) removed by pulling it off (a practice used in commercial fisheries) repeatedly touch the wound with their other appendages but not if they had been induced to autotomize the

cheliped without a wound (C. McCambridge, personal communication). Further, formalin injection into one cheliped of shore crabs, *H. sanguineus*, induces shaking and rubbing of the appendage and the use of that appendage is markedly reduced (Dyuizen et al., 2012). Thus prolonged attention and guarding are common in decapods (but see Puri & Faulkes, 2010). We note also that edible crabs that have had a claw pulled off causing tissue damage showed a marked prolonged physiological stress response whereas those induced to autotomize do not (Patterson, Dick, & Elwood, 2007).

Behavioural trade-offs between avoiding the noxious stimulus and retaining some other requirement have also been observed. Hermit crabs, for example, leave less preferred species of shell more readily compared with those in preferred species when subject to abdominal shock (Appel & Elwood, 2009b; Elwood & Appel, 2009) and are less likely to evacuate shells after shock when odours of predators are present (Magee & Elwood, n.d.).

Morphine has marked effects in reducing responsiveness to noxious stimuli in crabs, *Chasmagnathus granulatus* (Lozada, Romano, & Maldonado, 1988); however, this does not appear to be due to analgesia but rather a general lack of response to any stimulus (Barr & Elwood, 2011). Although an analgesic effect has repeatedly been demonstrated in vertebrates, widely differing animals might use different regulating pain/nociceptive systems (Barr & Elwood, 2011).

The prolonged rubbing and shaking of a cheliped injected with formalin noted above in *H. sanguineus* is accompanied by a gradual change in the central nervous system NO-ergic neurons that have been implicated in nociceptive reflexes in vertebrates and are present in primary sensory centres of crustaceans, insects and molluscs (reviewed in Dyuizen et al., 2012). These neurons were shown by expression of the enzyme nitric oxide synthase and this expression was earlier on the ipsilateral than on the contralateral side of the CNS indicating that it was due to a neuronal input from that side of the body. The earliest changes were seen in specific nerve fibres in the thoracic sensory neuropils and the most prominent in structures considered to modulate cheliped action. These changes occurred over a period of 30–60 min depending on location and showed far more than just an immediate reflex function. They are consistent with the idea of prolonged motivational change after noxious stimulation.

A recent study on crayfish, *Procambarus clarkii*, also noted long-term motivational change coupled with physiological change (Fossat, Bacqué-Cazenave, De Deurwaerdère, Delbecque, & Cattaert, 2014). Some subjects were exposed to electrical fields that were aversive and induced attempted escape responses and then allowed to choose where to walk in a cross-shaped apparatus that had two arms in the light and two in the dark. All animals preferred the dark arms but those recently subjected to the aversive stimulus showed a stronger preference for the dark than did controls. Those subject to the electric field also had higher brain serotonin levels and a higher level of blood glucose, which has previously been recognized as a component of a stress response (Patterson et al., 2007). Control animals injected with serotonin also showed strong avoidance of light and increased glucose. Further, an anxiolytic drug abolished the light avoidance of stressed animals. The authors concluded that the stress-induced avoidance is similar to vertebrate anxiety and indicates the ability of invertebrates to exhibit a state similar to mammalian emotion.

ARTHROPODS: INSECTS

Insects respond vigorously to noxious stimuli, but these responses can be suppressed (e.g. during sexual cannibalism, Sakaluk, Campbell, Clark, Johnson, & Keorpes, 2004) or intensified (e.g. after ultraviolet exposure, Babcock, Landry, & Galko, 2009).

The molecular mechanisms mediating these behaviours are at least partially known in some species (e.g. *D. melanogaster*) and appear to be homologous to the molecular mechanisms mediating nociception in mammals (Johnson & Carder, 2012). Nociception in insects, as in other invertebrates, is transduced by neurons dedicated to sensing damaging stimuli (nociceptors, Smith & Lewin, 2009). *Drosophila melanogaster* larvae have peripheral nociceptors that are studded with receptors sensitive to damaging stimuli (Tracey, Wilson, Laurent, & Benzer, 2003). The best studied of these receptors include transient receptor potential (TRP) channels such as the TRP channel 'Painless' (Tracey et al., 2003) which is an evolutionary homologue of the mammalian TRPA1 (Smith & Lewin, 2009). However, insects also differ from vertebrates in some ways in their responses to noxious stimuli. For example, insects tend to continue to use damaged limbs (Eisemann et al., 1984) and will self-cannibalize their own guts if injured (Lockwood, 2013).

How nociceptive information is processed within the insect central nervous system remains almost entirely unknown (Johnson & Carder, 2012), although there is evidence that nociceptive information reaches higher learning centres in the insect brain (e.g. Waddell, 2013). Nociception in insects can be modified using simple peripheral mechanisms, without the involvement of the central nervous system (Johnson & Carder, 2012). Therefore, simply showing that nociception is modifiable (e.g. by endogenous opioids or other molecules) is not a compelling argument that insects feel pain. Plasticity in response to noxious stimuli does not necessarily indicate that an animal has the complex central nociceptive processing power required for the experience of 'pain'.

As in vertebrates, noxious stimuli can be used to 'teach' insects a variety of tasks (Giurfa, 2013). For example, electric shocks have been used as a negative reinforcer in insect learning studies (Tedjakumala & Giurfa, 2013). Insects can also learn to avoid places associated with noxious stimuli (Giurfa, 2013). They can also learn to associate otherwise neutral stimuli with the cessation of electric shock and then prefer those stimuli, a phenomenon called 'pain relief learning' (Gerber et al., 2014). Therefore, insect nervous systems are capable of assessing motivational variables and noxious stimuli can act as a potent motivational force.

Whether insects have neural circuits capable of processing 'emotional' information (positive or negative) remains unclear. For example, a recent study in bees, *Apis mellifera*, suggests that negative stimuli can induce pessimistic cognitive biases, as is observed in vertebrates (Bateson, Desire, Gartside, & Wright, 2011). However, Giurfa (2013) pointed out that Bateson et al.'s (2011) data equally support the interpretation that bees became better discriminators of a food reward after shaking (i.e. the negative stimulus used in Bateson's study). This alternative explanation is appealing because shaking alters octopamine concentrations in the haemolymph (Bateson et al., 2011), and octopamine levels are known to influence sensory function (Orchard, Ramirez, & Lange, 1993; Roeder, 1999). At present there is no definitive evidence that insects have the prerequisite cognitive and emotional abilities to support a negative internal mental state.

With little neurobiological evidence for the existence of pain-like states in insects, we are left with trying to stretch the argument-by-analogy (Allen, 2011) to encompass the behaviour of this group. Insects may show behaviour that suggests an affective or motivational component (e.g. it has complex and long-lasting effects), but insects could do this, at least in some cases, by using mechanisms that require only nociception (e.g. long-term nociceptive sensitization) and/or advanced sensory processing (i.e. without any internal mental states). Moreover, recent advances in artificial intelligence (AI) have shown that robots can be programmed to express pain-like behaviour using relatively

simple processing systems (e.g. Castro-González, Malfaz, & Salichs, 2013). The behaviour of these robots fulfils the behavioural criteria for pain listed in Table 2 (e.g. see Castro-González et al., 2013; Lee-Johnson & Carnegie, 2010). For example, a robotic rodent has been programmed to experience 'discomfort' which can then be used as a learning motivator (Ames et al., 2012). AI researchers develop such 'emotional' robots because they recognize that affective processes give biological entities a great deal of cognitive flexibility (Lee-Johnson & Carnegie, 2010). These 'emotional' robots have no subjectively experienced emotions, but the robot's artificial emotions allow it to reprioritize its goals, modulate its behaviour and provide learning rewards (Castro-González et al., 2013; Lee-Johnson & Carnegie, 2010). Similarly, insects, and possibly other animals, could use simple processing rules to produce pain-like behaviour, without any internal experience of pain.

PRINCIPLE OF TRIANGULATION

Pain in animals has been assessed using a wide range of indices, and it has been argued that none of these indices when taken in isolation can be considered as definitive evidence of 'pain' in animals (e.g. Rose et al., 2014). However, we, along with other authors, (e.g. Bateson, 1991; Mason & Mendl, 1993; Nicol, Caplen, Edgar, & Browne, 2009; Sneddon, 2004, 2009, 2011, 2013; Weary et al., 2006; Elwood, 2012; Flecknell et al., 2011) are advocating not that these individual isolated indices should be taken as evidence of pain, but that together they represent an increasing level of complexity of responses to pain that go beyond simple and acute detection and reflex responses and begin to demonstrate a level of behavioural complexity that would require some form of experience. Pain is a complex multidimensional phenomenon (Rutherford, 2002); therefore, effectively identifying and then assessing the severity of pain may require a multimodal approach. Ultimately we are advocating applying the 'principle of triangulation', where all of the indices are taken together as evidence of underlying affective state (M. Bateson, personal communication). Such a principle forms the foundation in many different scientific fields where a definitive answer cannot be directly measured. For example, dark matter in the universe cannot be measured directly but its existence is inferred from gravitational effects of visible matter, radiation and the large-scale structure of the universe (Trimble, 1987).

CONCLUSIONS

Our summary of the evidence supports the conclusion that many animals can experience pain-like states by fulfilling our definition of pain in animals, although we accept that 100% certainty cannot be established for any animal species. Nevertheless, the 'precautionary principle', the idea that it is better to err on the side of more protection for a group of animals if it is beyond reasonable doubt that they experience pain (e.g. Andrews, 2011), proposes that we should act as if at least some animals experience pain. From an ethical (Bateson, 2005) and often a legal perspective we must ensure the welfare of animals. Thus here we provide a basis for future studies to direct the investigation of pain in animals where evidence is lacking or inconclusive. This does not preclude the use of animals in scientific research but careful consideration of the assessment and alleviation of pain during such research is vital (Bateson, 2005). However, even if we cannot be certain that some species experience pain, they should be treated with respect for reasons that do not hinge on whether or not they experience pain (Harvey-Clark, 2011; Lockwood, 2013; Mather, 2011).

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