

Effects of Voluntarily-ingested Buprenorphine on Plasma Corticosterone Levels, Body Weight, Water Intake, and Behaviour in Permanently Catheterised Rats

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Abstract. *This study investigated the peri- and postoperative effect of pre-emptive analgesia through voluntary ingestion of buprenorphine in Nutella[®], in male Sprague-Dawley rats. An arterial catheter was inserted and the rats were connected to an automated blood sampling device (AccuSampler[®]). Blood samples were drawn up to 18 h after surgery and the plasma concentrations of corticosterone were quantified. Postoperative changes in water intake and body weight were recorded, and the behaviour of the rats was analysed during two 30-min periods. Pre-emptive oral buprenorphine treatment reduced the plasma corticosterone levels in the postoperative period, compared to controls treated with local anaesthetics. Buprenorphine-treated rats consumed more water and maintained body weight better. Behavioural observations indicated that buprenorphine changed the behaviour in non-operated rats but there was no difference in the operated rats. The present study strengthens the hypothesis that pre-emptive oral buprenorphine in Nutella[®] is suitable for treatment of postoperative pain in rats.*

Anaesthesia and tissue injury during surgery, as well as postoperative pain, result in a stress response. This is characterized by activation of the hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic nervous system, which results in increased catabolism and retention of water and salt to maintain cardiovascular homeostasis. During this response, glucocorticoids are secreted from the adrenal cortex (1, 2). The major biologically active glucocorticoid in rodents is corticosterone, which is a frequently used marker of stress (3).

Laboratory animals are commonly used in experiments involving surgery and recovery from surgery. Since the surgical

stress response results in altered physiology, leading to a greater variation between animals, stress is a source of experimental error. Stress has a negative impact on the welfare of laboratory animals, with pathological lesions (4), immunosuppression with increased susceptibility to infectious diseases (5) and impaired wound healing (6). It is therefore important to reduce the stress response in connection to surgery.

To estimate the surgical stress response, clinical parameters like body weight, water consumption, food intake and behaviour (7-9) are often used. However, to study the activation of the HPA axis and correlate this to clinical signs, hormone secretion has to be quantified. For this purpose, it is important to use a sampling method that in itself does not stress the animal and thereby contribute to increased hormone secretion. Automated blood sampling equipment enables sampling without disturbing the animal. Earlier studies have demonstrated that blood sampling through an inserted catheter does not in itself affect the corticosterone levels (10-12).

Pre-emptive analgesic treatment can attenuate the stress response in the postoperative period and for example, morphine reduces postoperative corticosteroid plasma levels (13-15). Buprenorphine is a commonly used analgesic for treatment of postoperative pain in rats, and has been shown to be effective in analgesiometric tests and to reduce postoperative pain in clinical trials (16). When administering analgesics, it is preferable to use a route of administration that causes minimal stress, and oral voluntary ingestion can be used to avoid stressful restraint and injections. Lower postoperative plasma corticosterone levels were recorded in rats treated with oral buprenorphine through voluntary ingestion of the drug mixed in Nutella[®] compared to rats treated with subcutaneously administered buprenorphine (17). However, the efficacy of oral treatment of buprenorphine has been questioned (18, 19) and there are conflicting results in the literature (16), which is why the effects of oral voluntary ingestion of buprenorphine need to be further investigated. Measuring plasma corticosterone levels is an accurate method for assessing the acute stress response. However, it may be impractical to obtain sufficient

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numbers of serial blood samples in an experimental situation. Therefore, a combination of corticosterone data, clinical parameters such as body weight change, water consumption, and behavioural observations may serve as a robust strategy for stress assessment. Buprenorphine has been shown to modulate behaviour (20, 21), but the effects on behaviour after buprenorphine treatment in permanently catheterised animals have been sparsely investigated.

In the present study, we aimed to confirm our previous findings that oral voluntary ingestion of buprenorphine has beneficial effects on postoperative corticosterone levels and maintenance of body weight and water intake in rats. In addition, we aimed to investigate the behaviour of the animals during the early postoperative phase, in order to detect behavioural changes that could be useful to distinguish between untreated animals and animals treated with adequate postoperative analgesic treatment.

Materials and Methods

All animal experiments in this study were approved by the Animal Ethics Committee in Uppsala, Sweden.

Animals. In total, 49 male Sprague-Dawley rats (Scanbur, Sollentuna, Sweden) were used (28 of them were operated on), with mean body weight 390 ± 42 g (mean \pm SD) before surgery.

The rats were acclimatised for at least one week after arrival. They were kept in animal rooms with standard animal house conditions: room temperature $20 \pm 2^\circ\text{C}$, relative humidity 30-60%, 12 h light (06:00-18:00) and 12 h dark. The animals were housed in Makrolon type IV cages in groups of two or three and clean cages were provided twice a week. The animals had free access to pelleted food (R36, Lantmännen, Stockholm, Sweden) and tap water. Aspen chips (Finn Tapvei, Kortteinen, Finland) were used as bedding material. Two days before surgery, the rats were moved to the laboratory of the experiment and single housed in Makrolon type III cages. After surgery, the same cages and water bottles were used as before and food pellets were placed on the bedding to improve accessibility.

Analgesic treatment. The animals in the experimental group ($n=9$) were treated with buprenorphine (Temgesic[®]; Schering-Plough Europe, Brussels, Belgium), which was administered by voluntary oral ingestion at a dose of 0.4 mg/kg mixed with Nutella[®] (2 g/kg) one hour prior to surgery. All rats had consumed their buprenorphine-Nutella[®] mix prior to surgery. Two days before surgery, the rats had been given Nutella[®] to habituate them to the product. The animals in the control group ($n=19$) received the standard local anaesthetics lidocaine 4 mg/kg (Xylocain[®]; Astra Zeneca, Sweden) or bupivacaine 1 mg/kg (Marcain[®], Astra Zeneca, Sweden). The local anaesthetics were infiltrated in the skin and muscles of each incision site during surgery, commencing prior to opening of the skin. The doses were based on those recommended in the literature (22). The two local anaesthetics have different properties. Lidocaine has a very rapid onset but short duration (approximately 30-60 min), while bupivacaine has a slower onset but a longer duration (several hours) (23). There was no difference between these two treatments in any of the parameters studied in the present investigation, and the data were pooled into one control group.

Surgical procedure. Anaesthesia was induced with 5% isoflurane in 100% oxygen. The rats were shaved at the incision sites and attached to a Simtec anaesthetic mask for spontaneous respiration. The level of isoflurane was maintained at 2.5-3% to ensure adequate anaesthesia. The body temperature was maintained at $37\text{-}38^\circ\text{C}$ using a heated pad. A catheter filled with heparinised saline was inserted in the *arteria carotis communis* with its tip close to the heart. The catheter was led subcutaneously to the dorsal region of the neck and then through a metal spring and attached to the AccuSampler[®] (DiLab, Lund, Sweden).

Blood sampling and corticosterone analysis. Two blood samples were collected during surgery, the first after insertion of the catheter and the second just before finishing the surgery. Blood was then collected via the AccuSampler[®] at 0, 2, 6, 10, 14 and 18 h after the rat regained consciousness. The sampling period started between 11:30 and 12:15. After each sampling, the AccuSampler[®] injected saline into the rat at the same volume as that of the blood withdrawn. In total, eight samples each of 150 μl blood were collected. The total volume corresponds to less than 0.4% of the body weight. After the sampling, all experiments were terminated by intra peritoneal injection of 2 ml pentobarbitone (100 mg/ml, pentobarbital; Apoteket, Sweden).

The blood samples were collected in tubes and stored overnight at 4°C . The samples were centrifuged and plasma was then stored at -20°C until analysis. The plasma concentration of corticosterone was quantified with enzyme-linked immunosorbent assay (ELISA), using a commercial correlate ELISA kit (Assay-Designs Inc., Ann Arbor, MI, USA). The intra-assay coefficient was 6% and the inter-assay coefficient was 8%.

Body weight and water intake. All animals were weighed on the day they arrived at the laboratory, on the day of surgery and at the end of the experiment. The daily water intake was measured by weighing the water bottles.

Behavioural observations. For behavioural monitoring, the rats were filmed with a video camera for 30 minutes at both 25 min and 5 h after they regained consciousness. The first five minutes were not included in the analyses to exclude behaviours related to the camera being activated. Two groups of non-operated rats were also included, one treated with buprenorphine ($n=8$) and one control group without analgesic treatment ($n=16$). Half of the control group rats received Nutella[®] and the rest were untreated. There were no differences in the behaviour of these rats and hence the data for these were pooled into one group. The non-operated rats were filmed at the same time of day as those operated, at approximately 12:00 and 16:30. To estimate the level of locomotor activity of the rat, the following behaviours were scored: exploration, walking, digging and rearing. The frequencies of these behaviours were monitored, as well as the duration of resting.

Statistical analyses. Statistical analyses were performed using GraphPad Prism 5.01 and SPSS Statistics 17.0. The corticosterone levels during surgery were compared between groups with Student's *t*-tests. To determine the difference in plasma corticosterone levels after surgery between treatment groups, the general linear model (GLM) was used. The behavioural data were non-parametric and were analysed with Kruskal-Wallis tests. Changes in body weight and water consumption between treatment groups were compared using *t*-tests. *P*-values <0.05 were considered significant.

Results

Plasma corticosterone levels. The plasma corticosterone levels during surgery are illustrated in Figure 1A. There were no significant differences in the plasma corticosterone levels between groups. The plasma corticosterone levels during 18 h after surgery are presented in Figure 1B. Corticosterone levels in rats treated with oral buprenorphine were compared to levels in control rats treated with local anaesthetics using GLM. Buprenorphine-treated rats exhibited lower plasma levels of corticosterone during the entire postoperative period than did the control rats.

Changes in body weight and water consumption. The changes in body weight and water consumption one day after surgery compared to preoperative values are presented in Figure 2. There was no significant difference in pre-operative body weight or water intake between the treatment groups.

In the buprenorphine group, neither the body weight nor the water intake were reduced one day after surgery compared to postoperative levels. By contrast, in the control group, a reduction in both body weight and water intake was recorded. Both the postoperative change in body weight and water intake were significantly different between buprenorphine-treated and control rats.

Behaviour. The mean values of the frequency of active behaviours and percentage of time of resting are illustrated in Figure 3. During the first period of filming, the non-operated buprenorphine-treated rats spent less time resting compared to both non-operated control and operated buprenorphine-treated rats. The non-operated buprenorphine-treated rats were also more active than non-operated control during this period. During the second period of filming, there was no observed difference between groups.

Discussion

Oral treatment with buprenorphine mixed in Nutella[®], offered in a tray in the home cage, is an easy and non-invasive method to apply the analgesic. No handling of the animal is necessary and the rats always consume all of the mix given, if they are offered the Nutella[®] one or two days prior to surgery.

During surgery, the corticosterone levels remained high in the circulation showing an immediate activation of the HPA-axis and there was no significant difference between the treatment groups. The levels were still high (above 170 ng/ml) when the rats regained consciousness. The normal levels for male rats during this time of the day (approx. 12:00) are 0-30 ng/ml (24, 25) and thus, the elevation observed after surgery is substantial. At two hours postoperatively, the levels had declined in all rats regardless of treatment. During normal conditions, corticosterone is secreted in a diurnal rhythm, with the highest levels at the

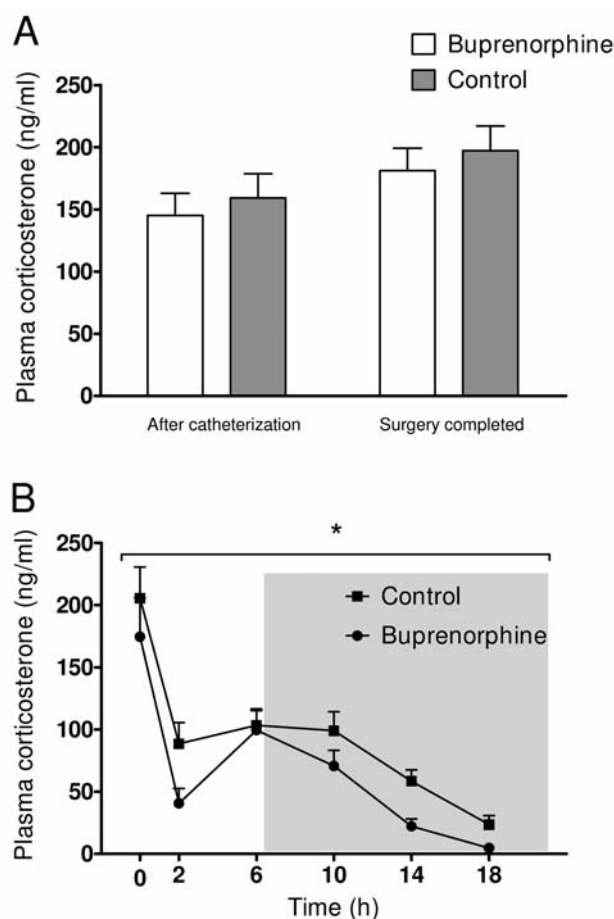


Figure 1. A: Plasma corticosterone levels during surgery (mean±standard error of the mean (SEM)). B: Plasma corticosterone levels during 18 h postoperatively (mean±SEM). The shaded box indicates the dark period (18:00 to 06:00). * $p < 0.05$, significant difference between groups determined by GLM.

beginning of the dark phase when the rats are most active and the lowest levels at the beginning of the light phase. During the onset of the dark period, corticosterone is secreted in a pulsatile manner, with plasma levels ranging from 0 to 70 ng/ml. The levels decline during the middle of the night (24). In the present study, the high levels after six to ten hours are probably due to this diurnal variation. Treatment with buprenorphine resulted in a faster decline in the plasma corticosterone concentrations compared to controls. This demonstrated that oral buprenorphine reduced the stress response in the postoperative period more effectively than did the local anaesthetics. This result is consistent with our previous findings where oral buprenorphine treatment reduced corticosterone levels more effectively compared to subcutaneous buprenorphine treatment and control (17).

Changes in body weight and water intake are commonly used parameters to assess postoperative recovery (7, 9). The rats were gaining weight during the two days before surgery.

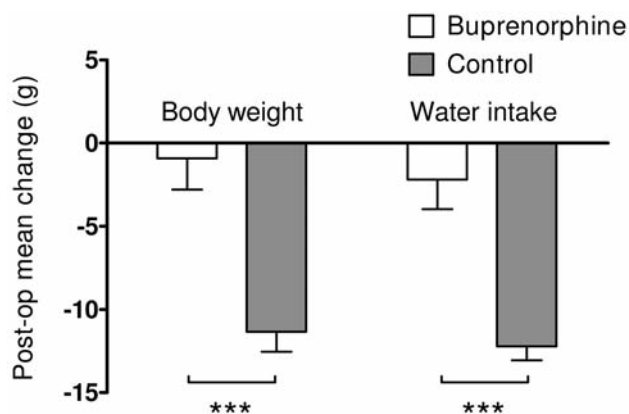


Figure 2. Changes in body weight and water intake from preoperative to postoperative weight (mean±SEM). *** $p < 0.0001$, significant difference between groups.

After the surgery, the body weight gain ceased, but in buprenorphine-treated rats, the body weight was not reduced, contrary to control rats. The control group also drank less water postoperatively compared to buprenorphine-treated rats. This supports our hypothesis that pre-emptive oral voluntary buprenorphine treatment improves postoperative recovery in rats. The changes in water intake and body weight agree with results from a previous study of postoperative treatment with buprenorphine and bupivacaine (26).

Behavioural observations are commonly used to assess postoperative recovery (8, 27). In the present study, active behaviours and time spent resting were observed at 0.5 and 5 hours postoperatively. There were large variations between rats within each treatment group, and no significant difference was observed in duration of time spent resting or in locomotor activity between rats in the operated groups in spite of the difference in plasma corticosterone levels and changes in body weight and water intake. The large variations in the data of the present study illustrate one of the difficulties with the interpretation of the behavioural data. Previous studies have suggested that buprenorphine alters the normal behaviour in non-operated rats, with an increase in the frequency of active as well as inactive behaviours (20, 21). However, in a study by Ilbäck *et al.* (28) there was only a minor increase in locomotor activity after subcutaneous treatment with a high dose of 0.15 mg/kg buprenorphine. In the present study, buprenorphine treatment of the non-operated rats caused a higher frequency of active behaviours and less time spent resting during the first period of filming, approximately three hours after treatment. During this period, there was also a difference in time spent resting between buprenorphine-treated non-operated and buprenorphine-treated operated rats. The non-operated rats rested less than the operated ones, indicating that buprenorphine affects behaviour more in non-operated rats than in operated rats.

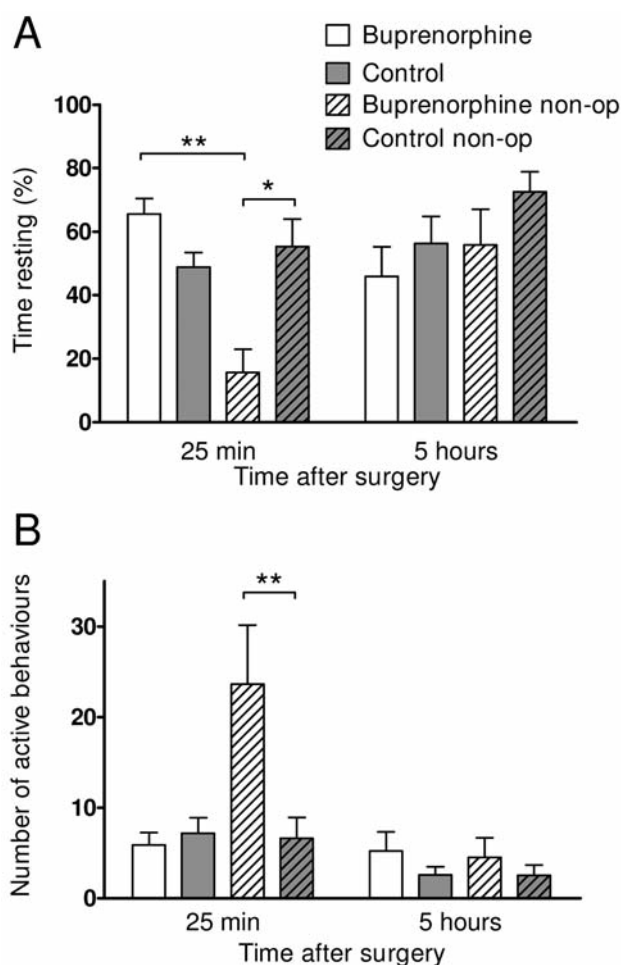


Figure 3. A: Percentage of time the rats spent resting during two periods of 25 min each (mean±SEM). B: Number of active behaviours during two periods of 25 min each (mean±SEM). * $p < 0.05$, ** $p < 0.01$, significant difference between groups.

During the second period of filming, there was no longer any difference between groups. This may be because the behavioural effect of buprenorphine had declined.

In the study of postoperative recovery, measurements of corticosterone, body weight and water intake changes appear to be more sensitive methods than the behavioural observations studied in the present investigation, at least in connection to the specific catheterization surgery and single-housing during automated blood sampling. Development of sensitive methods for stress assessment is essential, since they may assist not only in the assessment of postoperative recovery, but also in increasing our understanding of the mechanisms of postoperative pain and the efficacy of analgesic treatments.

In conclusion, it is evident from the present data that pre-emptive oral voluntary ingestion of buprenorphine reduces the plasma corticosterone levels during the first 18 hours after surgery compared to controls receiving local anaesthetics

during surgery only. The buprenorphine-treated rats also consumed more water and maintained body weight better than did control rats. Buprenorphine treatment seems to result in increased locomotor activity only in non-operated rats. This strengthens the hypothesis that pre-emptive oral buprenorphine in Nutella® is suitable for pre-emptive treatment of postoperative pain. However, the behaviours associated with awakening from anaesthesia, or during the approach of the dark period chosen for analysis in the present study, did not seem to be sufficiently sensitive to distinguish animals treated with analgesia from non-treated animals.

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References

- Silverthorn DU: Human Physiology: An Integrated Approach. San Francisco, Benjamin-Cummings, 2001.
- Traynor C and Hall GM: Endocrine and metabolic changes during surgery – anesthetic implications. *Br J Anaesth* 53: 153-160, 1981.
- Woodman DD: The adrenal glands. *In: Laboratory Animal Endocrinology: Hormonal Action, Control Mechanism and Interactions with Drugs*. Chichester, John Wiley and Sons Ltd., pp. 253-286, 1997.
- Suleman MA, Wango E, Farah IO and Hau J: Adrenal cortex and stomach lesions associated with stress in wild male African green monkeys (*Cercopithecus aethiops*) in the post-capture period. *J Med Primatol* 29: 338-342, 2000.
- Glaser R, Rice J, Sheridan J, Fertel R, Stoot J, Speicher C, Pinsky D, Kotur M, Post A and Beck M: Stress-related immune suppression: health implications. *Brain Behav Immun* 5: 7-20, 1987.
- Christian LM, Graham JE, Padgett DA, Glaser R and Kiecolt-Glaser JK: Stress and wound healing. *Neuroimmunomodulation* 13: 337-346, 2006.
- Martini L, Lorenzini RN, Cinotti S, Fini M, Giavaresi G and Giardino R: Evaluation of pain and stress levels of animals used in experimental research. *J Surg Res* 88: 114-119, 2000.
- Roughan JV and Flecknell PA: Behavioural effects of laparotomy and analgesic effects of ketoprofen and carprofen in rats. *Pain* 90: 65-74, 2001.
- Shavit Y, Fish G, Wolf G, Mayburd E, Meerson Y, Yirmiya R and Beilin B: The effects of perioperative pain management techniques on food consumption and body weight after laparotomy in rats. *Anesth Analg* 101: 1112-1116, 2005.
- Abelson KSP, Adem B, Royo F, Carlsson HE and Hau J: High plasma corticosterone levels persist during frequent automatic blood sampling in rats. *In Vivo* 19: 815-819, 2005.
- Royo F, Björk N, Carlsson HE, Mayo S and Hau J: Impact of chronic catheterization and automated blood sampling (AccuSampler) on serum corticosterone and fecal immunoreactive corticosterone metabolites and immunoglobulin A in male rats. *J Endocrinol* 180: 145-153, 2004.
- Vachon P and Moreau JP: Serum corticosterone and blood glucose in rats after two jugular vein blood sampling methods: comparison of the stress response. *Contemp Top Lab Anim Sci* 40: 22-24, 2001.
- Desborough JP: The stress response to trauma and surgery. *Br J Anaesth* 85: 109-117, 2000.
- Page GG, McDonald JS, and Ben-Eliyahu S: Pre-operative versus postoperative administration of morphine: impact on the neuroendocrine, behavioural, and metastatic-enhancing effects of surgery. *Br J Anaesth* 81: 216-223, 1998.
- Shavit Y, Weidenfeld J, DeKeyser FG, Fish G, Wolf G, Mayburd E, Meerson Y and Beilin B: Effects of surgical stress on brain prostaglandin E(2) production and on the pituitary–adrenal axis: Attenuation by preemptive analgesia and by central amygdala lesion. *Brain Res* 1047: 10-17, 2005.
- Roughan JV and Flecknell PA: Buprenorphine: A reappraisal of its antinociceptive effects and therapeutic use in alleviating post-operative pain in animals. *Lab Anim* 36: 322-343, 2002.
- Goldkuhl R, Carlsson HE, Hau J and Abelson KSP: Effect of subcutaneous injection and oral voluntary ingestion of buprenorphine on postoperative serum corticosterone levels in male rats. *Eur Surg Res* 41: 272-278, 2008.
- Martin LBE, Thompson AC, Martin T and Kristal MB: Analgesic efficacy of orally administered buprenorphine in rats. *Comp Med* 51: 43-48, 2001.
- Thompson AC, Kristal MB, Sallaj A, Acheson A, Martin LBE and Martin T: Analgesic efficacy of orally administered buprenorphine: methodologic considerations. *Comp Med* 54: 293-300, 2004.
- Liles JH, Flecknell PA, Roughan J and Cruz-Madorran I: Influence of oral buprenorphine, oral naltrexone or morphine on the effects of laparotomy in the rat. *Lab Anim* 32: 149-161, 1998.
- Roughan JV and Flecknell PA: Effects of surgery and analgesic administration on spontaneous behaviour in singly housed rats. *Res Vet Sci* 69: 283-288, 2000.
- Hedenqvist P and Hellebrekers LJ: Laboratory animal analgesia, anaesthesia, and euthanasia. *In: Handbook of Laboratory Animal Science, Vol. 1. Essential Principles and Practices*. Hau J and van Hoosier Jr. GL (eds.). Boca Raton, CRC Press, pp. 413-455, 2003.
- Nolan AM: Pharmacology of analgesic drugs. *In: Pain Management in Animals*. Flecknell PA and Waterman-Pearson A (eds.). London, Saunders, pp. 21-52, 2000.
- Atkinson HC, Wood SA, Kershaw YM, Bate E, and Lightman SL: Diurnal variation in the responsiveness of the hypothalamic–pituitary–adrenal axis of the male rat to noise stress. *J Neuroendocrinol* 18: 526-533, 2006.
- Siswanto H, Hau J, Carlsson HE, Goldkuhl R and Abelson KSP: Corticosterone concentrations in blood and excretion in faeces after ACTH administration in male Sprague-Dawley rats. *In Vivo* 22: 435-440, 2008.
- Hayes JH and Flecknell PA: A comparison of pre- and post-surgical administration of bupivacaine or buprenorphine following laparotomy in the rat. *Lab Anim* 33: 16-23, 1999.
- Roughan JV and Flecknell PA: Evaluation of a short duration behaviour-based post-operative pain scoring system in rats. *Eur J Pain* 7: 397-406, 2003.
- Ilbäck NG, Siller M and Stålhandske T: Effects of buprenorphine on body temperature, locomotor activity and cardiovascular function when assessed by telemetric monitoring in rats. *Lab Anim* 42: 149-160, 2008.

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