Malik A, Leach M.

**How do we assess pain in rodents in veterinary practice, what do we know
and why it is important?**

Veterinary Nursing Journal 2017, 32(4), 103 - 108

Copyright:

This is an Accepted Manuscript of an article published by Taylor & Francis in Veterinary Nursing Journal on 09*03/2017, available online: <https://doi.org/10.1080/17415349.2017.1291318>

DOI link to article:

<https://doi.org/10.1080/17415349.2017.1291318>

Date deposited:

15/12/2017

Embargo release date:

28 March 2018

This work is licensed under a

[Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International licence](https://creativecommons.org/licenses/by-nc-nd/4.0/)

Title:

How do we assess pain in rodents in veterinary practice, what do we know and why it is important?

Author:

Aneesa Malik

Email address: aneesa.vn@gmail.com

Co – Author:

Dr. Matt Leach

Member of Pain & Animal Welfare Science (PAWS) Group

School of Agriculture, Food & Rural Development

Agriculture Building

Newcastle University

Newcastle upon Tyne NE1 7RU

Tel: [+44 \(0191\) 2085494](tel:+441912085494)

Fax: [+44 \(0191\) 2087811](tel:+441912087811)

Email: Matthew.Leach@newcastle.ac.uk

Web: <http://www.ncl.ac.uk/afrd/staff/profile/matthew.leach>

ResearchGate: www.researchgate.net/profile/Matthew_Leach2/

Keywords: Pain behaviours and pain assessment, rats, mice

How do we assess pain in rodents in veterinary practice, what do we know and why it is important?

Abstract

Without the ability to effectively recognise pain and assess its severity in animals, there is no way of assessing the efficacy of analgesia administered. Therefore, we have no way of ensuring that pain is adequately managed (Leach et al. 2012). Despite the extensive use of rodents in medical research that often involves the assessment of pain and distress, translation of this knowledge to veterinary medicine has been relatively neglected, and original veterinary research in pain assessment in rodents is lacking. This article aims to provide a broad overview of common behaviours that could be used to assess pain in rodents.

Keywords:

Rats, mice, pain, pain assessment, pain behaviours

Introduction

Effective pain management is central to good veterinary practice (Epstein et al. 2015). A huge numbers of animals undergo painful procedures every day, in the form of routine veterinary practice, research, or food production. Pain is likely to cause emotional and physical suffering, therefore pain management is paramount for ethical and welfare reasons. Pain has been described by people as ‘one of the most negative experiences possible’ (Mellor, 2011). Pain can adversely affect clinical outcome in veterinary practice (Robertson, 2002) and has been shown to reduce the validity of data in research (e.g. Leach et al. 2012), and has economic implications where animals are kept or treated as a business (e.g. for research or food production).

Pain assessment in rodents is critical: they are a popular pet species (Bament, 2015) and they represent the most common animals used in research. In the UK, last year, over 3 million mice and 300,000 rats were used in research (Home Office 2014). These rodents undergo some of the most invasive procedures but receive analgesia less often than other species (e.g. Lascelles et al. 1999, Stokes et al. 2009, Makowska and Weary, 2013). Recent studies of research using rodents showed that only 10% and 20% of rats and mice received post-operative analgesia in a period between 2000 - 2001 and 2005 - 2006 respectively (Stokes et al. 2009), compared to a much greater 50% and 63% of ‘large’ animals receiving post-operative analgesia in the same periods (Coulter et al. 2009).

While many veterinary professionals are competent and effective at recognising and treating pain in familiar species such as dogs (Hansen and Hardie 1993), or horses (Lorena et al. 2013), pain recognition in many other species appears to be less effective. This may be due to many different factors including: [1] A lack of knowledge of what a non-painful rodent looks like, particularly as most vets and nurses only ever see painful animals. [2] Our own human perception or opinion of the animal’s ‘value’ or ‘worth’ (Henke and Erhardt 2001, cited in Landa 2012) or its ability to experience pain (e.g. Sneddon et al. 2014). [3] Concerns over the side - effects associated with many pain - relieving drugs (Richardson & Flecknell 2005). [4]

Our inability to effectively recognise and assess the severity of pain in animals we are less familiar with (e.g. Sneddon et al. 2014).

From a veterinary perspective, clinical signs in exotic animals such as rodents, often goes unnoticed until the condition is so far advanced that the animal presents in a critical state (McLaughlin and Strunk, 2016). The most common rodent species have been shown to exhibit pain behaviours when observed (e.g. Roughan et al. 2001, Leach et al. 2012). This appears to be in contrast to other prey species (e.g. rabbits), which may hide signs of a painful event as a strategy for survival (Underwood, 2002). However, the use of behaviour to effectively assess pain is further complicated by the variation observed in pain behaviours exhibited between species, and how the type of pain (i.e. acute or chronic) and location (i.e. somatic, visceral, or neuropathic) influences the behaviours observed.

Although many of the pain behaviours described in rats and mice arises from research for human benefit, they remain directly applicable to veterinary medicine. Examples of these behaviours are outlined in Table One. Currently these behaviours have only been validated for the procedures detailed in each study. Therefore they should be used with caution when assessing pain relating from other procedures, and should always be implemented as part of a 'holistic' assessment of pain that incorporates a wide range of measures.

(Table One: Common pain behaviours in rats and mice).

Despite this research into identifying pain behaviours in rodents, our ability to differentiate between pain, and other conditions such as 'sickness', and 'malaise' remains a challenge. However, it must be remembered that these states are likely to be concurrent in many cases. After all, other emotions will be present in pain states, such as depression accompanying chronic pain (Blackburn - Munro and Blackburn – Munro, 2001) and fear, anxiety and distress accompanying pain.

In conclusion, this article provides a brief overview of some common pain behaviours in rats and mice. This will hopefully help nurses build their own evidence base of pain behaviours in these two species, to aid them in their recognition and treatment of pain. For further information on the use of behaviour for assessing pain in rodents and example video sequences please contact Dr Matt Leach (matthew.leach@newcastle.ac.uk).

References

- Andrews, N., Legg, E., Lisak, D., Issop, Y., Richardson, D., Harper, S., Pheby, T., Huang, W., Burgess, G., Machin, I. & Rice, A.S. (2012) Spontaneous burrowing behaviour in the rat is reduced by peripheral nerve injury or inflammation associated pain. *European Journal of Pain*, 16, 485–495.
- Bament W. (2015) Critical care of the small rodent: a veterinary nurse's guide. *The Veterinary Nurse*. 6 (9) 548 - 557
- Blackburn – Munro G., Blackburn – Munro R.E. (2001) Chronic pain, chronic stress and depression: coincidence or consequence? *Journal of Neuroendocrinology*, 13, 1009 – 1023
- Coulter C.A., Flecknell P.A., Richardson C.A., (2009) Reported analgesic administration to rabbits, pigs, sheep, dogs, and non-human primates undergoing experimental surgical procedures. *Laboratory Animals*. 43, 232–238
- Cuiffreda M.C, Tolva V., Casana R., Gnechhi M., Vanoli E., Spazzolini C., Roughan J., Calvillo L. (2014) Rat experimental model of myocardial ischaemia/ reperfusion: an ethical approach to set up the analgesic management of acute post – surgical pain. (2014), *PLoS ONE*, e95913, doi: 10.1371/journal.pone.0095913.
- De Rantere D., Schuster C.J., Reimer J.N., Pang D.S.J. (2015) The relationship between the Rat Grimace Scale and mechanical hypersensitivity testing in three experimental pain models. *European Journal of Pain*. doi: 10.1002/ejp.742
- Epstein M.E., Rodan I., Griffenhagen G., Kadrik J., Petty M.C., Robertson S.A., Simpson W. (2015) AAHA/ AA FP Pain management guidelines for dogs and cats. *Journal of Feline Medicine and Surgery*. 17, (3) 251 – 272
- Giamberardino M.A., Affaitati G., Lerza R., Vecchier L. (1995) Artificial uteral calculus in rats: behavioural characterization of visceral pain episodes and their relationship with referred lumbar muscle hyperalgesia. *Pain* 61, 459 – 469
- Girling S. *Veterinary Nursing of Exotic Pets*. (2013) Blackwell Publishing, (2nd ed.), Chichester, UK.
- Hansen B. and Hardie E., (1993) Prescription and use of analgesics in dogs and cats in a veterinary teaching hospital: 258 cases (1983 – 1989). *Journal of American Veterinary Medicine Association*. 202, 1485 – 1494
- Home Office Data, (2014). *Statistics of scientific procedures on living animals*, Great Britain. Updated July 2014.
- Jablonski P. and Howden B.O. (2002) Oral buprenorphine and aspirin analgesia in rats undergoing liver transplantation. *Laboratory Animals* 36 (2) 134
- Jaggi A.S., Jain V., Singh N. (2009) Animal models of neuropathic pain. *Fundamental and Clinical Pharmacology*, 25, 1-28

Jirkof P., Cesarovic N., Rettich A., Nicholls F., Seifert B., Arras B. (2010) Burrowing behaviour as an indicator of post – laparotomy pain in mice. *Frontiers in Behavioural Neuroscience*, 4, 165

Kurejova M., Nattenmüller U., Hildebrandt U., Selvaraj D., Stösser S., Kuner R. (2010). An improved behavioural assay demonstrates that ultrasound vocalisations constitute a reliable indicator of chronic cancer pain and neuropathic pain. *Molecular Pain*. 26 (6) 18. doi: 10.1186/1744-8069-6-18

Landa L. (2012) Pain in domestic animals and how to assess it: a review. *Veterinary Medicine*, 57 (4) 185 - 192

Langford D.J., Bailey A.L., Chanda M.L., Clarke S.E., Drummond T.E., Echols S., Glick S., Ingrao J., Klassen – Ros T., La Croix – Fralish M.L., Matsumiya L., Sorge R.E., Sotocinal S.G., Tabaka J.M., Wong D., Van den Maagdenberg A.M.J.M., Ferrari M.D., Craig K.D., Mogil J.S. (2010). Coding of facial expressions of pain in the laboratory mouse. *Nature Methods*, 7, 447 – 449

Lascelles B.D.X., Capner C.A., Waterman – Pearson A.E., (1999). Current British veterinary attitudes to peri – operative analgesia for cats and small mammals. *Veterinary Record* 145, 601 – 604

Leach M.C., Klaus K., Miller A.L., di Perrotolo M.S. Sotocinal S.S., Flecknell P.A., (2012). The assessment of post-vasectomy pain in mice using behaviour and the mouse grimace scale. *PLoS One* 7(4) e35656.

Lorena S.E.R.S., Luna S.P.L., Lascelles B.D.X., Corrente J.E., (2013). Attitudes of Brazilian veterinarians in the recognition and treatment of pain in horses and cattle. *Veterinary Anaesthesia & Analgesia*. 40, 410 – 418

Makowska I.J. and Weary D.M., (2013). Assessing the emotions of laboratory rats. *Applied Animal Behaviour Science*. 148, 1 -12

Matsumiya L.C., Sorge R.E., Sotocinal S.G., Tabaka J.M., Wiestopf J.S., Zaloum A., King O.D., Mogil J.S. (2012). Using the mouse grimace scale to re - evaluate the efficacy of post – operative analgesia in laboratory mice. *Journal American Association of Laboratory Animal Science*. 51, 42 – 49

McLaughlin A, Strunk (2016) Common emergencies in small rodents, hedgehogs, and sugar gliders. *Veterinary Clinics of North America: Exotic Animal Practice*, 19, (2) 465 – 499. Emergency and Critical Care.

Mellor D.J. and Bayvel A.C.D. (2011) The scientific assessment of animal welfare. *First OIE global conference on evolving veterinary education for a safer world. Paris, France*. 167 – 174

Miller A.L., Kitson G.L., Skalkoyannis B., Flecknell P.A., Leach M.C. (2016) Using the mouse grimace scale and behaviour to assess pain in CBA mice following vasectomy. *Applied Animal Behaviour Science*. 181, 160 - 165

- Oyama T., Homan T., Kyotani J., Oka M. (2012) Effect of tramadol on pain – related behaviours and bladder overactivity in rodent cystitis models. *European Journal of Pharmacology*, 676, 75 – 80
- Parent A.J., Beaudet N., Beudry H., Bergeron J., Berube P., Drolet G., Sarret P., Gendron L. (2012) Increased anxiety like behaviours in rats experiencing chronic inflammatory pain. *Behavioural Brain Research*, 229 (1) 160 – 167
- Richardson C.A. and 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
- Robertson, S.A. (2002) What is pain? Animal Welfare Forum: Pain Management. *Journal of the American Veterinary Association*, 2, 202–205.
- Roeska K., Doods H., Arnt K., Treede R.D., Ceci A. (2008) Anxiety – like behaviour in rats with mononeuropathy is reduced by the analgesic drugs morphine and gabapentin. *Pain*, 139 (2), 349 – 357
- Roughan J.V., Bertrand H.G.M.J. and Isles H.M. (2015) Meloxicam prevents COX – 2 mediated post surgical inflammation but not pain following laparotomy in mice. *European Journal of Pain*. doi: 10.1002/ejp.712
- Roughan J. and Flecknell P.A. (2001) Behavioural effects of laparotomy and analgesic effects of ketoprofen and carprofen in rats. *Pain*, 90, 65 - 74
- Roughan J., Flecknell P.A., Davies A.B.R. (2004) Behavioural assessment of the effects of tumour growth in rats and the influences of the analgesic effects of carprofen and meloxicam. *Laboratory Animals*, 38, 286 – 296
- Sneddon L.U., Elwood R.W., Adamo S.A., Leach M.C. (2014.) Defining and assessing animal pain. *Animal Behaviour*, 97, 201 – 212
- 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., McDougall J.J., King O.D., Mogil J.S., (2011) The rat grimace scale: a partially automated method for quantifying pain in the laboratory rat via facial expressions. *Molecular Pain*. 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
- Underwood W. (2002) Pain and distress in agricultural animals. *Journal of the American Veterinary Medical Association*, 221, 208 - 211
- Van Loo P.L.P., Everse L.A., Bernsen M.R., Baumans V., Hellebrekers L.J., Kruitwagen C.L.J.J., den Otter W. (1997) Analgesics in mice used in cancer research: reduction of discomfort? *Laboratory Animals*, 31. 318 – 325

Vermeer L.M.M., Gregory E., Winter M.K., McCarson K.E., Berman N.E.J. (2013). Exposure to bisphenol A exacerbates migraine – like behaviours in a multi – behaviour model of rat migraine. *Toxicological Sciences*, 137 (2) 416 – 427

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 post surgical pain in rats. *Journal of the American Association for Laboratory Animal Science*. 54 (4) 420 - 425

Whittaker A.L. and Howarth G.S. (2014) Use of spontaneous behaviour measures to assess pain in laboratory rats and mice. How are we progressing? *Applied Animal Behaviour Science*, 151, 1 – 12

Whittaker A.L., Leach M.C., Preston F.L., Lymn K.A., Howarth G.S. (2016) Effects of acute chemotherapy induced mucositis on spontaneous behaviour and the grimace scale in laboratory rats. *Laboratory Animals* 50 (2) 108 - 118

Wieseler J., Ellis A., Springer D., Brown K., McFadden A., Mahoney J., Rezvani N., Maier S.F., Watkins L.R. (2010) A novel method for modelling facial allodynia associated with migraine in awake and freely moving rats. *Journal of Neuroscience Methods*. 185 (2), 236 – 245

Wolfensohn S. and Lloyd M. (2003) *Handbook of Laboratory Animal Management and Welfare*. 3rd Ed. Blackwell publishing, Oxford, UK.

Wright – Williams S.L., Courade J.P., Richardson C.A., Roughan J.V., Flecknell P.A. (2007) Effects of vasectomy surgery and meloxicam treatment on faecal corticosterone levels and behaviour in two strains of laboratory mouse. *Pain*, 130, 108 – 118

Yalcin I., Bohren Y., Waltisperger E., Sage – Ciocca D., Yin J.C., Freund – Mercier M.J., Barrot M. (2011) A time dependent history of mood disorders in a murine model of neuropathic pain. *Biological Psychiatry*, 70 (10), 946 – 953

Procedure	Species	Supposed type of pain	Behaviour
Laparotomy	Rat	Acute	Increased stretch Increased stretch – walk Increased abdominal licking Increased twitch Increased back arch Increased stagger – fall Increased writhe Decreased feeding Decreased high and low rearing
Uteral calculus	Rat	Acute	Increased frequency of ‘visceral’ behaviours <ul style="list-style-type: none"> • Back arch • Lick lower abdomen/ flank • Abdominal press • Stretch • Muscular contraction • Supine position (with ad limbs)
Vasectomy	Mouse	Acute	Increased writhe Increased flinch Increased rear leg lift Increased abdominal pressing to floor Decreased dig Decreased high rear
Cyclophosphamide – induced cystitis	Mouse	Acute	Increased eye closure Increased rounded back position Increased stretch posture Increased hip shake during micturition Increased abdominal lick
Various procedures (see paper)	Mouse	Acute	Grimace facial expression characterized by the presence of five facial action units, scored on a scale from 0 (no pain), 1 (moderate pain) to 3 (severe pain) <ol style="list-style-type: none"> 1. Orbital tightening 2. Nose bulging 3. Cheek bulging

Laparotomy	Mouse	Acute	<p>4. Ear position changes</p> <p>5. Whisker position changes</p> <p>Grimace scale measured efficacy of different doses of meloxicam: 1 (no pain), 2 (moderate pain), 3 (severe pain) (probe measured inflammation results).</p>
Vasectomy	Mouse	Acute	<p>Mouse Grimace Scale (MGS) was greater at 30 mins and 5 hours post-surgery than baseline. Frequency of pain behaviours (stagger, belly press, flinch, twitch and writhe) were significantly higher at 30 mins post surgery compared to baseline time points.</p>
Vasectomy	Mouse	Acute	<p>Grimace Scale scores were significantly higher post-surgery compared to baseline and significantly lower in the bupivacaine treated surgical group compared to control (saline). Frequency of pain behaviours were significantly higher in saline treated animals than in bupivacaine treated animals.</p>
Laparotomy	Mouse	Acute	<p>Uses the Grimace Scale to determine the efficacy of various analgesics at different time points (see paper for results).</p>
Intraplantar Freud's adjuvant, intra-articular kaolin-carrageenan, laparotomy	Rat	Acute	<p>Grimace facial expression characterized by four facial action units, scored from 0 (no pain), 1 (moderate pain) to 4 (severe pain).</p> <ol style="list-style-type: none"> 1. Orbital tightening 2. Nose/ cheek flattening 3. Ear position changes 4. Whisker position changes
Hot plate assay, laparotomy	Rat	Acute	<p>Grimace scale measured efficacy of various analgesic doses either given pre-operatively (hot plate) or prophylactically/ intra-operatively (laparotomy).</p>
Intraplantar Freud's adjuvant, intraplantar carrageenan, or plantar incision	Rat	Acute	<p>Peak grimace scores coincided with peak mechanical hypersensitivity (latency to withdrawal)</p>

Various procedures: see paper	Mice	Neuropathic/ acute/ chronic	Autotomy (self removal of pain) Increased scratching Increased hyperalgesia (mechanical, thermal, cold) Postural asymmetry Increased licking Increased palpation evoked flinching Increased spontaneous flinching Dropping head to injured side Orofacial flinch in orofacial nerve injury
Sham unilateral vasectomy or sham unilateral embryo transfer	Mice	Acute	Decreased burrowing Sunken flanks Hunched back Neglected grooming Piloerection of hair Immobility
Peripheral nerve injury	Rat	Neuropathic/ acute/ chronic	Decreased burrowing
Bladder cancer	Rat	Chronic	Increased abdominal lick Increased circular ambulation Increased shaking Increased coprophagy Decreased digging
Syngeneic lymphoma induction	Mouse	Acute/ chronic	Decreased grooming Decreased exploration Decreased food and water intake Decreased fur quality Increased sitting in a hunched posture
Liver transplant	Rat	Acute/ chronic	Decreased activity
Thoracotomy and ischaemia	Rat	Acute	Increased 'transient stop' Increased twitch

reperfusion injury (coronary artery ligation)			Increased stagger/ fall
Migraine model	Rat	Chronic	Decreased locomotion Increased light and sound avers Altered grooming habits Increased startle reflex
Chemotherapy induced mucositis	Rat	Acute	Increased writhe Increased twitch Increased back arch Increased gnawing of gnaw toy
Migraine model	Rat	Chronic	Facial allodynia: measured by v paw swipe at mechanical stimul Hind paw allodynia: measured l paw withdrawal caused by diffe of a mechanical stimulus
General	Rats, mice	General symptoms	Increased self mutilation: <ul style="list-style-type: none"> • Excessive grooming • Excessive licking, scratc Raised tail (mice) Abnormal appearance Decreased grooming Staring coat Teeth grinding Guarding behaviour
General	Rats, mice	General symptoms	Decreased exploratory behavior Increased aggression Increased vocalisation Increased hunched posture Increased struggling during rest Disturbed sleep Poryphorin staining (rats)
General	Rodents	General symptoms	Changes to daily behaviour Unkempt coat and piloerection Poryphorin staining (rats)

Chronic constriction nerve injury	Rats, mice	Neuropathic	<p>Depression and anxiety:</p> <ul style="list-style-type: none"> • Nerve injury mice show latency to groom after b with liquid (and reduced frequency) • Nerve injury mice have latency to try a novel fo • Nerve injury mice had i immobility time (at 8 an forced swim test • Nerve injury mice burie marbles (at 4 and 6 weel • Nerve injury mice had r spent in lit compartment test.
Intraplantar Freud's adjuvant	Rat	Chronic inflammatory pain	<p>Anxiety:</p> <ul style="list-style-type: none"> • Reduced active social in social test <p>Morphine abolished both pain (allodynia) and anxiety behaviour for tests used), whereas diazepam anxiety behaviours only: showing can be concurrent with pain.</p>
Intraplantar Freud's Adjuvant, Sciatic nerve ligation	Rats, mice	Inflammatory, neuropathic	<p>Anxiety:</p> <ul style="list-style-type: none"> • Chronic constriction inju showed increased anxiety using an elevated plus m • Midazolam reduces anxiety without reducing pain be (tested by mechanical hy • Morphine and gabapenti reduced both pain behav by mechanical hypersen anxiety behaviours (test elevated plus maze).
Injected fibrosarcoma cells (bone metastases	Mouse	Cancer pain, neuropathic pain	Ultrasonic vocalisation* at high (tested at 37 and 50 kHz)

cancer pain) or sciatic nerve ligation			

* Care must be taken interpreting vocalisation as pain, as vocalisation, as with humans and most other animals, occurs for different purposes in many different situations and emotional states. Research about pain – specific vocalisation in rodents is on – going.

Table One: Overview of pain behaviours in rats and mice (adapted from Whittaker and Howarth, 2014).