REVIEW ARTICLE

Refinement of animal use—assessment and alleviation of pain and distress

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Summary

The refinement of experimental techniques represents an important opportunity to improve the welfare of laboratory animals. Objective methods for the assessment of pain and distress in animals are needed before procedures that are claimed to be refinements can be evaluated. The methods currently used for assessment of pain and distress are unsatisfactory, and are often based on uncritical anthropomorphic assumptions. Future developments may enable the establishment of well validated clinical scoring systems, or identification of biochemical or physiological indices of pain or distress. If reliable methods of pain assessment can be developed, then a critical evaluation of the methods available for the alleviation of pain and distress can be undertaken. This article reviews methods of clinical pain assessment in animals, with reference to the techniques used in man. Techniques for pain alleviation are briefly reviewed.

Keywords Pain; distress; animal; analgesics

The reprinting of Russell and Burch's classic text *The Principles of Humane Experimental Technique* in 1992 serves to highlight the problems that still remain in implementing the 'three Rs' of Replacement, Reduction and Refinement. Russell and Burch defined refinement as 'simply to reduce to an absolute minimum the amount of distress imposed on those animals that are still used'. Although recognizing that refinement is important, the text devotes significantly less space to this concept, and the authors noted that of the three Rs, refinement presented more formidable difficulties to the experimenter. More significantly, throughout the discussion of refinement, an underlying assumption appears to have been made that procedures which are distressing to humans will also be distressing to animals. Although few would disagree with the general implications of this view, it is important that the assumption is not extended to assert either that procedures which cause distress in humans will cause an equal degree of distress in animals or that procedures which do not cause distress in humans will not cause distress in animals. This view is almost certainly unwarranted, and in some instances could lead to a reduction in the welfare of an individual animal. The promotion of refinement of experimental techniques requires more than the adoption of uncritical anthropomorphic views. A critical approach based upon careful assessment of pain and distress in animals is necessary.

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The importance of assessment of pain and distress

It is perhaps not immediately apparent why assessment of pain and distress is so

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central to the question of refinement of experimental techniques. It is helpful to consider 2 examples. Blood sampling of animals is required as part of a wide range of different research projects. The potential for refinement in blood sampling techniques has been the subject of a recent working party report (BVA/FRAME/RSPCA/UFAW 1993), and the use of atraumatic, minimally invasive procedures advocated. Many of the opinions concerning the desirability or otherwise of different blood sampling techniques are based not upon an objective assessment of the degree of pain or distress caused by each technique, but upon a subjective evaluation using anthropomorphic criteria. This makes evaluation of new techniques difficult, particularly when attempting to compare an invasive technique carried out under anaesthesia, with a technique which appears less invasive but requires manual restraint of an animal. If an objective method of assessing the pain or distress caused by each technique could be developed, then such comparisons could be undertaken more reliably, and agreed improvements adopted by all concerned.

A second example of the need to assess the degree of pain or distress arises when an intervention designed to alleviate pain is contemplated, for example administration of an analgesic following experimental surgery. The decision of whether to administer an analgesic may initially have been made on anthropomorphic criteria—if a surgical procedure will cause pain in man, it will also cause pain in an animal. However, the choice of analgesic should be influenced by the degree of pain that is actually present. If inappropriate use is made of a potent analgesic, then the undesirable side-effects of the agent may outweigh any potential pain alleviating effects. To determine the degree of pain that is present, and hence an appropriate analgesic regimen, some form of assessment is required. Without a scheme of assessment, it is necessary to assume that the degree of pain present will be identical in humans and animals after identical procedures. A consideration of the differences in anatomy, posture and behaviour between animals and man illustrates that this assumption is unlikely to be correct. Furthermore, in order to provide effective pain relief for as long as required, if no assessment scheme is used then it is necessary to assume that the duration of pain following a procedure is identical in animals and man, and that the rate of decrease in the magnitude of the pain is also the same. Even if these assumptions were to be true, it is also necessary to assume that all animals will experience an identical level of pain after undergoing a particular procedure, and that each animal will have an identical response to a particular dose of analgesic. In man, it is well established that different individuals have different analgesic requirements after apparently identical surgical procedures (Alexander & Hill 1987). In man, the dose of analgesic administered, and the frequency and duration of treatment can be adjusted by assessing pain in each individual patient. In animals, it seems reasonable to assume that effective pain relief can be achieved only by making a similar assessment. Selection of an arbitrary initial dose of analgesic is unlikely to prove uniformly effective. The response to a particular dose of a compound has been shown to vary considerably between animals of different strains, ages and sexes (Lovell 1986). Studies of the responses to opioids using experimental analgesiometry to assess the agents' efficacy have demonstrated that this variation in response occurs with morphine and other opioids (Frommel & Joye 1964, Katz 1980, Moskowitz *et al.* 1985). The degree of variation is considerable (Fig 1), and it is clear that selection of a particular dose regimen is likely to result in over-dosage of some animals, and provision of inadequate analgesia for others.

A final complication lies in the information which has been used to provide the dose rates of analgesics recommended for laboratory animals. In most instances these have been derived from studies using analgesiometric tests such as the hot-plate or tail-flick test (Flecknell 1984, ILAR 1992,

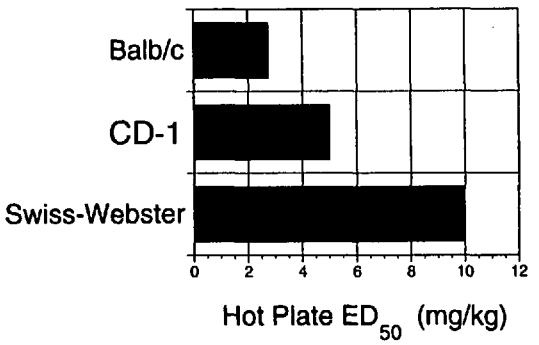

Fig 1 Hot plate ED_{50} of morphine in 3 strains of laboratory mice (data redrawn from Pick *et al.* 1991)

Liles & Flecknell 1992b). Although efficacy in analgesiometric tests provides a reasonable prediction of the potency of an analgesic in man (Taber 1974), the dose required to alleviate post-operative pain may vary considerably from the effective dose in an analgesiometric test. Data from such tests thus provide a useful indication of the relative potency of analgesic agents in animals, but some means of assessing clinical pain is required to determine appropriate dose rates following particular experimental procedures.

It is therefore of fundamental importance that objective methods of assessment of pain and distress are developed. This will enable comparison of different experimental techniques or modifications of existing techniques and selection of those modifications or methods which cause least pain or distress to the animal. It will also enable refinements such as analgesic administration, aimed at alleviating pain or distress, to be developed and evaluated.

Methods of assessment of pain and distress

The assessment of stress and distress in laboratory animals has been extensively reviewed (ILAR 1992, Manser 1992), hence this paper will consider primarily the assessment of pain. Pain has been defined as an 'Unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage' (IASP 1979). A key element in this definition is the subjectivity of the experience. In man, verbal or written communication of emotional states can at least be attempted, but in animals the lack of any direct means of communication prevents such interchange. It is helpful to consider how pain is assessed in those humans who cannot undertake written or verbal communication—babies and young infants. It is only relatively recently that the issue of pain in human neonates has been addressed as a significant issue (Anand 1990). Simple humanitarian considerations, together with a realization that pain results in detrimental stress responses, has led to a growth of interest in providing post-operative pain relief in human infants (Anand *et al.* 1987, Beyer & Bournaki 1989)

A range of different approaches have been adopted to assess the degree of pain and the efficacy of different analgesic regimens in infants (McGrath & Unruh 1989). The most widely used techniques have used pain scoring systems based upon criteria such as crying, facial expression, behaviour and posture. A trained observer, for example a paediatric nurse, uses a scoring system either to generate a numerical score, or to complete a visual analogue chart on behalf of the infant (McGrath & Unruh 1989). Visual analogue scales (VAS) have been widely used for pain assessment in man, and consist simply of an horizontal or vertical line, marked at one end with the phrase 'no pain' and at the other by 'worst possible pain' or similar wording. The patient marks on the line their current pain intensity. Using these techniques, a range of studies have assessed pain in infants and compared different analgesic treatments (Mather 1983).

Use of this type of scoring system for pain and distress in animals was proposed by Morton and Griffiths (1985), and these authors' proposals influenced numerous other working party reports and reviews over the succeeding years (Association of Veterinary Teachers and Research Workers 1986, LASA 1990, Flecknell 1991, ILAR 1992, Sanford 1992). As was pointed out in

their initial publication (Morton & Griffiths 1985), the problem with assessment of pain and distress lies both in the lack of specific indicators of pain, and the subjective nature of the assessment system. Although successful application of pain scoring has been reported (Leese *et al.* 1988), rigorous investigation of this type of scheme has highlighted the practical difficulties associated with its implementation. Particular problems noted were the considerable between-observer variation and the poor predictive value of certain clinical signs in particular circumstances (Beynen *et al.* 1987, 1988). Variation in pain scores between observers is a common problem in human pain assessment, but can be overcome to some extent by careful training for a specific clinical research project. Unless a small number of easily assessed indices of pain can be developed, introduction of such training for each potentially painful procedure carried in research animal units is unlikely to be practicable.

Despite these difficulties, pain scoring has been used in a number of investigations in veterinary clinical practice involving companion animals. For example the relative efficacy of several different analgesic regimens has been assessed in dogs: intercostal nerve block, intrapleural bupivacaine and systemic morphine following thoracotomy (Thompson & Johnson 1991); epidural or intravenous morphine after thoracotomy (Popilskis *et al.* 1993); and flunixin, carprofen and papaveretum following a variety of surgical procedures (Reid & Nolan 1991, Nolan & Reid 1993). These studies illustrate a second important consideration in pain scoring techniques. The clinical signs used in these studies were assumed to indicate pain, this assumption generally being based upon previous clinical experience. Administration of an effective analgesic would therefore be expected to reduce the overall pain score. Central to this approach is the need to validate the scoring system used by inclusion of appropriate control groups. In many of the studies of post-operative pain in veterinary clinical practice, the investigators have not considered it ethical to include a group of animals which received no post-operative analgesic (Taylor & Houlton 1984). In addition, many studies are unable to include control data from normal animals which do not undergo surgery, but which receive the analgesic agents, and some studies do not include pre-operative scoring of the animals. It is therefore uncertain what scores untreated animals would have shown post-operatively and whether the non-specific effects of analgesics might influence the scoring system used. The inclusion of an untreated control group which has undergone surgery poses an ethical dilemma, but it is important to consider that many animals still receive little or no post-operative analgesia either in veterinary clinical practice or in research institutes in many European countries, including the UK. Establishing a validated pain scoring scheme could lead to the adoption of more widespread use of analgesics. It is also important to note that some of the detrimental effects of surgery in rats, such as loss of body weight and suppression of food and water intake can also be produced in normal, unoperated rats by administration of opioid analgesics (Liles & Flecknell 1992a). Inappropriate use of analgesics may therefore be detrimental, highlighting the importance of administering these potent agents only when required.

In order to implement refinement effectively, it therefore seems reasonable to examine each procedure where analgesia may be required, carry out a monitoring or assessment scheme and compare the scores after the administration of an analgesic. Although this will require withholding of an analgesic initially, it will enable the potential beneficial effect of analgesic treatment to be evaluated critically. Once the effects of a particular treatment regimen have been established in a particular procedure, the influence of more frequent dosing or extended treatment periods can be assessed. During this evaluation, inclusion within the study protocol of carefully designed criteria for intervention and administration of an

analgesic should ensure that animals are not subjected to severe or prolonged pain.

A further difficulty associated with the use of clinical scoring systems has been recognized in studies of human subjects. When pain scoring carried out by the patient, using a visual analogue scale (VAS) was compared with VAS scores made simultaneously by various medical staff, the correlation between scores was poor, especially for patients who reported they had significant pain (Grossman *et al.* 1991). The considerable variation reported between child self-reporting of pain, parent ratings and nurse ratings (Manne *et al.* 1992) also has implications for pain scoring in animals. These and other similar investigations in man highlight the importance of careful and thorough validation of pain scoring systems, something which has yet to be achieved in studies of clinical pain in animals.

Objective assessment of pain

As discussed earlier, animals are unable to communicate directly their experiences of pain with us, so reliance must be made on behavioural and physiological indices of pain. In an attempt to avoid the subjective nature of clinical scoring systems, various workers have used more objective behavioural scoring systems, or have used biochemical, physiological or other measurements as indices of pain. In lambs, treatment with local anaesthetic was shown to block the cortisol and behavioural responses to tail docking and castration (Wood *et al.* 1991). In rats undergoing surgical procedures, administration of nalbuphine (Flecknell & Liles 1991) or buprenorphine (Liles & Flecknell 1993a,b,c) was shown to reduce the depressant effects of surgery on food and water consumption and body weight.

Adjuvant-induced arthritis has been extensively studied in the rat, and is believed to represent a model of chronic pain. This conclusion has been based upon behavioural changes, changes in vocalizations, changes in weight gain and hyperventilation that occur in this model, coupled with the response of these variables to analgesics (Colpaert 1987).

Rats with chronic arthritis have also been shown to self-administer both a non-steroidal antiinflammatory drug (NSAID) (suprofen) (Colpaert *et al.* 1980) and an opioid (oral fentanyl) (Colpaert *et al.* 1982). Unfortunately, the conditioning period required for self-administration studies make similar investigations of acute post-operative pain in animals impracticable.

Changes in exploratory behaviour in a novel environment were proposed by Barclay *et al.* (1988) as an index of pain or stress, and were used to evaluate a range of experimental techniques. So far this approach has not been used to assess possible changes following surgical procedures.

In certain specific circumstances, other physiological parameters can be used. For example, following thoracotomy, indices of respiratory function can be used as possible indicators of pain. In man, provision of effective analgesia following thoracotomy has been shown to have a beneficial effect on pulmonary function (Kaplan *et al.* 1975, Toledo-Pereyra & DeMeester 1979). In the dog following thoracotomy, improvements in arterial pO_2 occurred after administration of an analgesic (Flecknell *et al.* 1991), however the positive effects of analgesics may have been detectable in these animals only because their pulmonary function was seriously impaired as a result of lung transplantation.

In all of these animal studies, and in other attempts which have been made to validate clinical scoring systems, the assumption is made that if changes to a variable occur after a procedure that would cause pain in man, then that change may be related to the presence of pain in an animal. If administration of an analgesic reverses the changes associated with the procedure, this is taken as evidence that the changes were, at least in part, pain related. This is, of course, a somewhat circular argument, in essence stating that pain associated changes are those that are reduced by administration of analgesic drugs. The analgesics are, of course, only known to have a pain alleviating effect in humans, since there is no objective measure

of pain in animals. It is important to emphasize that the term 'pain' is used here to describe the conscious perception of noxious stimuli, and not measurement of peripheral nociceptive activity.

The definition of pain proposed by the International Association for the Study of Pain (IASP 1979) is helpful in making this distinction: 'Pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage'.

Although it has not been possible to produce a direct measure of animal pain, the validity of these indirect measures can be strengthened in a number of ways. If the underlying assumption that procedures that would cause pain in man will also cause pain in animals is accepted, then the magnitude of the change in any postulated index of pain should increase with procedures of increasing severity. This has been demonstrated when food and water and body weight, and locomotor activity were recorded in rats, which were then subjected to 3 surgical procedures of varying severity (Liles & Flecknell 1993b). Changes in the indices used should also be reduced by several different methods of analgesia, in order to confirm that the effects are not due to other, non-analgesic effects of a particular class of drug. The effects of surgery on food and water intake and body weight in rats have been shown to be reduced both by an opioid analgesic, buprenorphine, and a potent NSAID, carprofen (Liles & Flecknell 1993c), but the effects of surgery on locomotor activity were not affected by the NSAID. It is clearly important that the analgesics used do not have a positive effect on the proposed indices of pain in normal animals. In this respect, buprenorphine has been shown to stimulate activity (Liles & Flecknell 1992a), so making interpretation of the positive effects after surgery difficult. In contrast, NSAIDs and opioids have either little effect or a depressant effect on food and water consumption in normal animals, and hence positive effects on these variables following surgery are perhaps more convincing evidence of a specific analgesic action.

When considering post-surgical or post-trauma pain, it is possible that the behavioural and other changes observed as part of a pain scoring system may be related to a general surgical stress response, and so may not indicate the degree of pain which is present. Furthermore, the normal endorphin release which occurs following surgery or trauma might itself be responsible for some of the effects observed. If the effects are endorphin-mediated, then administration of a mixed opioid agonist/antagonist such as nalbuphine (Flecknell & Liles 1991) or a partial agonist such as buprenorphine (Liles & Flecknell 1993a,b) might be expected to have a beneficial effect by antagonizing the effects of endorphins. This hypothesis can be tested by administering a pure opioid antagonist such as naltrexone or naloxone. These agents are devoid of analgesic effect, so a positive effect on the proposed indices of pain would suggest that these indices are not pain related. If an opioid antagonist had either no effect, or increased the detrimental effects of surgery, then this would support the view that the variables assessed were related to pain. This type of study has been carried out in lambs (Wood *et al.* 1991), in which it was shown that the proposed pain-related behaviours were either enhanced or unchanged after naloxone administration. This type of investigation clearly has important ethical implications, since reversal of endogenous opioids by a μ antagonist could result in increased pain sensation. As with other use of animals, however, the detrimental effects on those animals studied must be balanced against the very considerable benefits to substantial numbers of other animals.

Methods of alleviation

If reliable methods of pain assessment can be developed, then a wide range of different techniques for pain alleviation can be employed. As mentioned earlier, virtually all analgesic agents are assessed for efficacy and toxicity in small laboratory animals, so considerable information is available to

form a basis for use of these agents to alleviate pain. A variety of different methods of administration of analgesics have been developed in man, and these have frequently been used in laboratory animals in studies relating to the mechanisms of pain sensation. For example epidural and intrathecal administration of opioids has been used to provide effective pain relief in man and in larger animal species in veterinary practice to alleviate post-operative pain (Dodman *et al.* 1992), although lack of a validated method of pain assessment has prevented full evaluation of its efficacy. The technique has been used as a tool to investigate the role of spinal nociceptive mechanisms in laboratory animals (Yaksh *et al.* 1988). Although the practicality of these techniques may be questionable in small rodents, percutaneous intrathecal (spinal) or epidural injection of local anaesthetics has been described in the rabbit (Kero *et al.* 1981, Hughes *et al.* 1993). In this and larger species, intrathecal or epidural administration of opioids and other analgesics may represent an important method of providing prolonged pain relief. This is of particular importance since the provision of prolonged analgesia to animals remains difficult.

In the absence of reliable methods of pain assessment it has been assumed that following some surgical procedures animals will require analgesics for 24 to 48 h. If this is proven to be necessary, then it will require a radical reorganization of the level of aftercare provided to both research animals and veterinary clinical patients in many establishments (Torrance 1993). One obvious solution to this difficulty is to administer analgesics by continuous infusion, a technique which has been shown to provide particularly effective pain relief in man (Hull 1985). Either an indwelling vascular catheter, implanted as part of the study requirements, could be used, or a percutaneous line set up specifically to administer analgesics. The wide availability of catheter and tether systems has increased the feasibility of using this technique, providing that the efficacy of treatment can be monitored in some way. Even more attractive is the prospect of allowing an animal to administer its own analgesic therapy. Buprenorphine administered continuously in the drinking water has been shown to be effective using analgesiometry in rats (Kistler 1988). Providing the animal is drinking sufficient water, this could provide long-term analgesia without the need for attendance at unsocial hours. A system analogous to human patient-controlled analgesia is never likely to be feasible for the control of acute pain, since it requires the animal to associate analgesic administration with the absence or reduction of pain sensation. Although it has been reported that this can be achieved with acute painful stimuli such as foot-shock (Dib 1985), the conditioning stimuli needed must of itself be painful to the animal, and may also differ qualitatively from the pain sensations which occur following surgery. Self-administration for the relief of chronic pain may be feasible in some circumstances, since, as discussed earlier, it has been shown that rats can develop a preference for anti-arthritic drugs when developing experimental arthritis (Colpaert *et al.* 1980).

If the requirement for repeated analgesic administration is met by attendance of research staff for prolonged periods, repeated injection of analgesics may still present practical difficulties and may be resented by the animal. Administration in a palatable food-stuff could resolve these difficulties. Several institutes in the USA have advocated the use of 'buprenorphine Jell-O', a flavoured gelatine preparation containing the opioid analgesic buprenorphine (Peckow 1992). Fruit-flavoured jelly is readily accepted by rats, and in our laboratory we have found that after a few days administration, rats will eat pellets of jelly immediately. Buprenorphine, like most opioids, undergoes significant first pass hepatic extraction when administered orally, and so only approximately 5–10% of the administered dose will be available (Cowan *et al.* 1977). This technique has been

evaluated, and shown to be effective in a rat laparotomy model, using food and water consumption and body weight changes as indicators of post-operative pain (Liles & Flecknell, in preparation).

Opioids have a range of other effects in addition to their analgesic action. These side-effects may interfere with particular research protocols and may restrict the use of opioids for pain control. It is therefore important to consider the use of alternative classes of compounds, such as the NSAIDs. A number of newer, more potent agents of this type are becoming available, and although evaluation of their efficacy is largely limited to clinical opinion, there are some studies that suggest they may be suitable for providing post-operative analgesia. In the dog, flunixin and carprofen have been reported as providing effective analgesia (Reid & Nolan 1991, Nolan & Reid 1993) and in rats, carprofen appeared to be an effective analgesic following laparotomy (Liles & Flecknell 1993c). NSAIDs also have a range of effects on different body systems and metabolic processes and, like the opioids, these may preclude their use in some research protocols. It is therefore worth considering a further option, the use of local anaesthetic preparations. These have been shown to be effective in man (Buckley 1985), and in animals (Thompson & Johnson 1991, Flecknell *et al.* 1991).

In addition to considering the use of analgesics, it is important to emphasize that the degree of pain and distress caused by any procedure will be markedly influenced by the expertise of the operator. This highlights the need for careful and extensive training of all those involved in research work, a process which is only just beginning to be formalized in many European countries.

Conclusions

Refinement of research is an ongoing process which requires input from all those involved in the use of experimental animals. In order to build on the current high level of awareness of the need to refine experimental procedures, and to evaluate procedures which are claimed to represent refinements, objective methods for the assessment of pain and distress are required. If efforts are not made to develop these assessment techniques, continued reliance on inadequate anthropomorphic criteria will delay progress in this area. Although some additional use of animals may be necessary to validate new techniques, the overall end result should be a significant reduction in the pain and distress caused to those animals which are still needed for research.

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