The Old School, Brewhouse Hill, Wheathampstead,
Hertfordshire AL4 8AN, UK

Animal Welfare 2009, 18: 49-63
ISSN 0962-7286

Painful dilemmas: the ethics of animal-based pain research

M Magalhães-Sant'Ana*[†‡], P Sandøe[§] and IAS Olsson[†§]

[†] Laboratory Animal Science, IBMC – Instituto de Biologia Molecular e Celular, Universidade do Porto, Rua do Campo Alegre 823, 4150-180 Porto, Portugal
[‡] Department of Veterinary Sciences, University Vasco da Gama, Quinta de S Jorge, Estrada da Conraria, 3040-714 Coimbra, Portugal
[§] Danish Centre for Bioethics and Risk Assessment, Faculty of Life Sciences, University of Copenhagen, Rolighedsvej 25, 1958 Frederiksberg C, Denmark
* Contact for correspondence and requests for reprints: mdsantana@euvg.net

Abstract

While it has the potential to deliver important human benefits, animal-based pain research raises ethical questions, because it involves inducing pain in sentient beings. Ethical decision-making, connected with this variety of research, requires informed harm-benefit analysis, and the aim of this paper is to provide information for such an analysis. We present an overview of the different models and their consequences for animal welfare, showing that, of the many animal models available, most have a considerable welfare impact on the animal. While the usual approach to pain control through administration of analgesic substances is usually unsuitable in pain research, refinement remains an option, both within the experimental protocol and in general husbandry and handling. Drawing on the overview, we develop a discussion of the ethical acceptability of animal-based pain research against the background of the kinds of harm done to the animals involved, the potential for refinement, and the expected benefits of the research.

Keywords: *animal models, animal welfare, ethics, laboratory animals, pain, Three Rs*

Introduction

Pain is connected with a number of fundamental biological processes in humans and other animals and probably evolved to provoke an appropriate reaction by the animal in various damaging or life-threatening situations. Although it possesses evolutionary value, pain is an aversive experience for the individual, especially when it is intense or prolonged, and humans are prepared to go to great lengths to avoid it. Therefore, there is, in our society, a demand for greater understanding of pain and the medications which relieve it. Most of the research answering this demand involves laboratory animals — a practice which is, in itself, controversial, and one that is frequently challenged by both the general public and animal protection organisations.

Pain research on animals involves inducing pain in the studied animals and, hence, the research can only take place at some expense to animal welfare. Consequently, the research poses an ethical dilemma: on the one hand, animals are made to feel pain; on the other hand, the outcome of the research may identify new ways to prevent or alleviate pain in humans (and animals as well). In this dilemma, either outcome will involve harm to at least some living beings: the use of the animals means that harm is done to them, but abolition of the research would place a burden on people (and animals) suffering from medical conditions for which a better treatment is desired. When compared with other areas of animal-based research, pain research is likely to present particular welfare problems. Firstly, pain is an experience to which the sufferer is strongly averse, especially when it occurs beyond a certain level of intensity and/or beyond a certain duration. Secondly, because the pain itself is the object of study, typical refinement tools such as analgesics and anaesthetics are only available in special cases.

In the present paper, we examine the use of animals in pain research through a review of the various models and research methods with a special focus on the impact on animal welfare. Drawing on this review, we discuss the ethical acceptability of such research against a background of the harm to the involved animals, the potential for refinement, and the expected benefits of the research.

Using animals in pain research — the ethical issue

Pain has been defined by the International Association for the Study of Pain (IASP) Task Force on Taxonomy as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage" (IASP 1994). Pain that is not successfully controlled causes significant loss of quality of life for sufferers and also has socio-economically important consequences (eg Smith *et al* 2001; Wenig *et al* 2008). With the therapies available today, acute pain associated with trauma or acute infection can generally be successfully controlled. Effective pain control for chronic pain in conditions such as cancer (Pacharinsak & Beitz 2008), chemotherapeutic-

https://doi.org/10.1017/S0962728600000063 Published online by Cambridge University Press

induced neuropathy (Tanner *et al* 1998; Pasharinsak & Beitz 2008) and peripheral and central neural injuries (Eaton 2003) is more problematic. In the search for better pain control methods, the use of animals in research plays an important role; however, it also raises ethical questions.

The issue of animal pain has been controversial among scientists and philosophers. The main aspect of that controversy has to do with the perceived difficulty of accessing the subjective experience of animals. Nociception — that is, the physiological response to noxious stimuli that cause or potentially cause tissue damage — can be measured objectively, but describing the subjectively-experienced unpleasantness of nociception is more difficult. In humans, the assessment of felt pain mostly relies strongly on verbal self-reporting, a tool which is unavailable where animals are concerned. Nevertheless, evolutionary arguments support the assumption that subjective experience in non-verbal humans (Anand & Craig 1996) and, at least, vertebrate animals (Rollin 1998) is similar to that in normal human speakers. Seeking a more generally applicable definition than that originally put forward by IASP, Molony (1997) defined animal pain as "an aversive sensory and emotional experience representing an awareness by the animal of damage or threat to the integrity of its tissues. It changes the animal's physiology and behaviour to reduce or avoid the damage, to reduce the likelihood of recurrence and to promote recovery". More than ten years later, there is still no universally accepted scientific approach to assessing pain in animals (Viñuela-Fernández *et al* 2007).

Animal pain is widely recognised as having ethical significance (Rollin 2003; Short 2003). Animal protection legislation requires that animals are not subject to unnecessary pain, and position papers and codes of conduct lay down the duty of veterinarians to prevent, treat and alleviate animal pain (eg AVMA 2003; RCVS 2008). Moreover, specific guidelines and legislation on animal research underline the importance of pain control: witness current European regulations, where Article 8 of Directive 86/609/EEC states that "All experiments shall be carried out under general or local anaesthesia". (Of course, ensuing sub-paragraphs introduce exceptions to that rule, but the emphasis on pain control is nevertheless strong).

Pain research is not the only discipline in which laboratory animals are subjected to painful experiences, but it appears to be in a special area as the research normally requires pain to be actively induced in animals. *Prima facie*, that requirement is ethically problematic, and the stronger the aversive experience, the more troublesome the issue of animal pain becomes from an ethical viewpoint. But pain is a complex phenomenon. Depending on its nature, location, duration and intensity, it ranges from a negligible nuisance to a completely debilitating condition. Whether the animal can control it, either fully by moving away from the painful stimulus or partly by changing its behaviour (eg by not placing weight on a painful joint), is also important. This complex variability must always be borne in mind because, as we will see later in the paper, the amount of pain animals experience in pain research varies widely.

Ethical decision-making on research with animals is usually guided by two principles. Firstly, there is the widely accepted principle of the Three Rs, which requires that the harm caused to animals is minimised through the use of *replacement* alternatives wherever possible, a *reduction* in the number of animals used to a minimum, and *refinement* of the methods. Secondly, people also agree that ethical decisions require an evaluation, or balancing, of the harm done to animals and the benefits to be gained by humans (eg Animal Procedures Committee 2003).

To apply the refinement element of the Three Rs principle, and to assess the harm imposed on animals in pain research, it is necessary to ask how different pain models affect the animals involved. A leading aim of the present paper is that of characterising the information that is relevant to this kind of assessment. Therefore, below, we give a detailed overview of pain models, focusing on issues associated with harm. Furthermore, we try to make some suggestions about the assessment of the benefits of pain research. However, it is important to be aware that even where it is possible to provide valid assessments of harm to animals and benefits to humans, the ethical dilemma in pain research still persists. The general dilemma is that pain is inflicted in animals without any corresponding benefit for them. In addition, specific cases might arise, for example, when we seek to identify the animal model that causes the least pain. Should long-lasting moderate pain be regarded as preferable to acute but excruciating pain? Is it more acceptable to inflict a large amount of pain on a small number of animals or a small amount of pain on a large number of animals?

In the following, we present an overview of various animal models and tests used in pain research and their impact on animal welfare. This is followed by a critical discussion of the applicability of the Three Rs principle and other relevant ethical principles, and an examination of factors affecting the potential benefits of animal-based research on pain. We conclude with a discussion of the overall ethical acceptability of this type of research.

Models and methods used in pain research

In the study of the pathophysiology of pain, many animal models have been designed over the years. Many more will certainly follow. As pain is a complex, multifaceted phenomenon, it has to be assessed by a range of approaches. Acute, inflammatory, visceral or neuropathic forms of pain differ in noxious stimulus and the nociceptors or neural pathways involved. Therefore, their perception and evaluation also differs. It is not feasible to design an animal model capable of replicating the full range of pain mechanisms that are of clinical interest.

Animal models of pain are developed to represent clinically-relevant pathological conditions (Walker *et al* 1999). Thus, pain is artificially induced with traumatic, chemical, surgical or other lesions designed to mimic actual human painful diseases. The sensitivity of modelled animals to pain can then be evaluated using algesiometric tests. Research protocols help to ensure that there is a controlled and homogeneous stimulus-response (eg Luo 2004; Crawley *et al* 2007).

https://doi.org/10.1017/S0962728600000063 Published online by Cambridge University Press

There is, therefore, an important distinction between pain (algesiometric) tests and pain models. The former measure animal responses to an acute nociceptive stimulus. They quantify the difference between the experimental group and the control group. Their utility resides in the opportunity they offer of measuring the effect of anti-nociceptive drugs (eg NSAIDs) or of studying hypersensitivity responses (allodynia and hyperalgesia) when used in combination with other pain models. A pain model, on the other hand, involves inducing pain and/or nociception in an animal, usually through tissue damage that results in a more persistent nociceptive activation than happens in the transitory pain tests.

In Tables 1 and 2 we present a number of animal tests and models of pain, with a focus on rats and mice. Most were initially developed for rats (*Rattus norvegicus*). Many models are simpler and more reliable to use in rats because their larger body size allows greater surgical precision, although mice (*Mus musculus*) are becoming more important with the application of gene technology (Wilson & Mogil 2001). Both tables give an overall picture of each assay, including a short description, the first published reference, the quality of the induction stimulus and its correlation with human pain.

To obtain a more complete understanding of the way in which the tests and models affect animal welfare, we have considered three aspects of their impact: i) how invasive the induction protocol is; ii) how severe the tissue damage is and iii) how intense the pain is and how long it lasts. For each of these aspects, we have assigned a severity scale of 3–5 degrees (see Table 3). Using a combination of the severity attributed to different models and the description of the histopathological and behavioural changes in relevant literature, each test and model was classified in respect of each of the three impact aspects (Tables 1 and 2).

A few observations can be made on the development of the different scales. Information about the severity of tissue damage is often difficult to find in descriptions of the models; that severity will also depend on what endpoints are applied in the actual experiment. For example, in a severe progressive condition, pain may not reach its later, more serious stages if animals are humanely killed at an earlier time-point (eg Slart *et al* 1997; Shimoyama *et al* 2002). Verbal self-report methods cannot be used with animals and, therefore, the determination of animal pain relies on behavioural information. Behavioural guidelines exist for this purpose (Wang & Wang 2003). However, the current guidelines, despite good intentions, often fail to cover detection of the onset of pain. They also give no reliable indication of pain's intensity (Roughan *et al* 2004). In 1985, Morton and Griffiths proposed guidelines on the recognition of pain in laboratory animals, but these proved to be difficult to follow in practice because there was little difference between affected animals and controls and the scoring system was not sufficiently characterised (Beynen *et al* 1987; Flecknell & Roughan 2004). Recognition, assessment and evaluation of chronic pain in experiments still depend on algesiometric tests of elicited acute nociception.

We were unable to find universally accepted criteria governing description of the persistence and intensity of pain inflicted on the experimental animal. We therefore developed our own categories. Following Kruger (1991) one may refer to acute pain as lasting for as long as one day and chronic pain as lasting for at least several days. Below (see Table 3) we make use of a more fine-grained distinction between pain of short (< 3 h), medium (3 h–3 days) and long (> 3 days) duration. For comparison, we have applied the human chronic pain classification presented by von Korff *et al* (1992). This is a brief and simple classification that measures the severity of pain in terms of a combination of intensity (low or high) and disability (low, moderate and severe) resulting in four grades together with a no-pain grade. Although it does not refer to the duration of pain, this grading could be useful in assessing pain in animals.

Welfare impact and the potential for refinement

Pain research does not always require the animals to be subjected to ethically problematic levels of pain. As can be seen in Table 1, the various algesiometric tests pose few ethical problems, partly because the pain inflicted in them is usually of short duration and limited intensity (see also Gebhart 1999), but also because the stimulus is induced in a non-invasive way, the endpoints are well defined (and, in several cases, even determined by the animal itself when its behaviour allows it to withdraw from the nociceptive stimulus) and the severity of damage ranges from none to rapidly reversible inflammation. The exceptions are the chemically-induced Formalin test and Writhing test, in which the injection of irritating substances does indeed cause tissue damage. The use of these tests also differs as they are long-duration stimulus tests (that measure tonic pain) and not acute phasic pain models (Eaton 2003; Mogil & Crager 2004).

By contrast, all of the pain models have a considerable impact on the animals (Table 2). Here, pain induction always implies that the animals are subject to relatively invasive procedures, ranging from single injections to multiple interventions, sometimes including surgery under general anaesthesia (the exception being cancer pain in animals genetically modified for spontaneous tumour development). There is often considerable tissue damage, which means that the pain will not be transitory but persist as long as the damaged tissue has not healed (or until the animal is humanely killed). How intense the pain is, will depend on several factors in the experimental protocol — in particular, the size of the pain-inducing stimulus (which agent is chosen, how much is administered, how large the pain-inflicting tumours are, etc). In most models, however, the pain is considerable, qualifying for grades III or IV on the von Korff scale of human chronic pain.

One type of pain model seems to be particularly controversial and has been questioned on ethical grounds by researchers in the field (Riopelle 1992): the type involving denervation of a neural segment. The denervation may be complete, as in the original Axotomy-autotomy model (AXO) (Wall *et al* 1979), or partial, as in the Bennet and

https://doi.org/10.1017/S0962728600000063 Published online by Cambridge University Press

Table 1 List of common algesiometric tests used in pain research.

Pain measured	Assay	First description	Induction stimulus	Short description	Utility (human-related pain)	Endpoint	Invasiveness of induction	Severity of damages	Level of pain induced	von Korff *et al* (1992)
Acute	Hot-plate test	Woolfe & MacDonald (1944)	Thermal	Enclosure on a radiant heated surface	Anti-nociceptive assay	Animal and operator	1	None	Short & weak	–
	Paw-flick test	Hargreaves *et al* (1988)		Focused beam of radiant heat on hind paw	Anti-nociceptive assay	Animal	1	None	Short & moderate	–
	Tail-flick test	D'Amour & Smith (1941)		Focused beam of radiant heat on tail	Anti-nociceptive assay	Animal	1	None	Short & intense	–
	Tail-withdrawal test	Ben-Bassat *et al* (1959)		Immersion of the tail in hot/cold water	Anti-nociceptive assay	Animal	1 to 2	None	Short & weak to moderate	–
	Escape test	Mauderli *et al* (2000)		Enclosure on a hot/cold surface with the possibility to escape	Anti-nociceptive assay	Animal	1	None	Short & weak	–
	Head withdrawal test	Ren & Dubner (1996)	Thermal, chemical, mechanical	Several	Anti-nociceptive assay	Animal	2	None	Short & variable intensity	–
	Paw pressure test	Randall & Selitto (1957)	Mechanical	Device which applies increasing pressure to the paw	Anti-nociceptive assay	Operator	2	None	Short & intense	–
	Tail-clip test	Haffner (1929)	Mechanical	Placement of a clamp at the base of the tail	Anti-nociceptive assay	Operator	2	None	Short & weak	–
	von Frey test	von Frey (1922)	Mechanical	Body surface stimulation using nylon monofilaments of increasing stiffness	Anti-nociceptive assay	Animal	1	None	Short & weak	–
	Flinch-jump test	Kimble (1955)	Electrical	Body surface stimulation using electrical shocks of increasing intensity	Anti-nociceptive assay	Animal	1	Mild	Short & intense	–
	Eye-wiping test	Farazifard *et al* (2005)	Chemical	NaCl eye drop	Acute trigeminal nociception	Cannot be ended other than by natural resolution	2	Mild	Short & moderate	–
	Tooth stimulation	Goetzl *et al* (1943)	Electrical	Electrode implanted in tooth	Acute trigeminal nociception	Operator	4	None	Short & weak	Grade I
Tonic	Formalin test	Dubuisson & Dennis (1977)	Chemical	Intradermal or subcutaneous injection of formalin	Hypersensitivity and analgesic assays	Cannot be ended other than by natural resolution	3	Mild to moderate	Long & moderate	Grade III
	Writhing test	Vander Wende & Margolin (1956)	Chemical	Abdominal constrictions after injection of chemicals	Visceral nociception	Cannot be ended other than by natural resolution	3	Mild to moderate	Long & variable intensity	Grade III

https://doi.org/10.1017/S0962728600000063 Published online by Cambridge University Press

Table 2(a) List of common animal models used in pain research.

Pain measured	Model	First description	Induction stimulus	Short description	Utility (human-related pain)	Endpoint	Invasiveness of induction	Severity of damages	Level of pain induced	von Korff *et al* (1992)
Inflammatory	Formalin, CFA, capsaicin, carrageenan, turpentine, zimosan, kaolin, mustard oil, honeybee venom, acetic acid	Various	Chemical	Injection/topical application of inflammatory agents	Cutaneous, sub-cutaneous, joint, muscular, orofacial injuries	Undefined	3 to 4	Mild to lethal	Medium to long lasting & variable intensity	Grades I–IV
	Induced bone injury	Houghton *et al* (1997)	Mechanical	Drilling a hole through the tibia or calcaneus	Bone lesions	Undefined	4	Moderate	Medium & weak to moderate	Grade II
Visceral	Endothelin, bradykinin, acetyle-choline, magnesium sulphate, hypertonic saline, iodinated radiocontrast agents, cyclophos-phamide, phenyl-quinone, acetic acid	Various	Chemical	Topical, via 'physiological' pathways, intravascular, intra-abdominal, peritoneal injec-tion of irritants	Visceral pain	Undefined	3 to 4	Mild to severe; possibly lethal	Long & variable intensity	Grades I–IV
	Artificial kidney stones	Giamber-ardino *et al* (1990)	Surgical	Surgical injec-tion of cement into ureter	Visceral pain	Undefined	4	Severe to lethal	Long & moderate	Grade IV
	Distension of hollow organs	Various	Mechanical	Surgical or ret-rograde place-ment of fluids or foreign bodies	Visceral pain	Undefined	4	Moderate to lethal	Long & variable intensity	Grades III–IV
	Ischaemia	Sutton & Lueth (1930)	Mechanical	Surgical occlusion of vasculature	Visceral pain	Undefined	4	Severe to lethal	Long & intense	Grade IV
Central neuropathic	Weight drop-contusion (Allen technique)	Allen (1911)	Mechanical	Contusion of sciatic nerve by dropping weight	Spinal cord injury	Undefined	4	Severe to lethal	Long & moderate to intense	Grade IV
	Photo-chemical spinal cord injury	Watson *et al* (1986)	Photo-chemical	Ischaemia induced by iv injection of erythrosin B, excited by argon ion laser	Spinal cord injury	Undefined	3	Lethal	Long & moderate to intense	Grade IV
	Excitotoxic spinal cord injury (ESCI)	Larson & Wilcox (1984)	Neuro-chemical	Intra-spinal or intra-thecal microinjection of neurochemicals	Spinal cord injury	Undefined	4	Severe to lethal	Long & moderate to intense	Grade IV

CFA: Freund's Complete Adjuvant.

https://doi.org/10.1017/S0962728600000063 Published online by Cambridge University Press

Table 2(b) List of common animal models used in pain research.

Pain measured	Model	First description	Induction stimulus	Short description	Utility (human-related pain)	Endpoint	Invasiveness of induction	Severity of damages	Level of pain induced	von Korff *et al* (1992)
Peripheral Neuro-pathic	Diabetic neuropathic pain (DNP)	Sima (1980)	Chemical or genetic	Intra-peritoneal injection of streptozotocin/ transgenic diabetic strains	Peripheral neuro-pathy induced by disease	Undefined	Genetic = 1 Chemical = 3	Lethal	Long & weak to moderate	Grade III
	Post-herpetic neuralgia (PHN)	Sadzot-Delvaux *et al* (1990)	Infectious	Latent varicella-zoster virus infection	Peripheral neuro-pathy induced by disease	Undefined	3	Moderate	Long & weak to moderate	Grade I–II
	Axotomy-autotomy model (AXO) (Neuroma)	Wall *et al* (1979)	Mechanical	Sciatic nerve multiple transection and ligation	Phantom pain (*anestesia dolorosa*)	Undefined	4	Lethal	Long lasting unknown intensity	Grade IV
	Chronic constriction injury (CCI or Bennett model)	Bennett & Xie (1988)	Mechanical	Four loose ligatures on sciatic nerve	Peripheral nerve injury	Undefined	4	Severe	Long & moderate to intense	Grade IV
	Partial sciatic nerve ligation (PSL or Seltzer model)	Seltzer *et al* (1990)	Mechanical	Partial tight ligature of sciatic nerve	Peripheral nerve injury	Undefined	4	Severe	Long & weak to moderate	Grade IV
	L5 or L5/L6 spinal nerve ligation (SNL or Chung model)	Kim & Chung (1992)	Mechanical	Tight ligature of L5 or L5 and L6 spinal nerves	Peripheral nerve injury	Undefined	4	Severe	Long & weak to moderate	Grade IV
	Sciatic cryoneur-olysis (SCN)	Wagner *et al* (1993)	Thermal	Freezing of sciatic nerve	Peripheral nerve injury	Undefined	4	Moderate to severe	Long & weak to moderate	Grade II–IV
	Inferior caudal trunk resection (ICTR)	Na *et al* (1994)	Mechanical	Unilateral resection of the ICT between S3-S4 nerves	Peripheral nerve injury	Undefined	4	Moderate	Long & weak to moderate	Grade III
	Sciatic inflammatory (SIN)	Eliav *et al* (1999)	Neuro-chemical	Injection of zymosan around the sciatic nerve	Peripheral nerve injury	Undefined	4	Mild to moderate	Medium & weak to moderate	Grade III
	Spared nerve injury (SNI)	Decosterd & Woolf (2000)	Mechanical	Transection of two of the three terminal branches of sciatic nerve	Peripheral nerve injury	Undefined	4	Severe	Long & weak to moderate	Grade IV
	Chronic constriction injury to the infraorbital nerve	Vos *et al* (1994)	Mechanical	Two loose chromic ligatures of infraorbital nerve	Trigeminal nerve injury	Undefined	4	Severe	Long & intense	Grade III
	Anti-GD2 ganglioside antibody injection	Slart *et al* (1997)	Immuno-therapy induced	Repeated anti-body injections via catheter	Auto-immune disorders	Undefined	5	Unknown	Medium & weak to moderate	Grade III

https://doi.org/10.1017/S0962728600000063 Published online by Cambridge University Press

Table 2(c) List of common animal models used in pain research.

Pain measured	Model	First description	Induction stimulus	Short description	Utility (human-related pain)	Endpoint	Invasiveness of induction	Severity of damages	Level of pain induced	von Korff *et al* (1992)
Cancer	Vincristine-induced peripheral neuropathy (VIPN)	Aley *et al* (1996)	Chemo-therapy induced	Repeated iv injections of vincristine	Chemo-therapy related peripheral neuro-pathy	Undefined	5	Moderate	Long & moderate	Grade III
	Taxol-induced peripheral neuropathy (TIPN)	Cavaletti *et al* (1995)	Chemo-therapy induced	Repeated intra-peritoneal injections of taxol	Chemo-therapy related peripheral neuro-pathy	Undefined	5	Moderate	Long & moderate to intense	Grade III
	Cisplatin-induced peripheral neuropathy (CIPN)	De Koning *et al* (1987)	Chemo-therapy induced	Repeated intra-peritoneal injections of cisplatin	Chemo-therapy related peripheral neuro-pathy	Undefined	5	Mild to moderate	Long & moderate to intense	Grade I–II
	Cancer invasion pain model (CIP)	Shimoyama *et al* (2002)	Cancer invasion	Implantation of malignant cells around the sciatic nerve	Cancer peripheral neuro-pathy	Undefined	4	Lethal	Long & weak to moderate	Grade IV
	Femur bone cancer (FBC)	Schwei *et al* (1999)	Bone cancer invasion	Injection of fibrosarcoma cells into femur	Bone cancer neuro-pathy	Undefined	4	Lethal	Long & moderate to intense	Grade IV
	Pancreatic cancer	Lindsay *et al* (2005)	Spontaneous pancreatic cancer	Transgenic mouse model with spontaneous pancreatic cancer development	Pancreatic cancer neuro-pathy	Undefined	1	Lethal	Long & moderate to intense	Grade IV
	Squamous cell carcinoma (SCC)	Nagamine *et al* (2006)	Cancer invasion	Injection of squamous carcinoma cells in sub-periostal tissue of lower gingiva	Orofacial cancer neuro-pathy	Undefined	4	Lethal	Long & moderate to intense	Grade IV

Undefined: Cannot be ended other than by natural resolution if that occurs.
In addition to the original papers describing the different models, we used a number of review papers of animal models of pain (Ness 1999; Ren & Dubner 1999; Walker *et al* 1999; Le Bars 2001; Eaton 2003; Wang & Wang 2003; Pasharinsak & Beitz 2008) to provide the information for this table.

Chung models, which are adaptations of the AXO/Neuroma model (Ma 2007). In either case, denervation results in limb deafferentation, which is often accompanied by autotomy (self-mutilation). There has been considerable debate about the real relevance of autotomy as a nociceptive response, with some authors describing the eliciting stimulus as chronic neuropathic pain (Mogil & Crager 2004) and others considering the possibility of complete absence of pain (Vierck *et al* 2008). In addition to the pain with which it is allegedly associated, the process of self-damage involves more than just "alarming aesthetics" (Mogil & Crager 2004), as an auto-mutilating animal is more prone to infections as well as other secondary pathologies such as dehydration, hypovolaemia and additional neuropathies.

As happens in other types of animal experimentation, scientists who carry out pain studies on animals should follow

https://doi.org/10.1017/S0962728600000063 Published online by Cambridge University Press

Table 3 Severity scales for the different aspects of impact on animals in pain research.

Category	Definition
Invasiveness of induction	1 Algesiometric test without restraint
	2 Algesiometric test with brief restraint
	3 Restraint and/or single injection of substance
	4 As 3 plus one event of surgery under general anaesthesia
	5 As 3 but repeated interventions
Severity of tissue damage	
None	1 No damage in a healthy/control animal
Mild	2 Acute or sub-acute revertive inflammation or lesion
Moderate	3 Inflammation or lesion that lasts several days but resolves completely
Severe	4 Severe and long-lasting injuries resulting in chronic disease but not necessarily death
Lethal	5 Progressive disorder leading up to spontaneous death if no earlier endpoint is applied
Pain, duration	
Short	$\leq$ 3 h
Medium	3 h–3 days
Long	$\geq$ 3 days
Pain, intensity	
Weak	Weak intensity
Moderate	Intermediate intensity
Intense	Strong intensity
Pain according to von Korff et al *(1992)**	
Grade 0	No pain
Grade I	Low disability-low intensity
Grade II	Low disability-high intensity
Grade III	High intensity-moderately limiting
Grade IV	High intensity-severely limiting

* Chronic pain scale for human clinical use. Included in order to compare our own classification with one that was already used and could be easily understood. As said, we were unable to find universally accepted criteria governing description of the persistence and intensity of the pain inflicted on experimental animals.

the principle of the Three Rs. As a supplement to replacement, reduction and refinement, Tannenbaum (1999) has suggested several specific principles of pain research on animals. First of all, Tannenbaum introduces the principle of *equality*, meaning that it should be assumed that pain is equally aversive for any animal, irrespective of species, unless there is evidence that the specific type of animal (eg invertebrates lacking a central nervous system) experiences less pain. The principles of *justification* and *value* lay down that no pain research may be carried out unless it can be sufficiently justified in terms of expected gain, and that the more pain is inflicted on the animals, the more important must the gains be to justify the experiment. This is essentially the same idea as the one underlying harm-benefit analysis. The principle of *minimisation* is essentially the same as that of reduction, aiming to reduce the number of animals experiencing pain, but it is complemented by an important additional principle of *fairness* to the individual animal which establishes that it is not morally desirable to reduce the number of animals if this leads to a situation where the remaining animals are made to feel pain beyond their capacity to adapt. IASP has laid down ethical guidelines for pain experiments using animals. These underscore several of the previous principles and add that pain relief should be made available or the animals should be able to self-select analgesia whenever this does not interfere with the aim of the investigation; and that, where possible, researchers should try the pain stimulus on themselves before applying it on animals.

There is often considerable potential for refinement within the experimental protocols themselves, as both the intensity and the duration of the pain will depend on factors in the

https://doi.org/10.1017/S0962728600000063 Published online by Cambridge University Press

experimental protocol. In algesiometric tests, the animal's ability to control exposure to the painful stimulus is important. The two most commonly employed tests — the Hot-plate test and the Tail-flick test (Le Bars *et al* 2001; Eaton 2003; Farazifard *et al* 2005) — differ in this respect, as it is only in the latter that the animal has total control of the endpoint of the experiment. In the former, the animal can lift its paws, but it cannot leave the plate, and it is the experimenter who, observing the animal's behaviour, decides whether the animal should be immediately removed or the temperature lowered in order to prevent injury or unnecessary pain. The typical sequence of behaviours starts with grooming of the forepaws and ends with jumping (Allen & Yaksh 2004). Successful application of the test without undue animal distress depends on a careful selection of criteria: the early responses may not be related to nociception (Wilson & Mogil 2001), whereas later responses, such as frantic agitation or jumping, are indicators of potential distress (Allen & Yaksh 2004). Mauderli *et al* (2000) propose the Escape test as an alternative. This is also a thermal nociceptive test, but allows the experimental animal to control exposure by being able to leave the heated or frozen surface, thus minimising the risk of thermal injury.

In models of pain, the duration of the painful stimulus usually depends directly on how long the experiment lasts. Intensity, on the other hand, depends on a number of factors. In the case of models provoking an inflammatory response, the agent chosen, the amount injected, and the site and area for injection, will all have an effect. When tumour development underlies the pain, the size to which the tumour is allowed to develop will, in part, determine the pain. Another approach is to develop tests which cause less animal distress. The Eye-wiping test (Farazifard *et al* 2005) (see Table 1) has been suggested as a non-invasive, short-lasting and non-damaging alternative to other orofacial inflammatory pain models such as the Orofacial capsaicin test (Pelissier *et al* 2002). Again, the CIPN model (Authier *et al* 2000) (see Table 2) shows that it is possible to develop models of chronic pain that induce moderate levels of pain and without causing severe body damage. Measures outside the experimental situation will also affect animal welfare. Animals in pain often become hypersensitive, which may result in even normal handling being painful; and so minimised and more careful handling is recommended (Roughan *et al* 2004). If pain affects the animal's ability to move around, facilitation of water and food access will reduce secondary effects on welfare. Softer bedding may also help, particularly if the painful stimulus has been applied to locomotory body parts, such as foot pads or joints.

It should be noted here that there is a need to establish more sophisticated behavioural techniques that allow specific signs of pain to be detected rather than obliging the experimenter to wait until the general state of the animal is affected. Roughan and Flecknell (2000, 2001, 2003) have pioneered protocols for detecting pain after abdominal surgery in rats and, in the area of pain research, such techniques would enable earlier detection of the onset of pain caused by neoplasia and, subsequently, better control of any pain animals experience before the experimental endpoint. Many experiments use vocalisations as the parameter to quantify the nociceptive threshold (eg Authier *et al* 2000). However, rats and mice can vocalise at frequencies well above the range of human hearing (ie greater than 20 KHz), and there may be a gap between audible vocalisation thresholds and nociceptive thresholds (Wilson & Mogil 2001). Some recent research in rats has focused on the way in which calls of different wavelength may reflect the caller's emotional state (Burman *et al* 2007; Portfors 2007). It has also been suggested that ultrasound vocalisation should be used as a valuable additional non-reflex behavioural measure in the Formalin test (Oliveira & Barros 2006) or in arthritic pain models (Han *et al* 2005), although Williams *et al* (2008) argue that ultrasonic vocalisations do not provide any more information than audible vocalisations do for assessing responses to potentially painful procedures. Potential uses of vocalisation thresholds with animals other than rodents have also been studied (eg Taylor & Weary 2000; Taylor *et al* 2001).

In humans, the diversion of the patient's attention away from his or her own pain can have an analgesic effect: listening to music, hypnosis, relaxation training, cognitive behaviour therapy and virtual reality techniques (see reviews in Gentle 2002 and Ford *et al* 2008) all illustrate this. Similarly, engaging in a cognitively demanding task has been found to reduce human volunteers' perception of stimulus-induced pain (Wiech *et al* 2005). The phenomenon of pain alleviation by a shift of attention has been studied in animals, measuring nociceptive response to the induction of inflammatory pain. When exposed to a novel arena or a novel object, rats showed less nociceptive behaviour after an intra-plantar injection of formalin, while non-contact exposure to an unfamiliar conspecific did not change nociceptive behaviour (Ford *et al* 2008). During post-deprivation feeding and pre-laying nest searching, laying hens showed less nociceptive behaviour in response to an intra-articular injection of sodium urate. When the hens were exposed to a novel pen, the reduction in nociceptive behaviour was accompanied by reduced inflammation measured as skin temperature over the injected joint (Gentle 2002). While this requires more research, these ideas may in the future serve as the basis of refinement measures for animals involved in pain research.

Elements of the principle of the Three Rs will interact with each other, and sometimes in a negative way. Among the potential conflicts, that between reduction and refinement has been pointed out by many authors (eg Hansen *et al* 1999; de Boo *et al* 2005). Such a conflict may occur when reductions in the number of animals results in a heavier burden on each individual animal (see also the discussion above about the principle of fairness). One example of the often complex forms of interaction is the speculation that, as a result of "learning, habituation or anticipatory escape behavior" (Lai & Chan 1982), repeated administration of the same acute pain algesiometric test results in a shortening of the pain response time. This systematic impediment conditions both experimental design and the reading of results and may lead to an increase in the number of animals used. The only

https://doi.org/10.1017/S0962728600000063 Published online by Cambridge University Press

exception to the general concern about repetition is provided by the Tail-flick test; but opting for this test may, on the other hand, compromise animal welfare more, as the very reason habituation does not occur is the high intensity of the noxious stimulus induced (Wilson & Mogil 2001).

Reduction in the number of animals subjected to pain can also be addressed statistically in situations where treatment and control groups experience different levels of pain. If an analgesic drug is tested, the treatment group will receive pain relief but the control group will not. If one treatment group is expected to experience (considerably more) suffering, the sample size, in this group, may be reduced, and this can be compensated for by increasing the sample size of the other group(s). Although this may increase the total number of animals, it will reduce the number of animals suffering without altering the burden carried by each individual animal (Sedcole 2006).

What about the first R, replacement? Approaches involving alternatives to laboratory animals definitely have a role to play in pain research. Data can, of course, be gathered from human patients during the course of clinical interventions, and studies inducing transitory pain without tissue damage can also be carried out on human volunteers (eg Wiech *et al* 2005). In addition to sidestepping the problem of translation between species, studies in humans also allow the researcher to address a wider range of aspects of pain, particularly through the use of different neuroimaging techniques (Langley *et al* 2008). While there are many scientific, practical and ethical obstacles to the introduction of studies with humans as a replacement, *in vitro* (or, more accurately, *ex vivo*) and *in silico* approaches are replacing animals in early stages in the drug discovery process in other disciplines — as evidenced by Monga and Sausville (2002) for cancer drugs and De Groot and Martin (2003) for vaccines, to give two examples. Wang and Wang (2003) list a number of cell models of potential interest in the study of chronic pain. While the study of the cognitive aspects of pain requires a whole-animal model, *ex vivo* models may offer a useful complement in the development of therapeutics, as is already happening in other disciplines.

Maximising the benefits of pain research with animals

Plainly, the Three Rs principle is a response to the second horn of the ethical dilemma that arises from our interest in securing human (and animal) benefits and our recognition that harm to animals ought to be avoided where possible. However, the first horn can also be addressed. It can always be asked, in other words: how probable is it that a research programme will deliver the benefits it is expected to deliver? While it is, of course, impossible to make guaranteed predictions about the outcomes of a research project, the difficulty of accurate prediction should not be regarded as a reason not to address this issue. Today, there is growing discussion of experimental benefit in a number of disciplines, including pain research.

The observation that some substances have proved effective in animals but not in humans and vice versa (Villanueva 2000) raises questions about the difficulty of translating preclinical research with animals into the clinical applications on humans the research is intended, ultimately, to produce. In pain research, this discussion has focused on the choice of models in relation to the biomedically most relevant types of pain and on the choice of measures of pain in animals. Several authors question reliance on stimulus-induced nociception in animal studies where the clinical need is to develop treatments for persistent pain conditions that are generally spontaneous rather than stimulus-dependent (Besson 1999; Villanueva 2000; Mao 2002).

Similar remarks can be made about the problem of preclinical research focusing on early responses to tissue damage in which it is assumed, but not scientifically established, that this will reflect the cellular basis of a persistent pain state (Mao 2002). Vierck *et al* (2008) stress that, since pain depends on cerebral processing, reflex responses are insufficient as a measure of pain. Instead, they advocate that an improved understanding of the abnormal activity in pain transmission systems in chronic pain patients should be used to design allodynia/hyperalgesia tests in animals that are specific to the human condition they are intended to model. This approach would also be in line with Villanueva's (2000) observation that several forms of chronic pain in humans, such as migraine and fibromyalgia, are not associated with any known tissue damage.

In this context, one might also note a discussion of the potential interaction between the anti-nociceptive drug being studied and the tests to determine its efficacy. The same algesiometric test can give different results depending on the administration procedure of the drug (Dogrul *et al* 2007); on the other hand, different algesiometric assays can give different anti-nociceptive responses with the same NSAID (Miranda *et al* 2001). The selection of the behavioural endpoint response may also be influenced by drug effects, especially sedatives and stimulants. For example, certain drugs, such as morphine, increase motor activity in mice without provoking hypersensitivity, while others, including haloperidol and amphetamine, disrupt motor function and leave animals unable to respond to nociceptic stimulus without provoking anti-nociception (Allen & Yaksh 2004).

If research with animals into treatments for human ailments is to be successful, it is important that the human condition is modelled appropriately in the experimental animal, in biological terms, and that the model has been proven to be effective in predicting effects in humans (van der Staay 2006). Observations, such as those reviewed above, point to the possibility that animal models and tests of pain are too far away from the human condition to be of clinical interest. However, an appropriate model is not the only important consideration.

In addition to the choice of model and test, an appropriate experimental design is crucial in designing a successful research programme involving animals. In other fields of neurobiology, researchers, concerned about the poor translation of preclinical research results into effective human treatments, carried out several systematic reviews of earlier animal experiments and found a number of critical shortcomings in experimental design. The most studied area here is that of experimental stroke, where it was found that in many

https://doi.org/10.1017/S0962728600000063 Published online by Cambridge University Press

of the animal experiments, for example, the efficacy of the prospective treatment was probably overestimated as a result of bias in the design. Often, animals were not randomly allocated to treatments; and researchers who were not blinded when they administered treatment (drug or control), or assessed its outcome, may have unknowingly influenced the measurements (van der Warp *et al* 2005; Crossley *et al* 2008).

No similarly systematic review of pain research is known to us. Nor is a specialist on systematic reviews aware of any such review (M Macleod, personal communication 2008). However, the field is sensitive to the same factors as those influencing other areas of neurobiological research. Despite highly standardised housing and testing conditions, unintended and unidentified local factors have been found to affect test outcomes (Crabbe *et al* 1999), leading to concern over the reproducibility of behaviour test results. It has been argued that while standardisation is effective in increasing internal validity (ie reproducibility within the same environment), this may be achieved at the expense of applicability (ie reproducibility in different environments) (Würbel 2000, 2002). Consequently, for a behavioural difference to be relevant, it should be reasonably robust across a range of environments (Würbel & Garner 2007). Specifically in pain research, Leo *et al* (2008) report obvious differences in experimental results between various studies in behavioural nociception response in mice. Differences in the experimental conditions, the observer's interpretation of behavioural cues, the established periods of observation, the definition of nociceptive response, and the methodological procedures, may explain these differences.

Even more serious than inter-laboratorial variation is the risk of biasing results. Research that relies on manual application of treatment, as happens when mechanical-induction stimulus is used (see Table 1), is especially prone to subjective measurement bias and errors (Bove 2006; Grigg *et al* 2007), as is research using behavioural measures scored by a human observer. To avoid such biases, researchers and technicians ought to be blinded as to the experimental treatment when they administer treatments and assess outcomes (Macleod *et al* in press).

An additional problem in the translation of animal research into human benefits is publication bias. Publication in peer-reviewed journals is a central feature of modern academic research and, as is well known, the performance of today's researchers is measured largely on the basis of the number of publications they have in influential journals. However, it is generally difficult to get negative results (no effect of treatment) published. As a direct consequence of this, publications are likely to reflect only part of the research that has actually been carried out in the field. This has wide-ranging ethical consequences. Of particular note is that fact that it affects the number of animals used in research (van der Staay 2006).

The difficulty of translating animal research into clinical applications has been appealed to by anti-vivisectionists for many years as an argument for abandoning the use of animals in research. What is new today is the fact that the difficulties are now being highlighted by clinicians who are concerned that they are not obtaining the expected benefits from research with animals. Of course, science operates, not under ideal conditions, but under economic and practical constraints. Therefore, any decision over which model to use is very unlikely to be based on scientific arguments only: money and time invested in acquiring or developing a particular technique or model will also be part of the decision-making. But keeping the critical discussion alive will be a crucial part of the iterative improvement of research methods. Moreover, many methodological improvements, such as randomisation and blinded treatment allocation and outcome assessment, can be implemented without any costly investments.

Opportunities and obstacles to easing the dilemma of pain research

While the acceptance of principles is certainly a good thing, putting them into practice is more important. Most countries have a legally entrenched system of ethics or animal use committees whose job it is to evaluate proposed experiments to ensure that the research is carried out according to official guidelines and principles (see Smith *et al* 2007 for a recent overview). It is our hope that this review paper will assist the work of such committees by providing a systematic and comprehensive overview of the available models, and their animal welfare impact within one scientific discipline. Such information is presently difficult to obtain for reasons we discuss below. However, legal and other regulatory mechanisms are not the only way to ensure good practice and, ultimately, responsibility for the way in which animals are used rests with the researchers themselves. This is true, not just in moral terms, but also practically since many decisions regarding the Three Rs can only be made at the research planning stage; hence the attitude of the researcher will be decisive in the choice of approach (eg animal or non-animal, a less severe or more severe model).

How scientists think and act is, of course, influenced by the culture (scientific and institutional) in which they operate, and therefore critical discussion and self-regulation within the scientific community will also be important (see also Vorstenbosch 2005 for further discussion of this). One aspect of this would be to bring considerations of ethics, including the Three Rs, into the review, both of funding applications and of manuscripts submitted for publication. In the European Framework Programmes, animal ethics review is included (but restricted to projects with non-human primates and those flagged up by the scientific review as potentially problematic, ethically speaking) and a proposal may be rejected on ethical grounds. In reviewing manuscripts submitted for publication, most journals continue merely to require a statement affirming that the research complies with official recommendations, or relevant legislation, or an ethics committee's decision (local, regional or national), rather than encouraging, or requiring, those involved in the peer-review procedure to seriously consider whether the submitted study was indeed carried out with the smallest achievable negative impact on the animals. Of the ten journals specifically dedicated to

pain research, indexed in ISI Web of Knowledge, six require a statement of compliance with guidelines, but only one (the open-access journal, *Molecular Pain*; 2007 impact factor 4.127) states that manuscripts may be rejected if "the research has not been carried out within an ethical framework, eg if the severity of the experimental procedure is not justified by the value of the knowledge gained".

In this context, it is noteworthy that information about the unexpected, adverse effects of inducing lesions are rarely reported in scientific papers and that it is usually very difficult to find information about the relationship between the induction method and any impact on the animals. It is also significant that there has been little progress in this respect over the more than 15 years that have passed since Morton (1992) called for a "fair press for animals" — and this, despite the important role that journals are in a position to play in promoting the Three Rs (Olsson *et al* 2007, 2008; Würbel 2007).

Animal welfare implications

The use of animals in research gives rise to ethical controversies. Today, many people regard it as problematic that, in order to protect humans from disease-related suffering, similar suffering is imposed on animals. Here, often, it is possible to conduct such research in ways that spare the animals of much, or all, of the anticipated suffering. However, this is rarely an option in pain research — here, it is often necessary to cause animals to feel pain when studying different ways of preventing or alleviating pain of that kind. It is therefore widely recognised that animal-based pain research poses a significant ethical dilemma. Some have gone so far as to propose a complete ban on such research. The authors of the present paper agree that, before pain research is initiated, careful consideration ought to have confirmed the likelihood that the research will actually deliver the hoped for benefits. This includes considerations of experimental design (Morton 1998, 2002) and systematic review, as this will help to ensure that the benefits flowing from the research will be maximised. The main aim of the present paper is, however, to discuss the impact on animal welfare of research, the potential to limit this impact by attending to the third of the Three Rs (refinement) and to other related principles such as the principle of fairness. Whenever animal-based pain research is undertaken, a serious effort should be made to choose a model and an experimental design in which the amount of distress and suffering imposed on the animals is as small as possible. To be in a position to make this kind of effort, however, it is necessary to have an overview of the ways in which different pain models affect animal welfare. This paper is offered as a first attempt to provide such an overview.

Acknowledgements

The authors wish to thank Miguel Santos Pais Vieira for useful input on the practical and scientific issues of using animals in pain research, Paul Robinson for language revision and two anonymous referees for comments to a previous version of the manuscript. Also we would like to acknowledge Mia Buehr and Peter Hjorth for their work on a related project that gave rise to the present paper.

References

Aley KO, Reichling DB and Levine JD 1996 Vincristine hyperalgesia in the rat: a model of painful vincristine neuropathy in humans. *Neuroscience 73*: 259-265

Allen AR 1911 Surgery of experimental lesion of spinal cord equivalent to crush injury of fracture dislocation of spinal column. *Journal of the American Medical Association 57*: 878-880

Allen JW and Yaksh T 2004 Assessment of acute thermal nociception in laboratory animals. In: Luo ZD (ed) *Pain Research: Methods and Protocols* pp 11-23. Humana Press: Totowa, NJ, USA

Anand KJS and Craig KD 1996 Editorial: New perspectives on the definition of pain. *Pain 67*: 3-6

Animal Procedures Committee 2003 *Review of Cost-Benefit Assessment in the Use of Animals in Research.* Home Office: London, UK

Authier N, Fialip J, Eschalier A and Coudore F 2000 Assessment of allodynia and hyperalgesia after cisplatin administration to rats. *Neuroscience Letters 291*: 73-76

AVMA 2003 *Principles of Veterinary Medical Ethics.* American Veterinary Medical Association: Schaumburg, IL, USA. http://www.avma.org/issues/policy/ethics.asp (accessed 25 July 2008)

Ben-Bassat J, Peretz E and Sulman FG 1959 Analgesimetry and ranking of analgesic drugs by the receptacle method. *Archives of International Pharmacodynamics and Therapeutics 122*: 434-447

Bennett GJ and Xie Y-K 1988 A peripheral mononeuropathy in rat that produces disorders of pain sensation like those seen in man. *Pain 33*: 87-107

Besson JM 1999 The neurobiology of pain. *The Lancet 353*: 1610-1615

Beynen AC, Baumans V, Bertens APMG, Havenaar R, Hesp APM and van Zutphen LFM 1987 Assessment of discomfort in gallstone-bearing mice: a practical example of the problems encountered in an attempt to recognize discomfort in laboratory animals. *Laboratory Animals 21*: 35-42

Bove G 2006 Mechanical sensory threshold testing using nylon monofilaments: the pain field's 'tin standard'. *Pain 124*: 13-17

Burman OHP, Ilyat A, Jones G and Mendl M 2007 Ultrasonic vocalizations as indicators of welfare for laboratory rats (*Rattus norvegicus*). *Applied Animal Behaviour Science 104(1-2)*: 116-129

Cavaletti G, Tredici G, Braga M and Tazzari S 1995 Experimental peripheral neuropathy induced in adult rats by repeated intraperitoneal administration of taxol. *Experimental Neurology 133*: 64-72

Crabbe JC, Wahlsten D and Dudek BC 1999 Genetics of mouse behavior: Interactions with laboratory environment. *Science 284*: 1670-1672

Crawley JN, Gerfen CR, Rogawski MA, Sibley DR, Skolnick P and Wray S 2007 *Short Protocols in Neuroscience – Systems and Behavioural Methods.* John Wiley & Sons: Hoboken, NJ, USA

Crossley NA, Sena E, Goehler J, Horn J, van der Worp B, Bath PMW, Macleod M and Dirnagl U 2008 Empirical evidence of bias in the design of experimental stroke studies - A metaepidemiologic approach. *Stroke 39*: 929-934

D'Amour FE and Smith DL 1941 A method for determining the loss of pain sensation. *Journal of Pharmacology and Experimental Therapeutics 72*: 74-79

de Boo MJ, Rennie AE, Buchanan-Smith HM and Hendriksen CFM 2005 The interplay between replacement, reduction and refinement: considerations where the Three Rs interact. *Animal Welfare 14*: 327-332

De Groot AS and Martin W 2003 From immunome to vaccine: epitope mapping and vaccine design tools. *Novartis Foundation Symposium 254*: 57-252

De Koning P, Neijt JP, Jennekens FG and Gispen WH 1987 Evaluation of cisdiamminedichloroplatinum (II) (cisplatin) neurotoxicity in rats. *Toxicology and Applied Pharmacology 89*: 81-87

https://doi.org/10.1017/S0962728600000063 Published online by Cambridge University Press

Decosterd I and Woolf CJ 2000 Spared nerve injury: an animal model of persistent peripheral neuropathic pain. *Pain 87*: 149-158

Dogrul A, Gülmez SE, Deveci MS, Gul H, Ossipov MH, Porreca F and Tulunay FC 2007 The local antinociceptive actions of nonsteroidal antiinflammatory drugs in the mouse radiant heat tail-flick test. *Anesthesia and Analgesia 104*: 927-935

Dubuisson D and Dennis SG 1977 The formalin test: a quantitative study of the analgesic effects of morphine, meperidine, and brain stem stimulation in rats and cats. *Pain 4*: 161-174

Eaton M 2003 Common animal models for sapasticity and pain. *Journal of Rehabilitation Research and Development 40(4)*: S41-S54

Eliav E, Herzberg U, Ruda MA and Bennett GJ 1999 Neuropathic pain from an experimental neuritis of the rat sciatic nerve. *Pain 83*: 169-182

European Community 1986 *Council Directive 86/609 on the Approximation of Laws, Regulations and Administrative Provisions of the Member States Regarding the Protection of Animals used for Experimental and Other Scientific Purposes. OJL 358.* Official Journal of the European Communities, Luxembourg

Farazifard R, Safarpour F, Sheibani V and Javan M 2005 Eye-wiping test: A sensitive animal model for acute trigeminal pain studies. *Brain Research Protocols 16*: 44-49

Flecknell PA and Roughan JV 2004 Assessing pain in animals – putting research into practice. *Animal Welfare 13*: S71-75

Ford GK, Moriarty O, McGuire BE and Finn DP 2008 Investigating the effects of distracting stimuli on nociceptive behaviour and associated alterations in brain monoamines in rats. *European Journal of Pain 12(8)*: 970-979

Gebhart GF 1999 Animal Models of Pain. *ILAR Journal 40(3)*. http://dels.nas.edu/ilar_n/ilarjournal/40_3/40_3Introduction.html

Gentle MJ 2002 Attentional shifts alter pain perception in the chicken. *Animal Welfare 10*: S187-S194

Giamberardino MA, Vecchiet L and Albe-Fessard D 1990 Comparison of the effects of ureteral calculosis and occlusion on muscular sensitivity to painful stimulation in rats. *Pain 43(2)*: 227-234

Grigg P, Robichaud II DR and Bove GM 2007 A feedback-controlled dynamic linear actuator to test foot withdrawal thresholds in rat. *Journal of Neuroscience Methods 163*: 44-51

Goetzl FR, Burril DY and Ivy AC 1943 A critical analysis of algesiometric methods with suggestions for a useful procedure. *Quarterly Bulletin Northwestern University Medical School 17*: 280-291

Haffner F 1929 Experimentelle Prüfung schmerzstillender Mittel. *Deutsche Medizinische Wochenschrift 55*: 731-733. [Title translation: Experimental test of analgesic means]

Han JS, Bird GC, Li WD, Jones J and Neugebauer V 2005 Computerized analysis of audible and ultrasonic vocalizations of rats as a standardized measure of pain-related behavior. *Journal of Neuroscience Methods 141*: 261-269

Hansen AK, Sandøe P, Svendsen O, Forsmann B and Thomsen P 1999 The need to refine the notion of reduction. In: Hendriksen CFM and Morton DB (eds) *Humane Endpoints in Animal Experiments for Biomedical Research* pp 139-144. Proceedings of the International Conference, 22-25 November 1998, Zeist, The Netherlands. RSM Press: London, UK

Hargreaves K, Dubner R, Brown F, Flores C and Joris J 1988 A new and sensitive method for measuring thermal nociception in cutaneous hyperalgesia. *Pain 32*: 77-88

Houghton AK, Hewitt E and Westlund KN 1997 Enhanced withdrawal responses to mechanical and thermal stimuli after bone injury. *Pain 73*: 325-337

IASP 1994 Part III: Pain terms: A current list with definitions and notes on usage. In: Merskey H and Bogduk N (eds) *Classification of Chronic Pain, Second Edition* pp 209-214. IASP Press: Seattle, USA

Kim SH and Chung JM 1992 An experimental model for peripheral neuropathy produced by segmental spinal nerve ligation in the rat. *Pain 50*: 355-363

Kimble GA 1955 Shock intensity and avoidance learning. *Journal of Comparative and Physiological Psychology 48*: 281-284

Kruger L 1991 The idiosyncratic problems associated with pain research. In: Kruger L (ed) *Methods in Pain Research* pp 1-10. CRC Press: Boca Raton, Florida, USA

Lai Y-Y and Chan SHH 1982 Shortened pain response time following repeated algesiometric tests in rats. *Physiology & Behavior 28*: 1111-1113

Langley CK, Aziz Q, Bountra C, Gordon G, Hawkins P, Jones A, Langley G, Nurmikko T and Tracey I 2008 Volunteer studies in pain research — opportunities and challenges to replace animal experiments. The report and recommendations of a focus on alternatives workshop. *NeuroImage 42*: 467-473

Larson AA and Wilcox GL 1984 Synergistic behavioral effects of serotonin and tryptamine injected intrathecally in mice. *Neuropharmacology 23*: 1415-1418

Le Bars D, Gozariu M and Cadden SW 2001 Animal models of nociception. *Pharmacological Reviews 53*: 597-652

Leo S, Straetemans R, D'Hooge R and Meert T 2008 Differences in nociceptive behavioral performance between C57BL/6J, 129S6/SvEv, B6 129 F1 and NMRI mice. *Behavioural Brain Research 190*: 233-242

Lindsay TH, Jonas BM, Sevcik MA, Kubota K, Halvorson KG, Ghilardi JR, Kuskowski MA, Stelow EB, Mukherjee P, Gendler SJ, Wong GY and Mantyh PW 2005 Pancreatic cancer pain and its correlation with changes in tumor vasculature, macrophage infiltration, neuronal innervation, body weight, and disease progression. *Pain 119*: 233-246

Luo ZD 2004 *Pain Research: Methods and Protocols*. Humana Press: Totowa, NJ, USA

Ma C 2007 Animal models of pain. *International Anesthesiology Clinics 45*: 121-131

Macleod MM, Fisher M, O'Collins V, Sena ES, Dirnagl U, Bath PMW, Alastair M, Buchan AM, van der Worp B, Traystman R, Minematsu K, Donnan GA and Howells DW Good laboratory practice: Preventing introduction of bias at the bench. *Stroke*, in press; doi:10.1161/STROKEAHA.108.525386

Mao J 2002 Translational pain research: Bridging the gap between basic and clinical research. *Pain 97*: 183-187

Mauderli AP, Acosta-Rua A and Vierck CJ 2000 An operant assay of thermal pain in conscious, unrestrained rats. *Journal of Neuroscience Methods 97*: 19-29

Miranda HF, Lopez J, Sierralta F, Correa A and Pinardi G 2001 NSAID antinociception measured in a chemical and a thermal assay in mice. *Pain Research and Management 6*: 190-196

Mogil JS and Crager SE 2004 What should we be measuring in behavioral studies of chronic pain in animals? *Pain 112*: 12-15

Molony V 1997 Comments on Anand and Craig (Letters to the Editor) *Pain 70*: 293

Monga M and Sausville EA 2002 Developmental therapeutics program at the NCI: molecular target and drug discovery process. *Leukemia 16(4)*: 520-526

Morton DB 1992 A fair press for animals. *New Scientist 1816*: 28

Morton DB 1998 The importance of non-statistical design in refining animal experimentation. *ANZCCART Facts Sheet. ANZCCART News 11(2)*. http://www.adelaide.edu.au/ANZCCART/publications/fs17.pdf

Morton DB 2002 The importance of non-statistical experimental design in refining animal experiments for scientists, IACUCs, and other ethical review panels. In: Gluck JP, DiPasquale T and Barbara Orlans F (eds) *Applied Ethics in Animal Research. Philosophy, Regulation, and Laboratory Applications* pp 149-178. Purdue University Press: West Layfayette, Indiana, USA

Morton DB and Griffiths PH 1985 Guidelines on the recognition of pain, distress and discomfort in experimental animals and an hypothesis for assessment. *Veterinary Record 116*: 431-436

Na HS, Han JS, Ko KH and Hong SK 1994 A behavioral model for peripheral neuropathy produced in rat's tail by inferior caudal trunk injury. *Neuroscience Letters 177*: 50-52

Nagamine K, Ozaki N, Shinoda M, Asai H, Nishiguchi H, Mitsudo K, Tohnai I, Ueda M and Sugiura Y 2006 Mechanical allodynia and thermal hyperalgesia induced by experimental squamous cell carcinoma of the lower gingiva in rats. *Journal of Pain 7*: 659-670

Ness TJ 1999 Models of visceral nociception. *ILAR Journal 40(3)*: 119-128

Oliveira AR and Barros HMT 2006 Ultrasonic rat vocalizations during the formalin test: A measure of the affective dimension of pain? *Anesthesia and Analgesia 102*: 832-839

Olsson IAS, Hansen AK and Sandøe P 2007 Ethics and refinement in animal research. *Science 317*: 1680

Olsson IAS, Hansen AK and Sandøe P 2008 Animal welfare and the refinement of neuroscience research methods – a case study of Huntington's disease models. *Laboratory Animals 42(3)*: 277-283

Pacharinsak C and Beitz A 2008 Animal models of cancer pain. *Comparative Medicine 58*: 220-233

Pelissier T, Pajot T and Dallel R 2002 The orofacial capsaicin test in rats: effects of different capsaicin concentrations and morphine. *Pain 96*: 81-87

Portfors CV 2007 Types and functions of ultrasonic vocalizations in laboratory rats and mice. *Journal of the American Association for Laboratory Animal Science 46*: 28-34

Randall LO and Selitto JJ 1957 A method for measurement of analgesic activity on inflamed tissue. *Archives of International Pharmacodynamics and Therapeutics 111(4)*: 409-419

RCVS 2008 *Guide to Professional Conduct*. Royal College of Veterinary Surgeons: London, UK. http://www.rcvs.org.uk/Templates/Internal.asp?NodeID=89642 (accessed on 25 July 2008)

Ren K and Dubner R 1996 An inflammation/hyperalgesia model for the study of orofacial pain. *Journal of Dental Research 75*: 217

Ren K and Dubner R 1999 Inflammatory models of pain and hyperalgesia. *ILAR Journal 40(3)*: 111-118

Riopelle JM 1992 The ethics of using animal models to study treatment of phantom pain. *Anesthesiology 76*: 1069

Rollin B 1998 *The Unheeded Cry: Animal Consciousness, Animal Pain, and Science*. Iowa State University Press: Iowa, USA

Rollin B 2003 Oncology and ethics. *Reproduction in Domestic Animals 38*: 50-53

Roughan JV and Flecknell PA 2000 Effects of surgery and analgesic administration on spontaneous behaviour in singly-housed rats. *Research in Veterinary Science 69*: 283-288

Roughan JV and Flecknell PA 2001 Behavioural effects of laparotomy and analgesic effects of ketoprofen and carprofen in rats. *Pain 90*: 65-74

Roughan JV and Flecknell PA 2003 Evaluation of a short duration behaviour-based post-operative pain scoring in rats. *European Journal of Pain 7*: 397-406

Roughan JV, Flecknell PA and Davies BR 2004 Behavioural assessment of the effects of tumor growth in rats and the influence of the analgesics carprofen and meloxicam. *Laboratory Animals 38*: 286-296

Sadzot-Delvaux C, Merville-Louis MP, Delree P, Marc P, Piette J, Moonen G and Rentier B 1990 An *in vivo* model of varicella-zoster virus latent infection of dorsal root ganglia. *Journal of Neuroscience Research 26*: 83-89

Schwei MJ, Honore P, Rogers SD, Salak-Johnson JL, Finke MP, Ramnaraine ML, Clohisy DR and Mantyh PW 1999 Neurochemical and cellular reorganization of the spinal cord in a murine model of bone cancer pain. *Journal of Neuroscience 19*: 10886-10897

Sedcole JR 2006 Experimental design: minimizing the number of subjects that suffer may not mean minimizing total suffering. *Animal Behaviour 71*: 735-738

Seltzer Z, Dubner R and Shir Y 1990 A novel behavioral model of neuropathic pain disorders produced in rats by partial sciatic nerve injury. *Pain 43*: 205-218

Shimoyama M, Tanaka K, Hasue F and Shimoyama N 2002 A mouse model of neuropathic cancer pain. *Pain 99*: 167-174

Short CE 2003 The management of animal pain. Where have we been, where are we now, and where are we going? *The Veterinary Journal 165*: 101-103

Sima AA 1980 Peripheral neuropathy in the spontaneously diabetic BB-Wistar-rat. An ultrastructural study. *Acta Neuropathologica 51*: 223-227

Slart R, Yu AL, Yaksh TL and Sorkin LS 1997 An animal model of pain produced by systemic administration of an immunotherapeutic anti-ganglioside antibody. *Pain 69*: 119-125

Smith BH, Elliott AM, Chambers WA, Smith WC, Hannaford PC and Penny K 2001 The impact of chronic pain in the community. *Family Practice 18*: 292-298

Smith JA, van den Broek FAR, Cantó Martorell J, Hackbarth H, Ruksenas O and Zeller W 2007 Principles and practice in ethical review of animal experiments across Europe: summary of the report of a FELASA working group on ethical evaluation of animal experiments. *Laboratory Animals 41*: 143-160

Sutton DC and Lueth HC 1930 Pain: Experimental production of pain on excitation of the heart and great vessels. *Archives of Internal Medicine 45*: 827-867

Tannenbaum J 1999 Ethics and pain research in animals. *ILAR Journal 40(3)*: 97-110

Tanner KD, Reichling DB and Levine JD 1998 Nociceptor hyper-responsiveness during vincristine-induced painful peripheral neuropathy in the rat. *The Journal of Neuroscience 18(16)*: 6480-6491

Taylor AA and Weary DM 2000 Vocal responses of piglets to castration: identifying procedural sources of pain. *Applied Animal Behaviour Science 70*: 17-26

Taylor AA, Weary DM, Lessard M and Braithwaite LA 2001 Behavioural responses of piglets to castration: the effect of pig age. *Applied Animal Behaviour Science 73*: 35-45

van der Staay FJ 2006 Animal models of behavioral dysfunctions: Basic concepts and classifications, and an evaluation strategy. *Brain Research Reviews 52*: 131-159

van der Warp HB, de Haan P, Morrema E and Kalkman CJ 2005 Methodological quality of animal studies on neuroprotection in focal cerebral ischaemia. *Journal of Neurology 252*: 1108-1114

Vander Wende C and Margolin S 1956 Analgesic tests based upon experimentally induced acute abdominal pain in rats. *Federation Proceedings 15*: 494

Vierck CJ, Hansson PT and Yezierski RP 2008 Clinical and pre-clinical pain assessment: Are we measuring the same thing? *Pain 135*: 7-10

Villanueva L 2000 Is there a gap between preclinical and clinical studies of analgesia? *Trends in Pharmacological Sciences 21*: 461-462

Viñuela-Fernández I, Jones E, Welsh EM and Fleetwood-Walker SM 2007 Pain mechanisms and their implication for the management of pain in farm and companion animals. *The Veterinary Journal 174*: 227-239

von Frey M 1922 Zur Physiologie der Juckempfindung. *Archives Néerlandaises de Physiologie de l'Homme et des Animaux 7*: 142-145. [Title translation : On the physiology of skin perception]

https://doi.org/10.1017/S0962728600000063 Published online by Cambridge University Press

von Korff M, Ormel J, Keefe FJ and Dworkin SF 1992 Grading the severity of chronic pain. *Pain 50*: 133-149

Vorstenbosch JMG 2005 The ethics of the Three Rs principle: a reconsideration. *Animal Welfare 14*: 339-345

Vos BP, Strassman AM and Maciewicz RJ 1994 Behavioral evidence of trigeminal neuropathic pain following chronic constriction injury to the rat's infraorbital nerve. *Journal of Neuroscience 14*: 2708-2723

Wagner R, DeLeo JA, Coombs DW, Willenbring S and Fromm C 1993 Spinal dynorphin immunoreactivity increases bilaterally in a neuropathic pain model. *Brain Research 629*: 323-326

Walker K, Alyson JF and Urban LA 1999 Animal models for pain research. *Molecular Medicine Today 5*: 319-321

Wall PD, Devor M, Inbal R, Scadding JW, Schonfeld D, Seltzer Z and Tomkiewicz MM 1979 Autotomy following peripheral nerve lesions: experimental anaesthesia dolorosa. *Pain 7*: 103-111

Wang LX and Wang ZJ 2003 Animal and cellular models of chronic pain. *Advanced Drug Delivery Reviews 55*: 949-965

Watson BD, Prado R, Dietrich WD, Ginsberg MD and Green BA 1986 Photochemically induced spinal cord injury in the rat. *Brain Research 367*: 296-300

Wenig CM, Schmidt CO, Kohlmann T and Schweikert B 2008 Costs of back pain in Germany. *European Journal of Pain*, in press

Wiech K, Seymour B, Kalisch R, Stephan KE, Koltzenburg M, Driver J and Dolan RJ 2005 Modulation of pain processing in hyperalgesia by cognitive demand. *NeuroImage 27(1)*: 59-69

Williams WO, Riskin DK and Mott KM 2008 Ultrasonic sound as an indicator of acute pain in laboratory mice. *Journal of the American Association for Laboratory Animal Science 47(1)*: 8-10

Wilson SG and Mogil JS 2001 Measuring pain in the (knockout) mouse: big challenges in a small mammal. *Behavioural Brain Research 125*: 65-73

Woolfe G and MacDonald AD 1944 The evaluation of the analgesic action of pethidine hydrochloride. *Journal of Pharmacology and Experimental Therapeutics 80*: 300-307

Würbel H 2000 Behavior and the standardization fallacy. *Nature Genetics 26*: 263

Würbel H 2002 Behavioural phenotyping enhanced: beyond (environmental) standardization. *Genes Brain and Behavior 1*: 3-8

Würbel H 2007 Publications should include an animal welfare section. *Nature 446*: 257

Würbel H and Garner JP 2007 Refinement of rodent research though environmental enrichment and systematic randomization. *NC3Rs 9*: 1-9

https://doi.org/10.1017/S0962728600000063 Published online by Cambridge University Press