ASSESSING PAIN IN ANIMALS

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Abstract

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Assessing the experience of pain in animals is a difficult task, yet one that is important in animal welfare research. Some approaches to pain assessment in animals are reviewed here. General qualities of pain scales and specific parameters suitable for clinical and experimental pain assessments are discussed. It is argued that pain assessment will progress through an integration of objective and subjective observations of behaviour coupled with multiple measures in various other areas. Such multidimensional pain scales allow an adequate characterisation of the complexity of an individual animal's pain experience to be made. This knowledge improves the recognition and treatment of pain and will allow informed moral debate on the acceptability of practices such as castration and tail-docking of lambs.

Keywords: *animal pain, animal welfare, beak trimming, canine ovariohysterectomy, lamb castration, pain assessment*

Introduction

Assessment and alleviation of pain is a fundamentally important area of animal welfare research and veterinary practice. In welfare research, pain assessment is carried out in order to identify when pain is likely to occur and to quantify its intensity. In veterinary practice, accurate pain assessment allows action to be taken to treat the individual and to monitor the success of that treatment. This article reviews some recent work in both fields.

Pain is defined by the International Association for the Study of Pain (IASP) as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage" (IASP 1979, p 250). Notes associated with this definition emphasise the importance of verbal self-report in human pain assessment, but the requirement of self-report is not satisfied in such individuals as small children, mentally handicapped adults, or animals (Anand & Craig 1996). Consequently, the ability of these individuals to feel pain has often been underestimated or dismissed. However, there are strong parallels in the behavioural and physiological responses of humans and animals to noxious stimulation (Dubner & Ren 1999), suggesting that rather than dismissing animal pain on the basis of the lack of verbal self-report, a different definition is required. The following definitions, which do not rely upon self-report, have been suggested:

"Pain in animals is an aversive sensory experience caused by actual or potential injury that elicits protective motor and vegetative reactions, results in learned avoidance behaviour, and may modify species specific behaviour, including social behaviour" (Zimmermann 1986, pp 16–17).

"Animal pain is an aversive sensory and emotional experience representing an awareness by the animal of damage or threat to the integrity of its tissues; (note, there may not be any damage) it changes the animal's physiology and behaviour to reduce or avoid the damage, to reduce the likelihood of recurrence and to promote recovery; non-functional pain occurs when the intensity or duration of the experience is not appropriate for the damage sustained (especially if none exists) and when physiological and behavioural responses are unsuccessful in alleviating it" (Molony 1997, p 293).

Molony (1992) also notes that although animal pain may serve the same function as pain in humans, and that similar damage should have similar significance, pain in animals and humans is not necessarily experientially the same. This view sidesteps any opposition to an extrapolation of experience from humans to animals. It also guards against an over-reliance on comparisons with human behaviour and physiology, which may cause some animal pain to be overlooked (Flecknell & Molony 1997).

It has been suggested that animals may experience the sensation of pain without the suffering component (Iggo 1984). If this were indeed the case, there would be fewer ethical dilemmas concerning the treatment of animals. However, pain should be viewed from an evolutionary perspective. If it is hypothesised that suffering from pain is a functional adaptation to life in a potentially dangerous environment, rather than a specific adaptation to the human niche or an emergent property of human cognitive abilities, it seems logical that other animals should share this adaptation. It can further be argued that the unpleasant suffering component of pain is an integral part of its evolutionary function (Dawkins 1998). This, combined with the close similarities that exist in the underlying mechanisms of pain in various animal species, including humans (Morton & Griffiths 1985), suggests that animal pain is likely to be as real an entity as human pain.

Classification of pain

Following injury, the pain felt by an individual may consist of various phases (Niv & Devor 1998). Acute pain occurs at the moment of injury, followed by sub-acute pain, which may develop into chronic pain. Acute pain functions as a warning of damage or potential damage and is likely to invoke withdrawal from and future avoidance of the stimulus, while the sub-acute phase provokes protective behaviour that aids healing following injury (Bateson 1991; Millan 1999).

The definition of what actually constitutes chronic pain varies. It is generally said that chronic pain lasts longer than the healing time of injury (Bonica 1953). Very often, pain is described as chronic purely in relation to its time-span, irrespective of the nature of the injury. The choice of a particular length of time after which pain is described as chronic is arbitrary. It may be most useful to view chronic pain as being the same as the "non-functional pain" described by Molony (1997). Chronic pain might therefore be seen as pain which remains "after pain can serve any useful function" (Melzack & Wall 1996, p 36).

Pain can also be classified in terms of its source within a body. The main distinction is between visceral, somatic and neuropathic pains, which may have different neurophysiological characteristics (Bennett 1994; McMahon 1997) and effects on an animal. Various tissues within the body differ in their sensitivity to pain. For instance, the following list is arranged in order of putatively decreasing sensitivity to pain: cornea, dental pulp, testicles, nerves, spinal marrow, skin, serous membrane, periosteum and blood vessels, viscera, joints, bones and encephalic tissue (Baumans *et al* 1994; Martini *et al* 2000).

It is important to note that pain is not the same as nociception — the two are distinct phenomena (Iggo 1984). Nociception is the physiological side of pain, without the aversive emotional component. It involves the relay of sensory information from nociceptors (tissue receptors that are sensitive to damaging or potentially damaging stimulation) to the spinal cord and then to the brain. Activation in nociceptive fibres may result in reflex withdrawal (controlled at the spinal cord level), autonomic activity (controlled by the brain) and, normally but not always, the presence of pain (Clark 1994). Note that reflex withdrawal and autonomic activity can occur without pain. The relationship between nociception and pain is further complicated by the fact that activity in nociceptive systems is not absolutely necessary for pain to occur (Wall 1992). If pain was more directly and reliably related to nociception, its assessment would be much easier (Hansen 1997).

Pain assessment studies

In this review, various approaches to animal pain assessment will be discussed mainly with reference to the following three examples.

Beak-trimming in poultry

Beak-trimming of poultry is a common practice used to prevent feather pecking and cannibalism, both of which can be major welfare problems (Gentle 1986a). It generally involves removal of part — typically one-third — of the upper beak and occasionally part of the lower beak through a combination of cutting and cautery.

Castration and tail-docking in lambs

Both castration and tail-docking are common farm practices. Castration may be carried out to produce untainted meat, or to avoid behavioural problems and indiscriminate breeding (Hosie *et al* 1996). Lambs are tail-docked to decrease the likelihood of blow fly strike (French *et al* 1994). In the UK, castration and tail-docking are predominantly achieved by the application of a rubber ring to the scrotum or tail. The ring blocks blood flow, causing ischaemia and the death of tissue distal to the ring. 'Bloodless' castrators such as the Burdizzo, which crush the spermatic cord, can also be used either alone or in combination with a rubber ring.

Postoperative pain in dogs

Consideration will also be given to clinical veterinary assessments of postoperative pain, primarily post-ovariohysterectomy pain in dogs. Ovariohysterectomy, which involves surgical removal of the ovaries and uterus, is one of the most common veterinary surgical procedures. It may either be performed as an elective surgery or for medical reasons.

Ethical justification for pain studies

Whenever animals are used in research their use should be justified and this should be clearly set out in any publications. This is particularly true for studies of animal pain. Ethical standards for animal pain studies have been suggested by the International Association for the Study of Pain (Zimmermann 1983) and these aim to minimise the duration and severity of experimentally induced pain. Some experimental methods allow animals a degree of control over the pain they experience, for example by using operant conditioning techniques or by studying withdrawal reflexes (Dubner & Ren 1999). However, in order to study severe or enduring pain, different approaches are required. In these cases it is more ethically justifiable to study the pain that animals experience as a result of natural disease, accidental trauma or necessary surgical treatment.

This rationale can be extended to the case of castration, where the justification for the work is partly that the procedure would be undertaken anyway on farms. Studies involving castration were designed initially to assess the severity of the procedure in order that the ethics of its routine use could be examined. As the body of work has moved on from establishing the severity of the procedure to assessing methods for reducing its severity, lambs castrated in experimental studies are considered to suffer less than those routinely castrated on farms. There is also the justification that these studies will advance the development of better methods for assessment of animal pain and the identification of pain-reduction methods, which may reduce the suffering of many animals (Molony & Kent 1997). Similar justification can be applied to beak-trimming studies; in both cases, large numbers of animals are, or will be, affected every year, so any advance in pain recognition and alleviation could potentially have extensive welfare benefits.

Similarly in veterinary medicine there is a need for improvement in pain assessment and alleviation which may occur only through studying animal pain itself. In such clinical studies, other ethical issues may arise. For instance, some treatment groups may not receive analgesia, and healthy animals may receive sham operations or anaesthesia. In studies relating to clinical problems it is less common for pain to be deliberately inflicted. Studying naturally occurring pain (ie animals presented for treatment) is easier to justify ethically but is less useful scientifically because there is more variation in the animals and their injuries, pain and previous experience (Waterman-Pearson 1999). In these cases, veterinarians are ethically obliged to take the best measures for the animals' well-being, and this often precludes pain being left untreated to provide negative controls. However, as research in different species expands, it becomes possible to test new analgesics against established ones rather than against placebo control groups. It is important that animals are withdrawn from a study and treated if the pain goes beyond certain set points (this is generally a legislative requirement).

The ethical framework within which decisions such as these are made is basically a utilitarian calculus of cost (a fairly well-defined level of suffering) against benefit (the potential but essentially unknown pain reduction). However, it is worth pointing out that from an animal-rights viewpoint, inflicting pain would never be justified despite potential benefits to other animals. A 'hybrid' of these differing views might suggest that within a cost–benefit analysis there are maximum levels of individual suffering which should not be passed irrespective of the potential benefits (Sandøe *et al* 1997). As with all other areas of animal research, the principles of reduction, refinement and replacement (Russell & Burch 1959) should be applied wherever possible, to reduce the total amount of suffering.

Measurement theory applied to pain assessment

Any particular pain scale has to meet appropriate standards of reliability, sensitivity and validity in order to be considered useful. It may be particularly important to demonstrate these attributes in animal pain studies. A reliable measure is one in which measures in similar individuals produce similar results in a reproducible manner (Streiner & Norman 1989), while sensitivity is a measure of how the parameter changes with changes in the measured quantity (Natelson *et al* 1987). Assessing reliability can be problematic, as the degree of pain felt by an individual can fluctuate and individuals vary in the pain they experience as a result of standard stimulation.

An effective pain-measurement scale should include parameters that sensitively co-vary with the degree of pain. To assess the sensitivity of various indicators of pain in lambs, Molony and Kent (1997) assigned lambs to the following groups: handled only; short scrotum castration with local anaesthetic; tail-docking; short scrotum castration; unilateral castration; bilateral castration and castration plus tail-docking. It was assumed that the increasing tissue damage provided increasingly painful stimuli. The value of particular indicators was then assessed by statistically assigning lambs to groups of increasing magnitude of these indicators and comparing these groups with the actual treatment groups of putatively increasing pain. If the assumption of increasing pain is correct, a perfect pain measure would place all lambs in the correct group. Using, in combination, a measure of particular abnormal postures and behaviours, 63 per cent of lambs could be placed in their correct group. Re-analysis of these data (Kent *et al* 2000b) shows that removing the tail-docked group and using more sophisticated statistics improves this result to 78.6 per cent, with incorrectly placed lambs always being misplaced by only one group. It is difficult to say whether the incorrectly assigned lambs are misplaced because of an inaccuracy in the scale or because the scale is correctly measuring pain and the original assumption of 'correct' group is flawed (perhaps because of variable effects of local anaesthetic or other individual differences in the pain experienced by the lambs). Note that in pain-assessment scales there is often a trade-off between measurement resolution and accuracy; using this scale for example, lambs could be very accurately (100 per cent) assigned to three less precise groups: mild, moderate, and severe pain. The challenge is to produce scales that can accurately discriminate between finer differences in pain. However, it is considered that 78.6 per cent is a reasonable level of accuracy at this level of resolution.

One problem with experiments testing sensitivity is that it is difficult to identify a series of stimuli that provides a linear increase in putative pain. Different noxious stimuli can result in qualitatively, as well as quantitatively, different pain sensations. However, for different treatments to be compared in terms of their severity they have to be measured on the same scale (Lester *et al* 1996). This means that the use of several different parameters in an index may be necessary to provide a more sensitive measure over a wide range of stimulation (Molony & Kent 1997).

Validity is defined as the extent to which a scale actually measures what it is intended to measure (Streiner & Norman 1989). It is important because a scale can be reliable and sensitive without actually measuring what it is purported to measure. When an established scale exists, a new parameter can be validated against it. In the case of animal pain, however, there are no established objective measurement scales against which to validate any new scale. This raises the question of whether measurements of animal pain can ever be truly validated. In humans, physiological and behavioural scales can be validated against verbal self-report of pain. Animals lack this ability, but certain constructs are measured in humans for which verbal report is not a reliable (or possible) source of validation (Streiner & Norman 1989), such as the Glasgow Coma Scale (Teasdale & Jennet 1974). Correlation between several independent measures can provide an alternative source of validation (Beyer & Wells 1989).

Perhaps the most important consideration in the development of animal pain scales is the determination of how significant the pain is to the animal. It is possible that a particular scale could be a reliable, sensitive and valid measure of something that is relatively insignificant to the animal. The magnitude of response may give some indication of the pain level — for example, it is assumed that extreme responses, which place a significant energetic cost on the animal, are associated with severe pain. In the case of behavioural changes, extreme responses may take up the majority of an animal's time budget, and extreme physiological responses may also use up substantial bodily resources, so this would seem to be a fair assumption. In some studies of pain from acute noxious stimulation, operant devices have been used to test the motivation of an animal to escape or avoid the stimulus (Chapman *et al* 1985). In this case, it is presumed that the greater the significance of the pain to the animal, the harder it will work to avoid it. Similarly, experiments that allow animals the opportunity to self-select an analgesic (Colpaert 1987; Danbury *et al* 2000) may be adapted to test the motivation of an animal to gain pain relief.

Behavioural responses to pain

Background and experimental analysis of behaviour

Behaviour (including postural changes) is the parameter most often used to assess animal pain. In the laboratory environment, behavioural analysis has mostly involved the use of standard tests (such as the tail-flick test) which measure withdrawal responses to acute noxious stimulation (Dubner & Ren 1999). More complex behavioural responses are assessed in models of longer-lasting pain (such as the formalin test) and changes in behaviour are used for clinical assessment by veterinarians and by scientists studying animal pain.

Behaviour is also commonly used in human neonate and infant pain assessment and it has even been suggested that pain behaviour in these individuals represents "infantile forms of self-report" (Anand & Craig 1996). It is also the case that, in some species, injured individuals may benefit from informing other members of their group that they are in pain (Fraser & Broom 1997). However, the vast variation in social organisation and prior selection pressure within the animal kingdom means that this will not always be the case. In some instances, animals — particularly prey animals — may have evolved ways of "disguising" responses to pain (Sanford *et al* 1986) or of suppressing both nociception and pain, and this can confound pain assessments.

Despite this, noxious stimulation frequently results in either quantitatively or qualitatively abnormal behaviour, which may or may not be adaptive. In the face of acute pain, escape and avoidance behaviours may be seen (Sanford *et al* 1986), as may vocalisation and defensive behaviours (Zimmermann 1986). For example, Schwartzkopf-Genswein *et al* (1998) quantified escape behaviour of steers following branding using a strain gauge. They found that hot branding caused stronger escape attempts than freeze branding, which in turn caused stronger attempts than sham branding. Castrated pigs show a greater level of high-frequency vocalisations in comparison to uncastrated controls (Weary *et al* 1998). Pain is a common cause of defensive aggression in cats and dogs (Hart & Hart 1985), both as a result of manipulation of painful areas and in unprovoked situations. Animals may direct attention towards the site of their pain, for example by licking or scratching (Short 1999). An increased incidence of licking directed to the scrotal area shown by calves in the 48 days following castration is a likely indication of chronic pain (Molony *et al* 1995).

With longer-lasting pain, various protective or guarding behaviours (where an animal protects an injury or sensitive body part from further environmental stimulation) may become apparent (Zimmermann 1986). For instance, chickens that have had one ankle joint injected with sodium urate crystals (as an experimental arthritis pain model) show a high frequency of standing on the untreated leg (Gentle & Corr 1995). Posture will often be changed as the animal adopts the position that causes it least pain (Hansen 1997). In response to colic pain, dogs may show a characteristic 'prayer' posture where they bow down on their front legs and stand on their hind limbs. In humans, chronic pain often causes depression (Fishbain *et al* 1997), and behaviours seen in animals suffering from chronic pain are sometimes taken as indicators of depression or 'learned helplessness' (Zimmermann 1986), where animals become unresponsive and apathetic following unsuccessful behavioural responses to pain. In chickens, treatments such as repeated feather removal can cause an immobile state that appears to be similar to learned helplessness (Gentle 1992).

As well as studying the undisturbed behaviour of an animal, the reaction of the animal to human approach, or to palpation of the injured area, can be useful in pain assessment (Morton & Griffiths 1985; Sanford *et al* 1986). This is especially true with milder pains where undisturbed behaviour may appear normal.

In some cases the behavioural response may be sufficient to remove the pain; for example, inactivity may ensure that damaged tissue is not stimulated. If the pain in these cases is relatively short-lived it can be argued that welfare is not reduced, because the animal is coping and is no longer feeling pain. However, if extended over a longer period, restriction in behaviour alone can reduce welfare. For instance, immobility resulting from pain associated with leg weakness may stop broiler chickens from accessing food and water as well as from performing other behaviours normally seen within their repertoire (Vestergaard & Sanotra 1999). Administering an analgesic can increase mobility, indicating that the previous lack of movement was due to pain rather than mechanical dysfunction (McGeown *et al* 1999).

Different methods of measuring behaviour as an indicator of pain can be categorised as subjective or objective (Hansen 1997). In practice, however, the situation is not so clearly polarised. Although in essence all assessments of animal pain by humans are epistemologically subjective, there is actually a continuum from subjective to objective in pain-assessment methods. This ranges from unquantified personal judgement, through visual analogue and descriptive scales to detailed quantification of behaviour. At the subjective end of the continuum are assessments that are primarily based on an observer's judgement and that cannot be absolutely verified. That is not to say that these judgements are necessarily less accurate than those that can be more readily verified.

The more objective assessments involve increasingly detailed quantification of clearly defined behaviours. The rigid definition of what is observed should increase inter-observer reliability (Caro *et al* 1979). These approaches may, however, be less sensitive than holistic, qualitative assessments of how an animal is behaving and the context in which it is being assessed, because of the focus on specific behaviours (Wemelsfelder 1997; Wemelsfelder *et al* 2000). In some cases, two instances of behaviour may both come under the same behavioural definition while at the same time appearing stylistically different. Even in studies where behaviour is rigidly quantified, interpretation of results is still subjective (Kavaliers 1988) and this is also the case with physiological parameters where measurement may be considered unequivocally objective.

Subjective methods may, however, rely on observers being very familiar with the species or, in some cases, the individual in question. There is also more reliance on the observer's empathy with the animal so that personality, mood or past experience (Sanford *et al* 1986) may confound the assessment. Any such confounding factor may decrease the reliability and sensitivity of these methods.

A commonly used method for subjective assessment of pain is the visual analogue scale (VAS), where the data collected are estimates (either through self-report or by an observer) of the pain being experienced. These estimates take the form of measured distances on a scale that represents a continuum of experience, commonly from no pain to the worst possible pain. A recent example of the use of VAS pain assessment was a study of the disruption of normal behaviour in castrated lambs (Thornton & Waterman-Pearson 1999). In this study, pain was scored on a scale ranging from "no pain whatsoever" to "the worst possible pain following castration". The results of this study are similar to those of the more objective behavioural assessments detailed below. This is not surprising as these behaviours formed the basis of one of their (Thornton & Waterman-Pearson 1999) VAS assessments. However, the point is that it may not be necessary to undertake the long and formal observations required in the objective experiments to achieve the same result.

Behaviour following castration and tail-docking was first quantified by Mellor and Murray (1989). They found a greater incidence of abnormal postures, such as ventral lying with partial or full hind-leg extension, and restlessness in castrated and docked lambs compared to controls. These observations provided the basis for developing these and other behaviours and postures as indicators of pain in lambs. More recently, the incidence of certain behaviours following treatment has also been quantified (Graham *et al* 1997; Molony *et al* 1997). Either alone or in combination, some of these behaviours are seen at an increased frequency following treatment. A combined index of restlessness, rolling, foot stamping/kicking and easing quarters has been shown to be particularly useful as an indicator of pain resulting from castration and tail-docking in lambs (Molony & Kent 1997; Kent *et al* 1998).

Immediately following beak-trimming in adult birds, behavioural observations suggest an apparently pain-free phase, which is followed (after roughly one day) by a period of acute pain and then a period of chronic pain (Gentle *et al* 1990). The presence of pain is suggested by the decreased willingness to use the beak (to feed, drink, and preen) in treated birds (Duncan *et al* 1989). This represents guarding behaviour. In the later phase, there are other, more general, disturbances in sleeping, eating, activity levels and social behaviour, which are taken as indicating chronic pain. Significant differences in behaviour are seen for many weeks following beak-trimming. These behavioural indicators of pain are reduced in birds beak-trimmed at one day old (Gentle *et al* 1997), suggesting that this is preferable to trimming older birds.

Clinical analysis of behaviour

Behaviour is likely to be the most practical tool for assessing pain in clinical situations (Hansen 1997). Using behaviour to assess pain has the major advantage of being non-invasive, so any effects of assessment on the animal are limited. However, when behaviour is quantified in detail it is often the case that it appears very specific to different types of pain (Lester *et al* 1996). For instance, the behaviour shown in response to the visceral component of castration pain (nociceptive signals from the testes) may be different to that from the somatic component (nociceptive signals from the scrotum) (Molony 1999). Different characteristic postures occur in response to pain in different areas of the body (Hansen 1997). This potential specificity means that detailed study of animals following particular procedures is necessary to identify general and specific responses to different pains. Studies that include anaesthesia and/or analgesia control treatment groups commonly find behavioural and physiological changes in these groups. These changes also have to be identified so that they do not confound results. However, detailed objective measurements of behaviour, of the type that have been undertaken in lambs, are rarely undertaken at the same time as subjective measures, which are probably the most practical method for clinical pain assessment. Detailed measurements of behaviour (over a range of pain severity) are needed to validate these scales and to improve their sensitivity, as quantification of behaviour may highlight differences that other scales miss.

For example, quantification of pain following ovariohysterectomy in dogs highlighted differences between dogs treated with analgesic and those which were not (Hardie *et al* 1997), whereas a numerical rating scale was not sensitive to these differences. Specifically, the benefits of analgesic treatment were shown as a quicker return to 'normal' behaviour, particularly in interactions with a handler. Another behavioural study of dogs following ovariohysterectomy (Fox *et al* 2000) identified particular non-interactive behaviours, such as 'drawing up the rear limbs', which occur at an increased frequency following surgery and which are absent or at a lower frequency after analgesic treatment. Such detailed observations are impractical in clinical situations but the behaviours identified can be inserted into 'day-to-day' pain scales.

In another study of postoperative pain in dogs, a VAS appeared more sensitive to different analgesic treatment than objective measures (Kyles *et al* 1998). It was concluded that the observers, being familiar with the dogs, "may have recorded subtle differences in behaviour based on the personality of each dog" (Kyles *et al* 1998). It is these subtle differences that need to be identified and incorporated into objective pain scales.

Because of individual variation, the best assessment of pain in a single animal would use that animal as its own 'control'. In clinical situations, however, such baseline assessments for comparison are only available from the keeper or owner (Sanford *et al* 1986), as the animal's behaviour is likely to be altered when it is presented for treatment. In the case of elective procedures, behaviour is also not likely to be stable or 'normal' in unfamiliar surroundings. Indicators of pain need to be clearly outwith (either qualitatively or quantitatively) the normal range for that particular species to be appreciated.

Physiological responses to pain

Background and experimental measurement of physiological indicators

Various real or potential threats to an animal result in stress responses. These are adaptive processes that allow an animal to allocate bodily resources quickly to resolve a problem (Wiepkema & Koolhaas 1993). Noxious stimulation normally results in stress responses, which are most commonly quantified by measuring levels of glucocorticosteroids such as cortisol. These are related to activity of the hypothalamo-pituitary-adrenocortical (HPA) axis. Although the hypothalamus does receive input from the nociceptive nerve system (Devey & Crowe 1997), the mechanisms linking pain and stress responses are unclear. Stress responses may occur in anaesthetised animals when nociceptive responses, although present, do not give rise to pain because of suppression in the cerebral cortex (Berkenbosch 1994). However, in animals capable of feeling pain and when other potential confounding factors are controlled for, measurements of changes in these neuroendocrine parameters are useful.

Animals show stress responses to a wide variety of situations and stimuli, and the magnitude of these responses provides measures of the associated distress. This distress can include pain as well as other adverse experiences (Mellor *et al* 2000). This generality of stress responses can limit their use as indicators of pain; for instance, measures of long-term stress caused by beak-trimming are confounded by the decreased social stress in groups of trimmed birds that results from decreased feather pecking (Struwe *et al* 1992). Generality means that measures of stress are too often affected by other factors (eg fear) to be very useful as clinical pain indicators when used on their own (Conzemius *et al* 1997). Physiological parameters may also change by reflex action in relation to trauma (eg the heart rate increases after haemorrhage to maintain tissue oxygen supply; Devey & Crowe 1997) or in response to anaesthetic/analgesic treatment, irrespective of pain (Livingston 1986). In dogs, induction of anaesthesia alone may (Fox *et al* 1994; Benson *et al* 2000) or may not

(Church *et al* 1994; Fox *et al* 1998) cause a rise in cortisol concentration. Certain types of analgesics can have unpleasant effects that cause increases in plasma cortisol on their own (Fox *et al* 1998).

The generality of the HPA axis stress response may, however, have some advantages for pain assessment. For instance, it has been proposed that the measurement of cortisol provides a more non-specific indicator of the degree of distress associated with different methods of castration than behaviour, which can be pain-specific (Lester *et al* 1996). The use of such measures of stress can therefore help in the validation of particular behaviours as pain indicators (Mellor & Murray 1989). It is, however, necessary to show that the behavioural and physiological changes are independent. Some physiological parameters may also relate to the affective component of pain — in other words, the significance of the pain to the animal (Möltner *et al* 1990).

Another problem is that acute stress responses can show 'ceiling' effects (Molony & Kent 1997), which limit their use in assessing severe pain. Such responses could indicate situations in which pain is as bad as it can get. However, reference to other less reactive indicators may show that the ceiling is in the indicator being measured, rather than in the pain experienced by the animal. The presence of such ceiling effects means that the duration of the response may be a better indicator of pain severity than the response magnitude (Berkenbosch 1994). Thus, the area under the response curve (integrated response), which combines magnitude and duration information, is a useful parameter to calculate. Although measurements of some stress responses may not vary sensitively over the full range of pain experience, they may improve the sensitivity of parts of a pain scale (Molony & Kent 1997).

Mellor and Murray (1989) were among the first to measure plasma cortisol following castration and tail-docking in lambs. They found that for the 30 min following castration and docking, cortisol release was maximal and was elevated above that of control animals for 60 min. Subsequent studies have found more prolonged increases in response to surgical castration (Lester *et al* 1996) or in older animals (Kent *et al* 1993). Patterns of cortisol release in lambs following castration and tail-docking often closely correspond to behavioural responses (Kent *et al* 1993; Molony *et al* 1993).

Shorter-term components of the stress response, sometimes called 'fight or flight' responses, include pupil dilation; changes in blood pressure; increased heart rate, respiration, body temperature and muscle tone; and defecation and urination (Sanford *et al* 1986). Some of these may be used as pain indicators. These effects are principally caused by catecholamine release as a result of sympathetic nervous and adreno-medullary hormonal activity. The quick response of these measures means that they can be useful indicators of short-term variations in pain, for instance at the onset of a painful procedure (Mellor *et al* 2000). However, the short latency between stressor onset and these responses means that experimenters will often be partly measuring responses to the sampling procedure itself.

Glatz and Lunam (1994) studied the heart rates of beak-trimmed and sham beak-trimmed chickens and found no difference, indicating another ceiling effect. Heart rate increase appears to be a sensitive measure only of mildly stressful or painful stimuli (Woolley & Gentle 1987) so the handling and restraint of sham beak-trimmed birds could have caused a maximal response. Changes in heart rate have been found following castration, but the influence of other variables means that these are unlikely to be practically useful (Molony & Kent 1997). Changes in the variability of heart rate, reflecting changes in the balance of parasympathetic–sympathetic control of heart function, have been suggested as an indicator of both acute and chronic pain (Lindh *et al* 1999; Storella *et al* 1999). Direct measurements of changes in catecholamine concentration in blood can be used to assist assessments of pain (Ley *et al* 1992), but they rarely are used for this purpose.

Although acute stress responses, such as activity in the HPA and sympatho-adrenomedullary systems, may be of little use in assessing persistent low levels of pain (Mellor & Stafford 1999), slower responses such as changes in immune function (Herzberg *et al* 1994) could be used in chronic pain assessment. For instance, beak-trimming may raise the heterophil : lymphocyte (H : L) ratio of chickens (McKee & Harrison 1995). However, Gentle and Seawright (1988) only found an altered H : L ratio 24 h after trimming and dismissed the measure as a potential indicator of chronic pain.

Clinical measurement of physiological indicators

As with behaviour, measurement of physiological variables has been an important means of human infant pain assessment (Abu-Saad 1998). Measurement of stress responses has also proven useful in assessing pain in some experimental veterinary studies. For instance, Benson *et al* (2000) found a reduced physiological stress response when dogs were given analgesic prior to ovariohysterectomy. However, such studies are based on comparisons of control and treatment groups; measurements such as cortisol concentration are unlikely to be useful for assessing pain in individual animals. In the clinical environment it is not feasible to control for the various factors, such as fear or normal circadian cycles, that can affect indicators of stress. Measurement of some parameters may also require invasive collection methods, such as a blood sample for cortisol testing (although cortisol can now be measured from saliva or urine samples in many animals; Beerda *et al* 1996). The normal physiological range of some parameters may also be altered during illness — not specifically because of pain (eg Church *et al* 1994). Even when a clear relationship between pain and parameters such as cortisol concentration exists, they are unlikely to be used on individual animals in the clinical setting because of the time necessary for analysis of samples.

However, in experimental studies of clinical problems, physiological parameters may prove useful by helping to validate behavioural indicators. Hansen *et al* (1997) studied physiological parameters in dogs following ovariohysterectomy and although cortisol levels were apparently lower in dogs that had undergone surgery and been given an analgesic compared to those not given an analgesic, the effect was limited. The behaviour of the same dogs was analysed in a separate paper (Hardie *et al* 1997); however, it would have been useful if the authors had compared behavioural and physiological responses in individual animals, in addition to describing behaviour and physiology separately. Similarly, Fox and co-workers could have compared their studies of physiological (Fox *et al* 1998) and behavioural (Fox *et al* 2000) responses following ovariohysterectomy. Such comparisons may be useful in the search for behavioural indicators for clinical pain assessment following ovariohysterectomy.

Some stress responses can be more easily measured and analysed and could potentially be used as clinical indicators of pain. However, Hansen *et al* (1997) appear to dismiss measurements of heart rate, body temperature and respiration rate as indicators of a postoperative stress response in dogs. Holton *et al* (1998) also apparently dismissed the use of heart and respiratory rates and note the wide variation in heart rate within their control group of healthy dogs. This individual variation is one of the main problems in clinical assessment of pain. Holton *et al* (1998) did identify a potential relationship between pupil dilation and pain, but they suggest that it is unlikely to be useful under clinical conditions. This is because pupil dilation is technically difficult to measure and may alter in relation to other factors. Despite the dismissal of heart rate, respiratory rate, body temperature and pupil dilation in these studies, these parameters have been found to be useful in clinical pain assessment as part of a multifactorial pain scale (Firth & Haldane 1999).

Neurophysiology

In the experimental setting, further evidence of pain and validation of other indicators may be provided by studies of activity in nociceptive afferent nerve fibres. These studies represent a basic step in demonstrating the existence of pain, rather than being practically useful in pain assessment.

Cottrell and Molony (1995) studied activity in the superior spermatic nerve of lambs following castration by application of a rubber ring. They found activity in the nerve for 90 min following application of the ring. This is a similar time course to the major behavioural and endocrine changes seen following rubber ring application (Kent *et al* 1993; Molony *et al* 1993).

Following beak-trimming, the temporal pattern of afferent nociceptive activity (Gentle 1991) relates well to the pattern of behaviour (Gentle *et al* 1991), which indicates a pain-free phase followed by a period of acute pain. The behavioural evidence for chronic pain is also backed up by longer-term evidence of spontaneous activity in nociceptive fibres (Breward & Gentle 1985).

Injury and pathology

The presence of obvious physical tissue damage can provide an indication that pain may be present (Dantzer 1986). This may provide a starting point for other methods of assessment. A problem with using physical evidence of damage, however, is the variable relationship between injury and pain (Wall 1979). In the extreme this means that injury can occur without pain, and that pain can occur without injury. Internal damage may also be missed by human observers and is difficult to assess (Dantzer 1986).

Neuromas, which form following injury as a result of regenerating nerves failing to connect to appropriate tissue (Breward & Gentle 1985), are one form of pathology that may cause pain. Tail-docking can lead to the formation of neuromas on the tail stump (French & Morgan 1992). In humans, amputation of limbs and the associated neuromas can cause 'phantom' neuropathic pain sensations, which may be extreme and long-lasting (Jensen & Nikolajsen 1999). This raises the question of whether lambs suffer from chronic neuropathic pain following docking (Jackson *et al* 1999). In poultry, neuromas formed following beak trimming may continue to develop for at least 70 days following the mutilation (Gentle 1986b) and may be responsible for the abnormal, spontaneous nociceptive activity recorded by Breward and Gentle (1985). Such long-term damage is reduced when birds are trimmed at one day old, which is consistent with behavioural evidence that pain is reduced with early trimming (Gentle *et al* 1997).

Both inflammatory and neuropathic pain can cause hyperalgesia ("increased sensitivity to noxious stimulation") and allodynia ("pain due to a non-noxious stimulus", such as a light touch) (IASP 1979). Measuring the nociceptive threshold (the point at which a noxious stimulus elicits a response) can give an indication of the pain that an animal may suffer as a result of stimuli in the environment and can therefore be useful in pain assessment. In dogs for instance, analgesic treatment prior to ovariohysterectomy was shown to reduce subsequent hyperalgesia, as measured by mechanical nociceptive threshold, reducing postoperative pain (Lascelles *et al* 1997). However, it should be noted that the nociceptive threshold may not be related to the pain that the animal is suffering at the time of the test.

When and how the tissue damage, or altered nociceptive threshold, is measured is important. For example, in the case of lamb castration with rubber rings, after the acute pain has passed the presence of the rubber ring around the neck of the scrotum causes a lesion and inflammation in the surrounding tissue (Kent *et al* 1999). These lesions could cause lambs to suffer longer-term chronic pain. However, nociceptive thresholds in lambs have only been measured in the 72 h following castration (Thornton & Waterman-Pearson 1999) and other assessments of the severity of inflammatory lesions do not correspond directly to behaviour patterns over the six weeks following castration (Kent *et al* 2000a). Because assessing behavioural alterations resulting from changes in pathology over time can provide evidence for the validity of behavioural indicators of pain, measuring longer-term changes in nociceptive threshold could help to validate behavioural indicators of chronic pain in lambs. The results of nociceptive threshold testing can differ depending on the type of stimuli used (ie thermal versus mechanical; Welsh & Nolan 1995), as different stimuli depend upon central or peripheral changes in nociceptive processing. Other possible measures of the severity of damage and associated inflammation include infrared thermography (Molony *et al* 1995), changes in levels of acute-phase proteins (Kent 1992), or laser-doppler velocimetry (Quinn *et al* 1991).

Relief from pain

Analgesic treatment in pain assessment

As well as being used to relieve pain, analgesics and anaesthetics are used in pain assessment. In horses, for instance, local anaesthetic nerve-blocks of particular parts of the leg or foot can help to identify the source of lameness (Wyn-Jones 1988). In lambs, administration of general anaesthesia during castration has helped to distinguish behaviour associated with the pain at the time of castration, which was abolished, from post-castration pain, which was unaffected (Thornton & Waterman-Pearson 1999). From this result, it was possible to confirm that although the application of a Burdizzo clamp causes pain at the time of castration, post-castration pain is reduced.

When assessing pain, it is necessary to combine analgesic treatment with the measurement of behavioural and physiological changes. If an animal showing abnormal behaviour or an abnormal physiological response is returned to 'normal' by the administration of an analgesic, the inference is made that the abnormal response was caused by pain. In lambs, administration of a local anaesthetic prior to castration and tail-docking removed the behavioural and endocrine changes normally seen (Wood *et al* 1991). There is also some evidence that analgesic treatment may reduce pain following beak-trimming (Glatz *et al* 1992).

There is, however, the danger that using the effects of analgesic treatment on behaviour to recognise pain becomes a circular argument (ie pain is something removed by an analgesic; an analgesic is something which removes pain; Bateson 1991). It is particularly important that substances that prevent the animal from expressing pain, rather than actually relieving the pain, are not mistaken for analgesics (Wall 1992). Ideally, the presence of pain should be validated with other indicators. It should also be noted that analgesic drugs may have behavioural effects unrelated to pain and nociception. For instance, some analgesic drugs also have general sedative effects, which decrease activity (Hardie *et al* 1997). Analgesic drugs may also have unpleasant side effects — for instance, putative dysphoric effects of butorphanol may be the cause of increased vocalisation in dogs (Fox *et al* 2000).

Self-selection of analgesic

The presence of pain may be inferred with even more certainty if an animal is given the opportunity to self-select an analgesic and does so (Colpaert 1987). Danbury *et al* (1997, 2000) have used analgesic self-selection experiments to assess pain due to lameness in broiler chickens. In these experiments, lame broilers have self-selected both morphine and the non-steroidal anti-inflammatory drug (NSAID) carprofen. However, healthy birds also showed a preference for morphine, so care must be taken with these sorts of experiments to ensure that the drug is selected for its analgesic properties and not for other reasons. In a clinical setting, the administration of analgesia orally within an animal's food has the potential to reduce postoperative stress (Flecknell 1996) and the opportunity for self-administration could be beneficial.

Endogenous pain relief

The pain felt by an individual following injury may be modulated by various mechanisms within the body. Some mechanisms, such as central sensitisation in the dorsal horn of the spinal cord (Doubell *et al* 1999), may act to increase the pain experienced. There are also various endogenous pain-relief mechanisms, and investigating these can be informative. For instance, Hao *et al* (1998) have shown in rats that individual variation in neuropathic-pain-related behaviour may be attributable to individual differences in the capacity for endogenous opioid analgesia. Assessing such individual variation is important in the validation of pain indicators because, in response to a painful stimulus, some animals may respond (in terms of the measure) and some may not; this could either be because the measure was not related to pain, or because not all individuals were in pain.

Evidence of ongoing endogenous-opioid-dependent analgesia can be obtained by the administration of opioid antagonists, such as naloxone, which should increase the severity of pain experienced. Using naloxone, Wood *et al* (1991) found that lambs may have only limited access to such pain relief in response to castration and tail-docking. This suggests that lambs do not use or do not have an opioid-based physiological way of coping with this pain, which conflicts with the suggestion that endogenous analgesia is an evolved adaptation to suppress pain in situations where pain reduces survival chances (eg by disrupting motor performance; Duggan 1992). However, the expression of pain-modulating mechanisms is highly variable: some noxious stimuli cause inhibition and some cause facilitation of nociception and pain (Fields & Basbaum 1999). For animals that have pain-inhibitory mechanisms, it is not valid to assume that if the mechanisms are not used following a given stimulus, pain is not being felt.

Recent work in the chicken has shown that motivational and attentional changes within an animal may influence the pain it experiences. For instance, feeding motivation may reduce the amount of pain-related behaviours shown by chickens (Wylie & Gentle 1998). Being placed in a novel environment may also focus attention such that pain is apparently no longer felt (Gentle & Tilston 1999). These effects are related because different motivational priorities guide the animal's focus of attention. In practice, this might affect animals brought to a novel environment such as a vet's surgery and could reduce behavioural indicators of pain (Hansen 1997).

The assessment of pain in animals could also be potentially confounded by a phenomenon such as 'predator-induced' analgesia, one of a wide variety of 'stress-induced' analgesias (Kavaliers & Colwell 1991). Many animals view humans as predators (Caine 1992) and the presence of a predator will hold an animal's attention. Predator avoidance is generally a high-priority behaviour, and might, temporarily at least, displace awareness of pain.

The activation of any endogenous pain-relief (coping) mechanism, while meaning that the animal may no longer be expressing pain, could still represent reduced welfare. This is because it may not be possible to sustain the coping mechanism and because the cause of the pain will remain. An injury or stressor severe enough to trigger a coping response always represents a challenge to the animal's welfare (Fraser & Broom 1997).

Conclusions

Combining objective and subjective assessments: the best of both worlds?

Current assessments of pain are often based on extremely limited scales or single parameters. However, pain is a complex multidimensional phenomenon and the responses of animals to it are also complex. When assessing behavioural changes induced by pain, it may be that qualitative observation by an experienced observer is the only method truly capable of capturing this complexity.

Although such assessments made by those familiar with the animal or species in question are often very useful in pain assessment (Flecknell 1996), this degree of familiarity is not always available to the observer. It is therefore necessary to attempt to pick out the relevant indicators by comparing these assessments with objective quantification of the animal's responses to pain. The relevant indicators can then be used to allow a closer approximation of the assessment of experienced observers to be made. Although such comparisons of objective and subjective measures on the same animals (eg Hardie *et al* 1997; Kyles *et al* 1998) often show differences between the two methods, these differences should be used as a base for progress by investigating their sources and picking out the useful information from each method.

Objective studies have value in their own right and can provide useful information for incorporation into more qualitative assessments. For instance, a subjective VAS assessment of lamb pain (Thornton & Waterman-Pearson 1999) was based on the active behaviours that had proved to be useful indicators in more objective studies (Mellor & Murray 1989; Molony *et al* 1997). The more objective nature of resulting scales should reduce the impact of observer bias, which could act to either underestimate or overestimate the degree of pain felt by an animal. Quantification of responses to pain may also be necessary for animals that are phylogenetically further from humans (eg amphibians; Machin 1999) and in which subjective assessments of pain may be less reliable.

Interaction between the alternative methods of data collection allows the best possible assessments to be made using either clinical qualitative scales that have an objective basis or objective scales that have included factors used by experienced observers. The method chosen will depend upon what is most appropriate in a given situation (ie clinical or experimental pain assessment) and what is possible in terms of observation time, the training required and other such considerations.

Pain assessment through multiple measures

Comprehensive study of different pains in different species allows the generality or specificity of particular responses to be assessed. These can then be used in pain assessment scales that are specific to the source of the pain (ie particular injuries or operations) and to the species (eg Morton 1999). Measurement of single parameters or several closely related parameters is unlikely to give a full account of the complex response to pain. A scale that allows measurement over the whole range of an animal's pain experience will need to include a range of measures that relate well to different portions of that experience, as it is unlikely that one measure will sensitively co-vary with pain over its entire extent.

A combination of behavioural and physiological measures is generally more comprehensive than either alone. These different responses may relate to different aspects of the pain experience and have different time-scales and sensitivities. An assessment of the degree of injury can act as a guide to the degree of likely pain. Although the relationship between pain and injury is variable (Wall 1979), very often pain does increase with increasing injury. So, the degree of injury (ie amount of tissue damage, tissue sensitivity to pain) gives an approximation for the amount of expected pain. For example, Mathews (2000) lists anticipated pain following various operations, illnesses and injuries. It is important to remember that pain may be greater or less than estimated in this way and fine scales for pain assessment are required to truly meet an animal's pain-relief requirement. In experimental studies, close correlation between several variables provides validation that pain is present (eg the close temporal correlation between nociceptive activity, behaviour and endocrine measures seen following lamb castration or the relationship between anatomical evidence of neuroma formation, nociceptive activity and behaviour following beak trimming in poultry).

For clinical utility, although simplicity is desirable, pain scales need to be comprehensive. In this setting it is crucial that the scale is useful for individual animals and can distinguish responses to pain from lingering effects of anaesthetic (Firth & Haldane 1999) while being sensitive to effective analgesic administration. It should also be quick to apply and require limited training (Hansen 1997) without the need for complex instrumentation.

Firth and Haldane (1999) have recently developed a numerical rating scale for use in dogs following ovariohysterectomy, which includes various physiological and behavioural parameters such as heart and respiratory rates, response to palpation, activity, and change in temperament, posture and vocalisation. Pain assessment using this method can be used to discriminate between different analgesic treatments in a consistent manner and appears to be more objective than visual analogue scales, while retaining practicality. However, this scale is a useful first attempt, rather than the finished article. There is considerable variability, which limits the value of this scale for assessing pain experienced by individual animals. Although there is good agreement between observers at the treatment-group level, the potential difference in pain scores assigned to individual animals is large in comparison to treatment differences. Also, many of the factors in the scale rely on comparison with pre-operation values. In an experimental study such as this, these may be useful comparisons; in clinical situations, however, there is a danger of underestimating postoperative pain if dogs were in pain before the operation. The behavioural parts of the scale could potentially be adapted to include parameters identified by detailed studies such as those by Hardie *et al* (1997) and Fox *et al* (2000).

Animal welfare implications

From analogy with human experience, both acute and chronic pain give rise to animal welfare problems when present. The presence of pain over very long time periods represents a particularly significant welfare problem. Such chronic pain may also reduce welfare in many other ways by causing disturbances in 'on-going' behaviours such as feeding, sleeping, exploration and social behaviour (Chapman *et al* 1985; Zimmerman 1986).

Ultimately, good pain assessment scales need to be applied to the problem of reducing the pain suffered by animals in various situations. In clinical situations, where all concerned would be expected to have an interest in reducing pain, the effect of a good pain scale is direct and potentially substantial. Despite this agreement, however, recent surveys of veterinary students and practitioners have identified significant variation in the clinical recognition and treatment of pain (Capner *et al* 1999; Hellyer *et al* 1999; Paul & Podberscek

2000). These studies highlight the need for further development of clinical pain-assessment tools.

There are likely to be substantial inter-individual differences in the experience of pain. These differences can be attributed to genetic factors (Mogil 1999) or the individual's early experience and environment (eg Melzack & Scott 1957; Pieretti *et al* 1991; Anand *et al* 1999). Individual differences also exist in the efficacy of analgesic treatment. This individual variation in the experience of pain means that a standard dose of a drug will be too much for some and too little for others (Flecknell 1996). Under-treated individuals will still suffer pain, and over-treatment should be avoided as both opioid and NSAID analgesics can have detrimental effects. Although experimental studies may identify suitable analgesic regimes for the average patient, pain scales are still required to assess the pain experienced by the individual animal in a clinical setting. Effective analgesic treatments can then be applied according to the needs of individual animals. As well as removing the aversive experience of pain, effective analgesia can reduce subsequent morbidity and mortality (Cousins & Power 1999).

In other situations, such as agriculture or laboratory research, there may often be an interest — very often financial — that runs counter to pain reduction. For example, methods for reducing pain associated with castration and tail-docking have been developed (Molony *et al* 1997) but would increase costs to farmers. In these cases, a better understanding of the pain experienced by animals in particular situations can inform moral debate on the acceptability of those situations. However, actual improvements in welfare are reliant upon the value that society places on the reduction of pain in animals.

Update

In the period between the acceptance and publication of this paper, two further references have become available. These papers are relevant to topics discussed in the review. Holton *et al* (2001) describe a behavioural pain-assessment scale developed for dogs and based on statistical analysis of behaviours considered by numerous veterinarians to be indicative of pain. The statistical techniques described provide a way of distilling down numerous descriptors to only the few most common and relevant. This study represents an attempt to produce a valid pain scale using subjective interpretations of behavioural alterations. (It is interesting to note that the authors have discounted physiological indicators altogether.) Wiseman *et al* (2001) catalogue the behaviours reported by owners as indicating chronic orthopaedic pain in dogs.

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