The Veterinary Journal 232 (2018) 70–77

Contents lists available at ScienceDirect

The Veterinary Journal

journal homepage: www.elsevier.com/locate/tvjl

Review

Rodent analgesia: Assessment and therapeutics

Paul Flecknell

Institute of Neuroscience, Newcastle University, Newcastle upon Tyne NE24HH, UK

ARTICLE INFO

Keywords:
Analgesia
Guinea pig
Mouse
Pain
Rat

ABSTRACT

Current use of analgesics to control procedure-related pain in laboratory rodents is unacceptably low. Almost all currently available analgesics were developed in small rodents, prior to use in man, so that safety and efficacy data in laboratory assays are available. Greater use of analgesics would be encouraged by critical evaluation of the potential interactions of these compounds with the outcomes of specific research studies. As in other species, effective post-procedural analgesia requires reliable 'cage-side' methods of assessing pain. Recent advances in pain assessment should lead to both more extensive and more effective use of analgesics in these species.

© 2017 Elsevier Ltd. All rights reserved.

Introduction

Rodents are the most widely used species in biomedical research, and it is estimated that approximately 4.6 million of these animals will experience procedure-related pain, which could be avoided by implementation of effective analgesia. Regrettably, the use of analgesics in laboratory rodents appears low, with various literature-based surveys suggesting that less than 25% of rodents receive specific analgesic treatment after surgical procedures (Stokes et al., 2009). This is somewhat surprising, since all of the analgesics currently available for medical and veterinary medical use were developed and assessed for safety and efficacy in rodents.

Responses from research workers indicated a number of reasons for this underuse of analgesics in laboratory animals: concern at the clinical side-effects of analgesics, concern that analgesic use could confound the results from their study, and an apparent uncertainty as to when and how to administer analgesics (Richardson and Flecknell, 2005). Issues related to side-effects of analgesics are relatively easily dealt with, and will be discussed in more detail later. Potential confounding effects on data is a legitimate concern. The justification for use of laboratory animals generally adopts a utilitarian approach. The harms caused to animals used in research need to be outweighed by the benefits to humans and other animals for the research to be permitted. If reducing the harms (in this case procedure related pain) prevents delivery of the benefits, then analgesic use would not be appropriate. However, careful consideration of the magnitude of the potential effects of analgesics on research outputs, and reviewing all of the therapeutic options, should almost invariably enable use of some measures to prevent or alleviate pain (Percie du Sert et al., 2017).

A much more significant problem is the difficulty of assessing pain in laboratory animals. This is, of course, a problem in all species of animals, in all areas of animal use by society. However, since rodents are used to study pain and nociceptive mechanisms, methods of pain assessment should be better developed in these species than in companion, farm, zoo or wild animals. Until relatively recently this was not the case, but we now have a range of approaches that are leading to major improvements in the management of pain.

The reason that pain assessment is central to the issue of providing pain relief is worth restating here. If we cannot identify the presence of pain, or its intensity, then we will not know when to give analgesics, nor what type of analgesic might be needed. We also will not be able to determine that the analgesics given have been effective, nor when to administer additional doses, nor when to stop treatment. The pitfalls of administering 'standard' doses of analgesics without proper pain assessment (Utting and Smith, 1979) were well recognized as contributing to the poor management of pain in people in the 1970s and 80s. The intensity of pain perceived after apparently identical surgical procedures varies in different individuals (Fillingim, 2005), and this variation also occurs in animals. In rodents, the genetics of this variation in both nociceptive thresholds, analgesic efficacy, and pain expression, have been extensively evaluated (Elmer et al., 1998; Mogil et al., 1999). It is reasonable to assume that similar variation occurs in all species, so the only means of effectively managing pain is to assess it accurately, so that appropriate treatment can be started, and the efficacy of that treatment assessed.

E-mail address: paul.flecknell@ncl.ac.uk (P. Flecknell).

https://doi.org/10.1016/j.tvjl.2017.12.017
1090-0233/© 2017 Elsevier Ltd. All rights reserved.

Pain assessment in rodents

Pain in humans is recognized to be a complex, multidimensional sensory and emotional experience. Although the nature of pain in animals almost certainly varies between species (Sneddon et al., 2014), methods of assessment should enable both the sensory and emotional components of pain to be identified. Newer techniques that attempt to assess the emotional component of pain in animals have been used in developing novel compounds intended for pain management in people. The drive to develop these methods arose from dissatisfaction with older techniques used in laboratory species which relied primarily on assessment of nociceptive thresholds. Neither of these approaches are suitable for 'cage-side' evaluation of animals that have undergone potentially painful procedures as part of other (non-pain research related) projects. However, they are of value in determining potentially effective dose rates of analgesic agents.

Nociceptive testing

Most laboratory based assessment techniques only evaluate the sensory component of pain, and many evaluate only nociceptive mechanisms (Mogil, 2009). Typical assessments apply a noxious stimulus and observe the animals' response, for example local heating in the Tail flick test, or when using the Hargreave's apparatus (Le Bars et al., 2001; Barrot, 2012). Other tests use different modalities of noxious stimulus (e.g. mechanical stimuli, cold, electric shock and chemical irritants). Assessments of the changes in sensory thresholds, for example after tissue injury, are also made, with response to light touch and other normally non-noxious stimuli being used to assess allodynia and hyperalgesia (Zahn and Brennan, 1999). These assays have been valuable in the study of mechanisms of nociception, and the modulation of sensory pathways, but analgesic doses effective in these systems cannot always be extrapolated to different clinical situations. However in some species, with some analgesics, this is the only data available (e.g. Collier et al., 1961)

The limitations of simple nociceptive assays has been recognized by research workers seeking to develop novel analgesics, particularly those investigating neuropathic pain (Rice et al., 2008). In an effort to evaluate responses that require central processing of pain, a variety of approaches have been developed.

Conditioned place preference and conditioned place avoidance

In humans, pain is aversive and unpleasant, and we assume this is also the case in animals. It follows, then, that alleviation of pain should be rewarding. This has been tested using conditioned place preference. This approach uses an apparatus consisting of two chambers which differ in visual and other sensory cues. For example one chamber might have a grid floor and striped walls, the other a solid floor and uniformly coloured walls. A treatment considered likely to induce pain, based on experience in people, is applied, for example induction of inflammation in a paw. The animal is then confined in one chamber, and given either saline or an analgesic. This is repeated, with the treatment and chamber being switched. The animal therefore associates one chamber with receiving pain relief, and one chamber with experiencing pain. After these training sessions, the animal is given a free choice and the time spent in each chamber is assessed. If relief from pain is rewarding, then the animal should choose to spend more time in the analgesic-paired chamber. In the first study to assess this (Sufka, 1994), inflammatory pain was induced and morphine administered as the analgesic. Appropriate controls for any reinforcing effects of morphine were included, and the design balanced for inherent preferences for one of the chambers over the other. Animals spent significantly more time in the analgesic paired chamber, supporting the hypothesis that reduction of pain was rewarding.

Conditioned place aversion studies take a similar approach, but one chamber is associated with a pain state, and the other with being pain-free. It is assumed that if pain is aversive, then animals will subsequently choose to spend more time in the chamber associated with a pain free state. This has been demonstrated in a number of studies, but more significantly, some research groups have compared normal animals with animals with deliberate lesions of the anterior cingulate cortex (Johansen et al., 2001), a brain area associated with the emotional component of pain in humans. Pain was induced by repeated injections of formalin into a footpad, a procedure that induces a short (20–30 min) period of pain. Lesioned animals developed significantly less conditioned place aversion, supporting the hypothesis that it is the emotional component of pain that is responsible for the learned avoidance in these types of assessment.

Subsequent studies have applied these approaches to examine the responses to different types of pain (for example neuropathic pain; Qu et al., 2011), and different analgesic agents. Dose rates established in these types of assessment are more likely to reflect clinically effective doses.

Digging tests, nest building

Other approaches to assessing more complex responses to pain have searched for parallels with human behaviours. People in chronic pain tend to show a variety of changes to their lifestyle, with a reduction, for example, in housekeeping, sociability etc. If pain disrupts these highly motivated and complex behaviours in man, then it is possible that similar effects may be seen in animals. Two behaviours have been evaluated in detail — digging and nest building. These are two highly motivated behaviours in most strains of rats and mice. Rats experiencing either neuropathic or inflammatory pain have been shown to reduce their digging behavior (Andrews et al., 2012) and this can be reversed by analgesic agents. This approach to assessing analgesic efficacy has now undergone a multicenter validation, and is likely to become widely used for assessment of novel analgesics (Wodarski et al., 2016). Burrowing behavior can also be used as a measure of pain in mice (Jirkof, 2014), although it was initially established as a general measure of well-being (Deacon, 2012).

Studies of disruption of nest building in mice experiencing pain have produced varying results. One of the first studies showed significant deterioration in the quality of nest building following surgery, which could be prevented by analgesic administration (Arras et al., 2007), but subsequent results were less clear cut (Jirkof et al., 2013; Negus et al., 2015). Alternative measures, such as the time taken to incorporate new material into an existing nest ('time to integrate to nest'), or the time taken to begin using new nesting material have also been used as metrics (Rock et al., 2014; Häger et al., 2015).

Since other causes of distress or ill-health can alter both nest building and digging behavior, care must be taken when using these measures as the sole indicator of pain, or of analgesic efficacy.

Operant testing

Operant testing of pain and nociception involves the animal making a learned response to avoid a potential noxious stimulus, or to gain access to a reward. A variety of approaches have been developed, some of which clearly require complex central processing of the task involved. One more recently developed assay, the operant orofacial pain assay (Neubert et al., 2008) appears to predict likely clinically effective dose rates of analgesics

(Ramirez et al., 2015). The apparatus used requires the rodent to poke its nose between metal bars, in order to obtain a preferred food reward. The bars can be heated, so that a noxious stimulus is applied to the nose. The animal then balances its desire for the reward against the aversive stimulus of the heat. Administering an analgesic increases the time spent obtaining the reward. In order to prevent tissue damage caused by repeated exposure to noxious heat, the animals' skin is sensitized with capsaicin, so that non-damaging lower temperatures can be used.

Cage-side assessments

The approaches described above are primarily useful when evaluating the nature of pain and analgesic efficacy, but are impracticable for routine use as measures of pain following a range of different types of research procedures. The exception may be nest building and burrowing, that could be integrated into more general assessments of animal welfare, but require background control data in the different strains of rodent and different environmental conditions used in research facilities.

Attempts to develop 'cage-side' pain assessment systems have adopted a number of different approaches. Some groups have assessed changes to normally exhibited behaviours, either alone or combined with changes in appearance thought to reflect pain (e.g. piloerection) (Oliver et al., 2017). Others have aimed to identify abnormal behaviours, that are only exhibited by animals that receive no pain relief (Roughan and Flecknell, 2004; Wright-Williams et al., 2013; Ellen et al., 2016). The hypothesis in both approaches is that analgesic administration should reverse the changes seen. Since these studies have generally assessed animals following surgical procedures, they should provide guidance as to clinically effective dose rates

As with all of the other approaches to pain assessment, and to assessments of analgesic efficacy, it is important that studies incorporate all appropriate control treatments. If this is not the case, then non-specific effects of analgesics or anaesthetics can alter the interpretation of study outcomes. The need for appropriate controls may also require use of a negative control group comprising animals that undergo a surgical procedure, for example, but receive no pain relief. This raises significant ethical concerns, but given the major gaps in our ability to assess and manage pain effectively in laboratory animals, such an approach can be justified. The degree and duration of pain should, of course, be the minimum required to obtain the data needed. The study design should also include use of intervention analgesia and withdrawal of animals from study if pain intensity is judged too great.

Many of the assessments used are either subjective, or likely to be sensitive to bias in scoring, so that good experimental design with blinding, randomization and inclusion of all relevant controls is particularly important (Kilkenny et al., 2010).

In mice, rats and guinea pigs, several behaviours that are associated with abdominal pain have been identified (Roughan and Flecknell, 2003; Wright-Williams et al., 2013; Ellen et al., 2016) and shown to be responsive to a number of different classes of analgesic. In rats, the simple metric of assessing food and water intake and consequent bodyweight changes has also been shown to be useful (Liles and Flecknell, 1994), however since this is a retrospective measure, it does not allow additional pain relief to be given to the animals being assessed.

Grimace scales

A number of facial expressions that appear to be associated pain have been identified (Langford et al., 2010). Changes in facial expression have been used to assess pain in humans for decades and a pain scale for infants using facial expression has been widely used (Grunau and Craig, 1990). Following the demonstration that mice have clearly identifiable changes in facial expression associated with different pain states, similar changes have been identified in a number of other species (Descovich et al., 2017). All species studied to date show some similar changes to their facial expressions, including contraction of the muscles around the eyes, producing a partial closure of the eye, together with changes in ear position, and the contour of the facial muscles. The changes in each individual component of the grimace score is rated on a numerical scale (usually 0, 1 or 2) and the sum of these scores comprises the "Grimace score". Increases in grimace scores following surgery, and the reversal of this increase by analgesic therapy have been demonstrated in rats and mice (Sotocinal et al., 2011; Leach et al., 2012; Matsumiya et al., 2012; Waite et al., 2015; Thomas et al., 2016).

The attraction of grimace scores is that they may be both an easier, more rapid assessment to make, and one which makes use of our tendency as humans to pay attention to faces (Leach et al., 2011). Changes in Grimace Scores may also occur following a range of different types and location of painful stimuli, whereas some of the other behavioural measures are only applicable following particular types of surgery (e.g., abdominal, Wright-Willams et al., 2007; orthopaedic, Foley, 2011).

Although Grimace Scoring is a potentially valuable means of scoring pain, we still lack a great deal of information about the triggers for grimacing in animals. Typical 'pain faces' can be detected during aggressive encounters in rats, before any injuries have occurred (Defensor et al., 2012). Sedation has been shown to be a potential confounding effect, and baseline Grimace scores, and scores in response to painful stimuli vary between different strains of mice (Miller and Leach, 2015a, 2015b; Miller et al., 2016).

Practical application of scoring systems

There is, as yet, no reference standard pain assessment scheme for rodents. Using a combination of methods to assess the behavior and appearance of animals, and their responses to analgesics, is most likely to be successful. Although many clinical observations, such as general activity, coat condition, eye colour and appearance etc are not specific signs of pain, it is useful to include them in any assessment as they indicate the animal's general welfare state following a potentially painful procedure such as surgery (Morton and Griffiths, 1985; Graf et al., 2016). Detecting these signs may trigger therapeutic actions, such as administration of fluids, soaked diet, and additional bedding, as well as considering the adequacy of pain relief. More specific signs of pain, such as behavioural changes and grimace scores can then be used to evaluate the need for additional analgesia.

Making these assessments requires at least 5–10 min per animal, and assessments may need to be repeated at least every 2–3 h if effective pain relief is to be achieved. This creates major resourcing issues, especially since rodent surgical procedures are often conducted on groups of 6–10 or more animals. The ideal solution, shown to be effective in people, is to allocate additional resources to pain management, although this may not be possible in all research facilities. Since research procedures are often conducted by the same personnel, on the same age, sex and strain of animal, individual variation in pain sensation and responses to analgesics should be relatively small. If an initial group of animals are assessed frequently, using detailed evaluations, and an apparently effective analgesic regimen established, then this regimen can be used with greater confidence in subsequent groups. Since procedures may change over time, repeating the more detailed assessments at intervals will enable further adjustments to the analgesic regimen to be made. It should be

recognized that this pragmatic approach is not ideal, because of the individual variation in pain intensity and analgesic requirements, but it is likely to represent a major improvement to current practice.

Therapeutics

As in all species, any analgesic regimen for rodents must be integrated with other general measures of care. For example, when managing post-surgical pain, the anaesthetic regimen, intra-operative care and pre- and post-operative management can all impact on the efficacy of pain assessment and pain management. For example, pain assessment may be difficult to undertake if recovery from anaesthesia is prolonged because of the choice of anaesthetic agents and the presence of hypothermia or other intra or post-operative complications. In addition, stress and fear may increase the degree of pain perceived by an animal.

There is still uncertainty about the efficacy of many analgesic agents, and particularly about their duration of action. However, likely effective dose rates can be suggested (Table 1) for a range of different agents. As in other species, it is probable that multimodal and preventive analgesia will be more effective than use of a single agent post-procedure. However, direct assessment of use of these regimens in post-surgical and other acute pain is lacking in rodents.

A major concern associated with the use of analgesics in laboratory animals is the potential interactions of these agents with research results. There are clearly situations where this can be demonstrated, for example administration of opioids changes infarct size in models of Stroke in rats (Ferland et al., 2007), and administration of opioids changes the degree of injury caused by coronary artery ligation (Ter Horst et al., 2017). In some cases, however, the potential effects are over-stated, and are based on studies in which analgesics were administered at relatively high dose rates, for prolonged periods. The judgements to be made are often complex, for example non-steroidal anti-inflammatory drugs (NSAIDs) can interact with bone healing but their effects in animal models can be contradictory (Pountos et al., 2012). When administered in lower doses, for shorter periods, they may have fewer direct effects, and promote earlier weight bearing in rodent models of fracture healing. Earlier weight bearing can, of course, influence bone healing. Unalleviated pain can reduce general activity, which can alter body temperature, which affects many metabolic processes, including wound healing. Pain can also prolong the catabolic state induced by surgery. To address these complex issues, careful consideration of all of these factors is required (Peterson et al., 2017), and this is often beyond the scope of individual research groups, veterinarians or ethics committees. The approach that can offer the greatest contribution to this area is the establishment of expert working groups, involving scientists actively working in the specific field of research, together with those with expertise in laboratory animal care and welfare. Such groups have already published constructive recommendations in some areas of research (Hawkins et al., 2015; Lidster et al., 2016; Percie du Sert et al., 2017), and it is to be hoped that other scientific disciplines adopt similar approaches.

If potential interactions between analgesics and research outcomes are a concern, this can be addressed by careful selection of the analgesic regimen, administration of analgesics only for the period required to provide pain relief, and titration of the dose used to be the lowest needed for effective pain relief.

Specific agents — NSAIDs

It is likely that all of the agents currently available for use in people and in companion and farm animals can be used safely in laboratory rodents, and dose rates effective in arthritis models are often available. Data on the use of NSAIDs for acute post-operative pain are available for a restricted range of agents, including meloxicam, carprofen and ketoprofen. There appears to be some

Table 1
Analgesic dose rates for rats, mice and guinea pigs. Note that considerable strain and sex variation is likely; the frequency of dosing, and dose rates, should be adjusted based on assessment of the intensity of pain.

Analgesic agent	Rat	Mouse	Guinea pig	Comments
Buprenorphine	0.03–0.05 mg/kg SC 0.5–0.6 mg/kg orally	0.1–0.2 mg/kg SC	0.05 mg/kg SC	Rat: laparotomy and behavioural assessment (Roughan and Flecknell, 2004), Rat Grimace Scale, orofacial operant assay (Taylor et al., 2016); mouse: post-vasectomy, behavioural measures (Wright-Williams et al., 2013), post-laparotomy, Mouse Grimace Score (Matsumiya et al., 2012); guinea pig: not adequately evaluated, based on clinical assessment
Buprenorphine SR (current US slow release formulation)	1.2 mg/kg SC	0.6 mg/kg SC	0.3–0.48 mg/kg SC[a]	Rat: orthopaedic surgery (Foley et al., 2011), incisional model (Chum et al., 2014); mouse: laparotomy with multiple measures of efficacy (Kendall et al., 2016), guinea pig: paw withdrawal and pk (Smith et al., 2016), laparotomy, multiple measures (Oliver et al., 2017)
Carprofen	5 mg/kg SC	20 mg/kg SC[b]	4 mg/kg SC (with buprenorphine slow release)	Rat: laparotomy, behavioural measures (Roughan and Flecknell, 2004), mouse: post-laparotomy, Mouse Grimace Score (Matsumiya et al., 2012), guinea pig: laparotomy, multiple measures (Oliver et al., 2017)
Ketoprofen	5 mg/kg SC	20 mg/kg SC[b]	[c]	Rat: laparotomy, behavioural measures (Roughan and Flecknell, 2004), mouse: post-laparotomy, Mouse Grimace Score (Matsumiya et al., 2012), guinea pig: no data
Meloxicam	1 mg/kg SC	5–20 mg/kg SC[b]	0.2 mg/kg	Rat: post-laparotomy, behavioural measures (Roughan and Flecknell, 2003), mouse: post-vasectomy, behavioural measures (Wright-Willams et al., 2007), guinea pig: orchidectomy, in combination with bupivacaine (Ellen et al., 2016)
Morphine	2–5 mg/kg SC	1–5 mg/kg SC	5 mg/kg SC	Rat: incisional model (Zahn et al., 1997), laparotomy and other assays, RGS (Sotocinal et al., 2011), mouse: reversal of acid induced nestbuilding suppression (Negus et al., 2015); guinea pig: antinociceptive test (Collier et al., 1961)
Tramadol	20–40 mg/kg orally 5 mg/kg SC	80 mg/kg SC	[c]	Rat: orofacial operant assay (Taylor et al., 2016), urethral calculi, behavioural measures (Affaitati et al., 2002); mouse: effective in post-laparotomy model only at dose rates close to toxic doses (Wolfe et al., 2015), guinea pig: no data

[a] Multi-modal analgesia with carprofen 4 mg/kg is more effective.
[b] Note these doses are close to, or exceed, the ulcerogenic dose.
[c] No data located.

marked species variation in the efficacy of NSAIDs, with very high dose rates being required in mice (20 mg/kg) in comparison with rats (1 mg/kg) for post-operative analgesia (Wright-Willams et al., 2007; Matsumiya et al., 2012; Miller and Roughan, 2012; Roughan et al., 2016).

Some NSAIDs have been associated with undesirable side effects. Although ketoprofen has been shown to be effective in rats (Roughan and Flecknell, 2001), it has been reported to cause gastrointestinal ulceration (Lamon et al., 2008; Shientag et al., 2012). There are anecdotal reports of toxicity of carprofen and meloxicam, but such reports are uncommon and the relatively short treatment period required for post-operative pain management is unlikely to result in undesirable side-effects. Dosing recommendations for carprofen and meloxicam are usually once daily, but this is based on relatively limited data, and twice daily administration may be required in mice (Engelhardt et al., 1996; Busch et al., 1998; Chen et al., 2016). In the guinea pig, meloxicam combined with local anaesthetic block at the surgical site provided effective pain relief (Ellen et al., 2016). The oral formulation of meloxicam is easy to administer to rats, mice, guinea pigs and other rodents.

Acetaminophen (paracetamol)

When assessed using a nociceptive model in mice, both direct analgesic effects of paracetamol and synergism with NSAIDs could be demonstrated (Miranda et al., 2006). However results when using this agent for post-surgical pain relief have been disappointing (Roughan, personal communication; Dickinson et al., 2009; Matsumiya et al., 2012). Although combinations of acetaminophen with NSAIDs or weak opioids may be more effective, few controlled studies have been undertaken to assess their efficacy for postoperative pain. Acetaminophen is availabile in a palatable solution and so is convenient for administration to rodents (Fleischmann et al., 2017), but until more conclusive demonstrations of efficacy are available, is probably better to use other analgesics.

Opioids

Opioids are the most widely used agents for provision of post-operative analgesia in laboratory rodents, with buprenorphine being the most commonly selected agent (Stokes et al., 2009). This is almost certainly because of the longer duration of action of this agent, which is dose dependent (Roughan and Flecknell, 2002), with higher dose rates providing a longer effect. When administered at the most commonly recommended dose rates (as in Table 1), the duration of action may range from 4 to 8 h, depending upon the species and the individual (Gades et al., 2000; Jirkof et al., 2014; Sauer et al., 2016).

Slow release formulations of buprenorphine are available and these provide sustained plasma concentrations of buprenorphine for 3 days (Foley, 2011; DiVincenti et al., 2016; Kendall et al., 2016). The slow release formulation may be particularly valuable when prolonged durations of analgesia are required. Repeated dosing with standard preparations of buprenorphine for several days at high dose rates has been associated with detrimental effects such as reduced appetite and weight loss (Gillingham et al., 2001), but his does not seem to occur with slow-release formulations (Jirkof et al., 2014). This may be because plasma concentrations of buprenorphine are more stable after use of the slow-release formulation, in contrast to the repeated peaks and troughs associated with intermittent dosing. Pharmacokinetic data is available in rats and mice, and of analgesic efficacy in various models (Clark et al., 2014; Jirkof et al., 2014; Kendall et al., 2014, 2016; Seymour et al., 2016; Smith et al., 2016). Although many surgical procedures would not result in pain of sufficient intensity and duration to require 3 days of opioid administration, if the slow release formulation can be used without causing undesirable side effects, then it will provide a very valuable addition to options for pain management in all species.

Buprenorphine administration has been associated with other adverse effects, notably pica (eating of non-nutritive materials, usually bedding) (Clark et al., 1997). This is a relatively uncommon side effect, and is a general side effect of opioids, thought to indicate nausea in species that are incapable of vomiting (Mitchell et al., 1977). In experimental models of opioid and chemotherapeutic agent-induced pica (Batra and Schrott, 2011; Davis, 2016), anti-emetics have been shown to be effective but there appear to be no reports of use of these agents to manage pica in a post-operative setting (Takeda et al., 1995). If pica is noted, either therapy with antiemetics can be attempted, or a different class of analgesics can be used to manage pain.

Other opioid side effects rarely cause clinically significant problems in rodents, for example the degree of respiratory depression is minimal in comparison to that seen in people. Concern has been expressed that the reduction in gut motility by opioids could be a problem in guinea pigs because of the sensitivity of this species to post-operative ileus, but this does not appear to be a significant problem. If there are concerns about ileus, prokinetics such as ranitidine or cisapride can be administered (Wenger, 2012).

As an alternative to systemic administration, opioids can also be administered epidurally or intrathecally to small rodents, as in larger species. The technique in guinea pig and rat is reasonably practicable (Turner et al., 2011), although a recent evaluation of intrathecal morphine in rats showed that, unlike in dog and cat, the duration of action of this opioid was not prolonged when administered by this route (Zahn et al., 1997; Thomas et al., 2016). Effective analgesia was, however, produced with a much reduced dose of morphine.

Tramadol

Tramadol has been shown to have analgesic efficacy in laboratory models of nociception (Oyama et al., 2012). However assessment of its efficacy for post-operative pain management in rats and mice have shown variable results (Affaitati et al., 2002; Zegre Cannon et al., 2011; Wolfe et al., 2015; Taylor et al., 2016). Its oral bioavailability make it a potentially useful agent for post-operative analgesia, but further studies to establish appropriate dose rates and dosing regimens are required.

Local anaesthetics

Local anaesthetics are used extensively in companion and farm animals to provide analgesia both during and following painful procedures. In contrast, they are used relatively infrequently in small rodents. This is presumably because of perceived practical issues, since safety and efficacy of the commonly used local anaesthetics is similar to that in other species. There are less data in guinea pigs, but clinical experience and laboratory studies indicate the agents can all be used successfully in this species.

Local anaesthetics can be administered as splash blocks, by local infiltration, by blocking specific sensory nerves and by the epidural or intrathecal route. Small mammals can only be given small volumes of commercial formulations of local anaesthetics, but both lidocaine and bupivacaine can be diluted 1:4 with only moderate effects on the duration of action (Grant et al., 2000). It is advisable to calculate the maximum safe dose, and prepare this in advance, since it is easy to inadvertently overdose when infliltrating a relatively large surgical field. Combining these two

agents has the advantage of providing a rapid onset of action due to the lidocaine, followed by more prolonged nerve block by bupivacaine. Although this combination reduces the duration of action of bupivacaine (Cuvillon et al., 2009), it is still often preferable to using either agent alone. The toxicity of these agents is additive, but in small rodents, higher doses have been proven to be safe and effective in the author's experience (10 mg/kg lidocaine plus 5 mg/kg bupivacaine).

Analgesics, efficacy and assessment

As discussed earlier, analgesics are relatively under-used in laboratory rodents. When they are administered, regimens often comprise a single agent, and pain assessment is rarely conducted. This contrasts markedly with the approach shown to be required in man, with use of multimodal analgesia and implementation of effective pain assessment tools. The need for adoption of this approach is evidenced most convincingly in people by the number needed to treat (NNT) data for analgesics. 'Number needed to treat' in the context of analgesic use is the number of patients that need to be given the agent in order for one patient to experience a 50% or greater reduction in pain intensity. Although there is some debate as to the best means of analyzing this data, most analgesics have an NNT of between 2 and 7 (McQuay et al., 2012; Katz et al., 2015). Most veterinarians make the assumption that if they administer an analgesic at the recommended dose rate, then it will be effective in controlling pain. If we accept that animals and people share similar mechanisms of pain perception and analgesic action, then the variation of analgesic efficacy in different individual people suggests that a similar variation will be seen in animals. This clearly reinforces the need for assessing pain regularly, so that poor-responders are given supplemental doses of analgesic, or alternative analgesic regimens.

Conclusions

Pain in small rodents can be managed effectively, just as it can be in other species. At present, pain management is limited by an apparent reluctance to implement multimodal approaches to pain relief and most significantly, by limited use of pain assessment tools. The rapid expansion of available data in this field should, however, lead to significant advances in preventing and alleviating pain in small rodents.

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

References

Affaitati, G., Giamberardino, M.A., Lerza, R., Lapenna, D., De Laurentis, S., Vecchiet, L., 2002. Effects of tramadol on behavioural indicators of colic pain in a rat model of ureteral calculosis. Fundamental and Clinical Pharmacology 16, 23–30.

Andrews, N., Legg, E., Lisak, D., Issop, Y., Richardson, D., Harper, S., Pheby, T., Huang, W., Burgess, G., Machin, I., et al., 2012. Spontaneous burrowing behaviour in the rat is reduced by peripheral nerve injury or inflammation associated pain. European Journal of Pain 16, 485–495. doi:http://dx.doi.org/10.1016/j.ejpain.2011.07.012.

Arras, M., Rettich, A., Cinelli, P., Kasermann, H.P., Burki, K., 2007. Assessment of post-laparotomy pain in laboratory mice by telemetric recording of heart rate and heart rate variability. BMC Veterinary Research 3, 16. doi:http://dx.doi.org/10.1186/1746-6148-3-16.

Barrot, M., 2012. Tests and models of nociception and pain in rodents. Neuroscience 211, 39–50. doi:http://dx.doi.org/10.1016/j.neuroscience.2011.12.041.

Batra, V.R., Schrott, L.M., 2011. Acute oxycodone induces the pro-emetic pica response in rats. Journal of Pharmacology and Experimental Therapeutics 339, 738–745. doi:http://dx.doi.org/10.1124/jpet.111.183343.

Busch, U., Schmid, J., Heinzel, G., Schmaus, H., Baierl, J., Huber, C., Roth, W., 1998. Pharmacokinetics of meloxicam in animals and the relevance to humans. Drug Metabolism and Disposition 26, 576–584.

Chen, P.H., Boyd, K.L., Fickle, E.K., Locuson, C.W., 2016. Subcutaneous meloxicam suspension pharmacokinetics in mice and dose considerations for postoperative analgesia. Journal of Veterinary Pharmacology and Therapeutics 39, 356–362. doi:http://dx.doi.org/10.1111/jvp.12297.

Chum, H.H., Jampachairsri, K., McKeon, G.P., Yeomans, D.C., Pacharinsak, C., Felt, S.A., 2014. Antinociceptive effects of sustained-release buprenorphine in a model of incisional pain in rats (Rattus norvegicus). Journal of the American Association for Laboratory Animal Science 53 (2), 193–197.

Clark, J.A., Myers, P.H., Goelz, M.F., Thigpen, J.E., Forsythe, D.B., 1997. Pica behavior associated with buprenorphine administration in the rat. Laboratory Animal Science 47, 300–303.

Clark, T.S., Clark, D.D., Hoyt, R.F., 2014. Pharmacokinetic comparison of sustained-release and standard buprenorphine in mice. Journal of the American Association for Laboratory Animal Science: JAALAS 53, 387–391.

Collier, H.O.J., Warner, B.T., Skerry, R., 1961. Multiple toe-pinch method for testing analgesic drugs. British Journal of Pharmacology 17, 28–40. doi:http://dx.doi.org/10.1111/j.1476-5381.1961.tb01101.x.

Cuvillon, P., Nouvellon, E., Ripart, J., Boyer, J.-C., Dehour, L., Mahamat, A., L'Hermite, J., Boisson, C., Vialles, N., Lefrant, J.Y., et al., 2009. A comparison of the pharmacodynamics and pharmacokinetics of bupivacaine, ropivacaine (with epinephrine) and their equal volume mixtures with lidocaine used for femoral and sciatic nerve blocks: a double-blind randomized study. Anesthesia & Analgesia 108, 641–649. doi:http://dx.doi.org/10.1213/ane.0b013e31819237f8.

Davis, T.G., 2016. Pica in rats as a preclinical model of emesis. Current Protocols in Neuroscience 77 doi:http://dx.doi.org/10.1002/cpns.12 9.53.1–9.53.6.

Deacon, R., 2012. Assessing burrowing, nest construction, and hoarding in mice. Journal of Visualized Experiments e2607. doi:http://dx.doi.org/10.3791/2607.

Defensor, E.B., Corley, M.J., Blanchard, R.J., Blanchard, D.C., 2012. Facial expressions of mice in aggressive and fearful contexts. Physiology & Behavior 107, 680–685. doi:http://dx.doi.org/10.1016/j.physbeh.2012.03.024.

Descovich, K.A., Wathan, J., Leach, M.C., Buchanan-Smith, H.M., Flecknell, P., Farningham, D., Vick, S.-J., 2017. Facial expression: an under-utilised tool for the assessment of welfare in mammals. ALTEX 34, 409–429. doi:http://dx.doi.org/10.14573/altex.1607161.

Dickinson, A.L., Leach, M.C., Flecknell, P.A., 2009. The analgesic effects of oral paracetamol in two strains of mice undergoing vasectomy. Laboratory Animals 43, 357–361. doi:http://dx.doi.org/10.1258/la.2009.009005.

DiVincenti, L., Meirelles, L.A.D., Westcott, R.A., 2016. Safety and clinical effectiveness of a compounded sustained-release formulation of buprenorphine for postoperative analgesia in New Zealand White rabbits. Journal of the American Veterinary Medical Association 248, 795–801. doi:http://dx.doi.org/10.2460/javma.248.7.795.

Ellen, Y., Flecknell, P., Leach, M., 2016. Evaluation of using behavioural changes to assess post-operative pain in the guinea pig (*Cavia porcellus*). PLoS One 11, e0161941. doi:http://dx.doi.org/10.1371/journal.pone.0161941.

Elmer, G.I., Pieper, J.O., Negus, S.S., Woods, J.H., 1998. Genetic variance in nociception and its relationship to the potency of morphine-induced analgesia in thermal and chemical tests. Pain 75, 129–140. doi:http://dx.doi.org/10.1016/S0304-3959(97)00215-7.

Engelhardt, G., Homma, D., Schlegel, K., Schnitzler, C., Utzmann, R., 1996. General pharmacology of meloxicam—part I: effects on CNS, gastric emptying, intestinal transport, water, electrolyte and creatinine excretion. General Pharmacology: The Vascular System 27, 673–677.

Ferland, C., Veilleux-Lemieux, D., Vachon, P., 2007. Effects of buprenorphine on intracerebral collagenase-induced hematoma in Sprague-Dawley rats. Journal of the American Association for Laboratory Animal Science 46.3, 13–16.

Fillingim, R.B., 2005. Individual differences in pain responses. Current Rheumatology Reports 7, 342–347. doi:http://dx.doi.org/10.1007/s11926-005-0018-7.

Fleischmann, T., Arras, M., Sauer, M., Saleh, L., Rülicke, T., Jirkof, P., 2017. Voluntary intake of paracetamol-enriched drinking water and its influence on the success of embryo transfer in mice. Research in Veterinary Science 111, 85–92.

Foley, P.L., Liang, H., Crichlow, A.R., 2011. Evaluation of a sustained-release formulation of buprenorphine for analgesia in rats. Journal of the American Association for Laboratory Animal Science: JAALAS 50, 198–204.

Gades, N.M., Danneman, P.J., Wixson, S.K., Tolley, E.A., 2000. The magnitude and duration of the analgesic effect of morphine, butorphanol, and buprenorphine in rats and mice. Contemporary Topics in Laboratory Animal Science 39, 8–13.

Gillingham, M.B., Clark, M.D., Dahly, E.M., Krugner-Higby, L.A., Ney, D.M., 2001. A comparison of two opioid analgesics for relief of visceral pain induced by intestinal resection in rats. Contemporary Topics in Laboratory Animal Science 40, 21–26.

Graf, R., Cinelli, P., Arras, M., 2016. Morbidity scoring after abdominal surgery. Laboratory Animals 50, 453–458. doi:http://dx.doi.org/10.1177/0023677216675188.

Grant, G.J., Piskoun, B., Lin, A., Bansinath, M., 2000. An in vivo method for the quantitative evaluation of local anesthetics. Journal of Pharmacological and Toxicological Methods 43, 69–72. doi:http://dx.doi.org/10.1016/S1056-8719(00)00079-4.

Grunau, R.V.E., Craig, K.D., 1990. Facial activity as a measure of neonatal pain expression. Advances in Pain Research and Therapy 15, 147–155.

Hawkins, P., Armstrong, R., Boden, T., Garside, P., Knight, K., Lilley, E., Seed, M., Wilkinson, M., Williams, R.O., 2015. Applying refinement to the use of mice and

rats in rheumatoid arthritis research. Inflammopharmacology 23, 131–150. doi:http://dx.doi.org/10.1007/s10787-015-0241-4.
Häger, C., Keubler, L.M., Biernot, S., Dietrich, J., Buchheister, S., Buettner, M., Bleich, A., 2015. Time to integrate to nest test evaluation in a mouse DSS-colitis model. PLoS One 10, e0143824. doi:http://dx.doi.org/10.1371/journal.pone.0143824.
Jirkof, P., 2014. Burrowing and nest building behavior as indicators of well-being in mice. Journal of Neuroscience Methods 234, 139–146. doi:http://dx.doi.org/10.1016/j.jneumeth.2014.02.001.
Jirkof, P., Fleischmann, T., Cesarovic, N., Rettich, A., Vogel, J., Arras, M., 2013. Assessment of postsurgical distress and pain in laboratory mice by nest complexity scoring. Laboratory Animals 47 doi:http://dx.doi.org/10.1177/0023677213475603 0023677213475603–161.
Jirkof, P., Tourvieille, A., Cinelli, P., Arras, M., 2014. Buprenorphine for pain relief in mice: repeated injections vs sustained-release depot formulation. Laboratory Animals doi:http://dx.doi.org/10.1177/0023677214562849.
Johansen, J.P., Fields, H.L., Manning, B.H., 2001. The affective component of pain in rodents: direct evidence for a contribution of the anterior cingulate cortex. PNAS 98, 8077–8082. doi:http://dx.doi.org/10.1073/pnas.141218998.
Katz, N., Paillard, F.C., Van Inwegen, R., 2015. A review of the use of the number needed to treat to evaluate the efficacy of analgesics. Journal of Pain 16, 116–123. doi:http://dx.doi.org/10.1016/j.jpain.2014.08.005.
Kendall, L.V., Hansen, R.J., Dorsey, K., Kang, S., Lunghofer, P.J., Gustafson, D.L., 2014. Pharmacokinetics of sustained-release analgesics in mice. Journal of the American Association for Laboratory Animal Science: JAALAS 53, 478–484.
Kendall, L.V., Wegenast, D.J., Smith, B.J., Dorsey, K.M., Kang, S., Lee, N.Y., Hess, A.M., 2016. Efficacy of sustained-release buprenorphine in an experimental laparotomy model in female mice. Journal of the American Association for Laboratory Animal Science: JAALAS 55, 66–73.
Kilkenny, C., Browne, W.J., Cuthill, I.C., Emerson, M., Altman, D.G., 2010. Improving bioscience research reporting: the ARRIVE guidelines for reporting animal research. PLoS Biology 8, e1000412. doi:http://dx.doi.org/10.1371/journal.pbio.1000412.
Lamon, T.K., Browder, E.J., Sohrabji, F., Ihrig, M., 2008. Adverse effects of incorporating ketoprofen into established rodent studies. Journal of the American Association for Laboratory Animal Science: JAALAS 47, 20–24.
Langford, D.J., Bailey, A.L., Chanda, M.L., Clarke, S.E., Drummond, T.E., Echols, S., Glick, S., Ingrao, J., Klassen-Ross, T., LaCroix-Fralish, M.L., et al., 2010. Coding of facial expressions of pain in the laboratory mouse. Nature Methods 7, 447–449. doi:http://dx.doi.org/10.1038/nmeth.1455.
Le Bars, D., Gozariu, M., Cadden, S.W., 2001. Animal models of nociception. Pharmacological Reviews 53, 597–652.
Leach, M.C., Coulter, C.A., Richardson, C.A., Flecknell, P.A., 2011. Are we looking in the wrong place? Implications for behavioural-based pain assessment in rabbits (*Oryctolagus cuniculi*) and beyond?. PLoS One 6, e13347. doi:http://dx.doi.org/10.1371/journal.pone.0013347.
Leach, M.C., Klaus, K., Miller, A.L., Scotto di Perrotolo, M., Sotocinal, S.G., Flecknell, P.A., 2012. The assessment of post-vasectomy pain in mice using behaviour and the Mouse Grimace Scale. PLoS One 7, e35656. doi:http://dx.doi.org/10.1371/journal.pone.0035656.
Lidster, K., Jefferys, J.G., Blümcke, I., Crunelli, V., Flecknell, P., Frenguelli, B.G., Gray, W.P., Kaminski, R., Pitkänen, A., Ragan, I., et al., 2016. Opportunities for improving animal welfare in rodent models of epilepsy and seizures. Journal of Neuroscience Methods 260, 2–25. doi:http://dx.doi.org/10.1016/j.jneumeth.2015.09.007.
Liles, J.H., Flecknell, P.A., 1994. A comparison of the effects of buprenorphine, carprofen and flunixin following laparotomy in rats. Journal of Veterinary Pharmacology and Therapeutics 17, 284–290.
Matsumiya, L.C., Sorge, R.E., Sotocinal, S.G., Tabaka, J.M., Wieskopf, J.S., Zaloum, A., King, O.D., Mogil, J.S., 2012. Using the Mouse Grimace Scale to reevaluate the efficacy of postoperative analgesics in laboratory mice. Journal of the American Association for Laboratory Animal Science: JAALAS 51, 42–49.
McQuay, H.J., Derry, S., Eccleston, C., Wiffen, P.J., Moore, R.A., 2012. Evidence for analgesic effect in acute pain—50 years on. Pain 153, 1364–1367. doi:http://dx.doi.org/10.1016/j.pain.2012.01.024.
Miller, A.L., Golledge, H.D.R., Leach, M.C., 2016. The influence of isoflurane anaesthesia on the Rat Grimace Scale. PLoS One 11, e0166652. doi:http://dx.doi.org/10.1371/journal.pone.0166652.
Miller, A.L., Leach, M.C., 2015a. The effect of handling method on the Mouse Grimace Scale in two strains of laboratory mice. Laboratory Animals 50, 305–307. doi:http://dx.doi.org/10.1177/0023677215622144.
Miller, A.L., Leach, M.C., 2015b. The Mouse Grimace Scale: a clinically useful tool? PLoS One 10, e0136000. doi:http://dx.doi.org/10.1371/journal.pone.0136000.
Miller, A.L., Roughan, J.V., 2012. A comparison of abdominal and scrotal approach methods of vasectomy and the influence of analgesic treatment in laboratory mice. Laboratory Animals 46, 304–310. doi:http://dx.doi.org/10.1258/la.2012.012078.
Miranda, H.F., Puig, M.M., Prieto, J.C., Pinardi, G., 2006. Synergism between paracetamol and nonsteroidal anti-inflammatory drugs in experimental acute pain. Pain 121, 22–28. doi:http://dx.doi.org/10.1016/j.pain.2005.11.012.
Mitchell, D., Krusemark, M.L., Hafner, E., 1977. Pica: a species relevant behavioral assay of motion sickness in the rat. Physiology & Behavior 18, 125–130. doi:http://dx.doi.org/10.1016/0031-9384(77)90103-2.
Mogil, J.S., 2009. Animal models of pain: progress and challenges. Nature Reviews Neuroscience 10, 283–294. doi:http://dx.doi.org/10.1038/nrn2606.
Mogil, J.S., Wilson, S.G., Bon, K., Eun Lee, S., Chung, K., Raber, P., Pieper, J.O., Hain, H.S., Belknap, J.K., Hubert, L., et al., 1999. Heritability of nociception I: responses of 11 inbred mouse strains on 12 measures of nociception. Pain 80, 67–82.
Morton, D.B., Griffiths, P.H., 1985. Guidelines on the recognition of pain, distress and discomfort in experimental animals and an hypothesis for assessment. Veterinary Record 116, 431–436. doi:http://dx.doi.org/10.1136/vr.116.16.431.
Negus, S.S., Neddenriep, B., Altarifi, A.A., Carroll, F.I., Leitl, M.D., Miller, L.L., 2015. Effects of ketoprofen, morphine, and kappa opioids on pain-related depression of nesting in mice. Pain 156, 1153–1160. doi:http://dx.doi.org/10.1097/j.pain.0000000000000171.
Neubert, J.K., King, C., Malphurs, W., Wong, F., Weaver, J.P., Jenkins, A.C., Rossi, H.L., Caudle, R.M., 2008. Characterization of mouse orofacial pain and the effects of lesioning TRPV1-expressing neurons on operant behavior. Molecular Pain 4, 43. doi:http://dx.doi.org/10.1186/1744-8069-4-43.
Oliver, V.L., Athavale, S., Simon, K.E., Kendall, L.V., Nemzek, J.A., Lofgren, J.L., 2017. Evaluation of pain assessment techniques and analgesia efficacy in a female guinea pig (*Cavia porcellus*) model of surgical pain. Journal of the American Association for Laboratory Animal Science 56.4, 425–435.
Oyama, T., Homan, T., Kyotani, J., Oka, M., 2012. Effect of tramadol on pain-related behaviors and bladder overactivity in rodent cystitis models. European Journal of Pharmacology 676, 75–80. doi:http://dx.doi.org/10.1016/j.ejphar.2011.12.002.
Peterson, N.C., Nunamaker, E.A., Turner, P.V., 2017. To treat or not to treat: the effects of pain on experimental parameters. Comparative Medicine 67, 1–14.
Percie du Sert, N., Alfieri, A., Allan, S.M., Carswell, H.V., Deuchar, G.A., Farr, T.D., Flecknell, P., Gallagher, L., Gibson, C.L., Haley, M.J., et al., 2017. The IMPROVE guidelines (ischaemia models: procedural refinements of in vivo experiments). Journal of Cerebral Blood Flow & Metabolism 9 doi:http://dx.doi.org/10.1177/0271678X17709185 0271678X1770918.
Pountos, I., Georgouli, T., Calori, G.M., Giannoudis, P.V., 2012. Do nonsteroidal anti-inflammatory drugs affect bone healing? A critical analysis. The Scientific World Journal doi:http://dx.doi.org/10.1100/2012/606404.
Qu, C., King, T., Okun, A., Lai, J., Fields, H.L., Porreca, F., 2011. Lesion of the rostral anterior cingulate cortex eliminates the aversiveness of spontaneous neuropathic pain following partial or complete axotomy. Pain 152, 1641–1648. doi:http://dx.doi.org/10.1016/j.pain.2011.03.002.
Ramirez, H.E., Queeney, T.J., Dunbar, M.L., Eichner, M.C., Del Castillo, D.I., Battles, A. H., Neubert, J.K., 2015. Assessment of an orofacial operant pain assay as a preclinical tool for evaluating analgesic efficacy in rodents. Journal of the American Association for Laboratory Animal Science: JAALAS 54, 426–432.
Rice, A.S.C., Cimino-Brown, D., Eisenach, J.C., Kontinen, V.K., LaCroix-Fralish, M.L., Machin, I., Mogil, J.S., Stöhr, T., on behalf of the Preclinical Pain Consortium, 2008. Animal models and the prediction of efficacy in clinical trials of analgesic drugs: a critical appraisal and call for uniform reporting standards. Pain 139, 243–247. doi:http://dx.doi.org/10.1016/j.pain.2008.08.017.
Richardson, C.A., Flecknell, P.A., 2005. Anaesthesia and post-operative analgesia following experimental surgery in laboratory rodents: are we making progress? Alternatives to Laboratory Animals 33, 119–127.
Rock, M.L., Karas, A.Z., Rodriguez, K.B.G., Gallo, M.S., Pritchett-Corning, K., Karas, R. H., Aronovitz, M., Gaskill, B.N., 2014. The time-to-integrate-to-nest test as an indicator of wellbeing in laboratory mice. Journal of the American Association for Laboratory Animal Science: JAALAS 53, 24–28.
Roughan, J.V., Bertrand, H.G.M.J., Isles, H.M., 2016. Meloxicam prevents COX-2-mediated post-surgical inflammation but not pain following laparotomy in mice. European Journal of Pain 20, 231–240. doi:http://dx.doi.org/10.1002/ejp.712.
Roughan, J.V., Flecknell, P.A., 2004. Behaviour-based assessment of the duration of laparotomy-induced abdominal pain and the analgesic effects of carprofen and buprenorphine in rats. Behavioural Pharmacology 15, 461.
Roughan, J.V., Flecknell, P.A., 2003. Evaluation of a short duration behaviour-based post-operative pain scoring system in rats. European Journal of Pain 7, 397–406. doi:http://dx.doi.org/10.1016/S1090-3801(02)00140-4.
Roughan, J.V., Flecknell, P.A., 2002. Buprenorphine: a reappraisal of its antinociceptive effects and therapeutic use in alleviating post-operative pain in animals. Laboratory Animals 36, 322–343.
Roughan, J.V., Flecknell, P.A., 2001. Behavioural effects of laparotomy and analgesic effects of ketoprofen and carprofen in rats. Pain 90, 65–74. doi:http://dx.doi.org/10.1016/S0304-3959(00)00387-0.
Sauer, M., Fleischmann, T., Lipiski, M., Arras, M., Jirkof, P., 2016. Buprenorphine via drinking water and combined oral-injection protocols for pain relief in mice. Applied Animal Behaviour Science 185, 103–112.
Seymour, T.L., Adams, S.C., Felt, S.A., Jampachaisri, K., Yeomans, D.C., Pacharinsak, C., 2016. Postoperative analgesia due to sustained-release buprenorphine, sustained-release meloxicam, and carprofen gel in a model of incisional pain in rats (*Rattus norvegicus*). Journal of the American Association for Laboratory Animal Science: JAALAS 55, 300–305.
Shientag, L.J., Wheeler, S.M., Garlick, D.S., Maranda, L.S., 2012. A therapeutic dose of ketoprofen causes acute gastrointestinal bleeding, erosions, and ulcers in rats. Journal of the American Association for Laboratory Animal Science: JAALAS 51, 832–841.
Smith, B.J., Wegenast, D.J., Hansen, R.J., Hess, A.M., Kendall, L.V., 2016. Pharmacokinetics and paw withdrawal pressure in female guinea pigs (*Cavia porcellus*) treated with sustained-release buprenorphine and buprenorphine hydrochloride. Journal of the American Association for Laboratory Animal Science: JAALAS 55, 789–793.

Sneddon, L.U., Elwood, R.W., Adamo, S.A., Leach, M.C., 2014. Defining and assessing animal pain. Animal Behaviour 97, 201–212. doi:http://dx.doi.org/10.1016/j.anbehav.2014.09.007.

Sotocinal, S.G., Sorge, R.E., Zaloum, A., Tuttle, A.H., Martin, L.J., Wieskopf, J.S., Mapplebeck, J.C., Wei, P., Zhan, S., Zhang, S., et al., 2011. The Rat Grimace Scale: a partially automated method for quantifying pain in the laboratory rat via facial expressions. Molecular Pain 7, 55. doi:http://dx.doi.org/10.1186/1744-8069-7-55.

Stokes, E.L., Flecknell, P.A., Richardson, C.A., 2009. Reported analgesic and anaesthetic administration to rodents undergoing experimental surgical procedures. Laboratory Animals 43, 149–154. doi:http://dx.doi.org/10.1258/la.2008.008020.

Sufka, K.J., 1994. Conditioned place preference paradigm: a novel approach for analgesic drug assessment against chronic pain. Pain 58, 355–366. doi:http://dx.doi.org/10.1016/0304-3959(94)90130-9.

Takeda, N., Hasegawa, S., Morita, M., Horii, A., Uno, A., Yamatodani, A., Matsunaga, T., 1995. Neuropharmacological mechanisms of emesis. I. Effects of antiemetic drugs on motion- and apomorphine-induced pica in rats. Methods and Findings in Experimental and Clinical Pharmacology 17, 589–590.

Taylor, B.F., Ramirez, H.E., Battles, A.H., Andrutis, K.A., Neubert, J.K., 2016. Analgesic activity of tramadol and buprenorphine after voluntary ingestion by rats (*Rattus norvegicus*). Journal of the American Association for Laboratory Animal Science: JAALAS 55, 74–82.

Ter Horst, E.N., Krijnen, P.A.J., Flecknell, P., Meyer, K.W., Kramer, K., van der Laan, A. M., Piek, J.J., Niessen, H.W.M., 2017. Sufentanil–medetomidine anaesthesia compared with fentanyl/fluanisone–midazolam is associated with fewer ventricular arrhythmias and death during experimental myocardial infarction in rats and limits infarct size following reperfusion. Laboratory Animals 59 doi:http://dx.doi.org/10.1177/0023677217724485 002367721772448.

Thomas, A., Miller, A., Roughan, J., Malik, A., Haylor, K., Sandersen, C., Flecknell, P., Leach, M., 2016. Efficacy of intrathecal morphine in a model of surgical pain in rats. PLoS One 11, e0163909–21. doi:http://dx.doi.org/10.1371/journal.pone.0163909.

Turner, P.V., Brabb, T., Pekow, C., Vasbinder, M.A., 2011. Administration of substances to laboratory animals: routes of administration and factors to consider. Journal of the American Association for Laboratory Animal Science: JAALAS 50, 600–613.

Utting, J.E., Smith, J.M., 1979. Postoperative analgesia. Anaesthesia 34, 320–332. doi:http://dx.doi.org/10.1111/j.1365-2044.1979.tb04929.x.

Waite, M.E., Tomkovich, A., Quinn, T.L., Schumann, A.P., Dewberry, L.S., Totsch, S.K., Sorge, R.E., 2015. Efficacy of common analgesics for postsurgical pain in rats. Journal of the American Association for Laboratory Animal Science: JAALAS 54, 420–425.

Wenger, S., 2012. Anesthesia and analgesia in rabbits and rodents. JEPM 21, 7–16. doi:http://dx.doi.org/10.1053/j.jepm.2011.11.010.

Wodarski, R., Delaney, A., Ultenius, C., Morland, R., Andrews, N., Baastrup, C., Bryden, L.A., Caspani, O., Christoph, T., Gardiner, N.J., et al., 2016. Cross-centre replication of suppressed burrowing behaviour as an ethologically relevant pain outcome measure in the rat: a prospective multicentre study. Pain 157, 2350–2365. doi:http://dx.doi.org/10.1097/j.pain.0000000000000657.

Wolfe, A.M., Kennedy, L.H., Na, J.J., Nemzek-Hamlin, J.A., 2015. Efficacy of tramadol as a sole analgesic for postoperative pain in male and female mice. Journal of the American Association for Laboratory Animal Science: JAALAS 54, 411–419.

Wright-Willams, S., Courade, J., Richardson, C., Roughan, J., Flecknell, P., 2007. Effects of vasectomy surgery and meloxicam treatment on faecal corticosterone levels and behaviour in two strains of laboratory mouse. Pain 130, 108–118. doi:http://dx.doi.org/10.1016/j.pain.2006.11.003.

Wright-Williams, S., Flecknell, P.A., Roughan, J.V., 2013. Comparative effects of vasectomy surgery and buprenorphine treatment on faecal corticosterone concentrations and behaviour assessed by manual and automated analysis methods in C57 and C3H mice. PLoS One 8, e75948. doi:http://dx.doi.org/10.1371/journal.pone.0075948.

Zahn, P.K., Brennan, T.J., 1999. Primary and secondary hyperalgesia in a rat model for human postoperative pain. Anesthesiology 90, 863–872.

Zahn, P.K., Gysbers, D., Brennan, T.J., 1997. Effect of systemic and intrathecal morphine in a rat model of postoperative pain. Anesthesiology 86, 1066–1077.

Zegre Cannon, C., Kissling, G.E., Goulding, D.R., King-Herbert, A.P., Blankenship-Paris, T., 2011. Analgesic effects of tramadol, carprofen or multimodal analgesia in rats undergoing ventral laparotomy. Lab Animal (New York) 40, 85–93. doi:http://dx.doi.org/10.1038/laban0311-85.