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Overview

Clinical Management of Pain in Rodents

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The use of effective regimens for mitigating pain remain underutilized in research rodents despite the general acceptance of both the ethical imperative and regulatory requirements intended to maximize animal welfare. Factors contributing to this gap between the need for and the actual use of analgesia include lack of sufficient evidence-based data on effective regimens, under-dosing due to labor required to dose analgesics at appropriate intervals, concerns that the use of analgesics may impact study outcomes, and beliefs that rodents recover quickly from invasive procedures and as such do not need analgesics. Fundamentally, any discussion of clinical management of pain in rodents must recognize that nociceptive pathways and pain signaling mechanisms are highly conserved across mammalian species, and that central processing of pain is largely equivalent in rodents and other larger research species such as dogs, cats, or primates. Other obstacles to effective pain management in rodents have been the lack of objective, science-driven data on pain assessment, and the availability of appropriate pharmacological tools for pain mitigation. To address this deficit, we have reviewed and summarized the available publications on pain management in rats, mice and guinea pigs. Different drug classes and specific pharmacokinetic profiles, recommended dosages, and routes of administration are discussed, and updated recommendations are provided. Nonpharmacologic tools for increasing the comfort and wellbeing of research animals are also discussed. The potential adverse effects of analgesics are also reviewed. While gaps still exist in our understanding of clinical pain management in rodents, effective pharmacologic and nonpharmacologic strategies are available that can and should be used to provide analgesia while minimizing adverse effects. The key to effective clinical management of pain is thoughtful planning that incorporates study needs and veterinary guidance, knowledge of the pharmacokinetics and mechanisms of action of drugs being considered, careful attention to individual differences, and establishing an institutional culture that commits to pain management for all species as a central component of animal welfare.

Abbreviations and Acronyms: BW, bodyweight; CFA, Complete Freund adjuvant; ED, Effective dose; FI, Food intake; PONV, postoperative nausea and vomiting;SX, surgical procedure; SR, sustained release.

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Clinical management of pain in research rodents remains an important ethical and moral issue for IACUC, researchers, and veterinarians today. This is not surprising—pain management in human patients is still poorly characterized and under managed and remains one of the most common reasons that patients seek medical attention.[7,22] Some aspects of inconsistency in rodent pain management may be attributable to unknown effective dosages of drugs for different strains of mice and rats, as well as challenges in assessing pain and pain mitigation in these animals. However, our review of the literature revealed that a large proportion of the inconsistent provision of adequate pain relief stems from either explicit or inferred socio-zoologic bias of the research community. For example, several studies have examined the methods of peer-reviewed papers that were published in highly ranked scientific journals and that involved surgery on research animals.[26,43,174,199] Repeatedly, these studies demonstrate a significant underuse of peri-operative analgesics in mice and rats[26,174,199] in contrast to much better reported use of analgesics in large animal (that is, primate, dog, and pig) surgical studies.[43,44] Follow-ups with authors of publications not reporting use of analgesics in mice and rats for painful surgical procedures has not significantly altered these findings, suggesting that it is unlikely to be due to under reporting of analgesic administration.[174,199]

To begin to address rodent pain consistently in research settings, there must be fundamental recognition that all mammals, at the very least, have near identical nociceptive pathways and pain signaling mechanisms.[158] Affective and cognitive processing of pain occurs as much in mice and rats as in primates and dogs, meaning that mice and rats are not somehow 'less sentient' species.[127,193] Recognizing and admitting this simple concept should give IACUC, researchers, and veterinarians pause before submitting or approving research protocols that do not specify adequate pain relief for mice and rats. An appropriate question to reflect upon in every instance should be, "Would this protocol be approved in a dog or a primate under these same conditions?". This question would go a long way toward improving consideration for pain management in mice and rats in research settings. An additional challenge for pain management in laboratory animal science has been the lack of objective pain indicators for some species.[84,137] Ongoing research has begun to address these gaps, resulting in the development of validated pain assessment tools for mice and rats.

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Considerations for types of pain based on underlying mechanisms

Recognition that mice and rats experience pain as much as other mammals is an important consideration when evaluating the types of pain (chronic or acute) to be managed. In both human and veterinary medicine, there is recognition of the importance of properly managing and treating acute pain, for ethical reasons and to prevent the condition from evolving into chronic pain— which is a more difficult condition to treat.[97,158] Working estimates are not available for the type, intensity, and duration of pain experienced with different research animal models; however, much of the pain that occurs in induced models is caused by acute peri-procedural pain. This would include most surgical models, models in which animals are instrumented with catheters, implants or other devices, initial injections of irritating substances, such as carrageenan, and many tissue biopsy or invasive sampling methods.

Strict guidelines do not distinguish acute and chronic pain, consistent with recognition that pain occurs along a continuum.[28] In human medicine, acute pain is considered to last up to 7 d after an initial event, but this limit can be modified by the severity, extent, and type of injury, and acute pain may last upward of 30 d or longer.[114] The pathophysiology of pain initiation and subsequent inflammation has been described previously, with no evidence of any biomarkers that distinguish acute from chronic pain, except that central sensitization is more common in chronic pain.[170]

Although acute pain may have evolved to provide a protective response to the host, a key distinguishing feature between acute and chronic pain is the lack of any physiologic benefits derived from chronic pain. Chronic pain, particularly when persistent and unrelieved, can severely and negatively impact quality of life[29] as a result of the onset of chronic maladaptive stress and hypothalamic-pituitary-adrenal gland axis activation, disruption of sleep, decreased functional and immune system performance, and impairment of social interactions.[29] Thus, unless chronic pain is the object of scientific study, this should be between this state and be avoided or minimized by managing pain in its acute stage.

General approaches to clinical pain management in rodents

Multiple pharmacologic agents are available to manage pain in research animals. These agents have different mechanisms and duration of action as well as varying potencies for providing pain relief (see below). This permits the veterinarian, research team, and IACUC to tailor treatments, based on the invasiveness of a given procedure and its potential to cause pain. While the use of standard operating procedures is helpful to ensure consistent pain management in research facilities, it can be counterproductive to take a 'one-size-fits-all' approach to pain management in rodents, for example, if only a specific nonsteroidal antiinflammatory drug (NSAID) is used for all painful studies in a facility.

The World Health Organization (WHO) provides a stepped approach to pain management for humans that can be useful to consider when treating research animals (Figure 1).[210] Certain steps in this 'pain ladder' can be skipped if the level of pain following a procedure is anticipated to be more severe, but it is useful to consider the full range of pharmacologic (and nonpharmacologic) options available for managing pain in research animals. Use of a systematic approach such as this allows treatment to be titrated to the amount of pain expected and observed. This type of approach helps to avoid both under and overuse of pain medications, both of which can be harmful to veterinary patients.

Setting realistic goals for pain management in laboratory rodents

Given the stated difficulties in managing pain adequately in humans, it may not be realistic to assume that all pain can be effectively treated in research animals all the time. This further emphasizes the importance of having a thoughtful plan that is tailored to the procedures being conducted. The plan should include anticipation of pain, early treatment to minimize sensitization, and evaluation of individual animals for a response to therapy. Companion animal pain management guidelines, such as the "PLATTER" approach, can provide a useful approach for systematic management of pain in research animals (see Figure 2).[53] Consistent use of this tool by the veterinary team for both clinical cases and research protocols would help to ensure better pain recognition and mitigation in laboratory rodents. Further, this approach could be built into the institutional animal user training program to ensure consistency in analgesia management. Where objective scoring tools do not exist, close observation of animal behavior is necessary and should be conducted noninvasively by an individual familiar with normal preprocedure behavior of the specific animals.

For new procedures or models with unknown outcomes, it can be useful to conduct detailed individual assessments on a few animals and then generalize these findings to develop a robust scoring system and appropriate pain treatment plan for the larger cohort.[58] Because pain and response to treatment can differ between sexes of animals, between animals of different ages, and even between genetically similar animals,[115,154] each rodent should be monitored directly after treatment for signs of comfort and wellbeing that indicate a pain-free state. This includes evaluating normal postures, social interactions, grooming, nest-building (in the case of mice), general activity, and food and water intake.

Evidence-based analgesia in rodents

Numerous formularies provide dosing regimens for pain management in rodents. These regimens are primarily based on studies evaluating analgesic efficacy, but also draw from commonly accepted historical practices. Analgesiometry assays used in these studies included variations of the tail flick assay, paw withdrawal, and the hot plate test. While these assays provide some information, they primarily test withdrawal reflexes indicative of nociception, and lack the ability to fully capture the more complex experience and central processing associated with surgical pain.[1] More recent studies have attempted to assess pain more comprehensively rather than just nociceptive responses.[14] These include assays such as behavioral assessments, grimace scales, vocalizations, and nesting behaviors (for a review, see reference 205).

Most analgesics used for mitigating pain in rodents fall into one of a few classes: opioids (or opioid-like), NSAID or local analgesics. Commonly used agents include buprenorphine, tramadol, meloxicam, carprofen, ketoprofen, ibuprofen, acetaminophen, lidocaine, and bupivacaine. Table 1). provides recommended dosing regimens for mice, rats and guinea pigs for each of these agents based on commonly referenced texts and guidelines.[60,83,119] An extensive literature review of pharmacokinetics of these drugs was also conducted. Table 2 presents data on establishment of therapeutic levels of

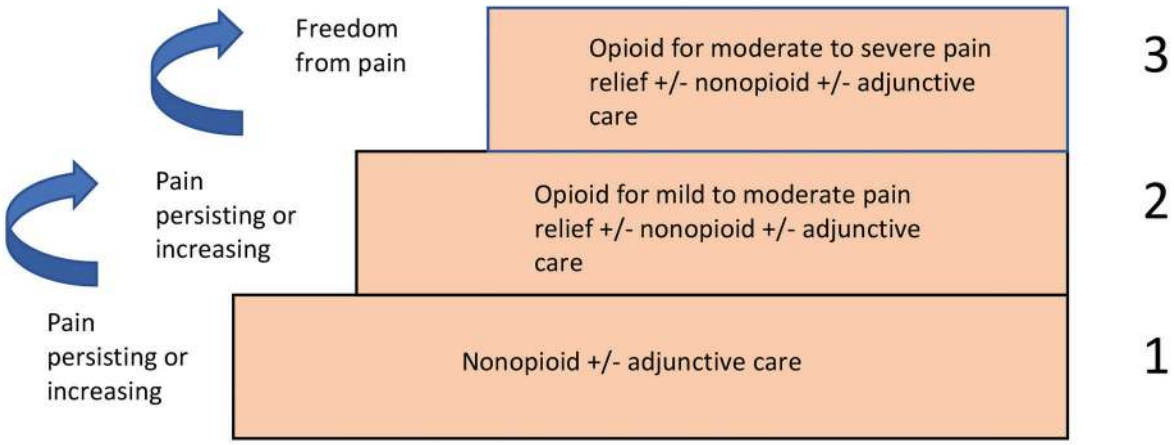

Figure 1. WHO's Pain Relief Ladder for Patient Management (modified from https://www.who.int/cancer/palliative/painladder/en/). This ladder models an approach for the veterinary clinician, IACUC, and research team to use for pain management in laboratory rodents, based on the anticipated level of invasiveness of procedures being conducted. For example, for a short recovery procedure, such as jugular vein cannulation being conducted by a skilled surgeon, the animal may require peri-operative NSAID treatment in addition to excellent postoperative nursing care. In all cases, regardless of the approved protocol or SOP, each patient should be assessed after the procedure to ensure that pain is being well managed. In the event that an animal appears uncomfortable, an escalation to the next higher level of care in the pain ladder should be considered.

*PL*an – every protocol or clinical case has a specific pain assessment and treatment plan

*A*nticipate – pain needs are anticipated, when possible, to minimize or prevent pain from occurring or, when pain is already present, to treat pain effectively

*T*rea*T* – appropriate treatments are given, considering the expected duration, severity, and type of pain

*E*valuate – treatment efficacy is evaluated using objective measures

*R*eturn – treatment is modified or discontinued based upon the clinical outcome

* Modified from 2015 AAHA/AAFP Pain Management Guidelines for Dogs and Cats (Epstein et al, 2015).

Figure 2. *PLATTER** Approach to Managing Pain in Research Animals.

commonly used analgesics. As shown, much of the literature has used "therapeutic levels" that are not based on well-proven studies in rodents, but rather extrapolated from other species, and the current dosing regimens do not appear to be based on achieving those therapeutic levels. The published ranges are also often very large, probably based on a range of analgesic efficacy from a small effect to a much more substantial dampening of pain responses. As such this information is currently of limited value and could benefit from more specific studies performed in rodents, but is included to provide currently available values.

An overview of the pharmacokinetic studies for mice, rats, and guinea pigs are summarized in Tables 3 to 5 respectively, and these are discussed in more detail below. The literature was further probed for efficacy studies, and results of these are summarized in a series of tables (Tables 6 to 12) , based on species (mouse, rat, or guinea pig) and analgesic drug or class (buprenorphine, nonopioid analgesics, and local anesthetics). The summary of the studies highlighted in these tables suggest, as discussed in more detail below, how the dosing regimens historically used for pain management in rodents may not be adequate,[66,105,112,113,130,149,163,211]

Buprenorphine

Buprenorphine, one of the most commonly used analgesics in rodents, is typically dosed subcutaneously twice a day, yet pharmacokinetic data demonstrate that mice and rats rarely achieve a plasma level greater than the purported therapeutic level beyond 4 to 6 h. Oral formulations provided continuously in feed or gels, and sustained-release formulations enhance the duration of action of buprenorphine. When buprenorphine is provided in MediGel or Nutella, the duration of effect can be up to 12 to 14 h [72,94]However, the mouse studies found considerable variation in the amount ingested.[94] Sustained-release formulations of buprenorphine regularly achieve a plasma level greater than therapeutic levels for more than 12 h, and often up to 24 h [37,103,112,202]However, manufacturer guidelines

Table 1. Common currently used analgesic dosing regimens for rodents

Species	Agent	Dose (mg/kg)	Route	Frequency
Mouse	Buprenorphine	0.05-0.1	SC	6-12 h
	Tramadol	5-40	SC, IP	ND
	Carprofen	2-5	SC	12-24 h
	Meloxicam	1-5	SC, PO	12 h
	Ketoprofen	2-5	SC	24 h
	Ibuprofen	30-40	PO	ND
	Acetaminophen	200	PO	ND
Rats	Buprenorphine	0.01-0.1	SC, IM	8-12 h
	Tramadol	5-20	SC, IP	ND
	Carprofen	2-5	SC	24 h
	Meloxicam	1-2	SC, PO	12-24 h
	Ketoprofen	2-5	SC	24 h
	Ibuprofen	15	PO	ND
	Acetaminophen	200	PO	ND
Guinea pig	Buprenorphine	0.05	SC	6-12 h
	Carprofen	2-5	SC, IM	12-24 h
	Meloxicam	0.1-0.3	SC, PO	24 h
	Ibuprofen	10	PO	4 h

ND = not determined.
Dosages drawn primarily from Flecknell 2016; Hawkins 2012; Kohn and colleagues 2007.

Table 2. Purported therapeutic plasma levels

Analgesic	Therapeutic plasma level (ng/mL)	Species studied	Reference
Buprenorphine	1.0	Human, rat	79
Carprofen	20,000-24,000	Human, cat, dog, in vitro	132
Ketoprofen	2,000-10,000	Human, rat	195
Meloxicam	390-911	Cat, dog, in vitro	69,102,131
Tramadol	100	mouse	56

(Zoopharm, Windsor, CO) suggest that dosing once every 72 h is sufficient. These findings suggest that the commonly used twice-daily dosing schedule of buprenorphine does not achieve an adequate duration of analgesia. Efficacy studies in rodents support these findings as they infrequently achieve clinical analgesia beyond 8 h, unless sustained-release formulations are used.

Nonsteroidal antiinflammatory drugs (NSAID)

The pharmacokinetics of nonopioid analgesics demonstrate similar pharmacokinetic and efficacy trends as buprenorphine. The commonly used dosages of NSAID in rodents fail to routinely provide plasma levels greater than therapeutic levels. Carprofen given at 5 mg/kg SC to mice has a duration of effect for 12 h;[112] however, the common dosing interval can be up to once daily. Efficacy studies demonstrate a minimal effect beyond the first 6 h postoperatively.[176] While carprofen administered in the drinking water achieved sustainable therapeutic levels up to 35 h, the study did not evaluate the efficacy of this route of administration in a postoperative model.[96] Meloxicam at 1 mg/kg SC in mice has a duration of effect of 4 h,[112] and when given orally at 10 mg/kg has a duration of action of 4 to 6 h.[21] However, when given at a higher oral dose of 20 mg/kg or in a sustained release formulation meloxicam has a duration of effect lasting up to 24 h.[96,112] Mice provided meloxicam in the drinking water refused to consume it.[96] Efficacy studies of meloxicam support pharmacokinetic studies in that 5 mg/kg appears to have no effect on postoperative analgesia in mice and clinically higher doses up to 20 mg/kg may be required for analgesia in mice.[130,212]Other NSAID have demonstrated similar findings of shorter duration of action in mice, which may be overcome with higher doses, such as ketoprofen at 10 to 20 mg/kg.[140] Doses of 1 or 4 mg/kg SC appear to be similarly ineffective in guinea pigs.[49,163] However, in rats, a 2 mg/kg SC dose reduced behavioral signs of pain in a laparotomy model.[160]

Local anesthetics

Local anesthetics have a short duration of action; 30 min with lidocaine and up to 60 min with bupivacaine. There are formulations that prolong the analgesic efficacy of local anesthetics, and these formulations can increase the duration of action to 24 to 48 h.

Recommendations

Dosing regimens for these analgesics should be carefully reconsidered in light of recent pharmacokinetic and efficacy studies . The frequency of dosing should be based on these pharmacokinetic studies as well as cage-side clinical assessments of pain, although clinical assessments should consider the ability of rodents to mask signs of pain. Table 13 provides our updated recommendations that address the inadequate dosing intervals that are widely used (and currently considered acceptable practice by many IACUC).[61] Given the inconsistent findings associated with the efficacy studies on NSAID, the dosing regimens recommended in Table 13 are based on current studies using more recent techniques to identify pain, such as

Table 3. Pharmacokinetics of analgesics used in mice

Analgesic	Dose (mg/kg)	Route	T_{max}	C_{max} (ng/mL)	Duration of action	Reference
Buprenorphine	5 µg/mL	PO-M	1 h	7.8	< 6 h	94
	15 µg/mL	PO-M	1 h	3.0	12 h	94
	0.03	SC	1 h	0.5	N/A	37
	0.05	SC	1 h	0.5	N/A	37
	0.1	SC	8 h	8.6	12 h	19
	0.1	SC	3 h	1.3	N/A	37
	0.1	SC	1 h	1.5	< 6 h	94
	0.1	SC	2 h	1.5	4 h	103
	0.6	SC	2 h	19.1	4 h	112
	2.0	SC	1 h	20.2	12 h	37
Buprenorphine SR	0.1[zp]	SC	4 h	14.5	24 h	112
	0.3[zp]	SC	6 h	0.8	N/A	37
	1.2[zp]	SC	0.5 h	5.0	12 h	37
	2.2[ih]	SC	2 h	11	24 h	103
	3.25[ag]	SC	6 h	16.3	72 h	202
	4.0[ih]	SC	24 h	ND	72-96 h	80
Carprofen	10	PO-G	2 h	20,300	N/A	96
	10	PO-W	12 h	17,000	N/A	96
	30	PO-W	24 h	32,000	N/A	164
	5	SC	2 h	525,000	12 h	112
Meloxicam	10	IV	5 min	365,000	4-6h	21
	10	PO	0.7 h	18,000	4-6h	21
	20	PO-G	4 h	16,700	24 h	96
	1	SC	2 h	4700	4 h	112
Meloxicam SR	6[zp]	SC	2 h	7300	12-24 h	112
Tramadol	25	IP	0.08 h	3010	4h	56
	25	IV	0.25 h	3710	2h	56
	25	PO-G	1 h	347	2 h	56
	25	PO	1 h	347	constant in water	56
	25	SC	0.25 h	1870	6 h	56
EMLA	18 mg/25g	Top	0.5 h	165	100 min Toxic at 21.2 mg/kg	6
	18 mg/25g	Top (open wound)	0.5 h	909	100 min	6
Bupivacaine	150 µL 0.5%	SC	1	1,000,000	approximately 4 h Toxic at >0.5%	73

Duration of action = time at which plasma level falls below therapeutic level (see Table 2)
N/A = plasma level did not exceed therapeutic level; ND = not determined
T_{max} = time to reach maximum concentration; C_{max} = maximum concentration
PO-W = oral in water; PO-M = oral in MediGel; PO-G = oral by gavage; Top = topical; SR= sustained release;
z*P* = manufactured by Zoopharm, Windsor, CO; ih= inhouse formulation; ag= manufactured by Animalgesics Laboratories, Millersville, MD

facial grimace score, and pharmacokinetic studies. Although several studies have evaluated voluntary ingestion of medical gels or feedstuff, routine use requires caution as rodents will reduce feed and water intake during the postoperative period and voluntary ingestion can be variable, resulting in inadequate dosing.

Multimodal analgesia

Another aspect of analgesic therapy that may overcome the current dosing challenges is multimodal analgesia. Multimodal analgesia combines multiple analgesics with different mechanisms of action into the treatment regimen, which often results in an increased efficacy while using lower dosages of the individual agents. Multimodal analgesia is commonly used in human and veterinary medicine for pain management.[12,13,17,42,50,126] Evidence that multimodal analgesia is effective in rodents is summarized in Table 14. In a tail-flick assay, the effects of ibuprofen were enhanced with opioids.[217] The effective dose of gabapentin and tramadol were both reduced when given in combination in a diabetic neuropathy model evaluating analgesia using the tail-flick assay, hot plate, and formalin test.[153] Similarly, the analgesic effect of tramadol was improved when ketoprofen was given concurrently using the writhing test, tail-flick assay, and formalin test.[150,152] **O**pioids also enhance the effects of tramadol.[59,175] In a murine laparotomy model, mice were treated with either buprenorphine alone or in combination with carprofen, administered in the drinking water.[164] The combination of buprenorphine and carprofen provided the best analgesia, compared with buprenorphine alone, and carprofen alone failed to provide any analgesia. A similar study was performed in a guinea pig ovariohysterectomy

Table 4. Pharmacokinetics of analgesics used in rats

Analgesic	Dose (mg/kg)	Route	T_{max}	C_{max} (ng/mL)	Duration of action	Reference
Buprenorphine	0.05	SC	0.5 h	1.5	2 h	72
	0.1	SC	4 h	2.7	8-24 h	64
	0.4	PO-N	2 h	1.25	14 h	72
	0.9 SR^{zp}	SC	4 h	2.8	24-48 h	64
	1.2 SR^{zp}	SC	4 h	2.8	24 h	64
	1.2 SR^{zp}	SC	24 h	1.01	24 h	160
Ketoprofen	2.5	IV	<5 min	10,000	48 h	181
	10	IV	<5 min	100,000	24 h	181
	3.2	PO	0.5 h	2730	24 h	143
	10	PO	0.5 h	11,700	90-360 min	4
	0.5	SC	ND	0.73	N/A	195
	1.0	SC	ND	1.79	N/A	195
	5.0	SC	ND	8.43	Measured at 2 h	195
Meloxicam	1	IV	< 0.25 h	5000	24 h	21
	0.3	PO	4.5-6.5 h	2300-3200	ND	21
Tramadol	20	IP	10 min	3187	300 min	186
	20	IV	< 10 min	23,314	300 min	186
Bupivacaine	2% 300 µL	SC	2 h	7000	Waned by 10 h	74

Duration of action = time at which plasma level falls below therapeutic level (see Table 2).
SR = sustained-release
N/A = indicates plasma level did not exceed therapeutic level
ND = not determined
T_{max} = time to reach maximum concentration
C_{max} = maximum concentration
PO-*n* = oral in Nutella
z*P* = manufactured by Zoopharm, Windsor, CO

Table 5. Pharmacokinetics of buprenorphine in guinea pigs

Dose (mg/kg)	Route	T_{max}	C_{max} (ng/mL)	Duration of action	Reference
0.2	IV	1.5 m	46.7	6 h	179
0.2	PO	1.2 h	2.4	3-6 h	179
0.05	SC	1 h	2.3	< 6 h	189
0.15 SR^{zp}	SC	1 h	2-2.3	6 h	216
0.3 SR^{zp}	SC	26 h	1.34	24-48 h	189
0.3 SR^{zp}	SC	1 h	6.9-11.5	48 h	216
0.48 SR^{ag}	SC	48 h	1.2	72-96 h	163
0.6 SR^{zp}	SC	1 h	64-71	72 h	216

Duration of action = time at which plasma level falls below therapeutic level (see Table 2).
SR = sustained-release formulation
T_{max} = time to reach maximum concentration
C_{max} = maximum concentration
z*P* = manufactured by Zoopharm, Windsor, CO; ag= manufactured by Animalgesics Laboratories, Millersville, MD

model.[163] Guinea pigs were treated at induction with an extended-release formulation of buprenorphine, carprofen or multimodal treatment. The frequency of behaviors indicative of pain was reduced in the multimodal treatment group compared with buprenorphine or carprofen alone.

Experiments assessing analgesic efficacy are challenging and complicated by species, strain, model, and environment. Nonetheless, studies evaluating alternative dosing regimens and multimodal therapies would further expand our knowledge base and provide better options for pain control. These studies must include proper control groups, including a "no treatment" group when not ethically precluded. However, sufficient data are available at this time to warrant the use of shorter dosing intervals for some of these drugs, and/or use of multimodal regimens. Many of the studies evaluating rodent pain have found that the most significant signs of pain occur within the first 12 to 24 h postoperatively. Multimodal therapies could be extremely beneficial during this critical postoperative time, including the administration of local anesthetic at the site of the incision, which could greatly reduce postoperative pain.[10,18]

Routes of administration

Administration of analgesic drugs to rodents must consider their small body size, stress associated with handling, the half-life of drugs, bioavailability, and factors that impact compliance with administration, such as difficulty in method of administration, time needed to administer the drug, and frequency of dosing required to achieve effective levels.

Table 6. Mouse efficacy studies of buprenorphine

Dose (mg/kg)	Route	Test	Duration of action	Comments	Reference
0.5-2	IP	HP, TF	105-135 min		3
0.5-6.8	IP	TF	ED70 at 0.5-2 mg/kg	Effective dose decreased with doses > 4.5 mg/kg	118
2.4	IP	SX	No effect	Dosed on day 1 and 7 postoperative	87
1	PO-N	SX		Reduced blood corticosterone	200
0.75	PO-F	HP, Lap	Up to 4 h	Suggest one SC dose followed by medicated feed for up to 20 h	155
4.2	PO-F	HP, Lap	Up to 4 h	Suggest one SC dose followed by medicated feed for up to 20 h	155
0.5-5.0	SC	HP, TF, WT		ED50 1.5 mg/kg	206
0.001-0.1	SC	Lap	Up to 90 min at 0.05-0.1 mg/kg		140
0.01, 0.05	SC	Lap		Partially effective at high dose	211
0.05	SC	Lap	5 h		148
0.05	SC	SX	Minimal effect	Dosed twice d for 2 d. Decrease BW, increase arterial pressure, decrease HR	173
0.1	SC	CLP	No effect		90
0.1	SC	HP, Lap	4 h	Dosed q8h for 24 h	103
0.1	SC	Lap	No effect	Dosed q12h for 3d	113
0.1	SC	Lap	No effect		124
0.1	SC	Lap, VF	2-8 h	Dosed q12h for 48 h. Suggest multimodal with carprofen	164
0.1	SC	SX	Partial efficacy to 12h	Dosed q12h for 3 d	203
0.25-5	SC	TF		ED50- 0.25 mg/kg ED30 1-5 mg/kg ED50- 10 mg/kg ED80- 50 mg/kg	171
0.3	SC	HP	No effect		25
0.5	SC	SX	No effect	Dosed q8h for 48 h	99
0.6	SC	SX		Low level pain up to 24 h	57
1.0	SC	HP	12 h		25
1.5	SC	HP, TF	4 h		88
2	SC	Lap	6 h	Dosed once, or q6h for 18 h. Increase in blood pressure at 6 h	70
2	SC	HP, TF	3-5 h		66
0.6 SR	SC	Lap	up to 24 h		113
1.0 SR	SC	CLP	24 h	Improved clinical score	90
1.5 SR	SC	HP, TF	up to 48 h		88
2.2 SR	SC	HP, Lap	24-48 h		103

Effects are based on a single dose of analgesic unless otherwise described in the comments.
PO-*n* = oral in Nutella; PO-F = oral in feed
CLP = cecal ligation and puncture; SX = surgical model; Lap = Laparotomy
HP = hot plate assay
TF = tail flick assay
VF = von Frey test
WT = writhing test
z*P* = manufactured by Zoopharm, Windsor, CO; ih= inhouse formulation

Parenteral administration

Parenteral routes remain the most common route of administration for analgesics. Based on retrospective reviews of analgesic administration reported in the literature, buprenorphine and carprofen are the most commonly used analgesics in rats and mice, and are most frequently administered subcutaneously.[91,199] Intraperitoneal and intramuscular injections have been reported but less commonly. Parenteral routes also offer more reliable and consistent rates of absorption and bioavailability, compared with oral administration.[204] While intraperitoneal injections might provide slightly faster absorption, subcutaneous injections are relatively easy for personnel to administer, can be performed with minimal and short-lasting restraint, and have less potential for adverse effects such as injection into an organ, and/or peritonitis. An often unrecognized characteristic of intraperitoneally administered substances is that absorption occurs largely through mesenteric vessels and are at least partially subject to first-pass hepatic metabolism.[136]

Buprenorphine, carprofen, and meloxicam, 3 commonly administered analgesics in rats and mice, are all available in injectable formulations but require dilution to be administered at appropriate dosages in mice. Carprofen and meloxicam were

Table 7. Mouse efficacy studies of nonopioid analgesics

Agent	Dose (mg/kg)	Route	Test	Duration of action	Comments	Reference
Acetaminophen	50	IP	Lap	1 h		149
	320	PO	SX		No effect on activity	87
	160,320	PO	CFA	Up to 90 min		156
	100-450	SC	Lap	No effect		140
Carprofen	30	PO-W	Lap, VF	In effective	Medicated water provided for 72 h	164
	5-25	SC	Lap	90 min at 20-25 mg/kg	Suggest 29 mg/kg	140
	5	SC	Lap		Burrowing latency similar to anesthesia alone	105
	5	SC	Lap		Activity and burrowing no different than anesthesia alone	104
	5	SC	Lap		Nest complexity improved slightly at high dose	106
	50					
Flunixin	2.5	SC	Lap	No effect		70
Gabapentin	1	IP	VF	3 h	Returned to baseline by 24 h	159
	3					
	3-100	IP	DN, FT, HP, TF		FT ED50 9.3 mg/kg	153
					HP ED50 16.5 mg/kg	
					TF ED50 17.6 mg/kg	
	50	IP	CCI, VF		ED50 7 mg/kg	45
Ibuprofen	200		TF	No effect		217
	40, 80	PO	CFA	150 min		156
	40	PO-W	SX		No effect on activity	87
	2.5-20	SC	CFA, VF		ED50 10 mg/kg	38
	200	SC	TF		In effective at 45 min	121
Ketoprofen			FT, WT		FT- ED50 100 mg/kg	68
					WT- ED80 10 mg/kg	
	30	IP	WT		ED50 30 mg/kg	151
	1-20	SC	Lap	90 min at 20 mg/kg	Suggest 65 mg/kg	140
Meloxicam	1	IP	HP, FT, WT		FT ED50 3 mg/kg, 10 mg/kg HP ED50 3 mg/kg,10 mg/kg	180
	3				WT- ED80 10 mg/kg	
	10					
	2	SC	Lap		Dosed once daily for 3 d. Reduced activity for 24 h postoperative	203
	2	SC	SX	Partially effective	Dosed with 2 mg/kg preoperative, then 1 mg/kg daily for 2 d. Improved BW, increase arterial pressures and HR	173
	5	SC	Lap	No effect		149
	5	SC	SX	No effect	Dosed once daily for 2 d	99
	5	SC	Lap	1 h		148
	5	SC	Lap		Corticosterone normalized at 20 mg/kg; All effective based on ethogram	212
	10					
	20					
	20	SC	SX	1 h	Reduced MGS and behaviors	130
Tramadol			FT, TF, WT		FT ED50 2.8 mg/kg	150
					TF ED25- 2.4 mg/kg	
					WT- ED50 1.86 mg/kg	
	20		Lap	No effect		123
	20	SC	SX	Minimal effect	Dosed daily for 2 d. Decrease BW, increase arterial pressure, decrease HR	173
	3-100	IP	DN, FT, HP, TF		FT ED50 3.5 mg/kg	153
					HP ED50 12.5 mg/kg	
					TF ED50 9.7 mg/kg	
	10-100	IP	CFA		ED50 25 mg/kg	152

Table 7. Continued

Agent	Dose (mg/kg)	Route	Test	Duration of action	Comments	Reference
	10-80	IP	HP	30-60 min	ED50 70 mg/kg	145
	10	IP	TF		Increased latency at 20 and 40 mg/kg	55
	20					
	40					
	50	IP	HP, TF	30-60 min	ED50 50 mg/kg; Trace minerals increased effectiveness	5
	40,80	PO	CFA	45-90 min		156
		SC	HP		ED50 14.8 mg/kg	175
					ED80 71.9 mg/kg	
	3.2	SC	WT		ED50 3.2 mg/kg	59

Effects are based on a single dose of analgesic unless otherwise described in the comments.
PO-W- oral in water; ED = effective dose; SX- surgical model; Lap- laparotomy
CLP = cecal ligation and puncture; CCI = chronic constriction injury
CFA = Complete Freund's adjuvant; DN = diabetic neuropathy; FT = Formalin test; HP = hot plate assay;
NP = neuropathic pain; PW = paw withdrawal test; TF = tail-flick assay; VF = von Frey test; WT = writhing test

Table 8. Mouse efficacy studies of local anesthetics

Agent	Dose	Route	Test	Duration of action	Comments	Reference
Bupivacaine	0.5%	Immer	TBX	No effect	Immersion for 30 s	48
	0.25% to 0.5% 50 µL	SC	HP, TF	5-15 min at 0.25 mg/kg 30-45 min at 0.5 mg/kg	Epinephrine at 1:200000 increased duration to 60 min	190
	0.5% 150 µL	SC	Electric	1-2 h		73
	10% in polymer	SC	HP, SNB	Up to 30 h		187
	333 mg/kg in polymer	SC	HP, SNB	Up to 48 h		192
	0.015% to 0.5% 150 µL	SC	Electric	15 min low dose; 60 min high dose		77
	0.12% 100 µL	SC	TF	30-45 min		191
	0.75% 20 µL	SC	TBX, TF	< 5 min	In effective for TBX	108
	1.1% 40 µL	SC	TF	45 min	Epinephrine increased duration to 80 min	75
	5 mg/kg	SC	Lap	Up to 60 min	Reduced mouse grimace scale	130
EMLA		Top	Tail vein injection	No effect		47
		Top	TBX	No effect		48
Lidocaine	2-4mM	Immer	TF	5 min		120
	0.5% 40 µL 1% 2%	SC	TF	5-30 min	Epinephrine at 1:200000 increased duration up to 100 min	76
	2% 20 µL	SC	TBX, TF	< 5 min	In effective for TBX	108

Effects are based on a single dose of analgesic unless otherwise described in the comments.
Immer = immersion; TBX = tail biopsy; SNB = sciatic nerve block; Electric = electrical stimulus

shown to be stable under a variety of environmental conditions (light compared with dark, and room temperature compared with 4 °C) for up to 7 d when diluted in reverse osmosis water.[96] Although this study evaluated oral administration, it provides evidence of the stability of these drugs, even after dilution.

Sustained-release formulations are increasingly available, and based on personal and listserv communications appear to be gaining widespread acceptance in the US. As early as 1994 investigators were exploring use of liposomal preparations to extend the duration of action of local anesthetics such as bupivacaine,[75] and systemic opioids such as morphine.[78] The first commercially available formulation of a systemically absorbed analgesic for use in rodents was Buprenorphine-SR-LAB (Zoopharm, Windsor, CO) and its use for analgesia in rats was first published in 2011.[64] Since that time, 14 other publications in rodents have included mice, rats, guinea pigs, and prairie dogs. Sustained-release meloxicam is also commercially available; however literature showing its efficacy and sustained plasma levels beyond 24 h in rodents are still lacking.[112,184] These sustained-release formulations, based on use of biodegradable polymers, offer many advantages including decreased handling (and thus stress) to the animal, decreased personnel time, and more consistent and sustained plasma and tissue drug levels, which decrease the potential for breakthrough pain that can occur if standard formulations are dosed too infrequently.[63] However, their use needs to be carefully considered and drawbacks weighed against their benefits. For example, current formulations require use of very small volumes for mice. This makes accurate dosing very challenging and over-dosing is a possibility. Also, absorption

Table 9. Rat efficacy studies of buprenorphine

Dose (mg/kg)	Route	Test	Duration of action	Comments	Reference
0.01	IM	Lap, TF	No effect	Dosed q12h for 72 h	41
0.1	IM	Lap, TF		Dosed q12h for 72 h. BW and food intake similar to saline treatment; TF increased latency	41
0.02-0.2	IP	TF	24 h at 0.2 mg/kg	Hyperalgesia at 0.01 mg/kg	207
8 µg/kg	IV	TF	4 h		161
0.4	PO	Lap	270-390 min	Observations limited to 390 min postoperative	176
0.5-10	PO-G	TF	2 h at 5-10 mg/kg		138
0.5	PO-G	HP	3-5 h		129
0.1-0.4	PO-J	Lap		Increased BW all treatment groups	62
0.5	PO-J	HP	1 h		129
0.5	PO-J	Lap		Dosed q12h for 36 h. Not effective based on BW	98
0.5- 2.0	PO-N	HP	60-120 at 1 mg/kg		92
0.4	PO-N	SX		No change in corticosterone; no change in activity 5h post op; BW loss less than control	72
0.3-3.0	SC	HP, TF		ED50 0.4 mg/kg	206
0.03	SC	Lap		Dosed q12h for 72 h. Decrease BW	20
0.03	SC	PW	24 h	Reduce RGS	133
0.05	SC	SX		Dosed q8-12h for 120 h. Improved gait	27
0.05	SC	HP, SX, VF	No effect	Dosed q12h for 72 h	34
0.05	SC	HP	1 h		107
0.05	SC	SX	No effect	Dosed preoperative and 18 h postoperative supplemented with 0.25 mg/kg POJ	117
0.05	SC	HP	3-5 h		129
0.05	SC		2 h		138
0.05	SC	HP		Dosed q12h for 60 h. PW latency increased; Minimal effect	141
0.05	SC	Lap	270-390 min postoperative	Observations limited to 390 min postoperative	176
0.05	SC	SX, VF, HP	Up to 96 h	Dosed q12h for 72 h. Reduced mechanical and thermal sensitivity.	184
0.05	SC	Lap		Lower ethogram score	160
0.1	SC	FT	6 h		1
0.1	SC	HP	30-240 min		92
0.1	SC	Lap		Dosed q12h for 72 h. Lower ethogram score	160
0.25-0.1	SC	VF		Increase threshold	196
0.2	SC	PW, SX		PW no effect at 24h; no effect on vertical rises	64
0.5	SC	SX		Increase corticosterone levels	72
0.5	SC	SX	No effect	Supplemented with 0.25 mg/kg POJ	117
0.5	SC	HP, TF	6-8 h		66
0.3 SR^{zp}	SC	HP, SX, VF	No effect		34
0.65 SR^{zp}	SC	HP	4-48 h		107
1.2 SR^{zp}	SC	HP, SX, VF		HP increase latency at 24h; VF no significant difference to baseline	34
1.2 SR^{zp}	SC	SX, PW	Up to 48 h	Increase vertical rises compared with buprenorphine	64
1.2 SR^{zp}	SC	HP	24-72h		107
1.2 SR^{zp}	SC	SX, VF, HP	Up to 96 h	Reduced mechanical and thermal sensitivity.	184
1.2 SR^{zp}	SC	Lap		Dosed q12h for 72 h. Lower ethogram score	160
4.5 SR^{zp}	SC	HP, SX, VF		HP increased latency at 24 h at 4.5 mg/kg; VF no effect; Sedative effect with 4.5 mg/kg	34

Effects are based on a single dose of analgesic unless otherwise described in the comments.
PO-*n* = oral in Nutella; PO-J = oral in gelatin; PO-G = oral by gavage
SX = surgical model; Lap = Laparotomy
HP = hot plate assay; PW = paw withdrawal test; TF = tail-flick assay; VF = von Frey test
z*P* = manufactured by Zoopharm, Windsor, CO

Table 10. Rat efficacy studies of nonopioid analgesics

Agent	Dose (mg/kg)	Route	Test	Duration of action	Comments	Ref
Acetaminophen	100, 300	PO	VF	No effect	Dosed daily for 2 d	196
	20-1000	PO	HP, TNT, VF	30-120 min at 100 and 1000 mg/kg	VF ED50 32.8 mg/kg	188
	4.48 mg/mL	PO-W	SX	No effect		27
Carprofen	2	PO-G	PW, VF	6-9 h		201
	5	PO-G	SX, VF, HP	Up to 48 h	Medicated feed provided 2 d preoperative and 2 d postoperative. Reduced mechanical pain, but not thermal.	184
	5	SC	Lap		Dosed preoperative and 4 and 24 h postoperative. Increased activity	23
	5	SC	Lap	270-390 min	Observation limited to 390 min postoperative	176
	5, 10	SC	CFA	No effect		166
Gabapentin	25-200	IP	FT		Effective at 100 and 200 mg/kg	157
	30-300	IP	CCI TF, VF		TF increase at 300 mg/kg; VF ED50 34 mg/kg; cold allodynia ED50 103 mg/kg	95
	5-20	IP	HP, VF		Increase thresholds 10-20 mg/kg	81
	300	PO	CFA	No effect		139
	30-300	PO-G	RS	1-4 h at 300 mg/kg 02-4 h at 100 mg/kg 3 h at 30 mg/kg		85
	10-100	SC	VF		Nominal effect at 100 mg/kg	167
	90	SC	TF	30-90 min		146
Ibuprofen	0.3-30	PO	CFA		No return to baseline gait	139
	20	PO	SX		Dosed q8-12h for 120 h. Improved gait	27
	31, 100	SC	CFA	WT bearing within 30-90 min;	Rearing increase at 100 mg/kg; Burrowing increased	178
Ketoprofen			HP, PW	6 h at 30-100 mg/kg		68
	3	IM	Lap, TF		Dosed q12h for 72 h. No effect	41
	3,5	IM	Lap		Dosed preoperative and 9-12 h postoperative. Reduced BW and FI; single and double dose have similar effect	40
	1,3.2,10	PO	HP	30-60 min	ED90 3.2 and 10 mg/kg	4
	0.5-10	SC	PW, VF		Guarding reduced 2-24 h at 5 and 10 mg/kg; no effect on PW or VF	195
	40	SC	Lap		Reduced RGS similar to morphine	111
Meloxicam	1	SC	Lap		Dosed daily for 3 d. Lower ethogram score; no difference from 2 mg/kg dose	160
	2	SC	Lap		Dosed daily for 3 d. Lower ethogram score	160
	2, then 1	SC	Lap		Dosed daily for 3 d. Improved BW, FI	20
	4.0 SR^{zp}	SC	SX, VF, HP	Up to 48 h	Reduced mechanical pain, but not thermal.	184
Naproxen	50-100	IP	CFA		Weight bearing increased at 30 min; increase burrowing	178
	50-150	IP	CFA		Effective at 50 mg/kg; higher dose no benefit	177
Tramadol	0.625-40	IP	HP, VF		ED50 10 mg/kg; ED80 40 mg/kg	144
	10	IP	HP		Dosed q12h for 60 h. In effective	141
	10-30	IP	HP		ED40 30 mg/kg	65
	10-40	IP	VF	15-30 min at 20 mg/kg; 15-120 min at 40 mg/kg		116
	11	IP	TF	75 min		218
	12.5	IP	Lap		Dosed preoperative and 4 and 24 h postoperative. No effect on activity, wheel running, BW	23

Table 10. Continued

Agent	Dose (mg/kg)	Route	Test	Duration of action	Comments	Ref
	1-25	IP	TF		Increase latency at 15 and 25 mg/kg; motor function impaired > 15 mg/kg	135
	4-50	IP	HP, TF		Increase latency at 12.5-50 mg/kg; heavy sedation > 25 mg/kg	24
	5-20	IP	CFA	60-90 min	Increase latency at 10 and 20 mg/kg	214
	5-40	IP	TF	30-120 min	ED50 20 mg/kg; ED80 40 mg/kg	100
	3-30	PO	HP, TNT, VF	30-120 min at 10 and 30 mg/kg	VF ED50 4.8 mg/kg	188
	4-50	PO-J	HP, TF	No effect		24
	0.45	SC	TF	30-90 min		162
	20	SC	FT		Reduced pain scores	67
	4-50	SC	HP, TF		Increased latency at 25-50 mg/kg; heavy sedation	24

PO-W = oral in water; PO-G = oral by gavage; PO-J = oral in gelatin;
SX = surgical model; Lap = Laparotomy; TNT = tibial nerve translocation; CCI = chronic constriction injury
CLP = cecal ligation and puncture; CFA = Complete Freund's adjuvant; DN = diabetic neuropathy; FT = Formalin test; HP = hot plate assay; RS = Randall-Selitto test; TF = tail-flick assay; VF = von Frey test; z*P* = manufactured by Zoopharm, Windsor, CO

Table 11. Rat efficacy studies of local analgesics

Agent	Dose	Route	Test	Duration of action	Comments	Ref
Bupivacaine		PN	SNB	7 h	Liposomal formulation increased duration to 21 h	54
	1-6 mg/kg liposomal formula	SC	VF	2 h		110
	2 mg/kg	SC	VF	—		110
	2% 300 µL	SC	VF	25 min		74
	2% liposomal Equation 300 µL	SC	VF	200 min		74
	5-15 mg/mL	SC	HP	120-200 min	Latency increased in dose dependent manner	93
Levobupivacaine	0.3% 50 µL	SC	SX	3-24 h		122
Lidocaine	2% 400 µL		HP, CCI		Reduced scratching behavior	15
	1.5-13.8 mmol/kg	SC	VF	15-30 min 13.8mmol	ED50 5.4 mmol/kg; ED75 8.0 mmol/kg	32
	2% 600 µL	SC	VF		ED50 0.13%	33
	4.4-62.2 mmol/kg	SC	VF	15-30 min at 62.2 mmol/kg	ED50 13.3 mmol/kg; ED80 36.7 mmol/kg	31
	2% gel	Top	TF	20 min		9
Pramoxine	12-120 mmol/kg	SC	VF	15-30 min at 120 mmol	ED50 42.1 mmol/kg; ED75 63.9 mmol/kg	32
Procaine	2% 600 µL	SC	VF		ED50 0.44%	33
Ropivacaine	2 mg/mL 300 µL	ID	Lap, VF	Up to 24 h	Less disturbed circadian rhythm, HR, BP	30

PN = perineural; SNB = sciatic nerve block; CCI = chronic constriction injury
ID = intradermal; Top = topical; HR = heart rate; BP = blood pressure; HP = hot plate assay; TF = tail-flick assay; VF = von Frey test

is variable and initial plasma concentrations can be quite high. Animals should be watched carefully during the first 4 to 8 h for signs of adverse opioid-induced effects, such as sedation, respiratory depression, and/or pica; however, other than pica in rats, other opioid-induced effects have not been appreciably seen in the authors' collective experiences. Lastly, the delay until an analgesic response is achieved must be factored into the pain management plan.

Regional anesthesia

Delivery of local anesthetics as a means of providing incisional or regional anesthesia and analgesia is a well-established and effective procedure. The relatively short duration of action and inability to redose in rodents has limited its utility to primarily 3 applications: (1) as part of a multimodal pain management plan, (2) as the sole pain management in minimally invasive procedures, such as small skin incisions for a subcutaneous implant, and (3) to provide some minimal analgesia when no systemic analgesia can be administered for scientific reasons.

Table 12. Guinea pig efficacy studies of buprenorphine, NSAIDs and local analgesics

Analgesic	Dose (mg/kg)	Route	Test	Duration of action	Comments	Reference
Buprenorphine						
	0.05	SC	RS	12-24 h	Dosed q12h for 72.	189
	1-5	SC	Pin prick		ED50 3.0 mg/kg; ED75 4-5 mg/kg at 30 min post administration	35
	0.3 SR[zp]	SC	RS	6 h		189
	0.48 SR[ag]	SC	Lap, VF	Up to 96 h	No change in behavior compared with analgesia only group	163
	0.6 mmol	IM	PW	4 h		213
Carprofen	1	SC	Lap, VF	Ineffective	Pain indices 2-8 h postoperative that resolved by 24 h	49
	4	SC	Lap, VF	Partially effective	Dosed daily for 3 d. Pain indices 8 h postoperative that resolved by 24 h	163
Meloxicam	0.2	SC	Lap		Dosed daily for 2 d. Received local bupivacaine and/or lidocaine. No effect.	52

Lap = laparotomy; PW = paw withdrawal assay; RS = Randall-Selitto test; VF = von Frey test;

Table 13. Updated analgesic dosing recommendations

Species	Agent	Dose (mg/kg)	Route	Frequency
Mouse	Buprenorphine	0.1-0.5	SC	4-6 h
	Buprenorphine SR[zp]	0.6	SC	48 h
	Tramadol	80	SC	24 h
	Carprofen	5	SC	12 h
		20	SC	24 h
	Meloxicam	5-10	SC	8-12 h
	Ketoprofen	20	SC	24 h
Rats	Buprenorphine	0.05-0.0.1	SC	6-8 h
		0.5-0.6	PO	24 h
	Buprenorphine SR[zp]	1.2	SC	48 h
	Tramadol	20-40	PO	24 h
		5	SC	24 h
	Carprofen	5	SC	24 h
	Meloxicam	1	SC	12-24 h
	Ketoprofen	5	SC	24 h
Guinea pig	Buprenorphine	0.05	SC	6 h
	Buprenorphine SR[zp,ag]	0.3-0.48	SC	48 h
	Carprofen	4	SC	12-24 h
	Meloxicam	0.2	SC	12-24 h

Modified from Flecknell 2018.[61]
SR = sustained release; # - provided in food treat, should be observed ingesting
z*P* = manufactured by Zoopharm, Windsor, CO; ag= manufactured by Animalgesics Laboratories, Millersville, MD.
Note: caution should be taken with higher doses of NSAIDs. Multimodal analgesia recommended to allow effective use of lower doses.

See Tables 8 and 11 for a summary of published efficacy studies in mice and rats respectively.

Oral administration

Bioavailability must be considered for any drug administered orally. Voluntary consumption will be variable between animals and both food and water consumption are often decreased after a surgical procedure.[8,87,197] If the drug is administered in a "treat" to encourage consumption, animals may need to be singly housed to ensure equal access and consumption. This could add another level of stress and an additional research variable. Absorption in the intestinal tract can be highly variable and affected by the amount of digesta in the tract, gastrointestinal motility, and other factors. The analgesics themselves may even impact GI motility.[125,165] Oral opioids are commonly used in humans but their primary use is for chronic pain, and there is a paucity of information on oral opioids in rodents. First pass metabolism is an impeding factor as opioids are degraded and lose a significant percentage of their bioavailability.

Oral gavage ensures exact dosing and delivery to all animals in the cohort. However, this method can be time consuming and the handling, restraint, and procedure itself may be stressful to the animals. Administration of analgesics in the drinking water is an attractive option and has been tested in a variety of paradigms in both mice and rats, but this method has numerous drawbacks to widespread use. Palatability and neophobia must be evaluated in each instance, as decreased

Table 14. Published multimodal analgesic efficacy studies

Species	Multimodal analgesics	Dose (mg/kg)	Route	Model	Comments	Reference
Mouse	Buprenorphine Carprofen	0.1	SC	Lap	Buprenorphine dosed q12h, carprofen medicated water provided for 72 h. Improved analgesia for 2-8 h postoperative	164
		30	PO-W			
	Gabapentin	3-100	IP	TF, HP, FT	Reduced ED50 for each analgesic	153
	Tramadol	3-100	IP			
	Tramadol	10-100	IP	TF, HP, FT	ED50 reduced with Keto	152
	Ketoprofen	30-250	IP			
	Buprenorphine	0.05	SC	Lap	Buprenorphine dosed once preoperative. Melox was given 24 h postoperative	148
	Meloxicam	5	SC			
	Meloxicam	5	SC	Lap	No effect	149
	Acetaminophen	50	IP			
	Ibuprofen	200	IP	TF	Opioids enhanced latency	217
	Tramadol		SC	WT, HP	Opioids reduced ED50	59,175
Rat	Buprenorphine	0.03	SC	PW	Similar effect to buprenorphine alone	133
	Meloxicam	2	SC			
	Buprenorphine	0.05	SC	SX	Buprenorphine dosed q8-12h, meloxicam daily. No effect; 8 h dosing resulted in pica	183
	Meloxicam	2	SC			
	Acetaminophen	20-1000	PO	HP, VF	ED50 reduced of each	188
	Tramadol	3-30	PO			
	Carprofen	5	SC	Lap	Dosed preoperative and 4 and 24 h postoperative. Increased activity with tramadol	23
	Tramadol	12.5	IP			
	Gabapentin	5-20	IP	HP, VF	Potentiates opioids	81,146,162,167
	Tramadol	10	SC	HP	Tramadol dosed q12h for 60 h, gabapentin dosed daily. Minimal effect	141
	Gabapentin	80	SC			
	Tramadol	10	SC	SX	Tramadol dosed q8-12h and gabapentin dosed daily for 120 h, No effect	27
	Gabapentin	80	SC			
	Levobupivacaine	0.3% 50 µL	SC	SX	Enhanced with ibuprofen and epinephrine	122
	Ibuprofen	2 mg/mL 50 µL	SC			
	Lidocaine	22.6 mmol/kg	SC	VF	Increased threshold	31
	Naloxone	43.2 mmol/kg				
Guinea pig	Meloxicam	0.2	SC	Lap	No effect	52
	Bupivacaine	1	SC			
	Lidocaine	1	SC			
	Buprenorphine SR[ag]	0.48	SC	Lap	Improved analgesia compared with carprofen alone	163
	Carprofen	4	SC			

PO-W = Oral by water
ag= manufactured by Animalgesics Laboratories, Millersville, MD

water consumption will significantly impact the analgesic dosing.[16,194] Further, decreased consumption may compound an already negative hydration state due to the surgery and associated blood/fluid loss. The solubility of oral solutions is another consideration. Ibuprofen and acetaminophen in pediatric suspensions tend to settle out of solution and both are relatively insoluble in water.[63] A study evaluating rats given acetaminophen in drinking water found no difference in paw pressure latency compared with control rats and treated rats consumed less.[39] This same study also compared buprenorphine in drinking water to intramuscular injection. An increased latency response was measured in high dose buprenorphine (2.9 mg/kg/day equivalent to 0.02mg/ mL water) in drinking water comparable to that seen with IM buprenorphine, and neophobia was not seen. However, one group measured a decreased response to hot plate sensitivity in rats provided acetaminophen elixir at a concentration of 4.48 mg/mL in drinking water.[147] While consumption of acetaminophen treated water was greater than 50% less than tap water on Day 1, the neophobic response decreased

substantially by Day 2. In addition, rats drank significantly more acetaminophen the day after surgery compared with no-surgery controls. In a study by Ingrao and colleagues male C57BL/6 mice consumed carprofen willingly when diluted in their drinking water but not meloxicam.[96] Buprenorphine added to drinking water at 0.009 mg/mL (calculated to deliver approximately 10 times published subcutaneous doses) did not negatively affect volume of water consumed in female C57BL/6 mice, and resulted in therapeutic blood levels at many of the time points evaluated.[182] This differs from the results obtained from a study in rats in which a measurable neophobic response was seen.[101] Despite those encouraging results, interindividual differences in water consumption were seen as well as sporadic consumption during the daytime (light phase), resulting in variability in serum concentrations.

Delivery of analgesia by consumption in diet or a food treat has met with some success and offers the advantage of less stress on the animals since they do not need to be handled and restrained for dosing. Buprenorphine has been administered to rats in gelatin,[62,134] in hazelnut chocolate spread to rats and mice,[2,71,109] and in commercially available gels such as Medi-Gel (Clear H2O, Portland, ME) in mice.[94] Indeed, in some studies, oral consumption provided longer lasting blood levels of drug than subcutaneous injection,[109] for which the duration of action in mice is not long enough to provide continuous analgesia when dosed only every 8 to 12 h. However, consistent themes in all of these studies were variability in consumption, both in quantity and time of day that led the authors to conclude that these methods may be unreliable for provision of consistent and continuous analgesia. Further, in almost all of these studies animals were singly housed. NSAID have also been provided in "treat" forms, including carprofen-containing tablets (Rodent MDs, Bio-Serv, Frenchtown, NJ), and carprofen containing sucralose gel (MediGel CPF, Clear H2O, Portland, ME).

Another consideration for self-administration in water or food is the time of consumption. Mice and rats consume most of their feed and water during the dark cycle.[198] If surgery occurs in the morning of any given day, and the animals do not consume significant quantities of the medication until that night, they will lack pain management during the most crucial initial 12-h postoperative period. Therefore, beginning drug administration prior to the painful procedure (for example surgery) is recommended to overcome both the neophobic response and circadian rhythm impact on consumption to ensure that sufficient blood levels are attained preemptively.

Transdermal administration: Transdermal patches are effective for delivery of analgesia in humans and larger animals, but their practical application to rats and mice is so far limited. Two studies have evaluated the Buprederm patch (Samyang Pharmaceutical Center, Daejeong, Korea) in mice.[168,215] Analgesia, as measured by tail-flick latency, was most pronounced 3 to 6 h after application and an effect was measurable for 24 h.[215]

Timing of administration

The concept of preemptive analgesia is now well established in the pain management of human patients. A PubMed search conducted in December 2018 with keywords "pre-emptive" and "analgesia" produced 412 results. Many of these related to dental, spinal, and other orthopedic procedures. The clinical justification for preemptive analgesia is based on preventing central sensitization of nerve fibers by noxious stimuli occurring peripherally. This excitation results in a lowered pain threshold and hyperalgesia.[11,209] Indeed, a number of studies in humans have demonstrated that preemptive use of local anesthetics decreased the amount of analgesia required postoperatively and decreased hyperalgesia associated with some injuries.[46,51,142] Preemptive analgesia should provide similar benefits in animals by enhancing ability to ameliorate pain resulting in faster recovery periods. Preoperative administration of buprenorphine 30 min prior to surgery in rats resulted in less reduction in food intake than those given buprenorphine after surgery.[86] Preoperative administration of pethidine to rats undergoing ovariohysterectomy surgery prevented postoperative hyperalgesia.[128]

Analgesia should be administered preoperatively whenever short surgical periods are anticipated and an inhalant such as isoflurane or sevoflurane is the sole anesthetic used. The time to onset of action of the analgesic must be considered in planning time of administration. Even drugs given subcutaneously or intraperitoneally are expected to take 15 to 30 min to achieve therapeutic levels. Orally administered drugs will take even longer due to time needed for intestinal absorption and first-pass metabolism in the liver. The increased use of sustained-release formulations offers many advantages, as previously discussed; however, these agents generally take longer to reach effective plasma levels than their standard formulations. An animal that is anesthetized with isoflurane for a 30-min surgical procedure and does not receive a dose of SR-buprenorphine until after the surgery is completed will likely experience unrelieved pain for 30 to 60 min during the postoperative recovery period.

If the surgical period is sufficiently long that an analgesic can be administered under anesthesia and reach effective tissue levels before anesthetic recovery, then this provides another reasonable option. The advantage is that the animal will not be subject to an additional handling (and thus stress) event prior to anesthesia. Another advantage of preoperative or perioperative analgesia is the anesthetic-sparing effect that many of these drugs provide.[89,169] Thus, incorporating administration of analgesics into the anesthetic management plan is another method of providing balanced anesthesia that reduces some of the adverse effects of individual anesthetic agents.

Adjunct (nonpharmacologic) considerations

The goal of pain management is to keep patients as comfortable as possible. Nonpharmacologic interventions that may reduce pain should be considered during postoperative recovery in mice and rats . Animals subjected to procedures resulting in more chronic discomfort or pain are also good candidates for adjunct care. Training researchers in gentle handling techniques and methods to evaluate animals in a nonintrusive manner will minimize incidental stress. Taking time to habituate animals (particularly rats) to handling in advance of the invasive period can further reduce handling stress at both the time of surgery and during postoperative recovery. Skilled surgeons will minimize the degree to which the surgical procedure itself contributes unnecessarily to pain caused by excess tissue trauma, secondary infections, tissue swelling, or other inflammatory responses.[119] Selection of appropriate materials such as synthetic suture material and implanted materials that evoke less tissue response and effective sterilization of surgical materials are also important considerations.

General principles of supportive nursing care apply to all species, including laboratory rodents. Providing additional external heat will help with anesthetic recovery and prevent discomfort associated with hypothermia; soft dry bedding in a solid bottom cage similarly will provide a more comfortable recovery period. Bedding material that does not stick to the

animal's eyes, nose or mouth, such as a paper chip or shredded paper nesting material, should be used.[60] Housing animals in a quiet area that is not heavily trafficked will minimize another potential source of stress. Food and water should be easily accessible to the animals without having to stand up on their hind limbs and stretch to reach it, particularly for orthopedic, invasive abdominal or spinal cord surgeries. Food pellets can be placed on the cage floor and soaked with water to encourage consumption. For animals needing even more supportive care, a variety of high-calorie supplements are available as well as gels as a ready source of hydration. Animals that have had a procedure impacting their mouth, jaw, and/or surrounding tissues may benefit from a soft diet until healed. Administration of fluids either subcutaneously or intraperitoneally may also be beneficial, both in anesthetic recovery and also in preventing dehydration during a period of inappetence. Recommended volumes are 1 to 2 mL for mice and 5 to 10 mL for rats depending on body weight.[60] Larger volumes should be divided into two doses and administered at 2 separate sites.

Side effects of analgesia use in rodents

Analgesic drugs should be administered with care because of inherent side effects that result from their structure, chemical characteristics, and mechanism of action, and because of the potential for overdose effects when administered at high or extra-label doses. Even correct doses of analgesics can have unintended side effects if animals are not managed appropriately after a procedure. Figure 3 shows an example of renal tubular injury induced by flunixin meglumine. In addition to renal effects, other unintended side effects of some classes of NSAIDs include gastric and duodenal ulcers, and even intestinal perforations.[208] The higher dosages of NSAID currently being recommended narrow the therapeutic window and caution should be taken when dosing beyond 3 consecutive days. In addition, hydration status should be assured to minimize risk of acute kidney injury. Opioid use is similarly confounded by side effects, and intoxication is generally associated with cardiorespiratory depression, sedation, constipation, and cognitive impairment.[82] Different classes of opioids will have different mechanisms of action, and different side effects. For example, buprenorphine has been associated with pica and obstruction in rodents when used at high doses.[36] A common side effect of postoperative opioid administration in humans and other animal species that is rarely considered in mice and rats is postoperative nausea and vomiting (PONV).[172,185] Although most rodents cannot vomit, they may experience nausea after opioid administration. If rodents experience some version of PONV after opioid administration, then this could be associated with acute postprocedural weight loss. Dogs develop PONV more frequently after morphine administration than buprenorphine.[172]

Pain on injection may occur with some analgesic drugs and in particular NSAID. The intramuscular route is best avoided for injection in small rodents because swelling, necrosis and subsequent sloughing has been associated with administering acidic agents into their small muscle mass.[204] Some sustained-release formulations of buprenorphine have been associated with skin irritation and necrosis.[64]

Adverse effects of analgesics can be reduced with a number of strategies. Combining analgesic drugs with different mechanisms of action to reduce the overall dose required for any single agent, by using topical and local anesthetics, and incorporating other adjunctive forms of care for animals (see adjunct considerations above) all reduce the deficits associated with analgesia administration. Research teams should work together with their clinical veterinarian to select the safest and most appropriate analgesia plan for their studies.

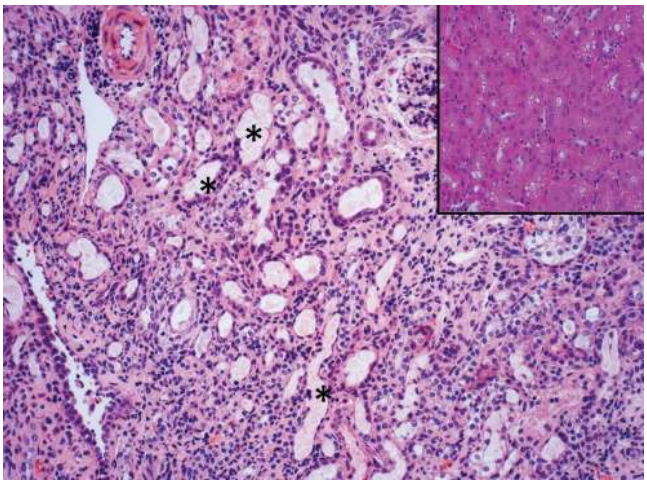

Figure 3. Photomicrograph of a mouse kidney demonstrating acute renal tubular dilatation and necrosis. This mouse was one of several animals that was treated with 2.5 mg/kg flunixin meglumine (Banamine) SC, a potent NSAID, following a 20-min surgical procedure conducted under ketamine/xylazine anesthesia. Fluids were not given after surgery, anesthesia was not reversed, and the cage of recumbent recovering mice was placed under a heat lamp. Animals were euthanized less than 24h after surgery following poor recovery. Renal injury was attributed to acute renal ischemia secondary to NSAID use that was compounded by mild to moderate clinical dehydration. Inset: Normal appearance of renal cortical tubules from an untreated mouse. (x100 H and E)

Conclusions

Like other mammals, laboratory rodents are sentient species and require the same considerations for peri-procedural treatment care that will minimize pain and suffering. The key to effective clinical management of pain is advance planning and anticipation of outcomes. A large number of pharmacologic and nonpharmacologic agents can be used alone or in combination to provide effective care while minimizing potential adverse effects. While the list of pharmacological agents considered most effective in rodents has not changed much in the last decade, we have presented an evidence-based approach to formulate our current recommendations, including consideration of dosing intervals, use of sustained-release formulations, and multimodal approaches to pain management. Based on the available evidence and dosing practices, rodents are often provided inadequate analgesia. However, a significant constraint is that more frequent dosing may require more frequent handling, restraint, and thus increased stress. Analgesics are potent agents with known side effects, and treatment plans should always be developed in conjunction with clinical veterinary input. More research is needed on the duration of effect of analgesia for many drugs and on better dose titrations for achieving and sustaining optimal analgesic blood drug levels. While valid scientific reasons may require withholding analgesic drugs to mice and rats after painful procedures, the vast majority of cases have no prohibitions to analgesic use. If potential experimental effects or interactions with specific analgesic agents are unknown or suspected, investigators and veterinarians should be encouraged to work collaboratively to design and conduct pilot studies before concluding that analgesia will not be provided. A commitment to appropriately managing pain in all research animals represents

a commitment to compassionate care and a goal that all those working with animals in research should be striving toward.

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