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Perioperative stress response in dogs undergoing elective surgery: variations in behavioural, neuroendocrine, immune and acute phase responses

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Abstract

The aim of this trial was to describe the behavioural, neuroendocrine, immune and acute phase stress responses in dogs undergoing elective surgery in normal, clinical practice conditions. Sixteen dogs were submitted to elective orchiectomy or ovariohysterectomy using a standardised surgical protocol. Each animal was confined to the Intensive Care Unit during pre- and post-surgery and perioperative behavioural, neuroendocrine, immune and acute phase responses were studied. Behavioural categories, cortisol, prolactin, white blood cell, C-reactive protein and haptoglobin variation were evaluated. Values at different times were compared with basal values shown by the dog in its usual environment. Communicative and explorative behaviours showed high occurrence pre-surgery and were inhibited post-surgery. Decreases in post-surgery activity, interactive behaviours and changes in waking/sleeping patterns were observed. The most sensitive marker of psychological stress, cortisol, in comparison with basal values, showed a significant increase both during pre- and post-surgery confinement in the ICU cage. Prolactin values were characterised by a significant decrease early into the post-surgery period. The immune response was characterised by long-term neutrophilia and monocytosis, but by short-term lymphopaenia and eosinopaenia, limited to the early post-operative period. With regard to the acute phase response, both C-reactive protein and haptoglobin showed a long-term increase, post-surgery. Changes in behavioural, haematological and biochemical markers showed that perioperative stress represents a major challenge to dog welfare.

Keywords: *animal welfare, behavioural response, dog, immune response, neuroendocrine response, perioperative stress*

Introduction

The perioperative stress response is a physiological reaction to surgery and various associated conditions such as pain, analgesia- and anaesthesia-induced dysphoria, human handling and confinement to a hospitalisation cage; all elements that may be perceived by the animal as physical and/or psychological threats (Hansen *et al* 1997; Hardie *et al* 1997; Mellor *et al* 2000; Moberg 2000; Väisänen *et al* 2002). Surgery trauma itself elicits a biological stress reaction that has been described in humans, horses and partially in dogs (Hansen *et al* 1997; Hardie *et al* 1997; Stover *et al* 1988; Taylor 1998; Väisänen *et al* 2002; Marrocco-Trischitta *et al* 2004). Of the factors described as perioperative stressors, those causing psychological stress have not been adequately evaluated. Confinement in an unfamiliar, size-restricted environment, usually in the form of an Intensive Care Unit (ICU) cage, together with social isolation and handling by unknown people, represents a major pre and postoperative threat, capable of activating the stress response (Hetts *et al* 1992; Wells 2004).

The stress response is a complex phenomenon in which four different components can be distinguished: the behavioural, the neuroendocrine, the immune and the autonomic nervous system responses (Matteri *et al* 2000).

The behavioural response normally represents the initial attempt at stressor control, therefore observing animal behaviour may provide a non-invasive tool for identification and rapid control of stressors where possible (Moberg 2000; Rushen 2000). As to the perioperative stress response, many recent studies on domestic and laboratory animals have attempted to identify behaviour correlated with stress perception caused by caging, hospitalisation and/or surgery (Hetts *et al* 1992; Beerda *et al* 1997, 1998, 1999; Hardie *et al* 1997; Mellor *et al* 2000; Roughan & Flecknell 2003; Väisänen 2005). Behavioural changes have also been proposed as good indicators of pain perception

during the post-surgery stress response (Morton & Griffiths 1985; Sanford *et al* 1986; Hardie *et al* 1997; Desborough 2000; Mathews 2000; Blackburn-Munro 2004).

The neuroendocrine stress response involves both the hypothalamic-pituitary-adrenal (HPA) and lactotropic axes (Matteri *et al* 2000). Cortisol and glucose have often been used as markers to assess HPA axis activation. An increase in serum or salivary cortisol and serum glucose has been described in dogs exposed to several stressors (Hetts *et al* 1992; Beerda *et al* 1996, 1997, 1998; Hansen *et al* 1997; Coppola *et al* 2006). The secretion of pituitary prolactin, used as a marker for lactotropic axis activation, is regulated by the suppressive effect of hypothalamic dopamine and the stimulatory effect of TRH, neurophysin, substance P and other factors (Matteri *et al* 2000). Dog prolactin is known to be involved in the emotional response and increases during positive interaction with humans (Odendaal & Meintjes 2003; Pageat 2005). Animals with generalised anxiety show hyperprolactinaemia, while dogs with phobias or mild anxiety do not (Pageat & Gaultier 2003; Pageat *et al* 2007).

The immune system response during stress is related mainly to HPA axis activation and an increase in circulating glucocorticoids. It can be assessed in a straightforward fashion by using total and differential white blood cell counts. Neutrophilia, monocytosis, lymphopaenia and eosinopaenia can thus be observed after exposure to different stressors (Blecha 2000; Moberg 2000; Schultze 2000; Stockham *et al* 2003).

Acute phase proteins may also be elevated in association with physical and psychological stress in humans, cattle, rats and mice (Marrocco-Trischitta *et al* 2004; Murata *et al* 2004; Cerón *et al* 2005). An increase in the hepatic synthesis of acute phase proteins in response to cytokine-mediated HPA axis activation has been proposed as the mechanism involved in the acute phase response to stress (Murata *et al* 2004).

Variations in postoperative stress biomarkers have been widely described in a variety of species. Postoperative increases in cortisol and glucose (Hansen *et al* 1997; Vaisanen *et al* 2002; Ambrisko *et al* 2005; Devitt *et al* 2005; Sibanda *et al* 2006), changes in immune function and variation of C-reactive protein (CRP) and haptoglobin (Hp) (Taylor 1998; Zahorec 2001; Murata *et al* 2004; Marrocco-Trischitta *et al* 2004; Cerón *et al* 2005) have been described in dogs and humans. Postoperative increases in prolactin have been reported in humans (Marrocco-Trischitta *et al* 2004). However, to our knowledge, there has yet to be a description of biochemical and/or haematological changes due to preoperative procedures.

The individual stress response is influenced by many factors such as genetics and early life experiences (Mason 2000; Rushen 2000). In light of this, the type of preoperative response shown by each subject, caused mainly by psychological stressors, should be considered when attempting to evaluate the corresponding post-surgery stress. Although separate studies have been devoted to pre- and post-surgery stress in dogs (Hardie *et al* 1997; Väisänen *et al* 2005), to our knowledge, no attempt has been made, to date, to evaluate pre and post-operative variations in the same group of animals.

The main aim of the present clinical study was to describe behavioural changes and variations in salivary cortisol, serum glucose, serum prolactin, total white blood cell count, white blood cell differential, neutrophil/lymphocyte ratio, serum haptoglobin and serum C-reactive protein occurring as a result of perioperative stress in dogs undergoing elective orchiectomy or ovariohysterectomy, while also attempting to assess differing sensitivity to psychological and physical perioperative stressors. Our ultimate goal is to identify useful tools for perioperative stress assessment in clinical conditions.

Materials and methods

Animals

A group of 16 adult dogs (7 females and 9 males, 2.6 [± 1.6] years old, both pure and mixed breed) underwent elective orchiectomy or ovariohysterectomy. All dogs had been kept in a public shelter for a minimum of 20 days, in a 6 m^2 pen (3 × 2 m; length × breadth) together with one or two other dogs, in accordance with local by-laws. A thorough physical examination, complete blood cell count, biochemistry panel and Leishmania antibodies serum concentration (ELISA test) was performed on all dogs and only healthy animals were included in our study. The reproductive status of each female was determined using a progesterone kit (Active® Progesterone EIA, DSL Inc, Webster, Texas, USA) and pseudopregnant, pregnant or lactating females were excluded. In addition, dogs presenting stereotyped behaviour or showing aggressiveness towards humans were not included. The animals enrolled in the study were fasted during the surgery day (for at least 18 h pre-surgery).

Sampling procedure

The timing of sample collection is summarised in Table 1. The study began every day between 1000 and 1100h. The first blood and saliva samples were collected from each dog in its usual environment (T0) the same day as surgery. Samples collected at this point were considered basal values. The dogs were then transferred, by walking, to the ICU, located in the same shelter holding facility, and placed in a 110 × 70 × 70 cm (length × breadth × height) cage, where behaviour was videotaped for 30 min (T1). The study period ended with a standardised, dynamic interaction test for pain evaluation (also videotaped). After this, further blood and saliva samples were obtained (T1). The dog was then transferred to the operating theatre. All surgeries were performed by the same graduate surgeon with the assistance of different veterinary undergraduates. Surgery was considered to be finalised with extubation of the animal.

A standardised anaesthetic and analgesic protocol was used for the surgical procedure. The dogs were pre-medicated with buprenorphine 0.01 mg kg^{-1} IM (Buprex®, Schering-Plough SA, Madrid, Spain), induced with thiobarbital 10 mg kg^{-1} IV (Tiobarbital®, B Braun Medical, Barcelona, Spain) and diazepam 0.5 mg kg^{-1} IV (Valium®, Roche Farma SA, Barcelona, Spain). The anaesthesia was maintained with isoflurane 1–2% (Isoflo®, Abbot Laboratories, Illinois, USA) vapourised in 100% oxygen 0.5–1 l min^{-1},

Table 1 Sample collection schedule.

Time	Definition	Samples	Parameters studied
T0 (basal)	Surgery day, dog in usual environment, pre-surgery	Saliva	Cortisol
		Blood	Glucose, PRL, CRP, Hp, WBC*
T1	Surgery day, dog in ICU cage, pre-surgery	Behaviour 30 min	Behavioural categories
		Interaction test	Behavioural categories, pain scoring
		Saliva	Cortisol
		Blood	Glucose, PRL, WBC*
T2	Surgery day, dog in ICU cage, post-surgery	Behaviour 30 min	Behavioural categories
		Interaction test	Behavioural categories, pain scoring
		Saliva	Cortisol
		Blood	Glucose, PRL, WBC*
T3	Surgery day, dog in usual environment, post-surgery	Saliva	Cortisol
T4	24 h post-surgery	Blood	CRP, Hp, WBC*
T5	48 h post-surgery	Blood	CRP, Hp, WBC*
T6	8 days post-surgery	Blood	CRP, Hp, WBC*

* Includes total white blood cell count and differential count.

delivered with a semi-disposable circle circuit (Burtons Medical Equipment Ltd, Kent, UK). The vapouriser setting was adjusted to maintain a surgical plane of anaesthesia as judged by eye position, jaw tone and lack of response to noxious stimuli. All dogs received intravenous crystalloid solution (Lactato de Ringer Braun, B Braun Medical, Barcelona, Spain) at 5–10 ml $kg^{-1}hour^{-1}$. Each dog was treated with an antibiotic, amoxicillin LA 11–22 mg kg^{-1} SC (Bivamox® LA, Boehringer Ingelheim España, Barcelona, Spain) and anti-inflammatory therapy, caprofen 4.4 mg kg^{-1} SC (Rimadyl, Pfizer España, Madrid, Spain) for four days after surgery. After extubation the dogs were transferred to the ICU cage, where every 30 min the degree of sedation was checked. When the animal was able to stand in the ICU cage, its behaviour was videotaped for 30 min, after which the dynamic interaction test for pain evaluation was performed and video-recorded (T2). At the end of this observation, blood and saliva samples (T2) were collected. The dog was then transferred to its usual pen where, after 30 min, a saliva sample (T3) was taken. No blood samples were collected at this point (T3) for ethical reasons, ie to offset the risk of post-surgical complications. Blood samples were also obtained at 24 h (T4), 48 h (T5) and 8 days (T6) after surgery. No more than two dogs were confined in the ICU at any given time, to minimise the variability of environmental influences on the dogs' behaviour.

Behavioural data collection and analysis

All behavioural samples were recorded with a digital video camera (Sony Handycam DCR-HF-40, Sony Corporation, Tokyo, Japan).

Behavioural data were divided into categories, evaluated for frequency of occurrence ('Events'; Table 2) and duration ('States'; Table 3), and collected on a checksheet. Categories evaluated as events were logged by continuous recording and their number of occurrences during the time of observation was considered, while those evaluated as states were logged by instantaneous sampling at 2 min intervals (15 instantaneous recording points in 30 min) (Martin & Bateson 1993). Behaviour scored in terms of frequency was recorded as occurring once every 5 s when the dogs displayed it in a continuous fashion (Beerda 1999).

Dynamic interaction test for pain evaluation

The interaction test was performed as follows: an operator knocked at the door of the ICU, opened it and entered. He reached for the cage, opened the door and greeted the dog gently. The operator then withdrew the dog from the cage, patting it gently from the chest to the flank and up to the ventral surgery site.

A single ethologist, familiar with the individual behaviour of the dogs enrolled in the study, carried out and recorded each of the tests, as well as later analysis and assessment. This strategy was designed to minimise the effect of individual behavioural variability of dogs.

The interactive behaviour of the dogs was analysed by using the Glasgow Pain Scale (GPS) (see Holton *et al* 2001 and Morton *et al* 2005). The behavioural categories shown by each dog were recorded. Both the frequency of occurrence of each behavioural category in pre- and post-surgery condi-

Table 2 Behavioural categories evaluated as events studied.

Category	Definition
Barking	Low frequency vocalisation, more or less soft or raucous.
Growling	A throaty rumbling vocalisation, usually low in pitch. It may be used in aggressive or defensive interaction.
Whining	Repeated, relatively brief 'exhalation vocalisations' of falling pitch.
Yelping	Loud, high pitched vocalisations.
Mouth opening	The dog opens and closes the mouth with rapid movements. The tongue is not visible. Could correspond to yawning.
Autogrooming	Behaviours directed at the subject's own body, eg scratching, licking or biting to groom. Includes taking care of wounds.
Tail chasing	The dog chases its own tail with continuous round movements.
Circling	Walking in a circle.
Pacing	Continuous movements from one cage extremity to the other.
Digging	Scratching the floor with the forepaws in a manner similar to that seen when dogs are digging holes.
Barrier manipulation	Chewing, touching with legs or licking the enclosure.
Jumping	Springing in the air, either spontaneously or in order to make contact with an object or a person.
Lip licking	The dog licks the lips exhibiting part of the tongue.
Nosing	The nose is moved along objects and/or clear sniffing movements are exhibited.
Paw lifting	A forepaw is lifted at an angle of approximately 45°.
Tail wagging	Repetitive wagging movements of the tail.

Adapted from Morton and Griffiths (1985), Beerda *et al* (1997, 1998), Hetts *et al* (1992), Hardie *et al* (1997) and Goodmann *et al* (2002).

Table 3 Behavioural categories evaluated as states studied.

Category	Definition
Panting	An increased frequency of inhalation and exhalation often in combination with the opening of the mouth.
Visual scanning	Visual exploration of the environment through the cage door.
Awake/alert	Dog with opened eyes.
Rest/sleep	Dog inactive with closed eyes.
Trembling	Body shaking with little, high frequency movements.
Walking	Displacement from one point to another with no clear exploratory movements.
Exploring	The dog moves slowly, sniffing and investigating the environment.
Lie on side	Positioned fully on side, one side of the dog in complete contact with the ground.
Lie half side/ventral	Positioned on side with body, but not head, in complete contact with the ground, or with ventrum and legs in contact with ground.
Lie dorsal	Positioned flat with back in contact with the ground.
Sitting	The pads of the front paws are on the ground with the front legs straight and the rump squarely on the ground.
Standing	Positioned with just four paws in contact with the ground, or two with the ground and two with the wall.
Against wall	The dog is against the walls of the enclosure, with the eyes opened or closed.
Against door	The dog is against the door of the enclosure, with the eyes opened or closed.

Adapted from Morton and Griffiths (1985), Beerda *et al* (1997, 1998), Hetts *et al* (1992), Hardie *et al* (1997) and Goodmann *et al* (2002).

tions and the corresponding total scores were compared, to obtain a qualitative and quantitative analysis.

Collection of blood and saliva samples

Blood samples were taken from the jugular vein using standard procedures. One ml of the sample was stored in an EDTA tube (Tapval Aquisel, Barcelona, Spain) and 3 ml transferred to tubes containing a coagulation activator (Tapval Aquisel, Barcelona, Spain). The samples were refrigerated during transport to the laboratory. After clot formation, the serum obtained was transferred to Eppendorf tubes and stored at –20°C.

Saliva samples were collected by the Salivette system (Sarstedt, Numbrecht, Germany) after salivary flow stimulation with 3% citric acid (Mandel 1990; Beerda *et al* 1997). Saliva collection always preceded blood sample collection, with the animal never being handled for more than 2 min, to avoid direct influence of handling on stress measures (Kobelt *et al* 2003). The tubes were kept refrigerated during transport to the laboratory and Salivette samples were then centrifuged at 3,500 rpm for 15 min and stored at –20°C.

Laboratory analysis

Saliva cortisol concentration was determined with a commercial human saliva ELISA test (Cortisol Saliva, BLK Diagnostics, Barcelona, Spain) which had been adapted in our laboratory to measure cortisol concentration in canine saliva.

Serum samples were analysed for glucose detection with an enzymatic UV test (hexokinase method) following manufacturer's instructions (Glucose Olympus, Hamburg, Germany). Serum prolactin concentration was measured with a commercial ELISA kit (Milenia® Canine Prolactin, Milenia Biotec, Bad Nauheim, Germany) following manufacturer's instructions.

EDTA blood samples were analysed within 6 h of collection with a laser-flow cytometer (ADVIA 120 Haematology System, Bayer, Fernwald, Germany), which provided total white blood cell (TWBC) and differential count (neutrophils, monocytes, lymphocytes and eosinophils).

Serum samples were analysed to measure haptoglobin and C-reactive protein levels. Hp concentration was determined with an automated biochemical assay (Tridelta phase range serum haptoglobin, Tridelta Development, Wicklow, Ireland) following manufacturer's instructions. CRP concentration was measured with a canine CRP-specific solid phase sandwich immunoassay (Tridelta phase range canine CRP kit, Tridelta Development, Wicklow, Ireland).

Immune and acute phase response markers were evaluated on a long-term basis (24, 48 h and 8 days post-surgery), according to previously published data (Ceron *et al* 2005) and because of the presumed major influence of the inflammatory response caused by tissue damage on marker variation. As glucose, cortisol and prolactin response to stress is known to be rapid (Matteri *et al* 2000), study of the markers was limited to the day of surgery to avoid influence of uncontrolled psychological stressors on response at 24, 48 h and 8 days post surgery.

Table 4a Pre- and post-surgery behavioural evaluation of events in dogs in confined environment.

Events	Pre-surgery Mean (± SE)*	Post-surgery Mean (± SE)*	*P*-value
Barking	11.09 (± 10.89)	0.09 (± 0.09)	0.180
Whining	22.91 (± 14.58)	46.27 (± 23.27)	0.953
Open mouth	3.82 (± 1.61)	0.00 (± 0.00)	0.017
Autogrooming	1.27 (± 0.91)	1.18 (± 0.81)	0.914
Circling	8.64 (± 4.75)	1.00 (± 0.70)	0.058
Pacing	0.00 (± 0.00)	0.18 (± 0.18)	0.317
Digging	0.64 (± 0.47)	0.00 (± 0.00)	0.180
Barrier manipulation	2.18 (± 1.30)	0.18 (± 0.18)	0.131
Lip licking	29.73 (± 11.81)	0.45 (± 0.21)	0.007
Nosing	24.09 (± 3.71)	2.82 (± 2.53)	0.004
Paw lifting	3.36 (± 2.20)	0.82 (± 0.82)	0.109
Tail wagging	10.82 (± 10.82)	0.45 (± 0.37)	1.000
Jumping	0.91 (± 0.64)	0.00 (± 0.00)	0.109

Table 4b Pre- and post-surgery behavioural evaluation of states in dogs in confined environment.

States	Pre-surgery Mean (± SE)**	Post-surgery Mean (± SE)**	*P*-value
Panting	3.36 (± 1.56)	1.18 (± 1.00)	0.249
Visual scanning	12.18 (± 0.84)	2.64 (± 0.79)	0.003
Awake/alert	13.00 (± 0.94)	3.82 (± 1.23)	0.006
Rest/sleep	2.00 (± 0.94)	11.18 (± 1.23)	0.006
Walking	0.36 (± 0.20)	0.00 (± 0.00)	0.102
Exploring	0.45 (± 0.20)	0.09 (± 0.09)	0.157
Lie on side	0.36 (± 0.36)	4.36 (± 1.80)	0.074
Lie half side/ventral	7.00 (± 1.89)	9.09 (± 1.84)	0.285
Sitting	5.18 (± 1.60)	0.45 (± 0.21)	0.009
Standing	1.64 (± 0.99)	1.0 (± 0.90)	0.066
Against wall	0.91 (± 0.25)	2.45 (± 1.42)	0.609
Against door	14.09 (± 0.25)	12.55 (± 1.42)	0.609

* Mean value of the total number of occurrences of the behavioural category throughout the 30 min of observation.

** Mean value of instantaneous sampling points in which the behaviour was detected during the 30 min of observation (15 points with intervals of 2 min), considered as an indicator of the duration (Martin & Bateson 1993).

Statistical analysis of behavioural data

Intra-observer reliability was determined by analysis of the correlation between two different observations of the same video-recording sample. Four independent 10-minute samples of different subjects were used to calculate the

Table 5 Pre- and post-surgery values on interactive behavioural categories.

GPS frequencies of behaviours	Pre-surgery (%)	Post-surgery (%)	*P*-value
Crying	0	8.3	0.307
Not vocal	100	91.6	0.307
Depressed	0	8.3	0.307
Nervous	33.3	8.3	0.132
Quiet	8.3	25	0.273
Content	8.3	58.3	0.009
Bouncy	50	0	0.005
Stiff	33.3	16.6	0.346
Reluctant	0	41.6	0.012
Cry	0	8.3	0.307
Flinch	0	8.3	0.307
Growl	8.3	8.3	1.00

Spearman Rank coefficient for the following behavioural categories: 'nosing', 'lip licking', 'mouth opening', 'visual scanning' and 'awake/alert' (Martin & Bateson 1993).

The influence of the type of surgery on behaviour was studied by comparing pre and postoperative values of dogs undergoing orchiectomy with those subjected to ovariohysterectomy — for all behavioural categories and GPS. Comparisons were made with a Mann-Whitney *U* test for all behavioural categories and GPS score, while the influence of surgery on the frequency of GPS categories was examined by using an χ^2 test. Differences were considered statistically significant when $P \leq 0.01$.

Pre- and post-surgery differences were studied by using a Wilcoxon test for the occurrence of all behavioural categories analysed and GPS scores, and by using an χ^2 test for the frequency of each GPS category. SPSS® 13.0 software (SPSS Inc, Chicago, USA) was used for the calculations. Differences were considered statistically significant when $P \leq 0.01$.

Statistical analysis of haematological and biochemical data

For the statistical analysis, the normal distribution of data was determined with a Shapiro-Wilk test. Data were considered to have a normal distribution when the test showed $P > 0.05$. To study the influence of tissue damage on stress response, basal (T0) and postoperative sampling times (T2, T3, T4, T5 and T6) of dogs undergoing orchiectomy and ovariohysterectomy were compared for all the markers analysed (eg T2 ovariohysterectomy compared with T2 orchiectomy), by using the *t*-test for normally-distributed data and the Mann-Whitney *U* test for non-parametric data. The values of parameters studied at different times and differences with basal values were analysed by a paired *t*-test for data showing a normal distribution and by a Wilcoxon test for those that did not. Differences were considered significant when $P < 0.05$. SPSS® 13.0 software (SPSS Inc, Chicago, USA) was used for the calculations.

Results

Behavioural results

Twelve (seven females and five males) of the 16 dogs enrolled in the study were suitable for behavioural analysis. Four dogs were excluded (three due to prolonged human presence and/or interaction that disturbed the study conditions and one escaped from its confinement cage).

Intra-observer reliability was high; the correlation, expressed by using a Spearman Rank coefficient, was 0.95 for 'nosing', 1.00 for 'lip licking', 0.95 for 'mouth opening', 0.94 for 'visual scanning' and 0.95 for 'awake/alert' ($P \leq 0.05$ for all categories).

No differences were found between dogs undergoing either ovariohysterectomy or orchiectomy for both pre and post-operative occurrence of behavioural categories and GPS score (data not shown). Male and female dogs were therefore grouped together for further analysis. The medium length of surgery, considered as the time during which the animal remained intubated, was 58.73 (± 5.04) min.

Behavioural results are shown in Table 4. The behavioural evaluation of dogs in the ICU cage showed statistically significant decreases between pre- and post-surgery frequency of occurrence for 'mouth opening', 'lip licking' and 'nosing', and between pre- and post-surgery duration for 'visual scanning', 'awake/alert' (with a corresponding increase in 'rest/sleep') and 'sitting' ($P < 0.01$ for all categories).

A post-surgery interaction test was performed at a mean time of 92.5 (± 50.29) min from the end of surgery. No statistically significant differences were observed at post-surgery time for the GPS score compared to pre-surgery (1.49 [±0.42] vs 1.74 [± 0.43] min). Regarding GPS behavioural categories (Table 5), a statistically significant decrease in frequency was seen between pre and postoperative periods for the category 'happy and bouncy' (tail wagging, jumping in kennel, often vocalising with happy excited noise), while 'happy and content' (interested in surroundings, positive interaction with observer, responsive and alert) and 'slow or reluctant to rise or sit' (slow to get up or sit down but not stilted in movement) showed a significant increase in frequency ($P \leq 0.01$ for all categories). See Holton *et al* (2001) for definition of GPS behavioural categories.

Haematological and biochemical results

All the 16 dogs enrolled in the study were suitable for haematological and biochemical analyses. Total white blood cells, neutrophils, monocytes, lymphocytes, eosinophils and glucose showed a normal distribution, therefore a *t*-test was used for statistical analysis. The distribution of all other parameters studied was not normal, so a statistical analysis was carried with Wilcoxon and Mann-Whitney *U* tests.

Dogs undergoing ovariohysterectomy showed a lower lymphocyte value (2.46 [± 0.69] × 10^3 cells μl^{-1}) than those

Figure 1

Variations of salivary cortisol at times T0, T1, T2 and T3 (mean value ± SE). Open marker indicates differences between marked value and T0 with $P < 0.05$; solid marker indicates differences between marked value and T0 with $P \leq 0.01$.

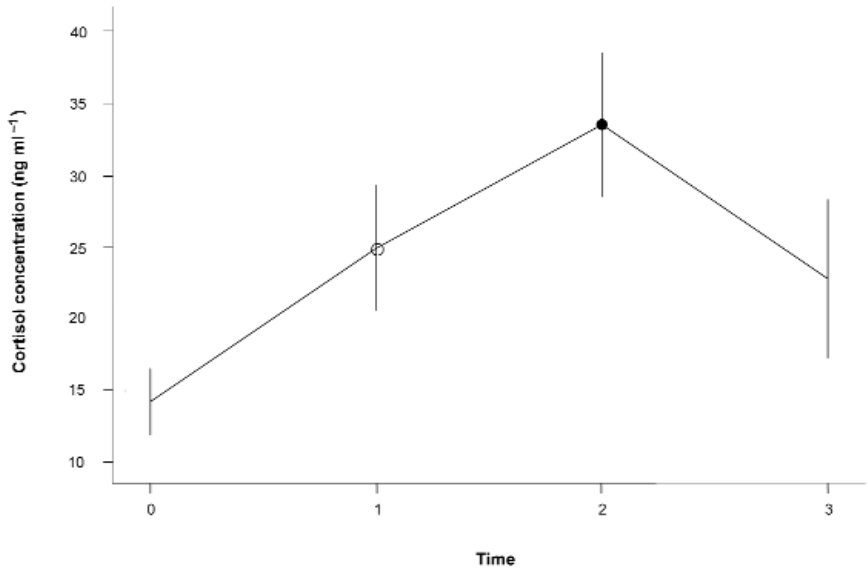
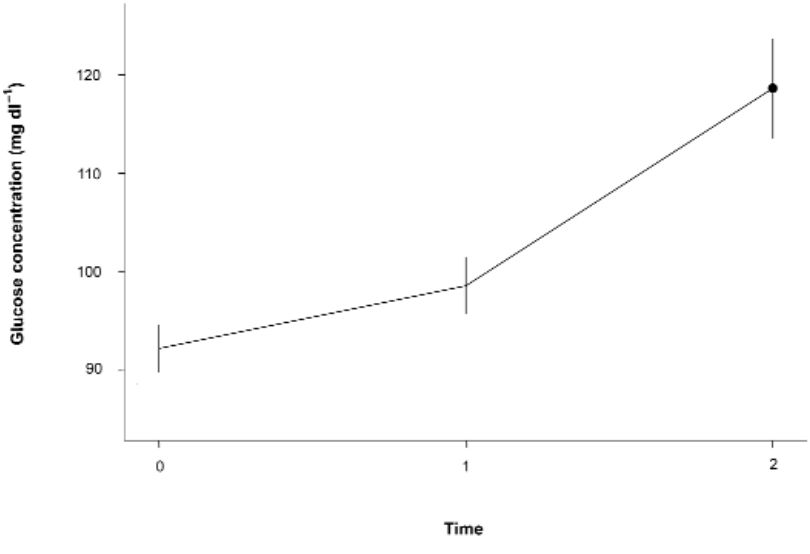


Figure 2

Variations of serum glucose at times T0, T1 and T2 (mean value ± SE). Open marker indicates differences between marked value and T0 with $P < 0.05$; solid marker indicates differences between marked value and T0 with $P \leq 0.01$.

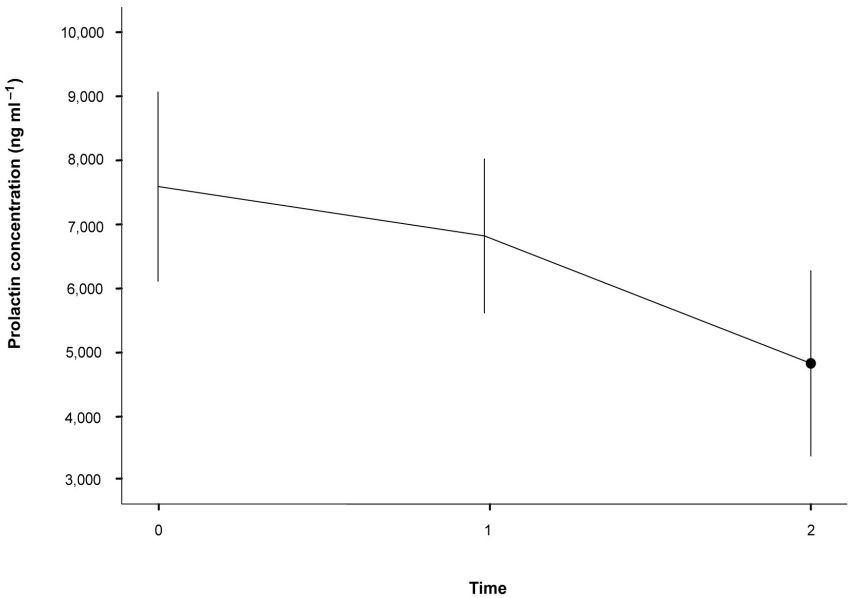


undergoing orchiectomy (3.76 [± 0.76] × 10^3 cells μl^{-1}; $P = 0.01$) 24 h after surgery (T4) and higher cortisol values (41.04 [± 24.09] ng ml^{-1} for ovariohysterectomy vs 14.80 [± 4.80] ng ml^{-1} for orchiectomy; $P < 0.05$) after return to their usual environment (T3). With the exception of these findings, no other differences in basal and postoperative mean values of dogs undergoing orchiectomy versus ovariohysterectomy were observed in any other parameter studied, and for this reason males and females were studied together.

Salivary cortisol concentrations showed significant differences between basal value (T0) and increased pre- ($P < 0.05$) and post-surgery ($P = 0.01$) values on the day of surgery (T1 and T2) (Figure 1). A statistically significant difference was seen in serum glucose variation between the basal value (T0) and the increased post-surgery value ($P < 0.01$) on the day of surgery (T2), even though a slight upward tendency began to become apparent before surgery (T1) (Figure 2). For serum prolactin, significant differences were detected by comparing the basal value (T0) with the decreased post-surgery value ($P < 0.01$) on day of surgery (T2), although a downward tendency began to be apparent at T1 (Figure 3).

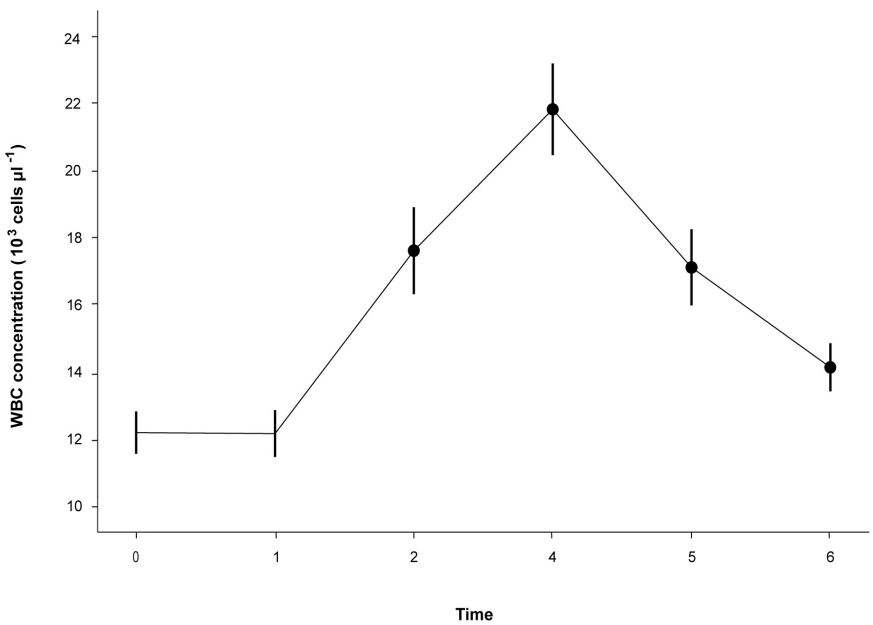
Total white blood cells and neutrophils showed a significant increase when basal values (T0) were compared with values on the day of surgery (T2), 24 h (T4), 48 h (T5) and 8 days

Figure 3



Variations of serum prolactin at times T0, T1 and T2 (mean value ± SE). Open marker indicates differences between marked value and T0 with $P < 0.05$; solid marker indicates differences between marked value and T0 with $P \leq 0.01$.

Figure 4



Variations of total white blood cells at times T0, T1, T2, T4, T5 and T6 (mean value ± SE). Open marker indicates differences between marked value and T0 with $P < 0.05$; solid marker indicates differences between marked value and T0 with $P \leq 0.01$.

(T6) post-surgery ($P < 0.01$ for all these comparisons) (Figures 4 and 5). Monocytes showed statistically significant differences between basal value (T0) and increased post-surgery value ($P < 0.01$) on the day of surgery (T2), as well as between basal value and 24 ($P < 0.01$) and 48 ($P < 0.01$) h post-surgery values (T4 and T5) (Figure 6). Lymphocytes and eosinophils showed statistically significant differences between basal value (T0) and decreased post-surgery value ($P < 0.01$) on the day of surgery (T2) (Figures 7 and 8). The neutrophil/lymphocyte ratio peaked at an early post-surgery time (T2) showing a statistically significant difference ($P = 0.001$) that was maintained for 8 days after surgery ($P < 0.05$ for all times) (Figure 9).

Serum CRP showed a significant increase when the basal value (T0) was compared with values at 24 h (T4), 48 h (T5) and 8 days (T6) after surgery ($P < 0.01$ for all comparisons) (Figure 10). Serum Hp showed statistically significant differences between the basal value (T0) and the increased values (T4 and T5) at 24 ($P < 0.01$) and 48 ($P < 0.01$) h (Figure 11). Peak values for CRP and Hp were reached at 24 and 48 h post-surgery, respectively.

Figure 5

Variations of neutrophils at times T0, T1, T2, T4, T5 and T6 (mean value ± SE). Open marker indicates differences between marked value and T0 with $P < 0.05$; solid marker indicates differences between marked value and T0 with $P \leq 0.01$.

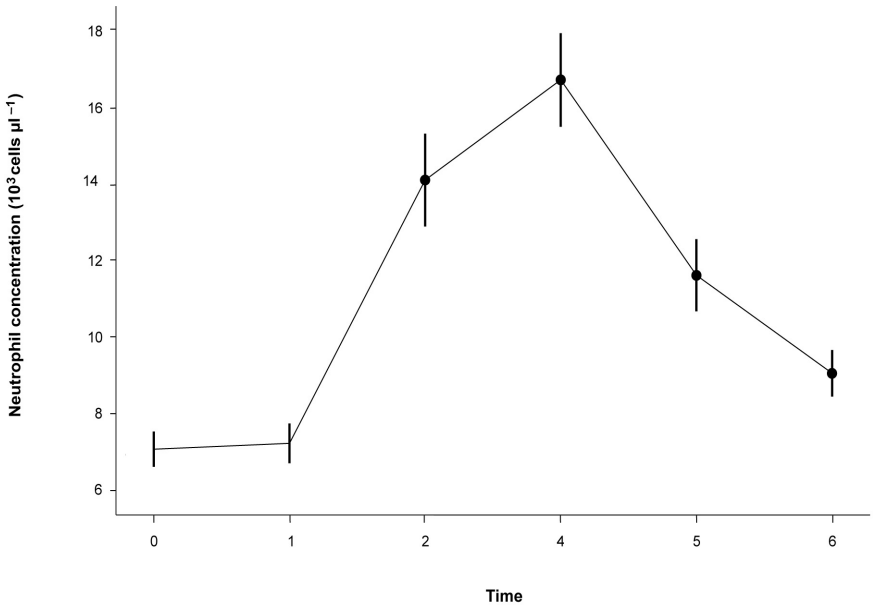
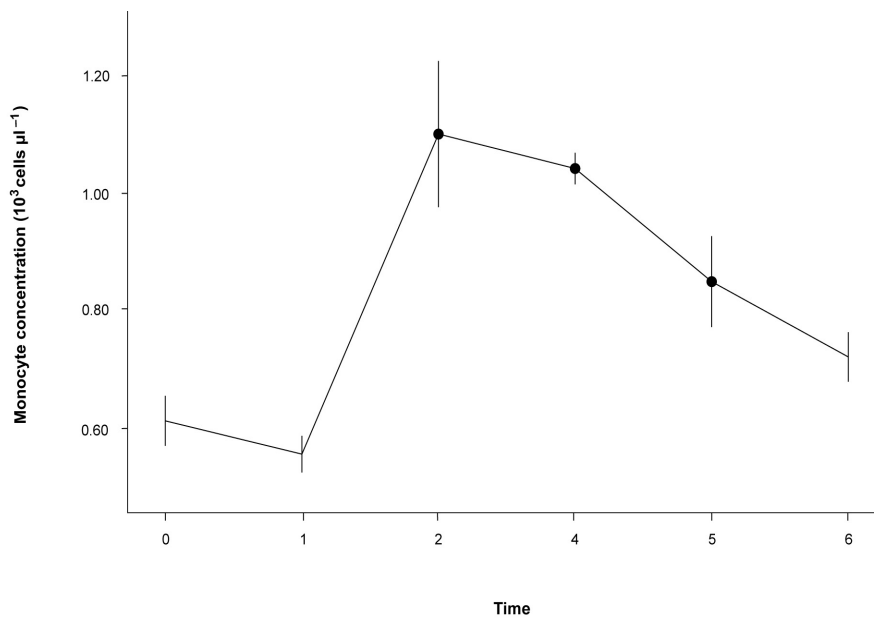


Figure 6

Variations of monocytes at times T0, T1, T2, T4, T5 and T6 (mean value ± SE). Open marker indicates differences between marked value and T0 with $P < 0.05$; solid marker indicates differences between marked value and T0 with $P \leq 0.01$.



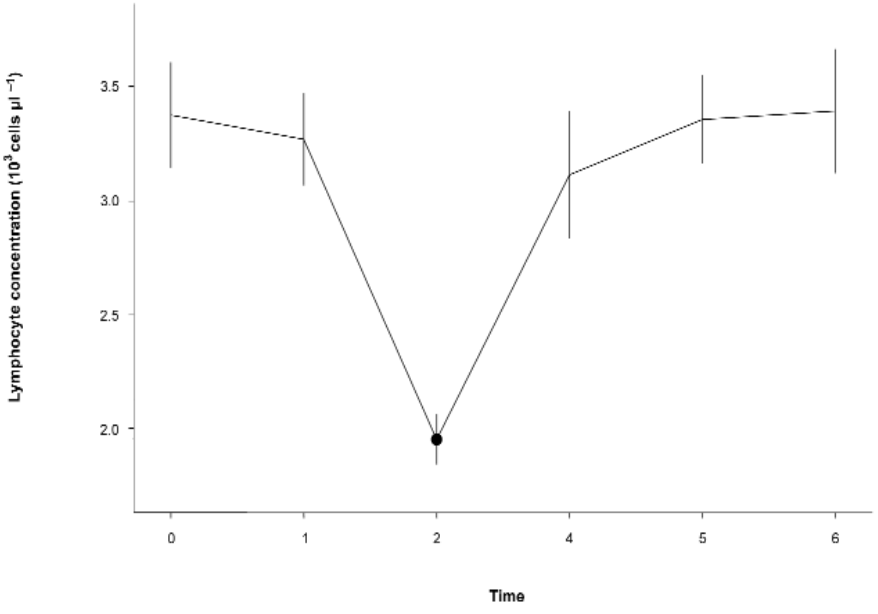
Discussion

Changes observed in behavioural, neuroendocrine, immune and acute phase responses appear to be useful tools for the perioperative stress assessment of dogs undergoing elective surgery. With regard to the behavioural response, explorative ('nosing' and 'visual scanning') and communicative ('mouth opening' and 'lip licking') behaviours were the categories most affected by postoperative stress, as they showed a high frequency of occurrence and duration in pre-surgery (T1) compared with post-surgery (T2). According to Hardie *et al* (1997), postoperative pain can create a decrease in communicative and explorative behaviours, but the use of opioids also played a role in altering communicative abilities.

We also observed an increase in the duration of 'rest/sleep' and a decrease in the duration of the 'sitting' position after surgery (T2) when compared to the preoperative period (T1). These changes may be related to the residual sedative effect of analgesia and anaesthesia and to the level of pain being perceived (Hardie *et al* 1997; Roughan & Flecknell 2002).

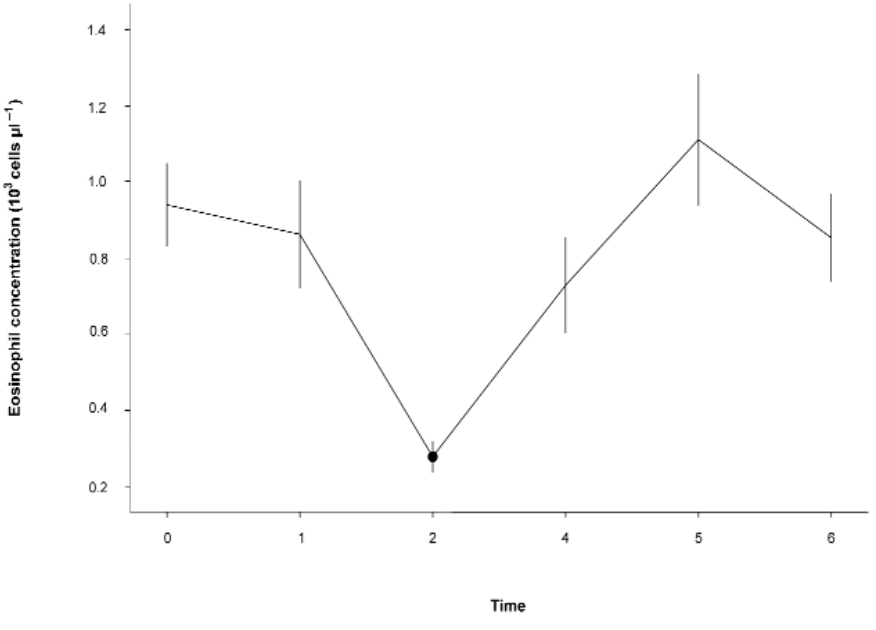
By analysing the postoperative (T2) frequency of interactive behavioural categories (GPS), we observed a reduced tendency for the animal to move and interact actively with the

Figure 7



Variations of lymphocyte cells at times T0, T1, T2, T4, T5 and T6 (mean value ± SE). Open marker indicates differences between marked value and T0 with $P < 0.05$; solid marker indicates differences between marked value and T0 with $P \leq 0.01$.

Figure 8



Variations of eosinophil cells at times T0, T1, T2, T4 , T5 and T6 (mean value ± SE). Open marker indicates differences between marked value and T0 with $P < 0.05$; solid marker indicates differences between marked value and T0 with $P \leq 0.01$.

handler, even though a positive attitude towards the handler persisted. The categories 'happy and content', implying a positive attitude but without active handler interaction, and 'slow or reluctant to rise or sit' increased, while 'happy and bouncy', which implies active interaction such as jumping, decreased. Postoperative pain and opioid administration were described as causes for similar changes, whereas thiobarbiturates and diazepam use was not seen to alter the dogs' capacity to move and actively interact, according to previous findings (Hart 1985; Hardie *et al* 1997; Pageat 1998; Thompson 1998; Crowell-Davis & Murray 2006).

The types of postoperative behavioural changes observed in this study have been described as pain-related in previous literature (Hansen *et al* 1997; Hardie *et al* 1997). Our haematological and biochemical results are also consistent with the effects of significant post-surgical pain. Although the GPS score suggests that post-surgery pain was controlled by analgesia, the pain scale is based on the subjective assessment of pain-related behaviour by veterinary surgeons (Morton *et al* 2005) and not on direct observational studies of dog behaviour. The absence of significant differences between pre- and post-surgery for the

Figure 9

Variations in the neutrophil/lymphocyte ratio at times T0, T1, T2, T4, T5 and T6 (mean value ± SE).

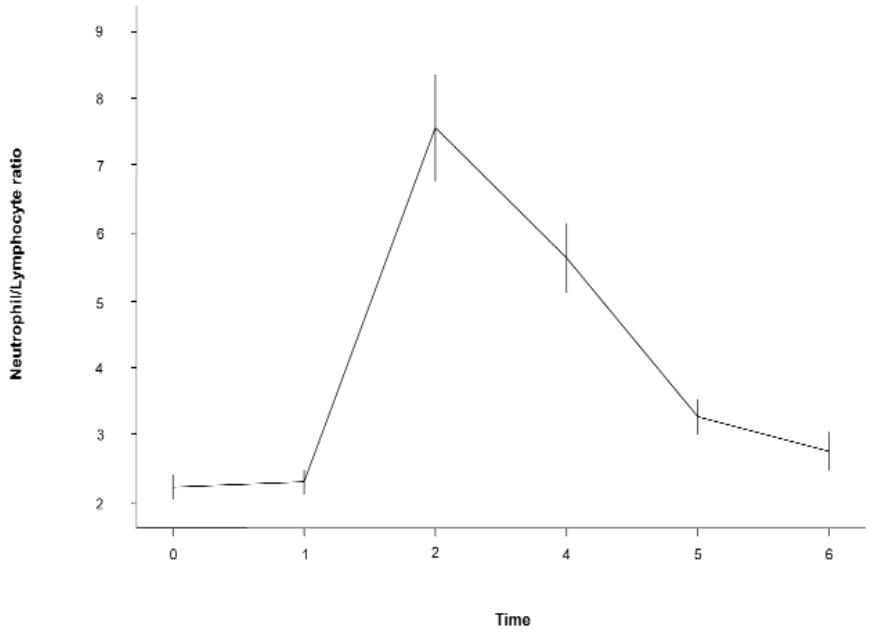
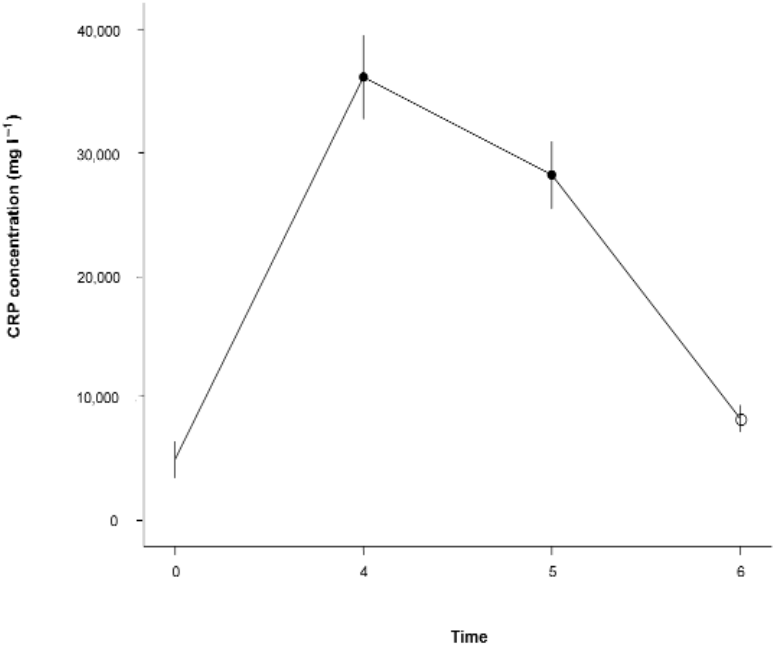


Figure 10

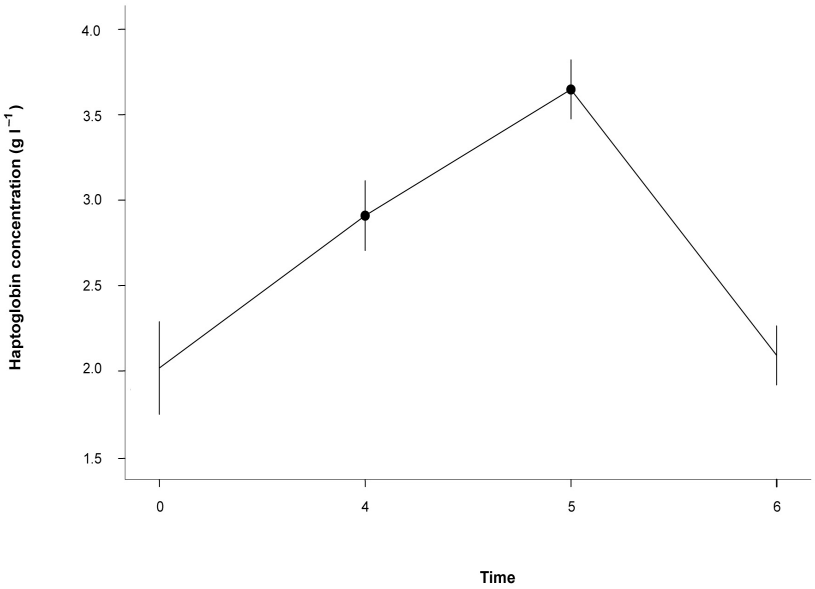
Variations in serum CRP at times T0, T4, T5 and T6 (mean value ± SE). Open marker indicates differences between marked value and T0 with $P < 0.05$; solid marker indicates differences between marked value and T0 with $P \leq 0.01$.



GPS scores in this study could be related to high frequency pre- and post-surgery behaviours (Table 5) such as 'nervous or anxious' or 'fearful and stiff', which supposedly have a high pain-related weight (1.13 and 1.17, respectively), but were not highly specific for low pain evaluation. Indeed, they were also displayed in cases of psychological stressors acting alone (T1). Moreover, administration of analgesia does not always result in the complete elimination of pain, and low pain perception may be undiagnosed using indirect pain assessment tools (Vetter & Heiner 1996).

Focusing our attention on the neuroendocrine response, cortisol was the most sensitive marker for psychological stress acting without any physical damage and/or pain, since it was the only one to show significant changes prior to surgery (T1). This rise in cortisol together with the simultaneous increase in glucose suggests an activation of the HPA

Figure 11



Variations in serum haptoglobin at times T0, T4 , T5 and T6 (mean value ± SE). Open marker indicates differences between marked value and T0 with $P < 0.05$; solid marker indicates differences between marked value and T0 with $P \leq 0.01$.

axis (Matteri *et al* 2000). This indicates that confinement in an unfamiliar and uncontrolled environment, together with handling by unknown people, represents a substantial source of stress for the animal that can be detected as early as 30 min after caging. The slight reduction in prolactin during preoperative time (T1), is related presumably to activation of the dopaminergic system (Matteri *et al* 2000) and can also be considered a sign of neuroendocrine sensitivity to preoperative stress. Since previous results have shown that the magnitude of the perioperative neuroendocrine response is directly proportional to postoperative analgesic requirements and morbidity in humans following major surgery (Anand *et al* 1992; Giesecke *et al* 1998), cortisol, glucose and prolactin could be useful biochemical markers in assessing psychological stress so as to guarantee animal welfare and rapid recovery.

After surgery (T2), both the HPA and lactotropic axes were substantially affected with significant increases in cortisol and glucose coupled with a significant decrease in prolactin. Post-surgery cortisol and glucose increases in dogs have been reported previously (Hansen *et al* 1997; Fox *et al* 1998; Matteri *et al* 2000; Väisänen *et al* 2002; Devitt *et al* 2005). The high post-surgery peak reached by cortisol (T2), followed by a rapid return to basal values (T3), confirms its sensitivity to psychological stress, but also shows that major intra and postoperative stressors, such as pain, are acting to heighten the stress response. The significant decrease in prolactin (T3) provides evidence of the major involvement of the lactotropic axis in postoperative stress and the presumed related activation of the dopaminergic axis. A postoperative increase in prolactin has been reported in humans (Marrocco-Trischitta *et al* 2004), but, to our knowledge, this is the first description of prolactin response as a perioperative stress marker in dogs. The divergent postoperative prolactin response between dogs and humans could be related to differences in the activation of prolactin feedback regulatory systems. When we consider that the increase in dopamine during an emotional challenge has an inhibitory effect on prolactin secretion (Matteri *et al* 2000), and that previous exposure to chronic stress increases dopamine release in response to acute stress (Cuadra *et al* 1999), the chronic stress experienced by the sheltered dogs enrolled in this study — kenneled in small structures with limited social contact (Hubrecht *et al* 1992; Beerda *et al* 1996) — could explain the prolactin decrease observed.

Regarding the immune response, no significant changes were detected before surgery (T1). Exposure to psychological stressors does not always result in alterations to immune functions and, when the immune response is activated, its magnitude tends to be mild in dogs (Dantzer & Mormede 1995; Stockham *et al* 2003). Furthermore, the hypothesis of an undetected long-term response of white blood cells to psychological stress should be considered, in a similar way to that which had been observed in the acute phase response. On the other hand, postoperative variations proved to be remarkable. Increases in total white blood cells, neutrophils and monocytes were evident soon post-surgery (T2) and persisted for several days, while falls in lymphocytes and eosinophils were limited strictly to the immediate postoperative time (T2), returning rapidly to basal values (T4). As previously reported by Schultze (2000), in a pure, psychological stress response without tissue lesions, neutrophilia usually resolves prior to lymphopaenia. In our study, postoperative psychological stress and tissue damage were acting simultaneously and the long-term neutrophil and monocyte responses detected,

compared with the extremely short lymphocyte and eosinophil responses, suggests that these cell types have different levels of sensitivity towards postoperative stressors. While the lymphocyte and eosinophil responses appear related to glucocorticoid increase rather than tissue damage (Schultze 2000; Stockham *et al* 2003), the persistent neutrophilia and monocytosis could be more indicative of tissue inflammatory stimulus. In accordance with these findings, a redistribution of lymphocytes from circulating blood to marrow or lymph nodes in response to glucocorticoids, has been described (Stockham *et al* 2003), as well as lymphocyte apoptosis after perioperative stress (Delogu *et al* 2001; Alleva *et al* 2003). The effect of pain on leukocyte response has also been reported (Griffis *et al* 2006). Neutrophilia and lymphopaenia following anaesthesia and surgery have been described in horses (Stover *et al* 1998). The neutrophil/lymphocyte ratio has been proposed as a useful and inexpensive indicator of sensitivity to postoperative stress in humans (Zakowsky 1992; Tayama *et al* 1999). The results obtained in this study suggest that similar conclusions may also be applicable to dogs; as the ratio increases, so does the stress experienced by the animal. In our study, the neutrophil/lymphocyte ratio is approximately four times the basal value (T0) at the moment at which the animal supposedly experiences the greatest challenge due to inflammatory and psychological postoperative stress (T2; see Figure 9).

The CRP and Hp tendencies seen in our study are in agreement with results reported in previous work (Yamamoto *et al* 1993; Conner & Eckersall 1998; Cerón *et al* 2005). The acute phase response has been shown to be sensitive to physical and psychological stress in humans and cattle (Murata *et al* 2004). An Hp increase following glucocorticoid treatment has been observed in dogs, while CRP appeared to be unaffected (Cerón *et al* 2005). Unfortunately, our study design could not usefully differentiate between the psychological and inflammatory perioperative response of acute phase proteins. The long-term nature of this response (Conner *et al* 1988; Yamamoto *et al* 1993; Cerón *et al* 2005) made it impossible to detect changes due to preoperative stress (T1) before the postoperative elements were added (T2). Nevertheless, the acute phase proteins showed a similar response to neutrophils and monocytes, suggesting that they could be a more reliable marker of inflammatory postoperative stress than a psychological one. In this case, they could be regarded as a valid alternative to WBC count, while offering the added advantage of being more stable for sample storage and analysis (Cerón *et al* 2005).

The difference in the amount of tissue damage between dogs undergoing orchiectomy versus ovariohysterectomy did not have any substantial influence on the inflammatory response observed in this study since no significant post-surgery differences were observed in the WBC, neutrophils, monocytes, CRP and Hp of the two groups of animals. Nevertheless, the dogs undergoing ovariohysterectomy tended to maintain more increased cortisol and decreased lymphocyte values after surgery than those undergoing orchiectomy, showing that ovariohysterectomy is a more stressful and painful experience than orchiectomy, but is not associated with a significant difference in the inflammatory response. Intra-operative pain experience could be related to a different activation of cortisol and lymphocyte responses, even if post operative pain perception appeared to be the same for both groups.

Although our results need to be considered with due caution, in view of the limited number of animals involved, the simultaneous study of several behavioural, haematological and biochemical stress markers provides an extensive description of perioperative stress in dogs undergoing elective surgery. Moreover, our behavioural analysis, carried out by using continuous observation and high intra-observer correlation, contributed in part to increased study reliability.

Conclusions and animal welfare implications

Behavioural changes together with haematological and biochemical markers of the neuroendocrine, immune and acute phase stress responses were shown to be sensitive tools for assessing perioperative stress in dogs undergoing elective orchiectomy and ovariohysterectomy. Changes in explorative and communicative behaviours, as well as alterations in waking/sleeping pattern, activity and active interaction with a handler appeared to be the most relevant postoperative behavioural variations; even though behavioural changes did not allow the effect of psychological stress to be differentiated from changes due to postoperative pain. Further studies comparing behaviours in typical environments with the ICU cage environment are required to quantify the effect of psychological stressors.

Cortisol was the most useful tool for assessing psychological stress, as it was the only marker to show a significant preoperative change. Although changes observed during preoperative periods were not always substantial, all biomarkers studied showed significant variations after surgery. Cortisol response suggests an important (but time-limited) activation of the HPA axis, while prolactin decline indicates activation of the dopaminergic-lactotropic system. Neutrophils, monocytes and the acute phase response proved to be good markers for inflammation, while lymphocytes and eosinophils showed greater sensitivity to early postoperative psychological stress. The neutrophil/lymphocyte ratio represents a useful and inexpensive tool for postoperative stress assessment. Changes observed showed that pain, analgesia- and anaesthesia-induced dysphoria, tissue damage, together with persistent psychological stressors, represented a major challenge for the animals' homeostatic balance.

Both preoperative psychological and postoperative multifactorial stresses appear to be involved in the perioperative response, confirming the importance of adequately considering all these factors from the perspective of animal welfare and recovery. We believe that the role of animal psychological stress in daily veterinary practice is not always properly evaluated, probably because it is characterised by subtle signs, particularly when the animal is adopting a passive coping strategy. Choosing the best caging conditions, taking care in handling, using adequate anaesthetic and analgesic drugs to alleviate pain and to reduce dysphoria as much as possible, are some of the

critical control points that can be easily managed to improve welfare in dogs undergoing elective surgery.

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References

Alleva R, Tomasetti M, Solenghi MD, Stagni F, Gamberini F, Bassi A, Fornasari PM, Fanelli G and Borghi B 2003 Lymphocyte DNA damage precedes DNA repair or cell death after orthopaedic surgery under general anaesthesia. *Mutagenesis 18*: 423-428

Ambrisko TD, Hikasa Y and Sato K 2005 Influence of medetomidine on stress-related neurohormonal and metabolic effects caused by butorphanol, fentanyl, and ketamine administration in dogs. *The American Journal of Veterinary Research 66*: 406-412

Anand KJS, Phil D and Hickey PR 1992 Halothane-morphine compared with high-dose sufentanil for anaesthesia and postoperative analgesia in neonatal cardiac surgery. *The New England Journal of Medicine 326*: 1-9

Beerda B, Schilder MBH, Janssen NSCRM and Mol JA 1996 The use of saliva cortisol and catecholamine measurements for a noninvasive assessment of stress response in dogs. *Hormones and Behaviour 30*: 272-279

Beerda B, Schilder MB, van Hooff JARAM and de Vries HW 1997 Manifestation of chronic and acute stress in dogs. *Applied Animal Behaviour Science 52*: 307-319

Beerda B, Schilder MB, van Hooff JARAM, de Vries HW and Mol JA 1998 Behavioural, saliva cortisol and heart rate responses to different types of stimuli in dogs. *Applied Animal Behaviour Science 58*: 365-381

Beerda B, Schilder MB, van Hooff JARAM, de Vries HW and Mol JA 1999 Chronic stress in dogs subjected to social and spatial restriction I. Behavioral response. *Physiology and Behavior 66*: 233-242

Blackburn-Munro G 2004 Pain-like behaviours in animals – how human are they? *Trends in Pharmacological Science 25*: 299-305

Blecha F 2000 Immune system response to stress. In: Moberg GP and Mench JA (eds) *The Biology Of Animal Stress* pp 111-122. CABI Publishing: Wallingford, UK

Ceròn JJ, Eckersall DP and Martinez-Subiela S 2005 Acute phase proteins in dogs and cats: current knowledge and future perspectives. *Veterinary Clinical Pathology 34*: 85-99

Conner JG and Eckersall PD 1988 Acute phase response in the dog following surgical trauma. *Research in Veterinary Science 45*: 107-110

Coppola CL, Grandin T and Enns RM 2006 Human interaction and cortisol: Can human contact reduce stress for shelter dogs? *Physiology and Behavior 87*: 537-541

Crowell-Davis S and Murray T 2006 *Veterinary Psychopharmacology*. Blackwell Publishing: Ames, Iowa, USA

Cuadra G, Zurita A, Lacerra CMN and Molina A 1999 Chronic stress sensitizes frontal cortex dopamine release in response to a subsequent novel stressor: reversal by naloxone. *Brain Research Bulletin 48*: 303-308

Dantzer R and Mormede P 1995 Psychoneuroimmunology of stress. In: Leonard B and Miller K (eds) *Stress, the Immune System and Psychiatry* pp 47-67. John Wiley and Sons Ltd: New York, USA

Delogu G, Moretti S, Famularo G, Marcellini S, Santini G, Antonucci A, Marandola M and Signore L 2001 Mitochondrial perturbations and oxidant stress in lymphocytes from patients undergoing surgery and general anesthesia. *Archives of Surgery 136*: 1190-1196

Desborough JP 2000 The stress response to trauma and surgery. *British Journal of Anaesthesia 85*: 109-117

Devitt CM, Cox RE and Hailey JJ 2005 Duration, complication, stress and pain of open ovariohysterectomy versus a simple method of laparoscopic-assisted ovariohysterectomy in dogs. *Journal of Veterinary Medical Association 227*: 921-927

Fox MS, Mellor DG, Lawoko CRO, Hodge H and Firth C 1998 Changes in plasma cortisol concentrations in bitches in response to different combinations of halothane and butorphanol, with or without ovariohysterectomy. *Research in Veterinary Science 65*: 125-133

Giescke K, Hamberger B, Jamberg PD, Klingstedt C and Pearson B 1988 High and low dose fentanyl anaesthesia: hormonal and metabolic response during cholecystectomy. *British Journal of Anaesthesia 61*: 575-582

Goodmann PA, Klinghammer E and Willard J 2002 *Wolf Ethogram, Ethology Series No 3*. EH Hess Institute of Ethology: Battleground, USA

Griffis CA, Compton P and Doering L 2006 The effect of pain on leukocyte cellular adhesion molecules. *Biological Research for Nursing 7*: 297-312

Hansen BD, Hardie EM and Carroll GS 1997 Physiological measurements after ovariohysterectomy in dogs: what's normal? *Applied Animal Behaviour Science 51*: 101-109

Hardie EM, Hansen BD and Carrol GS 1997 Behaviour after ovariohysterectomy in the dog: what's normal? *Applied Animal Behavioural Science 51*: 111-128

Hart BL 1985 Behavioural indications for phenotiazine and benzodiazepine tranquilizers in dogs. *Journal of American Veterinary Medical Association 186*: 192-194

Hetts S, Clark Derrel J, Calpin JP, Arnold CE and Mateo JM 1992 Influence of housing conditions on beagle behaviour. *Applied Animal Behavioural Science 34*: 137-155

Holton L, Reid J, Scott EM, Pawson P and Nolan A 2001 Development of a behaviour-based scale to measure acute pain in dogs. *Veterinary Record 148*: 525-531

Hubrecht RC, Serpell JA and Poole T 1992 Correlates of pen size and housing conditions in the behaviour of kennelled dogs. *Applied Animal Behavioural Science 34*: 365-383

Kobelt AJ, Hemsworth PH, Barnett JL and Butler KL 2003 Sources of sampling variation in saliva cortisol in dogs. *Research in Veterinary Science 75*: 157-161

Mandel I 1990 The diagnostic use of saliva. *Journal of Oral Phatology Medicine 19*: 119-125

Marrocco-Trischitta MM, Tiezzi A, Svampa MG, Bandiera G, Camilli S, Stillo F, Petasecca P, Sampogna F, Abeni D and Gurrini P 2004 Perioperative stress response to carotid endarterectomy: the impact of anaesthetic modality. *Journal of Vascular Surgery 39*: 1295-1304

Martin P and Bateson P 1993 *Measuring Behaviour: An Introductory Guide, Second Edition*. Cambridge University Press: Cambridge, UK

Mason WA 2000 Early developmental influences of experience on behavior, temperament and stress. In: Moberg GP and Mench JA (eds) *The Biology Of Animal Stress* pp 269-290. CABI Publishing: Wallingford, UK

Mathews KA 2000 Pain assessment and general approach to management. *Veterinary Clinics of North America: Small Animal Practice 30*: 729-755

Matteri RL, Carroll JA and Dyer CJ 2000 Neuroendocrine response to stress. In: Moberg GP and Mench JA (eds) *The Biology Of Animal Stress* pp 43-76. CABI Publishing: Wallingford, UK

Mellor DJ, Cook CJ and Stafford KJ 2000 Quantifying some responses to pain as a stressor. In: Moberg GP and Mench JA (eds) *The Biology Of Animal Stress* pp 171-198. CABI Publishing: Wallingford, UK

Moberg GP 2000 Biological response to stress: implications for animal welfare. In: Moberg GP and Mench JA (eds) *The Biology Of Animal Stress* pp 1-22. CABI Publishing: Wallingford, UK

Morton DB and Griffiths PHM 1985 Guidelines on the recognition of pain, distress and discomfort in experimental animals and a hypothesis for assessment. *Veterinary Record 20*: 431-436

Morton CM, Reid J, Scott EM, Holton LL and Nolan AM 2005 Application of a scaling model to establish and validate an interval level pain scale for assessment of acute pain in dogs. *American Journal of Veterinary Research 66*: 2154-2166

Murata H, Shimada H and Yoshioka 2004 Current research on acute phase proteins in veterinary diagnosis: an overview. *The Veterinary Journal 168*: 28-40

Odendaal JS and Meintjes RA 2003 Neurophysiological correlates of affiliative behaviour between humans and dogs. *The Veterinary Journal 165*: 296-301

Pageat P 1998 *Pathologie Du Comportement Du Chien, Second Edition*. Le Point Vétérinaire: Maisons-Alfort, France. [Title translation: Behavioural Pathology in the Dog]

Pageat P and Gaultier 2003 Using prolactin blood level in the diagnosis of anxiety related disorders in dogs. In: Heath S and DeKeuster T (eds) *Therapeutic Approaches in Veterinary Behavioural Medicine. Proceedings of the Ninth ESVCE Congress* pp 35-36. 19 September 2003, Salzburg, Austria. ESVCE: Lovendegem, Belgium

Pageat P 2005 Assessing prolactinemia in anxious dogs (*Canis familiaris*): interest in diagnostic value and use in the selection of the most appropriate psychotropic drug. In: Mills D, Levine E, Landsberg G, Horwitz D, Duxbury M, Mertens P, Meyer X, Huntley LR, Reich M and Willard J (eds) *Current Issues and Research in Veterinary Behavioural Medicine. Papers presented at the Fifth International Behaviour Meeting*. 14-16 July 2005, West Lafayette, Indiana, USA: Purdue University Press, USA

Pageat P, Lafont C, Falewée C, Bonnafous L, Gaultier E and Sillart B 2007 An evaluation of serum prolactin in anxious dogs and response to treatment with selegiline or fluoxetine. *Applied Animal Behaviour Science 105*: 342-350

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

Roughan JV and Flecknell PA 2003 Evaluation of a short duration behaviour-based post-operative pain scoring system in rats. *European Journal of Pain 7*: 397-406

Rushen J 2000 Some issues in the interpretation of behavioural response to stress. In: Moberg GP and Mench JA (eds) *The Biology Of Animal Stress* pp 23-41. CABI Publishing: Wallingford, UK

Sanford J, Ewbank R, Molony V, Tavernor WD and Uvarov O 1986 Guidelines for the recognition and assessment of pain in animals. *Veterinary Record 118*: 334-338

Schultze AE 2000 Interpretation of canine leukocyte responses. In: Feldman BJ, Zinkl JG and Jain NC (eds) *Shalm's Veterinary Ematology* pp 366-381. Lippincott Williams and Wilkins: Baltimora, USA

Sibanda S, Hughes JM, Pawson PE, Kelly G and Bellenger CR 2006 The effects of preoperative extradural bupivocaine and morphine on the stress response in dogs undergoing femoro-tibial joint surgery. *Veterinary Anaesthesia and Analgesia 33*: 246-257

Stockham SL, Keeton KS and Szlatovits B 2003 Clinical assessment of leukocytosis: distinguishing leukocytoses caused by inflammatory, glucocorticoid, physiologic and leukemic disorders or conditions. *Veterinary Clinics of North America: Small Animal Practice 33*: 1335-1357

Stover SM, Steffey EP, Dybdal NO and Franti CE 1998 Hematologic and serum biochemical alterations associated with multiple halothane anesthesia exposures and minor surgical trauma in horses. *American Journal of Veterinary Research 49*: 236-241

Tayama E, Hayashida N, Oda T, Tomoeda H, Akasu K, Kosuga T, Fukunaga S, Akashi H, Kawara T and Aoyagi S 1999 Recovery from lymphocytopenia following extracorporeal circulation: simple indicator to assess surgical stress. *Artificial Organs 23*: 736-740

Taylor PM 1998 Effects of surgery on endocrine and metabolic responses to anaesthesia in horses and ponies. *Research in Veterinary Science 64*: 133-140

Thompson SB 1998 Pharmacological treatment of phobias. In: Dodman NH and Shuster L (eds) *Psychopharmacology of Animal Behaviour Disorders* pp 141-184. Blackwell Science Inc: Malden, USA

Väisänen MN, Raekallio M, Kuusela E, Huttumen P, Leppäluoto J, Kirves P and Vainio O 2002 Evaluation of the perioperative stress response in dogs administered medetomidine or acepromazine as part of the preanesthetic medication. *American Journal of Veterinary Research 63*: 969-975

Väisänen MA, Valros AE, Hakaoja E, Raekallio MR and Vainio OM 2005 Pre-operative stress in dogs: a preliminary investigation of behaviour and heart rate variability in healthy hospitalized dogs. *Veterinary Anaesthesia and Analgesia 32*: 158-167

Vetter TR and Heiner EJ 1996 Discordance between patient self reported visual analogue scale pain scores and observed pain-related behavior in older children after surgery. *Journal of Clinical Anesthesia 8*: 371-375

Wells DL 2004 A review of environmental enrichment for kennel dogs, *Canis familiaris*. *Applied Animal Behaviour Science 85*: 307-317

Yamamoto S, Shida T, Miyaji S, Santsuka H, Fuise H, Mukawa K, Furukawa E, Nagae T and Naiki M 1993 Changes in serum C-reactive protein levels in dogs with various disorders and surgical traumas. *Veterinary Research Communications 17*: 85-93

Zahorec R 2001 Ratio of neutrophil to lymphocyte counts: rapid and simple parameter of systemic inflammation and stress in critically ill. *Bratislava Medical Journal 102*: 5-14

Zakowsi SG, McAllister CG, Deal M and Baum A 1992 Stress, reactivity, and immune function in healthy men. *Health Psychology 11*: 223-232