Pain and distress in laboratory rodents and lagomorphs

Report of the Federation of European Laboratory Animal Science Associations (FELASA) Working Group on Pain and Distress accepted by the FELASA Board of Management November 1992

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Introduction

The Working Group considered the nature of pain and distress in laboratory rodents and lagomorphs because they constitute the vast majority of subjects used in experimentation. In order to keep the document as practical and as easy to read as possible, the minimum number of references are given in the text. An extended bibliography of background material consulted by the Working Group is available from Dr V. Baumans, Rijksuniversiteit Utrecht, Bureau Proefdierdeskundige, PO Box 80.166, 3508 TD Utrecht, Netherlands. An attempt has been made to be consistent when using definitions of the sometimes vague terms that abound in this subject area. The information is arranged in sections, although these are not always mutually exclusive.

There is an inherent humanitarian desire to reduce pain and distress in laboratory animals to an absolute minimum, an aim which is recognized in European and national legislation. For example, the official guidance (Home Office 1990) attached to the UK *Animals (Scientific Procedures) Act 1986* requires licence holders 'to minimise any pain, suffering or distress'. One must, however, point out that pain and distress mechanisms are essentially devices for removing animals from potential sources of tissue damage or mortality: animals lacking such

mechanisms do not survive long in nature. Further, our desire to eliminate stimuli or situations producing pain or distress, could easily lead to deprivation, self-mutilation and environmental sterility (a lack of 'enrichment') if taken to extremes (Brain 1992, Dantzer 1991).

l. Definitions of pain, distress and suffering

Pain, distress and suffering are terms basically describing states of the human mind - human perceptions or experiences. It is difficult to transfer the definitions of 'mind' states to comparable states of laboratory animals. Researchers must, however, be familiar with the difficult concepts of pain, distress and suffering, and know how to recognize, assess, control and, preferably, to prevent this experience in their animals. This topic has also been discussed at length e.g. in Morton (1990). There is so far no consensus on defining these terms but, for the purposes of this document, the following definitions will be used.

Pain

The working definition of pain published by the International Association for the Study of Pain (1979) is 'Pain is an unpleasant sensory and emotional experience associated with actual or potential damage or described in terms of such damage.' This and other definitions of pain emphasize that it is an experience. Physiology and psychology suggest that this requires that a perception is evoked, in turn implying that the animal is conscious, with a functioning (alert) cerebral cortex. This seems true at least for mammals. It is also important that, when judging that an animal is in pain, the animal shows a *pain response* by some changes in behaviour (section VIII).

The definition of pain used here is limited to what is termed physical or nociceptive pain. Thus another important feature is that pain is the perception or experience of nociceptive stimuli, i.e. stimuli of a magnitude capable of causing or threatening to cause injury or tissue damage.

Distress

Distress is defined in the *Guidelines for the Recognition and Assessment of Pain in Animals* (UPAW 1989), as a state where the animal has to devote substantial effort or resources to the adaptive response to challenges emanating from the environmental situation, a definition very similar to that of emotional or mental pain (Spinelli & Markowitz 1987), perhaps only reflecting differences in English and American terminology. Stimuli potentially leading to distress are thus more or less extreme values or levels of the various factors constituting the animal's environment. This includes also the behaviour of researchers and technical staff to the animals in their care.

In this document, states that have been termed anxiety, frustration or depression are included within the definition of distress, as well as discomfort which is looked upon as a mild form of distress.

In attempting to assess the level of distress as mild, moderate or severe, it is important to realize that certain conditions might be more or less distressful to specific animals depending upon their opportunity and ability to cope with the situation. The better their opportunity and capacity to cope, the less severe the distress.

Suffering

Suffering is a specific state of 'mind', which is not identical to, but might be a consequence of, pain or distress. Physical pain or distress may result in suffering if they are of sufficient intensity or duration, or both. The greater the intensity, the less time is needed for pain or distress to lead to suffering. Suffering is reached when pain or distress is no longer tolerable to the individual animal. Physical pain has then reached a level beyond the pain tolerance threshold, or distress has passed the level that the animal is able to cope with. Detrimental effects including retarded growth, impaired breeding and inadequate body care are obvious at this stage. Clearly,

measures to prevent suffering include keeping any possible pain or distress to levels which the animal can tolerate or cope with.

II. Mechanisms of pain

Periphery

Nociceptive (pain-related) signals are generated in sense organs called nociceptors, in the skin connected to thin, myelinated A_-fibres (conduction velocity: 4-30m/s) or to unmyelfnated C-fibres (conduction velocity: 0.4 - 2 m/s). The existence of two sorts of afferent fibres (carrying information towards the central nervous system) may explain why brief cutaneous nociceptive stimuli are able to produce a double pain sensation. The first or fast pain is a well-localized, distinct pricking sensation evoked by activation of nociceptors connected to A_-fibres. The second or slow pain is a burning, more diffuse sensation ('ache') evoked by activity in C-fibres. C-fibres constitute the majority of cutaneous nociceptive and almost all visceral nociceptive afferents (Besson & Chaouch 1987, Campbell *et al*. 1989, Kitchell & Guinan 1990, Jeneskog 1991).

Several endogenous substances appear involved in pain sensations associated with tissue damage (traumatic or inflammatory), including potassium (K^+) and hydrogen (H^+) ions and bradykinin released by injured cells, histamine and serotonin released from degranulated mast cells, and serotonin also from aggregation of thrombocytes. Some prostaglandins and leukotrienes are produced during inflammation and sensitize the nociceptors leading to lower threshold for their activation. The A_- and C-fibres also contain neuropeptides, such as substance P, Calcitonin Gene-Related Peptide and neurokinin A, which are released at their central terminals within the dorsal horn of the spinal cord as well as at their peripheral terminals. These substances are implicated in 'neurogenic inflammation' through so-called axon reflexes.

Dorsal horn

The vast majority of fine afferent nerve fibres enter the spinal cord through the dorsal roots and split into ascending and descending branches which may run for some segments in a tract before terminating in the grey substance of the spinal cord's dorsal horn. Two main types of substances released from the nerve endings probably act as neurotransmitters and neuromodulators; these are excitatory amino-acids (e.g. glutamate) and peptides (substance P and several others). Two main classes of dorsal horn relay cells are directly or indirectly activated by nociceptive inputs, 'nociceptive specific' (NS) neurones, activated only by nociceptive stimuli, and 'wide dynamic range' (WDR) or 'convergent' neurones, activated, to some extent also by non-nociceptive stimuli.

As soon as it enters the central nervous system, the nociceptive message is subject to a variety of control mechanisms. These include segmental modulation (suppression) via activity in thick, myelinated skin fibres, and descending modulation through control systems of supraspinal origin, notably from the brain stem's periaqueductal grey substance. Another, seemingly global, system where one nociceptive input may inhibit another (even a remote nociceptive input) via segmental as well as supraspinal components is termed Diffuse Noxious Inhibitory Controls (DNIC).

The dorsal horn is rich in opioid receptors and their endogenous ligands, notably those derived from pro-enkephalin A and pro-dynorphin. The analgesic (pain-relieving) effects elicited by segmental as well as descending modulatory systems and also by administration of opioids at least partly depend on activation of such spinal receptors.

The axons (long nerve fibres) of the NS and WDR cells ascend, mainly contra-laterally, through the spinal cord. Although many interspecies differences exist, data related to the rat, the monkey and man appear to be to some extent homogeneous.

The main pathways are the spino-thalamic and the spino-reticular tracts. Other pathways, studied in animals, include the spino-(ponto)-mesencephalic, spino-solitarius, spino-cervical (Morin's) tracts and post-synaptic fibres in the dorsal columns. The nociceptive messages thus reach the brain through a multiplicity of pathways. The neo-spino-thalamic pathways, which terminate is the lateral thalamus, preserve a reasonable amount of somatotopy (localization). Signals are transmitted from the thalamus to the somatosensory cortex in the parietal lobes, where the sensory-discriminative dimension of pain is probably elaborated or at least initiated. Both the palaeo-spino-thalamic and the spino-reticulo-thalamic pathways terminate in the medial thalamus; somatotopy is no longer preserved in these systems.

The message eventually reaches wide areas of the frontal cerebral cortex and cortical and subcortical parts of the limbic system. The limbic system and the frontal cortical areas are implicated in the motivational-affective (emotional) dimension of all kinds of sensory information, including nociceptive information (Willis 1989, Willis & Coggeshall 1991).

Reticular formation

The medial ascending system of pathways largely connects the dorsal horn to medullary centres in the reticular formation regulating circulation and respiration. Consequently, autonomous (sympathetic) reflexes are elicited by nociceptive stimuli, such as increased heart rate and blood pressure, altered respiration, pupil dilation, and inhibition of gastrointestinal motility. These connections, furthermore, possibly activate the previously-mentioned descending modulatory systems.

Parts of the reticular formation are components of the so-called 'ascending reticular activating system' which is tonically active, being excited by different forms of ascending sensory (including nociceptive) information, and is indispensible for keeping the cerebral cortex alert. Nociceptive information seems particularly effective in arousing (alerting) the cerebral cortex. An aroused, alert cerebral cortex is the basis for consciousness and thus for perceptions – among them pain – to be produced.

Cerebral cortex

As mentioned earlier, the cerebral cortex is indispensible for all kinds of perceptions, including pain (Zieglgänsberger 1986). However, as a result of clinical observations in man, the role of the cortex in pain has been the subject of debate. First, during neurosurgical operations on patients who had not previously presented with deafferentation pain, pain was very rarely evoked by cortical stimulation, although it is very easy to evoke other somaesthetic sensations in this way. Second, most attempts (many years ago) to alleviate chronic pain by cortical ablation have failed. Interestingly, the use of magnetic resonance imaging and positron emission tomography have recently demonstrated that painful stimuli activate the contralateral sensory and, above all, anterior cingulate cortices (Jones *et a1*. 1991, Talbot *et al.* 1991, Kenshalo & Willis 1991).

Several different methods have been used to help people with chronic pain conditions, where conventional treatments have failed. These methods include prefrontal lobotomy and, more recently, cingulotomy. An interesting feature of using these kinds of lesions to treat a chronic pain condition is that patients report that they still feel pain but that it does not bother them, i.e. they no longer suffer from the pain. This indicates that such surgical interventions may separate the two dimensions of pain and that the motivational-affective component is dependent upon intact connections between the frontal cortex and underlying parts of the forebrain. It is, however, very uncertain if and how we can transfer this knowledge from human physiology to conditions in animals. The frontal lobes of the cerebral cortex are the parts which

have most conspicuously increased in size and development during vertebrate evolution. If the capacity for a motivational-affective interpretation of a nociceptive message increases with the size of these cortical areas, this ability would be highly developed in humans (and apes). However, all mammals may be assumed to perceive and experience pain, and furthermore remember situations associated with this sensation. This should be our basis for appropriate action, even if in mammals such as rodents and lagomorphs the capacity for making advanced interpretations of a pain situation are likely to be inferior to our own.

III. Measurement of analgesia and environmentally-induced analgesias

Many assays have been used in rodents to test the efficacy of putative analgesic compounds. These include: hot-water tail immersion (Janssen 1963); acetic acid writhing (Koster *et al.* 1959); exposure to unavoidable electroshock using ascending and descending current values; pressure methods (use of forceps or artery clamps); intra-plantar injection of yeast or carageenin (Taber 1974); radiant-heat tail flick (D'Amour & Smith 1941); and the hot-plate test (Woolfe & McDonald 1944). Some of these methods are severe, difficult to control and of very limited utility when assessing the impact of subtle changes in housing or scientific procedures involving animals. The last two are most likely to be useful in welfare research as they are particularly sensitive to opiates, easy to perform, require simple apparatus and have a well-defined end-point.

In the tail-flick assay, pain sensitivity is determined by focusing radiant-heat from a strong light bulb on to the rodent's tail tip until there is a reflexive flick away from the heat source or the appearance of small white blisters. Semi-automated devices are available. In the hot-plate test, individual animals are tested for nociceptive responses on a hot-plate maintained at 55 °C, a temperature which although uncomfortable will not cause serious damage. The end-points described are forepaw licking (animal sitting on hind legs licking forepaws in a washing action), hind paw lick (head orientated to hind paw with ventral surface angled upwards) and escape jumping (animal jumping upwards with both bind paws away from the hot-plate surface).

Using such tests, it has been possible to demonstrate that a wide range of environmental factors (some associated with animal husbandry or laboratory procedures) can influence pain sensitivity. This is of crucial importance in evaluating the impact of procedures on pain and distress. Amongst the diverse items shown to produce clear analgesias in rodent species (see Rodgers & Randall 1987) are acupuncture, anxiety, brain stimulation body pinch, centrifugal rotation, classical conditioning, copulation (males), electric footshock, exercise, electroconvulsive shock, food deprivation, forced swim, heat exposure, hypertonic saline stimulation, insulin, irradiation, novelty, opiates, pregnancy/parturition, presence of a predator, restraint, social conflict, social isolation, stress odours, tail pinch, tail shock, territorial scent marking, transcutaneous nerve stimulation and vaginal stimulation. Some analgesics involve endogenous opiods while others are not opiod dependent. Some are controlled by neural factors and others by hormones, so that there are many different forms of analgesia. In spite of the considerable technical difficulties mentioned earlier, their analysis seems essential in order to be able to specify and control pain levels associated with husbandry or with experimental procedures. The basic point which must be noted is that a wide range of experiences have dramatic influences on analgesiometric tests, and the possibility exists that they change an animal's perception of pain.

IV. Sensitivity of tissues and organs to pain

Sensitivity of particular tissues and organs depends on the innervation of tissues

(types of receptors, sensitivity to stimuli, density, size of the receptive field); the characteristics of the stimulus and possible sensitization at receptor level resulting from pathological tissue reactions (such as inflammation and ischaemia). Some variation of sensitivity can also occur because of processes affecting conductivity in nerves, such as nervous system maturation in the young animal, or alterations of nerves and of their myelin sheaths in metabolic diseases.

Information about tissue sensitivity can be obtained from clinical observations and from surgical experience in man and animals. More accurate knowledge can be expected from histological and electro-physiological studies of nociceptors, but there is still a gap between the experimental approach and the clinical data.

Skin

Cutaneous nociceptors include receptors activated by mechanical (e.g. pressure), thermal, and some chemical influences (Besson *et al.* 1986, Raja *et al.* 1988). These mechano-heat nociceptors, constitute two different groups, associated respectively with A_ and C fibres (p 5). The C fibre mechano-heat nociceptors are also stimulated by some chemicals and can show a sensitization phenomenon (their threshold is decreased when they are exposed to mediators of inflammation or other substances). Another type of nociceptor, the 'high threshold mechanoreceptor' is activated only by intense mechanical stimuli. Pain sensations arising from skin (superficial pain), which can be of high intensity, are diverse and provide accurate localization of the stimulation.

Muscles

Muscles are rarely very sensitive. The majority of receptors are mechanoreceptors and 75% originate from blood vessels, tendons and connective tissue (Raja *et al.* 1988). More than half of muscle receptors are stimulated by intravascular injection of pain-producing substances. Sensitivity of muscle receptors is increased by local inflammatory processes, and especially by ischaemia induced by sustained tonic contractions ('cramps').

Joints and bones

Joints and bones are normally relatively insensitive. Their receptors are activated only in inflammatory or degenerative pathological processes. Bones are sensitive to injuries particularly of their coating (the periosteum) which accounts for the sharp pains occurring after fractures or after surgical section. If, however, receptors of the periosteum are destroyed by the pathological process, pain sensitivity may be negligible (Crane 1987).

Teeth and cornea

Teeth and the cornea of the eye are amongst the most sensitive tissues. The density of nerve endings in dental pulp is 20 to 40 times greater than in skin while the cornea contains a still higher density of receptors (300-600 times that of skin) (Raja *et al.* 1988). The majority of corneal nerve endings (70%) are only sensitive to mechanical stimuli, others (17%) are activated only by cold and the remainder are activated by both.

Viscera

Viscera are less sensitive to pain than is the skin, explaining the clear-cut distinction between 'superficial' and 'visceral' pain. Parenchymatous organs (e.g. liver and kidney) do not produce pain except in the case of pathological injuries or inflammation. Hollow viscera (e.g. the digestive and urinary tracts) give rise to painful sensations only if a mechanical stimulus produced by distension or spasms occurs simultaneously with ischaemia. The existence of specific visceral nociceptors is not fully established: pain in the viscera can also result from paroxysmal stimulation of receptors and nerves involved in other functions, for instance regulating motility or producing vascular adjustments. Pain from viscera poorly discriminates type of stimulus and lacks precise localization. Referred pain is

frequently encountered with visceral injury. True nociceptors have been discovered only in two viscera: testis and gall bladder (Benson *et al*. 1986) areas, known to produce very sharp pain in man. In the bodily cavities (e.g. abdomen and chest), serous membranes (peritoneum and pleura) are considered sites of great sensitivity to pain, a feature which becomes obvious with injury (wounding or surgery) or inflammation.

Nervous tissue

Nervous tissue varies in its sensitivity to pain. The stimulation of peripheral receptors and of nerves (including A-_ and C afferents) induces a sharp pain sensation via the spinal cord. Stimulation of spinal cord dorsal columns elicits painful feelings as if to an electric discharge. In contrast, stimulation of encephalic (brain) tissue does not produce any pain, and one can perform stereotaxic surgery on conscious human patients.

General comments

A classification of tissues and organs in terms of a decreasing sensitivity can be generated: cornea, dental pulp, testis, nerves, spinal cord, skin, serous membranes, periosteum and blood vessels, viscera, joints, bones, and encephalic tissue. Although such a classification may seem useful, it is unrealistic because sensitivities can be greatly modified by pathological processes or experimental procedures. For these reasons, it is necessary to consider all data provided by practical experience. For instance, it is known that thoracotomy (thoracic surgery) is more painful in quadrupeds if performed by cutting the sternum than if an incision is made in muscles between two ribs (Haskins 1987, Johnson 1991, Sackman 1991). It is more important to evaluate overall severity of individual experimental procedures than to classify tissue sensitivity.

V. Effects of pain and distress

The affective-motivational (mood-related) and cognitive-evaluative (thought-related) dimensions of pain are of particular interest in assessing its effects on body function. Affective-motivational processes are related to release of neurotransmitters and ultimately adrenocortical and adrenomedullary hormones as well as endogenous opiates. The result may be stress or analgesia. Cognitive-evaluation processes may modulate these neuroendocrine responses, thereby altering pain detection thresholds. Little has been published on the effects of pain on physiological functions, but there is a substantial literature on the endocrine correlates of stress. Thus, pain is generally inferred from the activation of autonomic (= stress) responses (Manser 1992).

If experimenters cannot avoid using potentially painful or stressful procedures, they should be aware of the effects of stress on cardiovascular, respiratory, gastrointestinal and other functions. There is extensive evidence that different qualities (e.g. physical versus psychosocial) and durations (acute versus chronic) of stress may have variable influences (Adams *et al.* 1987, Cabib *et al.* 1988, Melia & Duman 1991). Moreover, the impact of these variables on the stress response may be modified by genetic background (Marek *et al.* 1991) or physiological state e.g. pregnancy (Pascoe *et al.* 1991). Such factors also influence pain sensitivity (Zamir *et al*. 1980, Gintzler & Bohan 1990) and, it must be assumed, alter effects of pain on body functions. Virtually contradictory outcomes of procedures in terms of pain and distress are therefore unsurprising. For instance, increased rates of major infections in individuals have been noted in patients subjected to pain (Benedetti 1990), perhaps due to the long-known immunosuppressive effects of corticosteroids (Berczi 1986). This contrasts with observations that pain (Fujiwara & Orita 1987) and stress (Jessup *et al.* 1987) may result in immunoenhancement. All these processes are time-dependent, a feature exemplified by contrasting effects of acute versus chronic stress (Cabib *et al*. 1988), as well as by studies showing that stress responses are dynamic events (Reznick 1989). Moreover,

Table 1 References to terms included in national legislations on the use of experimental animals by European countries*

	Pain	Suffering	Distress	Grading of severity	Cost-Benefit analysis
Austria	Yes	Yes	Yes	No	Yes
Belgium	Yes	No	No	No	No
Denmark	Yes	Yes	Yes	No	No
Finland	Yes	Yes	Yes	Yes	No
France	Yes	Yes	No	No	No
Germany	Yes	Yes	Yes	No	Yes
Ireland**	Yes	No	No	No	No
Italy	Yes	Yes	No	No	Yes
Luxembourg***	Yes	Yes	No	No	No
Netherlands	Yes	Yes	Yes	Yes	Yes
Norway****	Yes	Yes	Yes	No	No
Spain	Yes	Yes	Yes	No	Yes
Sweden	No	Yes	Yes	Yes	Yes
Switzerland	Yes	Yes	Yes	No	Yes
United Kingdom	Yes	Yes	Yes	Yes	Yes

*Greece & Portugal have legislation in preparation
**Uses1876 UK legislation
***Uses the general law on protecting animals
****The 1974 law is under revision

functional responses to pain and distress can be conditioned (Siegfried at al. 1984). If pain or stress is repeatedly matched with environmental cues, the functional response (analgesia or corticosterone release) may finally become elicited by presenting the environmental (or experimental) feature alone. In addition, putative antinociceptive neurotransmitters affect other systems e.g. endorphins influence immunological factors (Johnson & Tortes 1988) and substance P alters cardiovascular function (Urbanski *et al.* 1989).

Effects of impairment of muscular activity in the pain-affected area must also be taken into consideration. Other functions may suffer secondary impairment, depending on the muscular region affected (e.g. motor behaviour or respiration). Finally, recent investigations on postoperative pain in man show that morbidity and mortality are drastically reduced when proper postoperative analgesia is provided (Benedetti 1990).

In summary, it is difficult to predict the detailed effects of pain and distress in individuals or in specific experimental situations. One must consider individual cases. Pain and distress generally increase variability in experimental results, because of the various neurotransmitter and hormonal responses they elicit. Consequently, an animal in pain or distress is a poor research subject, except when pain itself is investigated. This practical feature must reinforce the ethical reasons for minimizing such conditions in experimentation.

VI. Legal obligations

A survey of national legislations of European countries on the protection of animals used for experimental purposes reveals that all texts include at least one of the terms 'pain', 'distress' or 'suffering' (Table 1). *All* European countries are, however, signatories to European Communities Directive 86/609/EEC or the Council of Europe *European Convention for the protection of vertebrate animals used for experimental and other purposes (1986)*. Both the Directive and the Convention use all three terms. Projects are generally required to avoid or minimize such experiences, consistent with the research aim. Alternatives to procedures that might

produce pain, suffering or distress must be considered and adopted whenever appropriate to the purposes of the investigation. Experiments should also be performed in species with the lowest degree of neurological development consistent with the procedure's aim.

Few of the legal tests surveyed required assessment and grading of severity of experimental procedures. Where it is required, assessment has to reflect the potential adverse effects the animals might experience. Examples of severity of different procedures are given in the guidance (Home Office 1990) to the UK *Animals (Scientific Procedures) Act 1986*. Collecting small blood samples, skin tests with substances expected to be only mildly irritant, conventional minor surgical procedures under anaesthesia such as laparoscopy, small superficial tissue biopsies or cannulation of principle blood vessels are considered mild unless they are repeated or combined in the same animal. Procedures described as moderate include: screening and developing of potential pharmaceutical agents, toxicity tests avoiding lethal endpoints, and most surgical procedures followed by post-operative analgesia and treatment. Substantial severity is assumed to be involved in procedures such as acute toxicity tests with significant morbidity or death as an endpoint, some efficacy tests of antimicrobial agents and vaccines, some models of disease and major surgery resulting in severe post-operative suffering.

Another requirement included in most European and national laws is that appropriate analgesia, sedation or anaesthesia have to be used in procedures causing actual or potential suffering to animals. In addition, some laws require a cost-benefit analysis in which painful and distressful procedures have to be balanced against expected positive results of the study.

VII. Sources of pain and distress

Various features of the operations of animal facilities can give rise to pain and distress. Some are obvious, others are less evident to all workers.

Transport

Using measures of mortality, condition, behaviour and endocrine changes it is clear that transport to animal houses from suppliers can be a significant source of stressful experiences. Obviously, factors such as cage and vehicle design, provision of food and water, time involved, care with handling and exposure to fumes, different temperatures or noises determine the degree to which transport constitutes a problem. Transport within a facility can also stress animals.

Physical factors associated with the macroenvironment

Major variations of ambient temperature, lighting (especially for nocturnal animals), relative humidity and noise can be important sources of distress in laboratory animals and it is conceivable that some procedures cause actual pain. Some apparently innocuous activities such as having mice in close proximity to rats (a natural predator) and use of unscreened (electronically) visual display units, operating vacuum cleaners or running taps (all sources of ultrasound) can cause distress and associated abnormal behaviour in laboratory rodents. The common practice of playing transistor radios in animal rooms is also a distinctly contentious activity.

Physical factors associated with the microenvironment

Cage design and construction can have a major impact on an animal's well-being. Factors such as opportunities for exercise or retreat out of direct contact with cagemates can be extremely important. Although cages have to be cleaned to prevent exposure to high levels of ammonia, the act of cleaning can cause great disturbance to some animals, especially those who leave odour patterns. Rehousing rodents from established groups in new associations is an intensely stressful procedure as measured by physiological and

behavioural changes. Cleaning is often followed by short bouts of fighting in group-housed male rodents. Certain times of the light/dark cycle minimize this response. Strangely, animals often appear even more distressed if existing substrates are disrupted but left in place than if the cage is cleaned. Cage-mates (especially males and lactating females) are potential sources of pain and distress for rodents and lagomorphs (Brain 1990). Again, one must understand requirements of particular species and use great care when housing animals together. Although fighting is relatively common in some species, most animals tend to use relatively non-injurious modes of attack on conspecifics, and have antinociceptive mechanisms which ameliorate the stress caused. Even 'good faith' procedures such as grouping animals for purposes of mating can generate fighting and chasing. As much of animal behaviour (whether one is looking at sexual or agonistic activity) involves initial ambivalence before the 'desired' activity, it is hardly remarkable that procedures can and do occasionally generate distress. The designating of individually-housed animals as abnormal is also dubious. The response to individual housing depends on the species and stage of development – a majority of group-housed male mice may be at a disadvantage *vis-a-vis* 'isolated' counterparts in that they show higher adrenocortical and lower gonadal activities (Brain 1975). Many workers fail to appreciate that rodents and lagomorphs often communicate between neighbouring cages using a variety of auditory (including ultrasound) and olfactory cues. It has, for example, been demonstrated that rats and mice can communicate pain and distress (presumably producing anxiety in neighbouring conspecifics) via a variety of olfactory messages. Some visual (probably a very minor sensory modality in rodents) separation is hardly 'isolation' in such species.

Factors associated with experimental procedures

Techniques used in husbandry and in laboratory practice may be sources of pain or distress. It is difficult to produce a definitive list of these very varied procedures but they obviously differ in severity. We might judge that efficient general and specialist husbandry, behavioural studies, and food and water deprivation for up to 24h are relatively painless and distress free. Tattooing and other methods of marking animals, administration of substances (see Table Al in LASA 1990), anaesthesia and recovery from anaesthesia, and surgical techniques (see Table A3 in LASA 1990) – are likely, however, to be painful and stressful. Collection of tissues and body fluids (see Table A2 in LASA 1990), post-surgical care, restraint (see Table A4 in LASA 1990), physiological, pharmacological and toxicological studies, and humane killing of animals – are difficult to evaluate. Better information is required on the severities associated with all scientific procedures.

VIII. Signs of pain and distress

A relatively simple means of accurately grading levels of pain and distress is needed that can be applied to the wide range of circumstances and procedures used in animal laboratories throughout Europe by animal care specialists, technicians and scientists. As rodent and lagomorph species lack the ability to communicate verbally their state of 'mind' or experience, we generally rely on modifications of behaviour, notably changes which are recognized as active, aversive (nocifensive) reactions to infer a state of pain in them. Although this might be regarded as crude *vis-a-vis* humans, the most effective medical screening systems for human pain are also visual rather than verbal.

Protective motor actions include genuine withdrawal reflexes as well as less specific fight or flight reactions. Such reactions are largely species-specific. The tendency to react to pain by inhibition of motor activity leading to a passive, immobile appearance rather than by withdrawal, must not be forgotten. This behaviour is more prevalent in rabbits and poultry, but does occur in rodents.

Viceral reactions, mainly caused by increased activity in parts of the sympathetic nervous system, may result in changes in heat rate, blood pressure, respiratory pattern, pupil size, sweating or gastrointestinal motility. These visceral reactions are not, however, conclusive evidence for a perception of pain, as they may be evoked by nociceptive stimuli in animals under an anaesthesia deep enough to suppress the cerebral cortex.

Changes in general or social behaviour or in learned avoidance reactions have been considered expressions of both physical and emotional pain. Great familiarity with normal behaviour and its variation in the particular species is needed in order to be able to recognize and assess such alterations. The Laboratory Animal Science Association (LASA 1990) has proved a valuable starting point in this consideration and their recommendation that evaluation of well-being necessitates workers becoming 'familiar with the normal behaviour, appearance, physiological and anatomical characteristics of each species' is extremely pertinent. One should add that individual strains (especially of mice) show enormous variability in behaviour and physiology, suggesting that one needs familiarity at the strain level (Jones & Brain 1985). It *is* important to understand differences between nocturnal and diurnal species and to recognize the simple importance of subjects being warm to the touch, showing good muscle tone, a well-groomed coat and maintenance of body weight. LASA (1990) record that most laboratory rodents will make positive attempts to avoid capture and handling when they are healthy. Subjects should make movements without reluctance, without favouring a particular limb and not show staggering or circling. Dantzer (1991) and Mason (1991) have recently challenged the view that stereotypes (repetitive movements) provide indices of distress in laboratory and farm species.

LASA (1990) suggest that many animals respond to acute pain by simple reflex actions including 'withdrawal from the source, accompanied by vocalization and followed by licking, shaking or scratching the affected part'. Although this implies that animals are consistent responders to specific stimuli, Rodgers (1989) has reviewed the wide range of analgesic mechanisms found in rodents, as well as the extremely diverse environmental factors associated with pain inhibition (see section III). The same stimulus may thus cause different perceptions of pain in different individuals. LASA (1990) suggest that a second stage of response involves 'some changes in behaviour, reluctance to move, combined with vocalization, irritability, short-term anorexia and abnormal posture'. Unusual noises such as 'chattering, bubbling or wheezing' may also be diagnostic. Ultrasound production might also be of utility here (Sales & Pye 1974) and pharmacological evidence has been produced that the ultrasounds produced by infant mice are reliable indices of 'anxiety' (Nastiti et al. 1991).

LASA (1990) Suggested that chronic pain or distress is usually more insidious in its early stages than acute pain and requires careful observation to detect changes in an animal's appearance and behaviour which indicate deterioration. Behaviour and appearance are actually promising candidates for routine assessments of pain and distress.

What other features might be used to assess pain and distress? It has been suggested that hormones (especially glucocorticoids) signify 'stress' (reviewed by Brain, 1990) but many hormonal factors change in a complex way and sampling itself can be stressful. Broom (1991) has suggested combining behavioural indices with physiological (hormones, heart rate) and immunological (stressed animals often show impaired antibody production) measurements, as well as injury, disease, mortality risk, growth and reproduction measures to obtain complete indications of welfare. One might also add that detailed pathological investigation can also prove useful in (retrospectively) establishing effects of housing conditions and procedures on distress. Several of these might be used to confirm the severity of

pain and distress. There is, however, a danger of intervening too much with the animal and one can make a convincing case for intelligently used behavioural indices, simple appearance and rate of body-weight change, combined with pathology, being the best indicators of health and welfare. Good stockmanship may, in the end, prove the best we can legitimately expect on a routine basis.
There is an urgent need for systematic evaluation of the usefulness of the postulated indicators, combined with an imperative to increase the sophistication of behavioural assessment in animal laboratories. Remote controlled videotape recording of activity can be very revealing in this respect.

IX. Grading of severity of pain and distress in animals

A variety of schemes for scoring pain and distress in laboratory animals have been presented. Such assessments may be important in three stages of experimental procedures namely: in gaining approval from ethical committees; during the experiment *per se*; and in postmortem examinations. The response to pain depends on age, sex, health status, species and strain of the animal. Criteria used in pain evaluation are applied differently in different schemes. All current methods are unsatisfactory in that they are relatively subjective but most workers feel that they are considerably better than nothing.

In general, discomfort can be assessed in a qualitative and a – more or less –quantitative way. In both the qualitative and the quantitative way, assessment of discomfort contains two steps: collection of data, which can be regarded as an objective process, followed by 'translation' into a degree of discomfort, which is a subjective process (Beynen *et al.* 1989*a,b,c*).

Morton & Griffiths (1985), Beynen *et al.* (1988*a,b,c,d*, 1989*a,b,c*), LASA (1990) and the Disturbance Index used by Barclay *et a1*. (1988) have tried to score signs of pain and distress. LASA identified components of severity and gave a numerical rating reflecting its potential range. Morton & Griffiths (1985) tried to correlate clinical signs and severity of pain and distress, while the Disturbance Index used changes in number of movements made by a normal laboratory rat or mouse introduced into an unfamiliar cage as a method for assessing severity of procedures. Other authorities (e.g. Wright & Woodson 1990) use qualitative criteria from clinical examination or physiological signs including pupillary dilation, degree of opening of the eyelids, transient increases or decreases in blood pressure, heart rate, increases and alterations in respiration (including panting and gasping), whisker movements, piloerection, increases in body temperature, increased muscle tone, sweating, changes in skin temperature, evacuation of the rectum, ungroomed appearance or excessive licking, decrease in appetite, and abnormal stances. All these schemes suggest an objectivity which is apparent rather than real as, in most cases, diverse items are assumed to be related to pain and/or distress without statistical weightings being applied. Flecknell (1986), Sanford et al. (1986) and Zimmermann (1986) have all provided additional material on behavioural indicators of pain in animals. Biochemical signs may also be used, including increases in plasma ACTH, corticosteroids and catecholamines as well as decreases in plasma sex steroids, but their correlation with pain is imprecise at best. Presumed 'mental' status may also be assessed with animals classified as depressed, unaware, unresponsive, anxious, alert, excitable or aggressive. These ratings have to be compared with previous behaviour and normal behaviour of the species, breed, or strain. The state of consciousness may be assessed in tests using e.g. visual or auditory threats and reflexes (palpebral or flexor withdrawal). Abnormal activity (inactivity to hyperactivity) may give clues about pain or distress as may posture, facial expression (where appropriate), gait (e.g. lameness), reluctance to accept handling (showing e.g. vocalization or attack) or vocalization (noises may characterize particular

physiological functions). Responses to analgesics – to reverse the signs of pain – may also be revealing. Some people differentiate between signs of acute pain such as guarding, crying, mutilation (licking, biting), restlessness, sweating, recumbency, ambulation (reluctance to move) and abnormal positions (head down etc.) and chronic pain expressed by features such as limping, licking area, reluctance to move, loss of appetite, changes in bowel and urinary activity, accumulation of body secretions (decrease in grooming) and changes of behaviour towards attendance.

Buckwell (1992) listed physical signs for rodents that can be related to mild, moderate and substantial severity, categories used in UK legislation. The approach is included in a slightly modified form below.

Mild	Moderate	Substantial
Reduced weight gain	Weight loss of up to 20%	Weight loss greater than 25%
Food and water consumption 40-75% of normal for 72 h	Food and water consumption less than 40% of normal for 72 h	Food and water consumption less than 40% for 7 days, or anorexia (total inappetence) for 72 h
Partial piloerection	Staring coat – marked piloerection	Staring coat – marked piloerection – with other signs of dehydration such as skin tenting
Subdued but responsive, animal shows normal provoked patterns of behaviour	Subdued animal shows subdued behaviour patterns even when provoked	Unresponsive to extraneous activity and provocation
Interacts with peers	Little peer interaction	
Hunched transiently especially after dosing	Hunched intermittently	Hunched persistently ('frozen')
Transient vocalization	Intermittent – vocalization when provoked	'Distressed' – vocalization unprovoked
Oculo-nasal discharge transient (typically signs of chromorhino-dacryorrhoea in rodents)	Oculo-nasal discharge persistent	Oculo-nasal discharge – persistent and copious
Normal respiration	Intermittent abnormal breathing pattern	Laboured respiration
Transient tremors	Intermittent tremors	Persistent tremors
No convulsions	Intermittent convulsions	Persistent convulsions
No prostration	Transient prostration (less than 1 h)	Prolonged prostration (more than 1 h)
No self-mutilation	No self-mutilation	Self-mutilation

A degree of retrospective assessment of distress is possible by using data from postmortem examination (Walvoort 1991). The following criteria can be used: anabolic/catabolic balance e.g. body weight, muscle volume, fluid balance, fatty depots, fur condition and gastro-intestinal contents; neuroendocrine balance e.g. gonad and adrenocortical sizes; immunological balance e.g. intactness of gastric mucosal barrier, lymphoid organ size and presence of infections as well as behaviour balance e. g. wear of toenails and chromorhino-dacryorrhoea.

Morton (1990) has also highlighted areas in which refinement, with the specific aim of reducing laboratory animal pain, distress and anxiety, can be achieved. He also suggested that good husbandry and housing are of paramount importance. It is believed that choice tests may have a role not only in determining preferred environments for laboratory animals but also in assessing the aversiveness of procedures [Baumans *et al.* 1987, 1990).

Conclusions

Pain, distress and suffering are all difficult concepts to apply to laboratory animals. However, as European workers have both an ethical and a legal requirement to limit the impact of such factors it seems timely to consider how much can be achieved. This report attempts to set out the nature of the problems and suggests ways in which progress can be made.

The essential problem is to find a relatively simple means of accurately grading the levels of pain and distress, a means that can be applied to the wide range of circumstances and procedures used in animal laboratories throughout Europe by animal care specialists, technicians and scientists. This is essential if one hopes to fulfil the natural humanitarian desire to minimize the pain and distress in laboratory animals that is enshrined in recent legislation and to perform the cost (in terms of pain and distress)/benefit (in terms of hoped for gains in knowledge) analyses of research that are advocated by many national authorities. Certainly, scientists require more quantifiable data on the welfare repercussions of many of the currently used procedures in husbandry and experimentation (Brain 1992).

In spite of improved laboratory techniques, it seems that good stockmanship and laboratory animal technology (generally based on appropriately interpreted behaviour, general condition, and body weight changes) combined with detailed pathology provides the best means of assessing attempts to improve the conditions of laboratory animals. This is not only ethically desirable but would tend to improve the quality of research.

It is essential to recognise the variations between species (and even strains of the same species) and for workers to avoid anthropocentrism where possible. Legislators and scientists also have a duty to warn the general public about the dangers of too readily applying human values to other species. Action based on human values is not always helpful to animals and can actually prove deleterious.

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