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

Animal Welfare 2011, 20: 591-596
ISSN 0962-7286

Voluntary ingestion of buprenorphine in mice

KR Jacobsen*†, O Kalliokoski†, J Hau† and KSP Abelson†‡

† Department of Experimental Medicine, University of Copenhagen, Blegdamsvej 3B, 2200 Copenhagen N, Denmark
‡ Department of Neuroscience, Division of Comparative Medicine, Uppsala University, Uppsala, Sweden
* Contact for correspondence and requests for reprints: kiroja@sund.ku.dk

Abstract

*Buprenorphine is a widely used analgesic for laboratory rodents. Administration of the drug in a desirable food item for voluntary ingestion is an attractive way to administer the drug non-invasively. However, it is vital that the animals ingest the buprenorphine-food-item mix as desired. The present study investigated how readily female and male mice (*Mus musculus*) of two different strains consumed buprenorphine mixed in a commercially available nut paste (Nutella®), and whether variation between genders and strains would affect the subsequent serum concentrations of buprenorphine. Buprenorphine at different concentrations mixed in Nutella® was given to male and female C57BL/6 and BALB/c mice in a complete cross-over study. Pure Nutella® or buprenorphine (1.0–3.0 mg kg^{-1} bodyweight [bw]) mixed in 10 g kg^{-1} bw Nutella® were given to the mice at 1500h. The mice were video recorded until the next morning, when blood was collected by submandibular venipuncture. The concentration of buprenorphine in the Nutella® mix did not affect the duration of ingestion in any of the groups. However, female mice consumed the Nutella® significantly faster than males. Repeated exposure significantly reduced the start time of voluntary ingestion, but not the duration of eating the mixture. These differences did not however affect the serum concentration of buprenorphine measured 17 h post administration.*

Keywords: *analgesia, animal welfare, buprenorphine, mice, refinement, voluntary ingestion*

Introduction

Pain in animals subjected to invasive procedures may be considered a 'contingent inhumanity' which is almost always detrimental to the object of the experiment (Russell & Burch 1959). Peri-operative treatment with an appropriate analgesic is thus an important refinement of invasive procedures.

Buprenorphine is a highly potent opioid. It acts as a partial agonist on the μ receptor subtype and is widely used as an analgesic in laboratory rodents subjected to mild or moderate invasive surgical procedures (Flecknell 2001). The recommended route of subcutaneous injection requires dosing every 8–12 h (Roughan & Flecknell 2002; Hedenqvist & Hellebrekers 2003; Flecknell 2009) which may stress the animals and result in fluctuating serum concentrations if not injected at correct intervals. In general, small animals subjected to injections with needles, often display symptoms of distress, and alternative non-invasive routes of administrations should be welcomed (Russell & Burch 1959). Furthermore, the duration of subcutaneously administered buprenorphine varies widely in different publications, and according to Gades *et al* (2000) the analgesic effect of buprenorphine only lasts 3–5 h in mice (*Mus musculus*) measured by tail-flick and hot-plate tests. In contrast, oral dosing of buprenorphine has been shown to result in a high and constant concentration in the circulation of mice (Kalliokoski *et al* 2011). However, oral dosing by gavage requires restraint of the animals. The potential stress of this procedure can be eliminated by allowing the animal to voluntarily consume the drug, a method which has gained some acceptance as an analgesic regimen in rats (*Rattus norvegicus*) (Liles *et al* 1998; Flecknell *et al* 1999; Goldkuhl *et al* 2010).

Voluntary ingestion of buprenorphine in rats has, however, had varying degrees of success. Doses 100× higher than those recommended for subcutaneous injection seem necessary to induce serum concentrations of buprenorphine providing effective analgesia in analgesiometric tests (Thompson *et al* 2004). Furthermore, some studies, using fruit-flavoured gel as the food item, demonstrated that oral administration of buprenorphine in concentrations inducing appreciable analgesia resulted in unpalatable mixtures not voluntarily consumed by the rats (Martin *et al* 2001; Thompson *et al* 2006). Despite variation in pain sensitivity according to the stage of the oestrous cycle, Thompson *et al* (2006) concluded that only the recommended dose of 0.05 mg kg^{-1} bw buprenorphine given by subcutaneous injection is successful in increasing the latency time measured by the hot-water tail-flick test. In contrast, Goldkuhl and colleagues (2008, 2010) found that oral doses of 0.4 mg kg^{-1} bw dissolved in 2 g kg^{-1} bw Nutella® reduced

https://doi.org/10.1017/S0962728600003225 Published online by Cambridge University Press

the post-surgical level of circulating corticosterone in rats subjected to permanent catheterisation (Goldkuhl *et al* 2008, 2010). In agreement with this, oral doses of 0.3 mg kg^{-1} bw have been demonstrated to be efficacious in inhibiting post-surgical bodyweight loss and reduced food and water intake (Flecknell *et al* 1999). The discrepancies may be due to differences in study design, pain-eliciting procedures, pain-assessment methods and strains tested. However, standard pharmacokinetic indices of buprenorphine suggest that oral dosage should be 10× the parenteral dose to compensate for the difference in bioavailability (Brewster *et al* 1981), which correlates well with the findings of Goldkuhl *et al* (2008) and Flecknell *et al* (1999). Furthermore, comparing results obtained from analgesiometric studies with data obtained from clinical post-operative pain studies could be misleading (Liles & Flecknell 1992; Elmer *et al* 1998). Even though analgesiometric tests provide valuable knowledge about pain sensitivity and analgesic potency, the mechanisms of post-surgical pain are very different. Doses of analgesia necessary to reduce pain sensitivity in analgesiometric tests are often higher than doses relevant in the clinical setting (Cooper *et al* 2005). In the clinical setting, behavioural parameters, changes in food and water consumption and bodyweight are often used to assess post-operative pain in many species. Despite criticism on the objectivity of these parameters and the possible interference of the analgesia on these, these parameters are well validated as measurements for pain, stress or discomfort in laboratory animals (Flecknell 1984; Liles & Flecknell 1993; Hawkins 2002).

A discrepancy in the negative consequences of buprenorphine on the animals' welfare (eg pica behaviour) is noted in the literature (Clark *et al* 1997; Gades *et al* 2000; Roughan & Flecknell 2004; Leach *et al* 2010). However, we have not observed any pica or abnormal behaviour in rats or mice when given buprenorphine by the voluntary ingestion method.

The aim of the present study was to investigate how readily mice of both genders of two different inbred strains would voluntarily ingest buprenorphine at two different doses (1.0 and 3.0 mg kg^{-1} bw), mixed in Nutella®. Subsequent serum concentrations of buprenorphine were quantified to assess the relationship between ingested amounts and resulting serum concentrations of the drug, and to evaluate the possibility of obtaining sufficient concentrations of buprenorphine in the morning, after the mixture being introduced to the animals the previous afternoon.

Materials and methods

The animal experiments performed in this study were approved by the Animal Experiments Inspectorate under the Danish Ministry of Justice (licence number 2005/561-1059). A complete cross-over design was used in order to reduce the number of animals needed in this study.

Study animals and housing conditions

Eight male and eight female BALB/c mice and eight male and eight female C57BL/6 mice, aged 10–11 weeks, weighing 21–32 g were used in a complete cross-over design composed of three experimental periods with three weeks washout inbetween. All mice were obtained from Taconic (Ry, Denmark). Male mice were housed with a female cage mate and female mice were housed in groups of six upon arrival for two weeks to acclimatise prior to the study. The mice were housed in Macrolon cages (Tecniplast, Varese, Italy) with food pellets (Altromin 1319, Brogaarden, Gentofte, Denmark) and acidified tap water provided *ad libitum*. Wooden chips (Tapvei Oy, Kortteinen, Finland) were used as bedding and cardboard houses were provided as environmental enrichment. Room temperature was maintained at 20 (± 2)°C, air humidity was 30–60% and the light regime was a 12:12 h dark: artificial light cycle with the light period starting at 0630h. During the dark period a red lamp illuminated part of the room in order to allow video recording.

All animals were individually housed during the experimental periods, but housed with female cage mates during the restitution periods. Two days before each experimental period the mice were separated in order to habituate to the individual housing condition. During the experimental period, no cardboard house was present in order to guarantee complete view of the mouse and the adhesive tape with Nutella® at all times. After the third treatment period all mice were euthanised.

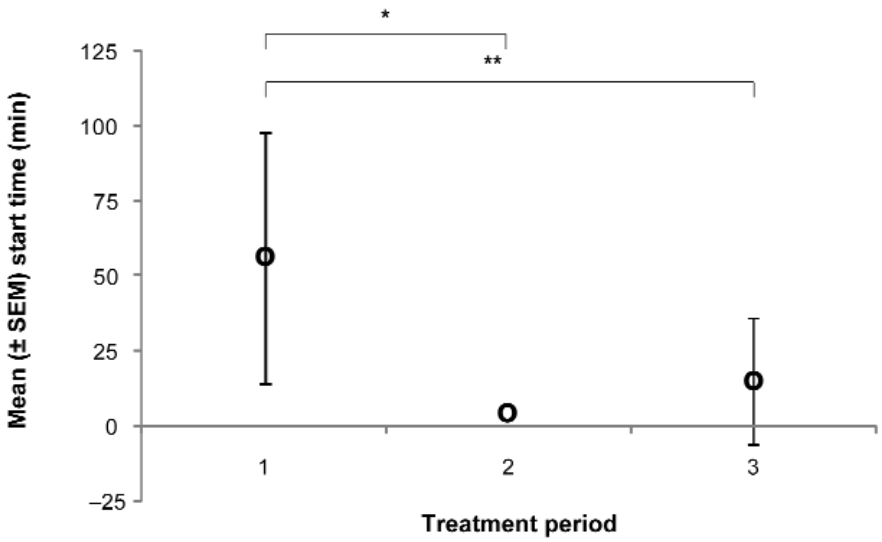
Experimental design

Three different treatments were tested on each mouse in a randomised order with three-week restitution periods between each experiment: (A) control treatment consisting of 10 g kg^{-1} bodyweight (bw) Nutella® (Ferrero, Pino Torinese, Italy); (B), a test treatment consisting of 10 g kg^{-1} bw Nutella® with 1.0 mg kg^{-1} bw buprenorphine (Temgesic, Schering-Plough Europe, Brussels, Belgium); and (C) a test treatment consisting of 10 g kg^{-1} bw Nutella® with 3.0 mg kg^{-1} bw buprenorphine. The buprenorphine tablets were crushed into a fine powder before being mixed with the Nutella® in order to assure even concentrations of the mixture.

The treatments A, B or C were presented to the mice at 1500h on a small piece of adhesive tape placed on the inside of the cage 4 cm above the bedding. The mice were given the treatment in a randomised order. The mice were video recorded immediately after the treatment was given and until the next morning at 0800h, when blood was collected via sub-mandibular venipuncture. A sample of approximately 200 µl of blood was collected from each animal per sampling. Serum was separated from the blood samples and analysed in duplicates using the Buprenorphine One-step ELISA (International Diagnotic Systems Corp, St Joseph, MI, USA) in accordance with the manufacturer's instructions. For improved accuracy, the provided standards were supplemented with additional dilutions to yield a seven-point standard curve consisting of concentrations 0, 0.5, 1, 2, 3, 6.5 and 10 ng ml^{-1}. All known cross-reactivities are reported by the manufacturer at < 0.06%, with the exception of norbuprenorphine, which cross-reacts at 1.1%. No analytical sensitivity was given by the manufacturer. The absorbencies were recorded at 450 nm (reference wave-

https://doi.org/10.1017/S0962728600003225 Published online by Cambridge University Press

Figure 1



Start time of voluntary ingestion for the three experimental periods. Between each experimental period is three weeks washout period. Circles display mean value of start time of voluntary ingestion (min). Data were pooled, since no difference was observed when comparing female and male mice or the two strains. $P < 0.05$ was considered significant. * $P < 10^{-5}$; ** $P < 10^{-3}$.

length: 650 nm) using a Thermo Multiskan ex microplate reader (Thermo Fisher Scientific, Waltham, MA, USA).

The duration of consuming the treatments (A, B or C) was determined by manually reading the videotapes. The manual reading was performed blinded to the three treatments. The first eating behaviour of each individual mouse noted was defined as the starting time and the duration of the voluntary ingestion was measured as the period between the starting time and the time where no Nutella® was present on the adhesive tape. If it was not possible to clearly define the time-point where no visible Nutella® was present due to the lighting, the duration of voluntary ingestion was excluded from the data.

All animals received all treatments (A, B and C) and no animal was presented the same treatment more than once. No animals had been given Nutella® or buprenorphine prior to the study.

Statistical analysis

Q-Q plots were performed to test for normal distribution. Log-transformations were used where appropriate. Start time and duration of voluntary ingestion of treatment A, B and C were analysed separately using a univariate general linear model with Tukey's multiple comparisons test with week of exposure, strain, sex and treatment as fixed factors. A Kruskal-Wallis analysis of variance test was performed to verify the results when the data deviated greatly from a normal distribution. The effects of treatment, sex and strain on serum concentrations of buprenorphine were analysed with univariate analysis of variance with Tukey's multiple comparisons *post hoc* test. Linear regression analyses were performed to investigate the influence of duration, start and end time of voluntary ingestion on the serum concentrations of buprenorphine. *P*-values < 0.05 were considered significant. All statistical tests were performed using PASW Statistics v18 (SPSS Inc, Chicago, USA).

Table 1 Mean and median values of start time of voluntary ingestion when providing male and female BALB/c and C57BL/6 mice doses of 1 or 3 mg kg^{-1} bw buprenorphine mixed in 10 mg kg^{-1} bw Nutella® or Nutella® without buprenorphine three times with three-week washout periods inbetween.

Exposure	Mean start time (min)	Median start time (min)	SD	N
1	56.2	6	104.6	32
2	0	0	0.3	32
3	15.1	0	60.7	32

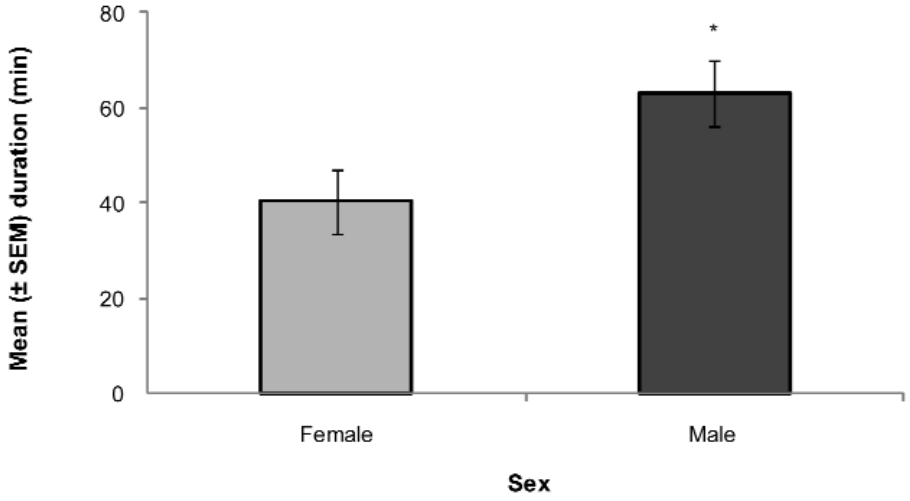
No sex, strain or dose-related difference in start time of voluntary ingestion time was observed when comparing the three treatments, and thus the groups were pooled. Zero represents eating behavior noted within the first minute after administration.

Results

Significant differences in start time of voluntary ingestion ($P < 10^{-5}$) were found when comparing the three treatment periods (period 1 > period 3 > period 2) (Figure 1). Mean and median values of start times are shown in Table 1. However, there was no difference in the start time of voluntary ingestion between the different doses of buprenorphine. Furthermore, repeated exposure to the Nutella® did not affect the duration of voluntary ingestion. There was no significant difference between the duration of eating the Nutella® containing the two different concentrations of buprenorphine, nor any difference when comparing these to the duration of consuming pure Nutella®. There were no differences in start and duration of voluntary ingestion when comparing the two strains. Female mice consumed the treatments significantly quicker than male mice ($P = 0.039$)

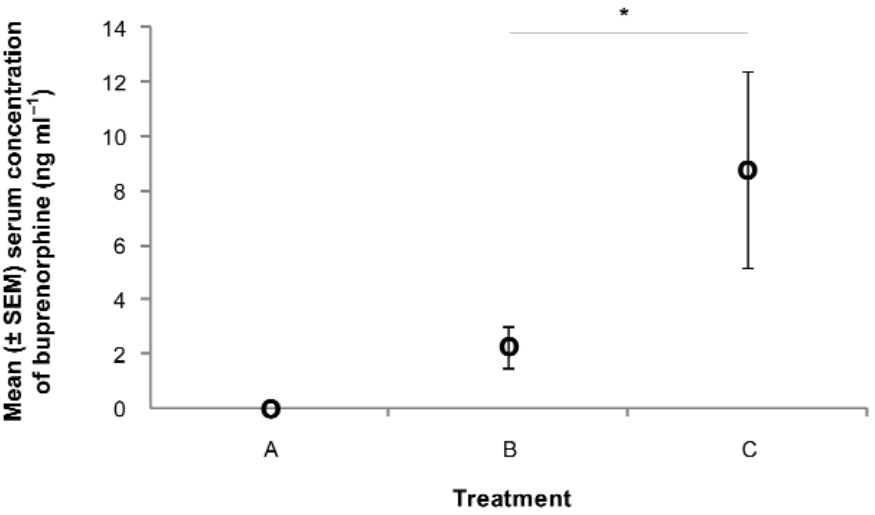
https://doi.org/10.1017/S0962728600003225 Published online by Cambridge University Press

Figure 2



Sex difference in duration of voluntary ingestion of the treatments (A, B and C) from the first noted eating behaviour until no visible treatment was present. Data were pooled, since no significant difference in duration of voluntary ingestion was observed between the two strains or between the three doses. * $P < 0.05$ was considered significant.

Figure 3



Serum concentrations of buprenorphine 17 h post administration by voluntary ingestion. Treatment group A was given 10 mg kg^{-1} bw Nutella®, B was given 1.0 mg kg^{-1} bw buprenorphine mixed in 10 mg kg^{-1} bw Nutella® and C 3.0 mg kg^{-1} bw buprenorphine mixed in 10 mg kg^{-1} bw Nutella®. Data were pooled, since no difference was seen between female and male mice or between the two strains. * $P < 0.05$ was considered significant.

Table 2 Mean and median values of duration of voluntary ingestion when providing female and male BALB/c and C57BL/6 mice doses of 1 or 3 mg kg^{-1} bw buprenorphine mixed in 10 mg kg^{-1} bw Nutella® or Nutella® without buprenorphine (10 mg kg^{-1} bw).

Sex	Mean duration (min)	Median duration (min)	SD	N
Female	56.2	6	104.6	32
Male	0	0	0.3	32
Total	15.1	0	60.7	32

No strain or dose-related difference in duration of voluntary ingestion time was observed when comparing the three treatments, and thus the groups were pooled.

(Figure 2). Mean and median duration of voluntary ingestion is presented in Table 2. Ingestion of 3.0 mg kg^{-1} bw buprenorphine resulted in significantly higher levels of circulating buprenorphine than did the lower dose of 1.0 mg kg^{-1} bw buprenorphine ($P < 10^{-7}$) (Figure 3). There were neither significant effects of the start, duration nor end time of ingestion on serum concentration of buprenorphine.

Discussion

Rats can be easily conditioned to salivate using chocolate as a treat (Guhad & Hau 1996) and chocolate has been used as an effective vehicle for drug administration in rats (Huang-Brown & Guhad 2002). Regarding mice, we recently demonstrated that the hazelnut spread, Nutella® with chocolate taste, serves as a useful vehicle for voluntary ingestion of buprenorphine in effective doses (Kalliokoski *et al* 2011). The present study demonstrated that even high concentrations of buprenorphine were effectively voluntarily ingested by BALB/c and C57BL/6 mice when administered in Nutella®, and mice of both strains voluntarily consumed all of the novel food within a few hours. A significant reduction in start time of ingesting the treatment was seen after repeated exposure. This phenomenon is well recognised in several inbred stains of mice due to their neophobic

https://doi.org/10.1017/S0962728600003225 Published online by Cambridge University Press

nature (Bolivar & Flaherty 2004; Sclafani 2006, 2007). However, after the mice had started to ingest, no difference in duration was observed between the three periods.

Female mice consumed the treatment significantly faster than males. In general, male mice are considered to be less neophobic than female mice and females show higher levels of 'emotional' or 'fear' responses than males during open-field testing (Archer 1977). Furthermore, male mice are considered superior to females in regard to localising and recognising new objects (Frick & Gresack 2003), although there are no clear tendencies of sex differences toward novel foods (Bolivar & Flaherty 2004). The reason for the sex difference seen in this experiment may therefore not be related to response to the novel food, but may reflect the females' preference for sweet food items, as demonstrated in several studies of rats (Valenstein *et al* 1967; Wade & Zucker 1969; Zucker 1969; Sclafani *et al* 1987). Similar studies have, to our knowledge, not been performed with mice. However, Forgie *et al* (1988) demonstrated strain differences in preferences for sweetened morphine between C57BL/6J mice and DBA72J mice, but no significant sex differences were seen (Forgie *et al* 1988). The sugar preference of female mice could also depend on the stage of the oestrous cycle with the highest levels of sugar intake occurring at the time of oestrus (Petersen 1976).

In contrast with previous studies on rats, where buprenorphine was mixed in a gel ('buprenorphine-jello'), there was no difference in the speed with which the mice ate the treatments when comparing the Nutella® treatment with the Nutella®-buprenorphine treatment. Even at the high dose of 3.0 mg kg^{-1} (60× the subcutaneous dose of 0.05 mg kg^{-1} bw) we were not able to detect any difference in duration of ingestion compared to the pure Nutella®.

The differences in start time of voluntary ingestion after repeated exposure, and the sex differences in voluntary ingestion time did not affect the subsequent serum concentration of buprenorphine. This is probably because these differences were too small to have any effect on the serum concentrations 17 h post administration. It is therefore possible to achieve high levels of circulating buprenorphine 17 h following presentation of the drug, regardless of mouse gender. This indicates that buprenorphine can be administered well in advance as a pre-emptive analgesic using the present voluntary ingestion scheme. However, further studies on this issue are needed to verify the observed serum concentrations of buprenorphine also have a significant biological effect in reducing post-surgical pain.

We have recently demonstrated that the dose of 0.4 mg kg^{-1} bw of voluntarily ingested buprenorphine mixed in Nutella® results in higher serum concentrations and with longer duration than the recommended dose of 0.05 mg kg^{-1} bw administered subcutaneously (Kalliokoski *et al* 2011). It is, however, not completely known whether this dose is sufficient to provide post-operative analgesia. Preliminary studies on BALB/c mice after surgical placement of carotid catheters, indicates that the buprenorphine doses necessary to reduce post-operative stress are higher than those studied by Kalliokoski *et al*. The present study demonstrates that even higher doses than those recommended will successfully be eaten by two commonly used strains of mice. The voluntarily ingested buprenorphine mixed in Nutella® is thus an effective and humane way of achieving high levels of circulating buprenorphine, since the palatability of the drug-Nutella® mix is high and since oral ingestion results in long-lasting, high serum concentration levels. The present voluntary ingestion method in mice is thus a refinement of a standard procedure and has the potential to improve the welfare of laboratory mice.

Animal welfare implications

The findings of the present study demonstrate that even high doses of buprenorphine mixed in Nutella® will be successfully eaten by both male and female BALB/c and C57BL/6 mice. The present voluntary ingestion method is thus a refined way of providing analgesic treatment to mice subjected to invasive procedures. Further research is needed to evaluate the efficiency in reducing post-surgical pain, but this study demonstrates that oral voluntary ingestion results in measurable serum concentrations of buprenorphine 17 h post administration.

Acknowledgements

The present investigation was supported by a generous grant from the Danish Strategic Research Council. The authors thank Trine Marie Nielsen and Karina Retsborg Pedersen for technical assistance.

References

Archer J 1977 Sex-differences in emotional behavior of laboratory mice. *British Journal of Psychology 68*: 125-131

Bolivar VJ and Flaherty L 2004 Genetic control of novel food preference in mice. *Mammalian Genome 15*: 193-198

Brewster D, Humphrey MJ and McLeavy MA 1981 The systemic bioavailability of buprenorphine by various routes of administration. *Journal of Pharmacy and Pharmacology 33*: 500-506

Clark JA, Myers PH, Goelz MF, Thigpen JE and Forsythe DB 1997 Pica behavior associated with buprenorphine administration in the rat. *Laboratory Animal Science 47*: 300-303

Cooper DM, Hoffman W, Wheat N and Lee HY 2005 Duration of effects on clinical parameters and referred hyperalgesia in rats after abdominal surgery and multiple doses of analgesic. *Comparative Medicine 55*: 344-353

Elmer GI, Pieper JO, Negus SS and Woods JH 1998 Genetic variance in nociception and its relationship to the potency of morphine-induced analgesia in thermal and chemical tests. *Pain 75*: 129-140

Flecknell PA 1984 The relief of pain in laboratory animals. *Laboratory Animals 18*: 147-160

Flecknell PA 2001 Analgesia of small mammals. *Veterinary Clinics of North America: Exotic Animal Practice 4*: 47-56

Flecknell PA 2009 *Laboratory Animal Anaesthesia, Third Edition.* Academic Press: USA

Flecknell PA, Roughan J and Stewart R 1999 Use of oral buprenorphine ('buprenorphine jello') for post-operative analgesia in rats: a clinical trial. *Laboratory Animals 33*: 169-174

Forgie ML, Beyerstein BL and Alexander BK 1988 Contributions of taste factors and gender to opioid preference in C57Bl and Dba mice. *Psychopharmacology 95*: 237-244

Frick KM and Gresack JE 2003 Sex differences in the behavioral response to spatial and object novelty in adult C57BL/6 mice. *Behavioral Neuroscience 117*: 1283-1291

Gades NM, Danneman PJ, Wixson SK and Tolley EA 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

Goldkuhl R, Carlsson HE, Hau J and Abelson KSP 2008 Effect of subcutaneous injection and oral voluntary ingestion of buprenorphine on post-operative serum corticosterone levels in male rats. *European Surgical Research 41*: 272-278

Goldkuhl R, Hau J and Abelson KSP 2010 Effects of voluntarily-ingested buprenorphine on plasma corticosterone levels, body weight, water intake, and behaviour in permanently catheterised rats. *In Vivo 24*: 131-135

Guhad FA and Hau J 1996 Salivary IgA as a marker of social stress in rats. *Neuroscience Letters 216*: 137-140

Hawkins P 2002 Recognizing and assessing pain, suffering and distress in laboratory animals: a survey of current practice in the UK with recommendations. *Laboratory Animals 36*: 378-395

Hedenqvist P and Hellebrekers LJ 2003 Laboratory animal analgesia, anesthesia, and euthanasia. In: Hau J and van Hoosier Jr (eds) *Handbook of Laboratory Animal Science, Essential Principles and Practices* pp 413. CRC Press: USA

Huang-Brown KM and Guhad FA 2002 Chocolate, an effective means of oral drug delivery in rats. *Lab Animal 31*: 34-36

Kalliokoski O, Jacobsen KR, Hau J and Abelson KSP 2011 Serum concentrations of buprenorphine after oral and parenteral administration in male mice. *The Veterinary Journal 187(2)*: 251-254

Kohn DF, Martin TE, Foley PL, Morris TH, Swindle MM, Vogler GA and Wixson SK 2007 Guidelines for the assessment and management of pain in rodents and rabbits. *Journal of the American Association for Laboratory Animal Science 46*: 97-108

Leach MC, Forrester AR and Flecknell PA 2010 Influence of preferred foodstuffs on the antinociceptive effects of orally administered buprenorphine in laboratory rats. *Laboratory Animals 44*: 54-58

Liles JH and Flecknell PA 1992 The effects of buprenorphine, nalbuphine and butorphanol alone or following halothane anaesthesia on food and water consumption and locomotor movement in rats. *Laboratory Animals 26*: 180-189

Liles JH and Flecknell PA 1993 The effects of surgical stimulus on the rat and the influence of analgetic treatment. *British Veterinary Journal 149*: 515-525

Liles JH, Flecknell PA, Roughan J and Cruz-Madorran I 1998 Influence of oral buprenorphine, oral naltrexone or morphine on the effects of laparotomy in the rat. *Laboratory Animals 32*: 149-161

Martin LBE, Thompson AC, Martin T and Kristal MB 2001 Analgesic efficacy of orally administered buprenorphine in rats. *Comparative Medicine 51*: 43-48

Petersen S 1976 Temporal pattern of feeding over estrous-cycle of mouse. *Animal Behaviour 24*: 939-955

Roughan JV and Flecknell PA 2002 Buprenorphine: a reappraisal of its antinociceptive effects and therapeutic use in alleviating post-operative pain in animals. *Laboratory Animals 36*: 322-343

Roughan JV and Flecknell PA 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*: 461-472

Russell WMS and Burch RL 1959 *The Principles of Humane Experimental Technique, Special 1992 Edition*. UFAW: Wheathampstead, UK

Sclafani A 2006 Enhanced sucrose and polycose preference in sweet 'sensitive' (C57BL/6J) and 'subsensitive' (129P3/J) mice after experience with these saccharides. *Physiology & Behavior 87*: 745-756

Sclafani A 2007 Fat and sugar flavor preference and acceptance in C57BL/6J and 129 mice: Experience attenuates strain differences. *Physiology & Behavior 90*: 602-611

Sclafani A, Hertwig H, Vigorito M and Feigin MB 1987 Sex-differences in polysaccharide and sugar preferences in rats. *Neuroscience & Biobehavioral Reviews 11*: 241-251

Thompson AC, DiPirro JM, Sylvester AR, Martin LBE and Kristal MB 2006 Lack of analgesic efficacy in female rats of the commonly recommended oral dose of buprenorphine. *Journal of the American Association for Laboratory Animal Science 45*: 13-16

Thompson AC, Kristal MB, Sallaj A, Acheson A, Martin LBE and Martin T 2004 Analgesic efficacy of orally administered buprenorphine in rats: methodologic considerations. *Comparative Medicine 54*: 293-300

Valenstein ES, Cox VC and Kakolews JW 1967 Further studies of sex differences in taste preferences with sweet solutions. *Psychological Reports 20*: 1231-1234

Wade GN and Zucker I 1969 Hormonal and developmental influences on rat saccharin preferences. *Journal of Comparative and Physiological Psychology 69*: 291-300

Zucker I 1969 Hormonal determinants of sex differences in saccharin preference, food intake and bodyweight. *Physiology & Behavior 4*: 595-602

https://doi.org/10.1017/S0962728600003225 Published online by Cambridge University Press